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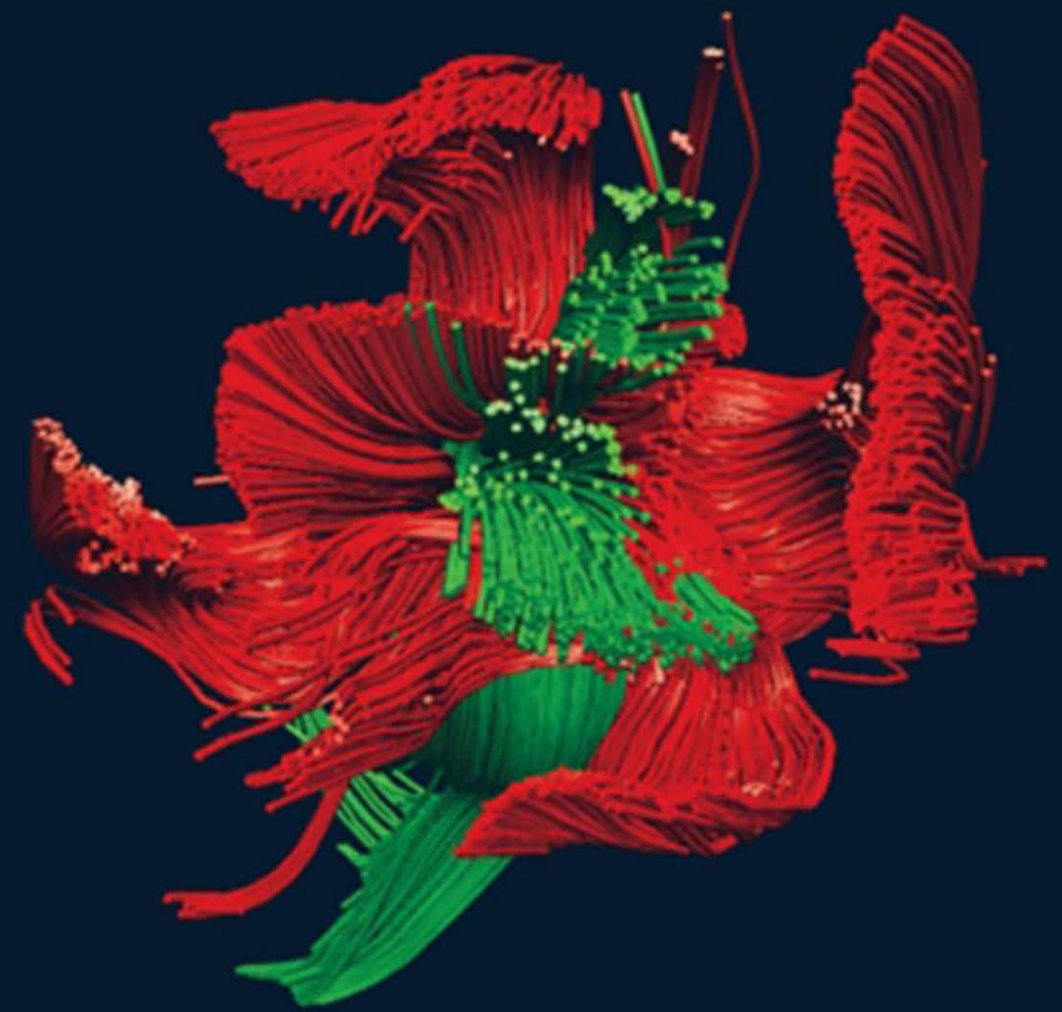
The Anatomical Basis of Clinical Practice



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FORTY-FIRST EDITION

Anatomy

The Anatomical Basis of Clinical Practice

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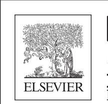

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COVER IMAGE

Reconstruction of the short and long connections of the hand region in the living human brain using advanced diffusion tractography developed by the NatBrainLab (www.natbrainlab.com). The connections of the hand region resemble a 'poppy flower' with a green stem representing the long projection fibres and four red 'petals' connecting the precentral cortex to postcentral and premotor cortices. This network is important for motor learning and execution of reaching and grasping hand movements (see chapter 25). (Image courtesy of Dr Marco Catani, originally published in Catani M, Dell'Acqua F, Vergani F, et al; Short frontal lobe connections of the human brain. 2012 *Cortex* 48:273–91.)

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PREFACE

'Anatomy is the basis of medical discourse.'

(Hippocrates, *De locis in homine* 2)

Looking through an almost complete set of the previous editions of *Gray's Anatomy*, I am struck by the marked difference in size between the first and fortieth editions. That progressive increase in girth has occurred *pari passu* with ground-breaking advances in basic science and clinical medicine over the past 155 years. Anatomy has become a far wider discipline than Henry Gray, Henry van Dyke Carter or any of their students could have envisaged. Fields such as cell biology, molecular genetics, neuroanatomy, embryology and bioinformatics either had not emerged or were in their infancy in 1858. Techniques that today inform our view of the internal landscape of the body – such as specialized types of light and electron microscopy; imaging modalities, including X-rays, magnetic resonance imaging, computed tomography and ultrasonography; the use of 'soft' perfusion techniques and frozen-thawed, unembalmed cadavers for dissection-based studies; and the advances in information technology that enable endoscopic and robotic surgery and facilitate minimally invasive access to structures previously considered inaccessible – were all unknown. As each development entered mainstream scientific or clinical use, the new perspectives on the body it afforded, whether at submicroscopic or macroscopic level, filtered into the pages of *Gray's Anatomy*: for example, the introduction of X-ray plates (twenty-seventh edition, 1938) and electron micrographs (thirty-second edition, 1958).

In the Preface to the first edition, Henry Gray wrote that *'This Work is intended to furnish the Student and Practitioner with an accurate view of the Anatomy of the Human Body, and more especially the application of this science to Practical Surgery'.* We remain true to his intention. An appropriate knowledge of clinically relevant, evidence-based anatomy is an essential element in the armamentarium of a practising clinician; indeed, 'If anything, the relevance of anatomy in surgery is more important now than at any other time in the past' (Tubbs, in Preface Commentary, which accompanies this volume).

In my Preface to the fortieth edition, I intimated that the book was quite literally in danger of breaking its binding if any more pages were added. In order to avoid this unfortunate occurrence, the forty-first edition contains a significant amount of material that is exclusively electronic, in the form of 77,000 words of additional text, 300 artworks and tables, 28 videos and 24 specially invited commentaries on topics as diverse as electron microscopy and fluorescence microscopy; the neurovascular bundles of the prostate; stem cells in regenerative medicine; the anatomy of facial ageing; and technical aspects and applications of diagnostic radiology. In keeping with the expectation that anatomy should be evidence-based, the forty-first edition contains many more references in the e-book than could be included in the thirty-ninth and fortieth printed editions.

Neel Anand, Rolfe Birch, Pat Collins, Alan Crossman, Michael Gleeson, Ariana Smith, Jonathan Spratt, Mark Stringer, Shane Tubbs, Alan Wein and Caroline Wigley brought a wealth of scholarship and experience as anatomists, cell biologists and clinicians to their roles as Section Editors. I thank them for their dedication and enthusiastic support, in selecting and interacting with the authors in their Sections and for meeting deadlines, despite the ever-increasing demands on their time from university and/or hospital managers. Pat Collins, Girish Jawaheer, Richard Tunstall and Caroline Wigley worked closely with many authors to update the text and artworks for organogenesis, paediatric anatomy, evidence-based surface anatomy and microstructure, respectively, across Sections 3 to 9. Jonathan Spratt acted as both a Section Editor (thorax) and an indefatigable 'go to' for sourcing images throughout the book; in the latter capacity, he has produced a superb collection of additional labelled images, available in the e-book (see Bonus imaging collection). Over a hundred highly experienced anatomists and clinicians contributed text, often extensively revised from the previous edition, and/or artworks, original micrographs or other images to individual chapters.

As a general rule, the orientation of diagrams and photographs throughout the book has been standardized to show the left side of the body, irrespective of whether a lateral or medial view is presented, and transverse sections are viewed from below to facilitate comparison with clinical images. Clinicopathological examples have been selected where the pathology is either a direct result, or a consequence, of the anatomy, or where the anatomical features are instrumental in the diagnosis/treatment/management of the condition. Wherever possible, the photomicrographs illustrate human histology and embryology; non-human sources are acknowledged in the captions.

In an ideal world, anatomical terminology would satisfy both anatomists and clinicians. For the avoidance of doubt, the same word should be agreed and used for each structure that is described, whether in the anatomy laboratory or the clinic. In the real world, this goal is achieved with varying degrees of success; alternative terms (co)exist and may (and frequently do) confuse or frustrate. Currently, *Terminologia Anatomica* (TA)[1] is the reference source for the terminology for macroscopic anatomy; the text of the forty-first edition of *Gray's Anatomy* is almost entirely TA-compliant. However, where terminology is at variance with, or, more likely, is not included in, the TA, the alternative term that is chosen either is cited in the relevant consensus document or position paper – e.g. 'European Position Paper on the Anatomical Terminology of the Internal Nose and Paranasal Sinuses'[2] and the International Interdisciplinary Consensus Statement on the 'Nomenclature of the Veins of the Lower Limbs'[3] – or enjoys widespread clinical usage: for example, the use of attitudinally appropriate terms in cardiology (see Chapter 57). The continued use of eponyms is contentious.[4] Proponents of their retention argue that some eponyms are entrenched in medical language and are (therefore) indispensable, that they facilitate communication because their use is so pervasive and that they serve to remind us of the humanism of medicine. Detractors argue that eponyms are inherently inaccurate, non-scientific and often undeserved. In this edition of *Gray's Anatomy*, synonyms and eponyms are given in parentheses on first usage of a preferred term and not shown thereafter in the text; an updated list of eponyms remains available in the e-book for reference purposes.

I offer my sincere thanks to the editorial team at Elsevier, initially under the leadership of Madelene Hyde and latterly of Jeremy Bowes, for their guidance, professionalism, good humour and unfailing support. In particular, I thank Poppy Garraway, Humayra Rahman Khan, Wendy Lee, Joanna Souch, Julie Taylor, Jan Ross and Louise Cook, for being at the end of a phone or available by e-mail whenever I needed advice or support.

I dedicate my work on the forty-first edition of *Gray's Anatomy* to the memory of my late husband, Guy Standring.

Susan Standring
January 2015

[1] *Terminologia Anatomica* (1998) is the joint creation of the Federative Committee on Anatomical Terminology (FCAT) and the Member Associations of the International Federation of Associations of Anatomists (IFAA).

[2] Lund VJ, Stammberger H, Fokkens WJ et al 2014 European position paper on the anatomical terminology of the internal nose and paranasal sinuses. Rhinol Suppl 24:1–34.

[3] Caggiati A, Bergan JJ, Gloviczki P et al; International Interdisciplinary Consensus Committee on Venous Anatomical Terminology 2005 Nomenclature of the veins of the lower limb: extensions, refinements, and clinical application. J Vasc Surg 41:719–24.

[4] Amarnani A, Brodell RT, Mostow EN 2013 Finding the evidence with eponyms. JAMA Dermatol 149:664–5; Fargen KM, Hoh BL 2014 The debate over eponyms. Clin Anat 27:1137–40; Lo WB, Ellis H 2010 The circle before Willis: a historical account of the intracranial anastomosis. Neurosurgery 66:7–18; Ma L, Chung KC 2012 In defense of eponyms. Plast Reconstr Surg 129:896e–8e.

The continuing relevance of anatomy in current surgical practice and research

PREFACE COMMENTARY

R Shane Tubbs

When our anatomy forebears embarked on the uncharted study of the human body, they did so without reference. Their focus was to chart and map the body simply to learn and describe intricacies never chronicled before. The anatomical 'map' we use today came about thanks to figures such as da Vinci, Vesalius, Cheselden and, more recently, Henry Gray. On the shoulders of these giants, we see farther than our predecessors. In *The Metalogicon*, published in 1159, John Salisbury recognized the profound observation of French philosopher Bernard of Chartres, who declared that *'...we are like dwarfs on the shoulders of giants, so that we can see more than they, and things at a greater distance, not by virtue of any sharpness of sight on our part, or any physical distinction, but because we are carried high and raised up by their giant size'*. So, with the gross anatomy of man presumed, by many scholars, to have been described and understood long ago, how does the modern anatomist bring relevance to the continued study of morphology? Is there any uncharted territory for the modern anatomist to plot in order to sustain our field of study and for it to continue to be perceived as relevant to an educational world, and to medical and dental curricula in which the time allotted to anatomical study has significantly waned? Simply put, yes. Henry Gray, based on the title of his original text, *Anatomy, Descriptive and Surgical*, knew very well that there was a need to refocus the lenses of teaching and research in the anatomical sciences, and to expand and explore their surgical relevance. Our gross anatomical map of the human body must continue to be updated and legends must continue to be placed on that map to incorporate modern advances in technology. New methods of surgery, such as laparoscopy and endoscopy, as well as the use of the surgical microscope, offer the opportunity to view the human form in a different light and in greater surgical detail than ever before. If anything, the relevance of anatomy in surgery is more important now than at any other time in the past. The modern surgeon must take what is learned macroscopically, in the dissection room, and apply this knowledge to structures seen under magnification and through instruments that provide a surgical field that is, at times, just millimetres in diameter. Therefore, attention to anatomical detail is of vital importance as references and anatomical landmarks are minimized in the surgical theatre of the new millennium.

As mentioned before, early anatomists dissected with curiosity about the unknown and gained knowledge that would become a prerequisite for proper surgical manœuvres. Today, as anatomists, our anatomical knowledge should create in us a curiosity about what we can do with the knowledge that we have gained. The ability to apply that knowledge offers an opportunity to be an integral part of the ever-progressing field of surgery. For example, today, surgical problems are often the impetus for dissection studies, which can influence the way in which surgery is performed and, moreover, can sway the way in which anatomy is taught (e.g. redefining a focus in condensed curricula and with decreased work hours for house officers). Surgically, dissection studies have allowed us to manipulate known human anatomy and to solve, for example, complex neurological problems. As an illustration of the surgical relevance of modern-day anatomical studies for neurological pathologies, we have conducted, in my laboratory, cadaveric feasibility studies that suggested that the phrenic nerve could be reinnervated in high quadriplegic patients who are ventilator-dependent (a morbid condition with an associated high mortality rate) by using the intact, adjacent accessory nerve (i.e. neurotization) (Tubbs et al 2008a) (**Fig. 1.6.1**). The theory behind this investigation was that the functioning accessory nerve would be used to form a new circuit between it and the dysfunctional phrenic nerve, and that this would allow recovery of diaphragm function. For this technique, a longitudinal incision was made along the lower half of the posterior border of sternocleidomastoid. Dissection was then performed in order to identify both the accessory nerve at this level, at its entrance into trapezius, and the phrenic nerve crossing anterior to scalenus anterior. The medial half of the accessory nerve was then split away from its lateral half and transected at its entrance into muscle. This distally disconnected medial half of the nerve was then swung medially to the phrenic nerve, which had been transected proximally. The two nerves were then sutured together without tension. This 'rearranging' of human anatomy has now been employed clinically with success. Yang et al (2011) used our study results to treat a 44-year-old man with complete spinal cord injury at the C2 level. Clinically, left diaphragm activity was decreased and the right diaphragm was completely paralysed. Four weeks after surgery, training of the synchronous activities of trapezius and inspiration was conducted. Six months after surgery, motion was observed in the previously paralysed right diaphragm. Evaluation of lung function indicated improvements in vital capacity and tidal volume. The patient was able to sit in a wheelchair and conduct activities without assisted ventilation 12 months after surgery. For the surgeon, such manipulation of anatomy requires a comprehensive understanding not only of normal anatomy but also of what might occur functionally by rewiring such nerves. For example, patients undergoing this surgery will initially need to think of moving their trapezius to activate their diaphragm. With time, this will not be the case. Similar illustrations of the plasticity of the brain have been seen in patients undergoing hypoglossal to facial nerve neurotization procedures; these patients at first need to think of moving their tongue in order for their facial muscles to contract.

Rewiring of nerves has been addressed in other studies. Thus, we have shown, first in a cadaveric study (Hansasuta et al 2001) and then clinically (Wellons et al 2009), that the medial pectoral nerve can be sectioned near its entrance into the deep surface of pectoralis major and swung round and sewn into the musculocutaneous nerve (**Fig. 1.6.2**). If this procedure is successful, axonal regrowth from the medial pectoral nerve into the musculocutaneous nerve (about 1 mm/day) will re-establish function in the anterior arm muscles; the loss of clinically significant function of the dually innervated pectoralis major is minimal and the functional gain of having the anterior arm muscles work is significant (Wellons et al 2009). Being able to bring the hand to the mouth and feed oneself is a task that most take for granted. In children with birth-related injuries to the upper brachial plexus (i.e. Erb's palsy), this movement is often the difference between waiting to be fed or feeding oneself. This method has been used at our institution for over 15 years with an 80% success rate, where success is measured as the patient regaining function of arm flexion.

Another example of what we have termed 'reverse translational research in anatomy' (i.e. from the bed to the bench and back) is the location of new anatomical diversionary sites (in this case, the medullary cavity of the ilium) that could be used in patients with cerebrospinal fluid absorption problems (i.e. hydrocephalus) and in whom the traditionally used receptacles for absorbing this diverted cerebrospinal fluid (e.g. peritoneal and pleural cavities, heart) are not options, as a consequence of e.g. malabsorption or local infection (Tubbs et al 2015) (**Fig. 1.6.3**). This alternative site has, for the first time, just been used and with success (unpublished data). Although not proven clinically, an earlier study in primates showed that the manubrium of the sternum could also be used as a distal receptacle for cerebrospinal fluid collection (Tubbs et al 2011). After tubing was tunnelled from the cannulated ventricle, the distal tubing was inserted subcutaneously into the superior aspect of the midline manubrium, where a small hole had been drilled. Up to 50 ml of saline per hour could be infused into the primate sternum without vital sign changes. This study, and the study using the ilium as a depository, both demonstrate the anatomical continuity between the bony medullary cavities and the vascular system. Such positive effects on patient outcomes not only make the study of human anatomy from a slanted perspective extremely gratifying, but are also practical since the results have direct application in the surgical theatre.

In addition to surgical anatomy playing a role in new uses of the normal anatomy, this field can also explore and direct new surgical approaches where the goals are to make surgery more effective and

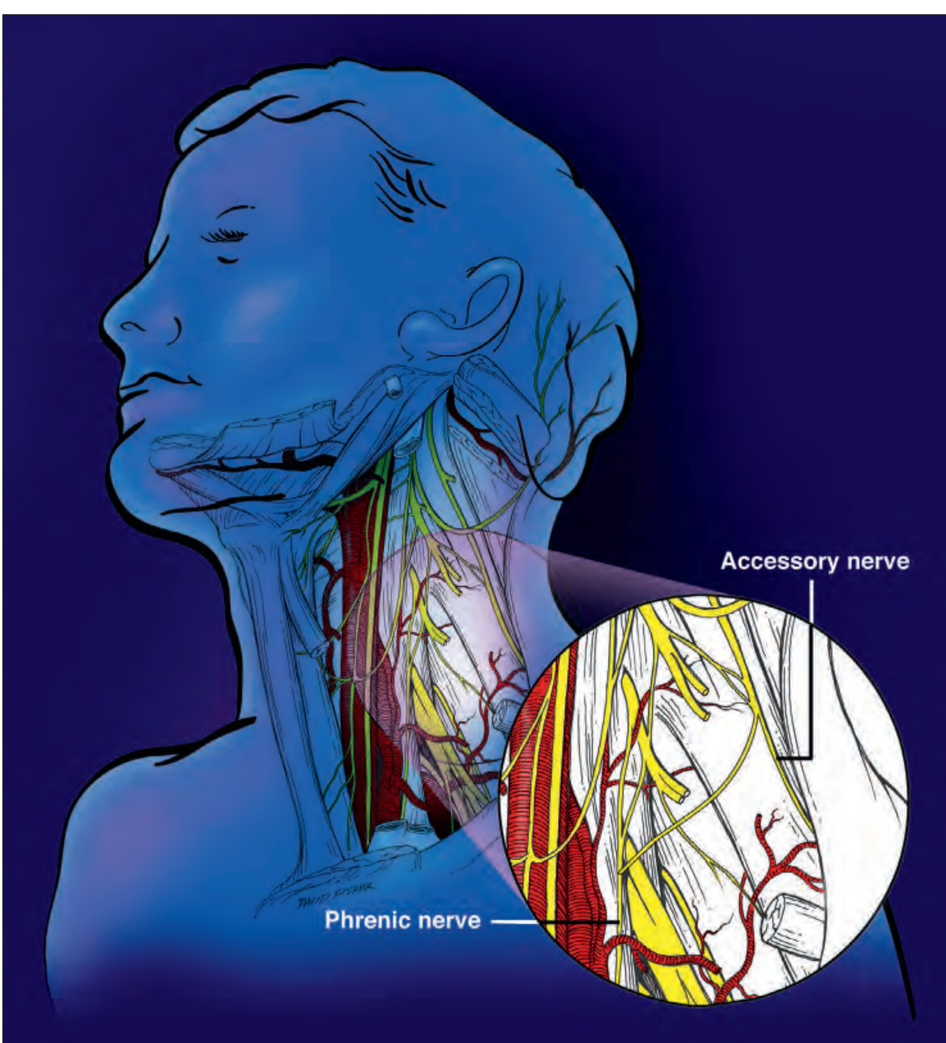

Fig. 1.6.1 A schematic representation of the anatomically defined technique of using the accessory nerve for neurotization of the phrenic nerve with application to patients with high cervical quadriplegia who are ventilator-dependent. With nerve regrowth, axons from the intact and functioning accessory nerve travel into the phrenic nerve to reinnervate this nerve and restore diaphragmatic function. In this example, only one-half of the accessory nerve is used in order to maintain some function of trapezius. (Drawn by Mr David Fisher.)

Fig. 1.6.2 The neurotization of the musculocutaneous nerve with the medial pectoral nerve (inset). Similar to the example illustrated in Figure 1.6.1, such a method of nerve repair is employed in the hope that a patient with an upper brachial plexus injury and anterior arm muscles that are dysfunctional can regain function by regrowth of axons from the intact medial pectoral nerve into and along the musculocutaneous nerve. (Drawn by Mr David Fisher.)

minimally invasive, and involve fewer complications. For example, we have performed feasibility studies looking at a wide range of novel approaches that might be used by the surgeon. These include a dorsal approach to the carpal tunnel for an entrapped median nerve (Tubbs et al 2005a); an anterior approach to the sciatic nerve potentially compressed by piriformis via the obturator foramen (Tubbs, unpublished data); an anterior approach to the upper thoracic vertebrae for spine fusion procedures (Tubbs et al 2010a); an intra-abdominal laparoscopic approach to decompress the pudendal nerve (Loukas et al 2008); and midline endoscopic approaches to the fourth ventricle with application to decompressing a 'trapped' fourth ventricle, as is seen in some cases of hydrocephalus (Tubbs et al 2004). We have also explored the feasibility in cadavers of using endoscopy for exploration of pathologies of the thecal sac (Chern et al 2011). In a series of children with intraspinal pathology (arachnoid cyst, spinal cord tumour, holocord syrinx and split cord malformation), intradural spinal endoscopy was a useful treatment, resulted in a more limited laminectomy and myelotomy, and, in one case, assisted in identifying a residual spinal cord tumour. It was also useful in the fenestration of a multilevel spinal arachnoid cyst and in confirming communication of fluid spaces in the setting of a complex holocord syrinx. Endoscopy aided the visualization of the spinal cord to ensure the absence of tethering in the case of split spinal cord malformation. These endoscopic approaches were only possible by knowing the normal anatomy and how it appears in a confined field of view, as first seen in the anatomy laboratory.

Lastly, the anatomist can add to the relevance of anatomy for the surgeon with studies that have an impact on the identification or avoidance of important structures during operative manœuvres (i.e. anatomical landmark studies). My group has defined surgical landmarks for anatomical structures such as the superior and inferior gluteal nerves (Apaydin et al 2013, Apaydin et al 2009); vein of Labbé (Tubbs et al 2012); sigmoid sinus (Tubbs et al 2009a); amygdala (Tubbs et al

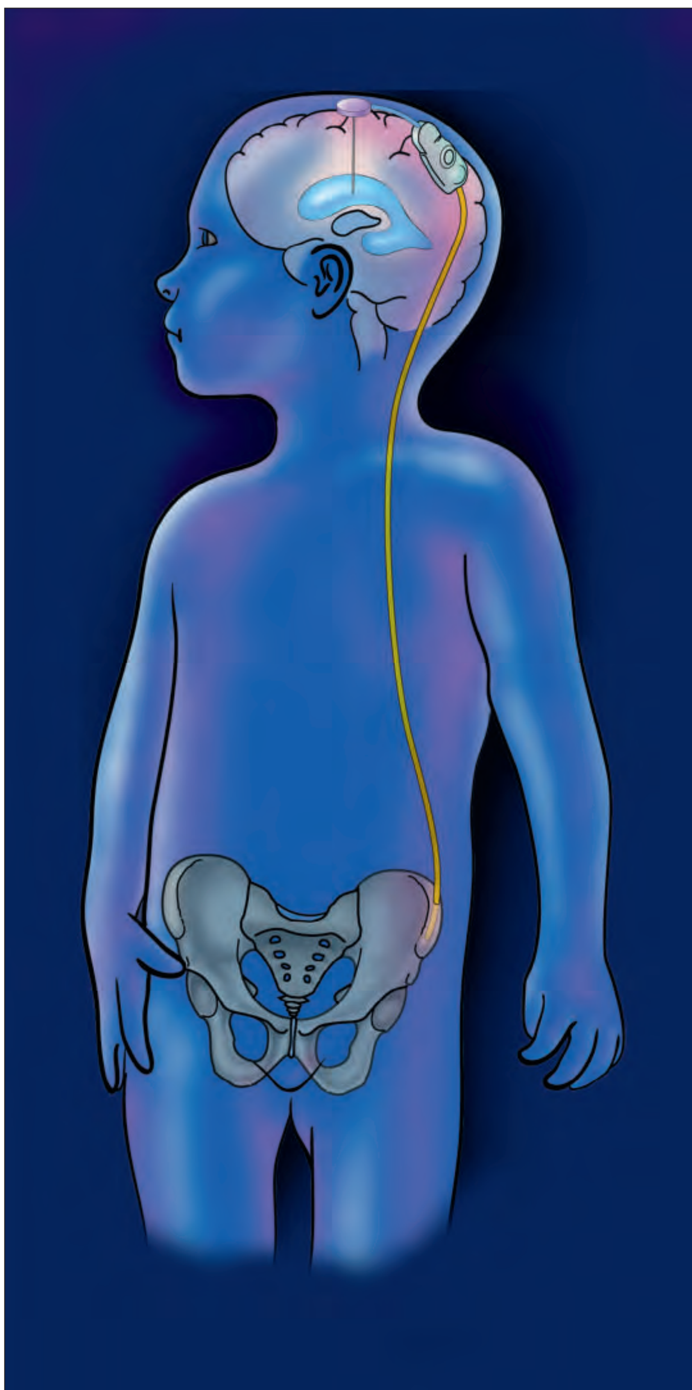

Fig. 1.6.3 The technique used in a patient with hydrocephalus to divert cerebrospinal fluid from the cerebral ventricles to the ilium. The enlarged ventricles are cannulated with a catheter connected to a subcutaneous valve that drains into tubing tunnelled under the skin and then implanted into the medullary cavity of the ilium; here, the cerebrospinal fluid is absorbed into the vascular system. The techniques described in Figures 1.6.2 and 1.6.3, based on surgical problems and manipulation of known anatomy for surgical benefit, were evaluated and studied in the anatomy laboratory, and have now been used clinically. (Drawn by Mr David Fisher.)

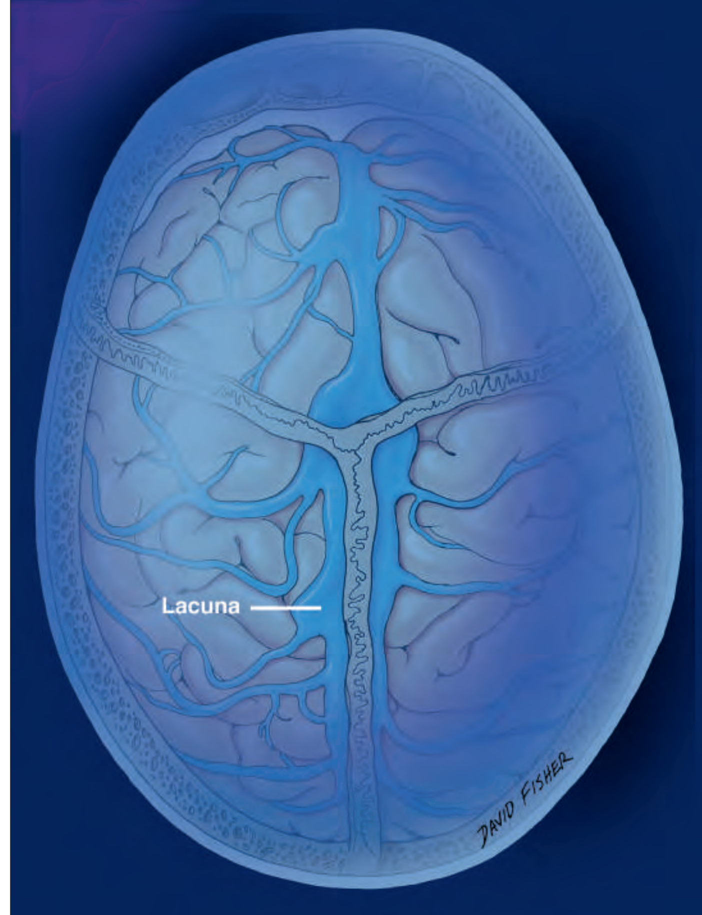

Fig. 1.6.4 A superior view of the cranium, with the underlying superior sagittal sinus, cortical veins and lateral lacunae illustrated. This study explored the relationship between the underlying lateral lacunae and the overlying coronal and sagittal sutures, and made measurements between these structures. Neurosurgically, the initial placement of burr-holes avoids the midline in order to prevent damage to the superior sagittal sinus. However, the intracranial entrance of the drill often injures more laterally placed lacunae. Using surface anatomy based on anatomical landmarks, a neurosurgeon can be more aware of the locations of these underlying structures while performing craniotomies. Such landmarks have now been used by neurosurgeons at our institution. (Drawn by Mr David Fisher.)

2010b); buccal branch of the trigeminal nerve (Tubbs et al 2010c); radial nerve and posterior interosseous branch (Cox et al 2010, Tubbs et al 2006a); perineal branch of the posterior femoral cutaneous nerve (Tubbs et al 2009b); lateral lacunae (Tubbs et al 2008b) (**Fig. 1.6.4**); basal vein of Rosenthal (Tubbs et al 2007); greater occipital nerve (Loukas et al 2006); long thoracic nerve (Tubbs et al 2006b); anterior interosseous nerve (Tubbs et al 2006c); accessory nerve (Tubbs et al 2005b); lumbar plexus and its branches (Tubbs et al 2005c); trochlear nerve (Tubbs and Oakes 1998); and frontal sinus (Tubbs et al 2002). Such studies might assist in decreasing the morbidity and increasing the efficiency of surgical approaches and certainly illustrate the surgical relevance of anatomy. Moreover, this list exemplifies the multitude of anatomical structures that may be given greater surgical relevance by addressing how they may be more accurately located in the operating theatre.

In this day and age, if anatomists are not to lose their footing and simply be considered teachers of an old and outdated discipline, the onus is on us to renew interest in our field with timely and salient studies that gird the loins of a profession that is in danger of becoming extinct. It is my opinion, and that of others, that one effective way to achieve this is to remind the world by demonstrations such as those listed here that the study of anatomy is as clinically relevant today as it was at its humble beginnings. Considering the adage that anatomy is the oldest child of Mother Medicine, the fact that surgical problems and anatomical studies go hand in hand is obvious – anatomical research is not a 'dead' science! The modern relevance of anatomy to surgical practice and research must not be underestimated.

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HISTORICAL INTRODUCTION

Gray's Anatomy is now on its way to being 160 years old. The book is a rarity in textbook publishing in having been in continuous publication on both sides of the Atlantic Ocean, since 1858. One and a half centuries is an exceptionally long era for a textbook. Of course, the volume now is very different from the one Mr Henry Gray first created with his colleague Dr Henry Vandyke Carter, in mid-Victorian London. In this introductory essay, I shall explain the long history of *Gray's*, from those Victorian days right up to today.

The shortcomings of existing anatomical textbooks probably impressed themselves on Henry Gray when he was still a student at St George's Hospital Medical School, near London's Hyde Park Corner, in the early 1840s. He began thinking about creating a new anatomy textbook a decade later, while war was being fought in the Crimea. New legislation was being planned that would establish the General Medical Council (1858) to regulate professional education and standards.

Gray was twenty-eight years old, and a teacher himself at St George's. He was very able, hard-working and highly ambitious, already a Fellow of the Royal Society, and of the Royal College of Surgeons. Although little is known about his personal life, his was a glittering career so far, achieved while he served and taught on the hospital wards and in the dissecting room (Fig. 1) (Anon 1908).

Gray shared the idea for the new book with a talented colleague on the teaching staff at St George's, Henry Vandyke Carter, in November 1855. Carter was from a family of Scarborough artists, and was himself a clever artist and microscopist. He had produced fine illustrations for Gray's scientific publications before, but could see that this idea was a much more complex project. Carter recorded in his diary:

> *Little to record. Gray made proposal to assist by drawings in bringing out a Manual for students: a good idea but did not come to any plan … too exacting, for would not be a simple artist* (Carter 1855).

Neither of these young men was interested in producing a pretty book, or an expensive one. Their purpose was to supply an affordable, accurate teaching aid for people like their own students, who might soon be required to operate on real patients, or on soldiers injured at Sebastopol or some other battlefield. The book they planned together was a practical one, designed to encourage youngsters to study anatomy, help them pass exams, and assist them as budding surgeons. It was not simply an anatomy textbook, but a guide to dissecting procedure, and to the major operations.

Fig. 1 Henry Gray (1827–1861) is shown here in the foreground, seated by the feet of the cadaver. The photograph was taken by a medical student, Joseph Langhorn. The room is the dissecting room of St George's Hospital Medical School in Kinnerton Street, London. Gray is shown surrounded by staff and students. When the photo was taken, on 27 March 1860, Carter had left St George's, to become Professor of Anatomy and Physiology at Grant Medical College, in Bombay (nowadays Mumbai). The second edition of *Gray's Anatomy* was in its proof stages, to appear in December 1860. Gray died just over a year later, in June 1861, at the height of his powers.

