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The *bicoid*-related Pitx gene family in development

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The important roles of homeobox genes in development of the hindbrain and axial body are well established. More recently, it has become clear that certain subfamilies of homeobox genes play particularly important roles in the development of more anterior structures. These have included the *paired* gene family in the eye (Gehring, 1996; Hanson and Van Heyningen, 1995; Macdonald and Wilson, 1996; Wehr and Gruss, 1996), the *orthodenticle* and *distalless* gene families in the fore- and midbrains (Acampora et al., 1996; Acampora et al., 1995; Price et al., 1991; Simeone et al., 1994; Williams, 1998), and the *Lhx* gene family in the pituitary gland (Sheng et al., 1997; Sheng et al., 1996). This review summarizes the newly identified *Pitx* gene family and its role in development. This family includes three vertebrate paralogues that have been cloned in multiple organisms, and a fly cognate. Mutations in two members of this gene family lead to human disease or birth defects affecting anterior structures.

The nomenclature for this gene family has been complicated by the fact that members have been cloned and uniquely named by more than one laboratory (Table 1). The first member of this family, mouse *Ptx1* (pituitary homeobox 1) was isolated as a transcription factor involved in pro-opiomelanocortin gene transcription in anterior pituitary corticotropes (Lamonerie et al., 1996). However, since some pentaxin genes in mouse and human had previously been assigned the *Ptx* gene symbol, the gene symbols for the three mouse paralogues for this new homeobox gene family are *Pitx1, Pitx2,* and *Pitx3* (Mouse Genome Database). In this review, we have adopted the official nomenclature of the MGD and propose that, for clarity, this nomenclature be adopted for other organisms.

Three vertebrate paralogues, *Pitx1, Pitx2,* and *Pitx3,* have all been cloned from mouse and human (Table 1 and references therein). Some paralogues have also been cloned from chicken (*Pitx1* and *Pitx2*), xenopus and zebrafish (*Pitx2*), and rat (*Pitx3*) (Table 1 and references therein). In two reports, mouse *Pitx1* was cloned in functional assays: in a two-hybrid screen using Pit-1 as bait (Szeto et al., 1996) and as noted above. Human *PITX2* was identified by positional cloning of the Rieger Syndrome gene (Semina et al., 1996). In the other reports, cloning was the result of using degenerate PCR or low stringency hybridization to detect expressed homeobox sequences in a variety of embryonic and adult tissues. The difficulty in cloning *Pitx1* from xenopus and zebrafish has suggested that this orthologue may not be as widely distributed in nature as *Pitx2* (Kitamura et al., 1997). However, the recent identification of a fly *Pitx* gene during a chromosome walk demonstrates that this gene family arose prior to the divergence of vertebrates and invertebrates (Vorbruggen et al., 1997). Each vertebrate paralogue has been mapped genetically in mouse and human (Table 1).

The Pitx proteins all belong to the *bicoid*-related subclass of homeodomain proteins because they encode the defining lysine at residue 50 within the homeodomain. This residue, at residue 9 within the recognition helix of the homeodomain, is the major determinant of DNA binding specificity (Gehring et al., 1994; Hanes and Brent, 1989). Several members of this small subfamily are essential for axis and pattern formation (Ang et al., 1996). *Pitx2* expresses multiple protein isoforms as a result of alternative splicing (Gage and Camper, 1997; Kitamura et al., 1997) and the use of different promoters (P. Gage and E. Semina, unpublished results) (Fig. 1). The three vertebrate paralogues are all highly conserved at the amino acid level (Fig. 1). For example, in mouse the Pitx2 and Pitx3 homeodomains are identical while Pitx1 differs by only two amino acids. The paralogues are also conserved C-terminal to the homeodomain (55–70%). In contrast, the N-termini of these proteins are essentially unrelated. The vertebrate orthologues are even more highly conserved. For example, there the mouse and chicken Pitx2a proteins are 96% identical with only ten amino acid substitutions between them. The *Drosophila* Pitx protein shows high conservation to the vertebrate proteins within the homeodomain (90–93%) and a short region near C-terminus that has been termed the OAR sequence (Furukawa et al., 1997) or the C-peptide (Kitamura et al., 1997). This sequence is present in several homeobox genes. In *Pitx2,* this domain appears to function as an intrinsic inhibitor of DNA binding activity whose function can be modulated by protein-protein interactions (Amendt et al., 1998).

