Pax genes and their roles in cell differentiation and development

Ahmed Mansouri*, Marc Hallonet† and Peter Gruss‡

Members of the *Pax* gene family are expressed in various tissues during ontogenesis. Evidence for their crucial role in morphogenesis, organogenesis, cell differentiation and oncogenesis is provided by rodent mutants and human diseases. Additionally, recent experimental *in vivo* and *in vitro* approaches have led to the identification of molecules that interact with Pax proteins.

Addresses
Department of Molecular Cell Biology, Max-Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany
*e-mail: amansou@gwdg.de
†e-mail: mhallon@gwdg.de
‡e-mail: pgruss@gwdg.de

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Abbreviations

BMP	bone morphogenetic protein
En	*Engrailed*
FGF	fibroblast growth factor
sey	small eye
Shh	Sonic hedgehog

Introduction

The murine *Pax* gene family was identified on the basis of sequence homology with *Drosophila* segmentation genes [1,2] and now consists of nine members. All Pax proteins contain a paired-box, DNA-binding domain of 128 amino acids located at the amino-terminal end. This domain has been highly conserved during evolution and is found in *Drosophila* and in human, mouse, rat, chicken, quail and zebrafish genes. Distinct classes or subgroups of *Pax* genes are defined by the presence or absence of a paired-type homeobox and of an octapeptide coding region, in addition to the paired box (see Fig. 1) [3,4]. Paired box containing proteins are transcription factors, as they display sequence-specific DNA-binding activity and can regulate transcription [5,6].

Pax genes display dynamic expression patterns during ontogenesis in a large variety of tissues, and mutant phenotypes correlate very well with the expression patterns (see Fig. 2). For example, *Pax1* is expressed in the sclerotome and is mutated in undulated mice suffering from skeletal abnormalities [7]. *Pax3* is expressed in the limb muscle, neural tube and neural crest, and is mutated in Splotch mice and human Waardenburg syndrome in which malformations of all these structures occur [8,9]. *Pax6* is expressed during eye formation and is mutated in small eye (sey) mice and rats [10–12], and in human aniridia [13]; in all of these, eye defects are displayed. *Pax2* is expressed during eye and kidney development [14] and is mutated in a human family with kidney and eye abnormalities [15]. *Pax* genes clearly play important roles during the formation of many structures. Furthermore, deregulated expression of *Pax* genes may lead to oncogenesis [16–18].

In this review, we focus on recent data documenting the role of *Pax* genes, and the interaction of the Pax proteins with other transcription factors, in the development of the nervous system, in organogenesis and in cell proliferation and differentiation.

Pax genes in the central nervous system

The roles of *Pax* genes are particularly documented at the level of the nervous system, which consequently may represent a model for the study of the function of the *Pax* genes. All *Pax* genes, except *Pax1* and *Pax9*, are expressed in various restricted territories in the neural tube. Unlike *Hox* genes, *Pax* genes are found in the more rostral domains of the brain: *Pax6* is found in the telencephalon [10,19], *Pax3* and *Pax7* in the mesencephalon [19–21], and *Pax2*, *Pax5* and *Pax8* at the midbrain–hindbrain boundary [19,22–24] (see Fig. 2). In the spinal cord, *Pax* genes display restricted expression patterns along the dorsoventral axis.

In the brain, comparison of the expression domains of *forkhead*, *Wnt*, *Engrailed* (*En*), and *Pax* genes with sites of neuronal differentiation suggest that some Pax proteins are morphoregulators of development of the brain [19,25,26]. Several *Pax6* semidominant mutations are documented in the mouse, rat and human, with various phenotypic severities. At the cellular level, *Pax6* mutations cause a delay in neuronal migrations in a gene-dose-dependent manner, and impair axonal growth and differentiation [27]. In mouse *sey* mutants, *Dlx1* gene expression, which is normally specific for the ganglionic eminence, ectopically extends into the cortex, where *Pax6* is normally expressed. Pax6 function is thus necessary for establishing and/or maintaining the frontier between two brain territories [28••]. Overexpression of the chicken *En1* gene in medulloblastoma cell lines results in the downregulation of *PAX6* expression, thus suggesting that these genes are inversely regulated [29]. In addition, grafting experiments of an *Engrailed*-expressing midbrain–hindbrain piece of neuroepithelium in which fibroblast growth factor (FGF)8 is expressed onto diencephalic structures can induce the expression of *En2* in the host adjacent tissues [30]. Moreover, FGF8 has been recently proposed as an organizer of this brain region and could be a major