Gray and Carter belonged to a generation of anatomists ready to infuse the study of human anatomy with a new, and respectable, scientificity. Disreputable aspects of the profession's history, acquired during the days of body-snatching, were assiduously being forgotten. The Anatomy Act of 1832 had legalized the requisition of unclaimed bodies from workhouse and hospital mortuaries, and the study of anatomy (now with its own Inspectorate) was rising in respectability in Britain. The private anatomy schools that had flourished in the Regency period were closing their doors, and the major teaching hospitals were erecting new, purpose-built dissection rooms (Richardson 2000).

The best-known student works when Gray and Carter had qualified were probably Erasmus Wilson's *Anatomist's Vade Mecum*, and *Elements of Anatomy* by Jones Quain. Both works were small – pocket-sized – but Quain came in two thick volumes. Both Quain's and Wilson's works were good books in their way, but their small pages of dense type, and even smaller illustrations, were somewhat daunting, seeming to demand much nose-to-the-grindstone effort from the reader.

The planned new textbook's dimensions and character were serious matters. Pocket manuals were commercially successful because they appealed to students by offering much knowledge in a small compass.

But pocket-sized books had button-sized illustrations. Knox's *Manual of Human Anatomy*, for example, was a good book, but was only 6 inches by 4 (15×10 cm) and few of its illustrations occupied more than one-third of a page. Gray and Carter must have discussed this matter between themselves, and with Gray's publisher, JW Parker & Son, before decisions were taken about the size and girth of the new book, and especially the size of its illustrations. While Gray and Carter were working on the book, a new edition of *Quain's* was published; this time it was a 'triple-decker' – in three volumes – of 1740 pages in all.

The two men were earnestly engaged for the following 18 months in work for the new book. Gray wrote the text, and Carter created the illustrations; all the dissections were undertaken jointly. Their working days were long – all the hours of daylight, eight or nine hours at a stretch – right through 1856, and well into 1857. We can infer from the warmth of Gray's appreciation of Carter in his published acknowledgements that their collaboration was a happy one.

> *The Author gratefully acknowledges the great services he has derived in the execution of this work, from the assistance of his friend, Dr. H. V. Carter, late Demonstrator of Anatomy at St George's Hospital. All the drawings from which the engravings were made, were executed by him.* (Gray 1858)

With all the dissections done, and Carter's inscribed wood-blocks at the engravers, Gray took six months' leave from his teaching at St George's to work as a personal doctor for a wealthy family. It was probably as good a way as any to get a well-earned break from the dissecting room and the dead-house (Nicol 2002).

Carter sat the examination for medical officers in the East India Company, and sailed for India in the spring of 1858, when the book was still in its proof stages. Gray had left a trusted colleague, Timothy Holmes, to see it through the press. Holmes's association with the first edition would later prove vital to its survival. Gray looked over the final galley proofs, just before the book finally went to press.

THE FIRST EDITION

The book Gray and Carter had created together, *Anatomy, Descriptive and Surgical,* appeared at the very end of August 1858, to immediate

acclaim. Reviews in *The Lancet* and the *British Medical Journal* were highly complimentary, and students flocked to buy.

It is not difficult to understand why it was a runaway success. *Gray's Anatomy* knocked its competitors into a cocked hat. It was considerably smaller and more slender than the doorstopper with which modern readers are familiar. The book held well in the hand, it felt substantial, and it contained everything required. To contemporaries, it was small enough to be portable, but large enough for decent illustrations: 'royal octavo' – 9½×6 inches (24×15 cm) – about two-thirds of modern A4 size. Its medium-size, single-volume format was far removed from Quain, yet double the size of Knox's *Manual*.

Simply organized and well designed, the book explains itself confidently and well; the clarity and authority of the prose are manifest. But what made it unique for its day was the outstanding size and quality of the illustrations. Gray thanked the wood engravers Butterworth and Heath for the 'great care and fidelity' they had displayed in the engravings, but it was really to Carter that the book owed its extraordinary success.

The beauty of Carter's illustrations resides in their diagrammatic clarity, quite atypical for their time. The images in contemporary anatomy books were usually 'proxy-labelled': dotted with tiny numbers or letters (often hard to find or read) or bristling with a sheaf of numbered arrows, referring to a key situated elsewhere, usually in a footnote, which was sometimes so lengthy it wrapped round on to the following page. Proxy labels require the reader's eye to move to and fro: from the structure to the proxy label to the legend and back again. There was plenty of scope for slippage, annoyance and distraction. Carter's illustrations, by contrast, unify name and structure, enabling the eye to assimilate both at a glance. We are so familiar with Carter's images that it is hard to appreciate how incredibly modern they must have seemed in 1858. The volume made human anatomy look new, exciting, accessible and do-able.

The first edition was covered in a brown bookbinder's cloth embossed all over in a dotted pattern, and with a double picture-frame border. Its spine was lettered in gold blocking:

GRAY'S
ANATOMY

… with 'DESCRIPTIVE AND SURGICAL' in small capitals underneath. *Gray's Anatomy* is how it has been referred to ever since. Carter was given credit with Gray on the book's title page for undertaking all the dissections on which the book was based, and sole credit for all the illustrations, though his name appeared in a significantly smaller type, and he was described as the 'Late Demonstrator in Anatomy at St George's Hospital' rather than being given his full current title, which was Professor of Anatomy and Physiology at Grant Medical College, Bombay. Gray was still only a Lecturer at St George's and he may have been aware that his words had been upstaged by the quality of Carter's anatomical images. He need not have worried: Gray is the famous name on the spine of the book.

Gray was paid £150 for every thousand copies sold. Carter never received a royalty payment, just a one-off fee at publication, which may have allowed him to purchase the long-wished-for microscope he took with him to India (Fig. 2).

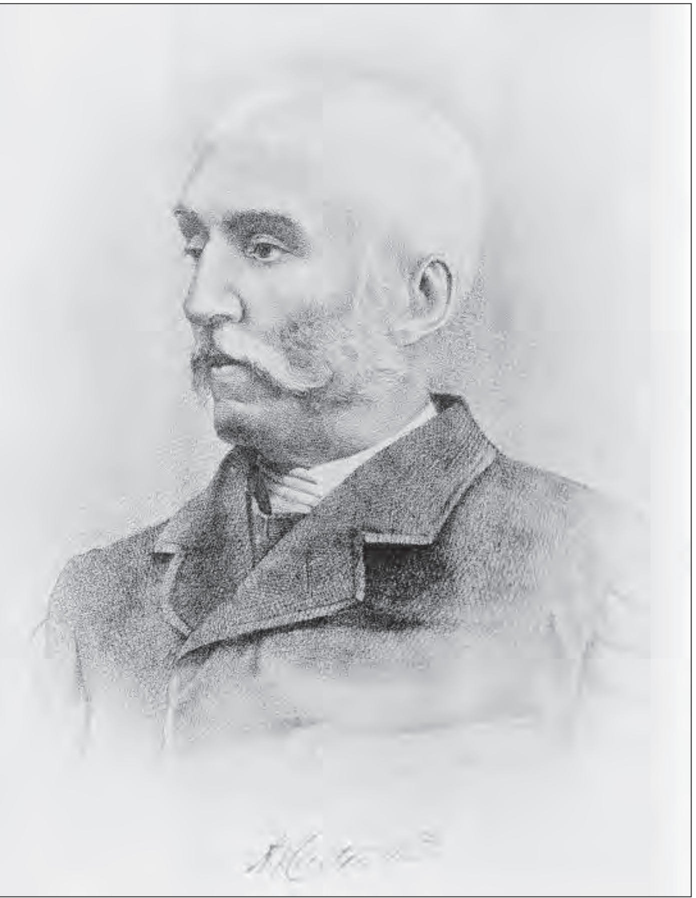

Fig. 2 Henry Vandyke Carter (1831–1897). Carter was appointed Honorary Surgeon to Queen Victoria in 1890.

The first edition print-run of 2000 copies sold out swiftly. A parallel edition was published in the United States in 1859, and Gray must have been deeply gratified to have to revise an enlarged new English edition in 1859–60, though he was surely saddened and worried by the death of his publisher, John Parker junior, at the young age of 40, while the book was going through the press. The second edition came out in the December of 1860 and it too sold like hot cakes, as indeed has every subsequent edition.

The following summer, in June 1861, at the height of his powers and full of promise, Henry Gray died unexpectedly at the age of only 34. Gray had contracted smallpox while nursing his nephew. A new strain of the disease was more virulent than the one with which Gray had been vaccinated as a child; the disease became confluent, and Gray died in a matter of days.

Within months, the whole country would be pitched into mourning for the death of Prince Albert. The creative era over which he had presided – especially the decade that had flowered since the Great Exhibition of 1851 – would be history.

THE BOOK SURVIVES

Anatomy Descriptive and Surgical could have died too. With Carter in India, the death of Gray, so swiftly after that of the younger Parker, might have spelled catastrophe. Certainly, at St George's there was a sense of calamity. The grand old medical man Sir Benjamin Brodie, Sergeant-Surgeon to the Queen, and the great supporter of Gray to whom *Anatomy* had been dedicated, cried forlornly: 'Who is there to take his place?' (Anon 1908).

But old JW Parker ensured the survival of *Gray's* by inviting Timothy Holmes, the doctor who had helped proof-read the first edition, and who had filled Gray's shoes at the medical school, to serve as Editor for the next edition. Other long-running anatomy works, such as Quain, remained in print in a similar way, co-edited by other hands (Quain 1856).

Holmes (1825–1907) was another gifted St George's man, a scholarship boy who had won an exhibition to Cambridge, where his brilliance was recognized. Holmes was a Fellow of the Royal College of Surgeons at 28. John Parker junior had commissioned him to edit *A System of Surgery* (1860–64), an important essay series by distinguished surgeons on subjects of their own choosing. Many of Holmes's authors remain important figures, even today: John Simon, James Paget, Henry Gray, Ernest Hart, Jonathan Hutchinson, Brown-Séquard and Joseph Lister. Holmes had lost an eye in an operative accident, and he had a gruff manner that terrified students, yet he published a lament for young Parker that reveals him capable of deep feeling (Holmes 1860).

John Parker senior's heart, however, was no longer in publishing. His son's death had closed down the future for him. The business, with all its stocks and copyrights, was sold to Messrs Longman. Parker retired to the village of Farnham, where he later died.

With Holmes as editor, and Longman as publisher, the immediate future of *Gray's Anatomy* was assured. The third edition appeared in 1864 with relatively few changes, Gray's estate receiving the balance of his royalty after Holmes was paid £100 for his work.

THE MISSING OBITUARY

Why no obituary appeared for Henry Gray in *Gray's Anatomy* is curious. Gray had referred to Holmes as his 'friend' in the preface to the first edition, yet it would also be true to say that they were rivals. Both had just applied for a vacant post at St George's, as Assistant Surgeon. Had Gray lived, it is thought that Holmes may not have been appointed, despite his seniority in age (Anon 1908).

Later commentators have suggested, as though from inside knowledge, that Holmes's 'proof-reading' included improving Gray's writing

style. This could be a reflection of Holmes's own self-regard, but there may be some truth in it. There can be no doubt that, as Editor of seven subsequent editions of *Gray's Anatomy* (third to ninth editions, 1864–1880), Holmes added new material, and had to correct and compress passages, but it is also possible that, back in 1857, Gray's original manuscript had been left in a poor state for Holmes to sort out. In other works, Gray's writing style was lucid, but he always seems to have paid a copyist to transcribe his work prior to submission. The original manuscript of *Gray's Anatomy*, sadly, has not survived, so it is impossible to be sure how much of the finished version had actually been written by Holmes.

It may be that Gray's glittering career, or perhaps the patronage that unquestionably advanced it, created jealousies among his colleagues, or that there was something in Gray's manner that precluded affection, or that created resentments among clever social inferiors like Carter and Holmes, especially if they felt their contributions to his brilliant career were not given adequate credit. Whatever the explanation, no reference to Gray's life or death appeared in *Gray's Anatomy* itself until the twentieth century (Howden et al 1918).

A SUCCESSION OF EDITORS

Holmes expanded areas of the book that Gray himself had developed in the second edition (1860), notably in 'general' anatomy (histology) and 'development' (embryology). In Holmes's time as Editor, the volume grew from 788 pages in 1864 to 960 in 1880 (ninth edition), with the histological section paginated separately in roman numerals at the front of the book. Extra illustrations were added, mainly from other published sources.

The connections with Gray and Carter, and with St George's, were maintained with the appointment of the next editor, T. Pickering Pick, who had been a student at St George's in Gray's time. From 1883 (tenth edition) onwards, Pick kept up with current research, rewrote and integrated the histology and embryology into the volume, dropped Holmes from the title page, removed Gray's preface to the first edition, and added bold subheadings, which certainly improved the appearance and accessibility of the text. Pick said he had 'tried to keep before himself the fact that the work is intended for students of anatomy rather than for the Scientific Anatomist' (thirteenth edition, 1893).

Pick also introduced colour printing (in 1887, eleventh edition) and experimented with the addition of illustrations using the new printing method of half-tone dots: for colour (which worked) and for new black-and-white illustrations (which did not). Half-tone shades of grey compared poorly with Carter's wood engravings, still sharp and clear by comparison.

What Henry Vandyke Carter made of these changes is a rich topic for speculation. He returned to England in 1888, having retired from the Indian Medical Service, full of honours – Deputy Surgeon General, and in 1890, he was made Honorary Surgeon to Queen Victoria. Carter had continued researching throughout his clinical medical career in India, and became one of India's foremost bacteriologists/tropical disease specialists before there was really a name for either discipline. Carter made some important discoveries, including the fungal cause of mycetoma, which he described and named. He was also a key figure in confirming scientifically in India some major international discoveries, such as Hansen's discovery of the cause of leprosy, Koch's discovery of the organism causing tuberculosis, and Laveran's discovery of the organism that causes malaria. Carter married late in life, and his wife was left with two young children when he died in Scarborough in 1897, aged 65. Like Gray, he received no obituary in the book.

When Pick was joined on the title page by Robert Howden (a professional anatomist from the University of Durham) in 1901 (fifteenth edition), the volume was still easily recognizable as the book Gray and Carter had created. Although many of Carter's illustrations had been revised or replaced, many others still remained. Sadly, though, an entire section (embryology) was again separately paginated, as its revision had taken longer than anticipated. *Gray's* had grown, seemingly inexorably, and was now quite thick and heavy: 1244 pages, weighing 5 lb 8 oz/2.5 kg. Both co-editors, and perhaps also its publisher, were dissatisfied with it.

KEY EDITION: 1905

Serious decisions were taken well in advance of the next edition, which turned out to be Pick's last with Howden. Published 50 years after Gray had first suggested the idea to Carter, the 1905 (sixteenth) edition was a landmark one.

The period 1880–1930 was a difficult time for anatomical illustration, because the new techniques of photo-lithography and half-tone were not as yet perfected, and in any case could not provide the bold simplicity of line required for a book like *Gray's*, which depended so heavily on clear illustration and clear lettering. Recognizing the inferiority of half-tone illustrations by comparison with Carter's wood-engraved originals, Pick and Howden courageously decided to jettison the second-rate half-tones altogether. Most of the next edition's illustrations were either Carter's, or old supplementary illustrations inspired by his work, or newly commissioned wood engravings or line drawings, intended 'to harmonize with Carter's original figures'. They successfully emulated Carter's verve. Having fewer pages and lighter paper, the 1905 (sixteenth edition) weighed less than its predecessor, at 4 lb 11 oz/2.1 kg. Typographically, the new edition was superb.

Howden took over as sole editor in 1909 (seventeenth edition) and immediately stamped his personality on *Gray's*. He excised 'Surgical' from the title, changing it to *Anatomy Descriptive and Applied*, and removed Carter's name altogether. He also instigated the beginnings of an editorial board of experts for *Gray's*, by adding to the title page 'Notes on Applied Anatomy' by AJ Jex-Blake and W Fedde Fedden, both St George's men. For the first time, the number of illustrations exceeded one thousand. Howden was responsible for the significant innovation of a short historical note on Henry Gray himself, nearly 60 years after his death, which included a portrait photograph (1918, twentieth edition).

THE NOMENCLATURE CONTROVERSY

Howden's era, and that of his successor TB Johnston (of Guy's), was overshadowed by a cloud of international controversy concerning anatomical terminology. European anatomists were endeavouring to standardize anatomical terms, often using Latinate constructions, a move resisted in Britain and the United States. *Gray's* became mired in these debates for over 20 years. The attempt to be fair to all sides by using multiple terms doubtless generated much confusion amongst students, until a working compromise was at last arrived at in 1955 (thirty-second edition, 1958).

Johnston oversaw the second retitling of the book (in 1938, twenty-seventh edition): it was now, officially, *Gray's Anatomy*, finally ending the fiction that it had ever been known as anything else. *Gray's* suffered from paper shortages and printing difficulties in World War II, but successive editions nevertheless continued to grow in size and weight, while illustrations were replaced and added as the text was revised.

Between Howden's first sole effort (1909, seventeenth edition) and Johnston's last edition (1958, thirty-second edition), *Gray's* expanded by over 300 pages – from 1296 to 1604 pages, and almost 300 additional illustrations brought the total to over 1300. Johnston also introduced X-ray plates (1938) and, in 1958 (thirty-second edition), electron micrographs by AS Fitton-Jackson, one of the first occasions on which a woman was credited with a contribution to *Gray's*. Johnston felt compelled to mention that she was 'a blood relative of Henry Gray himself', perhaps by way of mitigation.

AFTER WORLD WAR II

The editions of *Gray's* issued in the decades immediately following the Second World War give the impression of intellectual stagnation. Steady expansion continued in an almost formulaic fashion, with the insertion of additional detail. The central historical importance of innovation in the success of *Gray's* seems to have been lost sight of by its publishers and editors – Johnston (1930–1958, twenty-fourth to thirty-second editions), J Whillis (co-editor with Johnston, 1938–1954), DV Davies (1958–1967, thirty-second to thirty-fourth editions) and F Davies (co-editor with DV Davies 1958–1962, thirty-second to thirty-third editions). *Gray's* had become so pre-eminent that perhaps complacency crept in, or editors were too daunted or too busy to confront the 'massive undertaking' of a root and branch revision (Tansey 1995). The unexpected deaths of three major figures associated with *Gray's* in this era, James Whillis, Francis Davies and David Vaughan Davies – each of whom had been ready to take the editorial reins – may have contributed to retarding the process. The work became somewhat dull.

KEY EDITION: 1973

DV Davies had recognized the need for modernization, but his unexpected death left the work to other hands. Two Professors of Anatomy at Guy's, Roger Warwick and Peter Williams, the latter of whom had been involved as an indexer for *Gray's* for several years, regarded it as an honour to fulfill Davies's intentions.

Their thirty-fifth edition of 1973 was a significant departure from tradition. Over 780 pages (of 1471) were newly written, almost a third

of the illustrations were newly commissioned, and the illustration captions were freshly written throughout. With a complete re-typesetting of the text in larger double-column pages, a new index and the innovation of a bibliography, this edition of *Gray's* looked and felt quite unlike its 1967 (thirty-fourth edition) predecessor, and much more like its modern incarnation.

This 1973 edition departed from earlier volumes in other significant ways. The editors made explicit their intention to try to counter the impetus towards specialization and compartmentalization in twentieth-century medicine, by embracing and attempting to reintegrate the complexity of the available knowledge. Warwick and Williams openly renounced the pose of omniscience adopted by many textbooks, believing it important to accept and mention areas of ignorance or uncertainty. They shared with the reader the difficulty of keeping abreast in the sea of research, and accepted with a refreshing humility the impossibility of fulfilling their own ambitious programme.

Warwick and Williams's 1973 edition had much in common with Gray and Carter's first edition. It was bold and innovative – respectful of its heritage, while also striking out into new territory. It was visually attractive and visually informative. It embodied a sense of a treasury of information laid out for the reader (Williams and Warwick 1973). It was published simultaneously in the United States (the American *Gray's* had developed a distinct character of its own in the interval), and sold extremely well there (Williams and Warwick 1973).

The influence of the Warwick and Williams edition was forceful and long-lasting, and set a new pattern for the following quarter-century. As has transpired several times before, wittingly or unwittingly, a new editor was being prepared for the future: Dr Susan Standring (of Guy's), who created the new bibliography for the 1973 edition of *Gray's*, went on to serve on the editorial board, and has served as Editor-in-Chief for the last two editions before this one (2005–2008, thirty-ninth and fortieth editions). Both editions are important for different reasons.

For the thirty-ninth edition, the entire content of *Gray's* was reorganized, from systematic to regional anatomy. This great sea-change was not just organizational but historic, because, since its outset, *Gray's* had prioritized bodily systems, with subsidiary emphasis on how the systems interweave in the regions of the body. Professor Standring explained that this regional change of emphasis had long been asked for by readers and users of *Gray's*, and that new imaging techniques in our era have raised the clinical importance of local anatomy (Standring 2005). The change was facilitated by an enormous collective effort on the part of the editorial team and the illustrators. The subsequent and current editions consolidate that momentous change. (See Table 1.)

Table 1 ***Gray's Anatomy*** **Editions**

Edition	Date	Author/Editor(s)	Publisher	Title
1st	1858	Henry Gray *The drawings by Henry Vandyke Carter. The dissections jointly by the author and Dr Carter*	JW Parker & Son	*Anatomy Descriptive and Surgical*
2nd	1860	Henry Gray	JW Parker & Son	
3rd	1864	T Holmes	Longman	
4th	1866	T Holmes	Longman	
5th	1869	T Holmes	Longman	
6th	1872	T Holmes	Longman	
7th	1875	T Holmes	Longman	
8th	1877	T Holmes	Longman	
9th	1880	T Holmes	Longman	
10th	1883	TP Pick	Longman	
11th	1887	TP Pick	Longman	
12th	1890	TP Pick	Longman	
13th	1893	TP Pick *Gray's preface removed*	Longman	
14th	1897	TP Pick	Longman	
15th	1901	TP Pick & R Howden	Longman	
16th	1905	TP Pick & R Howden	Longman	
17th	1909	Robert Howden *Notes on applied anatomy by AJ Jex-Blake & W Fedde Fedden*	Longman	*Anatomy Descriptive and Applied*
18th	1913	Robert Howden & Blake & Fedden	Longman	
19th	1916	Robert Howden & Blake & Fedden	Longman	
20th	1918	Robert Howden & Blake & Fedden *First edition ever to feature a photograph and obituary of Henry Gray*	Longman	
21st	1920	Robert Howden *Notes on applied anatomy by AJ Jex-Blake & John Clay*	Longman	
22nd	1923	Robert Howden *Notes on applied anatomy by John Clay & John D Lickley*	Longman	
23rd	1926	Robert Howden	Longman	
24th	1930	TB Johnston	Longman	
25th	1932	TB Johnston	Longman	
26th	1935	TB Johnston	Longman	
27th	1938	TB Johnston & J Whillis	Longman	*Gray's Anatomy*
28th	1942	TB Johnston & J Whillis	Longman	
29th	1946	TB Johnston & J Whillis	Longman	
30th	1949	TB Johnston & J Whillis	Longman	
31st	1954	TB Johnston & J Whillis	Longman	
32nd	1958	TB Johnston & DV Davies & F Davies	Longman	
33rd	1962	DV Davies & F Davies	Longman	
34th	1967	DV Davies & RE Coupland	Longman	
35th	1973	Peter L Williams & Roger Warwick With a separate volume: *Functional Neuroanatomy of Man* – being the neurology section of Gray's Anatomy. 35th edition, 1975	Longman	
36th	1980	Roger Warwick & Peter L Williams	Churchill Livingstone	
37th	1989	Peter L Williams	Churchill Livingstone	
38th	1995	Peter L Williams & Editorial Board	Churchill Livingstone	
39th	2005	Susan Standring & Editorial Board	Elsevier	*The Anatomical Basis of Clinical Practice*
40th	2008	Susan Standring & Editorial Board	Elsevier	*The Anatomical Basis of Clinical Practice*
41st	2015	Susan Standring & Editorial Board	Elsevier	*The Anatomical Basis of Clinical Practice*

THE DOCTORS' BIBLE

Neither Gray nor Carter, the young men who – by their committed hard work between 1856 and 1858 – created the original *Gray's Anatomy*, would have conceived that so many years after their deaths their book would not only be a household name, but also be regarded as a work of such pre-eminent importance that a novelist half a world away would rank it as cardinal – alongside the Bible and Shakespeare – to a doctor's education (Sinclair Lewis 1925, Richardson 2008). From this forty-first edition of *Gray's Anatomy*, we can look back to appraise the long-term value of their efforts. We can discern how the book they created triumphed over its competitors, and has survived pre-eminent. *Gray's* is a remarkable publishing phenomenon. Although the volume now looks quite different to the original, and contains so much more, its kinship with the *Gray's Anatomy* of 1858 is easily demonstrable by direct descent, every edition updated by Henry Gray's successor. Works are rare indeed that have had such a long history of continuous publication on both sides of the Atlantic, and such a useful one.

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ANATOMICAL NOMENCLATURE

Anatomy is the study of the structure of the body. Conventionally, it is divided into topographical (macroscopic or gross) anatomy (which may be further divided into regional anatomy, surface anatomy, neuroanatomy, endoscopic and imaging anatomy); developmental anatomy (embryogenesis and subsequent organogenesis); and the anatomy of microscopic and submicroscopic structure (histology).

Anatomical language is one of the fundamental languages of medicine. The unambiguous description of thousands of structures is impossible without an extensive and often highly specialized vocabulary. Ideally, these terms, which are often derived from Latin or Greek, should be used to the exclusion of any other, and eponyms should be avoided. In reality, this does not always happen. Many terms are vernacularized and, around the world, synonyms and eponyms still abound in the literature, in medical undergraduate classrooms and in clinics. The *Terminologia Anatomica*,[1] drawn up by the Federative Committee on Anatomical Terminology (FCAT) in 1998, continues to serve as our reference source for the terminology for macroscopic anatomy, and the text of the forty-first edition of *Gray's Anatomy* is almost entirely TA-compliant. However, where terminology is at variance with, or, more likely, is not included in, the TA, the alternative term used either is cited in the relevant consensus document or position paper, or enjoys widespread clinical usage. Synonyms and eponyms are given in parentheses on first usage of a preferred term and not shown thereafter in the text; an updated list of eponyms and short biographical details of the clinicians and anatomists whose names are used in this way is available in the e-book for reference purposes (see Preface, p. ix, for further discussion of the use of eponyms).

PLANES, DIRECTIONS AND RELATIONSHIPS

To avoid ambiguity, all anatomical descriptions assume that the body is in the conventional 'anatomical position', i.e. standing erect and facing forwards, upper limbs by the side with the palms facing forwards, and lower limbs together with the toes facing forwards (Fig. 1). Descriptions are based on four imaginary planes – median, sagittal, coronal and horizontal – applied to a body in the anatomical position. The median plane passes longitudinally through the body and divides it into right and left halves. The sagittal plane is any vertical plane parallel with the median plane; although often employed, 'parasagittal' is therefore redundant and should not be used. The coronal (frontal) plane is orthogonal to the median plane and divides the body into anterior (front) and posterior (back). The horizontal (transverse) plane is orthogonal to both median and sagittal planes. Radiologists refer to transverse planes as (trans)axial; convention dictates that axial anatomy is viewed as though looking from the feet towards the head.

Structures nearer the head are superior, cranial or (sometimes) cephalic (cephalad), whereas structures closer to the feet are inferior; caudal is most often used in embryology to refer to the hind end of the embryo. Medial and lateral indicate closeness to the median plane, medial being closer than lateral; in the anatomical position, the little finger is medial to the thumb, and the great toe is medial to the little toe. Specialized terms may also be used to indicate medial and lateral. Thus, in the upper limb, ulnar and radial are used to mean medial and lateral, respectively; in the lower limb, tibial and fibular (peroneal) are used to mean medial and lateral, respectively. Terms may be based on embryological relationships; the border of the upper limb that includes the thumb, and the border of the lower limb that includes the great toe are the pre-axial borders, whilst the opposite borders are the post-axial borders. Various degrees of obliquity are acknowledged using compound terms, e.g. posterolateral.

When referring to structures in the trunk and upper limb, we have freely used the synonyms anterior, ventral, flexor, palmar and volar, and posterior, dorsal and extensor. We recognize that these synonyms are not always satisfactory, e.g. the extensor aspect of the leg is anterior with respect to the knee and ankle joints, and superior in the foot and digits; the plantar (flexor) aspect of the foot is inferior. Dorsal (dorsum) and ventral are terms used particularly by embryologists and neuroanatomists; they therefore feature most often in Sections 2 and 3.

Distal and proximal are used particularly to describe structures in the limbs, taking the datum point as the attachment of the limb to the trunk (sometimes referred to as the root), such that a proximal structure is closer to the attachment of the limb than a distal structure. However, proximal and distal are also used in describing branching structures, e.g. bronchi, vessels and nerves. External (outer) and internal (inner) refer to the distance from the centre of an organ or cavity, e.g. the layers of the body wall, or the cortex and medulla of the kidney. Superficial and deep are used to describe the relationships between adjacent structures. Ipsilateral refers to the same side (of the body, organ or structure), bilateral to both sides, and contralateral to the opposite side.

Teeth are described using specific terms that indicate their relationship to their neighbours and to their position within the dental arch; these terms are described on page 517.

[1]*Terminologia Anatomica* (1998) is the joint creation of the Federative Committee on Anatomical Terminology (FCAT) and the Member Associations of the International Federation of Associations of Anatomists (IFAA).

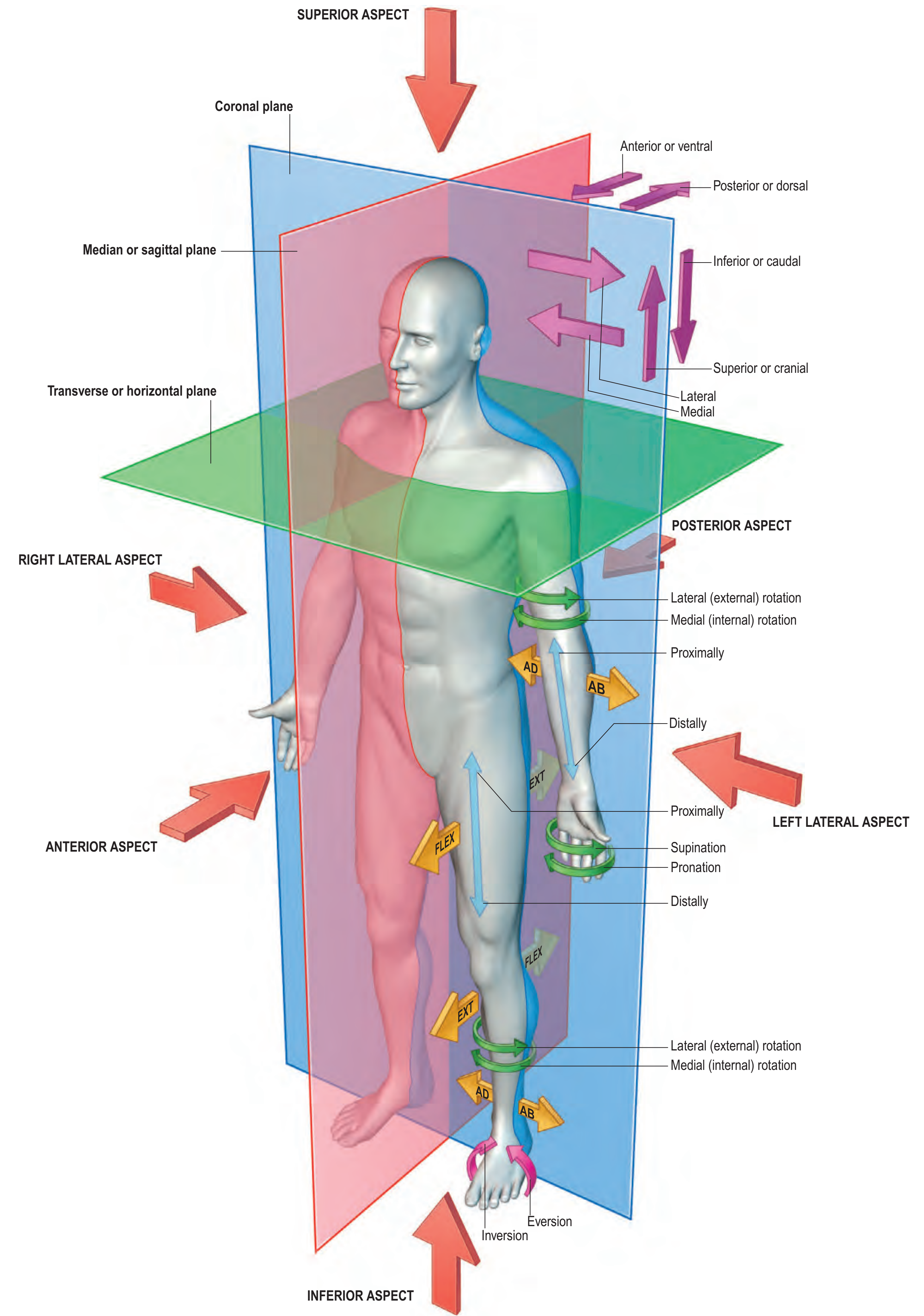

Fig. 1 The terminology widely used in descriptive anatomy. Abbreviations shown on arrows: AD, adduction; AB, abduction; FLEX, flexion (of the thigh at the hip joint); EXT, extension (of the leg at the knee joint).

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CHAPTER 1

Basic structure and function of cells

CELL STRUCTURE

GENERAL CHARACTERISTICS OF CELLS

The shapes of mammalian cells vary widely depending on their interactions with each other, their extracellular environment and internal structures. Their surfaces are often highly folded when absorptive or transport functions take place across their boundaries. Cell size is limited by rates of diffusion, either that of material entering or leaving cells, or that of diffusion within them. Movement of macromolecules can be much accelerated and also directed by processes of active transport across the plasma membrane and by transport mechanisms within the cell. According to the location of absorptive or transport functions, apical microvilli (**Fig. 1.1**) or basolateral infoldings create a large surface area for transport or diffusion.

Motility is a characteristic of most cells, in the form of movements of cytoplasm or specific organelles from one part of the cell to another. It also includes: the extension of parts of the cell surface such as pseudopodia, lamellipodia, filopodia and microvilli; locomotion of entire cells, as in the amoeboid migration of tissue macrophages; the beating of flagella or cilia to move the cell (e.g. in spermatozoa) or fluids overlying it (e.g. in respiratory epithelium); cell division; and muscle contraction. Cell movements are also involved in the uptake of materials from their environment (endocytosis, phagocytosis) and the passage of large molecular complexes out of cells (exocytosis, secretion).

