epididymis — a convoluted tube within the testes where sperm maturation occurs — acidifies its lumen using the V-ATPase H^+ pump[9]. Here, however, acidification has a different role: the acidic environment is necessary for sperm maturation and for making sperm cells dormant until they are 're-awoken' by the alkaline environment of the seminal vesicle. In this context, excessive ammonia secretion into the epididymal lumen would be detrimental, prematurely awakening sperm cells. The authors propose that Rhcg in the epididymis could be responsible for scavenging the potentially toxic ammonia from the lumen and transporting it into the cells lining the epididymal lumen where it can be converted to glutamine.

Trying to identify functional differences between knockout and normal mice is technically challenging, in part because of the small size of mice. What helped Biver *et al.* in their quest was their ability to adapt some classical physiological techniques to the mouse, including measurements of the composition of body fluids, of intracellular pH using fluorescent dyes, and of solute transport in isolated perfused renal tubules. These approaches have been part of the physiological armamentarium for years, but have gradually been displaced by modern molecular and cell-biological techniques. For example, the isolated perfused tubule technique developed[10] in the 1960s — like many other intricate physiological methods — has not been used extensively since the 1980s. With expertise in many physiological techniques in decline, Biver and colleagues' work is a testament that such methods are still of enormous value. It will be interesting to see whether physiologists' new role as functional annotators of the genome will spur a resurgence of training in traditional physiological techniques.

Biver and colleagues' data[2] resolve a long-standing question in kidney physiology: what is the molecular basis of ammonia transport into the urine? With the identification of Rhcg as the ammonia channel in the collecting duct, researchers can now address questions such as whether and how this channel may be regulated, and what its involvement is in disease processes associated with impaired regulation of blood pH. Reproductive biologists will undoubtedly soon be investigating the specific role of Rhcg in epididymal function and sperm maturation. ■

Mark A. Knepper is in the Division of Intramural Research, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-1603, USA.
e-mail: knep@helix.nih.gov

1. Clamp, M. *et al. Proc. Natl Acad. Sci. USA* **104,** 19428–19433 (2007).
2. Biver, S. *et al. Nature* **456,** 339–343 (2008).
3. Knepper, M. A., Packer, R. & Good, D. W. *Physiol. Rev.* **69,** 179–249 (1989).
4. Wall, S. M., Davis, B. S., Hassell, K. A., Mehta, P. & Park, S. J. *Am. J. Physiol. Renal Physiol.* **277,** F866–F874 (1999).
5. Pitts, R. F. *Fed. Proc.* **7,** 418–426 (1948).
6. Marini, A.-M., Urrestarazu, A., Beauwens, R. & André, B. *Trends Biochem. Sci.* **22,** 460–461 (1997).
7. Westhoff, C. M. & Wylie, D. E. *Transfus. Clin. Biol.* **13,** 132–138 (2006).
8. Chambrey, R. *et al. Am. J. Physiol. Renal Physiol.* **289,** F1281–F1290 (2005).
9. Pastor-Soler, N., Piétrement, C. & Breton, S. *Physiology* **20,** 417–428 (2005).
10. Burg, M., Grantham, J., Abramow, M. & Orloff, J. *Am. J. Physiol.* **210,** 1293–1298 (1966).

STRUCTURAL BIOLOGY

Enzyme knocked for a loop

Ronald L. Mellgren

Protein-digesting enzymes are kept on a tight leash to stop them from wantonly attacking targets. Two crystal structures show how an inhibitory protein domain gags one such enzyme without being chewed up itself.

All organisms have enzymes, known as proteinases, that break down other proteins, and have vital roles in such disparate processes as food digestion and blood clotting. These enzymes can't be allowed to run amok — if a protein has been mistakenly broken down, then a replacement has to be painstakingly re-synthesized from scratch. Several strategies have therefore evolved to ensure that proteinases are allowed to act only in appropriate circumstances. For example, inhibitor proteins block proteinase activity using various mechanisms[1,2]. In this issue, two crystallographic studies[3,4] show that the protein inhibitor calpastatin uses a previously undiscovered mechanism to block the activity of the calpain proteinase. Two helical regions of calpastatin bind to widely separated sites on calpain; this reinforces the wrapping of an intervening, unstructured region of the inhibitor around calpain's active site.

Calpains are calcium-dependent proteinases found inside cells[5]. In most mammalian cells, the two main forms are μ-calpain (calpain 1) and m-calpain (calpain 2), each of which consists of two protein chains: a large catalytic subunit and a smaller regulatory subunit. It has been shown that m-calpain is required for embryonic development in mice[6]. Calpains also cut several structural proteins that regulate cell shape, disrupting their association with other proteins and so affecting the organization of

50 YEARS AGO

Suspect Documents: Their Scientific Examination. By Dr. Wilson R. Harrison — The book under review is a very important contribution to the published knowledge on the examination of suspect documents ... Fraudulent documents have existed as long as genuine documents, and the problem of their detection has been of considerable importance to States and individuals. This problem existed before science was sufficiently advanced to assist in its solution and, therefore, there grew up a tradition of examination of documents based on superstitious credulity and with no scientific background whatsoever ... [T]he volume under review fills the gap from the British point of view ... and the book must therefore be read by all who are interested in these matters.