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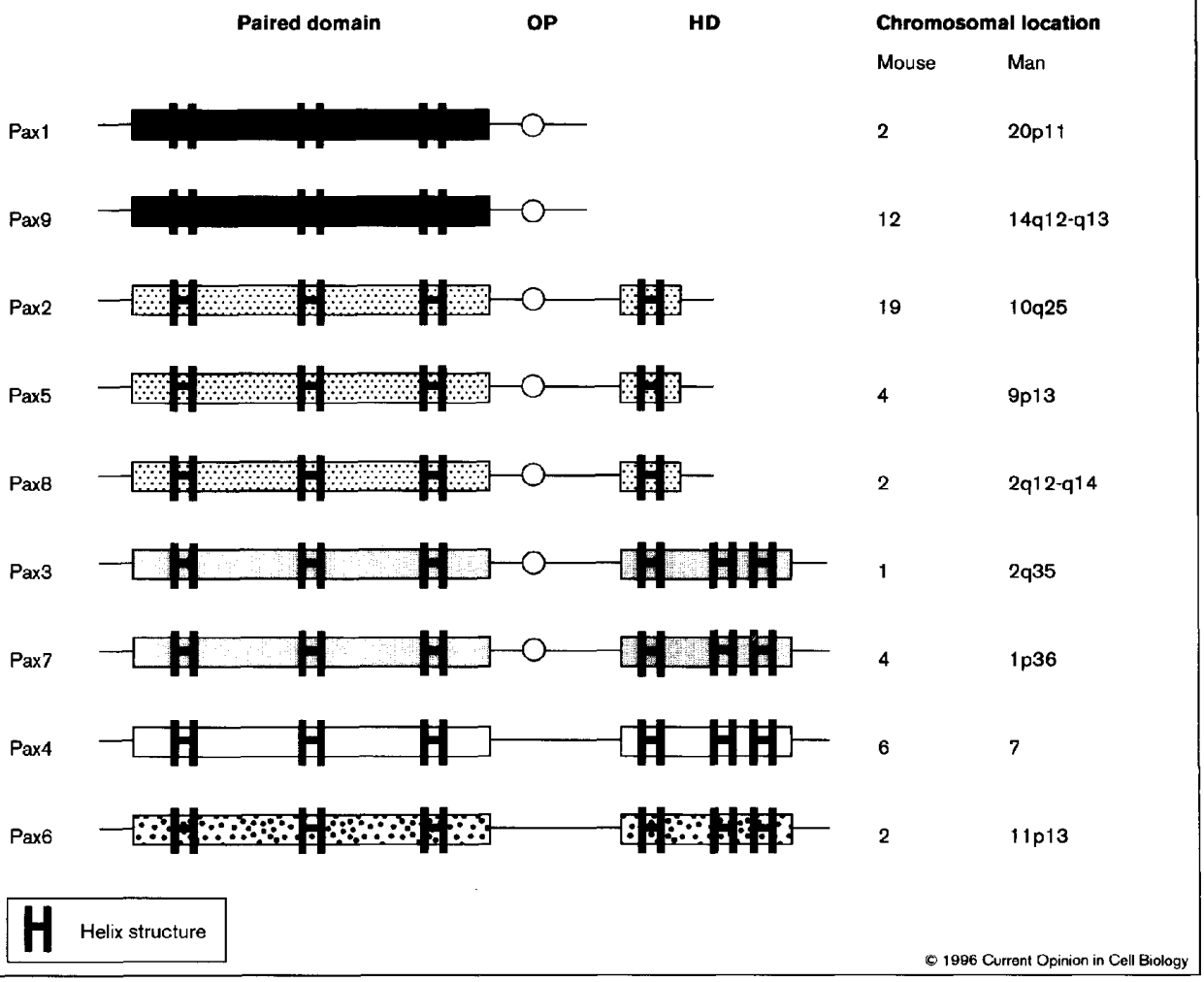