Epithelial cells rarely operate independently of each other and commonly form aggregates by adhesion, often assisted by specialized intercellular junctions. They may also communicate with each other either by generating and detecting molecular signals that diffuse across intercellular spaces, or more rapidly by generating interactions between membrane-bound signalling molecules. Cohesive groups of cells constitute tissues, and more complex assemblies of tissues form functional systems or organs.

Most cells are between 5 and 50 μm in diameter: e.g. resting lymphocytes are 6 μm across, red blood cells 7.5 μm and columnar epithelial cells 20 μm tall and 10 μm wide (all measurements are approximate). Some cells are much larger than this: e.g. megakaryocytes of the bone marrow and osteoclasts of the remodelling bone are more than 200 μm in diameter. Neurones and skeletal muscle cells have relatively extended shapes, some of the former being over 1 m in length.

CELLULAR ORGANIZATION

Each cell is contained within its limiting plasma membrane, which encloses the cytoplasm. All cells, except mature red blood cells, also contain a nucleus that is surrounded by a nuclear membrane or envelope (see Fig. 1.1; **Fig. 1.2**). The nucleus includes: the genome of the cell contained within the chromosomes; the nucleolus; and other subnuclear structures. The cytoplasm contains cytomembranes and several membrane-bound structures, called organelles, which form separate

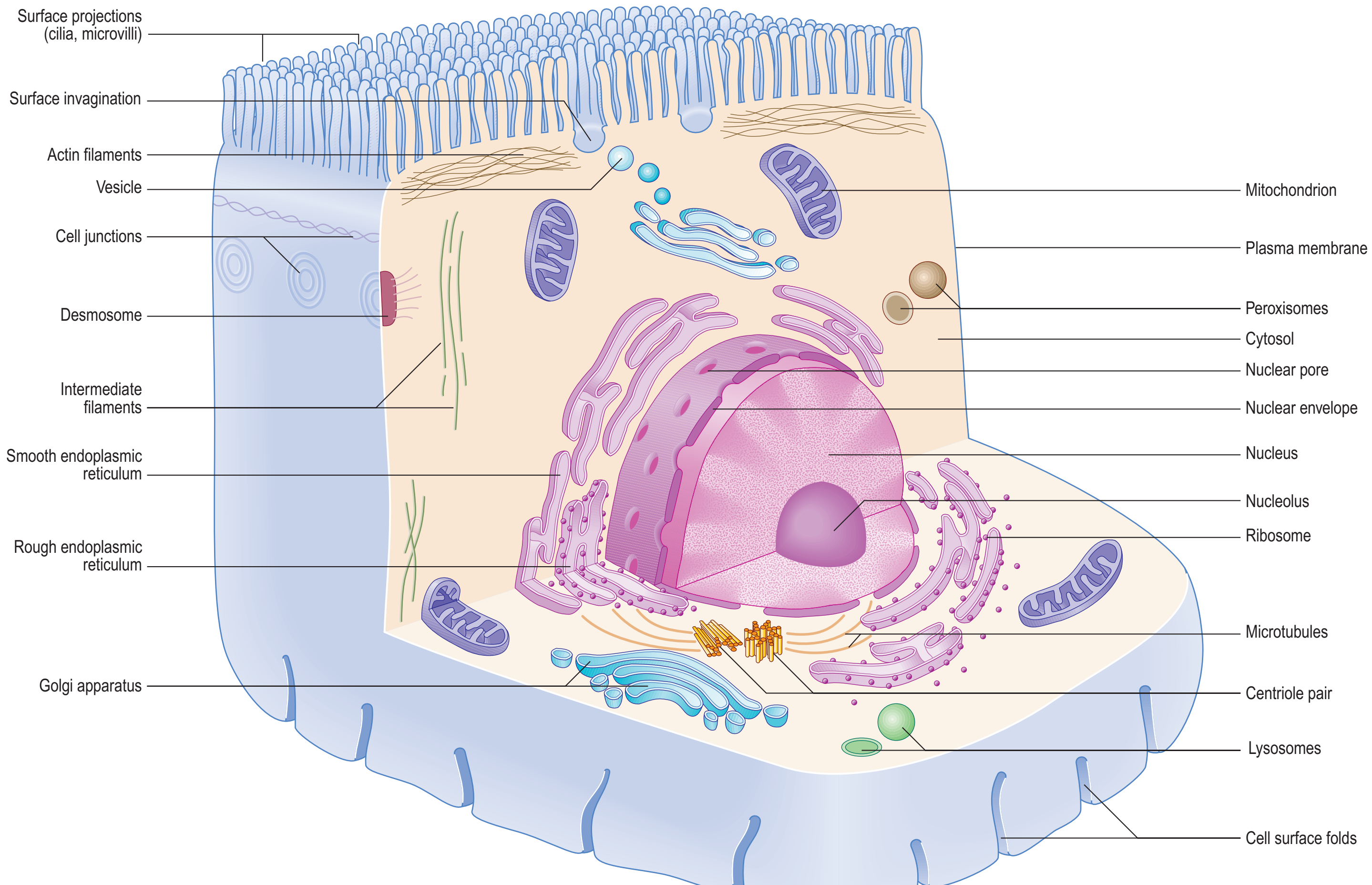


Fig. 1.1 The main structural components and internal organization of a generalized cell.

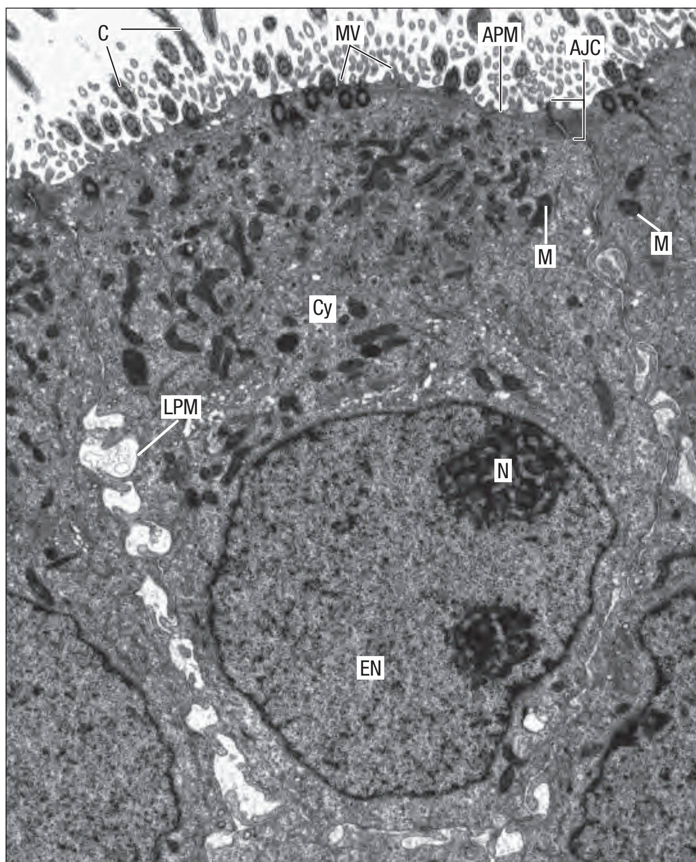


Fig. 1.2 The structural organization and some principal organelles of a typical cell. This example is a ciliated columnar epithelial cell from human nasal mucosa. The central cell, which occupies most of the field of view, is closely apposed to its neighbours along their lateral plasma membranes. Within the apical junctional complex, these membranes form a tightly sealed zone (tight junction) that isolates underlying tissues from, in this instance, the nasal cavity. Abbreviations: AJC, apical junctional complex; APM, apical plasma membrane; C, cilia; Cy, cytoplasm; EN, euchromatic nucleus; LPM, lateral plasma membrane; M, mitochondria; MV, microvilli; N, nucleolus. (Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

and distinct compartments within the cytoplasm. Cytomembranes include the rough and smooth endoplasmic reticulum and Golgi apparatus, as well as vesicles derived from them. Organelles include lysosomes, peroxisomes and mitochondria. The nucleus and mitochondria are enclosed by a double-membrane system; lysosomes and peroxisomes have a single bounding membrane. There are also non-membranous structures, called inclusions, which lie free in the cytosolic compartment. They include lipid droplets, glycogen aggregates and pigments (e.g. lipofuscin). In addition, ribosomes and several filamentous protein networks, known collectively as the cytoskeleton, are found in the cytosol. The cytoskeleton determines general cell shape and supports specialized extensions of the cell surface (microvilli, cilia, flagella). It is involved in the assembly of specific structures (e.g. centrioles) and controls cargo transport in the cytoplasm. The cytosol contains many soluble proteins, ions and metabolites.

Plasma membrane

Cells are enclosed by a distinct plasma membrane, which shares features with the cytomembrane system that compartmentalizes the cytoplasm and surrounds the nucleus. All membranes are composed of lipids (mainly phospholipids, cholesterol and glycolipids) and proteins, in approximately equal ratios. Plasma membrane lipids form a lipid bilayer, a layer two molecules thick. The hydrophobic ends of each lipid molecule face the interior of the membrane and the hydrophilic ends face outwards. Most proteins are embedded within, or float in, the lipid bilayer as a fluid mosaic. Some proteins, because of extensive hydrophobic regions of their polypeptide chains, span the entire width of the membrane (transmembrane proteins), whereas others are only superficially attached to the bilayer by lipid groups. Both are integral (intrinsic) membrane proteins, as distinct from peripheral (extrinsic) membrane proteins, which are membrane-bound only through their association with other proteins. Carbohydrates in the form of oligosaccharides and polysaccharides are bound either to proteins (glycoproteins) or to lipids (glycolipids), and project mainly into the extracellular domain (Fig. 1.3).

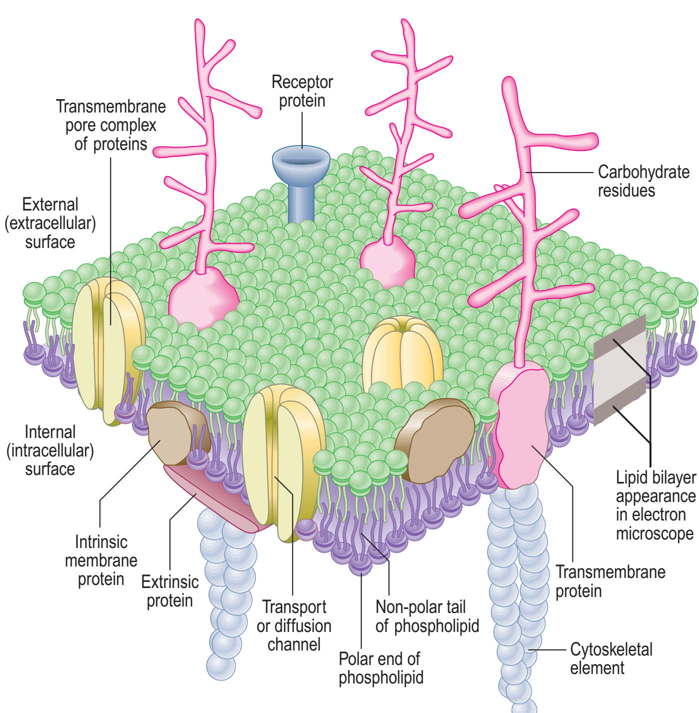


Fig. 1.3 The molecular organization of the plasma membrane, according to the fluid mosaic model of membrane structure. Intrinsic or integral membrane proteins include diffusion or transport channel complexes, receptor proteins and adhesion molecules. These may span the thickness of the membrane (transmembrane proteins) and can have both extracellular and cytoplasmic domains. Transmembrane proteins have hydrophobic zones, which cross the phospholipid bilayer and allow the protein to 'float' in the plane of the membrane. Some proteins are restricted in their freedom of movement where their cytoplasmic domains are tethered to the cytoskeleton.

In the electron microscope, membranes fixed and contrasted by heavy metals such as osmium tetroxide appear in section as two densely stained layers separated by an electron-translucent zone – the classic unit membrane. The total thickness of each layer is about 7.5 nm. The overall thickness of the plasma membrane is typically 15 nm. Freeze-fracture cleavage planes usually pass along the hydrophobic portion of the bilayer, where the hydrophobic tails of phospholipids meet, and split the bilayer into two leaflets. Each cleaved leaflet has a surface and a face. The surface of each leaflet faces either the extracellular surface (ES) or the intracellular or protoplasmic (cytoplasmic) surface (PS). The extracellular face (EF) and protoplasmic face (PF) of each leaflet are artificially produced during membrane splitting. This technique has also demonstrated intramembranous particles embedded in the lipid bilayer; in most cases, these represent large transmembrane protein molecules or complexes of proteins. Intramembranous particles are distributed asymmetrically between the two half-layers, usually adhering more to one half of the bilayer than to the other. In plasma membranes, the intracellular leaflet carries most particles, seen on its face (the PF). Where they have been identified, clusters of particles usually represent channels for the transmembrane passage of ions or molecules between adjacent cells (gap junctions).

Biophysical measurements show the lipid bilayer to be highly fluid, allowing diffusion in the plane of the membrane. Thus proteins are able to move freely in such planes unless anchored from within the cell. Membranes in general, and the plasma membrane in particular, form boundaries selectively limiting diffusion and creating physiologically distinct compartments. Lipid bilayers are impermeable to hydrophilic solutes and ions, and so membranes actively control the passage of ions and small organic molecules such as nutrients, through the activity of membrane transport proteins. However, lipid-soluble substances can pass directly through the membrane so that, for example, steroid hormones enter the cytoplasm freely. Their receptor proteins are either cytosolic or nuclear, rather than being located on the cell surface.

Plasma membranes are able to generate electrochemical gradients and potential differences by selective ion transport, and actively take up or export small molecules by energy-dependent processes. They also provide surfaces for the attachment of enzymes, sites for the receptors

Combinations of biochemical, biophysical and biological techniques have revealed that lipids are not homogenously distributed in membranes, but that some are organized into microdomains in the bilayer, called 'detergent-resistant membranes' or lipid 'rafts', rich in sphingomyelin and cholesterol. The ability of select subsets of proteins to partition into different lipid microdomains has profound effects on their function, e.g. in T-cell receptor and cell–cell signalling. The highly organized environment of the domains provides a signalling, trafficking and membrane fusion environment.

of external signals, including hormones and other ligands, and sites for the recognition and attachment of other cells. Internally, plasma membranes can act as points of attachment for intracellular structures, in particular those concerned with cell motility and other cytoskeletal functions. Cell membranes are synthesized by the rough endoplasmic reticulum in conjunction with the Golgi apparatus.

Cell coat (glycocalyx)

The external surface of a plasma membrane differs structurally from internal membranes in that it possesses an external, fuzzy, carbohydrate-rich coat, the glycocalyx. The cell coat forms an integral part of the plasma membrane, projecting as a diffusely filamentous layer 2–20 nm or more from the lipoprotein surface. The cell coat is composed of the carbohydrate portions of glycoproteins and glycolipids embedded in the plasma membrane (see Fig. 1.3).

The precise composition of the glycocalyx varies with cell type; many tissue- and cell type-specific antigens are located in the coat, including the major histocompatibility complex of the immune system and, in the case of erythrocytes, blood group antigens. Therefore, the glycocalyx plays a significant role in organ transplant compatibility. The glycocalyx found on apical microvilli of enterocytes, the cells forming the lining epithelium of the intestine, consists of enzymes involved in the digestive process. Intestinal microvilli are cylindrical projections (1–2 µm long and about 0.1 µm in diameter) forming a closely packed layer called the brush border that increases the absorptive function of enterocytes.

Cytoplasm

Compartments and functional organization

The cytoplasm consists of the cytosol, a gel-like material enclosed by the cell or plasma membrane. The cytosol is made up of colloidal proteins such as enzymes, carbohydrates and small protein molecules, together with ribosomes and ribonucleic acids. The cytoplasm contains two cytomembrane systems, the endoplasmic reticulum and Golgi apparatus, as well as membrane-bound organelles (lysosomes, peroxisomes and mitochondria), membrane-free inclusions (lipid droplets, glycogen and pigments) and the cytoskeleton. The nuclear contents, the nucleoplasm, are separated from the cytoplasm by the nuclear envelope.

Endoplasmic reticulum

The endoplasmic reticulum is a system of interconnecting membrane-lined channels within the cytoplasm (**Fig. 1.4**). These channels take various forms, including cisternae (flattened sacs), tubules and vesicles. The membranes divide the cytoplasm into two major compartments. The intramembranous compartment, or cisternal space, is where secretory products are stored or transported to the Golgi complex and cell exterior. The cisternal space is continuous with the perinuclear space.

Structurally, the channel system can be divided into rough or granular endoplasmic reticulum (RER), which has ribosomes attached to its outer, cytosolic surface, and smooth or agranular endoplasmic reticulum (SER), which lacks ribosomes. The functions of the endoplasmic reticulum vary greatly and include: the synthesis, folding and transport of proteins; synthesis and transport of phospholipids and steroids; and storage of calcium within the cisternal space and regulated release into the cytoplasm. In general, RER is well developed in cells that produce abundant proteins; SER is abundant in steroid-producing cells and muscle cells. A variant of the endoplasmic reticulum in muscle cells is the sarcoplasmic reticulum, involved in calcium storage and release for muscle contraction. For further reading on the endoplasmic reticulum, see Bravo et al (2013).

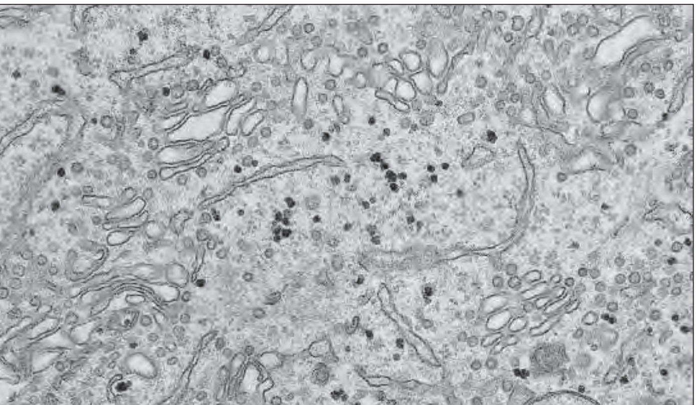


Fig. 1.4 Smooth endoplasmic reticulum with associated vesicles. The dense particles are glycogen granules. (Courtesy of Rose Watson, Cancer Research UK.)

Smooth endoplasmic reticulum

The smooth endoplasmic reticulum (see Fig. 1.4) is associated with carbohydrate metabolism and many other metabolic processes, including detoxification and synthesis of lipids, cholesterol and steroids. The membranes of the smooth endoplasmic reticulum serve as surfaces for the attachment of many enzyme systems, e.g. the enzyme cytochrome P450, which is involved in important detoxification mechanisms and is thus accessible to its substrates, which are generally lipophilic. The membranes also cooperate with the rough endoplasmic reticulum and the Golgi apparatus to synthesize new membranes; the protein, carbohydrate and lipid components are added in different structural compartments. The smooth endoplasmic reticulum in hepatocytes contains the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to glucose, a step in gluconeogenesis.

Rough endoplasmic reticulum

The rough endoplasmic reticulum is a site of protein synthesis; its cytosolic surface is studded with ribosomes (**Fig. 1.5E**). Ribosomes only bind to the endoplasmic reticulum when proteins targeted for secretion begin to be synthesized. Most proteins pass through its membranes and accumulate within its cisternae, although some integral membrane proteins, e.g. plasma membrane receptors, are inserted into the rough endoplasmic reticulum membrane, where they remain. After passage from the rough endoplasmic reticulum, proteins remain in membrane-bound cytoplasmic organelles such as lysosomes, become incorporated into new plasma membrane, or are secreted by the cell. Some carbohydrates are also synthesized by enzymes within the cavities of the rough endoplasmic reticulum and may be attached to newly formed protein (glycosylation). Vesicles are budded off from the rough endoplasmic reticulum for transport to the Golgi as part of the protein-targeting mechanism of the cell.

Ribosomes, polyribosomes and protein synthesis

Ribosomes are macromolecular machines that catalyse the synthesis of proteins from amino acids; synthesis and assembly into subunits takes place in the nucleolus and includes the association of ribosomal RNA (rRNA) with ribosomal proteins translocated from their site of synthesis in the cytoplasm. The individual subunits are then transported into the cytoplasm, where they remain separate from each other when not actively synthesizing proteins. Ribosomes are granules approximately 25 nm in diameter, composed of rRNA molecules and proteins assembled into two unequal subunits. The subunits can be separated by their sedimentation coefficients (S) in an ultracentrifuge into larger 60S and smaller 40S components. These are associated with 73 different proteins (40 in the large subunit and 33 in the small), which have structural and enzymatic functions. Three small, highly convoluted rRNA strands (28S, 5.8S and 5S) make up the large subunit, and one strand (18S) is in the small subunit.

A typical cell contains millions of ribosomes. They may form groups (polyribosomes or polysomes) attached to messenger RNA (mRNA), which they translate during protein synthesis for use outside the system of membrane compartments, e.g. enzymes of the cytosol and cytoskeletal proteins. Some of the cytosolic products include proteins that can be inserted directly into (or through) membranes of selected organelles, such as mitochondria and peroxisomes. Ribosomes may be attached to the membranes of the rough endoplasmic reticulum (see Fig. 1.5E).

In a mature polyribosome, all the attachment sites of the mRNA are occupied as ribosomes move along it, synthesizing protein according to its nucleotide sequence. Consequently, the number and spacing of ribosomes in a polyribosome indicate the length of the mRNA molecule and hence the size of the protein being made. The two subunits have separate roles in protein synthesis. The 40S subunit is the site of attachment and translation of mRNA. The 60S subunit is responsible for the release of the new protein and, where appropriate, attachment to the endoplasmic reticulum via an intermediate docking protein that directs the newly synthesized protein through the membrane into the cisternal space.

Golgi apparatus (Golgi complex)

The Golgi apparatus is a distinct cytomembrane system located near the nucleus and the centrosome. It is particularly prominent in secretory cells and can be visualized when stained with silver or other metallic

The glycocalyx plays a significant role in maintenance of the integrity of tissues and in a wide range of dynamic cellular processes, e.g. serving as a vascular permeability barrier and transducing fluid shear stress to the endothelial cell cytoskeleton (Weinbaum et al 2007). Disruption of the glycocalyx on the endothelial surface of large blood vessels precedes inflammation, a conditioning factor of atheromatosis (e.g. deposits of cholesterol in the vascular wall leading to partial or complete obstruction of the vascular lumen).

Protein synthesis on ribosomes may be suppressed by a class of RNA molecules known as small interfering RNA (siRNA) or silencing RNA. These molecules are typically 20–25 nucleotides in length and bind (as a complex with proteins) to specific mRNA molecules via their complementary sequence. This triggers the enzymatic destruction of the mRNA or prevents the movement of ribosomes along it. Synthesis of the encoded protein is thus prevented. Their normal function may have antiviral or other protective effects; there is also potential for developing artificial siRNAs as a therapeutic tool for silencing disease-related genes.

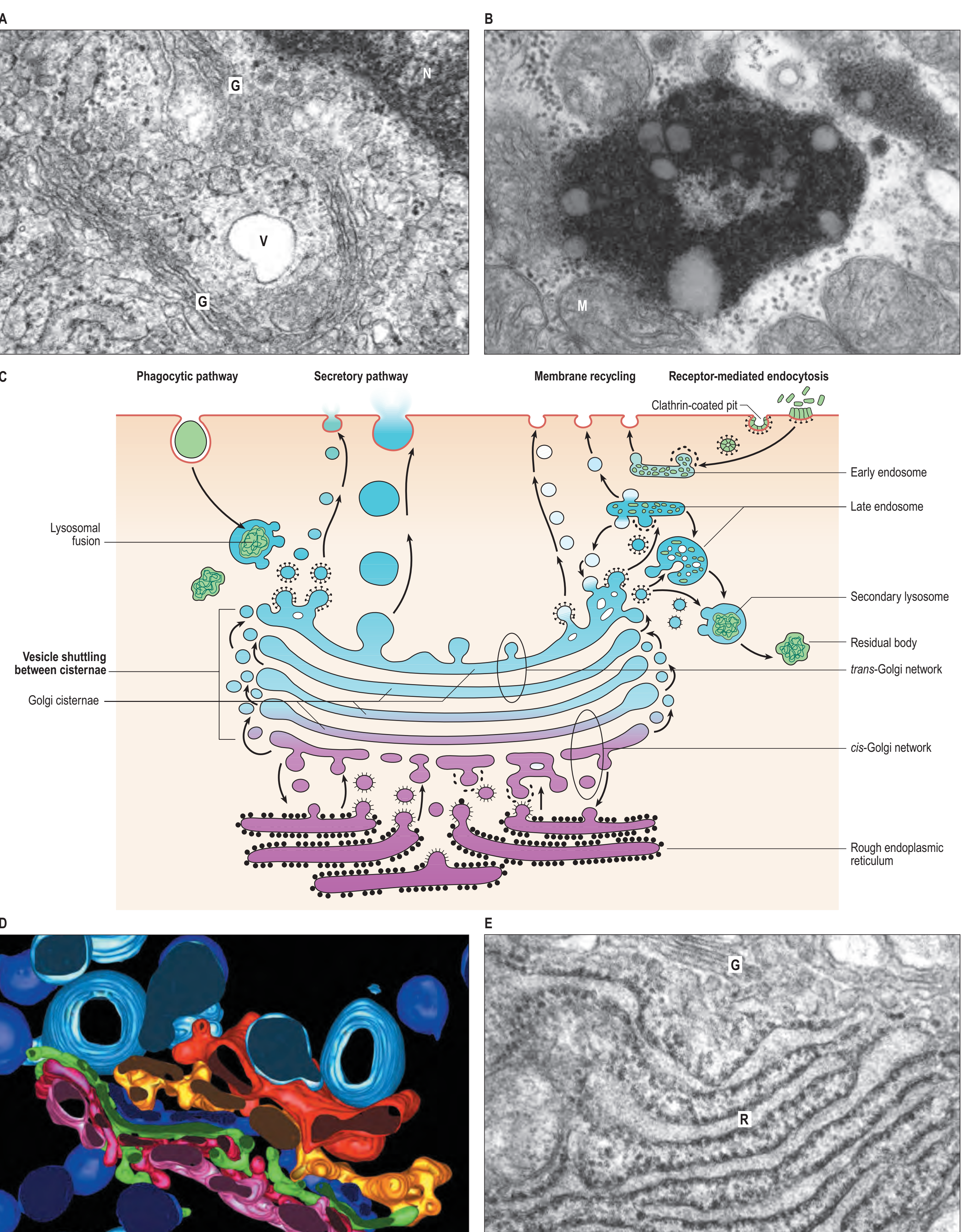


Fig. 1.5 The Golgi apparatus and functionally related organelles. **A**, Golgi apparatus (G) adjacent to the nucleus (N) (V, vesicle). **B**, A large residual body (tertiary lysosome) in a cardiac muscle cell (M, mitochondrion). **C**, The functional relationships between the Golgi apparatus and associated cellular structures. **D**, A three-dimensional reconstruction of the Golgi apparatus in a pancreatic β cell showing stacks of Golgi cisternae from the *cis*-face (pink) and *cis*-medial cisternae (red, green) to the *trans*-Golgi network (blue, yellow, orange–red); immature proinsulin granules (condensing vesicles) are shown in pale blue and mature (crystalline) insulin granules in dark blue. The flat colour areas represent cut faces of cisternae and vesicles. **E**, Rough endoplasmic reticulum (R), associated with the Golgi apparatus (G). (D, Courtesy of Dr Brad Marsh, Institute for Molecular Bioscience, University of Queensland, Brisbane. A,B,E From human tissue, courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

salts. Traffic between the endoplasmic reticulum and the Golgi apparatus is bidirectional and takes place via carrier vesicles derived from the donor site that bud, tether and fuse with the target site.

Golgins are long coiled-coil proteins attached to the cytoplasmic surface of cisternal membranes, forming a fibrillar matrix surrounding the Golgi apparatus to stabilize it; they have a role in vesicle trafficking (for further reading on golgins, see Munro 2011). The Golgi apparatus has several functions: it links anterograde and retrograde protein and lipid flow in the secretory pathway; it is the site where protein and lipid glycosylation occurs; and it provides membrane platforms to which signalling and sorting proteins bind.

Ultrastructurally, the Golgi apparatus (**Fig. 1.5A**) displays a continuous ribbon-like structure consisting of a stack of several flattened membranous cisternae, together with clusters of vesicles surrounding its surfaces. Cisternae differ in enzymatic content and activity. Small transport vesicles from the rough endoplasmic reticulum are received at one face of the Golgi stack, the convex *cis*-face (entry or forming surface). Here, they deliver their contents to the first cisterna in the series by membrane fusion. From the edges of this cisterna, the protein is transported to the next cisterna by vesicular budding and then fusion, and this process is repeated across medial cisternae until the final cisterna at the concave *trans*-face (exit or condensing surface) is reached. Here, larger vesicles are formed for delivery to other parts of the cell.

The *cis*-Golgi and *trans*-Golgi membranous networks form an integral part of the Golgi apparatus. The *cis*-Golgi network is a region of complex membranous channels interposed between the rough endoplasmic reticulum and the Golgi *cis*-face, which receives and transmits vesicles in both directions. Its function is to select appropriate proteins synthesized on the rough endoplasmic reticulum for delivery by vesicles to the Golgi stack, while inappropriate proteins are shuttled back to the rough endoplasmic reticulum.

The *trans*-Golgi network, at the other side of the Golgi stack, is also a region of interconnected membrane channels engaged in protein sorting. Here, modified proteins processed in the Golgi cisternae are packaged selectively into vesicles and dispatched to different parts of the cell. The packaging depends on the detection, by the *trans*-Golgi network, of particular amino-acid signal sequences, leading to their enclosure in membranes of appropriate composition that will further modify their contents, e.g. by extracting water to concentrate them (vesicles entering the exocytosis pathway) or by pumping in protons to acidify their contents (lysosomes destined for the intracellular sorting pathway).

Within the Golgi stack proper, proteins undergo a series of sequential chemical modifications by Golgi resident enzymes synthesized in the rough endoplasmic reticulum. These include: glycosylation (changes in glycosyl groups, e.g. removal of mannose, addition of *N*-acetylglucosamine and sialic acid); sulphation (addition of sulphate groups to glycosaminoglycans); and phosphorylation (addition of phosphate groups). Some modifications serve as signals to direct proteins and lipids to their final destination within cells, including lysosomes and plasma membrane. Lipids formed in the endoplasmic reticulum are also routed for incorporation into vesicles.

Exocytic (secretory) pathway

Secreted proteins, lipids, glycoproteins, small molecules such as amines and other cellular products destined for export from the cell are transported to the plasma membrane in small vesicles released from the *trans*-face of the Golgi apparatus. This pathway either is constitutive, in which transport and secretion occur more or less continuously, as with immunoglobulins produced by plasma cells, or it is regulated by external signals, as in the control of salivary secretion by autonomic neural stimulation. In regulated secretion, the secretory product is stored temporarily in membrane-bound secretory granules or vesicles. Exocytosis is achieved by fusion of the secretory vesicular membrane with the plasma membrane and release of the vesicle contents into the extracellular domain. In polarized cells, e.g. most epithelia, exocytosis occurs at the apical plasma membrane. Glandular epithelial cells secrete into a duct lumen, as in the pancreas, or on to a free surface, such as the lining of the stomach. In hepatocytes, bile is secreted across a very small area of plasma membrane forming the wall of the bile canaliculus. This region is defined as the apical plasma membrane and is the site of exocrine secretion, whereas secretion of hepatocyte plasma proteins into the blood stream is targeted to the basolateral surfaces facing the sinusoids. Packaging of different secretory products into appropriate vesicles takes place in the *trans*-Golgi network. Delivery of secretory vesicles to their correct plasma membrane domains is achieved by sorting sequences in the cytoplasmic tails of vesicular membrane proteins.

Endocytic (internalization) pathway

The endocytic pathway begins at the plasma membrane and ends in lysosomes involved in the degradation of the endocytic cargo through the enzymatic activity of lysosomal hydrolases. Endocytic cargo is internalized from the plasma membrane to early endosomes and then to late endosomes. Late endosomes transport their cargo to lysosomes, where the cargo material is degraded following fusion and mixing of contents of endosomes and lysosomes. Early endosomes derive from endocytic vesicles (clathrin-coated vesicles and caveolae). Once internalized, endocytic vesicles shed their coat of adaptin and clathrin, and fuse to form an early endosome, where the receptor molecules release their bound ligands. Membrane and receptors from the early endosomes can be recycled to the cell surface as exocytic vesicles.

Clathrin-dependent endocytosis occurs at specialized patches of plasma membrane called coated pits; this mechanism is also used to internalize ligands bound to surface receptor molecules and is also termed receptor-mediated endocytosis. Caveolae (little caves) are structurally distinct pinocytotic vesicles most widely used by endothelial and smooth muscle cells, when they are involved in transcytosis, signal transduction and possibly other functions. In addition to late endosomes, lysosomes can also fuse with phagosomes, autophagosomes and plasma membrane patches for membrane repair. Lysosomal hydrolases process or degrade exogenous materials (phagocytosis or heterophagy) as well as endogenous material (autophagy). Phagocytosis consists of the cellular uptake of invading pathogens, apoptotic cells and other foreign material by specialized cells. Lysosomes are numerous in actively phagocytic cells, e.g. macrophages and neutrophil granulocytes, in which lysosomes are responsible for destroying phagocytosed particles, e.g. bacteria. In these cells, the phagosome, a vesicle potentially containing a pathogenic microorganism, may fuse with several lysosomes.

Autophagy involves the degradation and recycling within an autophagosome of cytoplasmic components that are no longer needed, including organelles. The assembly of the autophagosome involves several proteins, including autophagy-related (Atg) proteins, as well as Hsc70 chaperone, that translocate the substrate into the lysosome (Boya et al 2013). Autophagosomes sequester cytoplasmic components and then fuse with lysosomes without the participation of a late endosome. The 26S proteasome (see below) is also involved in cellular degradation but autophagy refers specifically to a lysosomal degradation–recycling pathway. Autophagosomes are seen in response to starvation and cell growth.

