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PAX PROTEINS IN EMBRYOGENESIS AND THEIR ROLE IN NERVOUS SYSTEM DEVELOPMENT

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ABSTRACT

The mammalian Pax genes encode a family of transcription factors, which play important roles in embryonic development and organogenesis. During the central nervous system development Pax genes have substantial roles in neural differentiation and regional specification. Pax proteins are expressed in populations of nerve cells within the developing forebrain, midbrain, hindbrain and spinal cord. In this article the fundamental roles of Pax proteins in the embryogenesis are outlined and the temporal and spatial influences of Pax on the formation of the central nervous system are discussed.

Keywords: *human embryos, Pax, brain, spinal cord*

INTRODUCTION

Regionalization of the neural tube is a fundamental event in the development of the central nervous system [40]. Early patterning of the central nervous system results in subdivision of the neural tube along the dorsal-ventral and cranial-caudal axes into the forebrain, midbrain, hindbrain and spinal cord. In the regional specialization of these central nervous system domains, different families of transcriptional regulators are involved, one of which is the *Pax* gene family [39]. Pax proteins are common to most or all animals, are organized in clusters, and define positional information along the axis of neural tube [7]. Studies in animals and human embryos have shown the important role of Pax proteins in the formation of main subdivisions of the developing brain [2, 14, 21, 37]. Specific mutations within the number of Pax genes lead to developmental abnormalities in both humans and mice [10, 23, 41].

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In this paper, the basic roles of Pax proteins in the embryogenesis are highlighted and the temporal and spatial influences of Pax in signaling from the early steps of nervous system formation are discussed.

BASIC ROLES OF PAX PROTEINS IN MAMMALIAN EMBRYONIC DEVELOPMENT

Pax proteins are characterized by the presence of the paired domain of 128 amino acids, which bind to DNA [8, 22]. In addition to the paired domain, many Pax proteins contain a further DNA binding domain of the paired class homebox family, which has been used to subdivide them into a subgroup comprising Drosophila and vertebrate genes [5]. Crucial functions during organogenesis have been ascribed to several Pax family members in a variety of organisms. Recent studies have provided relevant data suggesting that particular Pax proteins have specific roles at several stages of organ development [9, 10]. The mammalian Pax genes have been divided into four subgroups based on genomic structure, sequence similarity and conserved function (Table 1) [9]. Pax genes have been cloned from a diversity of metazoans, including nematodes, arthropods and many vertebrates. Their highly conserved function across much of the animals has prompted the recent cloning of a number of Pax homologs from more primitive organisms, such as corals, sponges and hydra [18, 24, 35]. More than 100 Pax protein sequences are now available in public databases.

Table 1. Pax proteins and their target tissues and organs in mammals

Subgroup	Pax proteins	Target organs and tissues
I	Pax1	Sclerotome, perivertebral mesenchyme, thymus
	Pax9	Sclerotome, perivertebral mesenchyme, cranio-facial development, tooth
II	Pax2	Central nervous system, kidney, eye, inner ear
	Pax5	Central nervous system, B-cells
	Pax8	Central nervous system, thyroid gland, kidney
III	Pax3	Central nervous system, dermomyotome, neural crest, muscle
	Pax7	Central nervous system, dermomyotome, muscle, neural crest, cranio-facial development
IV	Pax4	Pancreas
	Pax6	Central nervous system, eye, pancreas

The members of Pax proteins are important for maintaining the normal function of certain cells. To carry out these roles, the Pax genes provide instructions for making proteins that attach to specific DNA and help control the activity of particular genes. Mutations of Pax genes lead to disorders that involve the incomplete development of tissues in which a particular Pax gene is misexpressed [42]. Several Pax mutations have been identified in humans [1, 6, 12, 16, 17, 19, 20, 32, 36]. Additionally, overexpression of Pax proteins has been noted in a variety of cancers [11, 15, 41]. Mutations in these Pax genes which have been associated with human diseases indicate that their activity is important for proper development of the embryo. In Table 2 most known defects and diseases caused by Pax gene mutations in humans are briefly summarized.

