Pancreatic Development: Relevance to the Salivary Glands

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Summary: Besides the natural wonder that embryogenesis engenders, understanding the molecular and genetic mechanisms that control development has profound relevance to understanding normal physiology and aberrations of physiology in diseased states. Much of the impetus to understand embryonic development is the hope that lost or defective tissue can be restored by reviving quiescent developmental programs. In this regard, an overview of pancreatic development illustrates developmental themes relevant to the formation of mature organs and the potential for restoring defective tissue. This review includes a general overview of some of the fundamental mechanisms that control development, a description of these processes for pancreatic development, and comment on their relevance to salivary gland development.

Key words: transcription factors, morphogens, organogenesis

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INTRODUCTION

The embryonic development of all organs proceeds via a series of well-choreographed steps. This dance is controlled by a progressive, reiterative interplay between two partners (Fig 1): extrinsic signals that influence the fate of neighboring cells and intrinsic effectors that direct patterns of gene expression within a cell (Wolpert et al, 1998). Organogenesis is initiated by extrinsic signals - extracellular factors, generally diffusible growth factors and morphogens or cell-surface molecules that signal through cell-surface receptors. The action of these extracellular signals specifies the fate of a field of responding cells at a unique position in the 3-dimensional space of the embryo. Only cells in the right place to receive the signals and with the right receptors to recognize them become 'competent' to begin a developmental program. Competence is manifested by the induction of a particular pattern of gene expression that restricts the developmental options of these cells by defining whether and how they respond to subsequent extracellular signals. For example, the initial signaling may induce a cell-surface receptor for a different morphogen, or a new component of a signal transduction pathway that changes the way the cell responds to the same morphogen.

Competence establishes a particular pattern of gene expression by the induction of specialized DNA-binding transcription factors – both activators and repressors – that bind and activate specific developmental genes (Fig 1). The unique combination of transcription factors forms an interacting network that directs a particular, restricted pattern of gene expression. The state of the cells of the field is changed, so that subsequent extracellular signals (even chemically identical ones) may now elicit a different cellular response, one that further modifies the transcription factor network and further alters the possible developmental responses to the next signal(s). Receipt of successive extracellular signals transforms the transcriptional network gradually, so that each subsequent cell generation becomes progressively more differentiated and developmental options in response to later signals become more restricted.

This is, in fact, a branching process. From cells in a field with broad developmental potential, progressive restrictions increasingly limit the developmental decisions the cells can make. The distinct differentiated cell types in an organ arise when the options of the branches of the developmental program become limited to single differentiation programs. For example, consider a pool of identical proliferating precursor cells that have the potential to produce cells of four different types (B, D, F, E) that populate a particular mature organ (Fig 2). Sequential inductions enforce progressive binary decisions that restrict the developmental potential of a subset of cells and their progeny at each stage. Some of the

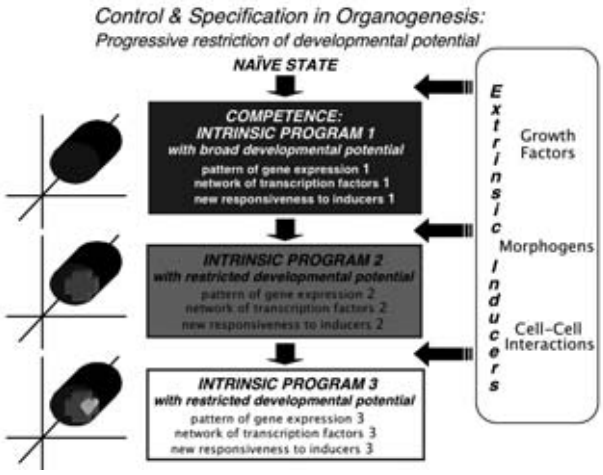


Fig 1 The response to intercellular (extrinsic) inducers is progressively restricted by the changing pattern of gene expression and is enforced by an interacting network of transcription factors. Left: the field of responding cells becomes progressively defined spatially, morphologically and biochemically. Centre: progressive changes in the patterns of gene expression in the cells of the field in response to extracellular signals also alters the nature of subsequent responses to similar signals. Right: A surprisingly small number of families of extrinsic signals induce a vast number of different cell fate decisions.