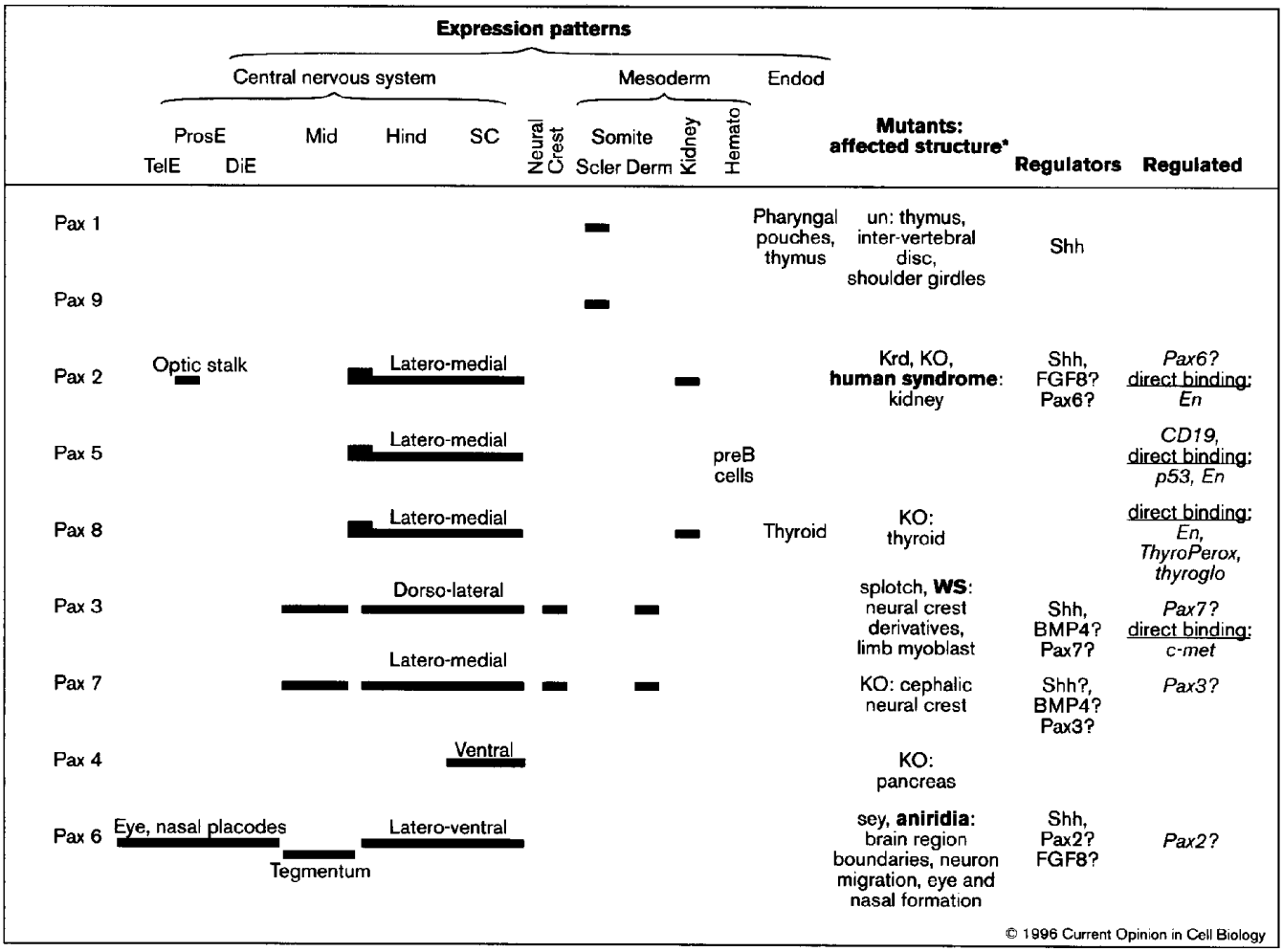
cture of the Pax proteins. All Pax proteins contain a paired-box, DNA-binding domain (paired domain) of 128 amino acids, located at amino-terminal ends. Distinct subgroups of proteins are defined by the presence or absence of a paired-type homeobox (HD) and of an peptide (OP) (related genes share the same patterning on the figure). In addition to the paired domain, Pax3, Pax4, Pax6 and Pax7 have cond DNA-binding domain, the paired-type homeobox (HD), at their carboxy-terminal ends. This homeobox is truncated and only carries first helix in Pax2, Pax5 and Pax8, and is totally absent in Pax1 and Pax9. In addition, all Pax proteins, except Pax4 and Pax6, carry an peptide of unknown function between the paired domain and the homeobox. Pax-related genes share overlapping expression patterns with ı other. Although some *Pax* genes may be present on the same chromosome, they are not clustered, as compared with the *Hox* genes :h are clustered.

