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Salivary Glands

Development, Adaptations and Disease

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Contents

Preface

Salivary glands are vital parts of the oral cavity, defects in which can cause major disruptions to our lifestyles. This book brings together basic science researchers and clinicians to produce a review of the latest developments in salivary gland research. The book is divided into four broad areas, which chart our current understanding of salivary gland morphology, development, regeneration and disorders.

In the chapters by Miletich and Tucker, the salivary glands are introduced and unusual adaptations are investigated. These chapters aim to introduce the complex nature of salivary glands and highlight their huge variation in size, shape and function across the animal kingdom. In the following three chapters by Pirraglia and Myat, Sequeira et al., and Wells and Patel, the development of the salivary gland is addressed from specification to branching morphogenesis and lumen formation. Here data is brought together from two diverse animal models, *Drosophila* and mouse, to provide an understanding of the basic steps of salivary gland development. These chapters introduce the genes and complex signalling pathways that direct development as the gland grows from initiation to differentiation. The book then turns to the prospect of regenerating salivary glands in adult tissue in the contributions by Lombaert and Hoffman and Carpenter and Cotroneo. Lombaert and Hoffman focus on the location of stem cells in embryonic glands, providing exciting new data on the role of growth factors in determining cell fate. The article by Carpenter and Cotroneo moves to a rat model of gland regeneration to study the molecular triggers and morphological changes involved in regeneration. These chapters highlight new areas of research that may shape the way salivary gland disorders are treated in the future. Finally, Thomas et al. look at the disorders of salivary glands from a clinical perspective, detailing how salivary gland disorders come about, and what techniques are being developed to help treat patients. It is hoped that together these chapters will provide an intriguing overview of salivary gland development, disorders and treatment, which will be of interest to developmental biologists, anatomists and clinicians.

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Introduction to Salivary Glands: Structure, Function and Embryonic Development

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Abstract

Salivary glands are a group of organs secreting a watery substance that is of utmost importance for several physiological functions ranging from the protection of teeth and surrounding soft tissues to the lubrication of the oral cavity, which is crucial for speech and perception of food taste. Salivary glands are complex networks of hollow tubes and secretory units that are found in specific locations of the mouth and which, although architecturally similar, exhibit individual specificities according to their location. This chapter focuses on the embryonic development of vertebrate salivary glands, which has been classically studied in the mouse model system since the 1950s. We describe here where, when and how major salivary glands develop in the lower jaw of the mouse embryo. Key mechanisms involved in this process are discussed, including reciprocal tissue interactions between epithelial and mesenchymal cells, epithelial branching morphogenesis and coordinated cell death and cell proliferation.

Salivary Glands as Multifunctional Organs

Terrestrial animals possess salivary glands, which are exocrine glands producing saliva, a watery substance that is excreted in the mouth. Salivary glands are either absent or very rudimentary in animals living in water [1]. For example, these glands are absent in aquatic animals such as fish, whose oral cavity fills with considerable amounts of liquid upon opening of the jaws. However, one pair of salivary glands exists in lampreys [2], parasitic jawless vertebrates that feed by boring into the flesh of various species of bony fishes to suck their blood. Lampreys have a sucking mouth that does not let water get into the oral cavity.

Saliva performs a wide array of physiologic and protective functions, some related to its fluid properties and others to its specific content of a variety of molecules [3]. Being a liquid, saliva primarily lubricates the oral mucosa lining the inside of the mouth and moistens food bites. As such, it cleans the oral cavity by flushing away food debris and bacteria, helps with mastication and swallowing of the food bolus, facilitates speech,