ALSO:

The control of diseases of livestock is an important feature in the general economy of most countries in improving animal production and in fostering international trade in livestock and animal products ... It is gratifying to learn in this connexion that one of the recent steps taken ... has been the establishment of a World Reference Laboratory for Foot-and-Mouth Disease at the Research Institute (Animal Virus Diseases), Pirbright, Surrey.
From *Nature* 22 November 1958.

100 YEARS AGO

A common criticism of the methods of teaching science adopted in schools for girls is that they are too academic and have little or no bearing upon the duties the girls will be called upon to perform in after life. This weakness is, we are glad to know, becoming less common, and earnest efforts are being made in several centres to arrange courses of work in which elementary science and the home arts are taught together, the latter being treated largely as applications of the former.
From *Nature* 19 November 1908.

50 & 100 YEARS AGO

the cell's protein 'skeleton' (the cytoskeleton). Studies on calpain-deficient cells show that calpains are important for remodelling the cytoskeletal structures that abut the cell membrane and regulate cell movement[7]. Furthermore, calpains contribute to several calcium-initiated cell-death programs.

Calpastatin inhibits both μ- and m-calpains, and is approximately the same size as the large calpain subunits. But unlike the proteinases, it is an intrinsically unstructured protein, adopting a defined structure only on binding to active calpain. Each calpastatin molecule contains four regions — the calpastatin inhibitory domains, CIDs I–IV — that bind calpains and block their activity (Fig. 1a). Each CID is, in turn, subdivided into three regions (A to C) that are predicted to interact with calpain. Because calpastatin molecules are largely unravelled in solution, they can potentially interact with four calpain molecules simultaneously, with one calpain at each CID.

The current papers[3,4] reveal for the first time a complete picture of how calpastatin shuts down calpain activity. Hanna *et al.*[3] (page 409) present the structure of calcium-bound m-calpain in complex with the CID-IV fragment of calpastatin, whereas Moldoveanu *et al.*[4] (page 404) report the structure of calcium-bound m-calpain in complex with the CID-I fragment. The two studies show that the binding of calcium ions to m-calpain causes changes in the positioning of the four catalytic subunit domains DI–DIV, and of a regulatory subunit domain, DVI (Fig. 1b). Calcium binding thus generates interaction sites in the proteinase to which CIDs bind; specifically, a site created in DIV is bound by CID region A, and a site in DVI is bound by CID region C. In this way, CIDs bind simultaneously to two widely separated domains of calpain. This strong binding allows CID region B — a largely unstructured area between the helices of regions A and C — to make several contacts with the DI–DIII regions of calpain, thus blocking the proteinase's active site. These observations[3,4] confirm and refine the findings of recent nuclear magnetic resonance studies[8], which also indicated that calpastatin wraps around calpain as described above.

This inhibitory mode of action by calpastatin seems like a dangerous trick — deliberately inserting an unfolded protein into the active site of a proteinase is a bit like putting one's head in a lion's mouth and hoping that it won't be bitten off. But the crystal structures[3,4] show that calpastatin has evolved a neat trick to avoid being cleaved by calpain: the short stretch of protein that would be expected to enter the active site actually loops away from the proteinase (Fig. 1b). Thus, calpastatin, although largely disordered on its own, is fine-tuned to generate a local structural motif that protects it from attack when attached to its calcium-bound substrate.

The new studies[3,4] tell us as much about calpains as they do about calpastatin. Although

