Pax8, a murine paired box gene expressed in the developing excretory system and thyroid gland

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Summary
Several mouse genes designated 'Pax genes' contain a highly conserved DNA sequence homologous to the paired box of Drosophila. Here we describe the isolation of Pax8, a novel paired box containing clone from an 8.5 day p.c. mouse embryo cDNA library. An open reading frame of 457 amino acids (aa) contains the 128 aa paired domain near the amino terminus. Another conserved region present in some other paired box genes, the octapeptide Tyr-Ser-De-Asn-Gly-Leu-Leu-Gly, is located 43 aa C-terminal to the paired domain. Using an interspecies backcross system, we have mapped the Pax8 gene within the proximal portion of mouse chromosome 2 in a close linkage to the surf locus. Several developmental mutations are located in this region. In situ hybridization was used to determine the pattern of Pax8 expression during mouse embryogenesis. Pax8 is expressed transiently between 11.5 and 12.5 days of gestation along the rostrocaudal axis extending from the myelencephalon throughout the length of the neural tube, predominantly in two parallel regions on either side of the basal plate. We also detected Pax8 expression in the developing thyroid gland beginning at 10.5 days of gestation, during the thyroid evagination. In the mesonephros and metanephros the expression of Pax8 was localized to the mesenchymal condensations, which are induced by the nephric duct and ureter, respectively. These condensations develop to functional units, the nephrons, of the kidney. These data are consistent with a role for Pax8 in the induction of kidney epithelium. The embryonic expression pattern of Pax8 is compared with that of Pax2, another recently described paired box gene expressed in the developing excretory system.

Key words: paired box, Pax cDNA, mouse chromosome 2, kidney development, thyroid development, mouse embryogenesis.

Introduction
The molecular mechanisms governing mammalian development are poorly understood. The investigation of these mechanisms is hampered mainly due to the lack of mutants in which the genes involved in the developmental control are affected. However, a number of developmental mutants have been identified in Drosophila (Lewis, 1978; Nusslein-Volhard and Wieschaus, 1980), facilitating the isolation of the corresponding genes (for review see Akam, 1987). A complex network of molecular interactions leading to the establishment of segmentation and segment identity of the Drosophila embryo is being revealed (for review see Scott and Carroll, 1987; Ingham, 1988).

One approach used to identify the molecules involved in mammalian development takes advantage of the fact that certain protein domains such as the homeobox, a common motif among the Drosophila developmental genes, are strongly conserved in evolution (McGinnis et al. 1984). Thus, numerous murine homeobox-containing genes have been identified by screening with Drosophila homeobox probes and the expression of these genes in the developing embryo has been studied (for review see Holland and Hogan, 1988). Another conserved domain of 128 amino acids, the paired box, was identified in the Drosophila segmentation genes paired (Kilchherr et al. 1986; Frigerio et al. 1986), gooseberry-distal and gooseberry-proximal (Bamgartner et al. 1987; Cote et al. 1987) suggesting a functional role for this domain in the process of Drosophila segmentation (Bopp et al. 1986). Subsequently, two additional Drosophila paired box genes of unknown function, Fox meso and Fox neuro, were cloned (Bopp et al. 1989). The paired box domain is also conserved in the evolution of fly, mouse and man (Dressler et al. 1988; Burri et al. 1989). Because various segmented structures, such as somites and mesonephric tubules, appear during the mouse development (Hogan et al. 1985), an intriguing possibility is that paired box genes are also involved in the process of mammalian segmentation. Indeed, Pax1, a murine paired box gene,
is expressed in a segmented manner in the intervertebral disk anlagen along the entire vertebral column (Deutsch et al. 1988). Furthermore, a point mutation in the highly conserved region of the paired box of Pax1 is associated with the undulated mutant, characterized by malformations in the vertebral column (Balling et al. 1988). Another recently described murine paired box gene, Pax2, is expressed in restricted regions along the entire developing spinal cord as well as in the mesonephros and metanephros (Dressler et al. 1990; Nornes et al. 1990). The mesonephric nephrons constitute a segmented structure. Hence, the expression of Pax2 in this tissue supports a possible role for this gene in the segmentation of the mouse embryo.

