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Program and Abstract book

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1. — CELSR1, 2, AND 3 GENES DIFFERENTIALLY REGULATE DIRECTION AND TRAJECTORY OF FACIAL BRANCHIOMOTOR NEURON MIGRATION IN MOUSE.

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During hindbrain development, facial branchiomotor neurons (FBMN) migrate from rhombomere (r)4 to lateral r6. In zebrafish, mutations in planar cell polarity (PCP) genes celsr2 and frizzled3a block FBMN caudal migration. Here, we investigated the role of cadherins Celsr1, 2, and 3, and Fzd3 in FBMN migration. In Celsr1 mutants, caudal migration was compromised and neurons often migrated rostrally from r4 to r2-3, a defect never observed previously in any vertebrate. This phenotype was not seen in Celsr1|Islet1 mice, indicating that effects of Celsr1 on FBMN migration are non cell autonomous. In Celsr2 mutants, FBMN migrated abnormally and prematurely into lateral r5. This phenotype was enhanced by inactivation of Celsr3, and mimicked by mutation of Fzd3. In contrast to Celsr1, Celsr3 acted cell autonomously, because anomalies were identical in Celsr2-/-,Celsr3-/-, and in Celsr2-/-,Celsr3|Islet1 embryos. In double Celsr1-/-,Celsr2-/- embryos, the migration phenotype was similar to that in Celsr2 -/-, indicating that Celsr2 is epistatic to Celsr1. These data suggest that Celsr1-3 regulate FBMN migration in two different ways : Celsr1 specifies the direction of FBMN migration along the rostrocaudal axis, whereas Celsr2, Celsr3 and Fzd3 regulate the trajectory of migrating neurons.

2. — SUPPORTING CELLS CYTOSKELETON DURING THE DEVELOPMENT OF THE ORGAN OF CORTI IN RAT.

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The mammalian auditory organ, the organ of Corti (OC), is composed of mechanosensory hair cells and nonsensory supporting cell types. Based on their morphology and physiology, at least four types of supporting cells can be identified in the OC : inner pillar cell, outer pillar cell, inner phalangeal cell and Deiters' cells. All supporting cells are highly specialized cells that are characterized by the presence of bundled microtubules with 15 protofilaments instead of 13.

Using antibodies against different proteins of cytoskeleton (tubulin, cytokeratin and vimentin), we investigated by confocal microscopy the setting up of supporting cells' cytoskeleton during the differentiation of the OC in rat from the embryonic day 18 (E18) to the postnatal day 15 (P15).

We showed that inner pillar cells are labelled with an anti-tubulin beta IV antibody from P0. Using an antibody to cytokeratin, a labelling appeared in Deiters'cells from E22. We also revealed that during the development of the OC, supporting cells were labelled with an anti-vimentin antibody from P0.

3. — ROLE OF EPHRIN-A5 IN THE SURVIVAL AND CONNECTIVITY OF SPIRAL GANGLION NEURONS.

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In mammals, degeneration of auditory neurons constitutes one of the prime causes of the sensorineural hearing loss. We believe that studying the developing auditory portion of the inner ear can provide important insights to mechanisms that would regulate regeneration after injury. In that context, our work has focused on ephrins and Eph receptors, since they have been shown to have an influence in regulating developmental functions such as axonal growth and guidance, synaptic formation and functions.

We have shown by in situ hybridization that ephrin-A5 is expressed in a small proportion of the auditory neurons, at different stages of the cochlear development. Most of them are located on the edge of the spiral ganglion.

We demonstrated that the amount of neurons falls of more than 50% in P5 ephrin-A5 knockout mice as compared to wild type. Moreover, the numbers of TUNEL and active caspase-3 positive cells in the spiral ganglion are significantly increased at P0 when ephrin-A5 is deleted. Finally, we established that lack of ephrin-A5 leads to a defective inner hair cell innervation.

Taken together, these results suggest a role for ephrin-A5 in regulating survival of auditory neurons and establishment of connectivity to sensory hair cells during development.

4. — SPATIOTEMPORAL DISTRIBUTION OF GLYCOGEN DURING THE MAMMALIAN AUDITORY ORGAN DEVELOPMENT.

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Although the structure of the auditory organ in mature mammals, the organ of Corti, is clearly established, its development is far to be elucidated. Using a PAS method at the light and electron microscope levels, we examined in great detail the spatiotemporal distribution of polysaccharides during Corti organ development in rat from embryonic day 16 (E16) to postnatal day 15 (P15).

At E16, small polysaccharide inclusions could only be detected in the cytoplasm of future inner pillar cells by electron microscopy. These became obvious at the light microscope level at E17. At E19, the PAS-positive material was large within inner pillar cells and polysaccharide deposits appeared in Hensen cell cytoplasm. From P3-P4, outer pillar and Deiters cells also exhibited polysaccharide accumulations. As the organ of Corti developped, the amount of polysaccharide inclusions within inner and outer pillar cells decreased. At P15, large amount of polysaccharide deposits were visible in Deiters cells whereas they have almost disappeared from inner and outer pillar cells.

Finally, we showed that the polysaccharide deposits present in the developing organ of Corti were digested with a salivary amylase suggesting that they would be essentially composed of glycogen.