and, last but not least, allows taste perception by solubilizing food chemicals, an essential step for the stimulation of receptor cells of the taste buds. Although mostly composed of water, saliva also contains electrolytes and an incredible variety of proteins and peptides fulfilling numerous functions. Specific components actively secreted in the saliva are key to maintaining the good health of the oral cavity. Saliva protects the teeth through the presence of negatively charged proteins that bind to hydroxyapatite minerals on the enamel surface of tooth crowns. Through its high bicarbonate concentration, it buffers acids produced by the dental plaque bacteria when carbohydrates are fermentated, thereby preventing tooth decay. Saliva also provides protection to the oral mucosa lining the inside of the mouth, via an array of antimicrobial agents including secretory immunoglobulin A, lysozyme and lactoperoxidase. In addition to its defensive role, saliva also initiates the digestion of starches and a small fraction of triglyceride lipids through α-amylase and lipase enzymes respectively. However, these two enzymes are considered to be of minor significance in healthy individuals since they are rapidly inactivated by gastric acidity. Apart from components having an obvious function, saliva also exhibits a tremendous variety of biologically active proteins in the form of growth factors and other small peptides, whose function remains largely unknown [4, 5]. The paramount importance of saliva is illustrated by the plethora of problems experienced by individuals with non-functional salivary glands, which produce decreased volumes of saliva leading to dry mouth (xerostomia). These include oral infections, dental caries, mastication and swallowing problems, loss of taste, pain or discomfort on eating or talking that have detrimental effects on the quality of life. Salivary gland disorders and their treatments are described by Thomas et al. [pp. 129–146] in this book.

Although salivary glands are thought of as organs whose function is mostly related to the maintenance of the oral cavity and the digestion of food substances, their secretions, in various animal species, have evolved to perform functions other than those previously cited. In particular, some birds, insect larvae, reptiles and small mammals have developed specialized uses for saliva that are described in detail by Tucker [pp. 21–31].

Structures and Cell Types of Adult Salivary Glands

Mature adult salivary glands consist of a parenchyma, the glandular secretory tissue, and a stroma, which is the supporting connective tissue. The parenchyma is composed of secretory units called secretory 'endpieces', which are connected to the oral cavity through a network of ducts. Salivary endpieces consist of secretory cells organized in round clusters, termed acini, or tubular clusters, called tubules. Secretory endpieces belong to three different types, mucous, serous, or seromucous, depending on the composition of their secretions. Mucous secretions are rich in complex carbohydrates, found as chains attached to mucin proteins, which represent most of the mass of these glycoproteins. Serous secretions are rich in proteins, with a notable absence of mucin

proteins, while seromucous secretions are a combination of both serous and mucous secretions. Consistency of acinar cell secretions varies with their composition; serous secretions are watery, whereas mucous secretions are viscous and adhere to oral structures, accounting for most of the lubrication effect of saliva. Interestingly, the different cell types found in secretory endpieces can be identified by their characteristic tissue organization and specific cell structure that are easily distinguished by histological stainings. Serous cells are pyramidal and as such form spheroidal clusters or acini. They display a large round central nucleus and small discrete apical granules that are darkly stained with haematoxylin. Mucous cells are columnar and organized in elongated tubular clusters. Resting mucous cells contain numerous large and close-packed granules that occupy their apical two thirds, pushing and flattening the nucleus at the base of the cell; their apical cytoplasm appears poorly stained with haematoxylin-eosin stain. In contrast, mucous cells are very distinctively stained blue with alcian blue staining. Secretory endpieces empty their secretions consecutively in intercalated ducts, striated ducts, excretory ducts and finally the main excretory duct that opens in the oral cavity. Intercalated ducts are lined by small cuboidal cells, striated ducts by columnar cells arranged in a simple or pseudostratified organization and excretory ducts are lined by a stratified columnar epithelium. The duct system is impermeable to water. However, it actively modifies the ionic content of the saliva in specific ductal areas, such as the striated ducts. Striated ducts are so called because the cells lining these ducts display basal striations due to cytoplasmic infoldings in which are located vertically aligned mitochondria that provide the energy necessary for active ion exchange at the apical membrane of these duct cells. Saliva production is a two-stage process. An isotonic plasma-like secretion is initially produced by secretory endpieces. When passing down the striated ducts, this primary fluid is rendered hypotonic as excess sodium ions are reabsorbed and potassium and bicarbonate ions are added. Although the cells located in secretory endpieces are the main producers of salivary proteins and glycoproteins, duct cells also secrete proteins. For example, striated ducts secrete immunoglobulin A and lysozyme. Many species of rodents, including mice and rats, exhibit an additional type of duct located between the intercalated and striated ducts and known as granular ducts or granular convoluted ducts [6]. The granules present in these cells are strongly stained by haematoxylin and basic dyes such as toluidine blue; they contain various growth factors and non-specific proteases. In humans, in which granular ducts are not present, these proteins are produced and secreted by striated ducts. Associated with secretory endpieces and proximal ducts (including intercalated ducts and, in rodents, granular ducts) are found myoepithelial cells. They are contractile cells, resembling smooth muscle cells, which are located between the basal lamina and the cytoplasmic membrane of either the cells of the secretory endpieces or the cells lining the salivary ducts. They interact with endpiece or ductal cells via desmosomes. Their shape depends on their location; at the level of endpieces, they appear as stellate, dendritic cells forming a basket around each endpiece, whereas in the wall of the ducts they are fusiform, with few cytoplasmic processes, and run parallel to the

