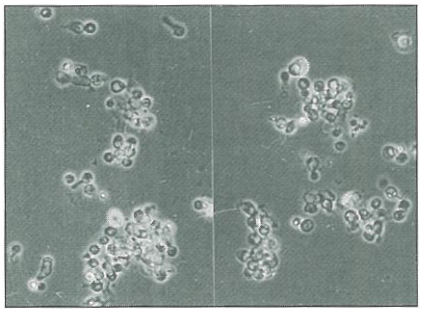
Elsewhere in biology

A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*, chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology October 1998, 5:R272–R275

☐ **Apparent caspase independence of programmed cell death in *Dictyostelium*.**
RA Olie, F Durrieu, S Cornillon, G Loughran, J Gross, WC Earnshaw and P Golstein (1998). *Curr. Biol.* **8**, 955–958.

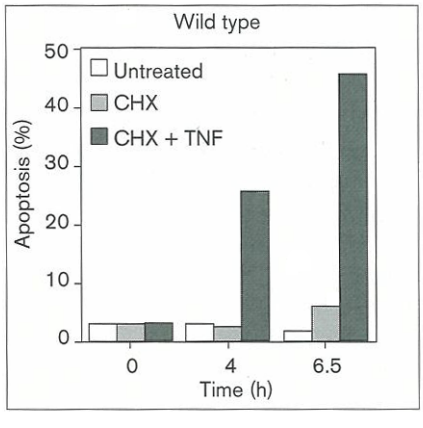
During normal development, cell elimination occurs by programmed cell death (PCD); the best known type of PCD is apoptosis. Activation of cysteine proteases termed caspases is required in many instances of animal PCD, but its



role outside the animal kingdom is, as yet, unknown. PCD occurs during developmental stages in the slime mold *Dictyostelium discoideum*. Under favorable conditions, *Dictyostelium* multiplies as a unicellular organism. Upon starvation, a pathway involving aggregation, differentiation and morphogenesis induces the formation of a multicellular fungus-like structure, consisting mainly of spores and stalk cells, the latter being a result of cell death. The authors examined whether caspase activity is required for *Dictyostelium* cell death. They found that caspase inhibitors, such as DEVD- fmk, did not affect cell death, although some caspase inhibitors that did not inhibit cell death impaired other stages in development and could block affinity labelling of soluble extracts of *Dictyostelium* cells with an activated caspase-specific reagent. The simplest interpretation of these results is that in *Dictyostelium*, whether or not caspase-like molecules exist and are required for some developmental steps, caspase activation is not required for cell death itself.
17 August 1998, Brief Communication, *Current Biology*.

☐ **Essential requirement for caspase-8/FLICE in the initiation of the Fas-induced apoptotic cascade.**
Peter Juo, Calvin J Kuo, Junying Yuann and John Bleniss (1998). *Curr. Biol.* **8**, 1001–1008.

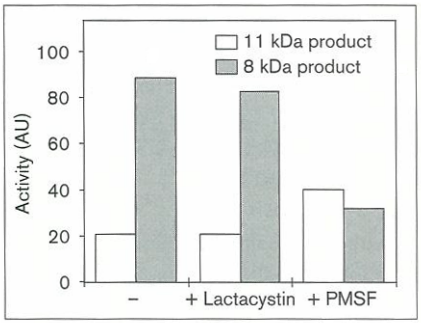
Fas (APO-1/CD95), a member of the tumor necrosis factor receptor (TNF-R) family, induces apoptosis when cross-linked with either Fas ligand or agonistic antibody (Fas antibody). The Fas–Fas ligand system has an important role in the immune system where it is involved in the downregulation of immune responses and the deletion of peripheral autoreactive T lymphocytes. The intracellular domain of Fas interacts with several proteins, including FADD (MORT-1), and the adaptor protein FADD can, in turn, interact with