Several paired box sequences have been detected in the mouse genome by hybridization (Dressler et al. 1988). As a first step to understanding the underlying mechanisms by which members of the Pax gene family function, we have sought to identify additional paired box genes of the mouse. In this report we describe the isolation and sequence of a novel paired box gene, Pax8. We show that Pax8 is closely linked to the surfet (surf) gene cluster near the centromere of chromosome 2. Using in situ hybridization, we demonstrate that Pax8 is expressed in restricted regions of the developing mouse embryo. The expression pattern is compared with that of the Pax2 gene, whose paired domain shows an extensive homology to the paired domain of Pax8. Comparison of the spatial pattern of expression of both genes in the developing metanephros is especially instructive. The morphogenesis of the metanephros results from mutual inductive interactions between the ingrowing ureteric bud and the metanephrogenic mesenchyme. During this process the branching ureter induces in the mesenchyme the cellular condensations that develop shortly thereafter to the S-shaped bodies, the precursors of the nephrons (for review see Potter, 1972; Saxén, 1987). We report here that Pax8 is expressed predominantly in the induced tissues, mesenchymal condensations and the S-shaped bodies. Pax2 is expressed in the ureter, condensations, and S-shaped bodies. The results suggest a function for both genes in the inductive process of kidney development.

Materials and methods

cDNA library screening

Initially, the cDNA clone c960 was isolated from an 8.5 days p.c. embryonic C57BL/6 mouse Agt10 cDNA library (Fahrner et al. 1987) in a low-stringency screen (hybridization in 7xSSC at 60°C with a subsequent washing in 2xSSC/0.2 % SDS at 42°C) using a mixture of Pax1, Pax2 and Pax3 paired box probes (Deutsch, unpublished). DNA sequencing revealed that this clone had part of a novel paired box. A 112 bp fragment containing the partial paired box sequence of c960 was labeled by random priming (Feinberg and Vogelstein, 1983) and used to screen 6x10^6 clones of the same cDNA library under high-stringency conditions (hybridization in 500 mM NaPi, pH 7.2/1 % SDS/1 mM EDTA at 65°C; washing several times in 40 mM NaPi, pH 7.2/1 % SDS at 65°C).

DNA sequencing

The overlapping restriction fragments of the Pax8 cDNAs were cloned into the plasmid vector Bluescript KS (Stratagene) and sequenced from both strands by the dideoxy method (Sanger et al. 1977) using commercial sequencing kits (Sequenase, US Biochemicals; T7, Pharmacia).

Embryos and tissues

Embryos for RNA isolation and in situ analysis were obtained from natural matings of female NMR1 mice. The day of the vaginal plug was designated as day 0.5 p.c. Tissues were isolated from adult NMR1 mice.

RNA isolation and Northern blot analysis

Total RNA was isolated by homogenizing tissues and embryos in guanidinium thiocyanate (Chirgwin et al. 1979) followed by centrifugation through a 5.7 M CsCl, 25 mM sodium acetate pH5.0 gradient in a Beckman SW40 rotor spun at 30000 revs-min^-1 for 24h. Poly (A) mRNA was isolated using oligo(dt)-cellulose columns. 5 µg of the respective RNA samples were electrophoresed through 1 % agarose gels containing 3.7 % formaldehyde and Mops buffer (20 mM morpholine propane sulfonic acid, 50 mM sodium-acetate, 10 mM EDTA, pH 7.0). RNA was blotted onto nylon membranes (Hybond-N) with 10xSSC and hybridized overnight in 50 % formamide, 5xSSC at 42°C. The membranes were then washed twice in 0.1xSSC, 1 % SDS at 65°C for 15 min each.