ducts. Contraction of myoepithelial cells helps secretory cells to discharge the content of their secretion granules, reduces the luminal volume of endpieces and ducts, resulting in increased salivary flow, and also aids to support and stabilize the glandular tissue against strong secretory pressures applied during periods of high saliva production. Other components of adult salivary glands include nerves, blood vessels and the fibrous connective tissue capsule covering each gland. The capsule projects septa into the salivary gland, dividing the parenchyma into lobules. Intercalated and striated ducts are located inside lobules, whereas excretory ducts are found in between lobules, within the connective tissue septa.

Different Salivary Glands, in Different Locations

Salivary glands are classically divided into major and minor salivary glands. Major salivary glands are large glands located at a distance from the oral mucosa, which empty their secretions in the oral cavity through long extraglandular ducts, whereas minor salivary glands are small secretory units contained inside the oral mucosa that open either directly in the mouth or indirectly through many short ducts. Unlike major salivary glands, minor salivary glands are not encapsulated by connective tissue, they are only surrounded by it. In humans, major salivary glands comprise three pairs of glands, namely the parotid, submandibular and sublingual glands, from which is derived 90% of the total saliva (fig. 1). In humans, parotid glands, which are the largest salivary glands, are located at the back of the mouth at the front and below of the ears. Saliva is excreted in the oral cavity through a 5 cm long duct (Stensen's duct) that opens opposite the second upper molar crown. Submandibular glands are located in the floor of the mouth in between muscle layers and touch the mandibular bone. They discharge their secretions each through a duct (Wharton's duct) opening in the floor of the mouth on the sublingual papilla located at the base of the lingual frenum posterior to the lower incisors. Submandibular secretions account for 70% of the saliva produced by major salivary glands. Sublingual glands are the smallest of the major salivary glands; they lie in the floor of the mouth just beneath the oral mucosa closer to the midline than the submandibular glands and empty through 8–20 excretory ducts that open under the tongue on the sublingual fold. Sometimes anterior acini of the sublingual gland drain into a main excretory duct (Bartholin's duct) that terminates with or near the orifice of the submandibular duct. In addition to these three pairs of large salivary glands, 600–1,000 minor salivary glands are scattered in the oral mucosa of the hard palate, tongue, floor of the mouth, inner side of the cheeks and lips and oropharynx. Minor salivary glands are not found within the gingiva, anterior part of the hard palate and dorsal surface of the anterior part of the tongue. These glands contribute only a small portion (10%) of total salivary secretions. Although closely related, major salivary glands exhibit differences in their architecture and secretions. The parotid glands are flat glands spread over a large area

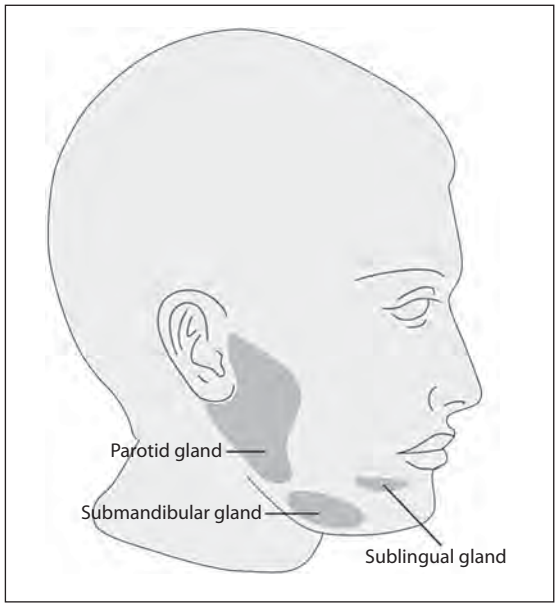

Fig. 1. Location of major salivary glands in humans.

