Vinod H. Nargund
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*Editors*

# Urological Oncology

Second Edition

Springer

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*Dedicated to our patients and teachers*

# Preface to Second Edition

We are delighted to present the second edition of *Urological Oncology* for our readership. When we collaborated with our fine team of authors initially, we felt that there was a need for a simple handbook focused on the interface between science and practical management that would allow the less experienced clinician or one with less specific knowledge of urological malignancies to understand clinical management practices and the basis for their implementation. We were gratified by the impact of the first edition of *Urological Oncology* and by the high level of feedback from readers requesting an update.

With this second edition, we have sought to maintain the didactic and easy-to-assimilate format and at the same time improve domains that might have been covered in more detail and include data from recent practice-changing clinical trials, innovations in laboratory diagnostics, surgery, radiation oncology, systemic therapy, and palliative/supportive care.

Medicine is changing at an ever-increasing pace, with a shifting focus on value rather than volume and an astounding amount of complex molecular and biostatistical information, daunting even to the most experienced clinician. We hope that our second edition will enable all to place these advances in the context of existing practices, so as to encourage more tailored management of patients. The second edition remains again compact, concise, and comprehensive.

We thank our contributors, staff at Springer, and, most of all, our readers, who have made this new edition possible.

September 2014

Vinod H. Nargund
Derek Raghavan
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# Preface to First Edition

*I keep six honest serving men*
*(They taught me all I knew);*
*Their names are What and Why and When*
*And How and Where and Who.*
*Rudyard Kipling ("The Elephant's Child," in The Just-So Stories, 1902)*

Clinical knowledge is based on three components: meticulous observation, detailed recording, and an understanding of basic science relevant to the clinical situation. The first two come with apprenticeship and the last one with personal research or inquisitive reading. It is the last component that is the basis for this book. Although most general urology books contain a fair amount of urological oncology, most of them are written by urologists for urologists. There is an increasing realization, however, that a multidisciplinary approach is required for the management of all cancers, including urological cancers. In particular, there is a need for surgeons and oncologists to have an integrated strategy for the management of complex cancer cases. A multidisciplinary team will include anesthetists, radiologists, minimally invasive surgeons, intensivists, nutritionists, and support and social work staff in addition to the cancer clinicians. We aim, in this book, to provide this integrated approach as it has contributions from specialists from these different disciplines. All these specialists should have a role in the management of patients to provide them with optimal chances of recovery. They have also a key role in counseling patients in a coordinated way, for otherwise, patients would gain piecemeal information of variable quality from a number of sources, including the Internet. The media and the Internet have increased cancer awareness among patients, who demand more and more answers to questions such as: What caused my cancer? How can I prevent a recurrence? Will my children get it? How do I get the best up-to-date treatment for my cancer?

Patients have a greater understanding that there may be choices in the management of their condition, and oncologists, both surgical and medical, have to listen to and include the patient's views in the decision-making process. We hope this book will assist in both the management and the counseling of patients with urological cancer. The book also includes chapters on basic science, research, and trials related to urological cancers, which will help those students with an interest in research. Relevant surgical anatomy and other details of basic science are included wherever necessary.

Initially, this book was intended to be a pocket guide on adult urological cancer, but it quickly metamorphosed into a minitextbook. The authorship is truly international and therefore reflects a consensus approach to investigation and treatment across the world. The text is didactic and should provide the basis for further reading from journals or more detailed review papers. The book is aimed at residents and urological specialists at all levels of training in urology and oncology.

London, UK — Vinod H. Nargund
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# Chapter 1
# Normal Cell

**Ray K. Iles**

Knowledge of normal cell biology is crucial for understanding the function of a normal cell and its deregulation in cancer. This chapter describes briefly the cellular and molecular features of a normal and malignant human cell with particular reference to genitourinary cancers.

## Cell Structure and Function

*Cell* (*plasma*) *membrane* is a bilayer consisting of amphipathic phospholipids, a polar hydrophilic head (phosphatidyl choline) and a lipid hydrophobic tail (commonly two long chain fatty acids). The phospholipids spontaneously form an effective bilayer barrier impermeable to most water-soluble molecules; the barrier also defines cellular internal environment. The membrane exchanges are regulated by proteins embedded within the lipid bilayer. *Cytoskeleton* is a complex network of structural proteins that regulates not only the shape of the cell but its ability to traffic internal cell organelles. The major components are microtubules, intermediate filaments, and microfilaments. *Cytoplasm* contains organelles and defines the interior of the cell. Although a fluid compartment, the organelles are held within a scaffolding or cytoskeleton that regulates the passage and direction in which the interior solutes and storage granules flow.

*Basement membrane* (BM) is a specialized form of extracellular matrix (ECM) that has been recognized as a key regulator of cell behaviour. In addition to structural support and cell compartmentalization, BM sends a signal to the cells about the extracellular microenvironment, thereby regulating cell behaviours [1]. The role of BM in

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angiogenesis is described later. The *nucleus* is an organelle containing the human genome and it is bound by two bilayer lipid membranes. The outer of the two is continuous with the endoplasmic reticulum (ER). Nuclear pores are present in the membranes, allowing the passage of nucleotides and DNA interacting proteins in and messenger RNA (mRNA) out. *Nucleoli* are dense areas within the nucleus rich in proteins and RNA chiefly concerned with the synthesis of ribosomal RNA (rRNA) and ribosomes.