In situ hybridization

The cDNA fragments chosen as templates for in vitro transcription were cloned into the Bluescript KS vector (Stratagene) and the resulting plasmids were linearized with restriction endonuclease. These templates were transcribed using T3 or T7 RNA polymerase (Promega Biotech), [35S]-UTP and [35S]-CTP (each approximately 100 µCi) and then degraded by DNase digestion. RNA probes were precipitated with 10 % trichloroacetic acid, collected on nitrocellulose filters (Millipore), eluted by brief boiling, and incubating at 65°C in 20 mM EDTA pH 8.0/1 % SDS. After ethanol precipitation, the probes were partially degraded in 0.2 M NaOH on ice for 30 min, neutralized with 1 M acetic acid and ethanol precipitated. The probes for in situ hybridization were resuspended at 5x10^6 cts min^-1 ml^-1 in hybridization buffer (50 % formamide; 2xSSC; 10 mM Tris, pH 7.5; 10 mM NaPi, pH 6.8; 5 mM EDTA; 10 % dextran sulfate; 10 mM DTT; 10 mM β-mercaptoethanol; 1 mM ADP-β-S; 0.1 mM UTP; 10 µM S-ATP; 150 µg ml^-1 salmon sperm DNA; 150 µg ml^-1 yeast tRNA).

Preparation of sections and in situ hybridization was done as described by Hogan et al. (1986) with modifications. Embryos were frozen in isopentane on dry ice and 8 µm cryosections were cut at -19°C. Sections were dried at 55°C, fixed in 4 % freshly dissolved paraformaldehyde, dehydrated in graded ethanol series, air dried and stored at -20°C until use. Prior to hybridization, slides were treated successively in water, 1 min; 2xSSC, 30 min at 70°C; H2O, 1 min; 0.125 mg ml^-1 pronase, 10 min at room temperature; 0.2 % glycine, 30s; PBS, 1 min; 4 % paraformaldehyde, 20 min; PBS, 1 min; 0.1 M triethanolamine with 1/400 volume acetic acid, 10 min; PBS, 1 min; followed by dehydridation in graded ethanol and air drying. Sections were treated overnight with 8 µl of boiled (2 min) hybridization mix under sonicated coverslips in a humidified atmosphere at 42°C, washed 2 h in 50 % formaldehyde/2xSSC/10 mM β-mercaptoethanol at 37°C, treated with 50 µg ml^-1 RNase for 15 min, washed again overnight, and dehydrated in graded ethanol. Slides
were dipped in Kodak NTB-2 emulsion, autoradiographed for about 10 days at 4°C, developed in Kodak D-19 for 3 min, washed in 1 % acetic acid for 1 min and fixed in 30 % sodium thiosulphate for 3 min. Sections were stained with Giemsa and visualized using a Leitz Labovert bright-field/dark-field microscope.

Results

Isolation and structure of the Pax8 cDNAs

Pax8 was identified by sequencing c960, a short cDNA isolated from an 8.5 day p.c. embryonic cDNA library (Fahrner and Hogan, 1985) using a low-stringency screen. This clone contained part of a new paired box sequence. A 112bp EcoRI-NciI fragment of c960 (indicated as probe 1 in Fig. 1) was used to screen the above cDNA library under high-stringency conditions. A 552 bp cDNA clone, c2A, was isolated. It contained more of the paired box as well as sequences down-stream. The entire c2A sequence was found to be present in the corresponding region of the genomic clone (data not shown). To isolate full-length Pax8 cDNA, the above cDNA library was screened again using the whole c2A clone as a probe. Two largely overlapping cDNAs, c3B and c27B, isolated in this screen, are shown schematically in Fig. 1. Together they span 2528 bp and include the entire 1371 bp coding region of Pax8 (Fig. 2).

The clone c3B has an AAUAAA polyadenylation signal (Proudfoot and Brownlee, 1976; Fitzgerald and Shenk, 1981) near its 3' end, followed 12 nucleotides further downstream by a stretch of 14 adenosine residues. Hence, c3B most likely represents the authentic 3' end of Pax8 mRNA. Because the Northern blot analysis indicates a Pax8 transcript of 3.1kb, approximately 600 nucleotides of the 5' untranslated region are still lacking in the full length Pax8 cDNA sequence.

