WHAT THE PAPERS SAY

Fibronectin in Early Embryonic Development of the Vertebrate

Jean Paul Thiery

Summary

During development some cells are migratory whilst others are stationary. However, the same cell may change its behaviour depending upon its environment. Recent evidence has implicated the extracellular matrix protein fibronectin in the regulation of migratory behaviour. As the structure of this molecule becomes elucidated, it is also becoming possible to interpret this regulation in precise molecular terms.

Many cells change their position during the embryogenesis of multicellular organisms. During translocation, cells interact with surrounding cells and/or with an extracellular matrix (ECM). In the last few years great progress has been made in analysing the composition and functions of the ECM. Fibronectin (FN) is one of the glycoproteins that, in association with collagens and proteoglycans, constitutes the three-dimensional meshwork of fibrils and interstitial bodies that make up the ECM. FN has been implicated in a variety of cell behaviours including adhesion and migration, and it transpires that this multiplicity of actions reflects the fact that FN is a family of closely related glycoproteins found both in the plasma in a soluble form and in the fibrillar structures of the ECM.[1]

In mammals, and possibly in other species, a single gene codes for FN.[2,3] In the chick, this gene extends over 50 kb and contains 48 exons.[4] A differential splicing mechanism, which could generate as many as ten different mRNAs, has been described recently for the exons of the FN gene, and thus could explain how different members of the FN family arise (refs. 5–8 and Kornblihtt, personal communication). For example, plasma FN synthesized in hepatocytes[8] is encoded by a messenger RNA lacking one complete exon.[5] Analysis of the protein structure of FN reveals two almost identical subunits of 2300 amino acids linked via disulphide bonds (Fig. 1). The amino acid sequence of the protein is expected to be available by early 1985, but already amino acid sequence analysis of part of the molecule has demonstrated three distinct types of homology internally.[9] Binding domains have been localized to specific regions of the FN molecule, those for heparin and fibrin being in the amino and carboxyl terminal regions of the molecule, while the binding sites for native or denatured collagen, actin, DNA, *Staphylococcus aureus* and the cell surface receptor(s) are located more centrally (see Fig. 1 and refs. 10 and 11).

From its structure and some of its binding functions *in vitro*, FN must be considered as a key element of the ECM mediating interactions between the cell surface and its environment. In particular, FN might mediate cell to substrate adhesion and consequently modulate cell motility. The nature of the interaction between cells and FN is not well understood; glycolipids, heparan sulphate and glycoproteins have all been