The vertebrate *Pitx* genes each have unique developmental and tissue-specific expression patterns (Fig. 2 and Table 2). However, there are several significant overlaps in expression pattern (Fig. 2). The most significant may be in the eye, where both *Pitx2* and *Pitx3* are expressed in the mesenchyme and its derivatives (Semina et al., 1998; Semina et al., 1996; Smidt et al., 1997). Demonstration in humans that mutations to *Pitx2* result in Rieger's Syndrome (Semina et al., 1996) and mutations to *Pitx3* result in anterior segment mesenchymal dysgenesis and dominant cataracts (Semina et al., 1998) confirmed the importance of these genes in eye development. These autosomal-dominant conditions each affect the development or maintenance of anterior structures of the eye. Interestingly, mouse *Pitx3* maps near *aphakia,* a recessive mutation resulting in small eyes that lack lenses and fail to develop beyond 11 days of gestation (Semina et al., 1997). Rieger's Syndrome patients frequently show defects in dental and umbilical development in addition to their ocular defects (Feingold et al., 1969; Rieger, 1935), and subsets of patients also present with isolated growth insufficiency (Feingold et al., 1969).

Several observations suggest that *Pitx* genes are also important for the development and function of other organs. The stomodeum is an ectoderm-derived layer of epithelium that derives from the anterior neural ridge and forms the earliest mouth structures (Couly and Le Douarin, 1985). *Pitx1* expression defines the stomodeum and continues within stomodial derivatives, including the nasal pit and Rathke's pouch (Lanctot et al., 1997). *Pitx1* is also expressed more caudally in the posterior lateral plate and extra-

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Table 1. Pitx gene family

Vertebrate	Species	Names	Mapping	Mutations[a]	References
Pitx1					
	Mouse	*Ptx1* *P-OTX* *Bft* *Brx2*	13	dumpy? mdac?	(Lamonerie et al., 1996) (Crawford et al., 1997) (Shang et al., 1997) (Szeto et al., 1996) (Kitamura et al., 1997)
	Human	*PTX1* *BFT*	5q31 5q22-q31		(Crawford et al., 1997) (Shang et al., 1997)
	Chicken	*cPtx1*			(Lanctot et al., 1997)
Pitx2					
	Mouse	*Ptx2* *Rieg* *Otlx2* *Brx1* *Arp1*	3		(Gage and Camper, 1997) (Semina et al., 1996) (Muccielli et al., 1996) (Kitamura et al., 1997) (Arakawa et al., 1998)
	Human	*RIEG*	4q25	Rieger's syndrome	(Semina et al., 1996)
		ARP1			(Arakawa et al., 1998)
	Chicken	cBrx1			(Kitamura et al., 1997)
	Xenopus	xBrx1			(Kitamura et al., 1997)
	Zebrafish	zBrx1			(Kitamura et al., 1997)
Pitx3					
	Mouse	*Pitx3*	19	aphakia?	(Semina et al., 1998; Semina et al., 1997)
	Rat	*Ptx3*			(Smidt et al., 1997)
	Human	*Pitx3*	10q25	Anterior segment mesenchymal dysgenesis and Dominant Cataracts	(Semina et al., 1998)
Nonvertebrate					
	Drosophila	*Ptx1*			(Vorbruggen et al., 1997)

[a] (?) Indicates that gene is a candidate in mouse based on genetic mapping.

