Pax Genes and Their Role in Organogenesis

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Abstract

Pax genes have been cloned on the basis of their homology to Drosophila segmentation gene paired. They share a common domain, the paired domain, that is sufficient to mediate sequence-specific DNA binding. Thus far, nine members have been characterized, which exhibit highly restricted temporal and spatial expression patterns. The analysis of mouse mutants has revealed their crucial role in the formation of a variety of tissues. In particular, they are involved in the regulation of early steps in organ development. They act to define the regional specification of distinct germ layers.

Introduction

Pax genes were identified on the basis of sequence homology to Drosophila segmentation genes (1, 2). They consist thus far of nine members. They share a common DNA-binding domain of 128 amino acids, the paired domain, located at the NH2-terminal end. The paired domain is highly conserved during evolution and is detected in different species including Drosophila, human, mouse, rat, chicken, and zebrafish. In addition to the paired domain, two other conserved motifs, the paired-type homeodomain and an octapeptide, are found in distinct classes of Pax genes (Fig. 1; Ref. 3). Pax proteins display sequence-specific DNA-binding activity to regulate transcription and are therefore transcription factors (4, 5).

During development, Pax genes are expressed in a highly specific spatial and temporal pattern. The analysis of mouse mutants and human syndromes has uncovered their important role as regulators of normal development. Phenotypes correlate closely with the expression patterns. Pax1 is mutated in undulated mice with defects in skeletal structures, derived from the sclerotome, where the gene is expressed (6). Pax3 is expressed in the somite, neural tube, and neural crest, and malformations in these structures are found in Splotch mice and Waardenburg syndrome, where Pax3 is mutated (reviewed in Ref. 7).

Pax6, which is expressed during eye development, is mutated in different species leading to eye abnormalities in mice (sey), rats (rs ey), humans (aniridia), and Drosophila (Eyeless; 8–12). Pax2, which is expressed early in eye and kidney development, was found to be mutated in a family with kidney and eye malformations (13–15). Additionally, mutant mice generated by homologous recombination clearly support the view that Pax genes are critical for the normal development in a variety of tissues. Furthermore, chromosomal translocations involving Pax3 or Pax7, which result in the expression of a Pax-forkhead fusion protein, are found in rhabdomyosarcoma (16, 17). In this review, we will focus on the role of Pax genes in cellular differentiation. We will emphasize the function in organogenesis, giving new insights into organ formation and uncovering a possible general mechanism of Pax gene function in a variety of tissues.

Organogenesis and Pax Genes

Pax genes are expressed in early steps of the generation of a number of organs outside of the nervous system. Pax1 is detected in the developing thymus (18), Pax2 and Pax8 in the kidney (13, 19), Pax2 in the eye and the inner ear (20, 21), Pax8 in the thyroid (19), Pax6 in the pancreas (22), and Pax6 in the eye and the pancreas (8, 23).

Thymus

Undulated mice suffer from thymus size reduction and impaired maturation of the thymocytes, indicating that Pax1 may be necessary for thymus epithelium differentiation (18).

Kidney

Mutations of the Pax2 gene in mice and men lead to kidney, eye, and inner ear defects. Heterozygous KrD mice (Kidney and Retinal defects) with a chromosomal deletion, including the Pax2 gene, exhibit a high incidence of kidney hypoplasia and retinal defects (15). Mice homozygous for a Pax2 mutation generated by homologous recombination have no kidneys and display eye and inner ear malformations (21, 24). In humans, a point mutation in the Pax2 gene is detected in a family with kidney hypoplasia and colobomas (15).

The analysis of Pax2 knock-out mice revealed that the mesonephric tubules are not formed. In contrast, the Wolfian duct appears normal at E9 to E10 of gestation but fails to extend caudally and starts to degenerate at E11. In summary, Pax2 appears to be required for the formation of the epithelial components of the urogenital system from the intermediate mesoderm (24).

Eye

At early stages of eye development, Pax2 and Pax6 seem to share overlapping domains of expression in the optic vesicle, which give rise to the developing eye. Later, Pax2 is restricted to the optic stalk, where it labels the prospective optic nerve, demarcating a boundary between it and the prospective outer retinal layer, which expresses Pax6 (21, 14). In Pax2−/− mice, a severe eye coloboma occurs, developing an outer pigmented layer, and neural retina extends into the Pax6-expressing domain; no differentiation of the glial cells surrounding the optic nerve is observed (21). Two possibilities may explain this defect in Pax2 loss of function conditions: (a) failure of restriction capacity of the border; or (b) transformation of the glial cells surrounding the optic nerve into Pax6-expressing cells (neural retina and pigmented layer). Several other examples for involvement of Pax genes at boundary formation have been described recently. In s3y mutant embryos, the Distal-less-1 (Dlx1) expression in basal ganglia heterotopically extends into the Pax6 cortical domain, thus compromising the boundary between the striatum and the cortex, as defined by the exclusive expression of both genes (25). Therefore, Pax genes appear to be involved in restricting boundaries of differentiation in the nervous system. It is likely that they act directly or indirectly on cell differentiation. In fact, in the ventral spinal cord, Pax6 has been shown to mediate sonic hedgehog signals to specify motor neurons and ventral interneurons (26).
The pancreas is also derived from the endoderm. Two main components constitute the pancreas. The major exocrine part is composed of secretory cells (acini) producing digestive enzymes. Scattered within the acini, are the islets of Langerhans, consisting of several types of endocrine cells. Four endocrine cell types are found in the adult pancreas, α, β, δ, and pp, that produce glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. Two Pax genes are expressed during early onset of pancreas development, Pax4 and Pax6 (22, 23, 28, 29). Pax4 is found at E10.5 in insulin-producing cells, and the expression persists in the mature pancreas (22). Pax6 is detected at E9.5 in glucagon-expressing cells and is later found in all endocrine cells (28, 29). Analysis of mutant mice lacking Pax4, Pax6, or both revealed that they play a critical role in the differentiation of the endocrine pancreas. In newborn Pax4−/− mice, insulin is not produced. Furthermore, the glucagon cells are numerous and abnormally organized. Normally, the majority of the endocrine cells are β-cells, whereas the glucagon-producing cells constitute only a small fraction, located at the periphery of the islet. However, in early Pax4−/− embryos, insulin-producing cells are detected only until E13.5. Accordingly, Pax4 is required for early differentiation of β-cells in the pancreas and may maintain their fate. The high number of glucagon-producing cells in mutant embryos most probably indicates that a high proportion of insulin-producing cells have changed their initial fate to become α-cells producing glucagon.