Comparison of the nucleotide sequence of the paired box of Pax8 with those of published paired box genes indicates that the Pax8 and Pax2 paired domains are very similar and constitute a separate class of paired domains (Table 1).

The open reading frame of the Pax8 gene

The Pax8 cDNA clone c27B contains a large open reading frame that could translate into a protein of 457 amino acids (aa) containing the paired domain near its amino terminus (Figs 1 and 2). This open reading frame begins at nucleotide 156 and ends with a stop codon UAG at position 1527. The sequence surrounding this first AUG codon does not match perfectly with Kozak's optimal mutation consensus (CC(A/G)CCAUGG; Kozak, 1986). However, the occurrence of stop codons in all reading frames upstream of the AUG codon at position 156 strongly indicates that this is the start of an open reading frame. Furthermore, the second AUG codon, at position 459, occurs in the paired domain, making it an unlikely candidate for a translational start site.

The conservation of Pax8 paired box sequences at the amino acid level is even stronger than at the nucleotide level, suggesting a conservation of protein domain

Table 1. Nucleotide (ntd) and amino acid (aa) identities between the paired domain of Pax8 and published paired box sequences (in %)

<table>
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<th>Gene</th>
<th>ntd 1-384</th>
<th>aa 1-128</th>
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<tr>
<td>Pax1</td>
<td>67.7</td>
<td>71.9</td>
</tr>
<tr>
<td>HuP48</td>
<td>68.7</td>
<td>71.9</td>
</tr>
<tr>
<td>Pox meso(P29)</td>
<td>66.7</td>
<td>70.3</td>
</tr>
<tr>
<td>Pox neuro(P4)</td>
<td>64.3</td>
<td>71.1</td>
</tr>
<tr>
<td>Pax2</td>
<td>80.2</td>
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</tr>
<tr>
<td>HuP2</td>
<td>65.9</td>
<td>71.1</td>
</tr>
<tr>
<td>prd</td>
<td>61.2</td>
<td>64.8</td>
</tr>
<tr>
<td>gsb-p(BSH4)</td>
<td>55.7</td>
<td>60.9</td>
</tr>
<tr>
<td>gsb-d(BSH9)</td>
<td>57.8</td>
<td>62.5</td>
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The published genes are Pax1 (Deutsch et al. 1988), Pax2 (Dressler et al. 1990), Pox neuro(P4)/Pox meso(P29) (Bopp et al. 1989), HuP48/HuP1/HuP2 (Burri et al. 1988), prd (Frigerio et al. 1986; Bopp et al. 1986) and gsb-p(BSH4)/gsb-d(BSH9) (Baumgartner et al. 1989).
function (Table 1). Similar to Pax1 (Deutsch et al. 1988) and Pax2 (Dressler et al. 1990), Pax8 protein contains no paired type homeodomain, a characteristic of paired and gooseberry proteins (Bopp et al. 1986). Another protein region conserved among several paired box genes, the octapeptide, was recently described by Burri et al. (1989). The octapeptide region is found 43 amino acids downstream from the paired domain in Pax8. The spacing between the paired domain and the octapeptide in the Pax2 protein (41aa) is very similar. The Pax8

![Diagram of Pax8 cDNA]

Fig. 2. Nucleotide and predicted amino acid sequences of Pax8 gene. The paired domain sequences are boxed. A stop codon (TAG) preceding the putative ATG initiation site in the same frame, the octapeptide and the polyadenylation site (AATAAA) are indicated by the solid underlines.
could be detected on the Northern blots of poly(A) pancreas or testis. Furthermore, no \textit{Pax8} transcripts detected in brain, liver, lung, spleen, muscles, ovary, from various adult tissues revealed a 3.1kb \textit{Pax8} message in kidney (Fig. 4). No transcripts were same pattern of expression. Only results obtained and 2, respectively; Fig. 1). Both probes revealed the cDNA fragments of were used as probes; the \textit{Pax8} hybridization analyses. Two different blot and \textit{in situ} (Fig. 5C,D). The expression of \textit{Pax8} gene (Dressier et al. 1987) is from mouse (Dressler et al. 1989). The consensus sequence is as proposed by Burri et al. (1989).