The *endoplasmic reticulum* (ER) is interconnecting branching tubules or flattened sacs (cisternae) of lipid membrane bilayer. It may contain ribosomes on the surface [rough endoplasmic reticulum (RER) when present, or smooth endoplasmic reticulum (SER) when absent]. ER is the site of production of transmembrane proteins and lipid and proteins for secretion or for other organelles. *Ribosomes* are complexes of protein and RNA that translate mRNA into a primary sequence of amino acids of a protein peptide chain. This chain is synthesized into the ER where it is first folded and modified into mature peptides.

The *Golgi apparatus* is characterized as a stack of flattened cisternae from which, vesicles bud off from the thickened ends. The primary processed peptides of the ER are exported to the Golgi for maturation into functional proteins (e.g. glycosylation of proteins, which are to be excreted, occurs here) before packaging into secretory granules and cellular vesicles, which bud off the end. *Lysosomes* are dense cellular vesicles containing acidic digestive enzymes.

*Mitochondria* are semiautonomous organelles responsible for cellular energy metabolism, free radical generation, and apoptosis [2]. They have two lipid bilayer membranes and a central matrix. The *outer membrane* contains gated receptors for the import of raw materials [pyruvate and adenosine diphosphate (ADP)] and the export of precursor of amino acids and sugars (oxaloacetate) and adenosine triphosphate (ATP). Proteins of the Bcl-2-Bax family are incorporated in this membrane and can release cytochrome C that triggers apoptosis [3]. The *inner membrane* is infolded (cristae) to increase its effective surface area, and it contains transmembrane enzyme complexes of the electron transport chain, generating an $H^+$ ion gradient. *The inner matrix* contains the enzymes of the Krebs' cycle. Mitochondria also possess several copies of their own DNA in a circular genome and thereby maintain genomic independence from the nucleus [4].

Mutations in mitochondrial DNA (mtDNA) have been identified in renal cell carcinoma (RCC) and prostate cancer. In RCC there is evidence to suggest alterations of mtDNA (mutation of the *ND1* gene) and mRNA coding for the subunit *ND3* gene [5, 6]. In prostate cancer there is evidence of mtDNA deletions that increase with advanced age [7]. The knowledge of cancer related mitochondrial abnormalities may help in devising novel anticancer therapies.

## Cell Dynamics

The cell component proteins and organelles are continually being formed and degraded. Old cellular proteins are mopped up by a small cofactor molecule called ubiquitin. Ubiquitination acts as a signal for destruction, and a complex containing more than three ubiquitin molecules is rapidly degraded by a macromolecule called

26S proteasome. Failure to remove worn protein can result in the development of chronic debilitating disorders. This is well demonstrated in von Hippel–Lindau (VHL) disease, which is caused by mutation of the *VHL* gene (3p26-p25). There is increased activity in hypoxia inducible factors 1 and 2 (HIF-1 and HIF-2) with *VHL* gene mutation [8]. The HIFs are transcription factors in angiogenesis and tumor growth. The VHL protein is thought to form an E3 ligase (ubiquitin-activating enzyme) [8].

## Cytoskeleton

As its name suggests cytoskeleton is a cellular skeleton and is made of protein. The major cytoskeleton components are microtubules, intermediate fi laments, and microfilaments. *Microtubules* are made up of polymerized α and β tubulin and continuously changing length. They form a "highway" transporting organelles through the cytoplasm. Two motor microtubule-associated proteins- dynein and kinesin allow antegrade and retrograde movements. During the *interphase*, the microtubules are rearranged by the microtubule-organizing center (MTOC), which provides a structure on which the daughter chromosomes can separate.

*Intermediate filaments* form a network around the nucleus and extend to the periphery of the cell. They make cell-to-cell contacts with the adjacent cells via desmosomes and basement matrix via hemidesmosomes. Their function appears to be in structural integrity, being prominent in cellular tissues under stress. The intermediate filament fiber proteins are specific; for example, keratin is intermediate fibers only found in epithelial cells, whereas vimentin is only found in mesothelial (fibroblastic) cells.

## Microfilaments

The muscle contractile actin and myosin filaments are also present throughout the nonmuscle cells, as truncated myosins (e.g., myosin 1), in the cytosol (forming a contractile actomyosin gel) beneath the plasma membrane. The calcium-dependent actin-binding proteins modulate the behaviour of microfilaments. Alterations in the cell's actin architecture are also controlled by the activation of small ras-like guanosine triphosphate (GTP)-binding proteins rho and rac. These are important in the rearrangement of the cell during division, and dysfunctions of these proteins are associated with malignancy.

## Intercellular Connections

The cytoskeleton and plasma membrane interconnect, and extracellular domains form junctions between cells to form tissues—tight, adherent, and gap junctions.

