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Pax Genes and Their Role in Organogenesis[1]

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Abstract

***Pax* genes have been cloned on the basis of their homology to the *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 NH_2-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 (rsey), 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.

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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), *Pax4* 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 sey 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).

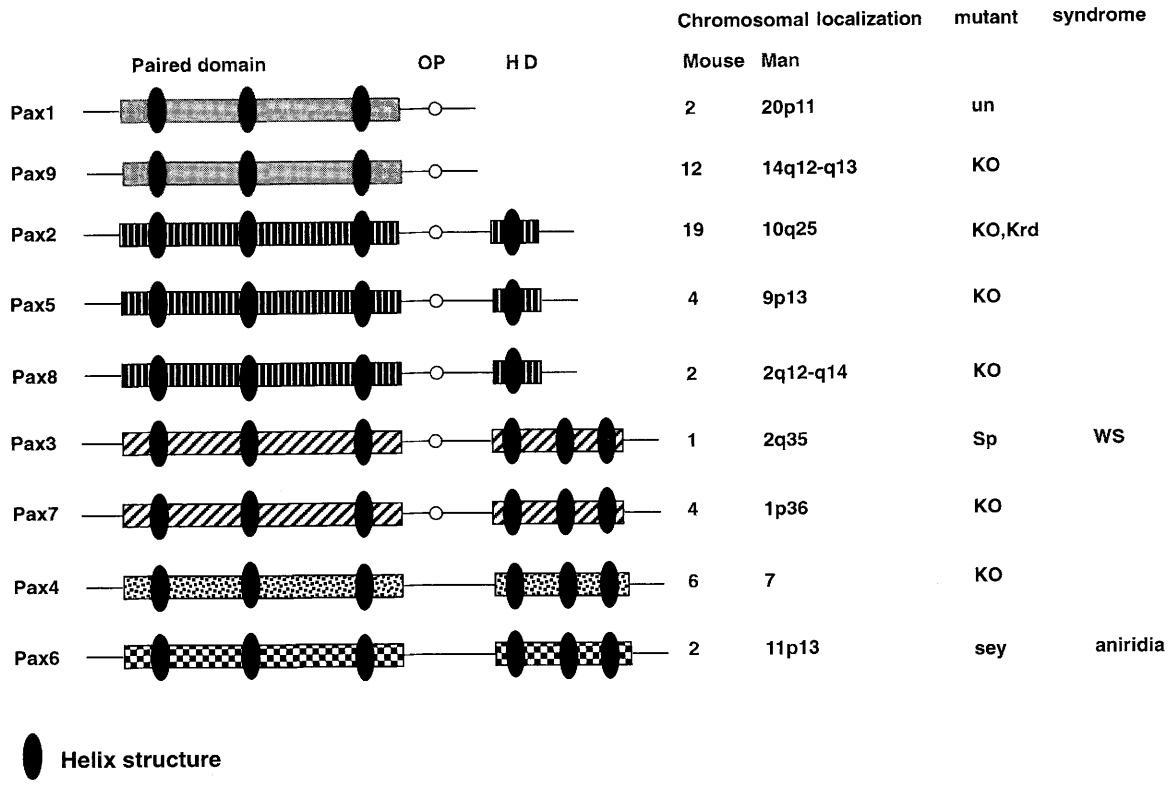

Fig. 1. Structure of the Pax proteins. All of the Pax proteins contain a conserved DNA-binding domain of 128 amino acids at the NH_2-terminal part, the paired box (*Paired domain*). The presence or absence of two other conserved domains, the octapeptide (*OP*) and a second DNA-binding domain (*HD*), the paired-type homeodomain, define distinct groups of Pax proteins. In the scheme, *Pax* genes of a distinct group are labeled in the same way. These genes share similar genomic organization and a common expression pattern. Chromosomal location and corresponding mutants or human syndromes are indicated. Concerning human *PAX2*, there is a family with a mutation in the octapeptide with kidney defects (see text). Also knock out mice (*KO*) are indicated. *sey*, small eye; *WS*, Waardenburg syndrome; *Sp*, Splotch; *Krd*, kidney and retinal defects mutant.

