Regulation of cell diversity in the developing spinal cord of the zebrafish

JOSÉ A. CAMPOS-ORTEGA*

Institut für Entwicklungsbiologie, University of Köln, Germany
* jose.campos@uni-koeln.de

We are interested in understanding how the composition of cell lineages in the developing central nervous system of the zebrafish is regulated. We follow a combination of three different approaches to this problem, the results of which will be the subject of my presentation. The first approach is clonal analysis, which involves either labelling individual cells within the neural plate by intracellular injection of fluorescein-dextran or transplanting cells that express GFP, and analysing the development and composition of the clones generated by them. We find that the cell lineages have a stereotypic, region-specific composition. Intracellular injection of rhodamine-dextran followed by repeated injections of BrdU indicates that progenitor cells divide asymmetrically with a stem cell-like pattern, giving rise to daughter cells that differentiate either as neurones or as glial cells, and daughter cells that continue to divide. In the second approach, transgenic embryos expressing GFP fused to histone 2A.F/Z are used to investigate various aspects of the pattern of mitotic divisions in the developing spinal cord *in vivo*. Particular attention is given to the orientation of the mitotic spindle in the ventricular zone and the behaviour of the daughter cells. We find that the mitotic spindle is always oriented parallel to the ventricular surface, irrespective of whether the daughter cells differentiate or continue to divide.

In the developing spinal cord, specification of progenitor cells and of the different cell types within lineages is mediated by diffusing morphogens and by direct cell-to-cell interactions. In the third approach, we use the Gal4-UAS technique for directed gene expression to analyse molecular genetic aspects of cell lineage development. We are currently studying the role played by Sonic hedgehog and Notch signalling in the specification of progenitor cells and of the various cell types. Zebrafish from activator strains, which express Gal4 in particular regions of the neural plate driven by specific promoters, are crossed with individuals of effector strains carrying *UAS:Shh* and *UAS:notch1a-intra* transgenes, and the effects on spinal cord development in the progeny are studied. Results from this work will be discussed.