Table 2. Pax genes associated with human diseases

Pax genes	Human diseases
Pax1	Vertebral malformations
Pax9	Oligodontia
Pax2	Renal-coloboma syndrome, Charge syndrome, Wilms tumor, Kaposi sarcoma
Pax5	Large cell lymphoma
Pax8	Congenital hypothyroidism, thyroid follicular carcinoma
Pax3	Waardenburg syndrome, rhabdomyosarcoma, Craniofacial-deafness-hand syndrome
Pax7	Rhambdomyosarcoma
Pax4	Type II diabetes
Pax6	Aniridia, microphthalmia, genitourinary anomalies, mental retardation syndrome

EXPRSSION OF PAX PROTEINS IN NEURAL TUBE DEVELOPMENT

Recent evidence from mouse and human embryos indicates that Pax proteins play an important role in early embryogenesis [15, 25, 33, 34, 38]. Development of the mammalian neural tube involves several temporally restricted processes: cell proliferation, migration, differentiation and cell death. Pax proteins have a restricted expression pattern along the craniocaudal and dorsoventral axes of the neural tube. The most caudal part of the neural tube develops during the process of secondary neurulation. By the end of Carnegie stage 14 (CS14), three layers differentiate in the lateral walls of the neural tube: the neuroepithelial, the mantel and the marginal layer [30, 37]. During following weeks, mitotic activity gradually ceases and the specific neurons differentiate according to their dorsal-ventral position, thus forming the definitive spinal

cord [38]. One of the first steps in the neural tube formation is the repression of Pax3 and Pax7 expression, which allows the neuroepithelial cells to form the ventral plate (floor plate) [8]. The cells of the floor plate itself then become sites of production of Shh. In the roof plate of the developing neural tube, BMP-s act as patterning signals and induce further dorsalizing molecules Pax3 and Pax7. In our studies on human embryos, expression of Pax2 proteins was seen in many types of early-differentiated neurons, located in the neuroepithelial (Figure 1) and marginal layers of the developing neural tube. In later developmental stages (CS16-20), expression of Pax2 is detected in the neuroepithelial, mantel and marginal layers of the developing spinal cord in human embryos (Figure 2), whereby Pax2 expression is more extensive in the alar plate than in the basal plate. In the early stages of development, weak expression of Pax6 has been identified in the middle part of the neuroepithelial layer of the developing neural tube, but at later developmental stages strong expression of Pax6 is seen in the ventral part of the neuroepithelial layer [38, 15]. In animals as well as humans, several abnormalities have been linked to deficiencies in Pax protein dosage, which indicates that the level of Pax transcription factor expression is important for the proper development of the nervous system.

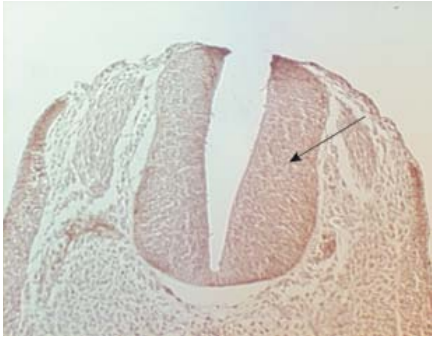

Figure 1. Transversal section of the developing neural tube in human embryo at CS12. Arrow indicates the expression of Pax2 in the neuroepithelial layer.

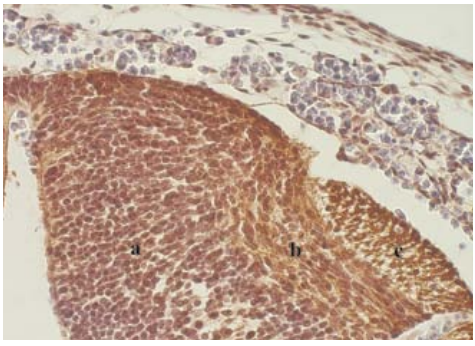

Figure 2. Transversal section of the developing spinal cord in human embryo at CS16. Expression of Pax2 in the neuroepithelial layer (a), mantel layer (b), and marginal layer (c)

