the function of a protein such as Mass1 will provide insight into cellular and molecular mechanisms controlling neuronal excitability in the mammalian nervous system. In turn these insights may suggest novel pharmacological approaches to regulating disorders of neuronal excitability such as epilepsy.

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Plugging the leaks

It has been known for over 20 years that vascular leakage in inflammation is dependent on neutrophils. The identification of the permeability-enhancing activity produced by neutrophils provides clues to the development of anti-inflammatory drugs with a new mechanism of action (pages 1123–1127).

KLAUS LEY

During inflammation, neutrophils and other leukocytes transmigrate from the vascular compartment into the inflamed tissue space. This process is initiated by leukocytes rolling along the vessel wall, followed by firm adhesion to the wall and migration across it. At first glance, it may come as no surprise that neutrophil transmigration is associated with increased vascular permeability, because the migrating leukocytes leave microvessels through holes in the wall, which may also allow plasma to leak[1]. However, careful investigations over the last two decades have unequivocally shown that the two processes can be dissociated. Neutrophil adhesion and transendothelial migration is possible with or without plasma leakage, and plasma leakage is possible with or without transmigration. Specifically, the permeability-increasing activity of neutrophils can be inhibited by polyanions like dextran sulfate[2] (Table 1). This means that the concept of leukocytes drilling holes through which plasma then escapes is incorrect; rather, neutrophils seem to have a specific way of regulating endothelial permeability. In this issue, Gautam *et al.*[3] identify neutrophil heparin-binding protein (HBP) as the key and possibly only mediator involved in the process.

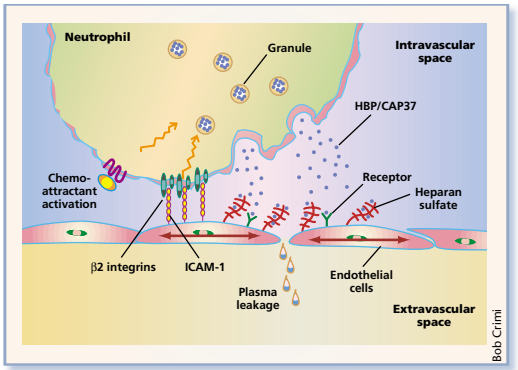

Fig. 1 At a site of inflammation, a neutrophil is activated by a chemoattractant (yellow circle), and adheres to an endothelial cell through β_2 integrins (green heterodimers on neutrophil surface; for example, leukocyte function associated molecule-1 (LFA-1)) becoming engaged to endothelial ligands such as intracellular adhesion molecule-1 (ICAM-1). The engagement of β_2 integrins triggers the release of granules containing HBP/CAP37 (blue dots), a molecule that binds to heparan sulfate (antenna-like structures on endothelial cells) and potentially to an unknown receptor. HBP/CAP37 causes endothelial cells to undergo cytoskeletal rearrangements (arrows) that lead to interendothelial gaps which allow plasma (drops) to leak into the extravascular space.

When rolling leukocytes become firmly adherent to the vessel wall, they engage a specialized class of integrins (β_2 or CD18 integrins) that are exclusively expressed on leukocytes. Somewhere during the process, most likely at multiple points, neutrophils become activated. Activation by chemokines, a class of peptides that bind to G-protein coupled receptors, or by other chemoattractants can promote neutrophil adhesion. In addition to adhesion, engagement of CD18 integrins mediates several other neutrophil responses, including the production of oxygen free radicals in the respiratory burst, expression of β_1 integrins, and the release of granules[4] (Fig. 1). Blocking CD18 integrins not only reduces neutrophil transmigration, but also vascular leakage[5]. This finding was initially interpreted to be a consequence of blocking transmigration, rather than a specific signaling event. Gautam *et al.* now show that engagement (by adhesion) or antibody-mediated cross-linking of CD18 integrins is necessary and sufficient to release a permeability-enhancing activity, which they identify as HBP, also known as CAP37 or azurocidin[6] (Fig. 1).

In this study, the authors use bovine aortic and human umbilical vein endothelial cells and peripheral blood neutrophils in a transwell system, a standard model for transmigration and permeability measurements, and also confirm that recombinant HBP/CAP37 increases microvascular permeability in an *in vivo* model. It appears that all of the permeability-increasing activity of human neutrophils, within experimental error, is caused by HBP/CAP37, a 28-kD molecule with homology to elastase but without protease activity. First, removal of HBP/CAP37 but not elastase or cathepsin G from the supernatant of activated neutrophils removes the permeability-increasing activity; second, a polyclonal antibody to HBP/CAP37 blocks most of the activity; and third, recombinant HBP/CAP37 shows identical activity as endogenous HBP/CAP37.

Notably, HBP/CAP37, although it has no protease activity, still binds inhibitors of Kunitz-type proteases such as aprotinin. Gautam *et al.* report that aprotinin neutralizes the permeability-increasing effect of HBP/CAP37. This finding raises the interesting possibility that the beneficial effects of aprotinin reported in clinical settings like cardiopulmonary bypass may be due to HBP/CAP37 blockade rather than anti-proteolytic effects.

