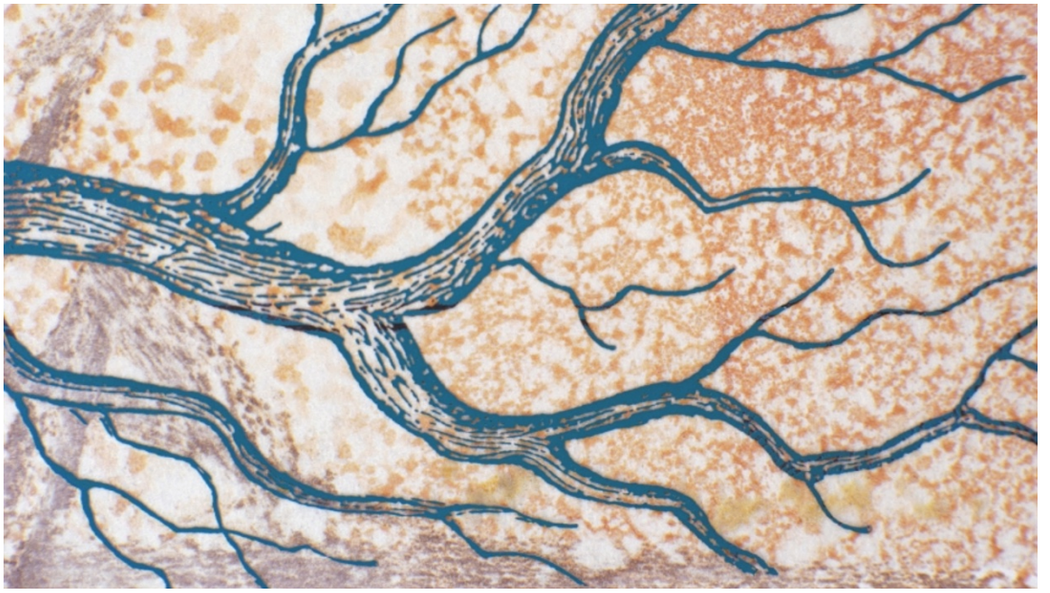

VASCULAR BIOLOGY

How not to clot

Blood clotting involves a complex signalling cascade, at the heart of which lies thrombin. This serine protease regulates the aggregation of platelets and blood-derived proteins to form a clot, which stems bleeding after injury and allows the wound to heal. Cellular responses to thrombin are mediated by a small family of G-protein-coupled receptors called the protease-activated receptors (PARs), and two reports from Shaun Coughlin and colleagues in *Nature* and *Science* now show several essential functions for these proteins.

Four members of the PAR family (PAR1–4) have been identified so far, and three of these (PAR1, 3 and 4) are activated by thrombin. Human platelets express PAR1 and PAR4, and blockade of PAR1 function decreases thrombin responses in human platelets in *ex vivo* studies. However, deletion of the *Par1* gene has no effect on platelet activation in mice, and this is because mouse platelets express PAR3 and PAR4, but not PAR1.

To study the steps that connect the activation of thrombin to platelet aggregation, Coughlin and colleagues deleted the mouse *Par4* gene. Platelets from these mice did not respond to thrombin and showed none of the characteristic changes in shape, secretion of ATP, aggregation or changes in the levels of cytoplasmic calcium, indicating that PAR4 is necessary for thrombin responses in mouse platelets. By contrast, expression of PAR3 in the $Par4^{-/-}$ mice was normal, indicating that PAR3 alone is not enough to allow platelet activation by thrombin. This supports a model in which PAR3 acts as a cofactor that helps to promote the cleavage and activation of PAR4 in response to a low concentration of thrombin.

Coughlin and colleagues then used the fact that the $Par4^{-/-}$ platelets are unresponsive to thrombin to investigate the importance of thrombin in haemostasis (appropriate clotting that stops bleeding) and thrombosis (inappropriate clotting that causes disease). Haemostasis was assessed by tail bleeding, and the $Par4^{-/-}$ mice lost 25 times as much blood as wild-type mice over 20 minutes. Thrombosis was monitored by injuring the mesenteric arterioles and then monitoring clot formation by videomicroscopy. Compared with wild-type mice, the $Par4^{-/-}$ mice were protected in this model of arteriolar thrombosis.

As the authors point out, although mice and humans differ in the subset of PAR proteins that they use, thrombin potently activates platelets from both species. So, in addition to establishing the importance of the thrombin signalling pathway in normal haemostasis and development, these results indicate that this pathway could be a target for preventing or treating thrombosis.

Although PAR1 is not involved in platelet activation in mice, embryos lacking this protein die at midgestation, often with signs of bleeding. What, then, is the function of PAR1? As Coughlin and colleagues report in their second paper, they have found a role for this protein in the development of blood vessels during embryogenesis.

To investigate why $Par1^{-/-}$ embryos bleed, the authors generated PAR1–*LacZ* 'knock-in' mice. They observed expression of PAR1 in the endocardium (the layer of cells that lines the chambers of the heart and forms the surface of the valves) and vascular endothelium. If loss of PAR1 signalling in the endocardial/endothelial cells were the main defect in $Par1^{-/-}$ embryos, Coughlin and colleagues reasoned that endothelial-specific expression of PAR1 should prevent death. They expressed a PAR1 transgene under the control of an endothelial-specific promoter and showed that this indeed reduced or prevented embryonic death.

How is endothelial PAR1 activated during normal embryonic development? Thrombin is a likely candidate, but further experiments raised the possibility that as-yet-unidentified activators might exist. These experiments also showed that PAR1 is not the only target of the coagulation cascade that is important for normal embryonic development, and candidates for additional targets include the other PARs. More generally, these studies indicate that the coagulation cascade might be involved in building blood vessels rather than just patching leaks as they occur.

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References and links

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WEB SITE UCSF Cardiovascular Research Institute: http://cvri.ucsf.edu/default.htm

IN THE NEWS

Stem cell funding suspended

The already beleaguered stem cell research community in the United States received another blow in August, when billionaire entrepreneur Jim Clark suspended payment towards a biomedical centre in protest against the US Government's restrictions placed upon stem cell research.

Clark stated in a letter to the New York Times that he was suspending his remaining $60 million pledge to Stanford University in response to the decision to restrict federally funded research to the 64 cell lines that are already known to exist.

"I can say that with no prospect of federal support, significant scientific inquiry in a field like stem cell research will stop. No research leader can forgo federal money", Clark wrote. The situation may, in fact, be worse as US Government officials now estimate there might only be around 25 lines that are actually available.

Clark, the founder of Netscape and a former electrical engineering professor at Stanford, had originally committed $150 million towards the James H. Clark Center for Biomedical Engineering and Sciences, for research into the convergence of engineering and molecular biology.

But restrictions on stem cell research means US scientists risk "being thrown into a dark age of medical research", Clark argues.

Many US scientists agree. Last month, a report on the state of human stem cell science by a National Academy of Sciences committee, headed by Bert Vogelstein, strongly supported federal financing for stem cell research, although it did not directly address the Government's policy.

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