Mapping the checkpoint

The spindle checkpoint blocks exit from mitosis until all kinetochores are bound by spindle microtubules. At that point, Cdc20 is released by the checkpoint proteins and activates the anaphase promoting complex (APC), a ubiquitin ligase, to initiate anaphase. On page 61, Casaletto et al. identify Xnf7 as the first APC regulator known to bind the core APC components, rather than Cdc20. On page 49, Kops et al. show that Zwint-1 acts as a physical bridge between the structural kinetochore proteins and other checkpoint proteins, including those implicated in sequestering Cdc20.

While looking for a regulator of cyclin B2, which is one of the major targets of the APC, Casaletto et al. identified Xnf7. Immunodepletion of Xnf7 accelerated meiotic exit after Ca\(^{2+}\) addition, which released a meiotic block in Xenopus egg extracts. Purification experiments indicated that Xnf7 interacts with the core APC proteins, although not with Cdc20. Moreover, Xnf7 has ubiquitin ligase activity and under ubiquitylating conditions blocked activation of purified APC. Addition of Xnf7 antibodies to egg extracts blocked spindle checkpoint function and allowed mitosis to proceed even when unbound kinetochores were present, suggesting that Xnf7 may link the spindle checkpoint proteins and APC core components.

Meanwhile, Kops et al. have started to map the physical links between the structural kinetochore proteins and the spindle checkpoint proteins. They started with several proteins that were known to participate in the checkpoint, but whose exact function remained unknown. Immunoprecipitation of tagged Zwint-1 indicated that it is in contact with both structural kinetochore proteins and ZW10, whereas ZW10 is complexed with Rod and Zwilch. Extracts lacking ZW10 were deficient in their checkpoint function, and Mad1 and Mad2 failed to accumulate at the kinetochores. Kops et al. hypothesize that Zwint1 attaches to core kinetochore proteins, and then ZW10 binds and this complex recruits Mad1, which in turn brings in Mad2, which is one component of the diffusible stop-anaphase signal.

ZW10 has been linked to dynein; its removal by dynein once microtubules contact the kinetochore may silence the checkpoint. Xnf7, by contrast, may help recruit the APC to the kinetochore for inhibition.

ECM controls flow-induced NF-κB

Orr et al. (page 191) show that the composition of the subendothelial extracellular matrix (ECM) controls whether NF-κB, a major inflammatory response protein, is activated by fluid shear stress.

Atherosclerosis typically occurs in regions of disturbed blood flow, such as vascular branch points. Integrins become activated in response to increased flow, converting the proteins to a high-affinity conformation. Once activated, integrins bind to the subendothelial ECM and initiate intracellular signaling. Additionally, the binding of some integrins, but not all, triggers NF-κB signaling. Others have observed that NF-κB signaling in the endothelium contributes to the initiation of atherosclerosis. Now, Orr et al. have connected these observations.

In an in vitro system, they found that NF-κB was activated in response to flow when cells were plated on fibronectin or fibrinogen, which are associated with damage or inflammation, but not when they were grown on collagen or laminin, which are the primary matrix components in healthy vasculature. In vivo, fibronectin and markers of NF-κB activation were found together in the subendothelial ECM in regions of disturbed flow even in mice that were resistant to atherosclerosis. Both ECM changes and expression of NF-κB markers were accelerated in ApoE-null mice as they developed atherosclerosis. If the team altered the structure of the matrix proteins, they reduced NF-κB signaling in response to flow.

The new results do not provide an immediate prevention strategy for atherosclerosis, but they do suggest a novel target for therapies. Knowing that different components of the subendothelial matrix result in different responses to flow means that, if researchers can find a way to maintain the innocuous matrix proteins or alter matrix properties to prevent NF-κB activation, they may be able to slow the disease.