class="source listing"

<table>
<thead>
<tr>
<th>Hup1</th>
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<td>Hup2</td>
<td></td>
</tr>
<tr>
<td>gsb-p (BSH9)</td>
<td>Tyr - Thc - Asn -</td>
</tr>
<tr>
<td>gsb-d (BSH9)</td>
<td>Tyr - - Asn - - -</td>
</tr>
<tr>
<td>\textit{Pax2}</td>
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</tr>
<tr>
<td>\textit{Pax8}</td>
<td>Tyr - - Asn - Leu - -</td>
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Consensus: Tyr Ser Ile Asp Gly Ile Leu Gly

\textbf{Fig. 3.} Compilation of homologous octapeptide sequences found in \textit{Pax8} and several other published paired box containing genes. The genes Hup1 and Hup2 are from man (Burri et al. 1989), gsb-p and gsb-d are from \textit{Drosophila} (Baumgartner et al. 1987), \textit{Pax2} is from mouse (Dressler et al. 1990). The consensus sequence is as proposed by Burri et al. (1989).

octapeptide differs from that of \textit{Pax2} by only one conservative amino acid substitution (Fig. 3). \textit{Pax8} protein has a repeat of leucine residues at aa positions 265, 272, 279 and 286. However, occurrence of two proline residues within this repeat makes it unlikely that this repeat forms the $\alpha$-helix, needed for a 'leucine zipper' configuration (Landschulz et al. 1988).

\textbf{The chromosomal localization of the \textit{Pax8} gene}

To determine the chromosomal location of \textit{Pax8}, we have used the mouse interspecies backcrosses (Guenet, 1986). Using an approx. 700bp genomic \textit{HindIII–NcoI} fragment from the \textit{Pax8} locus as a probe, we have demonstrated a \textit{Sau3A} restriction fragment length polymorphism (RFLP) between C57BL/6 mice and an inbred \textit{Mus spretus} line SPE/Pas (data not shown). This RFLP was used to probe DNAs from a panel of backcross progeny of (C57BL/6×SPE/Pas)$F_1$ females $\times$C57BL/6 males. The segregation pattern of \textit{Pax8} alleles was compared to the segregation patterns of all other genes analyzed in the same panel of backcrosses. \textit{Pax8} is closely linked to the surfet (surf) (0/30 recombinants scored) and HOX-5 (4/28 recombinants scored, linkage distance is approx. 14 cM organs) gene clusters within the proximal portion of chromosome 2 (Stubbs et al. 1990).

\textbf{Expression pattern of \textit{Pax8} RNA in the mouse embryo and adult tissues}

To determine the tissue specificity, temporal and spatial expression pattern of \textit{Pax8} RNA, we used Northern blot and \textit{in situ} hybridization analyses. Two different cDNA fragments of \textit{Pax8} were used as probes; the 112 bp \textit{EcoRI–NcoI} fragment from clone c960 containing the paired box sequences and the \textit{Pax8} specific 184 bp \textit{NcoI–EcoRI} fragment from clone c2A (Probe 1 and 2, respectively; Fig. 1). Both probes revealed the same pattern of \textit{Pax8} expression. Only results obtained with probe 2 are presented here.

Northern blot analysis of poly(A)$^+$ RNA samples from various adult tissues revealed a 3.1 kb \textit{Pax8} message in kidney (Fig. 4). No \textit{Pax8} transcripts were detected in brain, liver, lung, spleen, muscles, ovary, pancreas or testis. Furthermore, no \textit{Pax8} transcripts could be detected on the Northern blots of poly(A)$^+$ RNAs from 10 to 17 day p.c. embryos, even after prolonged exposure (data not shown). However, \textit{Pax8} mRNA may be scarce in samples of whole embryo poly(A)$^+$ RNA because its expression is restricted to small regions of the embryo and the level of this expression is low.