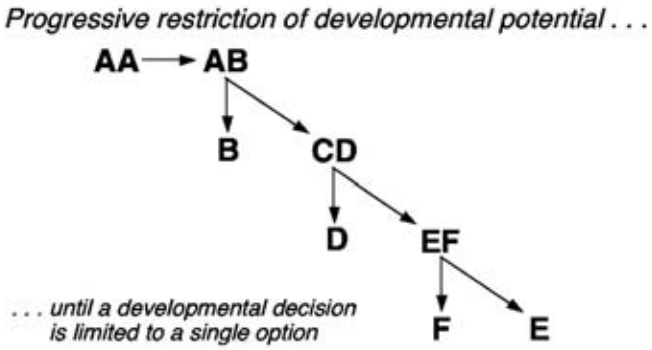


Fig 2 Progressive restriction of developmental potential in a branched pathway leads to multiple, distinct cell types during organogenesis.

cells make different decisions than others (i.e., A vs. B) because they occupy different positions in the field and therefore receive different signals or the same signals at different strengths. Because the response to a developmental inducer depends on the state of the cell – principally the specific network of transcription factors – the same inducers can be used over and over again in different regions of the embryo for the formation of very different organs. Thus the cellular composition of each organ is determined by the particular cellular responses to common inducers; the nature of the response is set by the developmental history of the responding cells. In the mature organ many of the transcription factors that compose the developmental network are often used to maintain the differentiated state (and therefore the function) of the mature organ.

THE INTEGRATION OF EXTRINSIC SIGNALS WITH TRANSCRIPTION FACTORS THAT IMPART LINEAGE AND SPATIAL INFORMATION

Surprisingly, only a few signaling pathways direct the formation of a wide variety of cells, their assembly into tissues, and the construction of organs (Pires-daSilva and Sommer, 2002). The principal pathways are TGFβ/BMP, DSL-Notch, Wingless, Hedgehog, receptor tyrosine kinase, nuclear hormone, and JAK/STAT (Fig 3). All control cell-fate decisions by transducing an extracellular signal into the activation of transcription factors bound to the promoters of target genes. Generally, in the absence of a signal, the bound transcription factors repress the target gene, and the presence of signaling converts the same transcription factor to an activator (Barolo and Posakony, 2002). For example, for the Notch-pathway the binding of a DSL-ligand to the Notch receptor causes the proteolytic cleavage and transfer of the receptor's intracellular domain into the nucleus, where it binds to the transcriptional mediator RBP-Jk. The binding of the Notch intracellular domain displaces co-repressors keeping the promoter inactive and recruits co-activators that activate the promoter. In the simplest case, nuclear hormone receptors are themselves transcription factors that respond to binding hormones.

The wide variation of developmental outcomes is due, in part, to the complexity of signaling pathways,

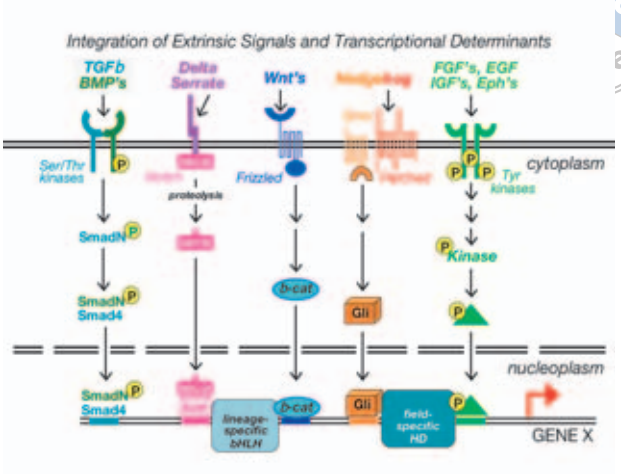
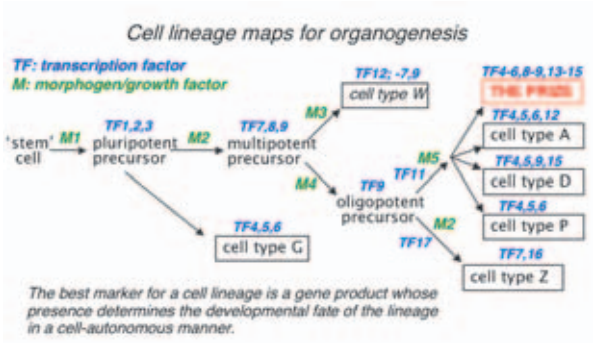