Thyroid Gland

The thyroid gland consists of two cell types of different embryological origins. The follicular cells, which secrete thyroid hormones, are derived from the endoderm of the floor of the pharynx. The parafollicular cells or C-cells produce calcitonin and are of neural crest origin. They constitute only a minor component of the thyroid gland. *Pax8* is expressed in the endoderm, at the time, when evagination starts from the floor of the pharynx. The expression persists until at least E16.5 of development in the mature organ (19). Mice lacking functional *Pax8* gene exhibit malformations in the thyroid, affecting only the follicular cells. In newborn mutant mice, the thyroid gland is composed of only C-cells. Analysis of early gestation mutant embryos revealed that the precursors of the follicular cells are able to evaginate from the endoderm, and development proceeds until E11.5 of gestation. However, at later stages, these cells are not detectable, and the remnant of the gland consists only of C-cells. Accordingly, the *Pax8* gene is required to maintain the differentiation of the endoderm into follicular cells during development of the thyroid gland (27).

Pancreas

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 (Sey^{Neu}) 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^{Neu} 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).

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 steps of organongenesis. 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 Sey^{Neu} 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 *Pax3* and *Pax7* in the differentiation of the dermomyotome, where a transition from epithelium to mesenchyme occurs to generate the myotome. In rhabdomyosarcoma, with fusion proteins of PAX3 or PAX7 with the transcription factor forkhead, this process may be disturbed, leading to transformation.