ulator of this cascade of regulations, which regionalizes embryonic neuroepithelium [31••,32].

2, *Pax5* and *Pax8* are expressed at the midbrain–hindbrain ındary level, as are members of the *Wnt*, *Engrailed*, and FGF gene families [33••]. Injection of antibodies ed against the zebrafish Pax[b] protein, a homologue Pax2, Pax5 and Pax8, causes specific and localized formations at this boundary that are associated with lecrease of *Wnt1* and *En2* RNA in this area [34]. ılarly, *Pax5*-knockout mice exhibit defects in tissues ived from the posterior midbrain region and the ral hindbrain; the inferior colliculus is partially deleted and the foliation of the cerebellum is perturbed in these mice [35]. These results, and the phenotypes observed in *Wnt1*$^{-/-}$ and *En1*$^{-/-}$ mice, in which the caudal midbrain and cerebellum are severely affected [33••], clearly indicate that Pax, Wnt and En act in concert during the development of the midbrain–hindbrain boundary region.

In the developing eye, *Pax2* is expressed exclusively in the optic stalk, and *Pax6* in the eye cup [36]. That these two genes are inversely regulated is confirmed by ectopic overexpression of the ventralizing factor Sonic hedgehog (Shh) which leads to overexpression of *Pax2*

Figure 2



Schematic summary of the expression patterns of *Pax* genes, the structures affected by mutations in the *Pax* genes, and the molecules regulating or being regulated by the Pax proteins. The patterns of expression indicated in this table are a simplification of the complex expression patterns of the *Pax* genes. The thick black horizontal lines represent expression patterns of the *Pax* genes. The expression of Pax2, Pax5 and Pax8 is more expanded at the midbrain–hindbrain boundary. Derm, dermomyotome; DiE, diencephalon; Endod, endoderm; Mid, midbrain; Hemato, haematopoietic system; Hind, hindbrain; KO, knockout; Krd, kidney and retinal defects; ProsE, prosencephalon; Scler, sclerotome; SC, spinal cord; TelE, telencephalon; thyroglo, thyroglobuline; ThyroPerox, thyroperoxidase; un, undulated mouse mutant; WS, human Waardenburg syndrome; ?, putative interacting candidates; direct binding, target DNA is directly bound by Pax proteins (see text for details). *In this column, human syndromes or diseases are indicated in bold type before the colon, whereas mouse mutants are indicated in normal type before the colon. Affected structures are indicated after the colon.

and downregulation of *Pax6* and provokes hypertrophia of the optic stalk and reduction of pigment epithelium and neural retina [37••,38]. *Pax2* and *Pax6* are thus crucial morphoregulators of the eye and are both under the regulation of ventralizing agents.

In the spinal cord, transplantation experiments in the chick indicate that the expression of *Pax3*, *Pax7* and *Pax6* is regulated by the notochord via a secretion of Shh and probably also by the roof plate and overlaying ectoderm, possibly via a secretion of bone morphogenetic proteins (BMPs) 4 and 7 [39,40]. *Pax3*, *Pax6* and *Pax7* are expressed prior to neural differentiation in mitotically active cells, as opposed to *Pax2*, *Pax5* and *Pax8* which are not expressed at this time. It has been reported that ectopic expression of *Pax3* in the entire spinal cord of the mouse, using the Hoxb4 enhancer, does not confer dorsal identities as expected but instead leads to lack of the floor plate in the affected areas of the spinal cord of the transgenic animals [41]. *Pax* genes may thus be important regulators of the dorsoventral patterning of the spinal cord.