Fig 3 The integration of extrinsic signals with transcriptional determinants is the key to cell-fate decisions during embryogenesis. Highly simplified forms of the major signaling pathways used during organogenesis are depicted. Not shown: steroid hormone receptor and JAK/STAT pathways. At the bottom, a hypothetical promoter of a developmentally regulated gene (X) illustrates the potential binding by a combination of factors mediating the transcriptional effects of extrinsic signaling, lineage-identity, and field- (or position) specificity. The transcriptional effectors of multiple signaling pathways might be required for the proper transcriptional activation of a developmentally regulated gene.

which are neither as simple nor as linear as first elucidated and portrayed in Fig 3. Complexity is derived from branching of individual pathways, with the branches leading to disparate regulatory targets. Some of the branches lead to cross-regulatory interactions with other pathways. The overall state of extrinsic signaling in a cell is the sum of all signals that have receptors in/on the cell, the strengths of each signal, and the status of the cross-regulatory contacts between signaling pathways.

This alone is not sufficient to specify a particular developmental outcome. Complex transcriptional enhancers and promoters may be viewed as biological microprocessors that receive and integrate inputs from multiple sources and respond with an output of their own, the rate of transcription of the target gene. Generally the binding of a transcriptional effector of signaling pathway is not sufficient to activate the promoter of a developmentally regulated gene. Additional promoter-binding transcription factors are required, some of which are common, but the key ones are specific to a cell-lineage or position in the embryo (e.g., Fig 3, bottom). The absence of these factors in other parts of the embryo prevents the activation of their target genes in inappropriate regions. In a complementary fashion, the binding of the lineage and field-specific factors in the absence of appropriate extrinsic signals is not sufficient to activate their target genes. Therefore, it is the consequence of the integrated inputs of the signaling pathways and the lineage/position-specific transcription factors, neither of which is sufficient alone, that determines the precise temporal and stage-specific activation of a developmental gene.

As is evident from the developmental pathway depicted in Fig 2, a cell-lineage map is critical to understanding the developmental program of an organ. Such a map (Fig 4a) identifies shared precursor cells; its branches denote the point of divergence of cellular differentiation programs and their relationships. Because key transcription factors often determine the developmental fates of progenitor cells, transcription factor genes can be powerful markers to trace cell-lineages. For example, the lineage map in Fig 4a suggests that transcription factor 7 (TF7) may convert the pluri-potent precursor pool to a more developmentally restricted multi-potent pool. Its absence may resolve cell type Γ from the rest of the lineages. If it were possible to indelibly mark cells that express TF7 and their descendants (regardless of whether they continue TF7 expression), it would be feasible to identify which cell types arise from TF7-expressing progenitors. For this particular example (Fig 4a), all mature cell types would be marked except type Γ. This lineage tracing strategy is currently feasible using Cre recombinase, a bacteriophage recombination enzyme that can activate non-reversibly the expression of an engineered lacZ marker transgene whose cell-specific expression can be readily monitored by cytochemical staining (Nagy, 2000). Using homologous recombination in embryonic stem cells to insert the Cre coding sequence into the endogenous *TF7* gene, developmental expression *in vivo* of the recombinase is made congruent with that of the *TF7* gene. Cells that express Cre activate the transgene by an irreversible chromosomal recombination event, and all descendents of the TF7-expressing cells are marked by their obligatory expression of lacZ. More recent advances using a conditionally activated Cre make it possible to 'pulse-mark' a lineage in a way that reveals when the expression of the factor in a precursor leads to a particular cell type and when not (Gu et al, 2002). A cell-lineage map is critical to elucidating programs of organogenesis.