with clearly visible epithelial branches, since these are separated from each other by large areas of mesenchymal tissue, whereas the submandibular and sublingual glands are globular, compact glands, with so densely packed epithelial branches that distal secretory endpieces touch each other, giving a 'bunch of grapes' appearance to these glands. Interestingly, each type of salivary gland produces a secretion with a distinctive composition suggesting that these organs function in a coordinated manner to generate a fluid adapted to environmental conditions. In humans, the parotid glands are exclusively serous, the sublingual glands mainly mucous in nature, and the submandibular glands seromucous mixed glands with a predominance of serous acini. In mice, the parotid, submandibular and sublingual glands are of the same type as in humans. In humans and mice, minor salivary glands are predominantly mucous in character, with exception of von Ebner's glands, a small group of salivary glands located on the posterior region of the tongue, beneath the circumvallate papillae, which are exclusively serous.

The largest volumes of saliva are by far produced by terrestrial mammals, which also display the largest salivary glands. The same major salivary gland configuration as described in humans can be observed in other mammals. An additional salivary gland, the zygomatic gland, which is located under the eye, has been described in carnivores including the domestic cat and dog, lagomorphs and some ruminants [7]. Rather than a proper salivary gland, it appears to be several dorsal buccal accessory glands grouped in a compact mass. This group of glands drains in the oral cavity through five major excretory ducts opening at the level of the last upper molar.

Variations in size, structure and secretions of salivary glands can be related to sex. In mice there is a pronounced sexual dimorphism of the submandibular gland. In males, submandibular glands are more voluminous, granular convoluted ducts containing secretory cells are more prominent and in mature glands, production of some enzymes such as aminotransferase can be as much as 10 times less than in females [8–10]. More recently, a microarray analysis has identified numerous sex-related differences in gene expression in all three major salivary glands of male and female mice [11]. Interestingly, importance and size of the different major salivary glands also appears to be related to the type of diet of mammalian species. For example, parotid glands, which, as serous glands, produce a fluid saliva that is mainly used to moisten food, are very developed in herbivores and less voluminous in carnivores. Horses and other mammals feeding on a diet of dried substances, such as hay, have indeed very large parotid glands and small submandibular glands. In contrast, other mammals such as anteaters, which are insectivores that use a long filiform tongue to catch ants and termites, exhibit unusually large submandibular glands and very small parotid glands. These animals collect insects by gluing them onto their tongue, which is coated with a very sticky saliva. This viscous saliva is secreted by the submandibular glands; it covers oral cavity structures including the tongue, and is therefore crucial for feeding. The voluminous submandibular glands extend along the long necks of these animals and adjacent chest area, where they even penetrate in the space between mammary glands, instead of being located in the floor of the mouth, as in humans. The type of diet also appears to influence the type of saliva secreted by the mixed submandibular glands; it is predominantly mucous in carnivores and mostly serous in herbivores and rodents.

Control of Saliva Production

Saliva production is continuous although the flow rate varies greatly during a day, following a circadian rhythm with an afternoon peak and a secretion near to zero during sleep [12]. Regulation of the volume and quality of saliva is achieved through the regulation of the activity of salivary effector cells, comprising myoepithelial cells, endpiece secretory cells and duct secretory cells, as well as through the regulation of the diameter of salivary gland blood vessels. Variations in salivary secretions are under neural control and more specifically under control of the autonomic nervous system (ANS) or vegetative nervous system, which is mainly concerned with the regulation of visceral functions and interactions with the internal environment. The ANS has two divisions, the parasympathetic autonomous system (PANS), aiming at homeostasis and conservation of body functions and the sympathetic autonomous system (SANS), providing answers to stress. PANS and SANS are both involved in the control of saliva production. Importantly they act synergistically, since stimulation of both PANS and SANS leads to an increase in saliva production. However,

