Murine genes with homology to Drosophila segmentation genes

GREGORY R. DRESSLER\(^1\), URBAN DEUTSCH\(^1\), RUDI BALLING\(^1\), DOMINIQUE SIMON\(^2\), JEAN-LOUIS GUENET\(^2\) and PETER GRUSS\(^1\)

\(^1\)Department of Molecular Cell Biology, Max Planck Institute of Biophysical Chemistry, 3400 Goettingen, FRG
\(^2\)Unité de Genetique de Manumiferes, Institut Pasteur, 25 rue de Dr Roux, Paris 75724 Cedex 15, France

Summary

As a potential method to define genes that control vertebrate segmentation processes during embryogenesis, murine genes containing a protein domain homologous to the Drosophila paired box sequence were isolated. The mouse genome contains at least three genes with highly conserved paired box like sequences, termed Pax genes, that are also conserved in other vertebrates. During embryogenesis, the PaxI gene is expressed in ventral sclerotome cells and in the intervertebral disks of the vertebral column. Thus, PaxI clearly shows a segmented pattern of expression. A second gene, Pax2, also is expressed in segmented structures of the intermediate mesoderm and in certain regions of ectoderm derived tissues. The PaxI and Pax2 genes map to chromosomes 2 and 7, respectively. Interestingly, several known mutations that affect vertebral column development map near the PaxI locus.

Key words: paired box genes, segmentation, vertebrate development, intervertebral disks.

Introduction

The genetic mechanisms controlling segmentation during mammalian embryogenesis still remain poorly understood. In contrast, many developmental genes and mutations have been characterized in Drosophila, which has led to a more thorough understanding of segmentation, pattern formation, and the interactions among these processes (for reviews see Akam, 1987; Scott & Carroll, 1987). The hypothesis that certain genetic mechanisms employed during development are highly conserved even between divergent species has been substantiated by the isolation of many vertebrate genes based on homology to conserved protein domains found among Drosophila homeotic and segmentation genes (McGinnis et al. 1984; Hart et al. 1985; Colberg-Poley et al. 1985; Joyner et al. 1985). Recently, mouse homologues to segmentation genes have been isolated based on a conserved sequence found among several Drosophila genes that specify inter- and intrasegmental boundaries (Deutsch et al. 1988). As in Drosophila, there appear to be at least three genes sharing this particular protein domain. Whether these murine segmentation homologues are also involved in the subdivision of the rostrocaudal axis during embryogenesis remains an intriguing possibility that, in light of the interspecies divergence between Drosophila and mouse, would be a tribute to the design and adaptability of such a genetic regulatory system.

The Drosophila paired box

It has been proposed that segmentation during Drosophila embryogenesis entails a progressive subdivision of the embryo into increasingly smaller units (Nüsslein-Volhard & Wieschaus, 1980). This subdivision is controlled by the gap, pair-rule and segment polarity genes whose respective mutations result in the deletion of adjacent segments, the deletion of alternate or parts of alternate segments and the deletion of segment parts with the mirror-image duplication of the remainder (Nüsslein-Volhard & Wieschaus, 1980). Recently, a 128 amino acid conserved protein domain, termed the paired box, was identified among the segmentation genes paired (prd), gooseberry-proximal (gsp-p), and gooseberry-distal (gsp-d) (Bopp et al. 1986; Cote et al. 1987). As
the *prd* gene is a member of the pair-rule class and the *gsb* genes are segment polarity genes, it was suggested that the conserved domains among these genes may provide a functional link between the generation of individual segments and the polarization of segments into anterior and posterior halves.

The spatial expression of the *prd* gene during *Drosophila* development undergoes a shift from a 7-band striped pattern with double segment periodicity to a 14-band striped pattern with single segment periodicity at the blastoderm stage (Kilchherr *et al.* 1986). The spatial expression of the *gsb-d* is similar to *prd*, initially showing 7 bands and dividing into 15 bands after germ band elongation (Bopp *et al.* 1986; Cote *et al.* 1987). The *gsb-p* gene, however, only exhibits the 15-band pattern with an additional stripe anterior to *gsb-d* (Bopp *et al.* 1986; Cote *et al.* 1987). Thus, the patterns of gene expression correlate directly with the nature of the mutant phenotypes.

