



pathogenesis are now known<sup>6,7</sup>.

The importance of Miele's study consists of having fished a single transcript that is consistently downregulated in infected spleens out of a pond of 10,000 non-specific mRNA transcripts. Analysis of the protein this transcript encodes yielded several big surprises. First, the transcript encodes erythroid differentiation-related factor (EDRF), a protein mainly found in erythroid precursors—a cell lineage that nobody would have suspected to be associated with prions. Miele *et al.*<sup>1</sup> observed that the EDRF downregulation can be detected in blood samples of mice and sheep. Interestingly, EDRF is not even expressed in brain, the organ that undergoes the most dramatic damage during the course of the disease. But the most unexplainable finding of the study is that EDRF downregulation is detectable in the bone marrow of BSE-affected cows. This is surprising because infectious prions cannot be detected in the lymphoreticular organs or bone marrow of cows. Therefore, it is difficult to argue that EDRF downregulation is a direct consequence of the involvement of lymphoreticular organs in pathogenesis. Prion infection of cows may modulate EDRF in a mysterious, indirect fashion.

What is the significance of this study? The gene *EDRF* has been identified using an unbiased 'transcriptomics' approach<sup>1</sup>. There was no underlying hypothesis about its possible involvement

in prion pathogenesis, and, for all we know, there is no specific reason to surmise that it has any. However, this study should stimulate a number of hypothesis-driven experiments that address this question. These may include challenging *EDRF*-null or *EDRF* transgenic animals with infectious prions and studying disease pathogenesis. Other experiments could include adoptive bone marrow transfer of EDRF-expressing cells, in order to identify the tissue compartment whose infection suppresses *EDRF* transcription.

Will it be possible to establish a diagnostic test for prion infection based on these observations? Judging from the results of the study, EDRF may be a good diagnostic marker, whether it actually participates in disease pathogenesis or not. Future studies are required to determine the point during the course of the disease at which downregulation of the transcript occurs in cows and—hopefully—in humans. It is possible that downregulation could indeed occur during early stages of disease progression, since infectious prion colonization of lymphoreticular tissues happens almost immediately after infection—in contrast to neuroinvasion, which is a late event.

However, several questions will need to be answered in order to establish the viability of an EDRF-based prion infection assay. For one thing, the normal cellular range of *EDRF* expression levels in a healthy, non-infected population

will have to be established and compared to that of infected individuals. The extent of variation among healthy individuals will determine the diagnostic accuracy of an EDRF-based screen. Also, it has not yet been investigated whether EDRF downregulation is a prion-specific phenomenon, or whether it occurs in other diseases. Given the current public and commercial interest in prion testing, and the desirability of a blood-based assay, the answers to these questions may come quickly.

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## A role for fibronectin in self-repair after ischemic injury

Plasma fibronectin has been proposed to play a role in wound healing. Studies with conditional knockout mice, however, indicate that fibronectin has more to do with protecting cells from ischemic damage after stroke (pages 324–330).