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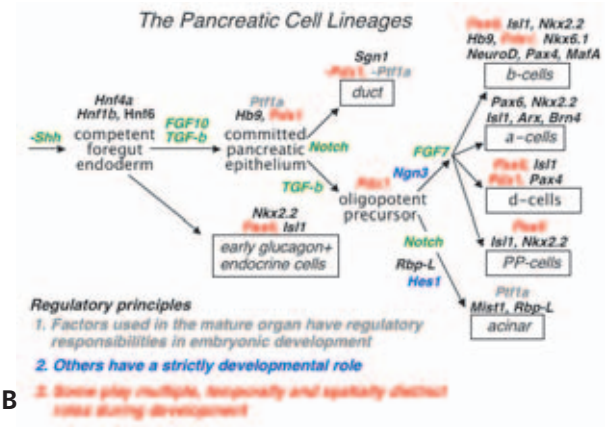

B

Fig 4 Cell lineage paths for organogenesis. **(a)** A detailed, though hypothetical, lineage map for the formation of an organ with seven cell types, showing the potential sites of action of anonymous extrinsic inducing morphogenetic factors (M#, green) and intrinsic transcription factors (TF#, blue). Because transcription factors often dictate the developmental fate of cells, they can be the most relevant markers for tracing the cell-lineage pathways. **(b)** The hypothetical map of panel **A** is actually a good representation of our current understanding of the cell lineages in pancreatic development.

REDIRECTING DEVELOPMENTAL PROGRAMS TO REPLENISH LOST OR DEFECTIVE CELL-TYPES

A current goal in medicine is to produce renewable sources of replenishment tissue *ex vivo* for transplantation therapy. Coupling knowledge of the extracellular signals and the transcription factor genes they control with a cell-lineage map provides the opportunity to intervene in development to favor a particular cell fate (e.g., the PRIZE cell type of Fig 4a). The pathway of Fig 4b is actually an anonymous version of the map of our current understanding of the pancreatic cell lineages (Schwitzgebel, 2001; Kim and MacDonald, 2002; Wilson et al, 2002; Servitja and Ferrer, 2004). For diabetic patients, the PRIZE would be the induced formation of glucose-responsive, insulin-producing cells to replace lost or dysfunctional β-cells. Some of the extracellular signals (green) have been identified for individual developmental decisions and many of the key transcription factors that effect lineage decisions are known. Excellent reviews by Wilson et al (2002) and by Servitja and Ferrer (2004) summarize current attempts to understand the network of transcription factors that define the pancreatic β-cell.

Cre-based lineage-tracing using the *Pdx1* gene showed that ductal, acinar and islet cells all derive from a 'multi-potent precursor' population (Gu et al, 2002). A pulse-marking experiment with conditional-Cre also using the *Pdx1* gene suggests that the multi-potent precursors may resolve first into a ductal lineage plus an oligo-potent pool that resolves into acinar and islet cell types (Fig 4b). Lineage tracing for Ptf1a-expressing cells showed that all exocrine as well as endocrine cells derive from PTF1a-expressing precursors (Kawaguchi et al, 2002). In contrast, lineage tracing of Ngn3-expressing precursor cells showed that only islet cells become marked; therefore, expression of Ngn3 commits cells to the endocrine lineage (Gu et al, 2002).