the cysteine protease caspase-8. As caspase-8 is recruited to the Fas complex through FADD and can autoactivate upon oligomerization, caspase-8 could, potentially, initiate the caspase cascade. In a genetic screen for essential components of the Fas-mediated apoptotic cascade, the authors isolated a Jurkat T lymphocyte cell line deficient in caspase-8 that was completely resistant to Fas-induced apoptosis. Complementation of this cell line with wild-type caspase-8 restored Fas-mediated apoptosis. Fas activation of multiple caspases and of the stress kinases p38 and c-Jun NH_2-terminal kinase (JNK) was completely blocked in the caspase-8-deficient cell line. The cell line was severely deficient in cell death induced by TNF-α. This study provides the first genetic evidence that caspase-8 is essential in the Fas signaling pathway and suggests that caspase-8 might participate in multiple apoptotic pathways.
26 August 1998, Research Paper, *Current Biology*.

☐ **A new large proteolytic complex distinct from the proteasome is present in the cytosol of fission yeast.**
Pawel A Osmulski and Maria Gaczynskas (1998). *Curr. Biol.* **8**, 1023–1026.

The proteasome, a eukaryotic proteolytic complex, is classed as the major nonlysosomal protease by its known and suspected functions, its size and its complexity. It seems improbable that other enzymes could be capable of substituting, even partially, for the potent proteasome, as this complex has a vital role in many cellular processes. It is possible, however, to adapt cultured EL-4 mouse lymphoma cells to survive

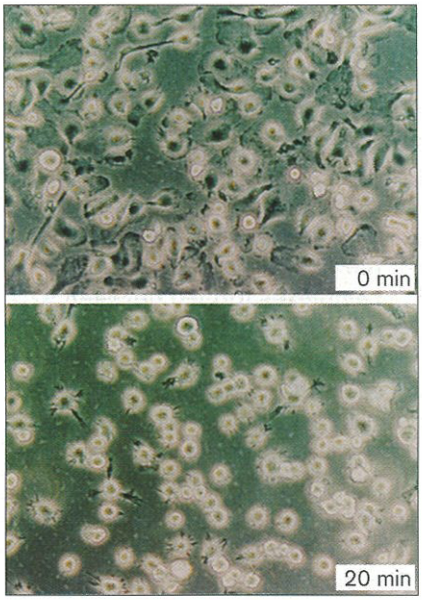


in the presence of a specific inhibitor of the proteasome. The inhibition of the

proteasome in these adapted EL-4 cells is accompanied by a dramatic increase in the activity of a new, as yet uncharacterized, large proteolytic complex. Here, the authors have presented evidence that a similar proteolytic activity is constitutively present in fission yeast, *Schizosaccharomyces pombe*, and that the yeast and mouse enzymes share basic physicochemical properties. The authors have shown that the *S. pombe* protease is found in two stable oligomeric forms, both of which are peptidases, although only the larger form acts as a proteinase. The authors refer to the new proteolytic complex as the multicorn to indicate its analogy to the archaebacterial tricorn protease.
31 August 1998, Brief Communication, *Current Biology*.

☐ **The protein tyrosine phosphatase SHP-1 regulates integrin-mediated adhesion of macrophages.**
Tamara IA Roach, Suzanne E Slater, Lynn S White, Xiaoling Zhang, Philip, W Majerus, Eric J Brown and Matthew L Thomas (1998). *Curr. Biol.* **8**, 1035–1038.