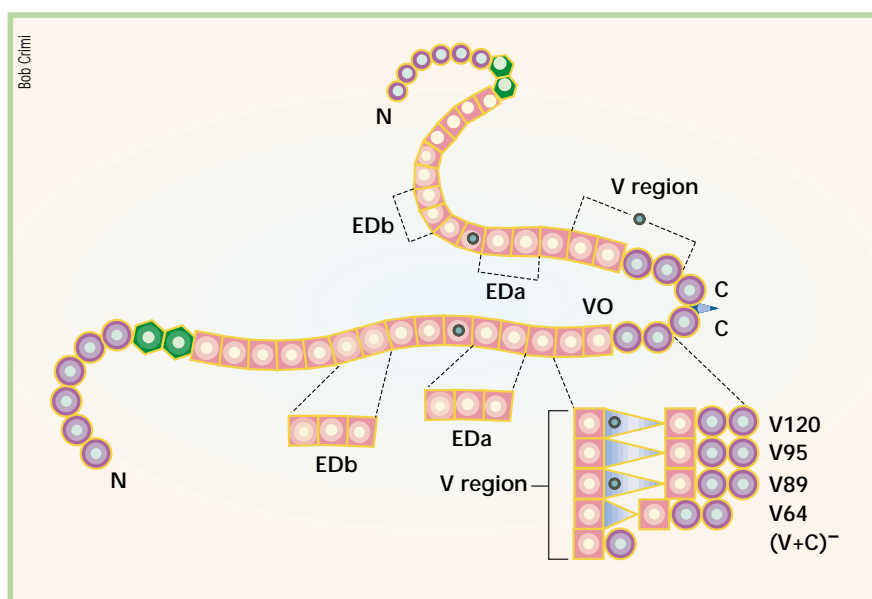
Fibronectin was characterized more than a half century ago as the plasma protein 'cold-insoluble globulin.' Research on this glycoprotein surged in the 1970s when it was found that its expression was lost during malignant transformation<sup>1</sup>. Fibronectin is a multifunctional protein that is now studied by many different types of biologists, including those who study integrin-mediated cell adhesion, composition and assembly of extracellular matrix, and multi-modular protein structure<sup>2</sup>. Analysis of fibronectin-null mice revealed that the protein is essential for development, as

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these mice die at embryonic day 8.5 due to severe defects in mesoderm-derived tissues<sup>3</sup>. This embryonic lethality has made it difficult to determine the *in vivo* functions of fibronectin.

Fibronectin can be categorized into two broad classes: cellular and plasma. Although both proteins are generated from a single gene, their isoforms differ due to alternative splicing (Fig. 1). Cellular fibronectin is expressed by fibroblasts, as well as other cell types, and is deposited locally into the extra-

cellular matrix. Plasma fibronectin is expressed at high levels by hepatocytes<sup>4</sup> and secreted in soluble form into the plasma, where it has a half-life of two days in the circulation. It is cross-linked to fibrin by activated blood coagulation factor XIII and is present in the hemostatic plug that forms after disruption of blood vessels. Some studies suggest that plasma fibronectin mediates cell adhesion and migration on the plug's provisional fibrin matrix, and thus supports wound healing. In this issue, Sakai *et al.*<sup>5</sup> describe investigations of the *in vivo* role of plasma fibronectin. They



**Fig. 1** Diagram of fibronectin. Fibronectin is a dimer composed of ~250-kD subunits. Each subunit is comprised of 3 types of repeating homologous sequences known as types I (Large Circles), II (Hexagons), and III (Squares) modules. 3 sites of alternative mRNA splicing (dashed lines) have been identified that lead to diversity in the stretch of tandem type III modules<sup>2,10</sup>. These include 2 type III modules known as extra domains, EDa and EDb. EDa and EDb are absent from plasma fibronectin, but not from cellular fibronectin. Near the C-terminus, sequences of 120, 95, 89, 64 or 0 residues can be inserted to give rise to V120, V95, V89, V64 and VO variants (V region). These are shown as a horizontal triangle or parts thereof. The sequence encoding the V-region can also be excluded to form the (V + C)<sup>-</sup> splice variant. The VO sequence is always present in one subunit of plasma fibronectin<sup>2</sup>. Thus, up to 24 different variably spliced subunits and up to 576 different homodimers or heterodimers can be formed. Different splice variants are expressed by different cells and in response to different stimuli<sup>7,10</sup>. Integrin binding sites are indicated by small circles.

report the surprising result that although it is dispensable for wound healing, plasma fibronectin has a role in neuronal protection following focal cerebral ischemia.