This view of the pancreatic lineage program and the transcription factors involved indicates three useful principles concerning the role of transcription factors (Fig 4b). First, cell-restricted factors that maintain the differentiated phenotype in adult cells generally play important and early regulatory roles during development as well. For example, PTF1a (*gray*), which activates transcription of all the acinar cell-specific genes encoding the digestive enzymes, is also required for the formation of the embryonic precursors that give rise to the islet and acinar lineages (Kawaguchi et al, 2002). Second,

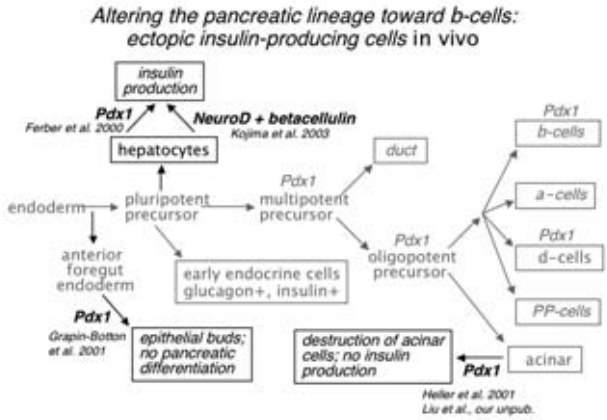

Fig 5 Attempts to change pancreatic as well as nonpancreatic cells to insulin-producing β-like cells in vivo using DNA transduction, viral transfection or transgenesis.

some factors (*blue*) have only a single, precise developmental role; for example precursor cells that express *Ngn3* are fated to form islet cells rather than acinar cells (Gu et al, 2002). Third, certain factors (*red*) may be reused to play critical roles at several major developmental junctures; for example, PDX1 is required early for epithelial cell proliferation (Jonsson et al, 1994; Offield et al, 1996), at mid-development for the appearance of the oligo-potent precursor cell population (Holland et al, 2002), probably later for the β-cell specific differentiation, and in the adult to maintain β-cell function (Ahlgren et al, 1998; Lottmann et al, 2001; Thomas et al, 2001).

Understanding the biology of pancreatic development has direct relevance to transplantation therapies for diabetics with deficient β-cell function. Recent advances in islet transplantation are remarkable (Shapiro et al, 2000), but not practical for the vast majority of the one million Type 1 diabetics in the United States. Current efforts are directed toward deriving renewable sources of differentiated, functional β-cells for transplantation. Several recent studies suggest that β-cell precursors with proliferation potential might be derived from embryonic stem cell lines (Hori et al, 2002; Rajagopal et al, 2003), from ductal tissue discarded from the purification procedures for human or mouse islets (Bonner-Weir et al, 2000; Ramiya et al, 2000), and from isolated human islets (Zulewski et al, 2001; Choi et al, 2004; Gershengorn et al, 2004; Seaberg et al, 2004).

The ability to coerce embryonic stem cells or adult precursor cells from these sources to differentiate along a particular path to produce functional β-cells *ex vivo* requires an understanding of the extrinsic morphogens and intrinsic transcriptional regulators that direct pancreatic development *in vivo*. Several recent experiments have examined the potential and the limitations to influence islet cell development *in vivo* through the forced expression of key transcriptional regulators (Fig 5). Because *Pdx1* has multiple, pleiotropic roles in pancreogenesis, its potential to reprogram pancreatic and nonpancreatic cells toward a β-cell fate has been tested extensively *in vivo*. Transient expression of PDX1 in liver using recombinant adenovirus induced a small subpopulation of hepatocytes to synthesize and secrete processed insulin in response to blood glucose and to reverse the phenotype of streptozotocin-induced diabetes in a mouse model (Ferber et al, 2000). It is conceivable that this approach may evolve into gene therapy. A more persistent induction of insulin expression in liver used a modified recombinant adenovirus vector to express beta-cellulin and NeuroD, a transcription factor required for proper β-cell differentiation *in utero* (Kojima et al, 2003). Expression of PDX1 in embryonic esophageal endoderm induced the formation of nascent pancreas-like epithelial buds, but no activation of pancreatic markers (Grapin-Botton et al, 2001). Whereas hepatic and pancreatic cells have common developmental origins (and can transdifferentiate under certain conditions), esophageal endoderm may be sufficiently different to require additional factors, such as NeuroD. Attempts to convert differentiating acinar cells to insulin-secreting cells *in vivo* by the forced overexpression of a PDX1 transgene were unsuccessful (Heller et al, 2001; Liu et al, our unpublished observations); either PDX1 is not sufficient, or the acinar phenotype is not susceptible to this change.