To precisely localize the spatial and temporal distribution of \textit{Pax8} RNA, \textit{in situ} hybridization analysis of tissue sections from mouse embryos of various developmental stages was done. Sections were hybridized with the $35^S$-UTP/CTP-labeled antisense RNA probes (material and methods). Sense RNA probes were used as negative controls.

\textbf{Fig. 4.} Northern blot analysis of \textit{Pax8} transcripts in the adult tissues of the mouse. Approximately 5 $\mu$g of polyadenylated RNA from corresponding tissues were hybridized with a unique \textit{Pax8} probe depicted in Fig. 1 as probe 2. The presence and integrity of RNA on the blots was confirmed by the hybridization with a $\beta$-actin probe.

\textbf{Fig. 5.} Illustrates the expression of \textit{Pax8} in the developing excretory system. Because \textit{Pax2} gene is also expressed in this system (Dressler et al. 1990), the patterns of expression of both genes were compared using parallel sections. The 527 bp \textit{BamHI–EcoRI} fragment unique for the \textit{Pax2} gene (Dressler et al. 1990) was used to produce RNA probes. Both \textit{Pax2} and \textit{Pax8} are expressed in the nephrogenic cord and in the more anterior mesonephric tubules of 10.5 day p.c. embryo (Fig. 5A–D). The mesonephric tubules are induced in the nephrogenic cord mesenchyme by the nephric (Wolfian) duct during its growth in the posterior direction. Only \textit{Pax2} was detected in the nephric duct (Fig. 5C,D).

During the next stage of kidney development, the ureteric bud emerges from the most caudal portion of the nephric duct, invades the metanephrogenic mesenchyme and branches. These branches induce the mesenchymal cellular condensations (Saxén, 1987).
Only Pax2 transcripts are detected in the branching ureter at 13.5 days gestation, whereas both Pax2 and Pax8 are expressed in the mesenchymal condensations and in the epithelial structures forming from the condensations (Fig. 5E–H). Thus, the inducing part of the secretory system, the nephric duct and the ureter, has no detectable Pax8 expression, whereas the responding part, the mesenchymal condensations and ultimately the S-shaped bodies, express Pax8. This is demonstrated more clearly in Fig. 5M,N; only the mesenchymal cells that have condensed around the ureteric duct express Pax8. The ureteric duct itself and the non-condensed mesenchymal cells show no Pax8 expression.

At day 16.5 of gestation morphogenesis proceeds in the cortex of the metanephros where Pax8 and Pax2 are expressed strongly (Fig. 5I–L). At this stage of gestation, Pax2 transcripts are also detected in the pancreas (Fig. 5K,L).

Fig. 6 shows the expression of Pax8 in the developing thyroid gland. Pax2 expression was not detected in this organ at any stage in development (data not shown). Pax8 transcripts first appear in the area of thyroid evagination from the floor of the pharynx at day 10.5 p.c. (Fig. 6A,B) and remain visible as the thyroid vesicle buds off from the floor of the pharynx and migrates caudally (Fig. 6C–H). Eventually, a two-lobed gland is formed. At this stage Pax8 is expressed in the lobes (Fig. 6L) and in the isthmus, which connects the lobes (Fig. 6K,L). No Pax8 expression is seen in the parathyroid gland, which develops independently from two pairs of pharyngeal pouches and subsequently embeds itself in the thyroid tissue (Fig. 6J).

Pax8 is expressed transiently in the myelencephalon (Fig. 71) and through the entire length of the neural tube at day 11.5 of gestation (Fig. 7A,B,E,F). The expression subsides at day 12.5 and is not detectable at day 13.5 of gestation (data not shown). The pattern of Pax8 expression in the neural tube is very similar, if not identical, to that of Pax2 at this stage of development (Fig. 7C,D,G,H). However, the genes differ in their temporal expression patterns in the CNS, because Pax2 expression in the spinal column continues at least to day 18.5 of gestation (Nornes et al. 1990).