Sakai *et al.*<sup>5</sup> used the *Cre/LoxP* system to disrupt fibronectin production by hepatocytes in adult mice. The authors were able to introduce *LoxP* elements around the fibronectin gene without affecting normal expression of mRNA or protein during development. Most experiments were carried out in mice expressing Cre recombinase under control of the interferon-inducible *Mx* promoter, a system that rapidly and completely eliminated fibronectin expression from liver. Expression was also decreased in spleen, hematopoietic cells (including megakaryocytes), kidney, heart and brain. As fibronectin production decreased in hepatocytes, the concentration of plasma fibronectin fell to less than 0.1% of control within several days. The authors also studied mice in which Cre recombinase was regulated by the hepato-

cyte-specific albumin promoter. These mice lost expression of hepatic fibronectin more gradually, and plasma levels of fibronectin dropped over the course of several months.

Fibronectin is stored in platelet-cell compartments known as  $\alpha$ -granules, which are secreted during platelet aggregation. An examination of platelets taken from both types of *Cre/LoxP* mice supports prior studies suggesting that fibronectin is stored in these granules as a mixture of cellular fibronectin, synthesized by megakaryocytes, and plasma fibronectin, taken up by endocytosis.

Contrary to what the literature would predict, mice lacking plasma fibronectin underwent normal healing of cutaneous wounds<sup>5</sup>. Immunofluorescence analysis demonstrated that platelet fibronectin was the only form of fibronectin that could be detected early after cutaneous wounding of the *Cre/LoxP* mice. However, locally produced cellular fibronectin appeared at the site of injury of within 12 hours

after wounding.

Previous studies had documented extravasation (blood vessel release) of plasma fibronectin, without subsequent local expression of cellular fibronectin, following ischemic brain injury. This led Sakai *et al.*<sup>5</sup> to study the response of the *Cre/LoxP* mice to occlusion of the middle cerebral artery for 30 minutes. Two days after the occlusion, the authors observed prevalent deposition of plasma fibronectin in the infarcted area of control mice, but no deposition of plasma fibronectin in that of the *Cre/LoxP* mice. At two and seven days after ischemia, infarction volumes in the *Cre/LoxP* mice were significantly greater compared with those of control mice. These results indicate that plasma fibronectin has the heretofore unappreciated role of minimizing the volume of affected tissue after reperfusion of ischemic brain.

How might deposition of plasma fibronectin protect neural tissue from ischemic injury? Sakai *et al.*<sup>5</sup> did not observe any differences in the numbers of phagocytic cells, amount of vascularization, or level of glial response between control and *Cre/loxP* mice. However, two days after ischemia was induced, the authors observed an increased number of caspase-3-positive apoptotic cells in the infarcted areas of *Cre/loxP* mice compared to controls, representing an increase in cell death. They propose that fibronectin binds to cell surface  $\alpha_5\beta_1$  and/or  $\alpha_v\beta_3$  integrins to cause upregulation of the anti-apoptotic protein Bcl-2, preventing caspase-3-mediated apoptosis. This apoptotic pathway occurs in both neuronal and non-neuronal cells.

Is there potential to use fibronectin to prevent neural cell death after stroke? Plasma fibronectin is able to cross the blood-brain barrier during infarction. The increase in permeability appears greatest in the center of infarction. Cells at the periphery of the infarcted area, which presumably receive the least damage, therefore may have the least opportunity to use the survival pathway afforded by plasma fibronectin. In other words, gradients of fibronectin release and cells able to respond to fibronectin likely oppose one another. In order to effectively use fibronectin for treatment of stroke, we may need to find ways to optimize its delivery. This may be done through addition of fibronectin to the clot-bust-



ing reperfusion mixture, or the addition of agents that increase the permeability fibronectin.

Sakai *et al.*<sup>5</sup> describe several interesting directions for future research. One important question to answer is whether plasma fibronectin is deposited after ischemic damage by copolymerization with fibrin or by some other mechanism. It will also be interesting to breed the Cre/*LoxP* mice with mice carrying disruptions in genes encoding proteins that interact with fibronectin. The authors propose examining the effects of triple-knock-out mice lacking plasma fibronectin, fibrinogen and von Willebrand factor, and determine whether hemostasis is impaired beyond that of mice lacking fibrinogen and von Willebrand factor<sup>6</sup>. This Cre/*LoxP* system can also be used to examine the effects of fibronectin deletion in other tissues such as cartilage, where the (V + C) splice variant (Fig. 1) of fibronectin is found<sup>7</sup>.

