

Cell-cell interactions and the cytoskeleton in organisation of the developing intervertebral disc

J.R. Ralphs

Connective Tissue Biology Labs, School of Biosciences, Cardiff University, Wales, GB

INTRODUCTION. The intervertebral disc develops from two key components: the embryonic notochord and sclerotomally derived mesenchyme. Initially, the mesenchymal cells surround the notochord and, then under the influence of a variety of embryonic patterning systems, form regular repeating dense annular condensations of sclerotomal cells interspersed with more widely spaced cartilage precursor cells of the early developing vertebral bodies. As the vertebral body cartilage differentiates, the annular condensations show evidence of differentiation into inner and outer regions. Shortly afterwards, the notochord rapidly bulges in the region of the developing disc, and thins in the vertebral bodies, from which it eventually disappears. The bulges form the foetal nucleus pulposus, and as they enlarge the annular condensations differentiate into the cartilaginous inner and fibrous outer annulus fibrosus. The ends of the vertebral bodies on either side form the cartilage endplates. The subsequent fate of the foetal nucleus pulposus is species dependent. Some species, eg mice and rats, retain notochordal cells as the predominant cell type in the nucleus pulposus throughout life. Others, eg horse, lose them before they are born. Many are intermediate between these extremes, including human beings, where notochordal cells are lost by about 8 years old, although a few may persist for longer. The nucleus pulposus becomes populated by a chondrocyte like cell population whose origin has not been conclusively demonstrated, but is likely to be from the surrounding cartilaginous inner annulus fibrosus and endplates. In the foetal annulus, cells have to organise the deposition of highly ordered arrays of collagenous lamellae to form the adult structure of the annulus fibrosus. The first stage of this process involves orientation of cells into sheets of oriented fibroblasts, with cell orientations being organised at angles of around 50-60 degrees of the cells in the preceding sheet. Work in our laboratories has examined how this orientation process occurs, and how the orientation of cells may be related to the oriented deposition of collagen.

METHODS. Rat intervertebral lumbar spines from fetuses, neonates and older animals, up to 2 years, were frozen, cryosectioned and labelled for a variety of molecules associated with the cytoskeleton, cell-cell and cell-matrix interactions and collagen production and examined by conventional and confocal microscopy.

RESULTS AND DISCUSSION. Early stages of cell orientation occur as cells assemble large actin stress fibres in their cytosol, coincident with cell elongation; within a cell layer all of the actin fibres are parallel, ensuring all of the cell have the same orientation. This organization is maintained for the remaining foetal period as the collagenous lamellae are deposited. In addition to orienting cells, the actin cytoskeleton may have a direct role in matrix orientation. Firstly, alpha5beta1 integrin at the cell surfaces is associated with stress fibres intracellularly and oriented fibronectin extracellularly, and secondly, examining another tissue with a high degree of collagen lamellar organization, the cornea, we have shown that intracellular vesicles containing type I procollagen localize along actin filaments, suggesting that collagen is trafficked along the stress fibres. At early stages of cell orientation, expression of gap junction proteins is prominent, suggesting coordination of cell behaviour in organizing the lamellae, along with cadherin expression showing cell-cell binding. Highly organized expression of vinculin and cadherins at slightly later stages suggests the maintenance and enhancement of cell-cell contacts using actin-associated adherens junctions, possibly retaining relative cell orientation of lamellae. Remarkably, the actin stress fibres are rapidly lost after birth, suggesting that cells now orient to the deposited extracellular matrix, rather than using their own intrinsic mechanisms.

Acknowledgements. I would like to thank Drs Tony Hayes and Claire Gealy for their efforts and the ARC and BBSRC for funding.