Late endosomes receive lysosomal enzymes from primary lysosomes derived from the Golgi apparatus after late endosome–lysosome membrane tethering and fusion followed by diffusion of lysosomal contents into the endosomal lumen. The pH inside the fused hybrid organelle, now a secondary lysosome, is low (about 5.0) and this activates lysosomal acid hydrolases to degrade the endosomal contents. The products of hydrolysis either are passed through the membrane into the cytosol, or may be retained in the secondary lysosome. Secondary lysosomes may grow considerably in size by vesicle fusion to form multivesicular bodies, and the enzyme concentration may increase greatly to form large lysosomes (**Fig. 1.5B**).

Lysosomes

Lysosomes are membrane-bound organelles 80–800 nm in diameter, often with complex inclusions of material undergoing hydrolysis (secondary lysosomes). Two classes of proteins participate in lysosomal function: soluble acid hydrolases and integral lysosomal membrane proteins. Each of the 50 known acid hydrolases (including proteases, lipases, carbohydrases, esterases and nucleases) degrades a specific substrate. There are about 25 lysosomal membrane proteins participating in the acidification of the lysosomal lumen, protein import from the cytosol, membrane fusion and transport of degradation products to the cytoplasm. Material that has been hydrolysed within secondary lysosomes may be completely degraded to soluble products, e.g. amino acids, which are recycled through metabolic pathways. However, degradation is usually incomplete and some debris remains. A debris-laden vesicle is called a residual body or tertiary lysosome (see Fig. 1.5B), and may be passed to the cell surface, where it is ejected by exocytosis; alternatively, it may persist inside the cell as an inert residual body. Considerable numbers of residual bodies can accumulate in long-lived cells, often fusing to form larger dense vacuoles with complex lamellar inclusions. As their contents are often darkly pigmented, this may change the colour of the tissue; e.g. in neurones, the end-product of lysosomal digestion, lipofuscin (neuromelanin or senility pigment), gives ageing brains a brownish-yellow colouration. Lysosomal enzymes

Carrier vesicles in transit from the endoplasmic reticulum to the Golgi apparatus (anterograde transport) are coated by coat protein complex II (COPII), whereas COPI-containing vesicles function in the retrograde transport route from the Golgi apparatus (reviewed in Spang (2013)).

The membranes contain specific signal proteins that may allocate them to microtubule-based transport pathways and allow them to dock with appropriate targets elsewhere in the cell, e.g. the plasma membrane in the case of secretory vesicles. Vesicle formation and budding at the *trans*-Golgi network involves the addition of clathrin on their external surface, to form coated pits.

Specialized cells of the immune system, called antigen-presenting cells, degrade protein molecules, called antigens, transported by the endocytic pathway for lysosomal breakdown, and expose their fragments to the cell exterior to elicit an immune response mediated initially by helper T cells.

may also be secreted – often as part of a process to alter the extracellular matrix, as in osteoclast-mediated erosion during bone resorption. For further reading on lysosome biogenesis, see Saftig and Klumperman (2009).

Lysosomal dysfunction

Lysosomal storage diseases (LSDs) are a consequence of lysosomal dysfunction. Approximately 60 different types of LSD have been identified on the basis of the type of material accumulated in cells (such as mucopolysaccharides, sphingolipids, glycoproteins, glycogen and lipofuscins). LSDs are characterized by severe neurodegeneration, mental decline, and cognitive and behavioural abnormalities. Autophagy impairment caused by defective lysosome–autophagosome coupling triggers a pathogenic cascade by the accumulation of substrates that contribute to neurodegenerative disorders including Parkinson's disease, Alzheimer's disease, Huntington's disease and several tau-opathies.

Many lysosomal storage diseases are known, e.g. Tay–Sachs disease (GM2 gangliosidosis), in which a faulty β-hexosaminidase A leads to the accumulation of the glycosphingolipid GM2 ganglioside in neurones, causing death during childhood. In Danon disease, a vacuolar skeletal myopathy and cardiomyopathy with neurodegeneration in hemizygous males, lysosomes fail to fuse with autophagosomes because of a mutation of the lysosomal membrane protein LAMP-2 (lysosomal associated membrane protein-2).

26S proteasome

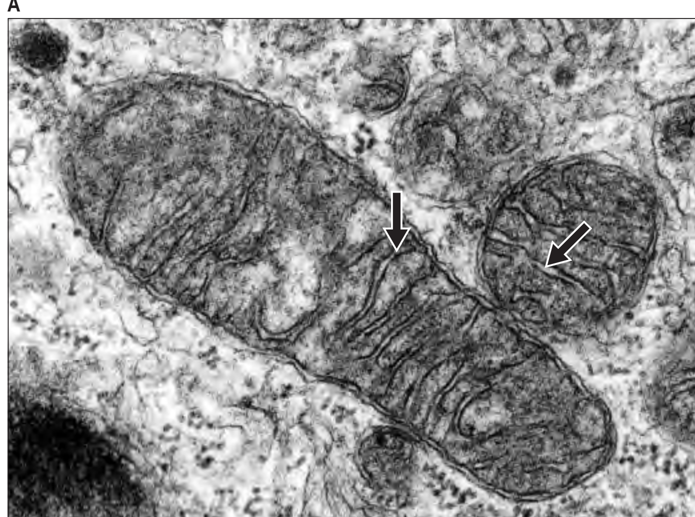
A protein can be degraded by different mechanisms, depending on the cell type and different pathological conditions. Furthermore, the same substrate can engage different proteolytic pathways (Park and Cuervo 2013). Three major protein degradation mechanisms operate in eukaryotic cells to dispose of non-functional cellular proteins: the autophagosome–lysosomal pathway (see above); the apoptotic procaspase–caspase pathway (see below); and the ubiquitinated protein–26S proteasome pathway. The 26S proteasome is a multicatalytic protease found in the cytosol and the nucleus that degrades intracellular proteins conjugated to a polyubiquitin chain by an enzymatic cascade. The 26S proteasome consists of several subunits arranged into two 19S polar caps, where protein recognition and adenosine 5′-triphosphate (ATP)-dependent target processing occur, flanking a 20S central barrel-shaped structure with an inner proteolytic chamber (Tomko and Hochstrasser 2013). The 26S proteasome participates in the removal of misfolded or abnormally assembled proteins, the degradation of cyclins involved in the control of the cell cycle, the processing and degradation of transcription regulators, cellular-mediated immune responses, and cell cycle arrest and apoptosis.

Peroxisomes

Peroxisomes are small (0.2–1 µm in diameter) membrane-bound organelles present in most mammalian cells. They contain more than 50 enzymes responsible for multiple catabolic and synthetic biochemical pathways, in particular the β-oxidation of very-long-chain fatty acids (>C22) and the metabolism of hydrogen peroxide (hence the name peroxisome). Peroxisomes derive from the endoplasmic reticulum through the transfer of proteins from the endoplasmic reticulum to peroxisomes by vesicles that bud from specialized sites of the endoplasmic reticulum and by a lipid non-vesicular pathway. All matrix proteins and some peroxisomal membrane proteins are synthesized by cytosolic ribosomes and contain a peroxisome targeting signal that enables them to be imported by proteins called peroxins (Braverman et al 2013, Theodoulou et al 2013). Mature peroxisomes divide by small daughter peroxisomes pinching off from a large parental peroxisome.

Peroxisomes often contain crystalline inclusions composed mainly of high concentrations of the enzyme urate oxidase. Oxidases use molecular oxygen to oxidize specific organic substrates (such as L-amino acids, D-amino acids, urate, xanthine and very-long-chain fatty acids) and produce hydrogen peroxide that is detoxified (degraded) by peroxisomal catalase. Peroxisomes are particularly numerous in hepatocytes. Peroxisomes are important in the oxidative detoxification of various substances taken into or produced within cells, including ethanol. Peroxin mutation is a characteristic feature of Zellweger syndrome (craniofacial dysmorphism and malformations of brain, liver, eye and kidney; cerebrohepatorenal syndrome). Neonatal leukodystrophy is an X-linked peroxisomal disease affecting mostly males, caused by deficiency in β-oxidation of very-long-chain fatty acids. The build-up of very-long-chain fatty acids in the nervous system and suprarenal glands determines progressive deterioration of brain function and suprarenal insufficiency (Addison's disease). For further reading, see Braverman et al (2013).

A



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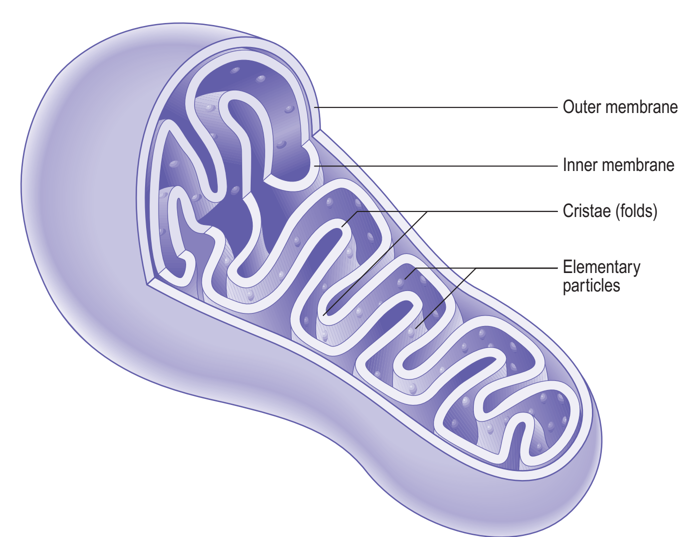


Fig. 1.6 **A**, Mitochondria in human cardiac muscle. The folded cristae (arrows) project into the matrix from the inner mitochondrial membrane. **B**, The location of the elementary particles that couple oxidation and phosphorylation reactions. (A, Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

Mitochondria

In the electron microscope, mitochondria usually appear as round or elliptical bodies 0.5–2.0 µm long (Fig. 1.6), consisting of an outer mitochondrial membrane; an inner mitochrondrial membrane, separated from the outer membrane by an intermembrane space; cristae, infoldings of the inner membrane that harbour ATP synthase to generate ATP; and the mitochondrial matrix, a space enclosed by the inner membrane and numerous cristae. The permeability of the two mitochondrial membranes differs considerably: the outer membrane is freely permeable to many substances because of the presence of large non-specific channels formed by proteins (porins), whereas the inner membrane is permeable to only a narrow range of molecules. The presence of cardiolipin, a phospholipid, in the inner membrane may contribute to this relative impermeability.

Mitochondria are the principal source of chemical energy in most cells. Mitochondria are the site of the citric acid (Krebs') cycle and the electron transport (cytochrome) pathway by which complex organic molecules are finally oxidized to carbon dioxide and water. This process provides the energy to drive the production of ATP from adenosine diphosphate (ADP) and inorganic phosphate (oxidative phosphorylation). The various enzymes of the citric acid cycle are located in the mitochondrial matrix, whereas those of the cytochrome system and oxidative phosphorylation are localized chiefly in the inner mitochondrial membrane.

The intermembrane space houses cytochrome *c*, a molecule involved in activation of apoptosis.

The number of mitochondria in a particular cell reflects its general energy requirements; e.g. in hepatocytes there may be as many as 2000, whereas in resting lymphocytes there are usually very few. Mature

The transcription factor EB (TFEB) is responsible for regulating lysosomal biogenesis and function, lysosome-to-nucleus signalling and lipid catabolism (for further reading, see Settembre et al (2013)). If any of the actions of lysosomal hydrolases, of the lysosome acidification mechanism or of lysosomal membrane proteins fails, the degradation and recycling of extracellular substrates delivered to lysosomes by the late endosome and the degradation and recycling of intracellular substrates by autophagy lead to progressive lysosomal dysfunction in several tissues and organs.

Experimentally, TFEB activation can reduce the accumulation of the pathogenic protein in a cellular model of Huntington's disease (a neurodegenerative genetic disorder that affects muscle coordination) and improves the Parkinson's disease phenotype in a murine model.

Cristae are abundant in mitochondria seen in cardiac muscle cells and in steroid-producing cells (in the suprarenal cortex, corpus luteum and Leydig cells). The protein steroidogenic acute regulatory protein (StAR) regulates the synthesis of steroids by transporting cholesterol across the outer mitochondrial membrane. A mutation in the gene encoding StAR causes defective suprarenal and gonadal steroidogenesis.

erythrocytes lack mitochondria altogether. Cells with few mitochondria generally rely largely on glycolysis for their energy supplies. These include some very active cells, e.g. fast twitch skeletal muscle fibres, which are able to work rapidly but for only a limited duration. Mitochondria appear in the light microscope as long, thin structures in the cytoplasm of most cells, particularly those with a high metabolic rate, e.g. secretory cells in exocrine glands. In living cells, mitochondria constantly change shape and intracellular position; they multiply by growth and fission, and may undergo fusion.

The mitochondrial matrix is an aqueous environment. It contains a variety of enzymes, and strands of mitochondrial DNA with the capacity for transcription and translation of a unique set of mitochondrial genes (mitochondrial mRNAs and transfer RNAs, mitochondrial ribosomes with rRNAs). The DNA forms a closed loop, about 5 µm across; several identical copies are present in each mitochondrion. The ratio between its bases differs from that of nuclear DNA, and the RNA sequences also differ in the precise genetic code used in protein synthesis. At least 13 respiratory chain enzymes of the matrix and inner membrane are encoded by the small number of genes along the mitochondrial DNA. The great majority of mitochondrial proteins are encoded by nuclear genes and made in the cytosol, then inserted through special channels in the mitochondrial membranes to reach their destinations. Their membrane lipids are synthesized in the endoplasmic reticulum.

It has been shown that mitochondria are of maternal origin because the mitochondria of spermatozoa are not generally incorporated into the ovum at fertilization. Thus mitochondria (and mitochondrial genetic variations and mutations) are passed only through the female line.

Mitochondria are distributed within a cell according to regional energy requirements, e.g. near the bases of cilia in ciliated epithelia, in the basal domain of the cells of proximal convoluted tubules in the renal cortex (where considerable active transport occurs) and around the proximal segment, called middle piece, of the flagellum in spermatozoa. They may be involved with tissue-specific metabolic reactions, e.g. various urea-forming enzymes are found in liver cell mitochondria. Moreover, a number of genetic diseases of mitochondria affect particular tissues exclusively, e.g. mitochondrial myopathies (skeletal muscle) and mitochondrial neuropathies (nervous tissue). For further information on mitochondrial genetics and disorders, see Chinnery and Hudson (2013).

Cytosolic inclusions

The aqueous cytosol surrounds the membranous organelles described above. It also contains various non-membranous inclusions, including free ribosomes, components of the cytoskeleton, and other inclusions, such as storage granules (e.g. glycogen), pigments (such as lipofuscin granules, remnants of the lipid oxidative mechanism seen in the suprarenal cortex) and lipid droplets.

Lipid droplets

Lipid droplets are spherical bodies of various sizes found within many cells, but are especially prominent in the adipocytes (fat cells) of adipose connective tissue. They do not belong to the Golgi-related vacuolar system of the cell. They are not membrane-bound, but are droplets of lipid suspended in the cytosol and surrounded by perilipin proteins, which regulate lipid storage and lipolysis. See Smith and Ordovás (2012) for further reading on obesity and perilipins. In cells specialized for lipid storage, the vacuoles reach 80 µm or more in diameter. They function as stores of chemical energy, thermal insulators and mechanical shock absorbers in adipocytes. In many cells, they may represent end-products of other metabolic pathways, e.g. in steroid-synthesizing cells, where they are a prominent feature of the cytoplasm. They may also be secreted, as in the alveolar epithelium of the lactating breast.

Transport across cell membranes

Lipid bilayers are increasingly impermeable to molecules as they increase in size or hydrophobicity. Transport mechanisms are therefore required to carry essential polar molecules, including ions, nutrients, nucleotides and metabolites of various kinds, across the plasma membrane and into or out of membrane-bound intracellular compartments. Transport is facilitated by a variety of membrane transport proteins, each with specificity for a particular class of molecule, e.g. sugars. Transport proteins fall mainly into two major classes: channel proteins and carrier proteins.

Channel proteins form aqueous pores in the membrane, which open and close under the regulation of intracellular signals, e.g. G-proteins, to allow the flux of solutes (usually inorganic ions) of specific size and charge. Transport through ion channels is always passive, and ion flow through an open channel depends only on the ion concentration gradient and its electronic charge, and the potential difference across the membrane. These factors combine to produce an electrochemical gradient, which governs ion flux. Channel proteins are utilized most effectively by the excitable plasma membranes of nerve cells, where the resting membrane potential can change transiently from about −80 mV (negative inside the cell) to +40 mV (positive inside the cell) when stimulated by a neurotransmitter (as a result of the opening and subsequent closure of channels selectively permeable to sodium and potassium).

Carrier proteins bind their specific solutes, such as amino acids, and transport them across the membrane through a series of conformational changes. This latter process is slower than ion transport through membrane channels. Transport by carrier proteins can occur either passively by simple diffusion, or actively against the electrochemical gradient of the solute. Active transport must therefore be coupled to a source of energy, such as ATP generation, or energy released by the coordinate movement of an ion down its electrochemical gradient. Linked transport can be in the same direction as the solute, in which case the carrier protein is described as a symporter, or in the opposite direction, when the carrier acts as an antiporter.

Translocation of proteins across intracellular membranes

Proteins are generally synthesized on ribosomes in the cytosol or on the rough endoplasmic reticulum. A few are made on mitochondrial ribosomes. Once synthesized, many proteins remain in the cytosol, where they carry out their functions. Others, such as integral membrane proteins or proteins for secretion, are translocated across intracellular membranes for post-translational modification and targeting to their destinations. This is achieved by the signal sequence, an addressing system contained within the protein sequence of amino acids, which is recognized by receptors or translocators in the appropriate membrane. Proteins are thus sorted by their signal sequence (or set of sequences that become spatially grouped as a signal patch when the protein folds into its tertiary configuration), so that they are recognized by and enter the correct intracellular membrane compartment.

Cell signalling

Cellular systems in the body communicate with each other to coordinate and integrate their functions. This occurs through a variety of processes known collectively as cell signalling, in which a signalling molecule produced by one cell is detected by another, almost always by means of a specific receptor protein molecule. The recipient cell transduces the signal, which it most often detects at the plasma membrane, into intracellular chemical messages that change cell behaviour.

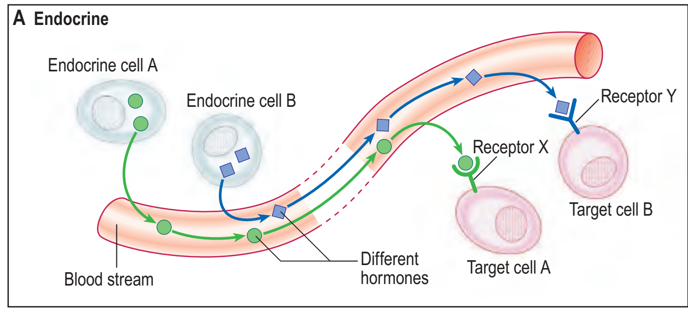
The signal may act over a long distance, e.g. endocrine signalling through the release of hormones into the blood stream, or neuronal synaptic signalling via electrical impulse transmission along axons and subsequent release of chemical transmitters of the signal at synapses or neuromuscular junctions. A specialized variation of endocrine signalling (neurocrine or neuroendocrine signalling) occurs when neurones or paraneurones (e.g. chromaffin cells of the suprarenal medulla) secrete a hormone into interstitial fluid and the blood stream.

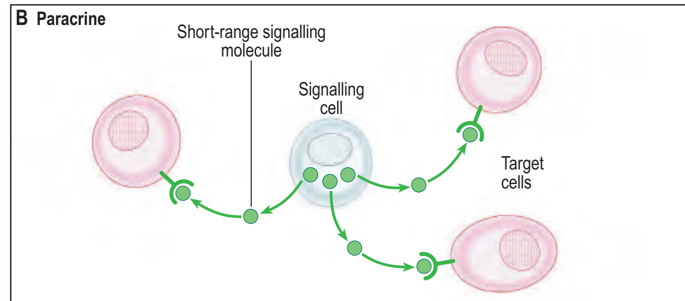
Alternatively, signalling may occur at short range through a paracrine mechanism, in which cells of one type release molecules into the interstitial fluid of the local environment, to be detected by nearby cells of a different type that express the specific receptor protein. Neurocrine cell signalling uses chemical messengers found also in the central nervous system, which may act in a paracrine manner via interstitial fluid or reach more distant target tissues via the blood stream. Cells may generate and respond to the same signal. This is autocrine signalling, a phenomenon that reinforces the coordinated activities of a group of like cells, which respond together to a high concentration of a local signalling molecule. The most extreme form of short-distance signalling is contact-dependent (juxtacrine) signalling, where one cell responds to transmembrane proteins of an adjacent cell that bind to surface receptors in the responding cell membrane. Contact-dependent signalling also includes cellular responses to integrins on the cell surface binding to elements of the extracellular matrix. Juxtacrine signalling is important during development and in immune responses. These different types of intercellular signalling mechanism are illustrated in **Figure 1.7**. For further reading on cell signalling pathways, see Kierszenbaum and Tres (2012).

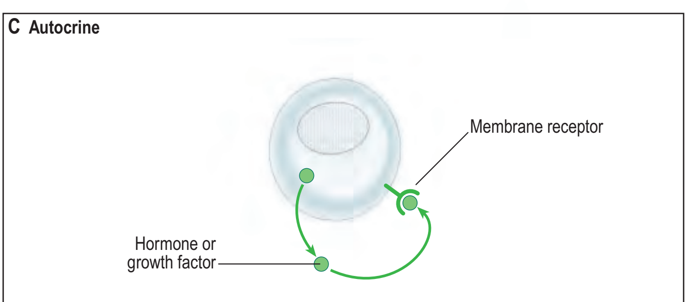
Signalling molecules and their receptors

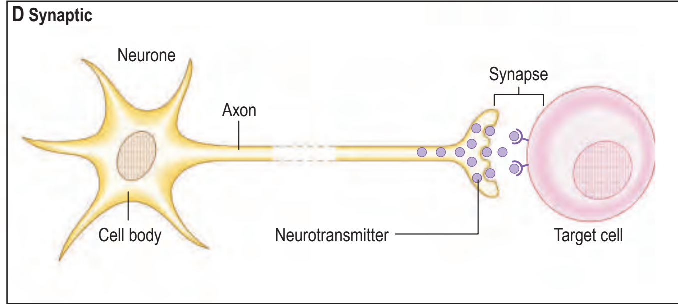
The majority of signalling molecules (ligands) are hydrophilic and so cannot cross the plasma membrane of a recipient cell to effect changes

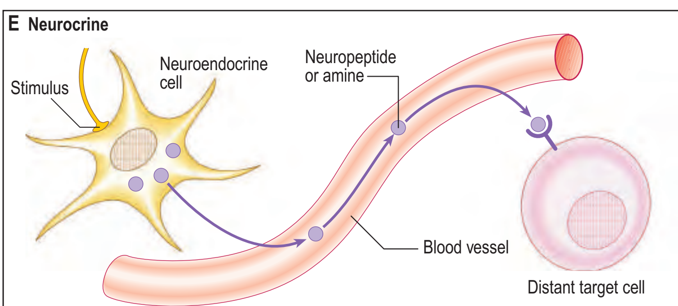
Mitochondrial ribosomes are smaller and quite distinct from those of the rest of the cell in that they (and mitochondrial nucleic acids) resemble those of bacteria. This similarity underpins the theory that mitochondrial ancestors were oxygen-utilizing bacteria that existed in a symbiotic relationship with eukaryotic cells unable to metabolize the oxygen produced by early plants. As mitochondria are formed only from previously existing ones, it follows that all mitochondria in the body are descended from those in the cytoplasm of the fertilized ovum.











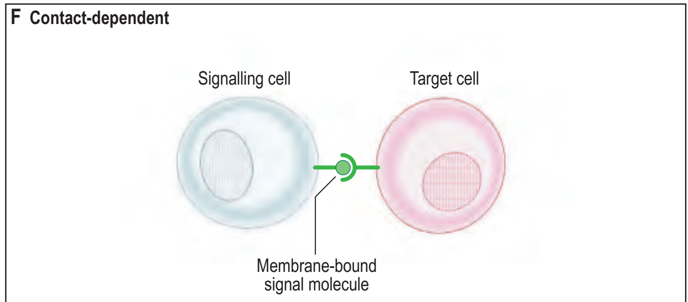


Fig. 1.7 The different modes of cell–cell signalling.

inside the cell unless they first bind to a plasma membrane receptor protein. Ligands are mainly proteins (usually glycoproteins), polypeptides or highly charged biogenic amines. They include: classic peptide hormones of the endocrine system; cytokines, which are mainly of haemopoietic cell origin and involved in inflammatory responses and tissue remodelling (e.g. the interferons, interleukins, tumour necrosis factor, leukaemia inhibitory factor); and polypeptide growth factors (e.g. the epidermal growth factor superfamily, nerve growth factor, platelet-derived growth factor, the fibroblast growth factor family, transforming growth factor beta and the insulin-like growth factors). Polypeptide growth factors are multifunctional molecules with more widespread actions and cellular sources than their names suggest. They and their receptors are commonly mutated or aberrantly expressed in certain cancers. The cancer-causing gene variant is termed a transforming oncogene and the normal (wild-type) version of the gene is a cellular oncogene or proto-oncogene. The activated receptor acts as a transducer to generate intracellular signals, which are either small diffusible second messengers (e.g. calcium, cyclic adenosine monophosphate or the plasma membrane lipid-soluble diacylglycerol), or larger protein complexes that amplify and relay the signal to target control systems.

Some signals are hydrophobic and able to cross the plasma membrane freely. Classic examples are the steroid hormones, thyroid hormones, retinoids and vitamin D. Steroids, for instance, enter cells non-selectively, but elicit a specific response only in those target cells that express specific cytoplasmic or nuclear receptors. Light stimuli also cross the plasma membranes of photoreceptor cells and interact intracellularly, at least in rod cells, with membrane-bound photosensitive receptor proteins. Hydrophobic ligands are transported in the blood stream or interstitial fluids, generally bound to carrier proteins, and they often have a longer half-life and longer-lasting effects on their targets than do water-soluble ligands.

A separate group of signalling molecules able to cross the plasma membrane freely is typified by the gas, nitric oxide. The principal target of short-range nitric oxide signalling is smooth muscle, which relaxes in response. Nitric oxide is released from vascular endothelium as a result of the action of autonomic nerves that supply the vessel wall causing local relaxation of smooth muscle and dilation of vessels. This mechanism is responsible for penile erection. Nitric oxide is unusual among signalling molecules in having no specific receptor protein; it acts directly on intracellular enzymes of the response pathway.

Receptor proteins

There are some 20 different families of receptor proteins, each with several isoforms responding to different ligands. The great majority of these receptors are transmembrane proteins. Members of each family share structural features that indicate either shared ligand-binding characteristics in the extracellular domain or shared signal transduction properties in the cytoplasmic domain, or both. There is little relationship either between the nature of a ligand and the family of receptor proteins to which it binds and activates, or the signal transduction strategies by which an intracellular response is achieved. The same ligand may activate fundamentally different types of receptor in different cell types.

Cell surface receptor proteins are generally grouped according to their linkage to one of three intracellular systems: ion channel-linked receptors; G-protein coupled receptors; and receptors that link to enzyme systems. Other receptors do not fit neatly into any of these categories. All the known G-protein coupled receptors belong to a structural group of proteins that pass through the membrane seven times in a series of serpentine loops. These receptors are thus known as seven-pass transmembrane receptors or, because the transmembrane regions are formed from α-helical domains, as seven-helix receptors. The best known of this large group of phylogenetically ancient receptors are the odorant-binding proteins of the olfactory system; the light-sensitive receptor protein, rhodopsin; and many of the receptors for clinically useful drugs. A comprehensive list of receptor proteins, their activating ligands and examples of the resultant biological function is given in Pollard and Earnshaw (2008).

Intracellular signalling

A wide variety of small molecules carry signals within cells, conveying the signal from its source (e.g. activated plasma membrane receptor) to its target (e.g. the nucleus). These second messengers convey signals as fluctuations in local concentration, according to rates of synthesis and degradation by specific enzymes (e.g. cyclases involved in cyclic nucleotide (cAMP, cGMP) synthesis), or, in the case of calcium, according to the activities of calcium channels and pumps. Other, lipidic, second

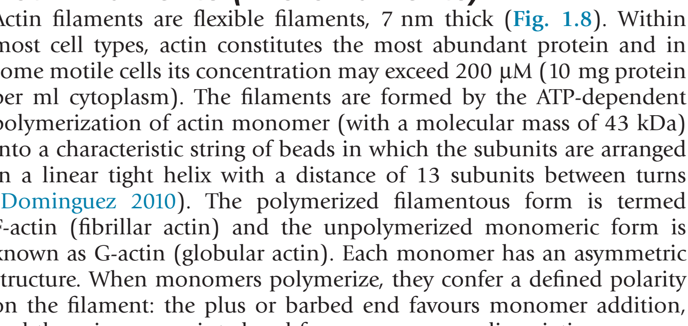
messengers such as phosphatidylinositol, derive from membranes and may act within the membrane to generate downstream effects. For further consideration of the complexity of intracellular signalling pathways, see Pollard and Earnshaw (2008).

Cytoskeleton

The cytoskeleton is a three-dimensional network of filamentous intracellular proteins of different shapes, sizes and composition distributed throughout the cytoplasm. It provides mechanical support, maintains cell shape and rigidity, and enables cells to adopt highly asymmetric or irregular profiles. It plays an important part in establishing structural polarity and different functional domains within a cell. It also provides mechanical support for permanent projections from the cell surface (see below), including persistent microvilli and cilia, and transient processes, such as the thin finger-like protrusions called filopodia (0.1–0.3 µm) and lamellipodia (0.1–0.2 µm). Filopodia consist of parallel bundles of actin filaments and have a role in cell migration, wound healing and neurite growth. The protrusive thin and broad lamellipodia, found at the leading edge of a motile cell, contain a branched network of actin filaments.

The cytoskeleton restricts specific structures to particular cellular locations. For example, the Golgi apparatus is near the nucleus and endoplasmic reticulum, and mitochondria are near sites of energy requirement. In addition, the cytoskeleton provides tracks for intracellular transport (e.g. shuttling vesicles and macromolecules, called cargoes, among cytoplasmic sites), the movement of chromosomes during cell division (mitosis and meiosis) or movement of the entire cell during embryonic morphogenesis or the chemotactic extravascular migration of leukocytes during homing. Examples of highly developed and specialized functions of the cytoskeleton include the contraction of the sarcomere in striated muscle cells and the bending of the axoneme of cilia and flagella.

The catalogue of cytoskeletal structural proteins is extensive and still increasing. The major filamentous structures found in non-muscle cells are microfilaments (7 nm thick), microtubules (25 nm thick) and intermediate filaments (10 nm thick). Other important components are proteins that bind to the principal filamentous types to assemble or disassemble them, regulate their stability or generate movement. These include actin-binding proteins such as myosin, which in some cells can assemble into thick filaments, and microtubule-associated proteins. Pathologies involving cytoskeletal abnormalities include ciliopathies (resulting from the abnormal assembly and function of centrioles, basal bodies and cilia); neurodegenerative diseases (a consequence of defective anterograde transport of neurotransmitters along microtubules in axons); and sterility (determined by defective or absent microtubule-associated dynein in axonemes, e.g. Kartagener's syndrome).



Actin filaments (microfilaments)

Actin filaments are flexible filaments, 7 nm thick (Fig. 1.8). Within most cell types, actin constitutes the most abundant protein and in some motile cells its concentration may exceed 200 µM (10 mg protein per ml cytoplasm). The filaments are formed by the ATP-dependent polymerization of actin monomer (with a molecular mass of 43 kDa) into a characteristic string of beads in which the subunits are arranged in a linear tight helix with a distance of 13 subunits between turns (Dominguez 2010). The polymerized filamentous form is termed F-actin (fibrillar actin) and the unpolymerized monomeric form is known as G-actin (globular actin). Each monomer has an asymmetric structure. When monomers polymerize, they confer a defined polarity on the filament: the plus or barbed end favours monomer addition, and the minus or pointed end favours monomer dissociation.

Treadmilling designates the simultaneous polymerization of an actin filament at one end and depolymerization at the other end to maintain its constant length.

See Bray (2001) for further reading.

Actin-binding proteins

A wide variety of actin-binding proteins are capable of modulating the form of actin within the cell. These interactions are fundamental to the

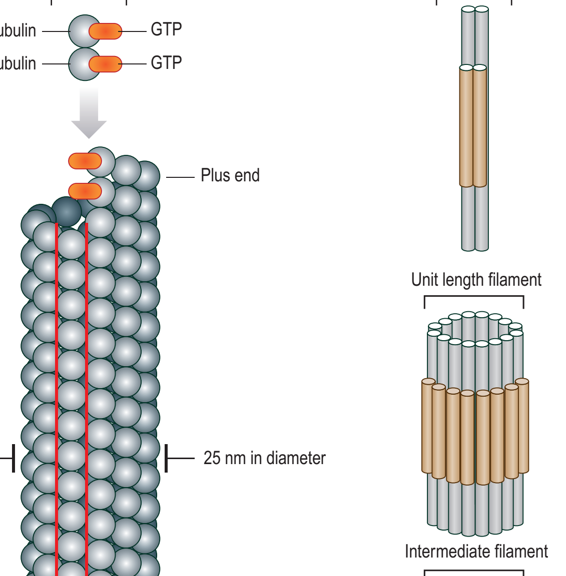
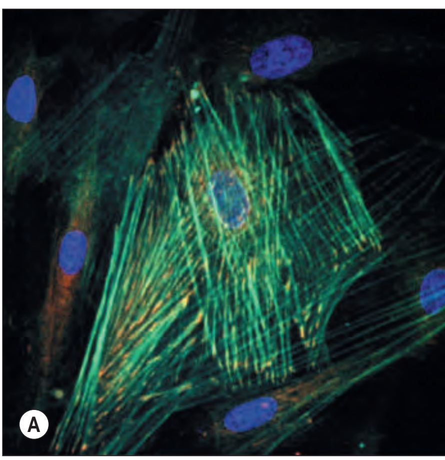


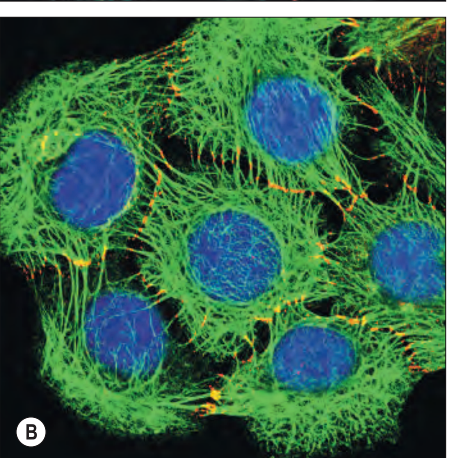
Fig. 1.8 Structural and molecular features of cytoskeletal components. **A**, The actin filament (F-actin) is a 7 nm thick polymer chain of ATP-bound G-actin monomers. F-actin consists of a barbed (plus) end, the initiation site of F-actin, and a pointed (minus) end, the dissociation site of F-actin. F-actin can be severed and capped at the barbed end by gelsolin. **B**, The microtubule is a 25 nm diameter polymer of GTP-bound α-tubulin and GTP-bound β-tubulin dimers. The dimer assembles at the plus end and depolymerizes at the minus end. A linear chain of α-tubulin/β-tubulin dimers is called a protofilament. In the end-on (top view), a microtubule displays 13 concentrically arranged tubulin subunits. **C**, Tetrameric complexes of intermediate filament subunits associate laterally to form a unit length filament consisting of eight tetramers. Additional unit length filaments anneal longitudinally and generate a mature 10 nm thick intermediate filament.