The murine Pax gene family

Using the *Drosophila* paired box sequence, Deutsch *et al.* (1988) have isolated a murine sequence from a genomic library that shows a high degree of amino acid conservation with all three *Drosophila* paired box sequences. This mouse sequence, termed Pax1 for paired box containing gene, was used to investigate the presence of paired box sequences in other vertebrates. Fig. 1 shows the results obtained when ³²P-labelled Pax DNAs were hybridized, under low stringency conditions, to restriction-enzyme-digested DNA from frog, turtle, chicken, hamster, rabbit, mouse and human. The multiple bands observed with both *PstI* and *BamHI*-digested DNA is evidence for multiple Pax sequences present in the genomes of all the vertebrates examined.

With the isolation of several independent cDNA clones from mouse embryonic libraries, the presence

---

Fig. 1. Southern blot of restriction-enzyme-digested DNA hybridized with murine paired box sequences. DNAs were digested with *PstI* (P) or *BamHI* (B), hybridization was done at 37°C in 43% formamide and the washing was done at 42°C in 2×SSC.
Fig. 2. A comparison of amino acid conservation between mouse and Drosophila paired box sequences. The numbers of identical amino acids between two sequences are shown in the corresponding squares. The intensity of shading reflects the degree of homology between the sequences. The deduced protein sequences for prd (Frigero et al. 1986), gsb-p and gsb-d (Bopp et al. 1986), and Paxl (Deutsch et al. 1988) are published. The protein sequence for Pax2 is unpublished.

of multiple Pax genes in the mouse was firmly established (G. Dressler & U. Deutsch, unpublished data). A comparison of amino acid conservation for the paired box domains, as deduced from DNA sequences, is shown in Fig. 2. The Drosophila paired box was originally described as being 128 amino acids in length, with the gsb-p box having a three amino acid internal deletion. The homologous mouse sequences, Paxl and Pax2, appear to be approximately six amino acids shorter. Thus, only 120 amino acids were used for the comparison. As might be expected, the three Drosophila genes show more homology to each other than to the mouse genes. However, Paxl and Pax2 are not significantly more homologous to each other than to the Drosophila sequences. In fact, Pax2 shows a slightly higher level of conservation (87/120) to the gsb-d gene when compared to the Paxl gene (85/120).

In addition to the paired box, the Drosophila genes prd, gsb-d and gsb-p also share a paired homebox domain (Bopp et al. 1986). It remains to be determined whether the murine Paxl and Pax2 genes also contain homeboxes, although a third murine gene, Pax3, appears to contain both a paired box and a homebox (U. Deutsch, unpublished data).

Segmentation in the mouse and the expression of Paxl

It is not altogether obvious that a mouse can be considered a segmented organism, as compared to an adult fruit fly for example. However, during the development of the mouse it is quite clear that segmentation plays an important role in the organization of the body plan and in the generation of individual tissues. Somitogenesis, the formation of metamerical units along the rostrocaudal axis lateral to the neural tube, is the most obvious example of segmentation in the mouse (reviewed by Hogan et al. 1985). The somites subsequently differentiate into dermatome, myotome and sclerotome, which ultimately generate the skin, skeletal muscles and axial skeleton, respectively. Ectoderm-derived tissues also show segmented characteristics, particularly the neural plate which is divided into neuromeres (Tuckett et al. 1985; Sakai, 1987) and the spinal ganglia, a neural crest cell derivative. Segmentation in tissues derived from the intermediate mesoderm is evident in the pro- and mesonephric tubules, the embryonic excretory organs.

The extent of Paxl expression during murine embryogenesis has been described in detail (Deutsch et al. 1988). Two representative micrographs and a summary of the Paxl transcription pattern are presented in Fig. 3. Paxl transcripts can first be detected at 9 days post coitum (p.c.) and are restricted to the sclerotome cells of the differentiated somite. Therefore, Paxl expression is initiated after the primary segmentation of the presomatic mesoderm. At 10 days p.c., Paxl transcripts can be detected as a continuous band of hybridization in ventral sclerotome cells along the entire rostrocaudal axis beginning at approximately the fourth occipital somite. Paxl-expressing cells appear to migrate ventromedially to surround the notochord. By 12 days p.c., the expression has undergone a shift and now exhibits a striped pattern, restricted to the anlagen of the intervertebral disks (Fig. 3A). By 14 days p.c., it is clear that the intervertebral disk cells are expressing Paxl (Fig. 3B). Expression is also detected in the sternum and the thymus. At later times, expression in
Fig. 3. A summary of Pax1 expression in the developing vertebral column as determined by Deutsch et al. (1988). (A) In situ hybridization of Pax1 to midsagittal section from a 12-day p.c. embryo showing dense labelling between the prevertebrae. (B) In situ hybridization of Pax1 to midsagittal section from a 14-day p.c. embryo showing dense labelling to the intervertebral disks. The dorsal side is at the top and rostral end is to the left. (C) Schematic representation of Pax1-expressing cells in a frontal view of the developing vertebral column.
the developing vertebral column decreases until it can no longer be detected in newborn animals.