*Tight junctions* (TJs) (zonula occludens) hold cells together with the proteins called c*laudins*. They show selective tissue expression and regulate what small ions may pass through the gaps between cells. These are particularly important in the lining urothelium as they create a physiological barrier between urine and blood [9]. Increased urinary concentration of hepatocyte growth factor/scatter factor (HGF/SF) is associated with high grade and muscle invasive bladder cancer [10, 11]; HGF/SF and interleukin-8 disrupt tight junctions and have been thought to cause progression of transitional cell carcinoma (TCC) [12]. *Adherent junctions* (zonula adherens) are continuous on the basal side of cells and contain cadherins. Cadherins comprise a family of calcium-dependent transmembrane cell-to-cell adhesion molecules, and reduced expression of subclass E-cadherin is associated with increased urothelial tumor recurrence and invasiveness [13].

Similarly aberrations in E-cadherin are associated with prostate cancer progression [14]. *Desmosomes* are apposed areas of thickened membranes of two adjacent cells attached to intermediate filaments of cytokeratin. Desmosomal adhesion inhibits invasive behaviour of cancer cells. Invasive TCCs show decreased desmosomal density compared to noninvasive TCCs [15]. *Gap junctions* allow substances to pass directly between cells without entering the extracellular fluids.

## Cell Adhesion Molecules

Adhesion molecules and adhesion receptors are essential for tissue structure organization. Differential expression of such molecules is implicit in the processes of cell growth and differentiation, such as wound repair and embryogenesis. There is increasing evidence to suggest that the adhesion properties of neoplastic cells play a key role in the development and progression of cancer [16]. Some of these molecules are involved in cell signalling and tumor suppression. There are four major families of adhesion molecules: cadherins, integrins, the immunoglobulin superfamily, and the selectins. The role of *cadherins* has already been described (zonula adherens). At desmosomal junctions cadherins mediate cell-to-cell connection. Integrins are essential for cell attachment, control cell migration, cell cycle progression, and programmed cell death. They are formed of α and β subunits that dimerize to yield different heterodimers, each with distinct ligand binding and signalling properties. They principally bind to extracellular matrix components such as fibrinogen, elastase, and laminin. Their intracellular domains connect to the actin cytoskeleton. They also affect migration, proliferation, and survival of both normal and neoplastic cells. The $\alpha_6\beta_4$ integrin is associated with collagen VII on the basement membrane of urothelium forming a hemidesmosomal anchoring complex, which acts as an effective barrier to cell migration [17]. Loss of the $\alpha_6\beta_4$ integrin is associated with collagen VII, which explains the defects in the loss of the urothelial barrier in bladder cancer [18].

The *immunoglobulin* superfamily cell adhesion molecules (CAMs) contain domain sequences, which are immunoglobulin-like in structures. The neural CAM (N-CAM) is found predominantly in the nervous system mediating a homophilic

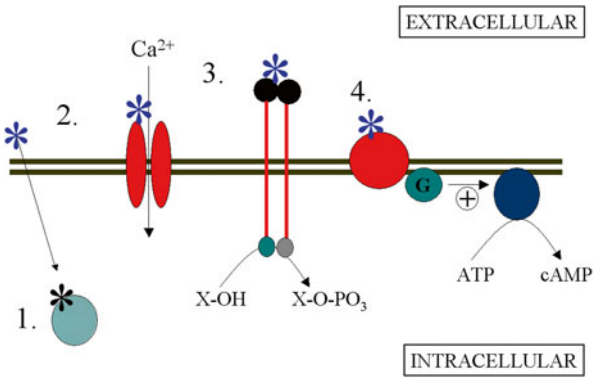


**Fig. 1.1** Illustration of the sites of cellular connections and the adhesion molecules found at the interface between the cell and a basement membrane and between cells. Diagrammatic illustration of the four types of receptor found in a cell: *1* cytoplasmic, *2* Ion channel, *3* enzyme linked, *4* G protein linked. (*represents receptor ligand)

(like with like) adhesion. Their function in the urinary tract is not completely understood. The *selectins*, unlike most adhesion molecules that bind to other proteins, interact with carbohydrate-ligands and mucin complexes on leukocytes and endothelial cells (vascular and hematological systems).

## Receptors

Cellular interpretation and translation of extracellular signals into an appropriate response is achieved through a diversity of receptors (Fig. 1.1). These signals could be soluble factors (e.g., chemicals, polypeptides, proteins, sugars), a ligand bound to another cell, or the extracellular matrix itself [19]. The receptors then transduce these extracellular signals across the cell wall to activate intracellular pathways, thereby bringing desired change [19]. There are three types of intercellular signalling: autocrine (cells respond to the substances secreted by themselves), paracrine (cells respond to signalling substance from adjacent cells); and endocrine (cells from distant sites). The malignant cells use signalling systems in all possible combinations.

## Secondary Messengers

### *Cyclic AMP, IP3/DAG, and $Ca^{2+}$ Ions*

Cyclic adenosine monophosphate (cAMP) is derived from ATP. It functions in intracellular signal transduction (e.g., effects of hormones like glucagon and adrenaline). It activates protein kinases and regulates the passage of $Ca^{2+}$ through ion channels. The G-protein–coupled transmembrane receptors (GPCs) form one of the largest families of cell receptors. G-protein complexes activate inner membrane-bound phospholipase complexes. These in turn cleave membrane phospholipid—polyphosphoinositide (PIP2)—into inositol triphosphate (IP3) (water soluble)

diacylglycerol (DAG) (lipid soluble). The former interacts with gated ion channels in the ER, causing a rapid release of $Ca^{2+}$, and the latter remains at the membrane, activating a serine/threonine kinase, protein kinase C.