stimulation of these two nervous systems differs in their effects on the fluid volume and protein content of the saliva secreted. Parasympathetic stimulation, which is most active during the day whilst eating, leads to the production of large volumes of saliva with low protein content. These watery secretions are predominantly produced by serous endpieces of the parotid and submandibular glands under chemical stimulation of the acetylcholine neuromediator. Stimulation of sympathetic nerves, leading to the release of noradrenaline, results in the secretion of low volumes of a protein-rich saliva. Depending on the noradrenergic receptor subtype, viscous secretion (α-receptor type) or amylase secretion (β-receptor type) is released. This thicker, mucous-rich saliva is mainly produced by the sublingual gland and partly by the submandibular gland. Such a secretion happens in certain situations when fear, stress or anger are aroused, or during hard physical exercise. PANS and SANS also affect salivary gland secretions indirectly by innervating the blood vessels that supply the glands. Water, the major component of salivary secretions, is obtained from the lymph filling the lymph spaces adjacent to the secretory endpieces. Since lymph exudes from blood vessels, any effect on the permeability of local blood vessels has repercussions on saliva production. As a matter of fact, abundant saliva secretion is usually associated with an abundant blood supply. PANS and SANS exert an antagonistic action on blood vessel diameter, and therefore permeability; stimulation of PANS results in vasodilatation while stimulation of SANS causes vasoconstriction. Accordingly, the volume of saliva secreted is large following PANS stimulation and low after SANS stimulation. Parasympathetic innervation of the salivary glands is achieved through cranial nerves originating in the brainstem, which is the lower part of the brain contiguous with the spinal cord. Parasympathetic innervation of the parotid gland is achieved via the glossopharyngeal nerve (cranial nerve IX), following the tympanic branch of this nerve, which connects in the otic ganglion with second-order neurons that synapse with parotid gland cells. The sublingual and submandibular glands receive their parasympathetic input from the facial nerve (cranial nerve VII) through the chorda tympani branch of this nerve, which connects in the submandibular ganglion with second-order neurons that innervate these two salivary glands [13]. In addition to the control exerted by these two kinds of autonomic nerves, unconditioned reflex pathways triggered by sensory stimuli also stimulate saliva production. Olfactory and gustatory stimuli, mastication, are peripheral inputs capable of stimulating salivation.

The Mouse Submandibular Gland as a Model for Vertebrate Salivary Gland Development

The mouse submandibular gland is classically used as a model system to study vertebrate salivary gland development. It is a mixed gland, and as such, terminal differentiation of secretory cells of endpieces is related to cytodifferentiation of both

the parotid and sublingual glands. In terms of early embryonic development, the submandibular, sublingual and parotid glands appear to follow similar morphogenetic events although these three glands start developing at different time-points. In mice, the submandibular gland (SMG) develops first, followed one day later by the sublingual gland (SLG). The parotid gland (PG) is last to develop and the minor salivary glands initiate their development even later. In humans, the SMG starts developing around week 6 of fetal life, the PG between the 6th and 7th week, followed by the SLG around the 8th week [14]. In mice, contrary to humans, the SMG and SLG develop in close association, ending up in the same anatomical location in adults where they share the same capsule of connective tissue. Despite the closeness of their developmental relationship, these two glands display in their mature state the same functional differences as those observed in humans; the adult mouse submandibular gland being predominantly serous and the adult mouse sublingual gland predominantly mucous.

Transgenic mice are very useful tools to understand the role of signalling pathways and molecules involved in the development of mammalian salivary glands (see table 1 for a list of salivary gland defects that have been observed in mouse mutants). Apart from mouse mutants, one important advantage of the mouse model is that the entire salivary gland rudiments of the SMG and SLG can be cultured in vitro in serum-free medium. The first organotypic cultures of mouse salivary glands were performed in the 1950s [15] and have since proved to be a very good model to study salivary gland development. In vitro development of these two glands recapitulates normal development in live embryos with few minor differences. Similar to other organ cultures, salivary gland development is slightly slower in vitro; when explants are cultured for more than 48 h, secretory endpieces appear to be grouped near the periphery of the gland whereas they are evenly scattered in vivo and finally, ducts undergo an abnormal dilatation in in vitro cultures that remains unexplained so far and hinders the study of regulation of lumen size in this culture system [15]. Despite these differences, advantages of this culture system are numerous. Importantly, salivary gland development can be observed. Epithelial branching can be readily followed in living explants and clear images of epithelial and mesenchymal tissues can be obtained in fixed preparations of these explants. Recent advances in cell-marking techniques have provided tools for live imaging of salivary gland development. Recombination of wild-type salivary mesenchyme with salivary epithelium taken from transgenic mice expressing Green Fluorescent Protein (GFP) [16] allows to follow epithelial morphogenesis and more specifically branching morphogenesis. Movements of individual epithelial cells have also been studied during branching morphogenesis by confocal time-lapse microscopy after labelling epithelial cells by microinjection of a GFP adenovirus construct [17]. Finally, in vitro culture of salivary glands combined with RNAi knockdown, small-molecule inhibitors and antibody-based blocking experiments is providing exciting results helping to dissect the signalling pathways that are involved in the morphogenetic events happening during salivary gland development.

