CELL CYCLE

Dual-purpose protein

It's not unusual for a protein to have different functions in different cellular contexts. But the discovery that an mRNA-export protein also helps to assemble the spindle during cell division does bring a surprise — that RNA itself is involved in spindle assembly.

In the work in question, Rebecca Heald, Karsten Weis and colleagues set out to characterize proteins that contribute to spindle assembly in the absence of centrosomes. Centrosomes generate microtubules and organize them into bipolar spindles — the machinery that segregates duplicated chromosomes during division in all eukaryotic cells. In some cases, however, spindles form without centrosomes, and microtubules nucleate around chromosomes, organizing themselves into bipolar arrays. This process seems to rely on the small GTPase Ran, which might promote the release of spindle-regulating factors from a nuclear import protein, importin-β. But the details remain unclear.

To investigate the process, Weis and colleagues aimed to identify proteins that function downstream of the active form of Ran (Ran•GTP) and importin-β. To do so, they used extracts of *Xenopus* eggs. Biochemical purification led the authors to Rae1 — a protein previously known for its ability to export mRNAs from the nucleus. Immunofluorescence studies showed that the protein localizes to spindles and around chromosomes in egg extracts.

So what does Rae1 do? To find out, Weis and colleagues used antibodies to deplete the protein from egg extracts; this markedly inhibited spindle assembly. A similar effect was observed in human cells *in vitro*, and resulted in defects in chromosome alignment or segregation. Rae1 cannot work alone, however; indeed, Weis and colleagues found that it binds to numerous other proteins in egg extracts. Many of these proteins can also bind RNA, so the authors used an RNA-digesting enzyme to find out whether RNA is

EXTRACELLULAR MATRIX

The matrix reloaded

Atherosclerosis causes significant mortality and morbidity in the Western World, and the prevention of this disorder is therefore an urgent healthcare priority. Now, a new report in *The Journal of Cell Biology* has shed light on the molecular interactions that initiate atherosclerotic plaque formation, and the authors also describe an important first step in the development of early interventional therapy.

Atherosclerotic plaques tend to develop at vascular sites that are exposed to turbulent blood flow, which indicates that local shear stresses are important in the development of these lesions. These fluid stresses induce signalling through the integrins — a family of adhesion molecules that are expressed by several cell types, including endothelial cells. Recent studies have revealed that the identity of the specific integrin–ligand pair is crucial in determining whether this signalling will be protective or will upregulate atherogenic genes by activating the proinflammatory nuclear factor-κB (NF-κB) transcription factor.

Integrin-binding proteins are located in the subendothelial extracellular matrix (ECM) and, in normal blood vessels, these comprise mainly collagen IV and laminin. However, when blood vessels are damaged, the so-called transitional matrix proteins — fibrinogen and fibronectin — are deposited in the ECM, and the binding of these proteins to their specific integrin partners might well trigger the activation of NF-κB.

To investigate the role of the ECM in atherosclerosis, Martin Schwartz and co-workers used *in vitro* assays to show that shear-stress-induced NF-κB activation was indeed dependent on the composition of the ECM — cells plated on fibronectin or fibrinogen showed enhanced activation of NF-κB and increased NF-κB target gene expression. *In vivo* assays validated these data — in a mouse model of atherogenesis, the ECM at atherosclerosis-prone sites comprised increased amounts of fibronectin and fibrinogen, and the endothelial cells at these sites expressed NF-κB-induced inflammatory markers.

By contrast, the protein components of normal subendothelium had a protective effect — the interaction of collagen IV with specific integrins activated the p38 mitogen-activated protein kinase (MAPK), which inhibits NF-κB activation. Importantly, in the cytoplasm of endothelial cells, activated p38 was localized to the site of integrin–ECM adhesion and, although this pathway inhibited shear-stress-induced activation of NF-κB, the global activation of NF-κB by cytokines was not affected. Furthermore, when Schwartz and his team treated cells that were plated on fibronectin with a peptide known to activate p38, shear-stress-induced upregulation of NF-κB was blocked.

The authors point out that this particular peptide would not be suitable for use in patients, but nevertheless, the identification of the p38 pathway as a target for therapeutic manipulation is particularly exciting — the local and selective action of p38 both focuses and restricts inhibition of NF-κB to the site of vascular damage, which ensures efficacy whilst avoiding the unacceptable side effects that would accompany global NF-κB inhibition.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Orr, W. A. *et. al*. The subendothelial extracellular matrix modulates NF-κB activation by flow: a potential role in atherosclerosis. *J. Cell Biol.* **169**, 191–202 (2005)