Septins are emerging as a novel cytoskeletal member because of their filamentous organization and association with actin filaments and microtubules. They are guanosine triphosphate (GTP)-binding proteins that form hetero-oligomeric complexes (see Mostowy and Cossart (2012) for additional information).

This polarity can be visualized in negatively stained images by allowing F-actin to react with fragments containing the active head region of myosin. Myosins bind to filamentous actin at an angle to give the appearance of a series of arrowheads pointing towards the minus end of the filament, with the barbs pointing towards the plus end.

It involves the addition of ATP-bound G-actin monomers at the barbed end (fast-growing plus end) and removal of ADP-bound G-actin at the pointed end (slow-growing minus end). Actin filaments grow or shrink by addition or loss of G-actin monomer at both ends. Essentially, actin polymerization *in vitro* proceeds in three steps: nucleation (aggregation of G-actin monomers into a 3–4-monomer aggregate), elongation (addition of G-actin monomers to the aggregate) and a dynamic steady state (treadmilling). Specific toxins (e.g. cytochalasins, phalloidins and lantrunculins) bind to actin and affect its polymerization. Cytochalasin D blocks the addition of new G-actin monomers to the barbed end of F-actin; phalloidin binds to the interface between G-actin monomers in F-actin, thus preventing depolymerization; and lantrunculin binds to G-actin monomers, blocking their addition to an actin filament.





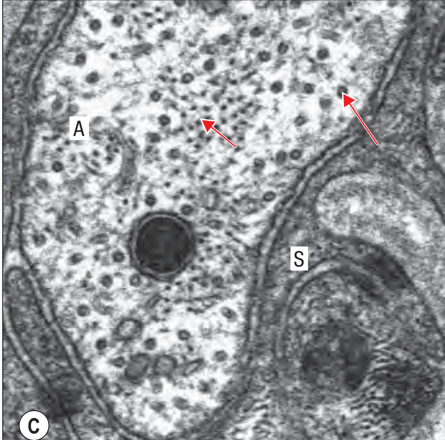


Fig. 1.9 The cytoskeleton. **A**, An immunofluorescence micrograph of α-actin microfilaments (green) in human airway smooth muscle cells in culture. The actin-binding protein, vinculin (red), is localized at the ends of actin filament bundles; nuclei are blue. **B**, An immunofluorescence micrograph of keratin intermediate filaments (green) in human keratinocytes in culture. Desmosome junctions are labelled with antibody against desmoplakin (red). Nuclei are stained blue (Hoechst). **C**, An electron micrograph of human nerve showing microtubules (small, hollow structures in cross-section, long arrow) in a transverse section of an unmyelinated axon (A), engulfed by a Schwann cell (S). Neuronal intermediate filaments (neurofilaments) are the solid, electron-dense profiles, also in transverse section (short arrow). (A, Courtesy of Dr T Nguyen, Professor J Ward, Dr SJ Hirst, King's College London. B, Courtesy of Prof. Dr WW Franke, German Cancer Research Centre, Heidelberg. C, Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

organization of cytoplasm and to cell shape. The actin cytoskeleton is organized as closely packed parallel arrays of actin filaments forming bundles or cables, or loosely packed criss-crossed actin filaments forming networks (Fig. 1.9A). Actin-binding proteins hold together bundles and networks of actin filaments. Actin-binding proteins can be grouped into G-actin (monomer) binding proteins and F-actin (polymer) capping, cross-linking and severing proteins. Actin-binding proteins may have more than one function.

Capping proteins bind to the ends of the actin filament either to stabilize an actin filament or to promote its disassembly (see Fig. 1.8).

Cross-linking or bundling proteins tie actin filaments together in longitudinal arrays to form bundles, cables or core structures. The bundles may be closely packed in microvilli and filopodia, where parallel filaments are tied tightly together to form stiff bundles orientated in the same direction. Cross-linking proteins of the microvillus actin bundle core include fimbrin and villin.

Other actin-bundling proteins form rather looser bundles of filaments that run antiparallel to each other with respect to their plus and minus ends. They include myosin II, which can form cross-links with ATP-dependent motor activity, and cause adjacent actin filaments to slide on each other in the striated muscle sarcomere, and either change the shape of cells or (if the actin bundles are anchored into the cell membrane at both ends), maintain a degree of active rigidity. Filamin interconnects adjacent actin filaments to produce loose filamentous gel-like networks composed of randomly orientated F-actin.

F-actin can branch. The assembly of branched filamentous actin networks involves a complex of seven actin-related proteins 2/3 (Arp2/3) that is structurally similar to the barbed end of actin.

See Rotty et al (2013) for further reading.

Branched actin generated by the Arp2/3 protein complex localizes at the leading edge of migrating cells, lamellipodia and phagosomes (required for the capture by endocytosis and phagocytosis of particles and foreign pathogens by immune cells). Formin can elongate pre-existing actin filaments by removing capping proteins at the barbed end.

Other classes of actin-binding protein link the actin cytoskeleton to the plasma membrane either directly or indirectly through a variety of membrane-associated proteins. The latter may also create links via transmembrane proteins to the extracellular matrix. Best known of these is the family of spectrin-like molecules, which can bind to actin and also to each other and to various membrane-associated proteins to create supportive networks beneath the plasma membrane. Tetrameres of spectrin α and β chains line the intracellular side of the plasma membrane of erythrocytes and maintain their integrity by their association with short actin filaments at either end of the tetramer.

Class V myosins are unconventional motor proteins transporting cargoes (such as vesicles and organelles) along actin filaments. Class I myosins are involved in membrane dynamics and actin organization at the cell cortex, thus affecting cell migration, endocytosis, pinocytosis and phagocytosis. Tropomyosin, an important regulatory protein of muscle fibres, is also present in non-muscle cells, where its function may be primarily to stabilize actin filaments against depolymerization.

Myosins, the motor proteins

The myosin family of microfilaments is often classified within a distinct category of motor proteins. Myosin proteins have a globular head region consisting of a heavy and a light chain. The heavy chain bears an α-helical tail of varying length. The head has an ATPase activity and can bind to and move along actin filaments – the basis for myosin function as a motor protein. The best-known class is myosin II, which occurs in muscle and in many non-muscle cells. Its molecules have two heads and two tails, intertwined to form a long rod. The rods can bind to each other to form long, thick filaments, as seen in striated and smooth muscle fibres and myoepithelial cells. Myosin II molecules can also assemble into smaller groups, especially dimers, which can cross-link individual actin microfilaments in stress fibres and other F-actin arrays. The ATP-dependent sliding of myosin on actin forms the basis for muscle contraction and the extension of microfilament bundles, as seen in cellular motility or in the contraction of the ring of actin and myosin around the cleavage furrow of dividing cells. There are a number of known subtypes of myosin II; they assemble in different ways and have different dynamic properties. In skeletal muscle the myosin molecules form bipolar filaments 15 nm thick. Because these filaments have a symmetric antiparallel arrangement of subunits, the midpoint is bare of head regions. In smooth muscle the molecules form thicker, flattened bundles and are orientated in random directions on either face of the bundle. These arrangements have important consequences for the contractile force characteristics of the different types of muscle cell.

Related molecules include the myosin I subfamily of single-headed molecules with tails of varying length. Functions of myosin I include the movements of membranes in endocytosis, filopodial formation in neuronal growth cones, actin–actin sliding and attachment of actin to membranes as seen in microvilli. As indicated above, molecular motors of the myosin V family are implicated in the movements of cargoes on actin filaments. So, for example, myosin Va transports vesicles along F-actin tracks in a similar manner to kinesin and cytoplasmic dynein-related cargo transport along microtubules. Each class of motor protein has different properties, but during cargo trafficking they often function together in a coordinated fashion. (See Hammer 3rd and Sellers (2012) for further reading on class V myosins.)

Other thin filaments

A heterogeneous group of filamentous structures with diameters of 2–4 nm occurs in various cells. The two most widely studied forms, titin and nebulin, constitute around 13% of the total protein of skeletal muscle. They are amongst the largest known molecules and have subunit weights of around 10^6; native molecules are about 1 μm in length. Their repetitive bead-like structure gives them elastic properties that are important for the effective functioning of muscle, and possibly for other cells.

Profilin and thymosin β4 are G-actin binding proteins. Profilin binds to G-actin bound to ATP; it inhibits addition of G-actin to the slow-growing (pointed) end of F-actin but enables the fast-growing (barbed) end to grow faster and then dissociates from the actin filament. In addition, profilin participates in the conversion of ADP back to the ATP–G-actin bound form. Thymosin β4 binds to the ATP–G-actin bound form, preventing polymerization by sequestering ATP–G-actin into a reserve pool.

Members of the F-actin capping protein family are heterodimers consisting of an α subunit (CPα) and a β subunit (CPβ) that cap the barbed end of actin filaments within all eukaryotic cells. Gelsolin has a dual role: it severs F-actin and caps the newly formed barbed end, blocking further filament elongation.

Fascin is an additional cross-linking protein. Villin is also a severing protein, causing the disassembly of actin filaments and the collapse of the microvillus.

In the presence of activated nucleation promotion factors, such as Wiskott–Aldrich syndrome protein (WASP) and WASP family verprolin-homologous protein (WAVE, also known as SCAR), the Arp2/3 protein complex binds to the side of an existing actin filament (mother filament) and initiates the formation of a branching actin daughter filament at a 70° angle relative to the mother filament utilizing G-actin delivered to the Arp2/3 complex site.

Spectrin-related molecules are present in many other cells. For instance, fodrin is found in neurones and dystrophin occurs in muscle cells, linking the contractile apparatus with the extracellular matrix via integral membrane proteins. Proteins such as ankyrin (which also binds actin directly), vinculin, talin, zyxin and paxillin connect actin-binding proteins to integral plasma membrane proteins such as integrins (directly or indirectly), and thence to focal adhesions (consisting of a bundle of actin filaments attached to a portion of a plasma membrane linked to the extracellular matrix).

Microtubules

Microtubules are polymers of tubulin with the form of hollow, relatively rigid cylinders, approximately 25 nm in diameter and of varying length (up to 70 µm in spermatozoan flagella). They are present in most cell types, being particularly abundant in neurones, leukocytes and blood platelets. Microtubules are the predominant constituents of the mitotic spindles of dividing cells and also form part of the axoneme of cilia, flagella and centrioles.

Microtubules consist of tubulin dimers and microtubule-associated proteins. There are two major classes of tubulin: α- and β-tubulins. Before microtubule assembly, tubulins are associated as dimers with a combined molecular mass of 100 kDa (50 kDa each). Each protein subunit is approximately 5 nm across and is arranged along the long axis in straight rows of alternating α- and β-tubulins, forming protofilaments (see Fig. 1.8). Typically, 13 protofilaments (the number can vary between 11 and 16) associate in a ring to form the wall of a hollow cylindrical microtubule. Each longitudinal row is slightly out of alignment with its neighbour, so that a spiral pattern of alternating α and β subunits appears when the microtubule is viewed from the side. There is a dynamic equilibrium between the dimers and assembled microtubules: dimeric asymmetry creates polarity (α-tubulins are all orientated towards the minus end, β-tubulins towards the plus end). Tubulin is added preferentially to the plus end; the minus end is relatively slow-growing. Microtubules frequently grow and shrink at a rapid and constant rate, a phenomenon known as dynamic instability, in which growing tubules can undergo a 'catastrophe', abruptly shifting from net growth to rapid shrinkage. The primary determinant of whether microtubules grow or shrink is the rate of GTP hydrolysis. Tubulins are GTP-binding proteins; microtubule growth is accompanied by hydrolysis of GTP, which may regulate the dynamic behaviour of the tubules. Microtubule growth is initiated at specific sites, the microtubule-organizing centres, of which the best known are centrosomes (from which most cellular microtubules polymerize) and the centriole-derived basal bodies (from which cilia grow). Microtubule-organizing centres include a specialized tubulin isoform known as γ-tubulin that is essential for the nucleation of microtubule growth.

Various drugs (e.g. colcemid, vinblastine, griseofulvin, nocodazole) cause microtubule depolymerization by binding the soluble tubulin dimers and so shifting the equilibrium towards the unpolymerized state. Microtubule disassembly causes a wide variety of effects, including the inhibition of cell division by disruption of the mitotic spindle. Conversely, the drug paclitaxel (taxol) is a microtubule depolymerization inhibitor because it stabilizes microtubules and promotes abnormal microtubule assembly. Although this can cause a peripheral neuropathy, paclitaxel is widely used as an effective chemotherapeutic agent in the treatment of breast and ovarian cancer.

Microtubule-associated proteins

Various proteins that can bind to assembled tubulin may be concerned with structural properties or associated with motility. One important class of microtubule-associated proteins (MAPs) consists of proteins that associate with the plus ends of microtubules. They regulate the dynamic instability of microtubules as well as interactions with other cellular substructures. Structural MAPs form cross-bridges between adjacent microtubules or between microtubules and other structures such as intermediate filaments, mitochondria and the plasma membrane. Microtubule-associated proteins found in neurones include: MAPs 1A and 1B, which are present in neuronal dendrites and axons; MAPs 2A and 2B, found chiefly in dendrites; and tau, found only in axons. MAP 4 is the major microtubule-associated protein in many other cell types. Structural microtubule-associated proteins are implicated in microtubule formation, maintenance and disassembly, and are therefore of considerable significance in cell morphogenesis, mitotic division, and the maintenance and modulation of cell shape. Transport-associated microtubule-associated proteins are found in situations in which movement occurs over the surfaces of microtubules, e.g. cargo transport, bending of cilia and flagella, and some movements of mitotic spindles. They include a large family of motor proteins, the best known of which are the dyneins and kinesins. Another protein, dynamin, is involved in endocytosis. The kinetochore proteins assemble at the chromosomal centromere during mitosis and meiosis. They attach (and thus fasten chromosomes) to spindle microtubules; some of the kinetochore proteins are responsible for chromosomal movements in mitotic and meiotic anaphase.

All of these microtubule-associated proteins bind to microtubules and either actively slide along their surfaces or promote microtubule assembly or disassembly. Kinesins and dyneins can simultaneously attach to membranes such as transport vesicles and convey them along microtubules for considerable distances, thus enabling selective targeting of materials within the cell. Such movements occur in both directions along microtubules. Kinesin-dependent motion is usually towards the plus ends of microtubules, e.g. from the cell body towards the axon terminals in neurones, and away from the centrosome in other cells. Conversely, dynein-related movements are in the opposite direction, i.e. to the minus ends of microtubules. Dyneins also form the arms of peripheral microtubules in cilia and flagella, where they make dynamic cross-bridges to adjacent microtubule pairs. When these tethered dyneins try to move, the resulting shearing forces cause the axonemal array of microtubules to bend, generating ciliary and flagellar beating movements. Kinesins form a large and diverse family of related microtubule-stimulated ATPases. Some kinesins are motors that move cargo and others cause microtubule disassembly, whilst still others cross-link mitotic spindle microtubules to push the two centriolar poles apart during mitotic prophase. See Bray (2001) for further reading.

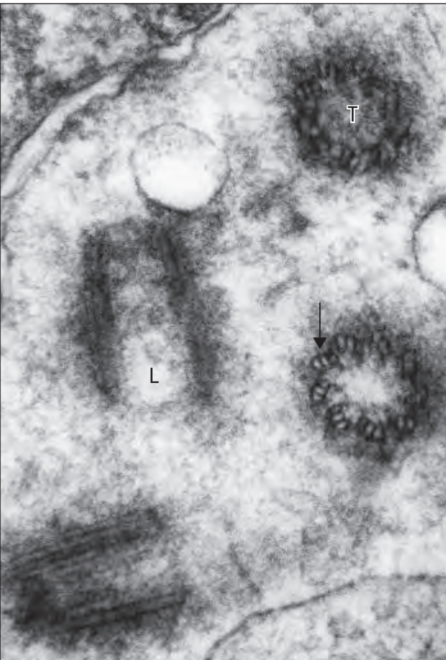


Fig. 1.10 A duplicated pair of centrioles in a human carcinoma specimen. Each centriole pair consists of a mother and daughter, orientated approximately at right angles to each other so that one is sectioned transversely (T) and the other longitudinally (L). The transversely sectioned centrioles are seen as rings of microtubule triplets (arrow). (Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

Centrioles, centrosomes and basal bodies

Centrioles are microtubular cylinders 0.2 µm in diameter and 0.4 µm long (**Fig. 1.10**). They are formed by a ring of nine microtubule triplets linked by a number of other proteins. At least two centrioles occur in all animal cells that are capable of mitotic division (eggs, which undergo meiosis instead of mitosis, lack centrioles). See Gönczy (2012) for further reading on the structure and assembly of the centriole. They usually lie close together, at right angles or, most usually, at an oblique angle to each other (an arrangement often termed a diplosome), within the centrosome, a densely filamentous region of cytoplasm at the centre of the cell. The centrosome is the major microtubule-organizing centre of most cells; it is the site at which new microtubules are formed and the mitotic spindle is generated during cell division. Centriole biogenesis is a complex process. At the beginning of the S phase (DNA replication phase) of the cell cycle (see below), a new daughter centriole forms at right angles to each separated maternal centriole. Each mother–daughter pair forms one pole of the next mitotic spindle, and the daughter centriole becomes fully mature only as the progeny cells are about to enter the next mitosis. Because centrosomes are microtubule-organizing centres, they lie at the centre of a network of microtubules, all of which have their minus ends proximal to the centrosome.

The microtubule-organizing centre contains complexes of γ-tubulin that nucleate microtubule polymerization at the minus ends of microtubules. Basal bodies are microtubule-organizing centres that are closely related to centrioles, and are believed to be derived from them. They are located at the bases of cilia and flagella, which they anchor to the cell surface. The outer microtubule doublets of the axoneme of cilia and flagella originate from two of the microtubules in each triplet of the basal body.

Microtubule-based transport of cargoes

The transport of cargoes along microtubules via the motor proteins kinesin and cytoplasmic dynein respectively is the means by which neurotransmitters are delivered along axons to neuronal synapses

The association of membrane vesicles with dynein motors means that certain cytomembranes (including the Golgi apparatus) concentrate near the centrosome. This is convenient because the microtubules provide a means of targeting Golgi vesicular products to different parts of the cell.

(anterograde axonal transport) and membrane-bound vesicles are returned for recycling to the neuronal soma (retrograde axonal transport) (p. 45). In addition to anterograde and retrograde motor proteins, the assembly and maintenance of all cilia and flagella involve the participation of non-membrane-bound macromolecular protein complexes called intraflagellar transport (IFT) particles. IFT particles localize along the polarized microtubules of the axoneme, beneath the ciliary and flagellar membrane. IFT particles consist of two protein subcomplexes: IFT-A (with a role in returning cargoes from the tip of the axoneme to the cell body) and IFT-B (with a role in delivering cargoes from the cell body to the tip of the axoneme). For further reading, see Scholey (2008) and Hao and Scholey (2009).

During ciliogenesis, IFT requires the anterograde kinesin-2 motor and the retrograde IFT-dynein motor to transport IFT particles–cargo complexes in opposite directions along the microtubules, from the basal body to the tip of the ciliary axoneme and back again (intraciliary transport). IFT is not just restricted to microtubules of cilia and flagella. During spermatid development, IFT particles–motor protein–cargo complexes appear to utilize microtubules of the manchette, a transient microtubule-containing structure, to deliver tubulin dimers and other proteins by intramanchette transport during the development of the spermatid tail (Kierszenbaum et al 2011). IFT also occurs along the modified cilium of photoreceptor cells of the retina. Mutations in IFT proteins lead to the absence of cilia and are lethal during embryogenesis. Ciliopathies, many related to the defective sensory and/or mechanical function of cilia, include retinal degeneration, polycystic kidney disease, Bardet–Biedl syndrome, Jeune asphyxiating thoracic dystrophy, respiratory disease and defective determination of the left–right axis. The seven-protein complex designated BBSome (for Bardet–Biedl syndrome, an obesity/retinopathy ciliopathy) is a component of the basal body and participates in the formation of the primary cilium by regulating the export and/or import of ciliary proteins. The transport of the BBSome up and down and round about in cilia occurs in association with anterograde IFT-B and retrograde IFT-A particles. For further reading on the BBSome, see Jin and Nachury (2009). For further reading on ciliogenesis, see Baldari and Rosenbaum (2010).

Intermediate filaments

Intermediate filaments are about 10 nm thick and are formed by a heterogeneous group of filamentous proteins. In contrast to actin filaments and microtubules, which are assembled from globular proteins with nucleotide-binding and hydrolysing activity, intermediate filaments consist of filamentous monomers lacking enzymatic activity. Intermediate filament proteins assemble to form linear filaments in a three-step process. First, a pair of intermediate filament protein subunits, each consisting of a central α-helical rod domain of about 310 amino acids flanked by head and tail non-α-helical domains of variable size, form a parallel dimer through their central α-helical rod domains coiled around each other. The variability of intermediate filament protein subunits resides in the length and amino-acid sequence of the head and tail domains, thought to be involved in regulating the interaction of intermediate filaments with other proteins. Second, a tetrameric unit is formed by two antiparallel half-staggered coiled dimers. Third, eight tetramers associate laterally to form a 16 nm thick unit length filament (ULF). Individual ULFs join end to end to form short filaments that continue growing longitudinally by annealing to other ULFs and existing filaments. Filament elongation is followed by internal compaction leading to the 30 nm thick intermediate filament (see Fig. 1.8). The tight association of dimers, tetramers and ULFs provides intermediate filaments with high tensile strength and resistance to stretching, compression, twisting and bending forces. In contrast to actin filaments and microtubules, intermediate filaments are non-polar (because of the antiparallel alignment of the initial tetramers) and do not bind nucleotides (as in G-actin and tubulin dimers), and ULFs anneal end to end to each other (in contrast to the polarized F-actin and microtubules, with one end, the plus end, growing faster than the other end, the minus end). See Herrmann et al (2007) for further reading.

Intermediate filaments are found in different cell types and are often present in large numbers, either to provide structural strength where it is needed (see Fig. 1.9B,C) or to provide scaffolding for the attachment of other structures. Intermediate filaments form extensive cytoplasmic networks extending from cage-like perinuclear arrangements to the cell surface. Intermediate filaments of different molecular classes are characteristic of particular tissues or states of maturity and are therefore important indicators of the origins of cells or degrees of differentiation, as well as being of considerable value in histopathology.

Intermediate filament proteins have been classified into five distinct types on the basis of their primary structure and tissue-specific expression. Of the different classes of intermediate filaments, keratin (cytokeratin) proteins are found in epithelia, where keratin filaments are always composed of equal ratios of type I (acidic) and type II (basic to neutral) keratins to form heteropolymers. About 20 types of each of the acidic and basic/neutral keratin proteins are known. For further reading on keratins in normal and diseased epithelia, see Pan et al (2012). Within the epidermis, expression of keratin heteropolymers changes as keratinocytes mature during their transition from basal to superficial layers. Genetic abnormalities of keratins are known to affect the mechanical stability of epithelia. For example, the disease epidermolysis bullosa simplex is caused by lysis of epidermal basal cells and blistering of the skin after mechanical trauma. Defects in genes encoding keratins 5 and 14 produce cytoskeletal instability leading to cellular fragility in the basal cells of the epidermis. When keratins 1 and 10 are affected, cells in the spinous (prickle) cell layer of the epidermis lyse, and this produces the intraepidermal blistering of epidermolytic hyperkeratosis. See Porter and Lane (2003) for further reading.

Type III intermediate filament proteins, including vimentin, desmin, glial fibrillary acidic protein and peripherin, form homopolymer intermediate filaments. Vimentin is expressed in mesenchyme-derived cells of connective tissue and some ectodermal cells during early development; desmins in muscle cells; glial fibrillary acidic protein in glial cells; and peripherin in peripheral axons. Type IV intermediate filaments include neurofilaments, nestin, syncoilin and α-internexin. Neurofilaments are a major cytoskeletal element in neurones, particularly in axons (see Fig. 1.9C), where they are the dominant protein. Neurofilaments (NF) are heteropolymers of low (NF–L), medium (NF–M) and high (NF–H) molecular weight (the NF–L form is always present in combination with either NF–M or NF–H forms). Abnormal accumulations of neurofilaments (neurofibrillary tangles) are characteristic features of a number of neuropathological conditions. Nestin resembles a neurofilament protein, which forms intermediate filaments in neurectodermal stem cells in particular. The type V intermediate filament group includes the nuclear lamins A, lamin B1 and lamin B2 lining the inner surface of the nuclear envelope of all nucleated cells. Lamin C is a splice variant of lamin A. Lamins provide a mechanical framework for the nucleus and act as attachment sites for a number of proteins that organize chromatin at the periphery of the nucleus. They are unusual in that they form an irregular anastomosing network of filaments rather than linear bundles. See Burke and Stewart (2013) for further reading.

Nucleus

The nucleus (see Figs 1.1–1.2) is generally the largest intracellular structure and is usually spherical or ellipsoid in shape, with a diameter of 3–10 µm. Conventional histological stains, such as haematoxylin or toluidine blue, detect the acidic components (phosphate groups) of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in cells and tissue sections. DNA and RNA molecules are said to be basophilic because of the binding affinity of their negatively charged phosphate groups to basic dyes such as haematoxylin. A specific stain for DNA is the Feulgen reaction.

Nuclear envelope

The nucleus is surrounded by the nuclear envelope, which consists of an inner nuclear membrane (INM) and an outer nuclear membrane (ONM), separated by a 40–50 nm perinuclear space that is spanned by nuclear pore complexes (NPCs). The perinuclear space is continuous with the lumen of the endoplasmic reticulum. The ONM has multiple connections with the endoplasmic reticulum, with which it shares its membrane protein components. The INM contains its own specific integral membrane proteins (lamin B receptor and emerin, both providing binding sites for chromatin bridging proteins). A mutation in the gene encoding emerin causes X-linked Emery–Dreifuss muscular dystrophy (EDMD), characterized by skeletal muscle wasting and cardiomyopathy.

The nuclear lamina, a 15–20 nm thick, protein-dense meshwork, is associated with the inner face of the INM. The major components of the nuclear lamina are lamins, the type V intermediate filament proteins consisting of A-type and B-type classes.

The nuclear lamina reinforces the nuclear membrane mechanically, determines the shape of the nucleus and provides a binding site for a range of proteins that anchor chromatin to the cytoskeleton. Nuclear lamin A, with over 350 mutations, is the most mutated protein linked to human disease. These are referred to as laminopathies, characterized by nuclear structural abnormalities that cause structurally weakened nuclei, leading to mechanical damage. Lamin A mutations cause a

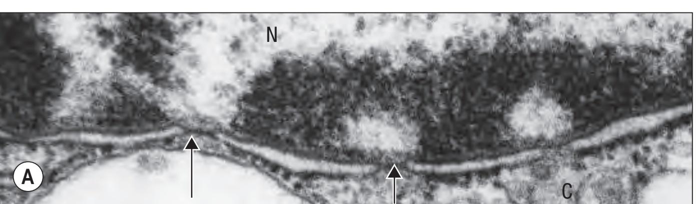
A-type lamins include lamin A (interacting with emerin), lamin C, lamin C2 and lamin AΔ10 encoded by a single gene (*LMNA*). Lamin A and lamin C are the major A-type lamins expressed in somatic cells, whereas lamin C2 is expressed in testis. B-type lamins include lamin B1 and lamin B2 (expressed in somatic cells), and testis-specific lamin B3. Lamin B1 is encoded by the *LMNB1* gene; lamin B2 is encoded by the *LMNB2* gene.

surprisingly wide range of diseases, from progeria to various dystrophies, including an autosomal dominant form of EDMD. A truncated farnesylated form of lamin A, referred to as progerin, leads to defects in cell proliferation and DNA damage of mesenchymal stem cells and vascular smooth muscle cells. Affected patients display cardiovascular disease and die at an early age. Mice lacking lamin B1 and lamin B2 survive until birth; however, neuronal development is compromised when lamin B1 or lamin B2 is absent. Overexpression of lamin B1 is associated with autosomal dominant leukodystrophy characterized by gradual demyelination in the central nervous system. See Worman (2012) and Burke and Stewart (2013) for additional reading on lamins and laminopathies.

Condensed chromatin (heterochromatin) tends to aggregate near the nuclear envelope during interphase. At the end of mitotic and meiotic prophase (see below), the lamin filaments disassemble by phosphorylation, causing the nuclear membranes to vesiculate and disperse into the endoplasmic reticulum. During the final stages of mitosis (telophase), proteins of the nuclear periphery, including lamins, associate with the surface of the chromosomes, providing docking sites for membrane vesicles. Fusion of these vesicles reconstitutes the nuclear envelope, including the nuclear lamina, following lamin dephosphorylation. See Simon and Wilson (2011) for further reading on the nucleoskeleton.

The transport of molecules between the nucleus and the cytoplasm occurs via specialized nuclear pore structures that perforate the nuclear membrane (**Fig. 1.11A**). They act as highly selective directional molecular filters, permitting proteins such as histones and gene regulatory proteins (which are synthesized in the cytoplasm but function in the nucleus) to enter the nucleus, and molecules that are synthesized in the nucleus but destined for the cytoplasm (e.g. ribosomal subunits, transfer RNAs and messenger RNAs) to leave the nucleus.

Ultrastructurally, nuclear pores appear as disc-like structures with an outer diameter of 130 nm and an inner pore with an effective diameter for free diffusion of 9 nm (**Fig. 1.11B**). The nuclear envelope of an active cell contains up to 4000 such pores. The nuclear pore complex has an octagonal symmetry and is formed by an assembly of more than 50 proteins, the nucleoporins. The inner and outer nuclear membranes fuse around the pore complex (see Fig. 1.11A). Nuclear pores are freely permeable to small molecules, ions and proteins up to about 17 kDa. See Raices and D'Angelo (2012) for further reading on nuclear pore complex composition. Most proteins that enter the nucleus do so as complexes with specific transport receptor proteins known as importins. Importins shuttle back and forth between the nucleus and cytoplasm. Binding of the cargo to the importin requires a short sequence of amino acids known as a nuclear localization sequence (NLS), and can either be direct or take place via an adapter protein. Interactions of the importin with components of the nuclear pore move it, together with its cargo, through the pore by an energy-independent process. A complementary cycle functions in export of proteins and RNA molecules from the nucleus to the cytoplasm using transport receptors known as exportins.



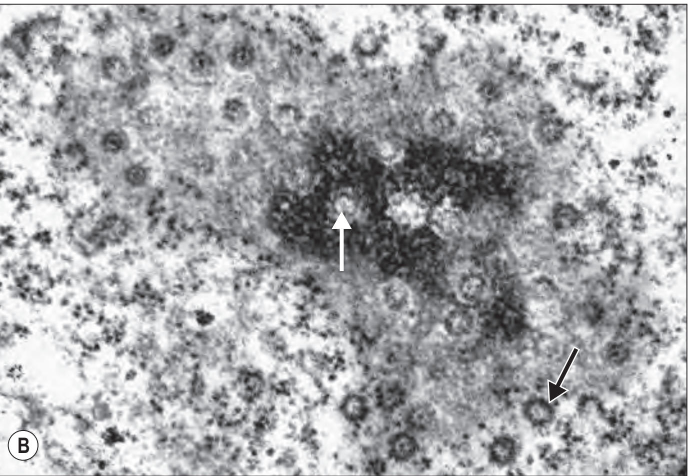


Fig. 1.11 **A**, The nuclear envelope with nuclear pores (arrows) in transverse section, showing the continuity between the inner and outer phospholipid layers of the envelope on either side of the pore. The fine 'membrane' appearing to span the pore is formed by proteins of the pore complex. Note that the chromatin is less condensed in the region of nuclear pores. Abbreviations: N, nucleus; C, cytoplasm. **B**, Nuclear pores seen '*en face*' as spherical structures (arrows) in a tangential section through the nuclear envelope. The appearance of the envelope varies in electron density as the plane of section passes through different regions of the curved double membrane, which is interrupted at intervals by pores through the envelope (see also Fig. 1.1). The surrounding cytoplasm with ribosomes is less electron-dense. Human tissues. (Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

A small GTPase called Ras-related nuclear protein (Ran) regulates the import and export of proteins across the nuclear envelope.

For further reading on the Ran pathway and exportins/importins, see Clarke and Zhang (2008) and Raices and D'Angelo (2012).

Chromatin

DNA is organized within the nucleus in a DNA–protein complex known as chromatin. The protein constituents of chromatin are the histones and the non-histone proteins. Non-histone proteins are an extremely heterogeneous group that includes structural proteins, DNA and RNA polymerases, and gene regulatory proteins. Histones are the most abundant group of proteins in chromatin, primarily responsible for the packaging of chromosomal DNA into its primary level of organization, the nucleosome. There are four core histone proteins – H2A, H2B, H3 and H4 – which combine in equal ratios to form a compact octameric nucleosome core. A fifth histone, H1, is involved in further compaction of the chromatin. The DNA molecule (one per chromosome) winds twice around each nucleosome core, taking up 165 nucleotide pairs. This packaging organizes the DNA into a chromatin fibre 11 nm in diameter, and imparts to this form of chromatin the electron microscopic appearance of beads on a string, in which each bead is separated by a variable length of DNA, typically about 35 nucleotide pairs long. The nucleosome core region and one of the linker regions constitute the nucleosome proper, which is typically about 200 nucleotide pairs in length. However, chromatin rarely exists in this simple form and is usually packaged further into a 30 nm thick fibre, involving a single H1 histone per nucleosome, which interacts with both DNA and protein to impose a higher order of nucleosome packing. Usually, 30 nm thick fibres are further coiled or folded into larger domains. Individual domains are believed to decondense and extend during active transcription. In a typical interphase nucleus, euchromatin (nuclear regions that appear pale in appropriately stained tissue sections, or relatively electron-lucent in electron micrographs; see Fig. 1.2) is likely to consist mainly of 30 nm fibres and loops, and contains the transcriptionally active genes. Transcriptionally active cells, such as most neurones, have nuclei that are predominantly euchromatic. See Luger et al (2012) for further reading on the nucleosome and chromatin structure.