In the pancreas of newborn Pax6−/− mice, only the α-cells are affected, and no glucagon is produced. In mutant embryos, few glucagon-producing cells could be detected (28), indicating that Pax6 is required for the differentiation of α-cells. The analysis of a second Pax6 mutant small eye (SeyN<sup>Neu</sup>) shows that these mice have a significant number of endocrine cells, but they are all significantly reduced (29). The conclusion made from this analysis was that Pax6 is necessary for the proliferation of the endocrine cells (29). The discrepancy between the two phenotypes may be due to Sey<sup>N<sub>Neu</sub></sup> being a hypomorphic allele. Also, genetic background variation may play a role (29). Both phenotypes rather point to a function for Pax6 in maintaining cell differentiation and proliferation. In the absence of Pax4 and Pax6, no endocrine cells are produced in the pancreas of double mutant animals (28).
Pax GENES IN ORGANOGENESIS

Discussion

Mutant mice have revealed new insights into the function of Pax genes. They have demonstrated that, besides the nervous system, they are controlling early stages of organogenesis. In the eye, Pax2 and Pax6 seem to regulate the specific differentiation of the optic stalk and the retina, respectively. They maintain a boundary between two regions of different differentiation pathways. This is consistent with Pax6 function in the brain and spinal cord. Accordingly, Pax genes act early to define the regional specificity of distinct germ layers. As a common dominator, Pax genes appear to directly or indirectly mediate the differentiation state of specific cell types in which they are expressed. It is not yet clear whether the Pax genes are maintaining a differentiation decision or are involved in its early specification. The analysis of the Pax4 knock out mice rather suggest that they are involved in both processes, because β-cells in the absence of Pax4 seem to change their fate to Pax6-expressing α-cells. It is also conceivable that Pax genes are involved in the maintenance of very early differentiation pathways in which cell precursors are still not fully committed. The absence of one Pax gene, therefore, may not necessarily lead to the lack of a certain cell type but rather to a change in cell fate. The analysis of the Pax6 knock-out mutant (28) and the SeyNew mice (29) clearly indicate that Pax6 is involved in cell differentiation and proliferation (28, 29).

It is interesting to notice that outside of the nervous system, Pax genes are involved in those cellular differentiation processes where epithelial-mesenchymal transitions take place. It is probable that the deregulation of these cellular events may lead to oncogenesis. In particular, it is worth mentioning the possible role of transcription factor forkhead, this process may be disturbed, leading to deregulation of these cellular events may lead to oncogenesis. In epithelial-mesenchymal transitions take place. It is probable that the differentiation and proliferation (28, 29).

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References


Dr. Phillip Sharp: You argue that the Pax genes are patterned by the homeotic genes, which we heard earlier about, and that they’re required to maintain the developmental state in pattern, or cell type in pattern, but do they not also contribute to the proliferation and cell type identity? Because you lose both, don’t you, if you have a mutation in a specific Pax gene?

Dr. Mansouri: Well, this is very difficult to separate. I mean, if you look at the Pax 4 mutation, for example, you see in Pax4 that they lose the insulin cells; you get glucagon cells instead, but you get a much larger number also. You can also argue there that the proliferation is enhanced in that case through direction of the proliferation of glucagon-producing cells. Also in Pax8 you can see that.

Dr. Sharp: But, in that case, is one of the Pax genes suppressing the expression of another? They seem to be alternatively interacting in terms of suppression. Does one Pax suppress the expression of another in given cell types, or is that just how you see it in the phenotypes?
Dr. Mansouri: I think in the Pax2, Pax6 story, this may be true, because there are binding sites for the Pax2 and Pax6 promoter, but for the other we cannot say.

Dr. Meinrad Busslinger: My question was exactly the same one. I wanted to know whether there will be physical interaction between the Pax2 and Pax6 promoter. Have you identified any of those signs, or even eliminated them, to show that in principle you then reactivate Pax6 in the optic stalk?

Dr. Mansouri: The binding sites for Pax2 and Pax6 are there, so we have to proceed to look. Then you get your interaction between the two.

Dr. Busslinger: Maybe it’s important to note here that the Pax binding sites are patterned, and so you can always find Pax binding sites within any DNA fragment. The big issue is, have you mutated those sites to show that their function is irrelevant?

Dr. Mansouri: We have not done yet that, but I mean, I can tell you something else. In the case of Pax3 and Pax7 for example, as we have in this knock-out, you see that when you have homozygous/heterozygous, for example, there is cross relation between those two genes. For example, Pax7 is never expressed in the roof plate, but in homozygous (Pax3)/heterozygous (Pax7), it gets expressed in the roof plate. So there is cross-regulation of genes.
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