### *Protein Phosphorylation*

Although phosphorylation of the cytoplasmic secondary messengers is often a consequence of secondary activation of cAMP, $Ca^{2+}$ and DAG, the principal route for the protein phosphorylation cascades is from the dimerization of surface protein kinase receptors. The tyrosine kinase receptors phosphorylate each other when ligand binding brings the intracellular receptor components in to close proximity. The inner membrane and cytoplasmic targets of these activated receptor complexes are Ras, protein kinase C, and ultimately the mitogen-activated protein (MAP) kinase, Janus Stat pathways (family of intracellular tyrosine kinase), or phosphorylation of IκB, causing it to release its DNA-binding protein nuclear factor κB (NF-κB). These intracellular signalling proteins usually contain conserved noncatalytic regions called the Serc homology regions 2 and 3 (SH2 and SH3). The SH2 region binds to phosphorylated tyrosine. The SH3 domain has been implicated in the recruitment of intermediates, which activate Ras proteins. Like G proteins, Ras (and its homologous family members Rho and Rac) switches between inactive GDP-binding state and active GTP-binding states. This starts a phosphorylation cascade of the MAP kinase, Janus-Stat protein pathways, which ultimately activate a DNA binding protein. This protein undergoes a conformational change, enters the nucleus, and initiates transcription of specific genes.

## Free Radicals

A free radical is any atom or molecule containing one or more unpaired electrons, making it more reactive than the native species. They have been implicated in a large number of human diseases. The hydroxyl (OHO) radical is by far the most reactive species, but the others can generate more reactive species as breakdown products. When a free radical reacts with a nonradical, a chain reaction ensues resulting in direct tissue damage by lipid peroxidation of membranes. Hydroxyl radicals can cause mutations by attacking DNA. Interestingly ionising radiation used in cancer treatment can activate this mechanism by the interaction with water molecules.

Superoxide dismutases O2O (SOD) convert superoxide to hydrogen peroxide. Glutathione peroxidases are major enzymes that remove hydrogen peroxide generated by SOD in cytosol and mitochondria. Free radical scavengers bind reactive oxygen species (ROS). α-Tocopherol, urate, ascorbate, and glutathione remove free radicals by reacting in a direct and noncatalytic way. There is a growing evidence that cardiovascular diseases and cancer can be prevented by a diet rich in substances that diminish oxidative damage. Principal dietary antioxidants include vitamins C and E, β-carotene, and flavonoids.

## Heat Shock Proteins

Heat shock proteins (HSPs) are induced by heat shock and other chemical and physical stresses [20], and their functions include the export of proteins in and out of specific cell organelles, acting as molecular chaperones (the catalysis of protein folding and unfolding), and the degradation of proteins (often by ubiquitination pathways). The unifying feature, which leads to the activation of HSPs, is the accumulation of damaged intracellular protein. Tumors have an abnormal thermotolerance, which is the basis for the observation of enhanced cytotoxic effect of chemotherapeutic agents in hyperthermic subjects. The HSPs are expressed in a wide range of human cancers and are implicated in cell proliferation, differentiation, invasion, metastasis, cell death, and immune response [20]. Although HSP detection by immunocytochemistry has been an established practice, serum detection of HSP and its antibodies is still a new research area. Various types of HSP have been demonstrated in urogenital cancers including kidney, prostate, and bladder. For example, HSP27 expression in prostate cancer indicates poor clinical outcome [21, 22].

## Programmed Cell Death

In necrotic cell death external factors damage the cell with influx of water and ions leading to the swelling and rupture of cellular organelles. Cell lysis induces acute inflammatory responses *in vivo* (Table 1.1). In apoptosis, cell death occurs through the deliberate activation of constituent genes whose function is to cause their own demise. Necrosis lacks the features of apoptosis and is an uncontrolled process. Another process is autophagy (self eating) which is lysosome mediated catabolic process. Apoptotic cell death has the following characteristic morphological features:

- Chromatin aggregation, with nuclear and cytoplasmic condensation into distinct membrane-bound vesicles, which are termed apoptotic bodies
- Organelles remain intact
- Cell blebs (which are intact membrane vesicles)
- No inflammatory response
- Cellular blebs and remains are phagocytised by adjacent cells and macrophages

**Table 1.1** Contrasting morphological features of necrotic and apoptotic cell death

| Necrosis | Apoptosis |
|---|---|
| Swelling of the cell | Cellular shrinkage |
| Ruptured plasma membrane | Intact plasma membrane |
| Intact nucleus | Condensation of chromatin and mDNA damage |
| Nonspecific proteolysis | Specific coordinated proteolysis |
| Swelling of cell organelles | Normal size cell organelles |
| Occurs because no energy available | Energy is required |