Heterochromatin (nuclear regions that appear dark in appropriately stained tissue sections or electron-dense in electron micrographs) is characteristically located mainly around the periphery of the nucleus, except over the nuclear pores (see Fig. 1.11A), and adjacent to the nucleolus (see Fig. 1.2). It is a relatively compacted form of chromatin in which the histone proteins carry a specific set of post-translational modifications, including methylation at characteristic residues. This facilitates the binding of specific heterochromatin-associated proteins. Heterochromatin includes non-coding regions of DNA, such as centromeric regions, which are known as constitutive heterochromatin. DNA becomes transcriptionally inactive in some cells as they differentiate during development or cell maturation, and contributes to heterochromatin; it is known as facultative heterochromatin. The inactive X chromosome in females is an example of facultative heterochromatin and can be identified in the light microscope as the deeply staining Barr body often located near the nuclear periphery or a drumstick extension of a nuclear lobe of a mature multilobed neutrophil leukocyte.

In transcriptionally inactive cells, chromatin is predominantly in the condensed, heterochromatic state, and may comprise as much as 90% of the total. Examples of such cells are mature neutrophil leukocytes (in which the condensed chromatin is present in a multilobular, densely staining nucleus) and the highly condensed nuclei of orthochromatic erythroblasts (late-stage erythrocyte precursors). In most mature cells, a mixture of the two occurs, indicating that only a proportion of the DNA is being transcribed. A particular instance of this is seen in the B lymphocyte-derived plasma cell, in which much of the chromatin is in the condensed condition and is arranged in regular masses around the perimeter of the nucleus, producing the so-called 'clock-face' nucleus (see Figs 4.6, 4.12). Although this cell is actively transcribing, much of

its protein synthesis is of a single immunoglobulin type, and consequently much of its genome is in an inactive state.

During mitosis, the chromatin is further reorganized and condensed to form the much-shortened chromosomes characteristic of metaphase. This shortening is achieved through further levels of close packing of the chromatin. The condensed chromosomes are stabilized by protein complexes known as condensins. Progressive folding of the chromosomal DNA by interactions with specific proteins can reduce 5 cm of chromosomal DNA by 10,000-fold, to a length of 5 µm in the mitotic chromosome.

Chromosomes and telomeres

The nuclear DNA of eukaryotic cells is organized into linear units called chromosomes. The DNA in a normal human diploid cell contains 6×10^9 nucleotide pairs organized in the form of 46 chromosomes (44 autosomes and 2 sex chromosomes). The largest human chromosome (number 1) contains 2.5×10^8 nucleotide pairs, and the smallest (the Y chromosome) 5×10^7 nucleotide pairs.

Each chromosomal DNA molecule contains a number of specialized nucleotide sequences that are associated with its maintenance. One is the centromeric DNA region. During mitosis, a disc-shaped structure composed of a complex array of proteins, the kinetochore, forms as a substructure at the centromeric region of DNA to which kinetochore microtubules of the spindle attach. Another region, the telomere, defines the end of each chromosomal DNA molecule. Telomeres consist of hundreds of repeats of the nucleotide sequence $(TTAGGG)_n$. The very ends of the chromosomes cannot be replicated by the same DNA polymerase as the rest of the chromosome, and are maintained by a specific enzyme called telomerase, which contains an RNA subunit acting as the template for lengthening the TTAGGG repeats. See Nandakumar and Cech (2013) for further reading on the recruitment of telomerase to telomeres. Thus telomerase is a specialized type of polymerase known as a reverse transcriptase that turns sequences in RNA back into DNA. The number of tandem repeats of the telomeric DNA sequence varies. The telomere appears to shorten with successive cell divisions because telomerase activity reduces or is absent in differentiated cells with a finite lifespan. In mammals, telomerase is active in the germ-cell lineage and in stem cells, but its expression in somatic cells may lead to or prompt cancer. A lack of telomere maintenance determines the shrinking of telomeres in proliferating cells to the point when cells stop dividing, a condition known as replicative senescence. See Sahin and DePinho (2012) for further reading on telomeres and progressive DNA damage.

The role of the telomere in ageing and cell senescence is further discussed at the end of this chapter.

Karyotypes: classification of human chromosomes

A number of genetic abnormalities can be directly related to the chromosomal pattern. The characterization or karyotyping of chromosome number and structure is therefore of considerable diagnostic importance. The identifying features of individual chromosomes are most easily seen during metaphase, although prophase chromosomes can be used for more detailed analyses.

Lymphocytes separated from blood samples, or cells taken from other tissues, are used as a source of chromosomes. Diagnosis of fetal chromosome patterns is generally carried out on samples of amniotic fluid containing fetal cells aspirated from the uterus by amniocentesis, or on a small piece of chorionic villus tissue removed from the placenta. Whatever their origin, the cells are cultured *in vitro* and stimulated to divide by treatment with agents that stimulate cell division. Mitosis is interrupted at metaphase with spindle inhibitors. The chromosomes are dispersed by first causing the cells to swell in a hypotonic solution, then the cells are gently fixed and mechanically ruptured on a slide to spread the chromosomes. They are subsequently stained in various ways to allow the identification of individual chromosomes by size, shape and distribution of stain (**Fig. 1.12**). General techniques show the obvious landmarks, e.g. lengths of arms and positions of constrictions. Banding techniques demonstrate differential staining patterns, characteristic for each chromosome type. Fluorescence staining with quinacrine mustard and related compounds produces Q bands, and Giemsa staining (after treatment that partially denatures the chromatin) gives G bands (**Fig. 1.12A**). Other less widely used methods include: reverse Giemsa staining, in which the light and dark areas are reversed (R bands); the staining of constitutive heterochromatin with silver salts (C-banding); and T-banding to stain the ends (telomeres) of chromosomes. Collectively, these methods permit the classification of chromosomes into numbered autosomal pairs in order of decreasing size, from 1 to 22, plus the sex chromosomes.

A summary of the major classes of chromosome is given in **Table 1.1**.

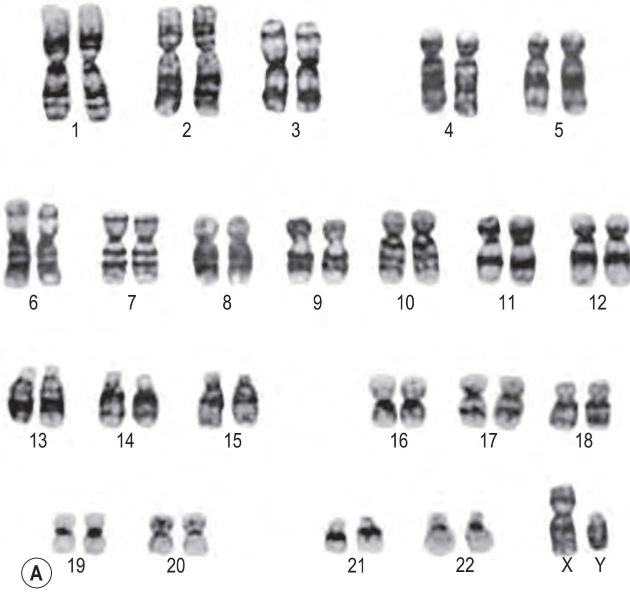
Methodological advances in banding techniques improved the recognition of abnormal chromosome patterns. The use of *in situ* hybridization with fluorescent DNA probes specific for each chromosome (**Fig. 1.12B**) permits the identification of even very small abnormalities.

Nucleolus

Nucleoli are a prominent feature of an interphase nucleus (see Fig. 1.2). They are the site of most of the synthesis of ribosomal RNA (rRNA) and assembly of ribosome subunits. Nucleoli organize at the end of mitosis

Table 1.1 Summary of the major classes of chromosome

Group	Features
1–3 (A)	Large metacentric chromosomes
4–5 (B)	Large submetacentric chromosomes
6–12 + X (C)	Metacentrics of medium size
13–15 (D)	Medium-sized acrocentrics with satellites
16–18 (E)	Shorter metacentrics (16) or submetacentrics (17,18)
19–20 (F)	Shortest metacentrics
21–22 + Y (G)	Short acrocentrics; 21, 22 with satellites, Y without



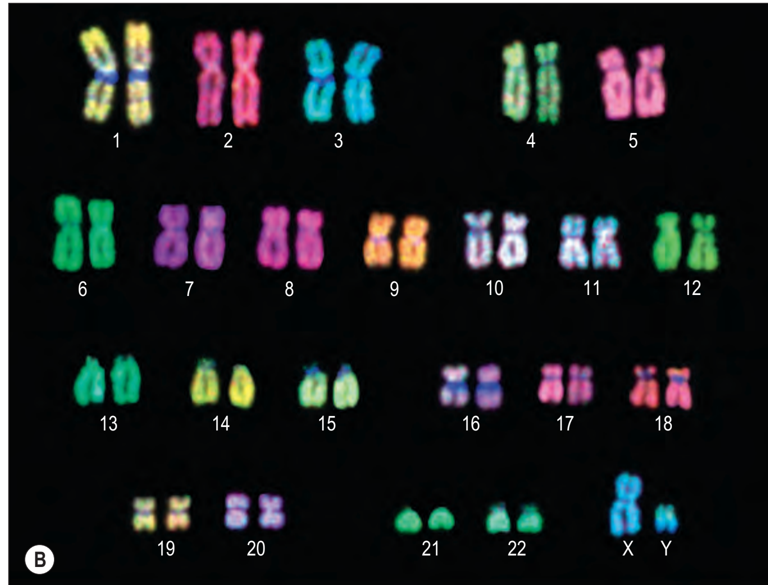


Fig. 1.12 Chromosomes from normal males, arranged as karyotypes. **A**, G-banded preparation. **B**, Preparation stained by multiplex fluorescence *in situ* hybridization to identify each chromosome. (Courtesy of Dr Denise Sheer, Cancer Research UK.)

Telomerase has been associated with ageing and cell senescence because a gradual loss of telomeres may lead to tissue atrophy, stem cell depletion and deficient tissue repair or regeneration. Mutations causing loss of function of telomerase or the RNA-containing template have been associated with dyskeratosis congenita (characterized by abnormal skin pigmentation, nail dystrophy and mucosal leukoplasia), aplastic anaemia and pulmonary fibrosis.

and consist of repeated clusters of ribosomal DNA (rDNA) genes and processing molecules responsible for producing ribosome subunits. The initial step of the assembly of a ribosome subunit starts with the transcription of rDNA genes by RNA polymerase I. The rDNA genes, arranged in tandem repeats called nucleolar organizing regions (NORs), are located on acrocentric chromosomes. There are five pairs of acrocentric chromosomes in humans. The initial 47S rRNA precursor transcript is cleaved to form the mature 28S, 18S and 5.8S rRNAs, assembled with the 5S rRNA (synthesized by RNA polymerase III outside the nucleolus) and coupled to small nucleolar ribonucleoproteins and other non-ribosomal proteins to form 60S (containing 28S rRNA, 5.8S rRNA and 5S rRNA) and 40S (containing 18S rRNA) preribosome subunits. These are then exported to the cytoplasm across nuclear pores as mature ribosome subunits. About 726 human nucleolar proteins have been identified by protein purification and mass spectrometry. For further reading on nucleolar functions, see Boisvert et al (2007).

Ribosomal biogenesis occurs in distinct subregions of the nucleolus, visualized by electron microscopy. The three nucleolar subregions are fibrillar centres (FCs), dense fibrillar components (DFCs) and granular components (GCs). Transcription of the rDNA repeats takes place at the FC-DFC boundary; pools of RNA polymerase I reside in the FC region; processing of transcripts and coupling to small nucleolar ribonucleoproteins take place in DFC; and the assembly of ribosome subunits is completed in the GC region.

The nucleolus is disassembled when cells enter mitosis and transcription becomes inactive. It reforms after nuclear envelope reorganization in telophase, in a process associated with the onset of transcription in nucleolar organizing centres on each specific chromosome, and becomes functional during the G_1 phase of the cell cycle. An adequate pool of ribosome subunits during cell growth and cell division requires steady nucleolar activity to support protein synthesis. Several DNA helicases, a conserved group of enzymes that unwind DNA, accumulate in the nucleolus under specific conditions such as Bloom's syndrome (an autosomal recessive disorder characterized by growth deficiency, immunodeficiency and a predisposition to cancer) and Werner's syndrome (an autosomal recessive condition characterized by the early appearance of various age-related diseases).

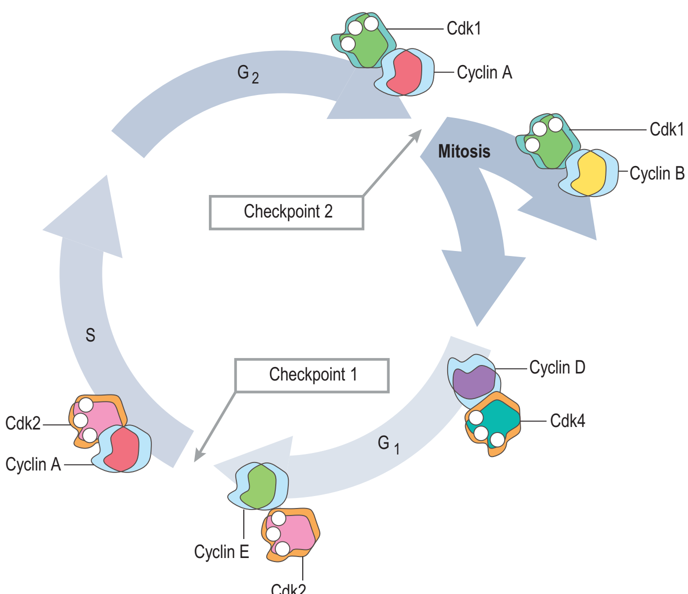


Fig. 1.13 The cell cycle consists of an interphase (G_1 phase, S phase and G_2 phase) followed by mitosis. The cyclin D/Cdk4 complex assembles at the beginning of G_1; the cyclin E/Cdk2 complex assembles near the end of G_1 as the cell is preparing to cross checkpoint 1 to start DNA synthesis (during S phase). The cyclin A/Cdk2 complex assembles as DNA synthesis starts. Completion of G_2 is indicated by the assembled cyclin A/Cdk1 complex. A cell crosses checkpoint 2 to initiate mitosis when the cyclin B/Cdk1 complex assembles. The cyclin B/Cdk1 complex is degraded by the 26S proteasome and an assembled cyclin D/Cdk4 marks the start of the G_1 phase of a new cell cycle. For details, see text. (Modified with permission from Kierszenbaum AL, Tres LL. Histology and Cell Biology: An Introduction to Pathology. 3rd ed, Philadelphia: Elsevier, Saunders; 2011.)

CELL DIVISION AND THE CELL CYCLE

During prenatal development, most cells undergo repeated division (see Video 1.1) as the body grows in size and complexity. As cells mature, they differentiate structurally and functionally. Some cells, such as neurones, lose the ability to divide. Others may persist throughout the lifetime of the individual as replication-competent stem cells, e.g. cells in the haemopoietic tissue of bone marrow. Many stem cells divide infrequently, but give rise to daughter cells that undergo repeated cycles of mitotic division as transit (or transient) amplifying cells. Their divisions may occur in rapid succession, as in cell lineages with a short lifespan and similarly fast turnover and replacement time. Transit amplifying cells are all destined to differentiate and ultimately to die and be replaced, unlike the population of parental stem cells, which self-renews.

Patterns and rates of cell division within tissues vary considerably. In many epithelia, such as the crypts between intestinal villi, the replacement of damaged or ageing cells by division of stem cells can be rapid. Rates of cell division may also vary according to demand, as occurs in the healing of wounded skin, in which cell proliferation increases to a peak and then returns to the normal replacement level. The rate of cell division is tightly coupled to the demand for growth and replacement. Where this coupling is faulty, tissues either fail to grow or replace their cells, or they can overgrow, producing neoplasms.

The cell cycle is an ordered sequence of events, culminating in cell growth and division to produce two daughter cells. It generally lasts a minimum of 12 hours, but in most adult tissues can be considerably longer, and is divided into four distinct phases, which are known as G_1 (for gap 1), S (for DNA synthesis), G_2 (for gap 2) and M (for mitosis). The combination of G_1, S and G_2 phases is known as interphase. M is the mitotic phase, which is further divided into four phases (see below). G_1 is the period when cells respond to growth factors directing the cell to initiate another cycle; once made, this decision is irreversible. It is also the phase in which most of the molecular machinery required to complete another cell cycle is generated. Centrosomes duplicate during S phase in preparation for mitosis. Cells that retain the capacity for proliferation, but which are no longer dividing, have entered a phase called G_0 and are described as quiescent even though they may be quite active physiologically. Growth factors can stimulate quiescent cells to leave G_0 and re-enter the cell cycle, whereas the proteins encoded by certain tumour suppressor genes (e.g. the gene mutated in retinoblastoma, *Rb*) block the cycle in G_1. DNA synthesis (replication of the genome) occurs during S phase, at the end of which the DNA content of the cell has doubled. During G_2, the cell prepares for division; this period ends with the onset of chromosome condensation and breakdown of the nuclear envelope. The times taken for S, G_2 and M are similar for most cell types, and occupy 6–8, 2–4 and 1–2 hours respectively. In contrast, the duration of G_1 shows considerable variation, sometimes ranging from less than 2 hours in rapidly dividing cells to more than 100 hours, within the same tissue.

The passage of a cell through the cell cycle is controlled by proteins in the cytoplasm: cyclins and cyclin-dependent kinases (Cdks; Fig 1.13). Cyclins include G_1 cyclins (D cyclins), S-phase cyclins (cyclins E and A) and mitotic cyclins (B cyclins). Cdks, protein kinases, which are activated by binding of a cyclin subunit, include G_1 Cdk (Cdk4), an S-phase Cdk (Cdk2) and an M-phase Cdk (Cdk1). Cell cycle progression is driven in part by changes in the activity of Cdks. Each cell cycle stage is characterized by the activity of one or more Cdk–cyclin pairs. Transitions between cell cycle stages are triggered by highly specific proteolysis by the 26S proteasome of the cyclins and other key components.

To give one example, the transition from G_2 to mitosis is driven by activation of Cdk1 by its partners, the A- and B-type cyclins; the characteristic changes in cellular structure that occur as cells enter mitosis are largely driven by phosphorylation of proteins by active Cdk1-cyclin A and Cdk1-cyclin B. Cells exit from mitosis when an E3 ubiquitin ligase, the anaphase promoting complex, also called cyclosome (APC/C), marks the cyclins for destruction. In addition, APC/C prompts the degradation of the mitotic cyclin B and the destruction of cohesins, thus allowing sister chromatids to separate.

There are important checkpoints in the cell cycle (see Fig. 1.13). Checkpoint 1 requires G_1 cyclins to bind to their corresponding Cdks to signal the cell to prepare for DNA synthesis. S-phase promoting factor (SPF; cyclin A bound to Cdk2) enters the nucleus to stimulate DNA synthesis. Checkpoint 2 requires M-phase promoting factor (mitotic cyclin B bound to M-phase Cdk1) to trigger the assembly of the mitotic spindle, breakdown of the nuclear envelope, arrest of gene transcription and condensation of chromosomes. During metaphase of mitosis, M-phase promoting factor activates APC/C, which determines the breakdown of cohesins, the protein complex holding sister chromatids together. Then, at anaphase, separated chromatids move to the opposite poles of the spindle. Finally, B cyclins are destroyed following

The targets for proteolysis are marked for destruction by E3 ubiquitin ligases, which decorate them with polymers of the small protein ubiquitin, a sign for recognition by the 26S proteasome.

their attachment to ubiquitin, targeting them for destruction by the 26S proteasome. As G_1 starts, cyclins D, bound to Cdk4, start preparation for a new cell cycle.

Quality control checkpoint 2 operates to delay cell-cycle progression when DNA has been damaged by radiation or chemical mutagens. Cells with checkpoint defects, such as loss of the protein p53, which is a major negative control element in the division cycle of all cells, are commonly associated with the development of malignancy. An example is Li Fraumeni syndrome, where a defective *p53* gene leads to a high frequency of cancer in affected individuals. In cells, p53 protein binds DNA and stimulates another gene to produce p21 protein, which interacts with Cdk2 to prevent S-phase promoting activity. When mutant p53 can no longer bind DNA to stimulate production of p21 to stop DNA synthesis, cells acquire oncogenic properties. The *p53* gene is an example of a tumour suppressor gene. For further reading on *p53* mutations and cancer, see Muller and Vousden (2013).

Mitosis and meiosis

Mitosis is the process that results in the distribution of identical copies of the parent cell genome to the two daughter somatic cells. In meiosis, the divisions immediately before the final production of gametes halve the number of chromosomes to the haploid number, so that at fertilization the diploid number is restored. Moreover, meiosis includes a phase in which exchange of genetic material occurs between homologous chromosomes. This allows a rearrangement of genes to take place, which means that the daughter cells differ from the parental cell in both their precise genetic sequence and their haploid state. Mitosis and meiosis are alike in many respects, and differ principally in chromosomal behaviour during the early stages of cell division. In meiosis, two divisions occur in succession, without an intervening S phase. Meiosis I is distinct from mitosis, whereas meiosis II is more like mitosis.

Mitosis

New DNA is synthesized during the S phase of the cell cycle interphase. This means that the amount of DNA in diploid cells has doubled to the tetraploid value by the onset of mitosis, although the chromosome number is still diploid. During mitosis, this amount is halved between the two daughter cells, so that DNA quantity and chromosome number are diploid in both cells. The cellular changes that achieve this distribution are conventionally divided into four phases called prophase, metaphase, anaphase and telophase (**Figs 1.14–1.15**, **Video 1.1**).

Prophase

During prophase, the strands of chromatin, which are highly extended during interphase, shorten, thicken and resolve themselves into recognizable chromosomes. Each chromosome is made up of duplicate chromatids (the products of DNA replication) joined at their centromeres. Outside the nucleus, the two centriole pairs begin to separate, and move towards opposite poles of the cell. Parallel microtubules are assembled between them to create the mitotic spindle, and others radiate to form the microtubule asters, which come to form the spindle poles or mitotic centre. As prophase proceeds, the nucleoli disappear, and the nuclear envelope suddenly disintegrates to release the chromosomes, an event that marks the end of prophase.

Prometaphase–metaphase

As the nuclear envelope disappears, the spindle microtubules extend into the central region of the cell, attaching to the chromosomes, which subsequently move towards the equator of the spindle (prometaphase). The spindle consists of kinetochore microtubules attached to the kinetochore, a multiprotein structure assembled at the centromeric DNA region, and polar microtubules, which are not attached to chromosomes but instead overlap with each other at the centre of the cell. The grouping of chromosomes at the spindle equator is called the metaphase or equatorial plate. The chromosomes, attached at their centromeres, appear to be arranged in a ring when viewed from either pole of the cell, or to lie linearly across this plane when viewed from above. Cytoplasmic movements during late metaphase effect the approximately equal distribution of mitochondria and other cell structures around the cell periphery.

Anaphase

By the end of metaphase every chromosome consists of a pair of sister chromatids attached to opposing spindle poles by bundles of microtubules associated with the kinetochore. The onset of anaphase begins with the proteolytic cleavage by the enzyme separase of a key subunit of protein complexes known as cohesins. The latter hold the replicated sister chromatids together to resist separation even when exposed to microtubule-dependent pulling forces. Proteolytic cleavage releases the cohesion between sister chromatids, which then move towards opposite spindle poles while the microtubule bundles attached to the kinetochores shorten and move polewards. At the end of anaphase the sister chromatids are grouped at either end of the cell, and both clusters are

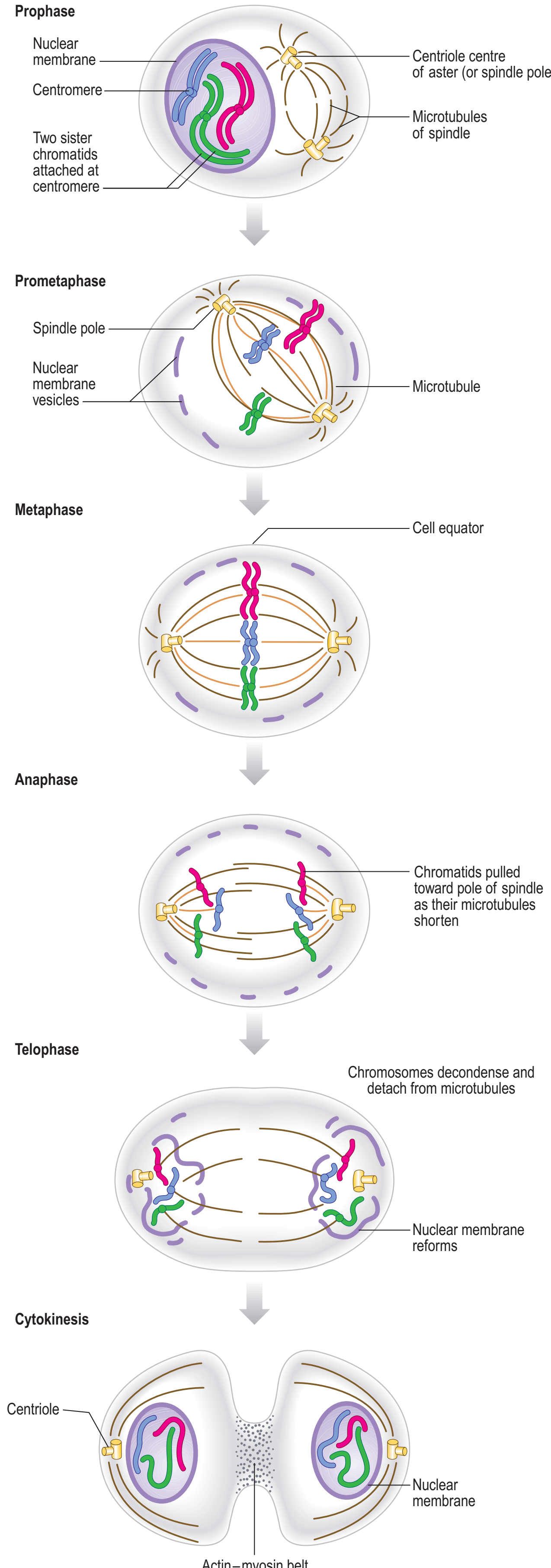
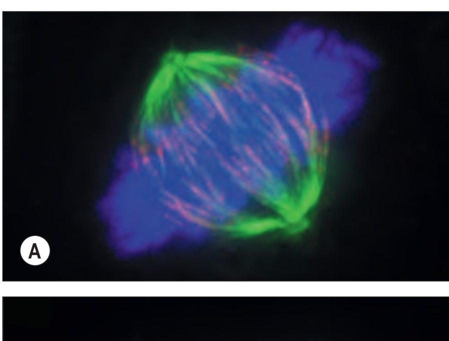
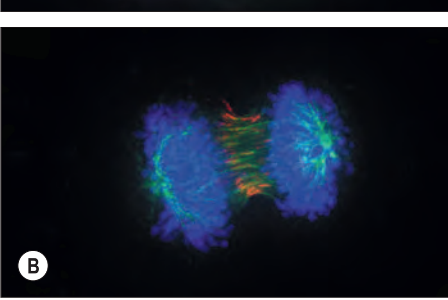


Fig. 1.14 The stages in mitosis, including the appearance and distribution of the chromosomes.





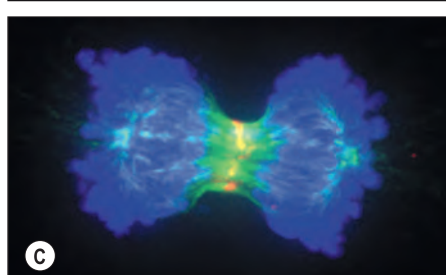


Fig. 1.15 Immunofluorescence images of stages in mitosis in human carcinoma cells in culture. **A**, Metaphase, with spindle microtubules (green), the microtubule-stabilizing protein (HURP; red) and chromosomal DNA (blue). **B**, Anaphase, with spindle microtubules (green), the central spindle (Aurora-B kinase, red) and segregated chromosomes (blue). **C**, Late anaphase, with spindle microtubules (green), the central spindle (Plk1 kinase, red, appearing yellow where co-localized with microtubule protein) and segregated chromosomes (blue). (Courtesy of Dr Herman Silljé, Max-Planck-Institut für Biochemie, Martinsried, Germany.)

diploid in number. An infolding of the cell equator begins, deepening during telophase as the cleavage furrow.

Telophase

During telophase the nuclear envelopes reform, beginning with the association of membranous vesicles with the surface of the chromosomes. Later, after the vesicles have fused and the nuclear envelope is complete, the chromosomes decondense and the nucleoli reform. At the same time, cytoplasmic division, which usually begins in early anaphase, continues until the new cells separate, each with its derived nucleus. The spindle remnant now disintegrates. While the cleavage furrow is active, a peripheral band or belt of actin and myosin appears in the constricting zone; contraction of this band is responsible for furrow formation.

Failure of disjunction of chromatids, so that sister chromatids pass to the same pole, may sometimes occur. Of the two new cells, one will have more, and the other fewer, chromosomes than the diploid number. Exposure to ionizing radiation promotes non-disjunction and may, by chromosomal damage, inhibit mitosis altogether. A typical symptom of radiation exposure is the failure of rapidly dividing epithelia to replace lost cells, with consequent ulceration of the skin and mucous membranes. Mitosis can also be disrupted by chemical agents, particularly vinblastine, paclitaxel (taxol) and their derivatives. These compounds either disassemble spindle microtubules or interfere with their dynamics, so that mitosis is arrested in metaphase.

Meiosis

There are two consecutive cell divisions during meiosis: meiosis I and meiosis II (Fig. 1.16). Details of this process differ at a cellular level for male and female lineages.

A Events preceding meiosis

Centromere
Premeiotic S phase
Paired sister centromeres
Meiotic prophase

B Meiotic prophase

Leptotene
Pairing of paternal and maternal homologues
Zygotene
Pachytene
Diplotene
Diakinesis
Meiosis I

C Meiosis I

Chiasmata
Metaphase I
Anaphase I
Meiosis I
Interphase (no S phase)
Meiosis II

D Meiosis II

Prophase II
Metaphase II
Anaphase II
Haploid gametes

Fig. 1.16 The stages in meiosis, depicted by two pairs of maternal and paternal homologues (dark and pale colours). DNA and chromosome complement changes and exchange of genetic information between homologues are indicated.

Meiosis I

Prophase I

Meiotic prophase I is a long and complex phase that differs considerably from mitotic prophase and is customarily divided into five substages, called leptotene, zygotene, pachytene, diplotene and diakinesis. There are three distinctive features of male meiotic prophase that are not seen during mitotic prophase: the pairing, or synapse, of homologous chromosomes of paternal and maternal origin to form bivalent structures; the organization of nucleoli by autosomal bivalents; and significant non-ribosomal RNA synthesis by autosomal bivalents (in contrast to the transcriptional inactivity of the XY chromosomal pair) (see Tres 2005). In the female, meiotic prophase I starts during fetal gonadogenesis, is arrested at the diplotene stage and resumes at puberty. In the male, meiosis starts at puberty.

Leptotene stage During leptotene, homologous chromosomes (maternal and paternal copies of the same chromosome), replicated in a preceding S phase and each consisting of sister chromatids joined at the centromere (see above), locate one another within the nucleus, and the process of genetic recombination is initiated. Cytologically, chromosomes begin to condense, appearing as individual threads that are attached via their telomeres to the nuclear envelope. They often show characteristic beading throughout their length.

Zygotene stage During zygotene, the homologous chromosomes initiate pairing or synapsis, during which they become intimately associated with one another. Synapsis may begin near the telomeres at the inner surface of the nuclear membrane, and during this stage the telomeres often cluster to one side of the nucleus (a stage known as the bouquet because the chromosomes resemble a bouquet of flowers). The pairs of synapsed homologues, also known as bivalents, are linked together by a tripartite ribbon, the synaptonemal complex, which consists of two lateral dense elements and a central, less dense, linear element.

The sex chromosomes also start to synapse during zygotene. In males, with distinct X and Y chromosomes, synapsis involves a region of shared DNA sequence known as the pseudoautosomal region. The XY bivalent adopts a special condensed structure, known as the sex vesicle, which becomes associated later at pachytene with migratory nucleolar masses originating in the autosomal bivalents.

Chromosome behaviour in meiosis is intimately linked with the process of genetic recombination. This begins during leptotene, as homologous chromosomes first locate one another at a distance. Synapsis, stabilized by the synaptonemal complex, facilitates recombination, as sites of genetic exchange are turned into specialized structures known as chiasmata, which are topological crossing-over points that hold homologous chromosomes together.

Pachytene stage When synapsis is complete for all chromosomes, the cell is said to be in pachytene. Each bivalent looks like a single thick structure, but is actually two pairs of sister chromatids held together by the synaptonemal complex. Genetic recombination between non-sister chromatids is completed at this point, with sites where it has occurred (usually one per chromosome arm) appearing as recombination nodules in the centre of the synaptonemal complex.

Diplotene stage During diplotene, the synaptonemal complex disassembles and pairs of homologous chromosomes, now much shortened, separate, except where crossing over has occurred (chiasmata). This process is called disjunction. At least one chiasma forms between each homologous pair, exchanging maternal and paternal sequences; up to five have been observed. In the ovaries, primary oocytes become diplotene by the fifth month *in utero* and each remains at this stage until the period before ovulation (up to 50 years).

Diakinesis Diakinesis is the prometaphase of the first meiotic division. The chromosomes, still as bivalents, become even shorter and thicker. They gradually attach to the spindle and become aligned at a metaphase plate. In eggs, the spindle forms without centrosomes. Microtubules first nucleate and are stabilized near the chromosomes; the action of various motor molecules eventually sorts them into a bipolar spindle. Perhaps surprisingly, this spindle is as efficient a machine for chromosome segregation as the spindle of mitotic cells with centrosomes at the poles.

Metaphase I

Metaphase I resembles mitotic metaphase, except that the bodies attaching to the spindle microtubules are bivalents, not single chromosomes. These become arranged so that the homologous pairs occupy the equatorial plane of the spindle. The centromeres of each pair of sister chromatids function as a single unit, facing a single spindle pole. Homologous chromosomes are pulled towards opposite spindle poles, but are held paired at the spindle midzone by chiasmata. Errors in chromosome segregation (known as non-disjunction) lead to the production of aneuploid progeny. Most human aneuploid embryos are non-viable and this is the major cause of fetal loss (spontaneous abortion), particularly during the first trimester of pregnancy in humans. The most common form of viable aneuploid progeny in humans is Down's syndrome (trisomy for chromosome 21), which exhibits a dramatic increase with maternal age.