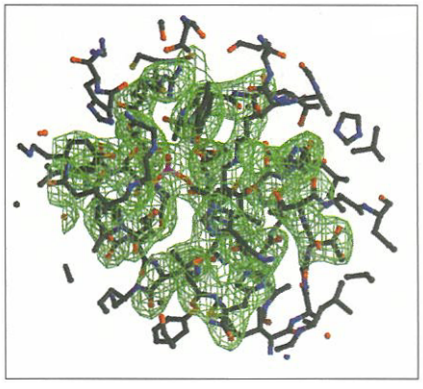
The Src homology 2 domain phosphatase-1 (SHP-1) is a tyrosine phosphatase containing two amino-terminal SH2 domains that is expressed primarily by hematopoietic-derived cells. The viable motheaten (*Hcph*$^{me\text{-}v}$) mutant mice (*me*^{v}) suffer from progressive inflammation due to a deficiency of SHP-1 enzyme activity and die at 3–4 months of age from macrophage and neutrophil accumulation in the lung. The mechanism by which SHP-1 deficiency leads to inflammation is unknown. The authors found that macrophages from *me*^{v} mice adhered and spread to a greater extent than normal macrophages through αmβ2 integrin-mediated contacts. Macrophages deficient in the transmembrane tyrosine phosphatase CD45 (*CD45–/–*) spontaneously detached from αmβ2 integrin contacts, whereas cells deficient in both CD45 and SHP-1 did not. In SHP-1-deficient macrophages there was a 10–15-fold



increase in D-3 phospholipid products of phosphatidylinositol (PI) 3-kinase. Concomitantly, there was a 2–5-fold increase in membrane-associated PI 3-kinase activity in mev macrophages relative to normal macrophages. Treatment of macrophages with the PI 3-kinase inhibitors wortmannin or LY294002 resulted in a dramatic detachment of cells, indicating that PI 3-kinase activity is required for adhesion. These data demonstrate that SHP-1 is necessary for detachment from αmβ2 integrin-mediated contacts in primary macrophages and suggest that a defect in this pathway could contribute to inflammatory disease.
31 August 1998, Research Paper, *Current Biology*.

☐ **The crystal structure of human cytosolic serine hydroxymethyltransferase: a target for cancer chemotherapy.**
Suzanne B Renwick, Keith Snell and Ulrich Baumann (1998). *Structure* **6**, 1105–1116.

Serine hydroxymethyltransferase (SHMT) is a ubiquitous enzyme found in all prokaryotes and eukaryotes. As an enzyme of the thymidylate synthase metabolic cycle, SHMT catalyses the retro-aldol cleavage of serine to glycine, with the resulting hydroxymethyl group being transferred to tetrahydrofolate to form 5,10-methylene-tetrahydrofolate. The latter compound the major source of one-carbon units in metabolism. Elevated SHMT activity has been shown to be coupled to the increased demand for DNA synthesis in rapidly proliferating cells, particularly tumour cells. Consequently, the central role of SHMT in nucleotide biosynthesis makes it an attractive target for cancer chemotherapy. The authors have solved the crystal structure of human cytosolic SHMT by multiple isomorphous replacement. The monomer has a fold

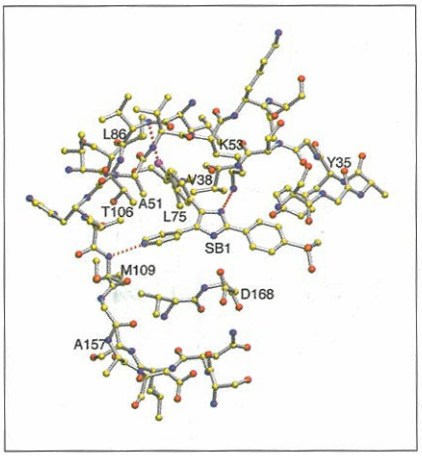


typical for α class pyridoxal 5′-phosphate (PLP)-dependent enzymes. The tetramer association is best described as a 'dimer of dimers' where residues from both subunits of one 'tight' dimer contribute to the active site. Many of the results of site-directed mutagenesis studies can easily be rationalised or re-interpreted in light of the structure presented here. The structure will also be of use for the rational design of inhibitors that could be useful in anticancer therapy.
15 September 1998, Research Paper, *Structure*.

☐ **Structural basis of inhibitor selectivity in MAP kinases.**
Zhulun Wang, Bertram J Canagarajah, Jeffrey C Boehm, Skouki Kassisà, Melanie H Cobb, Peter R Young, Sherin Abdel-Meguid, Jerry L Adams and Elizabeth J Goldsmith (1998). *Structure* **6**, 1117–1128.