## *Molecular Biology of Apoptosis*

Apoptosis is a highly regulated mechanism of cell death which helps in development process and getting rid of damaged cells. Most cells rely on a constant supply of survival signals without which they will undergo apoptosis. Neighbouring cells and the extracellular matrix provide these signals. Cancer, autoimmunity, and some viral illnesses are associated with inhibition of apoptosis and increased cell survival. Metastatic tumor cells circumvent the normal environmental cues for survival and can survive in foreign environments. The molecular basis of steps of apoptosis—death signals, genetic regulation, and activation of effectors—has been identified [23]. Apoptosis requires energy (ATP), and several $Ca^{2+}$ – and $Mg^{2+}$ dependent nuclease systems are activated, which specifically cleave nuclear DNA at the inter-histone residues. This involves the enzyme cysteine-containing aspartase-specific protease (CASPASE), which activates the caspase-activated DNAase (CAD)/inhibitor of CAD (ICAD) system. Apoptotic signals affect mitochondrial permeability, resulting in reduction in the membrane potential and mitochondrial swelling. The apoptotic trigger cytochrome c is released from mitochondria into cytosol [24].

## *Bcl-2, p53, and the Proapoptotic Gene Bax*

Several proteins including members of the Bcl-2 family regulate mitochondrial permeability. Bcl-2 (24 kd) is associated with the internal membrane of the mitochondria and the nucleus. Bcl-2 suppresses apoptosis by directly preventing mitochondrial permeability and by interacting with other proteins [25]. The other gene that has been studied extensively is the tumor suppressor gene *p53*. It has an important role in cell cycle regulation and acts as a transcription factor that controls other gene products. Normal wild-type *p53* limits cell proliferation after DNA damage by arresting the cell cycle or activating apoptosis [25]. Also, *p53* has a complex role in chemosensitivity; it can increase apoptosis or arrest growth, thereby increasing drug resistance [26]. Drugs like taxanes and vinca alkaloids induce apoptosis independent of *p53*, as they do not damage DNA [26]. The proapoptotic gene *bax* has also been extensively investigated. In contrast to Bcl-2, *bax* is a promoter of apoptosis. Different pathways are discussed later.

## *Molecular Genetics*

Genetic information is stored in the form of double-stranded deoxyribonucleic acid (DNA). Each strand of DNA is made up of a deoxyribose-phosphate backbone and a series of *purine* [adenine (A) and guanine (G)] and *pyrimidine* [thymine (T) and cytosine (C)] bases of the nucleic acid. For practical purposes, the length of DNA is generally measured in numbers of base pairs (bp). The monomeric unit in DNA

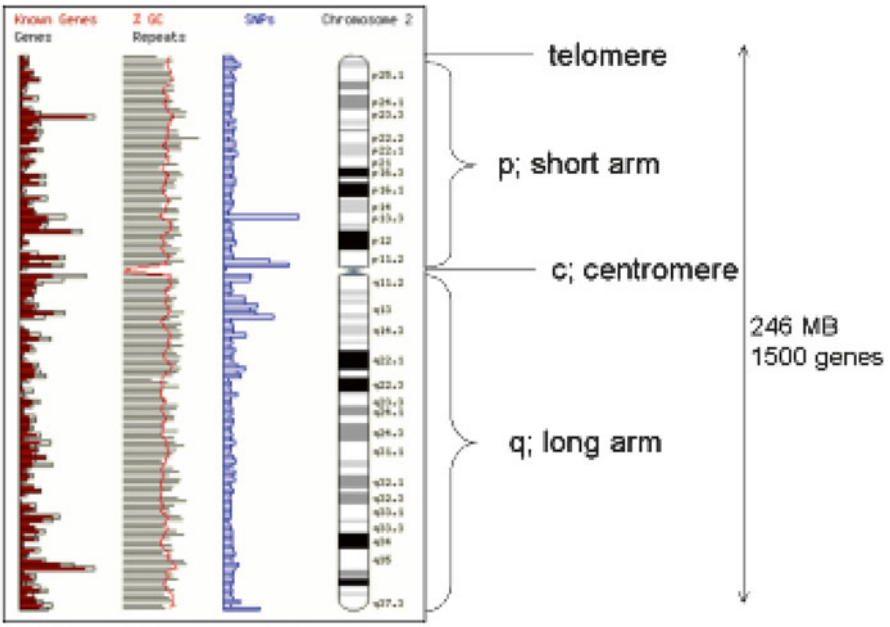


**Fig. 1.2** Chromosome structure (ch7) showing the position of the centromere telomeres, and short (*p*) and long arms (*q*). A G-banding pattern is shown and how this maps to known sites of genes and genetic markers such as single nucleotide polymorphisms (SNPs) and the CG rich island, which are characteristic of gene control elements

(and in RNA) is the nucleotide, which is a base joined to a sugar-phosphate unit. The two strands of DNA are held together by hydrogen bonds between the bases. There are only four possible pairs of nucleotides: TA, AT, GC, and CG. The two strands twist to form a double helix with major and minor grooves, and the large stretches of helical DNA are coiled around histone proteins to form nucleosomes and are further condensed into the chromosomes that are seen at metaphase.