These and other initial attempts to redirect pancreatic development toward β-cell fate have used a preliminary understanding of the cell-lineage pathways of pancreatic development, of the extrinsic signals (growth factors and morphogens) that drive cell-fate decisions, and of the intrinsic factors (DNA-binding transcriptional regulators) that mediate the changes in gene expression that carry out cell-fate decisions. Advances in the treatment of diabetes may rely on expanding and deepening this understanding of pancreatic development.

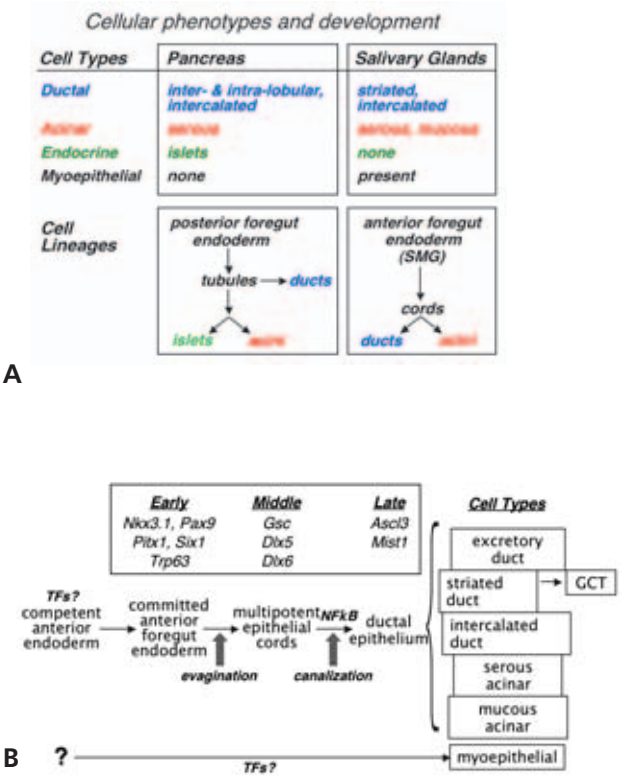

Fig 6 Lack of direct evidence for salivary gland developmental lineages and transcription factors that determine cell fate. **(a)** Superficial relationships between pancreatic and salivary gland cell types and development. **(b)** A potential cell lineage map for submandibular gland development. Boxed transcription factors have been implicated by their expression patterns, but developmental roles are unproven and unknown. The *bracket* reflects the lack of direct lineage evidence for the individual differentiated cell-types: cells of the excretory/interlobular ducts, the intralobular (striated and convoluted) ducts, the intercalated ducts, and the serous and mucous acini. TFs: unknown transcription factors acting at individual stages. Aspects of this developmental scheme were derived from several sources, especially Jaskoll and Melnick (1999), Melnick and Jaskoll (2000), and our unpublished observations.

REGENERATION *IN SITU*

It may be possible to treat some maladies, for example Type 2 diabetes or Sjögren's syndrome, by reactivation of quiescent programs of organogenesis or tissue-neogenesis that mimic or duplicate embryonic development. One example of potential therapeutic intervention is the treatment of ischemic heart disease. Chronic ischemia can cause arrhythmia and lead to cardiac arrest; cumulative effects can weaken the heart muscle and lead to cardiomyopathy. Cardiac ischemia is caused by reduced blood flow to the heart muscle due to defects in the coronary vasculature. Recent insights into cardiac angiogenesis have led to attempts to revitalize coronary circulation via gene therapy (Isner, 2002). Knowing the developmental growth factors and morphogens for cardiac vasculogenesis has encouraged intervention strategies using either transduced genes capable of expressing the factor (Isner, 2002) or the purified factor proteins (Post et al, 2001). Naked plasmid DNA and recombinant adenovirus have been used vectors to express a variety of growth factors. The proteins or vectors are introduced either by injection into the myocardium or into the intracoronary circulation. In a mouse model of ischemic heart, blood vessels formed at and surrounding the myocardial injection site of an adeno-associated viral vector expressing VEGF (Su et al, 2000). Results from clinical investigations of gene transfer in phase 1 trials, though preliminary, are also promising (Kastrup et al, 2005). Ectopic expression of FGF-4 or VEGF-2 may reduce angina and increased exercise tolerance and heart function (Symes et al, 1999; Isner, 2002). These encouraging results suggest that it may be possible to revascularize defective heart tissue, and by inference, other tissues, *in situ*.