embryonic mesoderm (Lanctot et al., 1997). *Pitx1* and *Pitx2* are the earliest known genetic markers for the incipient Rathke's pouch, the precursor to the anterior and intermediate lobes of the pituitary gland (Gage and Camper, 1997; Lanctot et al., 1997; Muccielli et al., 1996). *Pitx1* is expressed in cell lines representing the five anterior pituitary neuroendocine cell lineages (Tremblay et al., 1998), while *Pitx2* expression is absent only from corticotropes (Gage and Camper, 1997; Tremblay et al., 1998). Lineage specific expression of *Pitx1* and *Pitx2* protein will need to be confirmed using highly specific antibodies in immuno-colocalization experiments. The isolated growth hormone insufficiency in subsets of Rieger's patients is consistent with an essential role for *Pitx2* in the somatotrope lineage. *Pitx1* binds to and transactivates a *cis*-acting element required for activation of *Pomc* (Lamonerie et al., 1996). *Pitx1* also *trans*-activates several other pituitary-specific promoters (Szeto et al., 1996; Tremblay et al., 1998). Importantly, *Pitx1* appears to be required in cell culture for expression of *Lhx3*, which is essential for pituitary development (Tremblay et al., 1998).

Roles in brain development have been proposed for *Pitx2* and *Pitx3* based on their expression patterns. Early *Pitx2* expression patterns within the prosencephalon and mesencephalon are consistent with the prosomeric model of fore- and midbrain development (Kitamura et al., 1997; Muccielli et al., 1996; Rubenstein et al., 1994). Subsequently, *Pitx2* expression becomes limited to discrete brain nuclei (Kitamura et al., 1997; Muccielli et al., 1996). *Pitx3* expression is even more refined within the developing midbrain, being expressed specifically within neurons of the mesencephalic dopaminergic system (Smidt et al., 1997). Results from analysis of these cells in Parkinson's patients, as well as rat and mouse models for this disease, are consistent with a role for *Pitx3* in the determination and maintenance of this specific neuronal lineage (Saucedo-Cardenas et al., 1998; Smidt et al., 1997). Interestingly, *bicoid* is required for formation of anterior structures in the fly (Driever and Nusslein-Volhard, 1988; Driever et al., 1990), as are the closely related *Otx1* and −2 in both fly and vertebrates (Ang et al., 1996).

Pitx2 has recently been identified as a gene whose expression is down-regulated in *All1* double-knockout mouse embryonic stem cells (Arakawa et al., 1998). *ALL1*, the human homologue of *Drosophila trithorax*, is frequently rearranged in different human acute leukemias (Gu et al., 1992; Tkachuk et al., 1992). *PITX2* is expressed in normal bone marrow and expression in human acute leukemia cell lines correlates with rearrangement of *ALL1*. The ALL1 protein can bind a *Pitx2* promoter fragment and activates expression of linked reporter genes. Together, these data suggest a role for *Pitx2* in ontogeny of several hematopoetic lineages (Arakawa et al., 1998).

The most recent, exciting development in understanding the function of the *Pitx* gene family has been the demonstration that manipulation of *Pitx2* expression is sufficient to reprogram the left/right body asymmetry in vertebrates (Harvey, 1998; Yoshioka et al., 1988; Logan et al., 1988; Piedra et al., 1988; Ryan et al., 1998). In early mouse development, preceding the onset of visceral organogenesis, the normal pattern of *Pitx2* expression is asymmetric. The mouse mutation *iv*, which causes discordant reversals of heart and visceral organ situs (heterotaxia), perturbs the pattern of *Pitx2* expression and the pattern of other genes involved in establishing left-right asymmetry like *nodal* and *lefty-2*. Manipulation

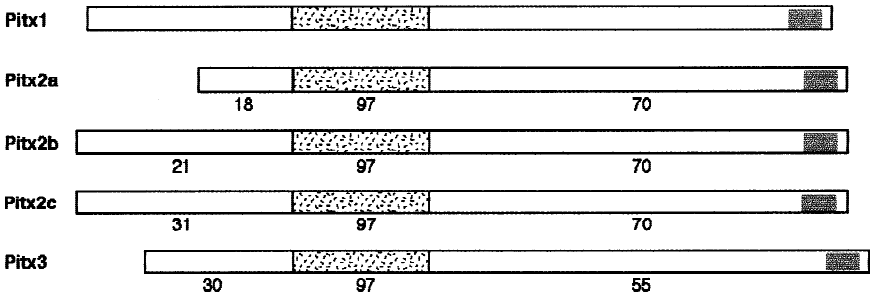

Fig. 1. Percent identity among mouse Pitx paralogues. Numbers represent the percent identity relative to Pitx1 within the homeodomains (stippled), as well as the regions N- and C-terminal to the homeodomain. The position of the OAR domain is also indicated (shading).