## Human Chromosomes

The nucleus of each diploid cell contains $3 \times 10^9$ bp of DNA (Fig. 1.2). Chromosomes are massive structures containing one linear molecule of DNA that is wound around histone proteins, which are further wound to make up the structure of the chromosome itself. Diploid human cells have 46 chromosomes, 23 inherited from each parent; 22 pairs of autosomes, and two sex chromosomes (XX female and XY male). The chromosomes can be classified according to their size and shape, the largest being chromosome 1. The constriction in the chromosome is the *centromere*, which may be in the middle of the chromosome (metacentric) or at one extreme end (acrocentric). The centromere divides the chromosome into a short arm (*p* arm) and

a long arm (*q* arm). In addition, chromosomes can be stained when they are in the metaphase stage of the cell cycle and are very condensed. The stain gives a different pattern of light and dark bands that is diagnostic for each chromosome. Each band is given a number, and gene mapping techniques allow genes to be positioned within a band within an arm of a chromosome. During cell division, *mitosis*, each chromosome divides into two so that each daughter nucleus has the same number of chromosomes as its parent cell. During gametogenesis, however, the number of chromosomes is halved by *meiosis*.

## Telomeres and Immortality

The ends of eukaryotic chromosomes, *telomeres*, do not contain genes but rather many repeats of a guanine-rich hexameric sequence TTAGGG. Telomeres are specialized DNA structures that protect the ends of chromosome from fusion and recombination events [27]. *Telomerase* is a ribonucleoprotein that is necessary to repair the telomeric losses. Replication of linear chromosomes starts at coding sites (origins of replication) within the main body of chromosomes and not at the two extreme ends. The extreme ends are therefore susceptible to single-stranded DNA degradation back to double-stranded DNA. As a consequence of multiple rounds of replication, the telomeres shorten, leading to chromosomal instability and ultimately cell death. Stem cells have longer telomeres than their terminally differentiated daughters. However, germ cells replicate without shortening of their telomeres because of telomerase. Most somatic cells (unlike germ and embryonic cells) switch off the activity of telomerase after birth and die as a result of apoptosis [28]. Many cancer cells, however, reactivate telomerase, contributing to their immortality. Conversely, cells from patients with progeria (premature ageing syndrome) have extremely short telomeres. Telomerase activity is detected in nearly all cancer cells [29]. Likewise, prostate cancer but not normal prostate or benign prostatic hyperplasia (BPH) tissue, expresses telomerase activity [30]. Inhibition of telomerase with DNA-damaging chemotherapy drugs seems a possibility in prostate cancer [31]. Telomerase from exfoliated transitional cell carcinoma cells has been used as a urinary marker in bladder cancer [32].

## The Mitochondrial Chromosome

The mitochondrial chromosome is a circular DNA (mtDNA) molecule (16.5 kb), and every base pair makes up part of the coding sequence. These genes principally encode proteins or RNA molecules involved in mitochondrial function. These proteins are components of the mitochondrial respiratory chain involved in oxidative phosphorylation (OXPHOS) producing ATP. The mtDNA mutations are generated during OXPHOS through pathways involving *reactive oxygen species* (ROS), and unlike the nucleus these mutations may accumulate in mitochondria because they lack protective

histones [33]. There are many reasons to believe that the biology of mitochondria could drive tumorigenesis: (1) mitochondria generate ROS, which in high concentrations are highly mitogenic to the nuclear and mitochondrial genomes; (2) mitochondria have a key role in effecting apoptosis; and (3) mitochondria accumulate in high density in some malignant tumors (renal cell carcinoma) as tumor cells have lesser dependence on mitochondria for their oxidative phosphorylation [34]. The mutations of mtDNA have been demonstrated in renal cell, prostate, and bladder cancers.

## Genes

A gene is a portion of DNA that contains the codes for a polypeptide sequence. Three adjacent nucleotides (a codon) code for a particular amino acid, such as AGA for arginine, and TTC for phenylalanine. There are only 20 common amino acids, but 64 possible codon combinations that make up the genetic code. This means that more than one triplet encodes for some amino acids; other codons are used as signals for initiating or terminating polypeptide-chain synthesis. Genes consist of lengths of DNA that contain sufficient nucleotide triplets to code for the appropriate number of amino acids in the polypeptide chains of a particular protein. In bacteria the coding sequences are continuous, but in higher organisms these coding sequences (exons) are interrupted by intervening sequences that are noncoding (introns) at various positions. Some genes code for RNA molecules, which will not be further translated into proteins. These code for functional ribosomal RNA (rRNA) and transfer RNA (tRNA), which play vital roles in polypeptide synthesis.

## Transcription and Translation

The conversion of genetic information to polypeptides and proteins relies on the transcription of sequences of bases in DNA to mRNA molecules; mRNAs are found mainly in the nucleolus and the cytoplasm, and are polymers of nucleotides containing a ribose phosphate unit attached to a base (Fig. 1.3). RNA is a single-stranded molecule but it can hybridize with a complementary sequence of single-stranded DNA (ssDNA). Genetic information is carried from the nucleus to the cytoplasm by mRNA, which in turn acts as a template for protein synthesis. Each base in the mRNA molecule is lined up opposite to the corresponding base in the DNA: C to G, G to C, U to A, and A to T. A gene is always read in the 5′-3′orientation and at 5′ promoter sites, which specifically bind the enzyme.

