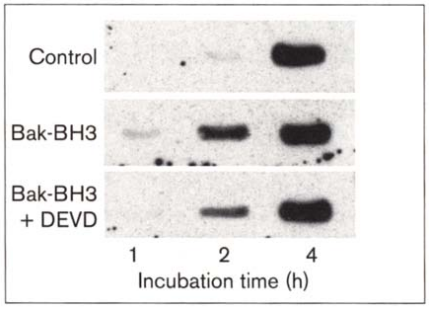
Elsewhere in biology

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

Chemistry & Biology April 1999, 6:R124–R127

☐ **Bcl-2 regulates amplification of caspase activation by cytochrome c.**
Sabina C Cosulich, Peter J Savory and Paul R Clarke (1999). *Curr. Biol.* **9**, 147–150.

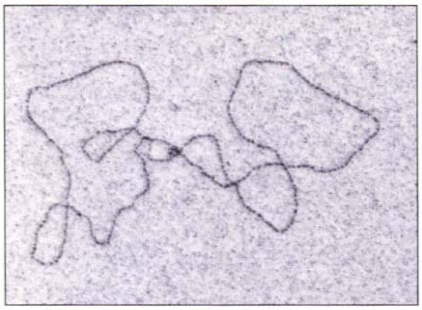
Caspases, a family of specific proteases, have central roles in apoptosis. Caspase activation in response to diverse apoptotic stimuli involves the relocalisation of cytochrome c from mitochondria to the cytoplasm, where it stimulates the proteolytic processing of caspase precursors. Cytochrome c release



is controlled by members of the Bcl-2 family of apoptosis regulators. The anti-apoptotic members Bcl-2 and Bcl-x_L may also control caspase activation independently of cytochrome c relocalisation or may inhibit a positive feedback mechanism. Here, the authors investigate the role of Bcl-2 family proteins in the regulation of caspase activation using a model cell-free system. The authors found that Bcl-2 and Bcl-x_L set a threshold in the amount of cytochrome c required to activate caspases, even in soluble extracts lacking mitochondria. Addition of dATP (which stimulates the procaspase-processing factor Apaf-1) overcame inhibition of caspase activation by Bcl-2, but did not prevent the control of cytochrome c release from mitochondria by Bcl-2. Cytochrome c release was accelerated by active caspase-3 and this positive feedback was negatively regulated by Bcl-2. These results provide evidence for a mechanism to amplify caspase activation that is suppressed at several distinct steps by Bcl-2, even after cytochrome c is released from mitochondria.
1 February 1999, Brief Communication, *Current Biology*.

☐ **Four dimers of λ repressor bound to two suitably spaced pairs of operators form octamers and DNA loops over large distances.**
Bernard Révet, Brigitte von Wilcken-Bergmann, Heike Bessert, Andrew Barker and Benno Müller-Hill (1999). *Curr. Biol.* **9**, 151–154.

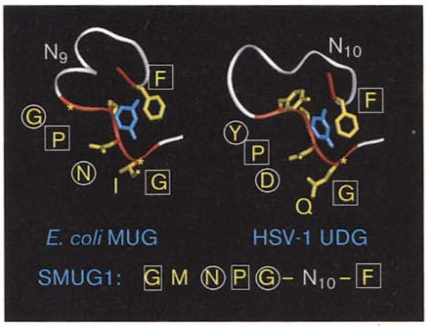
Transcription factors that are bound specifically to DNA often interact with each other over thousands of base pairs. Large DNA loops resulting from such interactions have been observed in *Escherichia coli*, but such interactions are not, as yet, well understood. The authors propose that unique protein complexes that are not present in solution might form specifically on DNA, making it possible for the complexes to interact tightly and



specifically with each other. The authors used the repressor and operators of coliphage λ to construct a model system in which to test their proposition. λ Repressor is a dimer at physiological concentrations, but forms tetramers and octamers at a hundredfold higher concentration. The authors predict that two λ repressor dimers form a tetramer *in vitro* when bound to two λ operators spaced 24 base pairs (bp) apart and that two such tetramers interact to form an octamer. The authors examined, *in vitro*, relaxed circular plasmid DNA in which such operator pairs were separated by 2850 bp and 2470 bp. Of these molecules, 29% formed loops as seen using electron microscopy. The loop increased the tightness of binding of λ repressor to λ operator. Consequently, repression of the λ PR promoter *in vivo* was increased fourfold by the presence of a second pair of λ operators, separated by a distance of 3600 bp.
1 February 1999, Brief Communication, *Current Biology*.

☐ **Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors.**
Karl A Haushalter, P Todd Stukenberg, Marc W Kirschner and Gregory L Verdine (1999). *Curr. Biol.* **9**, 174–185.

The cellular environment exposes DNA to a wide variety of endogenous and exogenous reactive species that can damage DNA, thereby leading to genetic mutations. DNA glycosylases protect the integrity of the genome by catalyzing the first step in the base excision-repair of lesions in DNA. Here, the authors report a strategy to conduct