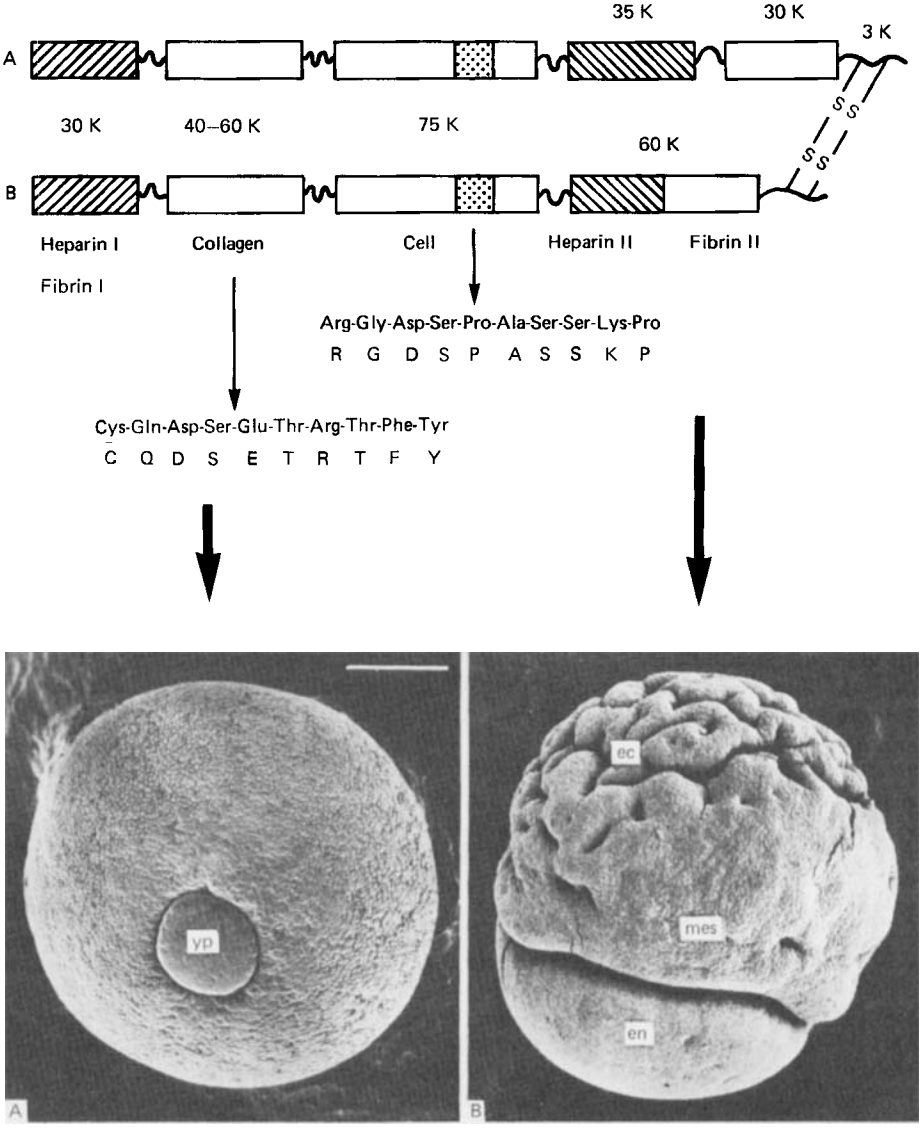

Fig. 1. *The general structure of the fibronectin molecule and the composition of two peptides representing the active parts of the collagen cell-binding domains. (A) Gastrulation occurs normally when a peptide corresponding to the collagen-binding domain is injected into the blastocoel (yp; yolk plug). (B) In contrast, when the peptide containing the FN-binding sequence is injected, embryos do not gastrulate. The mesoderm (mes) and the endoderm (en) remain on the exterior and the ectoderm (ec) becomes highly convoluted. For details see text and Boucaut* et al. *(1984b). Bar: 200 μm.*

considered as putative mediators of the interaction between FN and cells; but very recently a single class of 5×10^5 cell receptors, with a dissociation constant of $8{\cdot}4 \times 10^{-7}$ M, has been characterized in a fibroblastic cell line.[12] Binding to this receptor may be via a short, highly conserved hydrophilic amino acid sequence (Arg Gly Asp Ser) in the cell binding domain of FN.[13,14] Such a sequence has only been found in a few other proteins, two of which also have binding properties.[13] However, the receptor could be part of a molecular complex that may interact with other sites on FN or on other ECM molecules.

FN appears very early during development. In amphibians, FN can be detected in maturing oocytes,[15] and maternally derived FN mRNA are transcribed actively at the blastula stage.[16] However, although most cells of the blastula can produce FN, its assembly is restricted to the roof of the blastocoel cavity.[16,17] A dense network of FN-associated fibrils is already formed before the appearance in the marginal zone of the blastula of the blastoporal lip, marking the beginning of gastrulation. Several lines of evidence suggest that FN plays a pivotal role in this process of gastrulation with mesodermal cells, and possibly some endodermal cells, utilizing the FN-rich matrix as a substrate for adhesion and migration. First, scanning and electron microscopy showed that invaginating cells do interact with a fibrillar matrix both in anurans and urodeles.[18] Secondly, immunofluorescent and immunogold labelling allows a direct identification of FN in the fibrils that align with mesodermal cell filopodia.[19] Thirdly, inversion of the blastocoelic roof creates a zone lacking ECM which does not become occupied by migrating cells. Fourthly, monovalent antibodies to FN injected into the blastocoel prior to or at the onset of gastrulation inhibit completely the migration of mesodermal cells, both the endoderm and the mesoderm remaining at their original position.[20] Finally, peptides containing the cell binding site sequence (Arg Gly Asp Ser) also block gastrulation, possibly through competitive inhibition for the cell surface receptors with FN (Fig. 1).[21]

In addition to a role in gastrulation, FN has also been implicated in the migration of neural crest cells in birds. Crest cells migrate in FN-rich transient pathways which remain acellular for the most part.[22,23] In contrast to many embryonic cells, most crest cells do not produce and retain FN at their surfaces but do adhere and migrate on FN-containing ECM.[24,27] Crest cell movement *in vitro* and *in vivo* is perturbed in the presence of monovalent antibodies to FN or with the cell binding site peptide.[21,26]

There is evidence that other cell types require fibronectin for migration. Cells which are in direct contact with FN include: primordial germ cells in amphibians[28] and possibly in other vertebrates, myotomal cells,[29] endocardial cells[30] and endothelial cells and the Wolffian duct pioneer cells (Thiery, unpublished data).