The *Pax3* gene is inactivated by deletions or point mutations in various murine *splotch* or human Waardenburg syndrome semidominant alleles. *Splotch* mutants exhibit spina bifida, exencephaly, and neural-crest and limb-muscle defects [8,9,42]. Recent studies of *splotch delayed*, which produces a milder phenotype than other *splotch* alleles, revealed normal neuronal differentiation in dorsal and ventral regions of the affected neural tube

[43]. Similarly, *Pax7*$^{-/-}$ mice do not show any obvious neural tube defect [44•]. Functional redundancy between *Pax3* and *Pax7* could thus be responsible for the lack of abnormal neuronal differentiation phenotype in these mutants.

The *Drosophila gooseberry* (*gsb*) locus codes for two linked and structurally related genes, *gsb-proximal* and *gsb-distal*, which are homologues of the *Pax3* and *Pax7* genes (for review, see [45]). Experiments in which *Drosophila* embryos carried a deletion removing both *gsb* transcripts or in which embryos ectopically expressed only one of *gsb-distal* and *gsb-proximal* clearly indicate that Gsb specifies row five neuroblasts [46•,47]. Pax3 and Pax7 may also specify neuronal identity in the spinal cord, and only double mutations of both *Pax* genes may give us a definitive answer about their respective functions.

Pax genes in organogenesis

Pax genes are also expressed outside the central nervous system during organogenesis: *Pax1* is expressed in thymus, *Pax2* and *Pax8* in kidney, *Pax8* in thyroid, *Pax6* in pancreas and eye, and *Pax4* in pancreas ([14,24,48,49]; B Sosa-Pineda, P Gruss, unpublished data). Mutation of the *Pax1* gene in undulated mice leads to reduced thymus size and affects the maturation of the thymocytes [49]. Accordingly, *Pax1* seems to be required in thymus epithelium differentiation and hence influences T-cell maturation [49].

Krd (*kidney and retinal defects*) mice with a deletion of a chromosomal segment that includes the *Pax2* locus have kidney defects [50]. Kidney hypoplasia has been associated with heterozygosity of a human *PAX2* point mutation [15]. Experiments using antisense oligonucleotides to *Pax2* in kidney organ culture, and inactivation of *Pax2* by homologous recombination, demonstrate that this gene is essential for the development of the kidney epithelial components that are derived from the intermediate mesoderm [51,52•].

Pax8$^{-/-}$ mice suffer from a thyroid defect (A Mansouri, P Gruss, unpublished data). *Pax4*$^{-/-}$ mice suffer from a pancreas defect (B Sosa-Pineda, P Gruss, unpublished data).

Targeted ectopic expression of cDNA encoding *Drosophila* eyeless or its murine homologue, Pax6, induces morphologically normal eyes in the wings, legs and antennae, demonstrating that *eyeless* is the master control gene for eye morphogenesis in *Drosophila* [53•,54]. Analysis of homozygous *sey* mice, and studies of chimeric mouse embryos composed of wild-type and *sey*-mutant cells, indicates that *Pax6* acts directly and in a cell-autonomous manner in the optic cup and lens, and is essential for lens and nasal-placode specification from surface ectoderm [55,56•].

Pax proteins are thus crucial regulators of organogenesis in thymus, kidney, thyroid, pancreas and eye.

Pax genes in cell differentiation

Analysis of undulated mice demonstrates that *Pax1* is essential for the condensation of the mesenchymal sclerotome cells and for the initiation of chondrogenesis [57]. The effect of Pax1 on sclerotome differentiation is regulated by the notochord via secretion of Shh [58,59•]. Inactivation by gene targeting demonstrates that *Pax5* is essential for early B-cell differentiation [35]. The limb muscles, whose precursors express *Pax3*, are specifically disturbed in *splotch* mice [42]. Additionally, neural-crest derivatives, such as Schwann cells or melanocytes, are also affected in *splotch* mice [60–62]. *In vitro* experiments indicate that *Pax3* is exclusively expressed in differentiating and nonmyelinating Schwann cells (see [63]). Accordingly, Pax3 in involved in the migration, differentiation and possibly the proliferation of the neural-crest cells and myoblasts. Also, Pax7 has been shown to play a role in cephalic neural crest specification, differentiation and/or proliferation [44•].