The mitogen-activated protein (MAP) kinases are important signaling molecules that participate in diverse cellular events. They are potential

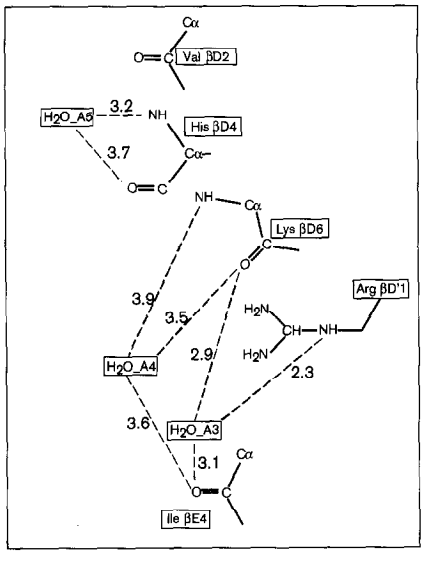
targets for intervention in inflammation, cancer, and other diseases. The MAP kinase p38 is responsive to environmental stresses and is involved in the production of cytokines during inflammation. In contrast, the activation of the MAP kinase ERK2 (extracellular-signal-regulated kinase 2) leads to cellular differentiation or proliferation.



The anti-inflammatory agent pyridinylimidazole and its analogs (SB compounds) are highly potent and selective inhibitors of p38, but not of the closely-related ERK2, or other serine/threonine kinases. Although these compounds are known to bind to the ATP-binding site, the origin of the inhibitory specificity towards p38 is not clear. The authors report the structural basis for the exceptional selectivity of these SB compounds for p38 over ERK2, as determined by comparative crystallography. The crystal structures of four SB compounds in complex with p38 and of one SB compound and olomoucine, a better inhibitor of ERK2, in complex with ERK2 are presented here. The SB inhibitors bind in an extended pocket in the active site and are complementary to the open domain structure of the low-activity form of p38. The relatively closed domain structure of ERK2 is able to accommodate the smaller olomoucine. The unique kinase–inhibitor interactions observed in these complexes originate from amino-acid replacements in the active site and replacements distant from the active site that affect the size of the domain interface. This structural information should facilitate the design of better MAP-kinase inhibitors for the treatment of inflammation and other diseases.
15 September 1998, Research Paper, *Structure*.

☐ **Mass spectrometric and thermodynamic studies reveal the role of water molecules in complexes formed between SH2 domains and tyrosyl phosphopeptides.**
Evonne Chung, Denise Henriques, Debora Renzoni, Marketa Zvelebil, J Michael Bradshaw, Gabriel Waksman, Carol V Robinson and John E Ladbury (1998). *Structure* **6**, 1141–115.

SH2 domains have a fundamental role in signal transduction. These domains interact with proteins containing phosphorylated tyrosine residues and, in doing so, mediate the interactions of proteins involved in tyrosine kinase signalling. The issue of specificity in SH2 domain interactions is therefore of great interest in terms of understanding tyrosine kinase signal-transduction pathways and in the discovery of drugs to inhibit them. Water molecules are found at the interfaces of many

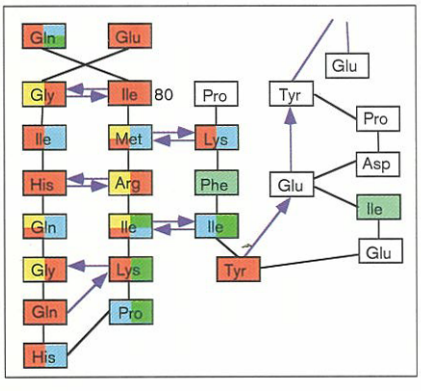


complexes, but, to date, little attention has been paid to their role in dictating specificity. The authors used a combination of nanoflow electrospray ionization mass spectrometry (ESI–MS), isothermal titration calorimetry and structural data to investigate the effect of water molecules in complexes formed between the SH2 domain of tyrosine kinase Src and tyrosyl phosphopeptides. Binding studies have been performed using a series of different peptides that were selected to allow changes in the water content at the complex interface and demonstrate changes in specificity. ESI–MS enables quantification of the number of water molecules that interact with a higher affinity than those generally found solvating the biomolecular complex. Comparing the interactions of different peptides, the authors show that an intricate network of water molecules have a key role in dictating specificity. The use of mass spectrometry to quantify tightly bound water molecules may prove of general use in structural biology, where an independent determination of the water molecules associated with a structure would be advantageous. Furthermore, the ability to assess whether given water molecules are important in high-affinity binding could make this method a precious tool in drug design.
15 September 1998, Research Paper, *Structure*.