Anaphase and telophase I

Anaphase I of meiosis begins with the release of cohesion between the arms of sister chromatids, much as it does during mitosis. As positioning of bivalent pairs is random, assortment of maternal and paternal chromosomes in each telophase nucleus is also random. Critically, sister centromeres, and thus chromatids, do not separate during anaphase I.

During meiosis I, cytoplasmic division occurs by specialized mechanisms. In females, the division is highly asymmetric, producing one egg and one tiny cell known as a polar body. In males, the process results in production of spermatocytes that remain joined by small cytoplasmic bridges.

Meiosis II

Meiosis II commences after only a short interval during which no DNA synthesis occurs. The centromeres of sister chromatids remain paired, but rotate so that each one can face an opposite spindle pole. Onset of anaphase II is triggered by loss of cohesion between the centromeres, as it is in mitosis. This second division is more like mitosis, in that chromatids separate during anaphase, but, unlike mitosis, the separating chromatids are genetically different (the result of genetic recombination). Cytoplasmic division also occurs and thus, in the male, four haploid cells, interconnected by cytoplasmic bridges, result from meiosis I and II.

CELL POLARITY AND DOMAINS

Epithelia are organized into sheets or glandular structures with very different environments on either side. These cells actively transfer macromolecules and ions between the two surfaces and are thus polarized in structure and function. In polarized cells, particularly in epithelia, the cell is generally subdivided into domains that reflect the polarization of activities within it. The free surface, e.g. that facing the intestinal lumen or airway, is the apical surface, and its adjacent cytoplasm is the apical cell domain. This is where the cell interfaces with a specific body compartment (or, in the case of the epidermis, with the outside world). The apical surface is specialized to act as a barrier, restricting access of substances from this compartment to the rest of the body. Specific components are selectively absorbed from, or added to, the external compartment by the active processes, respectively, of active transport and endocytosis inwardly or exocytosis and secretion outwardly. The apical surface is often covered with small protrusions of the cell surface, microvilli, which increase the surface area, particularly for absorption.

The surface of the cell opposite to the apical surface is the basal surface, with its associated basolateral cell domain. In a single-layered epithelium, this surface faces the basal lamina. The remaining surfaces are known as the lateral cell surfaces. In many instances, the lateral and basal surfaces perform similar functions and the cellular domain is termed the basolateral domain. Cells actively transport substances, such as digested nutrients from the intestinal lumen or endocrine secretions, across their basal (or basolateral) surfaces into the subjacent connective tissue matrix and the blood capillaries within it. Dissolved non-polar gases (oxygen and carbon dioxide) diffuse freely between the cell and the blood stream across the basolateral surface. Apical and basolateral surfaces are separated by a tight intercellular seal, the tight junction (occluding junction, zonula adherens), which prevents the passage of even small ions through the space between adjacent cells and thus maintains the difference between environments on either side of the epithelium.

Cell surface apical differentiations

The surfaces of many different types of cell are specialized to form structures that project from the surface. These projections may permit

movement of the cell itself (flagella), or of fluids across the apical cell surface (cilia), or increase the surface area available for absorption (microvilli). Infoldings of the basolateral plasma membrane also increase the area for transport across this surface of the cell. In most non-dividing epithelial cells, the centriole-derived basal body gives rise to a non-motile primary cilium, which has an important mechanosensory role.

Cilia and flagella

Cilia and flagella are motile, hair-like projections of the cell surface, which create currents in the surrounding fluid or movements of the cell to which they are attached, or both. There are two categories of cilia: single non-motile primary cilia and multiple motile cilia. Primary cilia are immotile but can detect physical and biochemical signals. Motile cilia are present in large numbers on the apical epithelial domain of the upper respiratory tract and oviducts, and beat in a wave-like motion to generate fluid movement. Cilia also occur, in modified form, at the dendritic endings of olfactory receptor cells, vestibular hair cells (kinocilium), and the photoreceptor rods and cones of the retina. Flagella, with a primary function in cell locomotion, are found on single-cell eukaryotes and in spermatozoa, which each possess a single flagellum 70 µm long.

A cilium or flagellum consists of a shaft (0.25 µm diameter) constituting most of its length, a tapering tip and a basal body at its base, which lies within the surface cytoplasm of the cell (**Fig. 1.17**). Other than at its base, the entire structure of the cilium is covered by plasma membrane. The core of the cilium is the axoneme, a cylinder of nine microtubule doublets that surrounds a central pair of single microtubules (see Fig. 1.17). Ciliogenesis of primary cilia and motile cilia involves distinct steps. A centriole-derived basal body migrates to the apical cell domain and axonemal microtubule doublets emerge from its distal region, called the transition zone. The continued elongation of the cilium requires the import and intraciliary transport of tubulin dimers to the distal tip by bidirectional motor-driven proteins of the intraflagellar transport complex.

The constant length of cilia is maintained by a steady-state balance between tubulin turnover and addition of new tubulin dimers at the ciliary tip.

Several filamentous structures are associated with the 9+2 doublet microtubule of the axoneme in the cilium or flagellum shaft, e.g. radial spokes extend inwards from the outer doublet microtubules towards the central pair, surrounded by an inner sheath (see Fig. 1.17). The outer doublet microtubules bear two rows of tangential dynein arms attached to the complete A subfibre of the doublet (consisting of 13 protofilaments), which point towards the incomplete B subfibre of the adjacent doublet (consisting of 10–11 protofilaments). Adjacent doublets are also linked by thin nexin filaments. Tektins are scaffolding filamentous proteins extending along the axonemal microtubules.

In motile cilia, arrays of dynein arms with ATPase activity cause outer microtubule doublets to move past one another, resulting in a large-scale bending motion. Microtubules do not change in length. Movements of cilia and flagella are broadly similar. In addition to the axoneme, spermatozoan flagella have outer dense fibres and a fibrous sheath surrounding the axoneme. Flagella move by rapid undulation, which passes from the attached to the free end. In human spermatozoa, there is an additional helical component to this motion. In cilia, the beating is planar but asymmetric. In the effective stroke, the cilium remains stiff except at the base, where it bends to produce an oar-like stroke. The recovery stroke follows, during which the bend passes from base to tip, returning the cilium to its initial position for the next cycle. The activity of groups of cilia is usually coordinated so that the bending of one is rapidly followed by the bending of the next and so on,

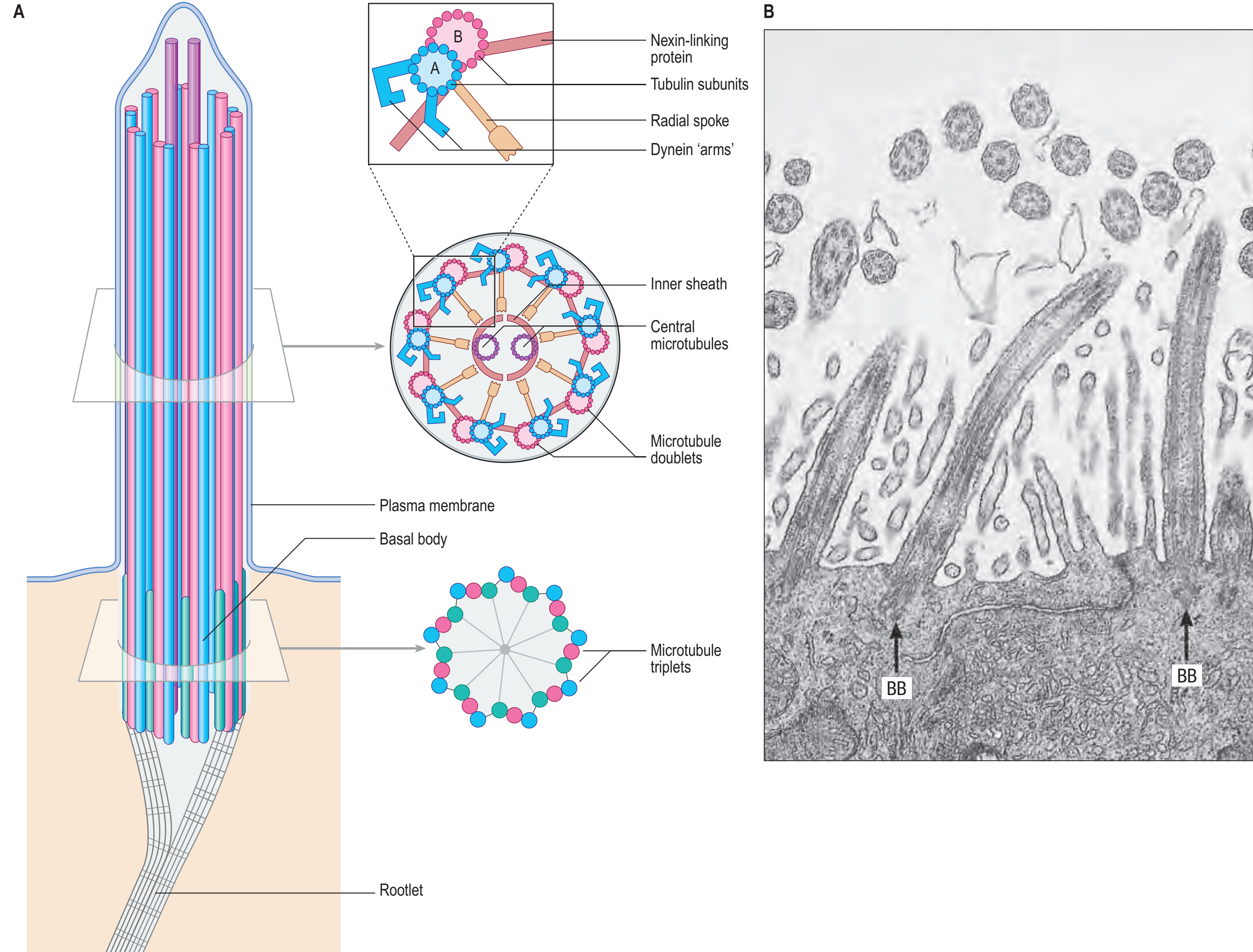


Fig. 1.17 **A**, The structure of a cilium shown in longitudinal (left) and transverse (right) section. A and B are subfibres of the peripheral microtubule doublets (see text); the basal body is structurally similar to a centriole, but with microtubule triplets. **B**, The apical region of respiratory epithelial cells, showing the proximal parts of three cilia sectioned longitudinally, anchored into the cytoplasm by basal bodies (BB). Other cilia project out of the plane of section and are cut transversely, showing the '9+2' arrangement of microtubules. (B, With permission from Young B, Heath JW. Wheater's Functional Histology. 4th ed. Edinburgh: Elsevier, Churchill Livingstone; 2000.)

As indicated on page 15, the IFT-B protein complex participates in intraciliary/intraflagellar anterograde transport of cargoes, a step essential for the assembly and maintenance of cilia and flagella; the IFT-A protein complex is required for retrograde transport of cargoes to the cell body for turnover. The movement of IFT proteins along microtubules is catalysed by kinesin-2 (towards the ciliary tip; anterograde direction) and cytoplasmic dynein-2 motor proteins (towards the cell body; retrograde direction). A cargo includes axonemal components, ciliary/flagellar membrane proteins (including the BBSome) and ciliary signal transduction proteins.

resulting in long travelling waves of metachronal synchrony. These pass over the tissue surface in the same direction as the effective stroke. Ciliary motion is important in clearing mucus from airways, moving eggs along oviducts, and circulating cerebrospinal fluid in brain ventricles. In the node of the developing embryo, cilium-driven flow is essential for determining left–right visceral asymmetry (developing patterning). Cilia also have a sensory function, determined by the presence of receptor and channel proteins on the ciliary membrane. Primary cilia in the collecting ducts of the uriniferous tubule sense the flow of urine and also modulate duct morphogenesis. Cilia are essential for signalling through the hedgehog pathway, a mechanism involved in organizing the body plan, organogenesis and tumorigenesis in vertebrates. For additional reading on hedgehog signalling and primary cilia, see Briscoe and Thérond (2013).

There is a group of genetic diseases in which cilia beat either ineffectively or not at all, e.g. Kartagener's immotile cilia syndrome. Affected cilia exhibit deficient function or a lack of dynein arms. Males are typically sterile because of the loss of spermatozoan motility, and half have an alimentary tract that is a mirror image of the usual pattern (*situs inversus*), i.e. it rotates in the opposite direction during early development. Defects in ciliary motility disrupt airway mucus clearance, leading to chronic sinusitis and bronchiectasis. Defects in sensory cilia determine polycystic kidney disease, anosmia and retinal degeneration.

Microvilli

Microvilli are finger-like cell surface extensions usually 0.1 µm in diameter and up to 2 µm long (**Fig. 1.18**).

Microvilli are covered by plasma membrane and supported internally by closely packed bundles of actin filaments linked by cross-bridges of the actin-bundling proteins, fascin and fimbrin. Other bridges composed of myosin I and calmodulin connect the filament bundles to the plasma membrane. At the tip of each microvillus, the free ends of microfilaments are inserted into a dense mass that includes the protein, villin. The actin filament bundles of microvilli are embedded in the apical cytoplasm amongst a meshwork of transversely running actin filaments stabilized by spectrin to form the terminal web, which is underlain by keratin intermediate filaments. The web is anchored laterally to the tight junctions and zonula adherens of the apical epithelial junctional complex. Myosin II and tropomyosin are also found in the terminal web, which may explain its contractile activity.

Microvilli greatly increase the area of cell surface (up to 40 times), particularly at sites of active absorption. In the small intestine, they have a very thick cell coat or glycocalyx, which reflects the presence of integral membrane glycoproteins, including enzymes concerned with digestion and absorption. Irregular microvilli, filopodia, are also found on the surfaces of many types of cell, particularly free macrophages and fibroblasts, where they may be associated with phagocytosis and cell motility. For further reading on the cytoskeleton of microvilli, see Brown and McKnight (2010).

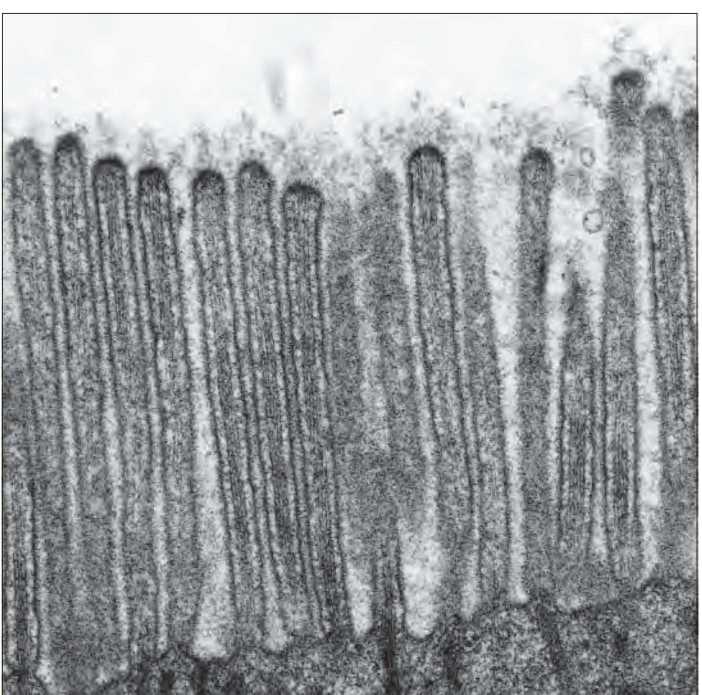


Fig. 1.18 Microvilli sectioned longitudinally in the striated border of an intestinal absorptive cell in a human duodenal biopsy specimen. Actin filaments fill the cores of the microvilli and insert into the apical cytoplasm. A prominent glycocalyx (formed by the extracellular domains of plasma membrane glycoproteins) is seen as a fuzzy coat at the tips of and between microvilli; it includes enzymes concerned with the final stages of digestion. (Courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK.)

Long and branching microvilli are called stereocilia, an early misnomer, as they are not motile and lack microtubules. An appropriate name is stereovilli. They are found on cochlear and vestibular receptor cells, where they act as sensory transducers, and also in the absorptive epithelium of the epididymis.

Intercellular junctions

The basolateral region of the plasma membrane of epithelial cells establishes junctions with adjacent cells and with structural components of the extracellular matrix. Intercellular junctions are resilient and dynamic, and prevent epithelial tissues from dissociating into their component cells. In adults, the epidermis withstands imposed deformations because of the interplay of two components of intercellular junctions, the junctional cytoskeleton and cell adhesion molecules (**Fig. 1.19**). The establishment and maintenance of cell polarity in an epithelial layer depends on two circumferential apical belts, the tight junctions and the zonulae adherentes, running in parallel to each other and associated with F-actin. These two belts control epithelial permeability and determine epithelial cell polarity. The apical cell domain resides above the belts; the basolateral cell domain resides below the belts. Desmosomes (maculae adherentes) are a third class of spot-like intercellular adhesion. In contrast to tight junctions and the zonulae adherentes, desmosomes do not form belts and link instead to intermediate filaments. The hemidesmosome, anchoring epithelial cells to the basal lamina, also links to intermediate filaments. Gap junctions are unique: they provide direct connection between adjacent cells and are not linked to the cytoskeleton. Molecular aspects of cell adhesion molecules will be considered first and then integrated with the junctional cytoskeleton to define specific structural and molecular aspects of different intercellular junctions.

Cell adhesion molecules

Cell adhesion molecules are transmembrane or membrane-anchored glycoproteins that bridge the intercellular space from the plasma membrane to form adhesive contacts. There are a number of molecular subgroups, which are broadly divisible on the basis of their dependence on calcium for function. Calcium-dependent cell adhesion molecules include cadherins and selectins. Calcium-independent cell adhesion molecules include the immunoglobulin-like superfamily of cell adhesion molecules (Ig-CAMs), including nectins, and integrins, the only cell adhesion molecules consisting of two subunits (α and β subunits).

Calcium-dependent cell adhesion molecules: cadherins and selectins

Cadherins are single-pass transmembrane glycoproteins, with five heavily glycosylated calcium-binding external domains and an intracellular catenin-binding cytoplasmic tail. Catenins are intracellular proteins linking cadherins to F-actin in the belt-arranged zonula adherens. The extracellular segment of cadherins participates in Ca^{2+}-dependent homophilic *trans*-interactions in which a cadherin molecule on one cell binds to an identical cadherin molecule on an adjacent cell. After binding, cadherins cluster laterally (*cis*-interaction) at cell–cell junctions to form a zipper-like structure that stabilizes tight adhesion between cells.

Different cell types possess different members of the cadherin family, e.g. N-cadherins in nervous tissue, E-cadherins in epithelia, and P-cadherins in the placenta. Two further members of the cadherin family are the desmogleins and the desmocollins. Cadherins are present in macula adherens and desmosomes but not in tight junctions or hemidesmosomes (see below). Alterations in the expression of cadherins in the epidermis produce pathological conditions such as blisters and ulcerations. See Brieher and Yap (2013) for further reading on cadherins and their associated cytoskeleton.

As with cadherins, selectins are Ca^{2+}-dependent. In contrast to cadherins, selectins do not establish homophilic *trans*-interactions. Instead, they bind to carbohydrates and belong to the group of lectins. Each selectin has an extracellular carbohydrate recognition domain (CRD) with binding affinity to a specific oligosaccharide attached to a protein or lipid. The molecular configuration and binding affinity of the CRD to carbohydrate moieties is Ca^{2+}-dependent. Selectins participate in the homing of leukocytes circulating in blood towards tissues by

When arranged in a regular parallel series, as typified by the absorptive surfaces of the epithelial enterocytes of the small intestine and the proximal convoluted tubule of the nephron of the kidneys, microvilli acquire a fuzzy appearance like the bristles of a paintbrush (the designations brush border or striated border are used at the light microscope level).

The cytoplasmic tail recruits proteins of the catenin complex: β-catenin is the first to be recruited and the cadherin–β-catenin complex rapidly recruits α-catenin; α-catenin binds directly to F-actin and coordinates the activity of actin nucleating proteins and actin binding partners (such as vinculin and α-actinin) to provide the dynamic forces to modulate cell–cell adhesion; p120-catenin binds to the cytoplasmic tail of cadherin and becomes a positive regulator of cadherin function.

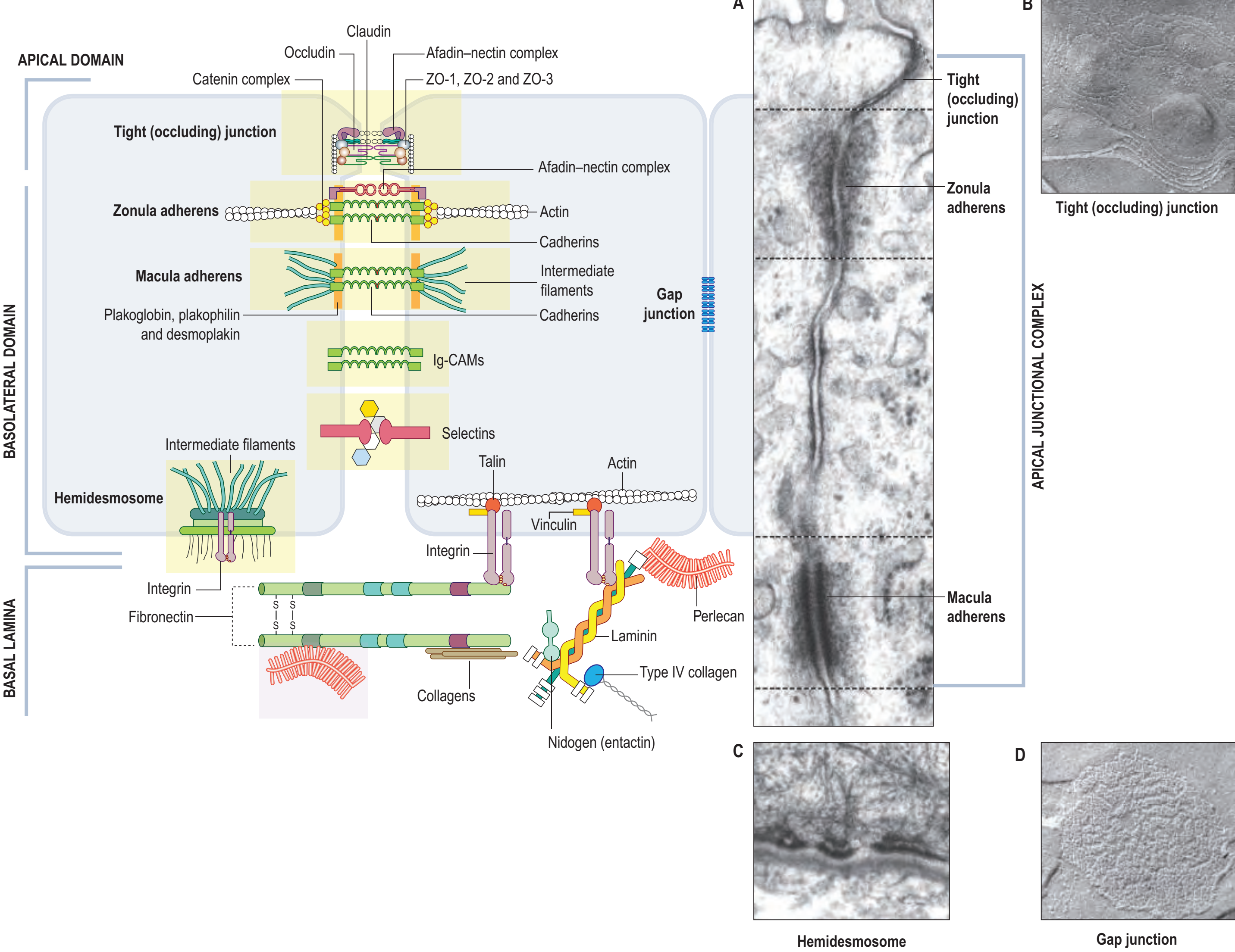


Fig. 1.19 Intercellular junctions: the apical junctional complex and other junctional specializations, illustrating the protein components of each junction and of the basal lamina. An anastomotic network of contacts between adjacent cell membranes forms a tight occluding junction. Basal plasma membrane is attached to a basal lamina at a hemidesmosome. In a gap junction, numerous channels (pores within connexons) are clustered to form a plaque-like junctional region between adjacent plasma membranes. (A and C are transmission electron micrographs; B and D are freeze-fractured preparations.) **A**, An apical junctional complex. **B**, A tight junction. **C**, A hemidesmosome. **D**, A gap junction. (B, Courtesy of Dr Andrew Kent, King's College London. D, Courtesy of Professor Dieter Hülser, University of Stuttgart. A,C, From human tissue, courtesy of Dr Bart Wagner, Histopathology Department, Sheffield Teaching Hospitals, UK. Diagram modified from Kierszenbaum AL, Tres LL. 2012. Histology and Cell Biology: An Introduction to Pathology. 3rd ed, Philadelphia: Elsevier, Saunders; 2011.)

extravasation across the endothelium. For additional reading on the significance and mechanism of homing, see Girard et al (2012).

Three major types of selectin include L-selectin (for lymphocytes), E-selectin (for endothelial cells) and P-selectin (for platelets).

Calcium-independent cell adhesion molecules: Ig-CAMs, nectins and integrins

Ig-CAMs are cell-surface glycoproteins with an extracellular domain characterized by a variable number of immunoglobulin-like loops. Most Ig-CAMs have a transmembrane domain; others are attached to the cell surface by a glycophosphatidyl inositol (GPI) anchor. As in cadherins, Ig-CAMs establish homophilic interactions contributing to cell–cell adhesion, although in a Ca^{2+}-independent manner. The cytoplasmic tail of Ig-CAMs also interacts with cytoskeletal components such as F-actin, ankyrins and spectrin. Ig-CAMs can directly or indirectly bind growth factor receptors and control their internalization.

Different types are expressed in different tissues. Neural cell adhesion molecules (N-CAMs) are found on a number of cell types but are expressed widely by neural cells. Intercellular adhesion molecules (ICAMs) are expressed on vascular endothelial cells. Cell adhesion molecule binding is predominantly homophilic, although some use a heterophilic mechanism, e.g. vascular intercellular adhesion molecule (VCAM), which can bind to integrins.

Nectins and nectin-like molecules (Necls) are members of the Ig-CAM superfamily (see Takai et al (2008) for further reading on nectins and Necls). They have an extracellular domain with three Ig-like loops, a transmembrane segment and a cytoplasmic tail. The nectins and Necls consist of four and five members, respectively. These are present in the belt-like tight junctions and zonula adherens.

The nectin–afadin complex initiates the formation of a zonula adherens and after cell–cell contacts are formed between adjacent cells, cadherins are recruited to these contact sites. Afadin and α-catenin interact with one another and also with F-actin through adaptor proteins.

Integrins mediate cell–extracellular matrix and cell–cell interactions, and integrate extracellular signals with the cytoskeleton and cellular signalling pathways. Because integrins can be activated by proteins binding to their extracellular or their intracellular domains, they can function in a bidirectional fashion by transmitting information outside-in (cues from the extracellular environment) and inside-out (cues from the intracellular environment) of the cell. The integrin family of proteins consists of α subunits and β subunits forming transmembrane heterodimers. The amino-acid sequence arginine–glycine–aspartic acid, or RGD motif, on target ligands (such as fibronectin, laminin and other extracellular matrix proteins) has binding affinity to the extracellular binding head of integrins. For further reading on integrins and their ligands properties, see Barczyk et al (2010).

The actin-binding protein talin binds the cytoplasmic domain of integrin β subunit and activates integrins. Vinculin interacts with talin and α-actinin cross-links two filaments of actin. Kindlins, named after the gene mutated in Kindler's syndrome, a skin blistering disease, interact with talin to activate integrins.

Homing, a process that also enables thymus-derived T cells (see Ch. 4) to home in on lymph nodes, consists of two phases. In the first, selectin phase, carbohydrate ligands on the surface of leukocytes adhere loosely to selectins present on the surface of endothelial cells. During the second, cooperative sequential integrin phase, strong adhesion permits the transendothelial migration of leukocytes into the extravascular space in cooperation with cell adhesion molecules of the Ig-CAM superfamily.

Nectins can interact homophilically or heterophilically with other nectins to mediate, primarily, adhesion. The intracellular domain of nectins binds to the cytoplasmic adaptor protein afadin, which links to actin, whereas Necls interact with scaffolding proteins but not to afadin. Necls are involved in a large variety of cellular functions, including axon–glial interaction, Schwann cell differentiation and myelination.

In humans there are about 18 α-subunit subtypes and 8 β-subunit subtypes, which produce 24 integrin heterodimers. The subunits are associated by non-covalent interactions and consist of an extracellular ligand-binding head, two multidomain segments, two single-pass transmembrane segments and two cytoplasmic tails. Upon binding of extracellular ligands, integrins undergo a conformational change (integrin activation), which allows the recruitment of several cytoplasmic F-actin activator proteins (such as talin, vinculin, α-actinin and kindlins) to their short cytoplasmic domain. This results in the formation of a protein complex that interacts with the actin cytoskeleton.

In addition, the protein complex promotes the recruitment and activation of several protein kinases (such as focal adhesion kinase), leading to the activation of signalling pathways essential for several cellular activities such as cell migration, proliferation, survival and gene expression.

Genetic mutations in integrins or integrin regulators have been associated with Glanzmann's thrombasthenia (caused by mutations in integrin β3 subunit), the immunodeficiency disorder leukocyte adhesion deficiency types I and III (determined by mutations in integrin β2 subunit and kindlin 3, respectively) and skin diseases (caused by mutations in kindlin 1 and integrin α2, α6 and β3 subunits). Integrins are essential in the homing process, following the selectin phase, and are also involved in tumour progression and metastasis.

Specialized intercellular junctions

Specialized cell–cell junctions are the hallmark of all epithelial tissues. There are two major categories: symmetric junctions and asymmetric junctions. Symmetric junctions may be subdivided into three types: tight junctions (also known as occluding junctions or zonulae occludentes); anchoring junctions (including zonulae adherentes, or belt desmosomes, and maculae adherentes, or spot desmosomes); and communication junctions, represented by gap junctions. Tight junctions and anchoring junctions are components of the epithelial apical junctional complex. Hemidesmosomes are asymmetric junctions (see Fig. 1.19).

Tight junctions (occluding junctions, zonulae occludentes)

Tight junctions are the most apical component of the epithelial apical junctional complex. The main functions of tight junctions are the regulation of the paracellular permeability of the epithelial layer and the formation of an apical–basolateral intramembrane diffusion barrier, the hallmark of epithelial cell polarity. Tight junctions form a continuous belt (zonula) around the cell perimeter, near the apical domain of epithelial cells, and are connected to the actin cytoskeleton. At the site of the tight junction, the plasma membranes of adjacent cells come into close contact, so that the space between them is obliterated. Freeze-fracture electron microscopy shows that the contact between these membranes is represented by branching and anastomosing sealing strands of protein particles on the P (protoplasmic) face of the lipid bilayer (**Fig. 1.19A,B**). A tight junction contains numerous proteins: occludins and claudins, members of the tetraspanin family of proteins, containing four transmembrane domains, two loops and two cytoplasmic tails – occludins and tetraspanins provide the molecular basis for the formation of the branching and anastomosing strands seen in freeze-fracture preparations; the afadin–nectin complex and junctional adhesion molecules (JAMs), each forming *cis*-homodimers and interacting with each other through their extracellular domains (forming *trans*-homodimers) – nectins and JAMs are members of the immunoglobulin superfamily, and the afadin component of the afadin–nectin complex interacts with F-actin; and cytosolic zonula occludens proteins 1, 2 and 3 (ZO-1, ZO-2 and ZO-3). ZO-1 protein is associated with afadin and the intracellular domain of JAMs. All three ZO proteins facilitate the reciprocal interaction of occludins, claudins and JAMs with F-actin. Defects in paracellular magnesium permeability and reabsorption in kidneys occur when there is a mutation in claudin 16 and claudin 19 (renal magnesium wasting). For further reading on claudins, see Escudero-Esparza et al (2011). For further reading on JAMs, see Bazzoni (2003).

Anchoring junctions

In contrast to tight junctions, zonulae adherentes and maculae adherentes are characterized by the presence, along the cytosolic sides of the plasma membranes of adjacent epithelial cells, of symmetric dense plaques connected to each other across the intercellular space by cadherins. They differ in that F-actin is associated with plaques in zonulae adherentes and intermediate filaments are linked to plaques in maculae adherentes.

Zonula adherens (belt desmosome)

A zonula adherens is a continuous belt-like zone of adhesion parallel and just basal to a tight junction and also encircling the apical perimeter of epithelial cells. Ca^{2+}-dependent cell adhesion molecules (members of the desmoglein and desmocollin families of cadherins) are key components of a zonula adherens. In addition to the cadherin–catenin complex, a zonula adherens also houses the afadin–nectin complex.

A specific component of a zonula adherens is a cytoplasmic dense plaque attached to the cytosolic side of the plasma membrane. It consists of desmoplakin, plakophilin and plakoglobin proteins (the latter is also known as γ-catenin). A similar plaque is seen in a macula adherens or spot desmosome (see below).

Fascia adherens

A fascia adherens is similar to a zonula adherens, but is more limited in extent and forms a strip or patch of adhesion, e.g. between smooth muscle cells, in the intercalated discs of cardiac muscle cells and between glial cells and neurones. The junctions involve cadherins attached indirectly to actin filaments on the inner side of the membrane.

Desmosomes (maculae adherentes)

Desmosomes are limited, plaque-like areas of particularly strong intercellular contact. In epithelial cells, they may be located subjacent to the tight junction and zonula adherens belts, forming collectively the epithelial apical junctional complex (see Fig. 1.19A). The intercellular gap is approximately 25 nm; it is filled with electron-dense filamentous material (the intercellular cadherins) running transversely across it and is also marked by a series of densely staining bands (the cytoplasmic dense plaques) running parallel to the cell surfaces. Adhesion is mediated by Ca^{2+}-dependent cadherins, desmogleins and desmocollins. Within the cells on either side, each cytoplasmic dense plaque underlies the plasma membrane and consists of the proteins plakophilin, desmoplakin and plakoglobin (γ-catenin), into which the ends of intermediate filaments are inserted. The type of intermediate filament depends on the cell type, e.g. keratins are found in epithelia and desmin filaments are found in cardiac muscle cells. Desmosomes form strong anchorage points, likened to spot-welds, between cells subject to mechanical stress, e.g. in the prickle cell layer of the epidermis, where they are extremely numerous and large.