Pax genes, cell proliferation and oncogenesis

Overexpression of *Pax* genes can transform fibroblasts into tumours in nude mice [18]. Similarly, the expression of *PAX2* and the paralogous gene *PAX8* are abnormally upregulated in Wilm's tumour, a paediatric renal carcinoma of mesenchymal origin [64,65].

Translocations of human *PAX3* or *PAX7* result in the expression of a PAX–forkhead fusion protein carrying the intact DNA-binding domains of the PAX3 or PAX7 molecules that are probably responsible for the generation of rhabdomyosarcoma [17,66]. In the case of the PAX3–forkhead fusion, the molecule produced is a more efficient transcription factor than PAX3 itself and would be expressed under the normal *PAX3* promoter [67]. It has not, however, been directly demonstrated that the fusion protein is driven by the *PAX* promoter.

Deregulation of *PAX5* expression has been reported in humans in malignant astrocytomas [68] and medulloblastomas [69]. In addition, the *Pax5* promoter is modified in large-cell lymphomas by insertion of the potent Eμ enhancer of the IgH gene [70]. It is not clear how Pax5 is acting in these tumours but it may be involved in regulating cell proliferation, as *in vitro* experiments indicate that Pax5 may regulate proliferation in B cells [71].

Thus, the overexpression of *Pax* genes in tissues in which they are normally expressed may lead to tumorigenesis, suggesting that doses of Pax proteins are critical for their normal function.

Pax protein target genes

Little is known about Pax target genes. However, *in vitro* protein–DNA binding assays and *in vivo* expression analysis indicate that a 1.0 kilobase fragment of the *En2* enhancer contains binding sites for Pax2, Pax5 and Pax8 [72•]. Mutation of these binding sites disrupts initiation and maintenance of expression of the β-galactosidase reporter gene in the midbrain–hindbrain boundary of transgenic mice [72•].

Pax3 and the tyrosine kinase receptor for hepatocyte growth factor/scatter factor that is encoded by the c-*met* proto-oncogene are expressed in the lateral dermomyotome, that is, the progenitors of limb muscle [73]. *Splotch* mice display defects in neural-crest derivatives, failure of neural-tube closure and lack of limb muscles [8,9,42]. *Splotch* and c-*met*$^{-/-}$ mice exhibit the same phenotype in the limb. Furthermore, c-*met* expression is greatly reduced in the dermomyotome of *splotch* embryos. In addition, Pax3 directly activates c-*met* expression by binding to the c-*met* promoter, thus demonstrating that c-*met* is a target gene of Pax3 [74•,75].

Similarly, *Pax5* was identified as a B cell specific transcription factor that regulates the expression of the CD19 gene which encodes a surface protein [76]. In addition, *Pax8*, which is expressed in the thyroid, binds to the promoter, and regulates the expression, of the thyroperoxidase and thyroglobuline genes [77].

In vitro experiments also show that Pax5 can directly inhibit transcription of the p53 gene by binding to the untranslated first exon, and could thereby participate in tumorigenesis [78].

Conclusions

Pax gene mutants are powerful tools for the study of *Pax* gene function. Nevertheless, functional redundancy between related members, alternative splicing and complex promoters complicate the analysis. Consequently, knockout mutants are greatly needed to study the effect of defined mutations. New techniques using conditional knockouts and knockins may be used to specifically inactivate the *Pax* gene of interest in a selected tissue at a defined time.

Pax protein doses play a crucial role in proper function of the proteins. The ectopic expression of *Pax* genes in transgenic mice may therefore give new insights into the mechanism of action of Pax proteins.

The possible regulation of cell adhesion by Pax proteins could explain some of the mutant phenotypes. In this context, *in vitro* approaches will most probably contribute to the functional analysis of the *Pax* genes.

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