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References

1. Bopp, D., Burri, M., Baumgartner, S., Frigerio, G., and Noll, M. Conservation of a large protein domain in the segmentation gene *paired* and in functionally related genes of Drosophila. Cell, *47:* 1033–1040, 1986.
2. Deutsch, U., Dressler, G. R., and Gruss, P. Pax1, a member of a paired box homologous murine gene family, is expressed in segmented structures during development. Cell, *53:* 617–625, 1988.
3. Gruss, P., and Walther, C. Pax in development. Cell, *69:* 719–722, 1992.
4. Chalepakis, G., Fritsch, R., Fickenscher, H., Deutsch, U., Goulding, M., and Gruss, P. The molecular basis of the *undulated/Pax-1* mutation. Cell, *66:* 873–884, 1991.
5. Czerny, T., Schaffner, G., and Busslinger, M. DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. Genes Dev., *7:* 2048–2061, 1993.
6. Balling, R., Deutsch, U., and Gruss, P. *Undulated*, a mutation affecting the development of the mouse skeleton, has a point mutation in the paired box of Pax-1. Cell, *55:* 531–535, 1988.
7. Mansouri, A., Stoykova, A., and Gruss, P. *Pax* genes in development. J. Cell Sci. Suppl., *18:* 35–42, 1994.
8. Walther, C., and Gruss, P. *Pax-6*, a murine paired box gene, is expressed in the developing CNS. Development (Camb.), *113:* 1435–1449, 1991.
9. Hill, R. E., Favor, J., Hogan, B. L. M., Ton, C. C. T., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D., and van Heyningen, V. Mouse *Small eye* results from mutations in a paired-like homeobox-containing gene. Nature (Lond.), *354:* 522–525, 1991.
10. Fujiwara, M., Uchida, T., Osumi-Yamashita, N., and Eto, K. Uchida rat (*rSey*), a new mutant rat with craniofacial abnormalities resembling those of the mouse *Sey* mutant. Differentiation, *57:* 31–38, 1994.
11. Jordan, T., Hanson, I , Zaletayev, D., Hodgson, S., Prosser, J., Seawright, A., Hastie, N., and van Heyningen, V. The human *PAX6* gene is mutated in two patients with aniridia. Nat. Genet., *1:* 328–332, 1992.
12. Quiring, R., Walldorf, U., Kloter, U., and Gehring, W. J. Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. Science (Washington DC), *265:* 785–789, 1994.
13. Dressler, G. R., Deutsch, U., Chowdhury, K., Nornes, H. O., and Gruss, P. *Pax2*, a new murine paired box-containing gene and its expression in the developing excretory system. Development (Camb.), *109:* 787–795, 1990.
14. Macdonald, R., and Wilson, S. W. Pax proteins and eye development. Curr. Opin. Neurobiol., *6:* 49–56, 1996.
15. Sanyanusin, P., Schimmenti, L. A., McNoe, L. A., Ward, T. A., Pierpont, M. E. M., Sullivan, M. J., Dobyns, W. B., and Eccles, M. R. Mutation of the *PAX2* gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat. Genet., *9:* 358–363, 1995.
16. Galili, N., Davis, R. J., Fredericks, W. J., Mukhopadhyay, S., Rauscher, F. R., Emanuel, B. S., Rovera, G., and Barr, F. G. Fusion of a fork head domain gene to *PAX3* in the solid tumour alveolar rhabdomyosarcoma. Nat. Genet., *5:* 230–235, 1993.
17. Shapiro, D. N., Sublett, J. E., Li, B., Downing, J. R., and Naeve, C. W. Fusion of *PAX3* to a member of the Forkhead family of transcription factors in human alveolar rhabdomyosarcoma. Cancer Res., *53:* 5108–5112, 1993.
18. Wallin, J., Eibel, H., Neubüser, A., Wilting, J., Koseki, H., and Balling, R. *Pax1* is expressed during development of the thymus epithelium and is required for normal T-cell maturation. Development (Camb.), *122:* 23–30, 1996.
19. Plachov, D., Chowdhury, K., Walther, C., Simon, D., Guénet, J-L., and Gruss, P. *Pax8*, a murine paired box gene expressed in the developing excretory system and thyroid gland. Development (Camb.), *110:* 643–651, 1990.
20. Nornes, H. O., Dressler, G. R., Knapik, E. W., Deutsch, U., and Gruss, P. Spatially and temporally restricted expression of *Pax2* during murine neurogenesis. Development (Camb.), *109:* 797–809, 1990.
21. Torres, M., Gómez-Pardo, E., and Gruss, P. Pax2 contributes to inner ear patterning and optic nerve trajectory. Development (Camb.), *122:* 3381–3391, 1996.
22. Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G., and Gruss, P. The *Pax4* gene is essential for the differentiation of insulin-producing β-cells in mammalian pancreas. Nature (Lond.), *386:* 399–402, 1997.
23. Turque, N., Plaza, S., Radvanyi, F., Carriere, C., and Saule, S. Pax-QNR/Pax-6, a paired box- and homeobox-containing gene expressed in neurons, is also expressed in pancreatic endocrine cells. Mol. Endocrinol., *8:* 929–938, 1994.
24. Torres, M., Gomez-Pardo, E., Dressler, G. R., and Gruss, P. Pax-2 controls multiple steps of urogenital development. Development (Camb.), *121:* 4057–4065, 1995.
25. Stoykova, A., Fritsch, R., Walther, C., and Gruss, P. Forebrain patterning defects in *Small eye* mutant mice. Development (Camb.), *122:* 3453–3465, 1996.
26. Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., Van Heyningen, V., Jessel, T. M., and Briscoe, J. Pax6 controls progenitor cell identity and neuronal Fate in response to graded Shh signaling. Cell, *90:* 169–180, 1997.
27. Mansouri, A., Chowdhury, K., and Gruss, P. Follicular cells of the thyroid gland require *Pax8* gene function. Nat. Genet., *19:* 87–90, 1998.
28. St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A., and Gruss, P. Pax6 is required for differentiation of glucagon-producing α-cells in mouse pancreas. Nature (Lond.), *387:* 406–409, 1997.
29. Sander, M., Neübuser, A., Kalamaras, J., Ee, H. C., Martin, G. R., and German, M. S. Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev., *11:* 1662–1673, 1997.

Discussion

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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