Hemidesmosomes

Hemidesmosomes are asymmetric anchoring junctions found between the basal side of epithelial cells and the associated basal lamina. The latter is a component of the basement membrane and contains laminin, an integrin ligand. The other component of the basement membrane is the reticular lamina, a collagen-containing layer produced by fibroblasts that also contains fibronectin, another integrin ligand. Hemidesmosomes resemble a single-sided desmosome, anchored on one side to the plasma membrane, and on the other to the basal lamina and adjacent collagen fibrils (**Fig. 1.19C**). The plaque has distinct proteins not seen in the plaques of a zonula adherens or a macula adherens: BPAG1 (bullous pemphigoid antigen 1), a member of the plakin family, and BPAG2 (bullous pemphigoid antigen 2), which possesses an extracellular collagenous domain. BPAG1 and BPAG2 were initially detected in patients with bullous pemphigoid, an autoimmune blistering disease. On the cytoplasmic side of the dense plaque there is a less dense plate into which keratin filaments are inserted, where they interact with the protein plectin associated with integrin α6β4. Hemidesmosomes use integrins and anchoring filaments (laminin 5) as their adhesion molecules anchored to the basal lamina, whereas desmosomes use cadherins.

Focal adhesion plaques

Less highly structured attachments with a similar arrangement exist between many other cell types and their surrounding matrices, e.g. between smooth muscle cells and their matrix fibrils, and between the ends of skeletal muscle cells and tendon fibres. The smaller, punctate adhesions resemble focal adhesion plaques, which are regions of local attachment between cells and the extracellular matrix. They are typically situated at or near the ends of actin filament bundles (stress fibres), anchored through intermediary proteins to the cytoplasmic domains of integrins. In turn, these are attached at their external ends to collagen or other filamentous structures in the extracellular matrix. They are usually short-lived; their formation and subsequent disruption are part of the motile behaviour of migratory cells. See Geiger et al (2009) for further reading on focal adhesions.

Gap junctions (communicating junctions)

Gap junctions resemble tight junctions in transverse section, but the two apposed lipid bilayers are separated by an apparent gap of 3 nm, which is bridged by a cluster of transmembrane channels (connexons). Each connexon is formed by a ring of six connexin proteins whose external surfaces meet those of the adjacent cell in the middle. A minute central pore links one cell to the next (**Fig. 1.19D**). Larger assemblies of many thousands of channels are often packed in hexagonal arrays. Gap junctions occur between numerous cells, including hepatocytes and cardiac myocytes.

AGEING, CELLULAR SENESCENCE, CANCER AND APOPTOSIS

Ageing is a universal feature of biological organisms, defined by a gradual decline over time in cell and tissue function that often, but not

Essentially, two molecules, cadherins and afadin, link to the actin cytoskeleton. In cultured cells, nectins appear to initiate the formation of a zonula adherens before the involvement of cadherins.

always, decreases the longevity of an individual. The hallmarks of ageing are reviewed in López-Otín et al (2013).

Cellular senescence is defined by an irreversible arrest in cell proliferation when cells experience DNA damage at telomeres and a decrease in mitogenic signalling. In contrast to reversibly arrested quiescent cells in G_0 of the cell cycle, senescent growth arrest is irreversible; cells in this state cannot be stimulated to proliferate by known stimuli and cannot be prompted to re-enter the cell cycle by physiological mechanisms. For further reading on senescence and the cell cycle, see Chandler and Peters (2013). Senescent cells can cause or foster degenerative diseases. In old age, cellular senescence in humans determines typical pathologies, including atherosclerosis leading to stroke, osteoporosis, macular degeneration, cardiopulmonary and renal failure, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

Senescent cells undergo changes in gene expression, which result in the secretion of proinflammatory cytokines, growth factors and proteases, activities that collectively define a senescence-associated secretory phenotype capable of triggering angiogenesis, inflammatory responses, stem cell renewal and differentiation, and which may also determine resistance to cancer chemotherapy. Senescent cells can be identified histochemically by their expression of either senescence-associated β-galactosidase, a lysosomal marker which is overexpressed in these cells, or the tumour suppressor protein $p16^{INK4a}$, which promotes the formation of senescence-associated chromatin. For further reading on ageing, cellular senescence and cancer, see Campisi (2013).

Cellular senescence can be caused by a disruption of metabolic signalling pathways, derived from mitogens and proliferation factors, and the activation of tumour suppressors, combined with telomere shortening and genomic damage. See Sahin and DePinho (2012) for further reading.

Cellular senescence suppresses tumorigenesis because cell proliferation is required for cancer development. However, senescent cells can stimulate the proliferation and malignant progression of adjacent premalignant cells by the release of senescence-inducing oncogenic stimuli. Cancer cells must harbour mutations to prevent telomere-dependent and oncogene-induced senescence, such as in the p53 and p16-retinoblastoma protein pathways. See López-Otín et al (2013) for further reading on the pathogenesis of ageing.

Apoptosis

Cells die as a result of either tissue injury (necrosis) or the internal activation of a 'suicide' programme (apoptosis) in response to extrinsic or intrinsic cues. Apoptosis (programmed cell death) is defined by the controlled demolition of cellular constituents and the ultimate uptake of apoptotic cell fragments by other cells to prevent immune responses. Some senescent cells become resistant to cell-death signalling, i.e. they are apoptosis-resistant. In effect, senescence blocks growth of damaged or stressed cells, whereas apoptosis quickly disposes of them. Apoptosis is a central mechanism controlling multicellular development. During morphogenesis, apoptosis mediates activities such as the separation of the developing digits, and plays an important role in regulating the number of neurones in the nervous system (the majority of neurones die during development). Apoptosis also ensures that inappropriate or inefficient T cells are eliminated in the thymus during clonal selection.

The morphological changes exhibited by necrotic cells are very different from those seen in apoptotic cells. Necrotic cells swell and subsequently rupture, and the resulting debris may induce an inflammatory response. Apoptotic cells shrink, their nuclei and chromosomes fragment, forming apoptotic bodies, and their plasma membranes undergo conformational changes that act as a signal to local phagocytes. The dead cells are removed rapidly, and as their intracellular contents are not released into the extracellular environment, inflammatory reactions are avoided; the apoptotic fragments also stimulate macrophages to release anti-inflammatory cytokines.

Apoptosis and cell proliferation are intimately coupled; several cell cycle regulators can influence both cell division and apoptosis. The signals that trigger apoptosis include withdrawal of survival factors or exposure to inappropriate proliferative stimuli. Three main routes to the induction of apoptosis have been established (Fig. 1.20). Two, the Fas ligand (FasL) pathway and the granzyme B pathway, are extrinsic, whereas the mitochondrial route is intrinsic. The Fas ligand (FasL) pathway involves binding of FasL to death receptors on the plasma membrane and recruitment of adaptor proteins, such as the Fas-associated death domain proteins, followed by the recruitment and activation of caspase 8. The granzyme B pathway involves creation of a perforin plasma membrane channel enabling the caspase-like granzyme B to

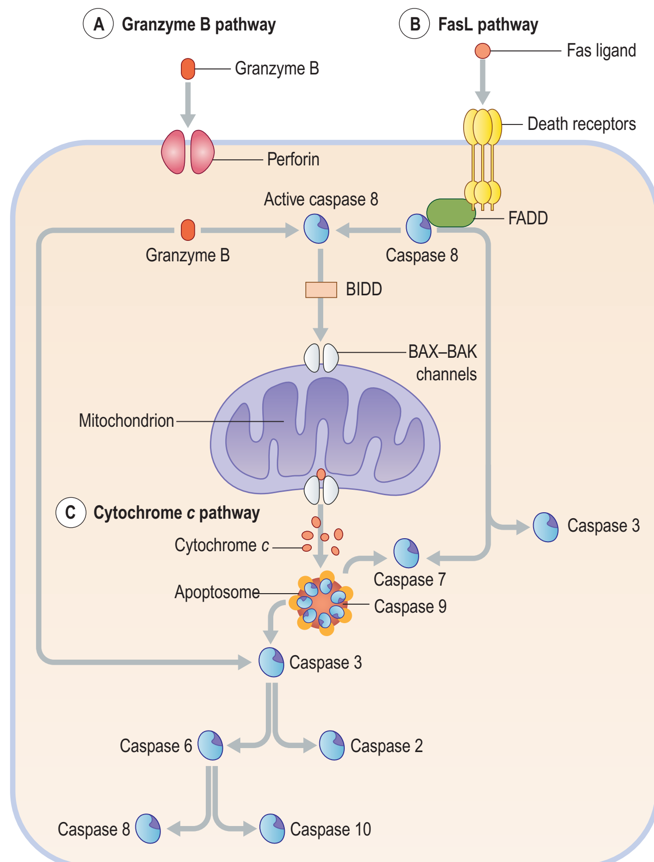


Fig. 1.20 Caspase activation pathways during apoptosis. **A**, The granzyme B extrinsic pathway activates caspase 8 and caspase 3 following entry of granzyme B across the plasma membrane pore-forming protein, perforin. This pathway is observed in cytotoxic T cells or natural killer cells for delivery of the protease granzyme B to target cells. **B**, The Fas ligand (FasL) extrinsic pathway is initiated by binding of FasL to clustered transmembrane death receptors that recruit adaptor proteins, such as the Fas-associated death-domain protein (FADD) to their intracellular domain, which in turn recruits and aggregates caspase 8 molecules, which become activated. Activated caspase 8 activates caspase 7 and caspase 3. **C**, The cytochrome *c* intrinsic pathway starts when granzyme B or activated caspase 8 causes the truncation by proteolysis of the protein BIDD (BH3-interacting domain death agonist), which penetrates a mitochondrion through BAX–BAK (BCL-2 associated X protein–BCL-2 antagonist killer) channel proteins on the outer mitochondrial membrane, causing the release of cytochrome *c*. Cytochrome *c* enables the assembly of the apoptosome (consisting of seven molecules of apoptosis protease-activating factor-1 (APAF1) and seven molecules of caspase 9), which in turn activates caspase 3 and caspase 7. Finally, the proteolytic activation cascade of caspase 6, caspase 2, caspase 8 and caspase 10 executes cell deconstruction.

enter the cell. The intrinsic mitochondrial route involves the release of cytochrome *c* from the space between the inner and outer mitochondrial membranes into the cytosol. Extrinsic and intrinsic pathways work cooperatively in the subsequent activation of a family of initiator-effector proteases, known as caspases (cysteine aspartic acid-specific proteases), which are present in healthy cells as inactive precursor enzymes or zymogens. Activation of caspases 3, 6 and 7 mediates apoptosis by initiating a cascade of degradative processes that target major constituents of the cell cytoskeleton, producing membrane blebbing, a distinctive feature of apoptosis caused by cytosolic and nuclear fragments flowing into the developing apoptotic bodies. Caspase cleavage inactivates many systems that normally promote damage repair and support cell viability, and activates a number of proteins that promote the death and disassembly of the cell. For further reading on apoptosis, see Taylor et al (2008).

Bonus e-book video

Video 1.1 Mitosis in a cell with fluorescently-labelled chromosomes and microtubules.

The ends of the chromosomes, or telomeres, become shorter and more dysfunctional with each DNA replication round. Telomere shortening has been shown to activate DNA damage responses, leading to mitochondrial dysfunction (a decrease in production of ATP and an increase in reactive oxygen species) and the activation of p53, which induces growth arrest, apoptosis and senescence of stem cells and progenitor cells. p53 interconnects with different longevity metabolic signalling pathways, including the insulin, insulin-like growth factor I (IGFI) and mammalian target of rapamycin (mTOR) pathways, which are known to regulate lifespan by increasing the expression of genes involved in stress resistance and energy balance. Mutations in TERC (the RNA component of telomerase) and TERT (the catalytic component of telomerase) are found in patients with the premature ageing syndrome, dyskeratosis congenita (poor growth of fingernails and toenails, skin pigmentation and oral leukoplakia). Other important contributors to cell senescence are dysregulated autophagy and lack of disposal of misfolded proteins by the ubiquitin–26S proteasome machinery. These responses are collectively designated telomere-initiated cellular senescence.

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CHAPTER

2

Integrating cells into tissues

Cells evolved as single, free-living organisms, but natural selection favoured more complex communities of cells, multicellular organisms, where groups of cells specialize during development to carry out specific functions for the body as a whole. This allowed the emergence of larger organisms with greater control over their internal environment and the evolution of highly specialized organic structures such as the brain. The human body contains more than 200 different cell types, sharing the same genome but with different patterns of gene expression.

Some cells in the body are essentially migratory, but most exist as cellular aggregates in which individual cells carry out similar or closely related functions in a coordinated manner. These aggregates are termed tissues, and can be classified into a fairly small number of broad categories on the basis of their structure, function and molecular properties. On the basis of their structure, most tissues are divided into four major types: epithelia, connective or supporting tissue, muscle and nervous tissue. Epithelia are continuous layers of cells with little intercellular space, which cover or line surfaces, or have been so derived. In connective tissues, the cells are embedded in an extracellular matrix, which, typically, forms a substantial and important component of the tissue. Muscle consists largely of specialized contractile cells. Nervous tissue consists of cells specialized for conducting and transmitting electrical and chemical signals and the cells that support this activity.

There is molecular evidence that this structure-based scheme of classification has validity. Thus the intermediate filament proteins characteristic of all epithelia are keratins (Pan et al 2012); those of connective tissue are vimentins; those of muscle are desmins; and those of nervous tissue are neurofilament and glial fibrillary acidic proteins. However, cells such as myofibroblasts, neuroepithelial sensory receptors and ependymal cells of the central nervous system have features of more than one tissue type. Despite its anomalies, the scheme is useful for descriptive purposes; it is widely used and will be adopted here.

In this chapter, two of the major tissue categories, epithelia and general connective and supporting tissues, will be described. Specialized skeletal connective tissues, i.e. cartilage and bone, together with skeletal muscle, are described in detail in Chapter 5 as part of the musculoskeletal system overview. Smooth muscle and cardiac muscle are described in Chapter 6. Nervous system tissues are described in Chapter 3. Specialized defensive cells, which also form a migrant population within the general connective tissues, are considered in more detail in Chapter 4, with blood, lymphoid tissues and haemopoiesis.

EPITHELIA

The term epithelium is applied to the layer or layers of cells that cover the body surfaces or line the body cavities that open on to it. The fate of embryonic epithelial populations is illustrated in Figure 12.3. Epithelia function generally as selective barriers that facilitate, or inhibit, the passage of substances across the surfaces they cover. In addition, they may: protect underlying tissues against dehydration, chemical or mechanical damage; synthesize and secrete products into the spaces that they line; and function as sensory surfaces. In this respect, many features of nervous tissue can be regarded as those of a modified epithelium and the two tissue types share an origin in embryonic ectoderm.

Epithelia (**Fig. 2.1**) are predominantly cellular and the little extracellular material they possess is limited to the basal lamina. Intercellular junctions, which are usually numerous, maintain the mechanical cohesiveness of the epithelial sheet and contribute to its barrier functions. A series of three intercellular junctions forms a typical epithelial junctional complex: in sequence from the apical surface, this consists of a tight junctional zone, an adherent (intermediate) junctional zone and a region of discrete desmosome junctions. Epithelial cell shape is most usually polygonal and partly determined by cytoplasmic features such as secretory granules. The basal surface of an epithelium lies in contact with a thin layer of filamentous protein and proteoglycan termed the basal lamina, which is synthesized predominantly by the epithelial cells. The basal lamina is described on page 34.

Epithelia can usually regenerate when injured. Indeed, many epithelia continuously replace their cells to offset cell loss caused by mechanical abrasion (reviewed in Blanpain et al (2007)). Blood vessels do not penetrate typical epithelia and so cells receive their nutrition by diffusion from capillaries of neighbouring connective tissues. This arrangement limits the maximum thickness of living epithelial cell layers. Epithelia, together with their supporting connective tissue, can often be removed surgically as one layer, which is collectively known as a membrane. Where the surface of a membrane is moistened by mucous glands it is called a mucous membrane or mucosa, whereas a similar layer of connective tissue covered by mesothelium is called a serous membrane or serosa.

CLASSIFICATION

Epithelia can be classified as unilaminar (single-layered, simple), in which a single layer of cells rests on a basal lamina; or multilaminar, in which the layer is more than one cell thick. The latter includes: stratified squamous epithelia, in which flattened superficial cells are constantly replaced from the basal layers; urothelium (transitional epithelium), which serves special functions in the urinary tract; and other multilaminar epithelia such as those lining the largest ducts of some exocrine glands, which, like urothelium, are replaced only very slowly under normal conditions. Seminiferous epithelium is a specialized multilaminar tissue found only in the testis.

Unilaminar (simple) epithelia

Unilaminar epithelia are further classified according to the shape of their cells, into squamous, cuboidal, columnar and pseudostratified types. Cell shape may, in some cases, be related to cell volume. Where little cytoplasm is present, there are generally few organelles and therefore there is low metabolic activity and cells are squamous or low cuboidal. Highly active cells, e.g. secretory epithelia, contain abundant mitochondria and endoplasmic reticulum, and are typically tall cuboidal or columnar. Unilaminar epithelia can also be subdivided into those that have special functions, such as those with cilia, numerous microvilli, secretory vacuoles (in mucous and serous glandular cells) or sensory features. Myoepithelial cells, which are contractile, are found as isolated cells associated with glandular structures, e.g. salivary and mammary glands.

Squamous epithelium

Simple squamous epithelium is composed of flattened, tightly apposed, polygonal cells (squames). This type of epithelium is described as tessellated when the cells have complex, interlocking borders rather than straight boundaries. The cytoplasm may in places be only 0.1 μm thick and the nucleus usually bulges into the overlying space (**Fig. 2.2A**). These cells line the alveoli of the lungs, where their surface area is huge and cytoplasmic volume correspondingly large, and they also form the outer capsular wall of renal corpuscles, the thin segments of the renal tubules and various parts of the inner ear. Because it is so thin, simple squamous epithelium allows rapid diffusion of gases and water across its surface; it may also engage in active transport, as indicated by the presence of numerous endocytic vesicles in these cells. Tight junctions (occluding junctions, zonulae adherentes) between adjacent cells ensure that materials pass primarily through cells, rather than between them.

Cuboidal and columnar epithelia

Cuboidal and columnar epithelia consist of regular rows of cylindrical cells (**Figs 2.2B, C**). Cuboidal cells are approximately square in vertical section, whereas columnar cells are taller than their diameter, and both

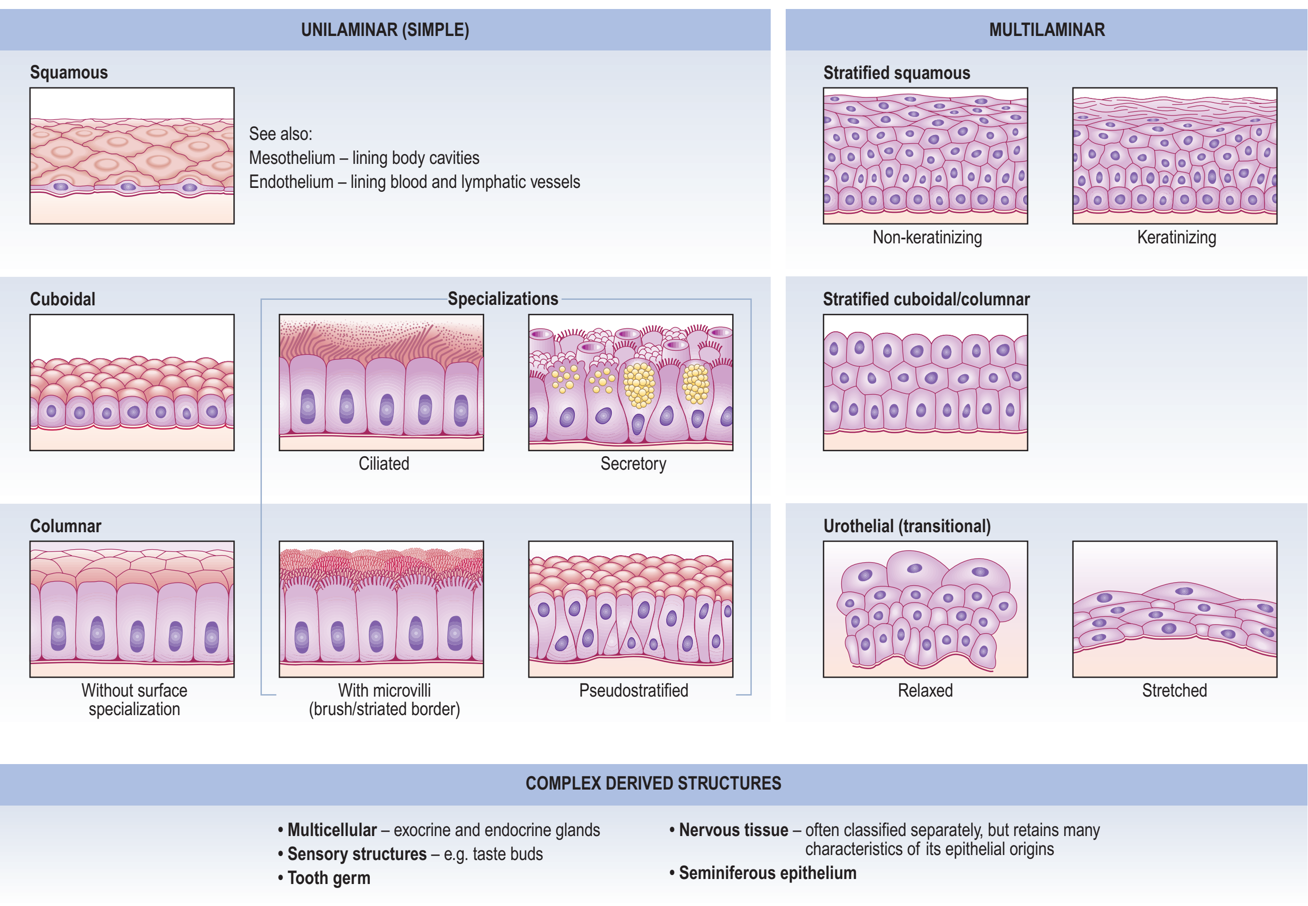


Fig. 2.1 Classification of epithelial tissues and cells.

are polygonal when sectioned horizontally. Commonly, microvilli are found on their free surfaces, which considerably increases the absorptive area, e.g. in the epithelia of the small intestine (columnar cells with a striated border of very regular microvilli), the gallbladder (columnar cells with a brush border of microvilli); proximal convoluted tubules of the kidney (large cuboidal to low columnar cells with brush borders); and the epididymis (columnar cells with extremely long microvilli, erroneously termed stereocilia).

Ciliated columnar epithelium lines most of the respiratory tract, except for the lower pharynx and vocal folds, and it is pseudostratified (**Fig. 2.2D**) as far as the larger bronchioles; it also lines some of the tympanic cavity and auditory tube; the uterine tube; and the efferent ductules of the testis. Submucosal mucous glands and mucosal goblet cells secrete mucus on to the luminal surface of much of the respiratory tract, and cilia sweep a layer of mucus, trapped dust particles and so on from the lung towards the pharynx in the mucociliary rejection current, which clears the respiratory passages of inhaled particles. Cilia in the uterine tube assist the passage of oocytes and fertilized ova to the uterus.

Some columnar cells are specialized for secretion, and aggregates of such cells may be described as glandular tissue. Their apical domains typically contain mucus- or protein-filled (zymogen) vesicles, e.g. mucin-secreting and chief cells of the gastric epithelium. Where mucous cells lie among non-secretory cells, e.g. in the intestinal epithelium, their apical cytoplasm and its secretory contents often expand to produce a characteristic cell shape, and they are known as goblet cells (see Fig. 2.2D). For further details of glandular tissue, see page 32, and for the characteristics of mucus, see page 40.

Pseudostratified epithelium

Pseudostratified epithelium is a single-layered (simple) columnar epithelium in which nuclei lie at different levels in a vertical section (Fig. 2.2D). All cells are in contact with the basal lamina throughout their lifespan, but not all cells extend through the entire thickness of the epithelium. Some constitute an immature basal cell layer of smaller cells, which are often mitotic and able to replace damaged mature cells. Migrating lymphocytes and mast cells within columnar epithelia may also give a similar, pseudostratified appearance because their nuclei are found at different depths. Much of the ciliated lining of the respiratory tract is of the pseudostratified type, and so is the sensory epithelium of the olfactory area.

Sensory epithelia

Sensory epithelia are found in special sense organs of the olfactory, gustatory and vestibulocochlear receptor systems. All of these contain sensory cells surrounded by supportive non-receptor cells. Olfactory receptors are modified neurones and their axons pass directly to the brain, but the other types are specialized epithelial cells that synapse with terminals of afferent (and sometimes efferent) nerve fibres.

Myoepithelial cells

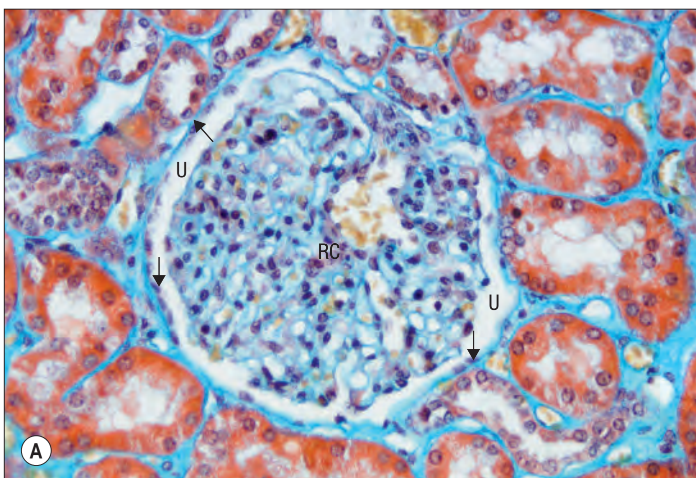
Myoepithelial cells, which are also sometimes termed basket cells, are fusiform or stellate in shape (**Fig. 2.3**), contain actin and myosin filaments, and contract when stimulated by nervous or endocrine signals. They surround the secretory portions and ducts of some glands, e.g. mammary, lacrimal, salivary and sweat glands, and lie between the basal lamina and the glandular or ductal epithelium. Their contraction assists the initial flow of secretion into larger conduits. Myoepithelial cells are ultrastructurally similar to smooth muscle cells in the arrangement of their actin and myosin, but differ from them because they originate, like the glandular cells, from embryonic ectoderm or endoderm. They can be identified immunohistochemically on the basis of the co-localization of myofilament proteins (which signify their contractile function (**Fig. 2.4**)) and keratin intermediate filaments (which accords with their epithelial lineage).

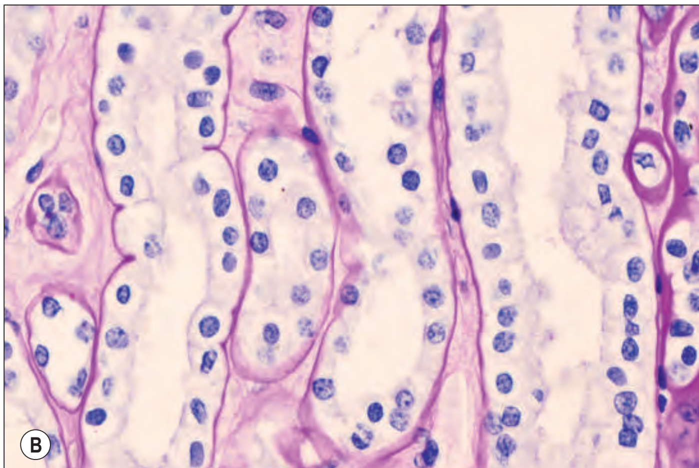
Multilaminar (stratified) epithelia

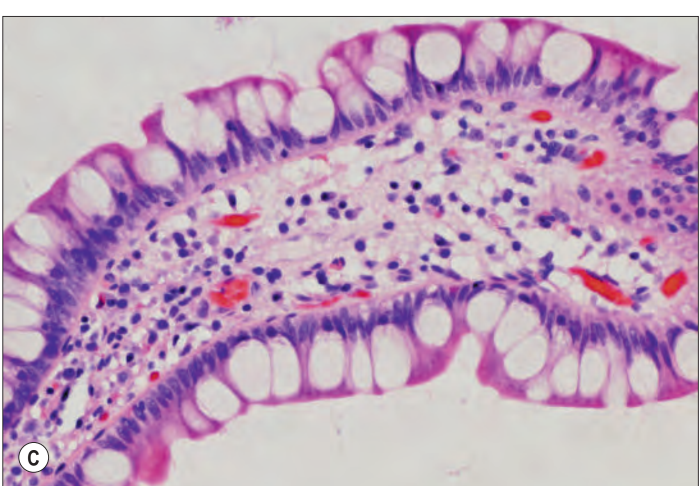
Multilaminar epithelia are found at surfaces subjected to mechanical damage or other potentially harmful conditions. They can be divided into those that continue to replace their surface cells from deeper layers, designated stratified squamous epithelia, and others in which replacement is extremely slow except after injury.

Stratified squamous epithelia

Stratified squamous epithelia are multilayered tissues in which the formation, maturation and loss of cells is continuous, although the rates of these processes can change, e.g. after injury. New cells are formed in the most basal layers by the mitotic division of stem cells and transit (or transient) amplifying cells. The daughter cells move more superficially, changing gradually from a cuboidal shape to a more







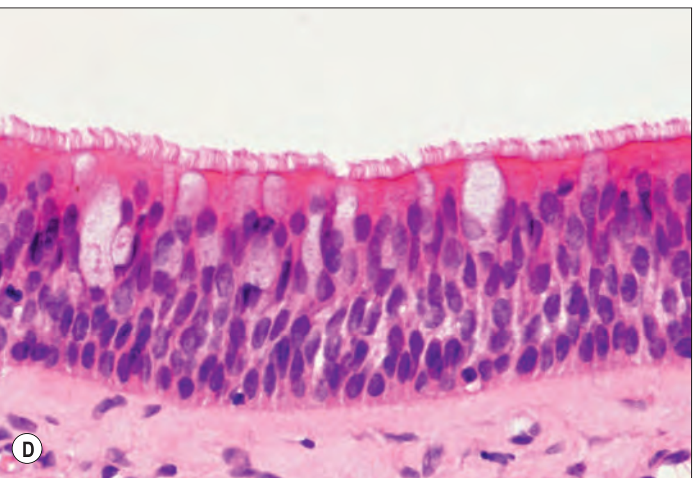


Fig. 2.2 **A**, Simple squamous epithelium lining the outer parietal layer (arrows) of a Bowman's capsule in the renal corpuscle (RC), stained with the trichrome, Martius Scarlet Blue (MSB). Oval epithelial nuclei project into the urinary space (U), within a highly attenuated cytoplasm. **B**, Simple cuboidal epithelium lining a group of collecting ducts sectioned longitudinally in the renal medulla. The basement membranes are stained magenta with periodic–acid Schiff (PAS) reagent. **C**, Simple columnar epithelium covering the tip (off field, right) of a villus in the ileum. Tall, columnar absorptive cells with oval, vertically orientated nuclei bear a striated border of microvilli, just visible as a deeper-stained apical fringe. Numerous interspersed goblet cells are present, with pale apical cytoplasm filled with mucinogen secretory granules and dark, flattened, basally situated nuclei. **D**, Ciliated columnar pseudostratified epithelium in the respiratory tract, and interspersed goblet cells, with pale, mucinogen granule-filled apical cytoplasm. All human tissues. (All human tissues, courtesy of Mr Peter Helliwell and the late Dr Joseph Mathew, Department of Histopathology, Royal Cornwall Hospitals Trust, UK.)

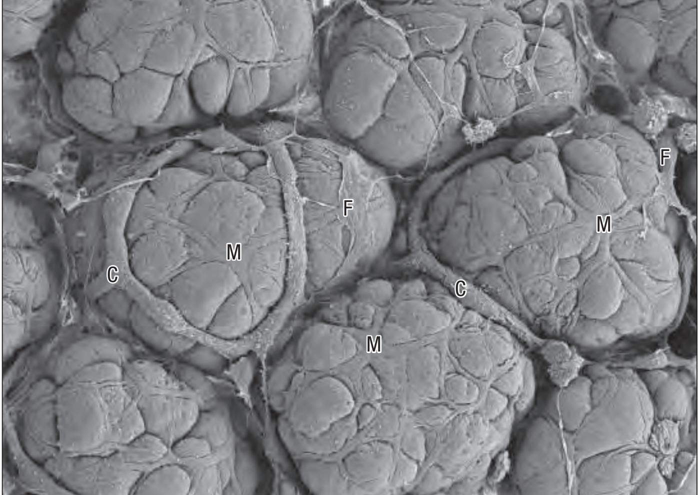


Fig. 2.3 Stellate myoepithelial cells (M) wrapped around secretory acini in the lactating mouse mammary gland, seen in the scanning electron microscope after enzymatic depletion of extracellular matrix. Blood capillaries (C) and fibroblasts (F) are also indicated. (Courtesy of Prof. Toshikazu Nagato, Fukuoka Dental College, Japan.)

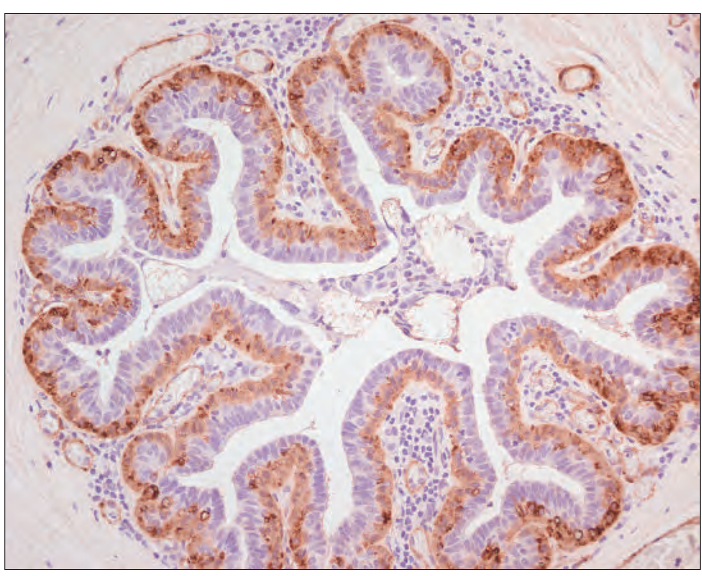


Fig. 2.4 Myoepithelial cells (stained brown), in a human breast duct, demonstrated immunohistochemically using antibody to smooth muscle actin. (Courtesy of Mr Peter Helliwell and the late Dr Joseph Mathew, Department of Histopathology, Royal Cornwall Hospitals Trust, UK.)