Table 1. Salivary gland defects in mouse mutants

Salivary gland defects	Genotype	Gene product	Reference (first author)
Submandibular/sublingual gland			
Agenesis – initiation, no branching	*FGF10–/–*	Fibroblast growth factor 10	Ohuchi, 2000 [42], Jaskoll, 2005 [43]
	FGFR2-IIIb–/–	Fibroblast growth factor receptor 2 (IIIb)	De Moerlooze, 2000 [44], Revest, 2001 [45], Jaskoll, 2005 [43]
	FGF8$^{C/N}$; AP2α-IRESCre/+	Fibroblast growth factor 8	Jaskoll, 2004 [46]
Agenesis	*p63*	p63 (homolog to p53 tumour suppressor)	Yang, 1999 [47]
	Ptx1	Pitx1 – transcription factor	Szeto, 1999 [48]
Agenesis or hypoplasia	*Tbx1–/–*	Tbx1 – transcription factor of T-box family	Jerome, 2001 [49]
Agenesis, hypoplasia or wild-type correlating with external craniofacial phenotype	*Twsg1–/–*	Twisted gastrulation – secreted protein	Melnick, 2006 [50]
Severe hypoplasia	*Shh–/–*	Sonic hedgehog – signalling molecule	Jaskoll, 2004 [51]
Hypoplasia	*FGF10+/–*	Fibroblast growth factor 10	Entesarian, 2005 Jaskoll, 2005 [43]
	FGFR2-IIIb+/–	Fibroblast growth factor receptor 2 (IIIb)	Jaskoll, 2005 [43]
	FGFR2-IIIc+/–	Fibroblast growth factor receptor 2 (IIIc)	Jaskoll, 2002 [20]
	Itga3–/–; Itga6–/–	Integrin α_3 and Integrin α_6	De Arcangelis, 1999 [53], Rebustini, 2007 [54]
	MMP14	Matrix metalloprotease 14	Oblander, 2005 [55]
	Scrb1–/–	Scribbled 1 – cytoplasmic protein	Murdoch, 2003 [56]
	Six1–/–	Six1 – transcription factor	Laclef, 2003, McCoy 2009 [58]
Hypoplasia + disorganized epithelium + reduced lumen formation	*Lama5–/–*	Laminin α_5	Rebustini, 2007 [54]
Hypoplasia + absence of lumen	*FGF8$^{H/N}$ (hypomorph)*	Fibroblast growth factor 8	Jaskoll, 2004 [46]

Table 1. Continued

Salivary gland defects	Genotype	Gene product	Reference (first author)
Hypoplasia + decrease in granular convoluted ducts and acini	*Eda^{Ta}/Y*	Ectodysplasin-A	Blecher, 1983 [59], Jaskoll, 2003 [60]
Hypoplasia + disorganized mesenchyme	*Pax6*	Transcription factor	Jaskoll, 1999 [20]
Disorganized mesenchyme + reduced branching and lumen formation	*Bmp7–/–*	Bone morphogenetic protein 7	Jaskoll, 2002 [20]
Impaired growth, branching and maturation of the epithelium	*EGFR–/–*	Epidermal growth factor receptor	Jaskoll, 1999 [61], Haara, 2009 [62]
Severe dysplasia – absence of duct and acini	*Edar^{dl}*	Ectodysplasin-A receptor	Jaskoll, 2003 [60]
Severe dysplasia – absence of acini, lumen occlusion and disorganized epithelium	*MMTV-Cre; p120fl/fl*	p120 catenin	Davies, 2006 [63]
Disorganized basement membrane + abnormal cytodifferentiation	*Itga3b1–/–*	Integrin $\alpha_3\beta_1$	Menko, [64]
Shortened main ducts	*RARα$_1$; RARγ*	Retinoic acid receptors	Lohnes, 1994 [65]
Defect in duct maturation	*Cp2l1–/–*	Grainyhead-related transcription factor	Yamaguchi, 2006 [66]
Parotid gland			
Hypoplasia	*FGF10+/–*	Fibroblast growth factor 10	Entesarian, 2005 [52]
Defects specific to sublingual gland			
Agenesis	*Lama5–/–*	Laminin α_5	Rebustini, 2007 [54]
Dysplasia – cystic epithelial formations in the parenchyma	*RARα$_1$; RARγ*	Retinoic acid receptors	Lohnes, 1994 [65]
Retarded maturation of mucous-secreting acinar cells + disruption of cellular architecture	*Nkx2-3–/–*	Transcription factor	Biben, 2002 [67]
Shortened main duct	*RARβ$_2$; RARγ*	Retinoic acid receptors	Lohnes, 1994 [65]
Minor salivary glands			
Smaller glands with narrow ducts	*Nkx3.1–/–*	Transcription factor	Schneider, 2000 [68]