EXPRESSION OF PAX PROTEINS IN THE DEVELOPING BRAIN

Increasing evidence demonstrates that the members of the Pax proteins family exhibit a distinct and spatial pattern during mammalian neurogenesis and play a critical role in the formation of different tissues during nervous

system formation [25, 28]. Early patterning of the central nervous system (CNS) results in subdivision of the neural tube along the anterior-posterior axis into the forebrain, midbrain, hindbrain and spinal cord [39]. Pax proteins have been shown to play an important role in the formation of the main parts of the developing brain [14, 15, 21]. Figure 3 shows the expression of Pax2 in the wall of the developing forebrain, midbrain and hindbrain. The boundaries between these regions can be recognized by the region-specific expression of certain genes as well as by morphological landmarks like constrictions of the developing brain [31].

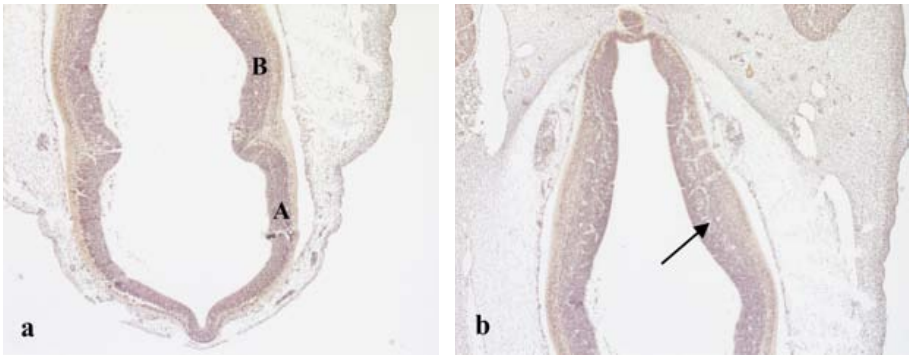

Figure 3. Transversal section of the brain of human embryos in CS12. (a) Expression of Pax2 in the wall of the hindbrain (A) and midbrain (B). (b) Expression of Pax2 in the wall of the forebrain (arrows).

PAX PROTEINS AT THE MIDBRAIN-HINDBRAIN BOUNDARY

The boundary between the midbrain and the hindbrain is of particular interest because it has been shown to control the development of the mesencephalon and metencephalon. A number of regulatory proteins are known to play an important role in the formation of the midbrain/hindbrain organizer. Genetic studies in mouse and zebrafish have revealed that the development of this isthmic organizer depends on secreted proteins (Wnt1, FGF8) and transcription factors (Otx2, Gbx2, En1, En2, Pax2, Pax5, Pax8) [21, 27]. All of these molecules have been studied in mice by means of targeted or naturally occurring mutations [31]. The formation of the isthmic organizer is a result of complex cross regulatory interactions between transcription factors and secreted proteins [3]. Pax proteins constitute a distinct class of Pax transcription factors which are all expressed in spatially overlapping patterns at the midbrain-hindbrain boundary [26]. Pax2 is the earliest known protein to be

expressed throughout the prospective midbrain-hindbrain region in the late gastrula embryos [28]. Pax2 is progressively refined to a narrow ring centered at the midbrain-hindbrain boundary by early embryonic days. In the developing human brain, expression of Pax2 is seen in the isthmic region, which is in accordance with the data described in mouse embryos [3, 14, 26, 31] and thus supporting the idea that Pax2 protein plays a key role in the formation of the isthmic organizer and controls the further development of the midbrain and hidbrain. These data indicate the role of Pax proteins, together with other transcription factors, in the establishment of the border between the two parts of the developing brain.

PAX PROTEINS IN DIENSEPHALON AND CEREBELLUM DEVELOPMENT

Besides the expression of Pax proteins in early parts of the developing brain, Pax2 protein has also been detected in the developing cerebellum and diencephalon. There are several models with different types of mutations in Pax genes which lead to the loss of the diencephalon and cerebellum in mouse and zebrafish embryos [4, 13, 14, 31, 39].