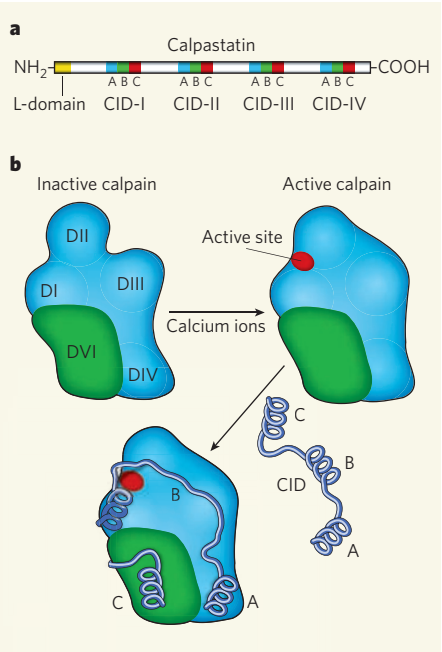

Figure 1 | It's a wrap for calpain. Calpain enzymes break down proteins, but are inhibited by calpastatin. **a**, Calpastatin is an elongated protein that contains three or four independent inhibitory domains (CIDs). The larger form of calpastatin (which contains four CIDs) also has a terminal L-domain that anchors the protein to cell membranes. Each CID is composed of three regions, A–C, which bind to different domains of calpain. **b**, Hanna *et al.*[3] and Moldoveanu *et al.*[4] report crystal structures of a calpain (m-calpain) in complex with calcium ions and with CID fragments of calpastatin. These structures show calpain's four catalytic subunit domains (DI to DIV, blue) and its regulatory subunit domain DVI (green). When m-calpain binds to calcium, it adopts a compact, active conformation in which DIII moves toward DII, and the active site (red) is formed. The A region of the CID associates with DIV, whereas the C region binds to DVI. The B region wraps around the remaining subunits, blocking the active site. The B region loops away from the catalytic site to avoid being cleaved by the enzyme.

the crystal structure of calcium-free (and thus inactive) m-calpain was solved several years ago[9], crystallization of calcium-bound calpain has been problematic. Structural studies have thus relied on the extrapolation of results from engineered calpain fragments that contain only DI and DII (ref. 10). Calpastatin does not bind calcium, and has little affinity for calpains in the absence of calcium. It therefore seems highly likely that, as the authors of the studies suggest[3,4], the structure of calcium-bound calpain in their calpastatin co-crystals is at least a close approximation of the active conformation of m-calpain.

The structures[3,4] show that, on calcium binding, four specific arginine amino-acid side chains in the DIII domain of m-calpain contact the DII domain, stabilizing the catalytically active conformation. Moldoveanu *et al.*[4] observe that mutations in m-calpain, in which the arginines are replaced with other amino acids, lower m-calpain's activity. These arginines are also found in a muscle-specific isoform of calpain known as calpain-3; mutation of the arginines in calpain-3 leads to loss of the proteinase's activity, an effect that has been associated with some cases of limb-girdle muscular dystrophy[11].

More broadly, the studies[3,4] provide further evidence for the involvement of m- and μ-calpains in continuous cellular processes. Calpastatin CIDs apparently evolved to remain intact as they inhibit calpain activity, even though resistance to cleavage is not necessary for this purpose[3]. But if cleaved and reused, CIDs would probably be less effective at blocking calpain activity than the intact inhibitors. It therefore seems that there is an evolutionary advantage in salvaging CIDs for future inhibition cycles, to avoid organisms using vital resources to make new molecules whenever the need arises. It is thus reasonable to conclude that calpains are activated within cells often enough to warrant this energy-saving measure.

Previous studies have shown that calpains can cleave calpastatin in the disordered regions between CIDs, even at relatively high concentrations of calpastatin[12]. The resulting calpastatin fragments are themselves calpain inhibitors. Some calpastatin is anchored to membranes through a terminal region (the L-domain, Fig. 1a), but the fragmentation mechanism could provide water-soluble calpain inhibitors that access different subcellular regions. Moldoveanu *et al.*[4] show that region B of CID-I does not seem to inhibit μ-calpain well — could this explain how apparently inhibited calpain can still find a way to cleave the calpastatin to which it is bound? By damaging calpastatin, calpain could paradoxically sow the seeds of its own defeat, because, once formed, the calpastatin fragments have the potential to effectively shut down the proteinase's activity throughout the cell. With such fascinating nuances still to be explained, the calpain–calpastatin system certainly deserves continued exploration. ■

Ronald L. Mellgren is in the Department of Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, Ohio 43614-2598, USA.
e-mail: ronald.mellgren@utoledo.edu

1. Borth, W. *FASEB J.* **6,** 3345–3353 (1992).
2. Estrada, S., Olson, S. T., Raub-Segall, E. & Bjork, I. *Protein Sci.* **9,** 2218–2224 (2000).
3. Hanna, R. A., Campbell, R. L. & Davies, P. L. *Nature* **456,** 409–412 (2008).
4. Moldoveanu, T., Gehring, K. & Green, D. R. *Nature* **456,** 404–408 (2008).
5. Goll, D. E., Thompson, V. F., Li, H., Wei, W. & Cong, J. *Physiol. Rev.* **83,** 731–801 (2003).
6. Dutt, P. *et al. BMC Dev. Biol.* **6,** doi:10.1186/1471-213x/6/3 (2006).
7. Franco, S. J. & Huttenlocher, A. *J. Cell Sci.* **118,** 3829–3838 (2005).
8. Kiss, R. *et al. FEBS Lett.* **582,** 2149–2154 (2008).
9. Hosfield, C. M., Elce, J. S., Davies, P. L. & Jia, Z. *EMBO J.* **18,** 6880–6889 (1999).
10. Moldoveanu, T. *et al. Cell* **108,** 649–660 (2002).
11. Jia, Z. *et al. Biophys. J.* **80,** 2590–2596 (2001).
12. Mellgren, R. L., Mericle, M. T. & Lane, R. D. *Arch. Biochem. Biophys.* **246,** 233–239 (1986).