RNA polymerase and so indicate where transcription is to commence. Eukaryotic genes have two AT-rich promoter sites. The first, the TATA box, is located about 25 bp upstream of (or before) the transcription start site, while the second, the CAAT box, is 75 bp upstream of the start site.

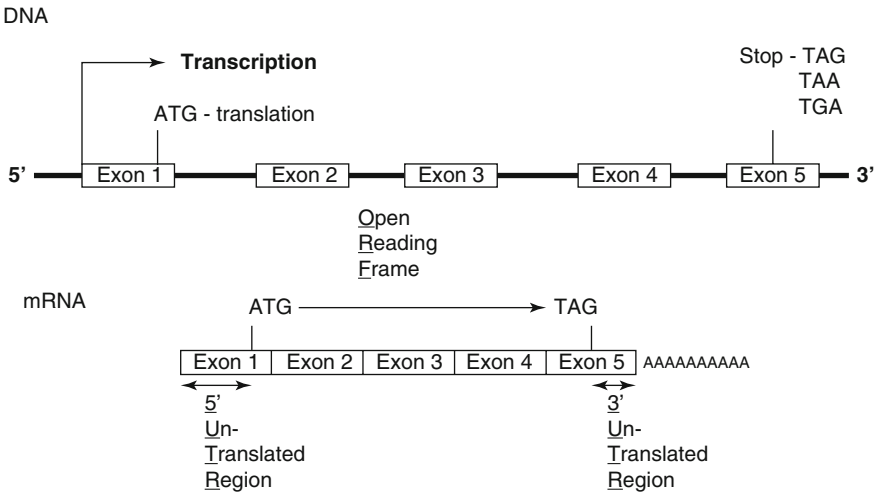


**Fig. 1.3** The intronic and exonic structure of a gene coded on chromosomal DNA and the structural correspondence as a processed mRNA

The initial or primary mRNA is a complete copy of one strand of DNA and therefore contains both introns and exons. While still in the nucleus, the mRNA undergoes posttranscriptional modification whereby the 5′and 3′ ends are protected by the addition of an inverted guanidine nucleotide (CAP) and a chain of adenine nucleotides (Poly A), respectively. In higher organisms, the primary transcript mRNA is further processed inside the nucleus, whereby the introns are spliced out. Splicing is achieved by small nuclear RNA in association with specific proteins. Furthermore, alternative splicing is possible whereby an entire exon can be omitted. Thus more than one protein can be coded from the same gene. The processed mRNA then migrates out of the nucleus into the cytoplasm. Polysomes (groups of ribosomes) become attached to the mRNA. Translation begins when the triplet AUG (methionine) is encountered. All proteins start with methionine, but this is often lost as the leading sequence of amino acids of the native peptides is removed during protein folding and posttranslational modification into a mature protein. Similarly the Poly A tail is not translated and is preceded by a stop codon, UAA, UAG, or UGA. The mRNA align on the endoplasmic reticulum and the ribosomal subunit synthesis the polypeptide chain in to the lumen of the ER.

## The Control of Gene Expression

Gene expression can be controlled at many points in the steps between the translation of DNA to proteins (Fig. 1.4). Proteins and RNA molecules are in a constant state of turnover; as soon as they are produced, processes for their destruction are at work. For many genes transcriptional control is the most important point of

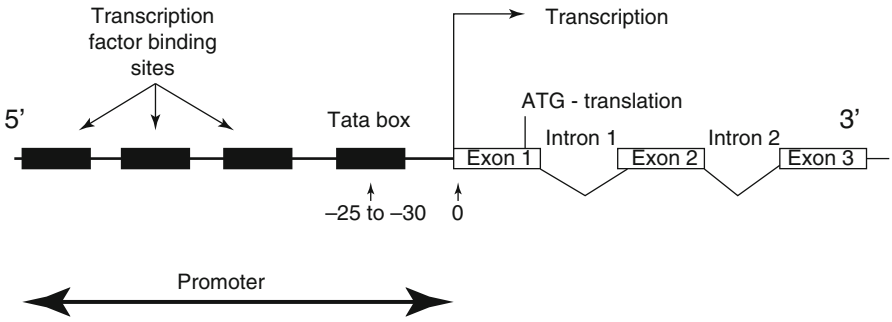


**Fig. 1.4** Diagrammatic representation of the location of DNA binding sequence, which constitute the promoter recognition and assembly site in relationship to the site of gene transcription

regulation. Deleterious, even oncogenic, changes to a cell's biology may arise through no fault in the expression of a particular gene. Apparent overexpression may be due to non-breakdown of mRNA or protein product.

## *Transcriptional Control*

Gene transcription (DNA to mRNA) is not a spontaneous event and is possible only as a result of the interaction of a number of DNA-binding proteins with genomic DNA. Regulation of a gene's expression must first start with the opening up of the double helix of DNA in the correct region of the chromosome. To do this, a class of protein molecules that recognize the outside of the DNA helix have evolved.

These DNA-binding proteins preferentially interact with the major groove of the DNA double helix. The base-pair composition of the DNA sequence can change the geometry of a DNA helix to facilitate the fi t of a DNA-binding protein with its target region: CG-rich areas form the Z-structure DNA helix; sequences such as AAAANNN cause a slight bend, and if this is repeated every 10 nucleotides it produces pronounced curves. DNA-binding proteins that recognize these distorted helices result either in the opening up of the helix so that the gene may be transcribed, or in the prevention of the helix being opened.