RELEVANCE TO THE DEVELOPMENT OF THE SALIVARY GLANDS

Superficially the pancreas appears to be a displaced, simple salivary gland of serous acini with the intrusion of a small amount (about 1% of the mass) of endocrine tissue organized into islets (Fig 6a). Indeed a longstanding belief, now dispelled (Pictet et al, 1976), held that the endocrine tissue is derived from migrating neural crest cells that invade the pancreatic parenchyma (Pearse, 1977). It is now clear that the exocrine and endocrine compartments of the pancreas arise from a common pool of precursor cells (Fig 4b; for a review see Kim and MacDonald, 2002). Indeed, there are major differences between salivary and pancreatic organogenesis: although the pancreas and submandibular gland arise from the endoderm, the parotid is from the ectoderm; mucous acini, myoepithelial cells, and uniquely

differentiated ducts are not present in the pancreas; the major salivary glands arise by the invagination of solid epithelial cell cords that subsequently canalize, whereas the pancreatic epithelial buds form as cellular tubes topologically linked to the duodenal mucosa. These differences extend to the fundamental controls of organogenesis: very few transcriptional regulators of pancreatic development are shared with the salivary glands (Schwitzgebel, 2001; Wilson et al, 2002; Kong et al, our unpublished observations).

Salivary gland development has been the classical example of branching morphogenesis and the role of inductive signals (Hieda and Nakanishi, 1997; Sakai et al, 2004); however, little is known of the molecular genetic controls involved. Moreover, although glandular morphogenesis appears to reveal an unambiguous pathway for the cellular lineages (Fig 6b), confirmation is lacking. Rigorous cell lineage tracing is necessary to verify the simple pathway for the genesis of the major epithelial cell types suggested by visual inspection of salivary gland development. The molecular regulatory consequences of salivary gland growth factors, morphogens and cell-cell contacts for developmental decisions must be elucidated. Crucial salivary gland transcription factors must be identified, their developmental roles defined (including their direct target genes), how they establish an interacting regulatory network, and how they are induced by extrinsic factors. By analyzing the expression pattern of 800 DNA-binding transcription factors in adult and fetal organs, we have identified thirty-four that are partly restricted to the submandibular gland, and therefore, may play important roles in development and maintenance (Kong et al, our unpublished results). Further characterization would be expected to identify those with developmental functions. Because the submandibular/sublingual and parotid glands derive from different germ layers, initiating events for organogenesis, the relationships of cell lineages and transcriptional regulators may differ between the glands.

CONCLUSIONS

New insights into the mechanisms that direct organogenesis of the pancreas, blood vessels and cardiac muscle point to intervention strategies to deal with diabetes, cardiac ischemia and cardiac hypertrophy, respectively. Can equivalent developmental insights be relevant to problems of salivary gland biology and disease? Certainly, Sjögren's autoimmune disease may be treatable by tissue regeneration or replenishment within the context of immunosuppression, as for islet transplantation for diabetics. Rebuilding salivary gland tissue will require coincident vascularization and may benefit from the success of therapeutic cardiac angiogenesis. To consider these possibilities for the major salivary glands, continued progress is necessary to define the molecular and genetic processes of salivary gland development. The natural beauty of salivary organogenesis and the drive to understand how things work are worthwhile additional incentives.

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