If the somite is considered the primitive segment, then subsequent somite differentiation and sclerotome division into vertebrae and disk cells might be considered segment polarization events. Since Pax1 expression is initiated after primary segmentation has occurred, Pax1 may function, by analogy to Drosophila, as a segment polarity gene specific for one half of the vertebral segment, the disk.

The Pax2 gene also exhibits a spatial and temporal expression pattern in segmented structures during embryogenesis. Pax2 can first be detected in the extending mesonephric duct and mesonephric tubules, as well as the developing metanephros (Dressler, unpublished). In addition, Pax2 transcripts can be detected in the neural tube at the border of the ependymal and mantle layers and in the ventral horns. It is of interest to note that Pax2-expressing cells in the intermediate mesoderm are adjacent to the mesoderm-derived ventral sclerotome cells which express Pax1.

The Pax gene expression patterns in the vertebral column and the intermediate mesoderm extend along the rostrocaudal axis. In contrast, expression patterns of some mouse homeobox genes (Dony & Gruss, 1987; Gaunt, 1987; Holland & Hogan, 1988; Le Mouellic et al. 1988; Breier et al. 1988) are region specific in mesoderm-derived tissues. This important distinction could reflect the functional differences between the Pax genes, required for segmentation along the entire rostrocaudal axis, and homeobox genes, required for positional specification along the axis.

The chromosomal location of murine Pax genes

In order to determine the chromosomal location of the Pax genes, the method of mouse interspecies crosses was used (reviewed by Guenet, 1986). Restriction fragment length polymorphisms (RFLPs), for Pax1 and Pax2, between C57BL/6 mice and an inbred Mus spretus line SPE/Pas (maintained in the laboratory of J.-L. Guenet) were used to probe DNAs from a panel of backcross progeny of (C57BL/6×SPE/Pas) F1 females × C57BL/6 males. The chromosomal assignment of the Pax genes was then determined by matching the RFLPs to known genetic markers. The Pax1 gene is closely linked to the agouti locus on chromosome 2. As 4/63 recombinants were scored, the linkage distance between agouti and Pax1 is approximately 6 ± 3 cM. Similarly, the Pax2 gene is linked to the cHa-ras gene on chromosome 7 (23 cM ± 6 cM).

Within 7 cM of the agouti locus on chromosome 2 are two known mutations that, in light of the expression pattern of Pax1, are worth mentioning. The recessive mutation diminutive (dm) results in macrocytic anaemia and consistent smaller body size. In addition, dm homozygotes have an additional rib at both ends of the thorax, additional presacral vertebrae, malformed vertebrae, and fused ribs (Stevens & Mackensen, 1958). Potentially more relevant is the recessive mutation undulated (un). Mice homozygous for un have malformed vertebrae along the entire body axis because anterior sclerotome cells fail to join the posterior cells from the adjacent sclerotome during vertebrae formation. The result is a smaller vertebra and a larger intervertebral disk (Gruneberg, 1954). Although the anomalies described, particularly for un, correlate with the time and position of Pax1 expression, any definitive correlation will require more precise linkage distance determinations.

Conclusions

The unusually high degree of amino acid conservation among Drosophila paired box domains and murine Pax domains suggests a conservation of function that may reflect similar genetic mechanisms. Thus, the isolation and characterization of murine paired box containing genes is a potential method for unravelling the morphogenetic basis of segmentation during embryogenesis. During the course of evolution, however, the paired box domain may have been adapted such that it retains its molecular function but in another context. The possibility that the Pax genes are involved in cellular migration, intercellular communication, or other physiological processes associated with the formation of structures such as the vertebral column cannot be ruled out. Although the Pax gene expression patterns during mouse development are revealing, conclusive evidence regarding the developmental nature of these genes will require mutational analysis, perhaps through the application of gene-targeting techniques currently under development.

References


Primary structure and developmental expression pattern of \textit{Hox}3.1, a member of the murine Hox 3 homeobox gene cluster. \textit{EMBO J.} 7, 1329–1336.