Cranially, the midbrain is separated from the diencephalon through a different set of molecular interactions. The diencephalon is thought to consist of a roof plate and two alar (dorsal) plates, but to lack floor and basal (ventral) plates [29]. The alar plate of the diencephalon is characterized by the expression of Pax6. Pax6 expression in human embryos also characterizes the infundibulum, the evagination of the floor plate of the diencephalon and Rathke's pouch [38]. Pax2 protein in human embryonic tissues may also be associated with the establishment of the developing diencephalon [37], as well as with the migration and setting of cells in the alar plates (Figure 4a). Results of investigations on human Pax proteins are in accordance with the described data in mouse embryos, which indicated that Pax proteins are important in the formation of diencephalic alar plates and formation of the diencephalic-mesencephalic border [40].

The cerebellum develops from the isthmus. The initially cerebellar plate consists of the neuroepithelial, mantel and marginal layers [29]. The data of experiments have shown that Pax2 is important for cerebellum development in mice. There are several mouse models with different types of mutations in Pax genes. Besides other defects of the developing brain, all these mutants display defects in the developing cerebellum [13, 14, 31, 39]. Pax2, Pax5, and Pax8

constitute a subgroup within the mammalian Pax family whose proteins are expressed in a partially overlapping manner in the developing cerebellum [39]. The data of human embryos have also demonstrated that Pax2 is important for the proper development of the cerebellum. Pax expression is seen in the neuroepithelial, mantel and marginal layers of the developing cerebellar plate (Figure 4b). During further development, a number of cells formed by the neuroepithelial layer migrate to the surface of cerebellum to form the external granular layer. In the fetal mouse brain, the Pax2 protein is expressed in Purkinje cells and the external granule cell layer of the cerebellum [28]. Hereby, Pax2 may have a crucial role in the formation of the cerebellar cortex.

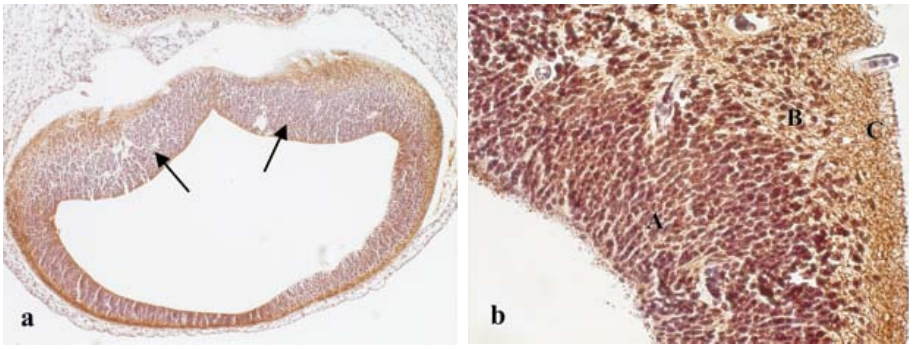

Figure 4. Transversal section in the brain of human embryos in CS18. (a) Expression of Pax2 in the wall of the diencephalon (arrows). (b) Expression of Pax2 in the (A) neuroepithelial, (B) mantle, (C) marginal layer of the cerebellar plate.

In summary, Pax proteins have many functions in the embryonic development, including nervous system formation in both vertebrates and invertebrates. Pax proteins are expressed in a population of nervous cells within the developing forebrain, midbrain, hindbrain and spinal cord. Pax3 and Pax7 are expressed throughout the forming neural tube and are involved in early dorsal-ventral patterning of the central nervous system. Diencephalon and cerebellum development depends on the organizing center that is located at the midbrain-hindbrain junction. Expression of the three closely related transcription factors Pax2, Pax5 and Pax8 overlaps temporally and spatially in this region of the developing embryo. During human embryonic development, Pax2, Pax3 and Pax6 seem to be involved in brain segmentation and establishment of the dorsal-ventral polarity of the spinal cord.

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