Researchers should also investigate

the roles of plasma and cellular fibronectin in response to ischemic damage of other organs, such as heart. Reperfusion is the standard therapy for acute myocardial infarction, even when the myocardium is considered beyond salvage<sup>8,9</sup>. Fibronectin has been observed to accumulate more rapidly in animal models of cardiac infarct/reperfusion than in hearts of animals with permanent ligation of a coronary artery<sup>8,9</sup>. Plasma fibronectin may therefore have the same protective effects after myocardial infarction that it does after cerebral infarction.

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## The neglected ion: HCO<sub>3</sub><sup>-</sup>

Most research into the function of the cystic fibrosis transmembrane conductance regulator has focused on its role in Cl<sup>-</sup> transport. New findings suggest that we may have been focusing on the wrong ion.

Cystic fibrosis (CF) is a recessive hereditary disease that results in the accumulation of thick mucus that clogs the lungs and interferes with the flow of digestive enzymes through the pancreas and to the small intestine. Other complications of CF are the abnormal secretions of sweat and saliva. The disease is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator<sup>1</sup> (CFTR), which was cloned over 12 years ago<sup>2</sup>. CFTR functions as a Cl<sup>-</sup> channel, and Cl<sup>-</sup> permeability defects are well documented in cells from CF patients<sup>3</sup>. However, despite extensive research focused on CFTR function, there is still no obvious explanation for how a defect in Cl<sup>-</sup> permeability leads to chronic refractory lung infections and several other symptoms of this fatal disease. Although almost all disease-associated mutations are believed to disrupt Cl<sup>-</sup> transport, there is no compelling explanation for the remarkable diversity in morbidity and mortality observed among CF pa-

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tients<sup>4</sup>. In the 1 March issue of *Nature*, Choi *et al.*<sup>5</sup> report that in addition to disrupting Cl<sup>-</sup> transport, CF-associated CFTR mutations also disrupt the transport of another biologically-important ion, HCO<sub>3</sub><sup>-</sup>.

Early studies in CF patients reported that HCO<sub>3</sub><sup>-</sup> secretion was barely detectable in pancreatic juice<sup>6</sup>. These findings were of interest, as 85% of CF patients develop pancreatic insufficiency (PI); that is, the pancreas cannot produce enough enzymes to support normal digestion<sup>7</sup>. Other studies reported that even in CF patients who retained pancreatic sufficiency (PS), pancreatic enzymes were secreted in abnormally low volumes because HCO<sub>3</sub><sup>-</sup> dependent fluid secretion was impaired<sup>8</sup>. Despite these findings, the role of HCO<sub>3</sub><sup>-</sup> anions in CF pathogenesis has received little attention compared to other aspects of this disease.

Like many scientists faced with unanswered questions, CF researchers have frequently asked themselves if they were overlooking something. Choi *et al.*<sup>5</sup> provide good evidence that we have indeed overlooked the role of aberrant HCO<sub>3</sub><sup>-</sup> transport in CF pathogenesis. The authors studied mutant CFTR function by transfecting human embryonic kidney cells with genes encoding 17 different CF-associated CFTR mutants. Of the mutants that were correctly processed and transported to the cellular membrane, those associated with PI retained less than 14% of the HCO<sub>3</sub><sup>-</sup> transport activity observed in control cells, which were transfected with the wild-type CFTR gene. In contrast, mutant forms of CFTR associated with PS were able to transport HCO<sub>3</sub><sup>-</sup> at greater than 30% of the wild-type levels. Remarkably, 7 of 9 PI mutations tested in cells were able to transport Cl<sup>-</sup> at wild-type levels or higher.

These findings suggest that CFTR is essential for more than just the transport of Cl<sup>-</sup>, and that the separate func-