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PAX6-gene expression during human embryonic development at the cellular level.

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Purpose: PAX-genes encode transcriptional factors important during embryogenesis. They are characterized by the presence of a highly conserved DNA-binding domain, the *Paired* box. PAX6-gene mutations have been identified in Aniridia and Peters anomaly.Our aim is to map PAX6 gene expression on human embryonic tissue sections at the cellular level.

Methods: We studied, by *in situ* hybridization, the pattern of PAX6-gene expression during embryonic human development (three to six weeks post-fertilization). This project was approved by the Hospital Necker Ethic Committee.

Results: At Carnegie stage 10, PAX6-gene is preferentially expressed throughout the entire neural tube. This confirms a major role of PAX6-gene during neurogenesis. At stages 15 and 17, PAX6-gene is expressed in the rostral embryonic brain (diencephalon and telencephalon), in the pons , in part of the rhombencephalon, the spinal cord but not in the future cerebellum nor in the mesencephalon. In the eye, the cellular pattern consists in a strong labelling of the neural retina, the lens and the ectoderm facing the optic vesicle (the future cornea). The specific labelling of the neural retina seems to be restricted to the inner and outer layers. The intermediary layer seems devoid of any labelling.

Conclusions: this study shows the feasability of *in situ* hybridization methodology to analyze the expression of PAX6-gene during early human embryogenesis at the cellular level. It shows for the first time at the cellular level the expression of PAX6 in the human embryonic optic vesicle , in the neural retina , in the lens and in the future cornea.

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CYTOSKELETON OF THE INTRAOCULAR EPITHELIAL AND MUSCLE CELLS OF THE FETAL HUMAN EYE

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Purpose To analyze cytoskeletal development in neuroectodermally derived epithelial and muscle cells of the human eye during the 2nd and 3rd trimesters of pregnancy.
Methods Nine formalin-fixed and paraffin-embedded autopsy eyes representing the 13th to 40th week of gestation were studied with 10 monoclonal antibodies (MAbs) to intermediate filaments and α-smooth muscle actin (αSMA) using the avidin-biotinylated peroxidase complex (ABC) method.
Results The *epithelia of the iris* reacted with MAbs Vim 3B4 and V9 to vimentin in all specimens. Reactivity with MAb CAM 5.2 to CK 8 and MAbs CY-90 and KS-B17.2 to CK 18 disappeared from its anterior and posterior layer by the 18th and 28th gestational week, respectively. The whole anterior layer reacted with MAb 1A4 to αSMA from the 28th week onward. MAbs DE-U-10 and D33 to desmin labeled its *dilator fibers* by the 37th week, and focal reactivity for CK 8 and 18 concurrently appeared in them. The future *iris sphincter* reacted for αSMA in all eyes. In the earliest specimens, this reactivity was still confined to cells of the ring sinus. The initial reactivity of the sphincter for vimentin gradually disappeared after the 22nd week, whereas MAbs to desmin increasingly labeled it from the 18th week onward. MAbs to vimentin, CK 8 and CK 18 labelled the *ciliary epithelium* in all specimens. The *ciliary muscle* reacted for vimentin and αSMA in all eyes studied, and from the 16th week onward increasingly strong immunoreactivity for desmin was present in it. The *retinal pigment epithelium* reacted with MAbs to vimentin, CK 8 and CK 18 throughout the fetal period studied.
Conclusions The results highlight the highly individual cytoskeletal development of the derivatives of the optic vesicle, and offer a framework for detecting pathological changes in the neurocectodermal cytoskeleton of the fetal human eye after the first trimester of pregnancy.

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NADPH DIAPHORASE REACTIVITY OF THE RABBIT RETINA DEPENDS UPON THE STATE OF ADAPTATION.

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Purpose: Nitric oxide is synthesized from L-arginine by Nitric Oxide Synthase (NOS). In the rabbit retina, NOS was be demonstrated in the inner segments of the photoreceptors, in horizontal cells and in two populations of amacrine cells. Our goal was to test the possibility that the activity of the nitric oxide system in the rabbit retina depended upon the state of adaptation. Methods: NADPH diaphorase histochemistry was performed on retinas from light- and dark-adapted rabbits and on retinas from rabbits that were injected intravitrealy with either L-arginine, L-NAME or L-glutamate. Results: A retina treated by L-arginine exhibited more intense NADPH diaphorase staining compared to that exposed to L-NAME probably due to activation of NOS by its substrate. We found that NADPH diaphorase reactivity of amacrine cells was most pronounced in retinas from light-adapted rabbits, while horizontal cells staining was enhanced by dark-adaptation. L-glutamate injected intravitreally effectively blocked synaptic transmission in the outer plexiform layer of the rabbit as judged from the electroretinogram. The retinas from the L-glutamate treated eyes exhibited dark-adapted like NADPH diaphorase reactivity even in light-adapted animals. Conclusions: The data indicate that activation of NOS in the rabbit retina depends upon its cellular origin and the state of adaptation.