

Development

Oral Presentation

Monday November 7

11.00am – 11.15am

O-26

WHIRLIN COMPLEXES WITH P55 AT THE STEREOCILIA TIP DURING HAIR CELL DEVELOPMENTP Mburu¹, Y Kikkawa², R Romero¹, S Townsend¹, H Yonekawa², S D M Brown¹¹ MRC Mammalian Genetics Unit, Harwell, Oxon, United Kingdom, ² Tokyo Metropolitan Institute of Medical Science (Rinshoken), Tokyo, Japan

Actin cytoskeleton remodelling is fundamental to a variety of cellular processes including morphological alterations at the cell surface. Hearing in mammals is dependent upon the proper development of actin-filled stereocilia at the surface of hair cells in the inner ear. Recent work has established that whirlin, a PDZ protein, localises to the stereocilia tips and by virtue of mutations in the whirlin gene has been shown to play an important role in stereocilia development. Myosin XVa interacts with whirlin and is responsible for localising whirlin at the stereocilia tip. We have previously demonstrated that whirlin shows an extraordinary expression pattern that traverses the stereocilia bundle during its development. We now show using yeast 2-hybrid screening, in vitro and in vivo pull-down assays that whirlin interacts with the MAGUK protein p55. p55 is known in erythrocytes to form a trimeric complex with protein 4.1R and the transmembrane protein glycophorin C promoting actin cytoskeleton assembly. We find that both p55 and protein 4.1R are expressed in both the developing stereocilia bundle and in the shorter microvilli-like stereocilia surrounding the graded stereocilia. In the whirler mutant, expression of p55 and protein 4.1R in hair cell stereocilia fades out prematurely from around P5. In the shaker2 (myosin XVA) mutant, expression of both p55 and 4.1R is completely abolished. We propose that whirlin forms a complex with p55 and protein 4.1R at the stereocilia tip that mediates actin polymerisation in response to some as yet unidentified external signal.

Development**Oral Presentation****Monday November 7****11.15am – 11.30am****O-27****THE NOTCH LIGAND DELTA1 INTERACTS WITH PDZ-DOMAIN CONTAINING PROTEINS**C Hoefler, S Pfister, J Adamski, G Przemeck, M Hrabé de Angelis

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Cell-cell signaling by the Notch-pathway is mediated by the interaction of the transmembrane receptor Notch with its ligands Delta and Serrate (Jagged in vertebrates) presented on adjacent cells. Whereas signal transduction to Notch expressing cells has been described, it is yet unclear whether Delta-dependent signaling may also exist within the Delta expressing cell. Recently, we report on the identification of proteins interacting with the intracellular domain of mouse Delta1 (Dll1cyto). Among others, we identified several PDZ-domain containing proteins as Dll1cyto interacting (Pfister et al., 2003). For example, the interaction with Magi2 (also known as Acvrin1 or S-SCAM) and Magi3 was confirmed by in vitro and in vivo systems. Interacting domains could be delimited to the fourth PDZ domain of Magi2, the fifth PDZ domain of Magi3 and to the C-terminal PDZ-binding motif of Dll1. In situ expression analyses as well as immunohistochemistry in mouse embryos revealed that Dll1 and newly identified interacting partners show partly overlapping but distinct expression patterns, for example, in the central nervous system. Here, we will present newest results on Dll1-interacting PDZ proteins and discuss possible functions of Dll1cyto-dependent mechanisms.

Literature:

Pfister, S. et al. 2003, JMB 333, 229-235

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Oral Presentation

Monday November 7

11.30am – 11.45am

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A MAJOR ENHANCER FOR LIMB-SPECIFIC EXPRESSION OF *SHH* RESIDES IN 1MB UPSTREAM OF THE CODING SEQUENCE, AND HAS BEEN LOST IN LIMBLESS SPECIEST Sagai¹, M Hosoya¹, Y Mizushima¹, H Masuya², M Tamura¹, T Shiroishi¹¹ National Institute of Genetics, Mishima, Shizuoka-ken, Japan, ² RIKEN Genome Sciences Center, Tsukuba Igaragi-ken, Japan

The paired fins of teleost fishes and tetrapod limbs have evolved from a common ancestral appendage. Our previous studies revealed that an intronic sequence of the mouse *Lmbr1*, localized in 1Mb upstream of the *Shh* coding sequence, is highly conserved among tetrapods and teleost fishes, and has a single base substitution in mouse preaxial polydactyly mutants. We examined the physical linkage of *Shh* and the conserved sequence in a teleost fish, medaka. The sequence was found in the intron 5 of the medaka *Lmbr1* homolog, and is placed in the same scaffold as the *Shh* coding. These facts suggest that the conserved sequence is *cis*-acting regulator for limb-specific *Shh* expression, and that the physical linkage of the *Shh* coding and the *cis*-acting regulator evolved prior to divergence of teleost fishes and tetrapods. We intended to directly examine the role of the conserved sequence by targeted mutation to eliminate the sequence in the mouse. The knockout mouse showed a complete loss of *Shh* expression in the limb buds and severe amputation of distal elements of the limbs, resembling *Shh* coding knockout mouse and human congenital deformity named Acheiropodia. Notably, this sequence has been lost in two independent lineages of limbless species, limbless newt and snakes. Thus, the conserved sequence contains a major enhancer for limb-specific *Shh* expression, and is indispensable for the development of distal limb structures. Furthermore, loss of this conserved sequence might be involved in evolution of the limbless species.

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Oral Presentation

Monday November 7

11.45am – 12.00pm

O-29

THM1 IS A NOVEL NEGATIVE REGULATOR OF MOUSE SONIC HEDGEHOG (SHH) SIGNALINGP V Tran¹, B J Herron¹, P J Scherz², H Qiu¹, A Turbe-Doan¹, K Parker¹, D R Beier¹¹ Brigham and Women's Hospital, Boston, MA, United States, ² Harvard Medical School, Boston, MA, United States

The SHH signaling pathway plays a fundamental role in mammalian embryonic development. The signaling cascade is triggered by binding of the SHH ligand to the transmembrane receptor, Patched (*Ptc*), which releases its repression of the signal transducer Smoothed (*Smo*). The SHH signal culminates on Glioblastoma (*Gli*) transcriptional regulators which activate target genes. We report the characterization of alien (*aln*), a novel mutant mouse, which displays a phenotype characteristic of inappropriate activation of SHH signaling; *aln* mutants exhibit preaxial polydactyly, craniofacial abnormalities and misexpression of SHH target genes in the neural tube and limb bud. Genetic analyses suggest that the *aln* gene product acts as a negative regulator downstream of *Smo* but upstream of *Gli2*. Using positional cloning, we have identified a missense mutation in an evolutionarily conserved N-terminal amino acid in a novel gene we call *Thm1* (Tetratricopeptide repeat (TPR)-containing Hedgehog modulator 1). We found GLI3 activator: GLI3 repressor ratios to be 10-fold higher in *aln* anterior limb buds relative to wild-type, suggesting a role for *Thm1* in regulating cleavage of the GLI3 transcriptional activator to its repressor form. We are currently investigating *in vitro* whether *Thm1* may have a direct role in this process. The predicted protein structure of the *Thm1* product reveals multiple TPR domains, which are known to enable the assembly of macromolecular complexes. It is possible that *Thm1* may function in the formation of complexes necessary to bring together different components required for GLI3 processing.