☐ **VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 Å resolution and mutational analysis of the interface.**
Yves A Muller, Yvonne Chen, Hans W Christinger, Bing Li, Brian C Cunningham, Henry B Lowman and Abraham M de Vos (1998). *Structure* **6**, 1153–1167.

Vascular endothelial growth factor (VEGF) is a highly specific angiogenic growth factor; anti-angiogenic treatment through inhibition of receptor activation by VEGF might have important therapeutic applications in diseases such as diabetic retinopathy and cancer. A neutralizing anti-VEGF antibody shown to suppress tumor growth in an *in vivo* murine model has been used as the basis for production of a humanized version. The authors present the crystal structure of the complex between VEGF and the Fab fragment of this

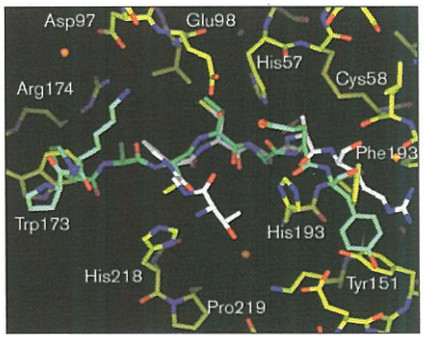
humanized antibody, as well as a comprehensive alanine-scanning analysis of the contact residues on both sides of the interface. Although the VEGF residues critical for antibody binding are distinct from those important for high-affinity receptor binding, they occupy a common region on VEGF, demonstrating that the neutralizing effect of antibody binding results from steric blocking of VEGF–receptor interactions. The findings suggest that the character of antigen–antibody interfaces is similar to that of other protein–protein interfaces, such as ligand–receptor interactions; in



the case of VEGF, the principal difference is that the residues essential for binding to the Fab fragment are concentrated in one continuous segment of polypeptide chain, whereas those essential for binding to the receptor are distributed over four different segments and span across the dimer interface.
15 September 1998, Research Paper, *Structure*.

☐ **The crystal structure of the novel snake venom plasminogen activator TSV-PA: a prototype structure for snake venom serine proteinases.**
Marina AA Parry, Uwe Jacob, Robert Huber, Anne Wisner, Cassian Bon and Wolfram Bode (1998).
Structure **6**, 1195–1206.

Trimeresurus stejnejeri venom plasminogen activator (TSV-PA) is a snake venom serine proteinase that specifically activates plasminogen. Snake venom serine proteinases form a subfamily of trypsin-like proteinases that



are characterised by a high substrate specificity and resistance to inhibition. Many of these venom enzymes specifically interfere with haemostatic mechanisms and have a long-circulating half-life. For these reasons, several of them have commercial applications and are potentially attractive pharmacological tools. The determination of the crystal structure of TSV-PA reveals unique structural elements such as the presence of a phenylalanine at position 193, a carboxy-terminal tail clamped via a disulphide bridge to the 99 loop, and a structurally conserved Asp97 residue. The presence of a *cis* proline at position 218 is in agreement with evolutionary relationships to glandular kallikrein. The authors postulate that Phe193 accounts for the high substrate specificity of TSV-PA, rendering it incapable of forming a stable complex with bovine pancreatic trypsin inhibitor and other extended substrates and inhibitors. The three-dimensional structure presented here is the first of a snake venom serine proteinase and provides an excellent template for modelling other homologous family members.
15 September 1998, Research Paper, *Structure*.