**Discussion**

In this report, we have described a novel murine paired box gene, Pax8, which is expressed in a tissue-specific manner during development. The 2528 bp Pax8 cDNA sequence encompasses the entire coding region for a 457aa protein. A paired domain is located near the amino terminus of this conceptual protein. Interestingly, all the paired box genes described so far in man, mouse and fly contain the paired domain near the amino end of their corresponding proteins. A conservation of this domain among such different species suggests conservation of function. However, this function is at present unknown. It has been shown that the products of the *Drosophila* segmentation gene paired, which contain both paired and homeodomains, can bind specific DNA sequences (Hoey and Levine, 1988; Treisman et al. 1989). Not all of these DNA-binding activities can be ascribed to the homeodomain of the paired protein (Treisman et al. 1989). Thus, the DNA-binding function of the paired domain remains an attractive hypothesis.

In *Drosophila*, two paired box genes that do not contain a homeodomain have been described (Bopp et al. 1989). Pax8 and two other murine genes, Pax1 (Deutsch et al. 1988) and Pax2 (Dressler et al. 1990), share this characteristic. Furthermore, the paired domains of Pax8 and Pax2 have a pronounced similarity. This structural similarity is especially noteworthy because both genes have similar tissue-specific expression. Using in situ hybridization, we have compared the expression of Pax8 and Pax2 in the developing excretory system. The cellular compartments of this system result from the reciprocal inductive interactions between the growing nephric duct and later ureter, and nephrogenic mesenchyme (for review see Saxén, 1987). The nephrogenic mesenchyme responds to the induction by formation of cellular condensations, which give rise to the epithelial structures of the nephrons. The expression of both genes has parallels with the morphogenetic changes during these inductive processes: whereas Pax8 expression is restricted to the responding tissues, Pax2 transcripts can be detected both in the inducing and in the responding tissues of the kidney.

During the first stage of kidney development, the nephric duct induces the formation of the mesonephric tubules where both Pax8 and Pax2 are expressed. The tubules are organized segmentally along the rostrocaudal axis. However, this segmental pattern does not correspond to the primary segmentation of the mouse embryo into somites. Thus, it is questionable whether Pax8 or Pax2 play the part in primary segmentation analogous to their *Drosophila* homologs. Accordingly, we could not detect Pax8 or Pax2 expression by in situ hybridization at 8.5 day of gestation when segment determination is occurring (Hogan et al. 1985; Hogan et al. 1986).

The molecular mechanisms of signal transduction underlying the inductive process of epithelium forma-
Fig. 6. Expression of Pax8 in the developing thyroid gland. Sagittal section of 10.5 day p.c. embryo, magnification ×100 (A,B). TD, thyroid diverticulum; P, pharinx. Sagittal section of 11.5 day p.c., ×100 (C,D), 13.5 day p.c., ×40 (E,F) and 14.5 day p.c., ×40 (G,H) embryo. Parasagittal section (I,J) and sagittal section (K,L) of 16.5 day p.c. embryo, magnification ×40. Tr, thyroid; Ptr, parathyroid; I, isthmus; Tm, thymus.
Fig. 7. Expression of Pax8 and Pax2 in the CNS of 11.5 day p.c. embryo. Cross sections, magnification ×100 (A–D). Sagittal sections, magnification ×40 (E–H). Expression of Pax8 (A,B,E,F) and Pax2 (C,D,G,H). Tr, thyroid. Parasagittal section through the myelencephalon, expression of Pax8 (I,J), magnification ×40.
tion in the metanephric kidney are not known. Two levels of determination can be distinguished in this process. First, the metanephrogenic mesenchyme isolated from an 11 day embryo is already predetermined to respond to the inducing agents by tubule formation (Grobstein, 1955; Saxén, 1970). It may imply that the cells of the metanephric blastema have already reached a state in which only few additional factors are needed to start morphogenesis. Hypothetically, transcription factors, Pax8 and Pax2 among them, that are induced in these cells during this period could play a crucial role in this process. Not only ureter but also neural tube can induce epithelialization of the mesenchymal cells (Grobstein, 1955). Interestingly, Pax2 is expressed in both of these tissues. The second level, an actual determination of the mesenchymal cells to become the components of nephron is achieved after approximately 24 h of contact with the inductor (Saxén and Lehtonen, 1978). The molecular events that take place during this time are completely obscure. Subsequently, irreversible morphogenetic changes occur including the formation of the basal membrane, establishment of the epithelial polarity and an increase in cellular adhesivity (Saxén, 1987).