In addition to the culture of whole salivary gland rudiments, it is also possible, in early developing salivary glands, to cleanly separate the epithelium from the mesenchyme by enzymatic treatment and culture the epithelium alone either in growth factor-reduced matrigel [18] or in a laminin matrix [19]. In vitro culture of isolated

salivary epithelium allows the researcher to study the effects of purified molecules on epithelial cell behaviour, in the absence of effects on the mesenchyme and from signals from the mesenchyme.

Early Embryonic Development of Salivary Glands: Initial Bud Formation

Although there is little doubt that the parotid gland derives from ectodermal tissue since it arises from the stomodeum, contradictory reports can be found in the literature regarding the origin of the sublingual and submandibular glands as to whether they are ectodermal or endodermal derivatives. In absence of a general endodermal marker, it is difficult to conclude either way. However, the endoderm is clearly capable of supporting salivary gland development given that minor salivary glands developing in the tongue, including von Ebner's glands, are undoubtedly of endodermal origin, as the epithelial layer covering the tongue is derived from the endoderm. Despite this controversy, major salivary glands are widely regarded as ectodermal organs, together with other exocrine glands such as mammary, sweat and sebaceous glands, and organs such as teeth, hair, scales, feathers and nails. All ectodermal organs originate from two adjacent tissues of distinct embryonic origin, the epithelium and the mesenchyme. Development of ectodermal organs proceeds through constant, sequential and reciprocal interactions between these two tissues translated at the molecular level by signalling molecules, which regulate cell proliferation, movements and differentiation. Although ectodermal organs exhibit great diversity in their appearance, molecular mechanisms involved in their development are strikingly similar, so what we learn about the development of salivary glands can help to reveal more general principles and conversely, mechanisms unravelled in other ectodermal organs can aid us to understand salivary gland development.

The mouse SMG develops through a series of interactions between the oral epithelium covering the first branchial arch, and a population of mesenchymal cells derived from the cranial neural crest, a migratory cell population that detaches from the embryonic neural epithelium [20]. The epithelial compartment will eventually give rise to the secretory endpieces of the salivary glands, the extensive network of ducts leading the salivary secretions into the oral cavity, and the myoepithelial cells. The mesenchymal compartment will produce the capsule surrounding the gland. Mouse SMG embryonic development is classically described in stages [21]. The first morphological sign of SMG development is observed around embryonic day 11.5 (E11.5). An epithelial thickening appears in the floor of the mouth, at the back of the first mandibular molar, adjacent to the developing tongue (fig. 2a, 1). This thickening develops at the bottom of the alveolo-lingual sulcus, a gutter-like groove that forms in the floor of the mouth as the result of the upward growth of the tongue rudiment (fig. 2b, top panel). This early stage is known as the prebud stage. SLG development starts one day later at E12.5 by a thickening of the oral epithelium located right next to the SMG on the buccal side (fig.