Major sites of developmental expression

Tissue	*Pitx1*	*Pitx2*	*Pitx3*
Branchial arches	+	+	
Eye		+	+
Pituitary	+	+	
Forebrain		+	
Midbrain		+	+
Teeth	+	+	
Heart		+	
Forelimb mesenchye		+	
Hindlimb mesenchyme	+	+	

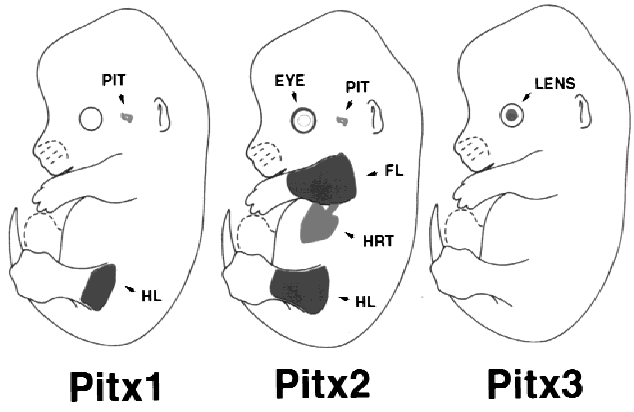

Fig. 2. Summary of major sites of developmental expression. Idealized expression patterns at e13.5.

Table 2. Additional sites of developmental expression

Vertebrate	
Pitx1	
	Stomodeum
	First branchial arch
	Tongue
	Palate
	Olfactory system
	Posterior lateral plate mesoderm
	Body wall muscle
	Bladder
	Stomach
	Hindgut
	Pancreas
Pitx2	
	Tongue
	Maxillary and mandibular epithelia
	Umbilicus
	Kidney
	Bone marrow
	Lung
Pitx3	
	Mesencephalic dopaminergic neurons of midbrain
	Tongue
	Mesenchyme around spinal column and sternum
Nonvertebrate	
Pitx1	
	Developing midgut and malpighian tubes
	Developing CNS
	Brain
	Head sensory organs
	Subset of somatic muscle precursors to ventral larval muscles

of chick and frog embryos has provided critical mechanistic information on left-right patterning. Sonic hedgehog (*Shh*) becomes restricted to the left at an early stage in the pathway through interaction with *activin*β*B*, and it acts upstream of *nodal* and *lefty-2*. *Pitx2* appears to be the most downstream gene in this pathway, because blocking *Shh* signaling and delivery of *Pitx2* on the right side by retroviral infection causes bilateral or randomized development. These experiments provide convincing evidence of a role for *Pitx2* in vertebrate patterning of laterality. It will be interesting to determine whether loss of *Pitx2* by gene targeting in mice influences patterning in a manner similar to that of ectopic expression in chick embryos, or whether other proteins can compensate.

Pitx2 and *Pitx3* mutations in humans have confirmed that *Pitx* gene family members are important developmental regulators. Further evidence suggests a broader role for these genes in the development of specific tissues. Importantly, the human conditions described to date are autosomal-dominant and are presumed to result from haplo-insufficiency (Semina et al., 1998; Semina et al., 1996). The effect of loss-of-function in these genes is likely to be much more severe in the homozygous state, as has been observed for other homeobox genes (e.g., *Pax6*, *Mitf*, *Pax3*). Interestingly, the lack of morphologically apparent phenotype in fly *Pitx1* gain- and loss-of-function has implied a role for this gene in controlling physiological cell functions rather than pattern formation (Vorbruggen et al., 1997). Thus, careful genetic dissection of these genes in model organisms will be required to fully understand their role in the development and maintenance of their respective tissues.

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