Whilst there is a good association between the migration and the presence of FN, many different areas of the embryo are not occupied by migrating cells even though they contain FN. For example, neural crest cells can utilize several of the FN pathways available at each axial level, but tend not to utilize all. Thus, factors other than the mere presence of FN must operate to control the ability of cells to migrate in FN-rich matrices. What might these be? First, the FN concentration itself, if too high and particularly if in the soluble form, may saturate the receptors and provoke either paralysis or loss of adherence. Secondly, components such as chondroitin sulphate may inhibit migration.[24] Finally, hyaluronic acid, produced by crest cells themselves, may reduce the binding of the cells to FN (Turley, personal communication).

It is tempting to suggest that cells can migrate in FN-rich matrices only if they do not produce and retain FN at their surfaces. These migratory cells may also carry a lower number and/or lower affinity FN receptors than other mesenchymal cells that will therefore remain stationary in the embryo (Fig. 2).[31] It is important to emphasize that FN does not provide either a signal for epithelial–mesenchymal cell conversion or cues for directionality of migration. However, all of the evidence so far does strongly suggest that FN is required for adhesion and migration of many embryonic cells.

Further support for a pivotal role of FN rather than collagen type I has been obtained with transgenic mice unable to synthesize the collagen polypeptide. These mice, in the homozygous state, die suddenly between days 12 and 14 of gestation due to a major blood vessel rupture and deficient erythropoiesis. Such mice do not have any obvious morphological defect, particularly with respect to gastrulation, neurulation and the migration and proper localization of neural crest cells (ref. 32 and Jaenish, personal communication).

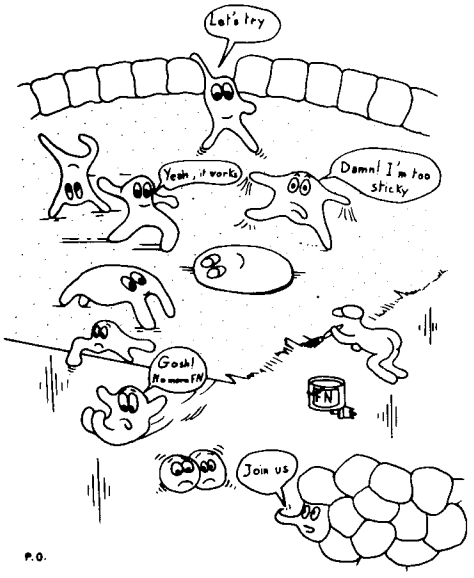

Fig. 2. *Interactions of cells with fibronectin in the extracellular matrix. After egression from an epithelium, cells adhere to FN-containing matrices. Many embryonic tissues contribute to the formation of the extracellular matrix. In the matrix, cells can be found in one of two states, i.e. locomotory or stationary. The transition between the two states may be controlled by the nature of the FN-receptors and/or the ability of the cells to produce and retain FN on their surfaces. If cells encounter a region lacking FN, migration may be arrested which, in turn, may lead to the formation of a new structure where adhesion between cells becomes predominant (drawn by Bruno Páult).*

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WHAT THE PAPERS SAY

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Torsional Stress in Eukaryotic Chromatin

Walter A. Scott

Summary

The bulk of the DNA in eukaryotic chromatin behaves as if it is topologically relaxed; however, a subfraction can be shown to be under supercoil tension. Endonuclease S1 cuts at specific hypersentive sites in chromatin (in the promoter regions of active genes) and this enzyme cuts in the same region in supercoiled plasmids, but not in relaxed or linearized molecules. A subfraction of the minichromosomes formed after SV40 infection or microinjection of plasmid DNA into oocytes contains supercoil tension and this subfraction is thought to play an important role in transcription.

DNA molecules isolated from natural sources are under torsional strain in the form of negative supercoil tension. This means that forces are present which tend to unwind the DNA double helix. Those forces favor the conversion of regions of the double helix into alternative structures (e.g.) cruciform structures or left-handed double helix (Z-DNA) which can take up some of the twist. These alternatives to the right-handed double helix (B-DNA) may be important in the recognition of functional elements along the DNA molecule by protein molecules.

It has been known for some time that torsional stress activates transcription of many *E. coli* genes.[1,2] In fact, DNA gyrase may play a critical role in prokaryotic gene expression by continually pumping negative supercoils into the DNA in order to maintain a stressed condition.

Until recently, however, it has seemed that eukaryotic chromatin was not maintained in such a state of tension. DNA molecules isolated from eukaryotic cells after removal of the protein component do contain negative supercoils, but each nucleosome takes up approximately one negative supercoil, so that the chromatin which exists in the cell is topologically relaxed. For example, topoisomerase I (relaxing en-