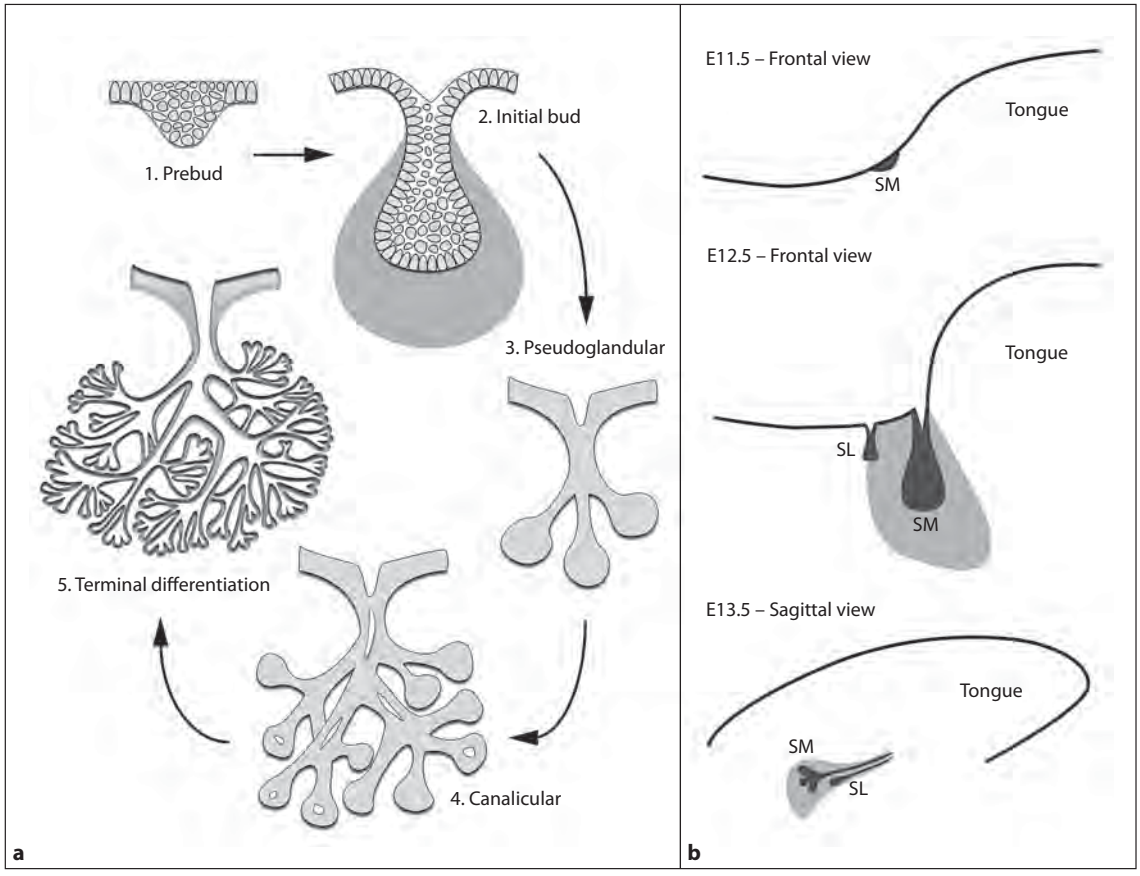

Fig. 2. Embryonic development of the submandibular (SM) and sublingual (SL) glands in the mouse. **a** Developmental stages of the SM gland. **b** Development of the SL gland relative to the SM gland. Condensed mesenchyme is indicated in dark grey around salivary gland epithelial rudiments in **a** and **b**.

2b, central panel). Importantly, both the SMG and SLG do not start developing in the location where their major excretory ducts open in the adult mouth. At E12.5, the SMG epithelial thickening invaginates in the underlying mesenchyme of the first branchial arch. Sustained epithelial proliferation in a downward direction leads to the formation of a thick solid epithelial stalk terminated by a bulge constituting the initial bud stage of SMG development (fig. 2a, 2). Concomitant to this process, mesenchymal cells condense around the SMG primordium. This well-defined mass of connective tissue represents the capsular rudiment of the gland. The initial bud, surrounded by condensed mesenchyme, will form the parenchyma of the SMG, whereas the main excretory duct of this gland is formed by closure, in a rostral direction, of the alveolo-lingual sulcus [22]. The SMG main excretory duct, as well as the one of the SLG, opens in the floor of the mouth at the sublingual caruncle, a mucosal fold located behind the lower incisors.