## *Structural Classes of DNA-Binding Proteins*

The regulation of gene expression is controlled by DNA binding proteins. There are four basic classes of DNA-binding protein, classified according to their structural motifs: helix-turn-helix, zinc finger, helix-loop-helix, and leucine zipper.

## *Transcription Factors*

The *promoter* is a modular arrangement of different elements that act as a binding site for RNA polymerase II and the initiation of transcription. The initiation of transcription involves a large complex of multimeric proteins [RNA polymerase (I or II)] plus the general transcription factors (GTFs). The GTFs can activate transcription of any gene that has a GTF recognition sequence such as the TATA box. The TATA box is a promoter element that is always located 25 to 30 base pairs from the start of transcription and serves to anchor RNA polymerase II.

*Operators* are proteins that bind to DNA sequences in the spatial areas where the large complex of proteins of the GTFs such RNA polymerase II complex assemble. Their mere presence stoichiometrically inhibits or enhances promoter protein assembly.

*Enhancers* are elements that can be at the 5′or the 3′ end of genes and can vary in distance from the coding sequence itself. Enhancers are not obligatory for the initiation of transcription but alter its efficiency in such a way as to lead to the upregulation of genes. Looping of the DNA helix allows distantly located enhancers to interact with the promoter site.

*Transcription factors* are proteins that bind to sequence specific regions of DNA at the 5′ end of genes called response elements to regulate gene expression. These elements can form a part of the promoters or enhancers. They can be divided into basal transcription factors, which are involved in the constitutive activation of so-called housekeeping genes, and inducible transcription factors, which are involved in the temporal and spatial expression of genes that underlie tissue phenotype and developmental regulation.

*Insulators* are DNA sequence elements which are the answer for the problem of enhancers inappropriately binding to and activating the promoter of some other gene in the same region of the chromosome as the target gene. Insulator binding regions of DNA (as few as 42 base pairs may do the trick) located between the enhancer(s) and promoter or silencer(s) and promoter of adjacent genes or clusters of adjacent genes. The function of the insulator protein is to prevent a gene from being influenced by the activation (or repression) of its neighbors.

The enhancer for the promoter of the gene for the delta chain of the gamma/delta T-cell receptor for antigen (TCR) is located close to the promoter for the alpha chain of the alpha/beta TCR (on chromosome 14 in humans). A T cell must choose between one or the other. There is an insulator between the alpha gene promoter and the delta gene promoter that ensures that activation of one does not spread over to the other.

All insulators discovered so far in vertebrates work only when bound by a protein designated CTCF (“CCCTC binding factor”; named for a nucleotide sequence found in all insulators). CTCF has 11 zinc fingers.

## *Genetic Disorders in Genitourinary Cancer*

Normal cell growth and survival require genomic stability. One of the hallmarks of all neoplasms is genetic instability [35]. Gross chromosomal rearrangements (GCRs) such

as translocations, deletions of a chromosome arm, interstitial deletions, inversions, and gene amplification have been consistently reported in different cancers [36]. Genomic instability in cells leads to the activation of proto-oncogenes or inactivation of tumor suppressor genes leading to transformation and development of cancer phenotypes (see below). Knowledge of the mechanism by which genome stability is maintained is crucial for the development of therapeutic applications in cancer management

## Definitions of Chromosomal Disorders

*Aneuploidy* refers to the state of an abnormal number of chromosomes (differing from the normal diploid number). Abnormalities may occur in either the number or the structure of the chromosomes.

### *Abnormal Chromosome Numbers*

#### Nondisjunction

If a chromosome or chromatids fail to separate either in meiosis or mitosis, one daughter cell would receive two copies of that chromosome and other cells receive no copies of the chromosome. Nondisjunction during meiosis can lead to an ovum or sperm having either [1] an extra chromosome (trisomic), resulting in three instead of two copies of the chromosome [e.g., trisomy 7 bladder and ureteral tumors [36, 37]]; or [2] no chromosome (monosomic), resulting in one instead of two copies of the chromosome. Full autosomal monosomies are extremely rare and deleterious. Sex chromosome trisomies (e.g., Klinefelter's syndrome, XXY) are relatively common. The sex-chromosome monosomy in which the individual has an X chromosome only and no second X or Y chromosome is known as Turner's syndrome. Occasionally, nondysjunction can occur during mitosis shortly after two gametes have fused. It will then result in the formation of two cell lines, each with a different chromosome complement. This occurs more often with the sex chromosome, and results in a *mosaic* individual. Rarely the entire chromosome set may be present in more than two copies, so the individual may be triploid rather than diploid and have a chromosome number of 69. Triploidy and tetraploidy (four sets) are nonviable.

### *Abnormal Chromosome Structures*

Abnormal constitution of chromosomes can lead to the disruption to the DNA and gene sequences, giving rise to genetic diseases. *Deletions* of a portion of a chromosome may give rise to a disease syndrome if two copies of the genes in the deleted region are necessary, and the individual will not be normal with the one copy