Profound molecular changes in the extracellular matrix and cell surface molecules during the nephron formation have also been reported recently. The expression of fibronectin and interstitial type I and type III collagens is replaced by the basement membrane components including type IV collagen and laminin chains (Ekblom, 1981; Ekblom et al. 1981b; Ekblom et al. 1980; Senior et al. 1988; Laurie et al. 1989; Ekblom et al. 1990). Antibodies against laminin A chain can inhibit the polarization of the mesenchymal cells, suggesting a functional role for laminin in this process (Klein et al. 1988b). Another extracellular matrix glycoprotein, tenascin, is induced around the mesenchymal condensations (Aufderheide et al. 1987). Furthermore, neural cell adhesion molecules (N-CAM) are replaced by uvomorulin, another primary CAM (Vestweber et al. 1985; Klein et al. 1988a). Another result of induction is that the mesenchymal cells acquire the responsiveness to transferrin (Ekblom et al. 1983) and express the desmosomal proteins (Garrod and Fleming, 1990). Being putative transcription factors, Pax8 and Pax2 may be involved in the described molecular processes.

It is also worth noting that the developing nephron itself becomes segregated into three segments, i.e., glomerulus, proximal and distal tubules. The markers specific for each of these segments have been described (Ekblom et al. 1981a). Pax8 and Pax2 are molecular markers characteristic of both early mesenchymal condensations and late epithelial structures resulting from condensations.

Using an interspecies backcross, we have mapped Pax8 to the centromeric region of mouse chromosome 2 in a close linkage to the surf locus. It would be interesting to determine whether the human homolog of Pax8 is located on human chromosome 9q which reveals a strong synteny to proximal mouse chromo-

some 2 (Yon et al. 1989; Stubbs et al. 1990). Several mouse developmental mutations including Danforth's short tail (Sd), stubby (stb), fidget (fi), lethargic (lh) and rachiterata (rh) are linked in the proximal portion of mouse chromosome 2 (Davisson et al. 1988). The semidominant Sd mutation is especially interesting in context of the present report. Recently, the surf cluster has been positioned within close proximity of the Sd locus (Stubbs et al. 1990). Thus, Pax8, surf and Sd map close to each other. The Sd mutation is characterized by the abnormalities of the axial skeleton and the reduction or absence of kidneys (Dunn et al. 1940). The phenotype of the skeleton and kidneys may both have a common origin from the abnormality of the notochord in the Sd mice (Grüneberg, 1958). However, the organ culture studies have revealed a reduction of the tubule formation intrinsic to the mutant metanephrogenic mesenchyme (Gluecksohn-Waelsch and Rota, 1963). Because Pax8 is expressed in the developing tubules, it would be interesting to investigate its association with the Sd mutation.

The expression of Pax8 in the developing neural tube, secretory system and thyroid gland, which originate from ectoderm, mesoderm and endoderm, respectively, may indicate pleiotropic functions of the Pax8 gene. Furthermore, we cannot exclude the possibility that Pax8 is also expressed in the mesenchymal component of the developing thyroid. Both in the mesenchymal condensations of the kidney and in the developing thyroid gland, Pax8 expression is associated with the zones of changes in cell proliferation (Saxén et al. 1983; Smuts et al. 1978). Common functions of Pax8 in the developing kidney and thyroid associated with the appearance and/or maintenance of the cell polarization also cannot be ruled out (Chambard et al. 1981).

Because of the overlapping expression pattern of Pax8 and Pax2, it would be interesting to find out whether these two gene products interact with each other at the molecular level.

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References


Pax8 expression during embryogenesis


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