The complete dependence of neutrophil-mediated permeability increase on HBP/CAP37, at least in the experimental

Table 1 Dissociation of leukocyte transmigration and plasma leakage

Experimental state	Leukocyte transmigration	Plasma leakage
normal	–	–
inflammation; for example LtB_4 or IL-1β	+	+
LtB_4 plus dextran sulfate administration	+	–
histamine administration	–	+

LtB_4: leukotriene β_4; IL-Iβ, interleukin Iβ

system employed by the authors, is quite remarkable, given the large number of proteins, peptides and lipid mediators released by activated neutrophils. Elastase, which has often been suspected to cause increased vascular permeability, indeed had no such activity, and removing elastase from neutrophil supernatant had no effect. The identification of the mediator of neutrophil-dependent permeability increase could have potentially far-reaching consequences. If it is possible to selectively and specifically inhibit HBP/CAP37, it could be a treatment that is additive or potentially synergistic with histamine blockers and other treatments aimed at reducing inflammatory exudates.

Gautam *et al.* open the door to research aimed at studying neutrophil-dependent leakage. First on the list will be the identification of an endothelial receptor for HBP/CAP37 and elucidation of how signals are transduced. Although HBP/CAP37 is known to bind to heparan sulfate proteoglycan, it is not clear that the relevant receptor itself is a proteoglycan. For example, many chemokines bind heparan sulfate, yet they transduce signals through their cognate G-protein–coupled receptors. Another issue to address is whether HBP/CAP37 is really the only or the most important mediator of neutrophil-induced vascular permeability in more complex systems and in different organs. To answer this question, it will be necessary to generate HBP/CAP37-deficient mice by homologous recombination or find effective and specific inhibitors that are applicable *in vivo*. Third, the regulation of HBP/CAP37 release is not entirely clear. Although ligation of CD18 integrins plays a role, other signals may be required.

As we await conclusive answers to these questions, the finding that HBP/CAP37 mediates neutrophil-induced vascular permeability has identified a potentially interesting target for therapeutic intervention. Inhibitors of HBP/CAP37 could become anti-inflammatory drugs that work through a novel mechanism of action.

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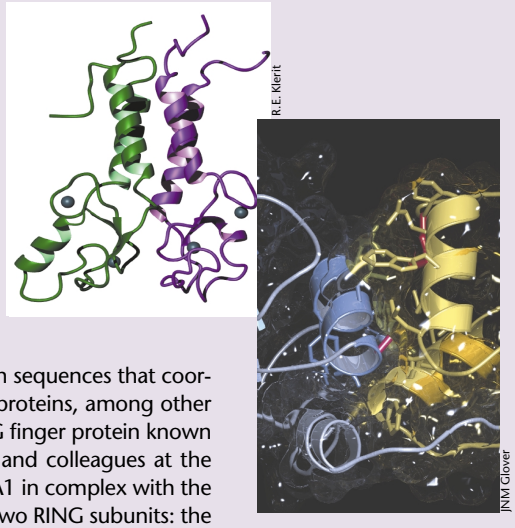
A close look at the ends of BRCA1

The first glimpses at the structures of the N- and C-terminal regions of BRCA1 provide important insights into the molecular properties of the protein and a framework for studying how inherited mutations in the *BRCA1* gene affect its tumor suppressor function, according to two papers published in this month's issue of *Nature Structural Biology*.



The *BRCA1* gene encodes a 1,863 amino-acid protein that has been implicated in several cellular processes, including DNA repair and cell-cycle checkpoint control. The protein contains two recognizable protein motifs: a RING finger domain near the N-terminus and two tandem copies of a BRCT domain at the C-terminus. Both types of motifs appear to be critical to tumor suppressor function of the gene as mutations in these regions are often seen in *BRCA1*-linked tumors.

RING fingers, which are found in a number of different proteins, are cysteine-rich sequences that coordinate the binding of two zinc ions and appear to promote the ubiquitination of proteins, among other functions. The RING finger in *BRCA1* specifically interacts with another similar RING finger protein known as BARD1, which was recently identified based on this interaction. Rachel Klevit and colleagues at the University of Washington report the solution structure of the RING domain of BRCA1 in complex with the RING domain of BARD1. The illustration to the left shows a ribbon diagram of the two RING subunits: the BRCA1 subunit (green) contains three α-helices and three β-strands, while the BARD1 subunit (purple) contains two α-helices and two β-strands. Two α-helices from each subunit pair in an antiparallel fashion to form a stable four-helix bundle, placing the sequences that bind zinc ions (shown as circles) in direct apposition to one another. The authors point out that several mutations that cause breast or ovarian cancer map to the zinc-binding regions or the BRCA1 –BARD1 dimerization surface.

Both BARD1 and BRCA1 also share another conserved sequence known as the BRCT domain, a phylogenetically conserved sequence found in proteins involved in DNA repair and cell-cycle regulation. In the second report, Mark Glover and colleagues at the University of Alberta, Canada, determine the crystal structure of the C-terminal BRCT region of BRCA1 at 2.5 Å resolution. The illustration to the right shows the structure of the two BRCT repeats (one in blue and the other in yellow), which have adopted similar structures and are packed together in a head to tail arrangement. The structure provides the first evidence that these repeats assemble into a stable unit that is resistant to limited proteolysis. Two of the best-characterized BRCA1 cancer-causing mutations occur at the interface between the two repeats and the authors show that these mutations reduce the proteolytic stability of the composite structure.

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