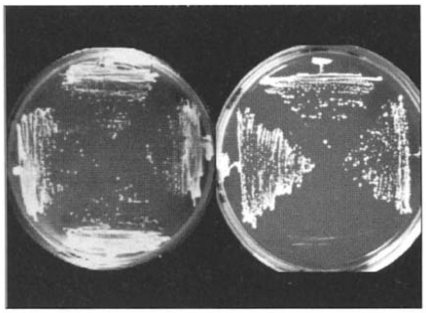


genome-wide screening for expressed DNA glycosylases, on the basis of their ability to bind to a library of four synthetic inhibitors that target the enzyme's active site. These inhibitors, used in conjunction with the *in vitro*

expression cloning procedure, led to the identification of novel *Xenopus* and human proteins, xSMUG1 and hSMUG1, respectively, that efficiently excise uracil residues from DNA. Despite a lack of statistically significant overall sequence similarity to the two established classes of uracil-DNA glycosylases, the SMUG1 enzymes contain motifs that are hallmarks of a shared active-site structure and overall protein architecture. The unusual preference of SMUG1 for single-stranded rather than double-stranded DNA suggests a unique biological function in ridding the genome of uracil residues, which are potent endogenous mutagens. The 'proteomics' approach described here has led to the isolation of a new family of uracil-DNA glycosylases. The three classes of uracil-excising enzymes (SMUG1 being the most recently discovered) represent a striking example of structural and functional conservation in the almost complete absence of sequence conservation.
11 February 1999, Research Paper, *Current Biology*.

☐ **Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast.**
Antonio Casamayor, Pamela D Torrance, Takayasu Kobayashi, Jeremy Thorner and Dario R Alessi (1999). *Curr. Biol.* **9**, 186–197.

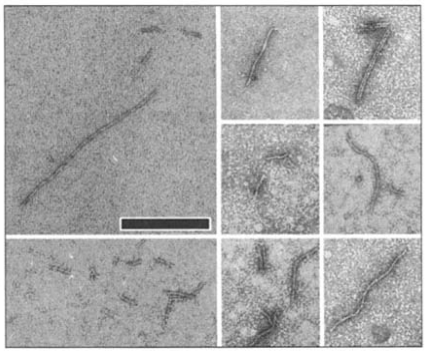
In animal cells, recruitment of phosphatidylinositol 3-kinase by growth factor receptors generates 3-phosphoinositides, which stimulate 3-phosphoinositide-dependent protein kinase-1 (PDK1). Activated PDK1 then phosphorylates and activates downstream protein kinases, including protein kinase B (PKB)/c-Akt, p70 S6 kinase, PKC isoforms, and serum- and glucocorticoid-inducible kinase (SGK), hereby eliciting physiological responses. The authors found that two previously uncharacterised genes of *Saccharomyces cerevisiae*, which they term *PKH1* and *PKH2*, encode protein kinases with catalytic domains closely resembling those of human and *Drosophila* PDK1. Both Pkh1 and Pkh2 were essential for cell viability. Expression of human PDK1 in otherwise inviable *pkh1Δ pkh2Δ* cells permitted growth. In addition, the yeast *YPK1* and *YKR2* genes were found to encode protein



kinases each with a catalytic domain closely resembling that of SGK; both Ypk1 and Ykr2 were also essential for viability. Otherwise inviable *ypk1Δ ykr2Δ* cells were fully rescued by expression of rat SGK, but not mouse PKB or rat p70 S6 kinase. Purified Pkh1 activated mammalian SGK and PKBα *in vitro* by phosphorylating the same residue as PDK1. Pkh1 activated purified Ypk1 by phosphorylating the equivalent residue (Thr504) and was required for maximal Ypk1 phosphorylation *in vivo*. Unlike PKB, activation of Ypk1 and SGK by Pkh1 did not require phosphatidylinositol 3,4,5-trisphosphate, consistent with the absence of pleckstrin homology domains in these proteins. The phosphorylation consensus sequence for Ypk1 was similar to that for PKBα and SGK. Pkh1 and Pkh2 function similarly to PDK1, and Ypk1 and Ykr2 to SGK. As in animal cells, these two groups of yeast kinases constitute two tiers of a signalling cascade required for yeast cell growth.
11 February 1999, Research Paper, *Current Biology*.

☐ **Self-regulated polymerization of the actin-related protein Arp1.**
James B Bingham and Trina A Schroer (1999). *Curr. Biol.* **9**, 223–226.

The actin-related protein Arp1 is the major subunit of dynactin, a key component of the cytoplasmic dynein motor machinery. Of the ubiquitously expressed members of the Arp superfamily, Arp1 is most similar to conventional actin and, on the basis of conserved sequence features, is predicted to bind ATP and possibly polymerize. The authors analyzed the behaviour of highly purified, native Arp1. Arp1 was found to polymerize rapidly into short filaments that were

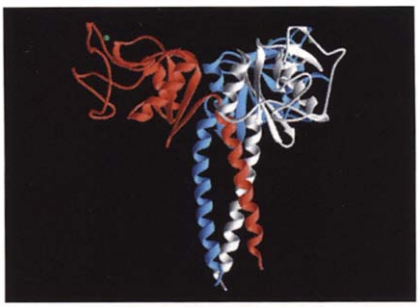


similar, but not identical, in length to those in dynactin. With time, these filaments appeared to anneal to form longer assemblies but never attained the length of conventional actin filaments.
15 February 1999, Brief Communication, *Current Biology*.

☐ **Crystal structure of the trimeric α-helical coiled-coil and the three lectin domains of human lung surfactant protein D.**
Kjell Håkansson, Nam Keung Lim, Hans-Jürgen Hoppe and Kenneth BM Reid (1999). *Structure* **7**, 255–264.

Human lung surfactant protein D (hSP-D) belongs to the collectin family of C-type lectins and participates in the innate immune surveillance against microorganisms in the lung through recognition of carbohydrate ligands present on the surface of pathogens. The involvement of this protein in innate immunity and the allergic response make it the subject of much interest. The authors have determined the crystal structure of a trimeric fragment of hSP-D and it comprises an α-helical coiled-coil and three carbohydrate-recognition domains (CRDs). An interesting deviation from symmetry was found in the projection of a single tyrosine sidechain into the centre of the coiled-coil; the asymmetry of this residue

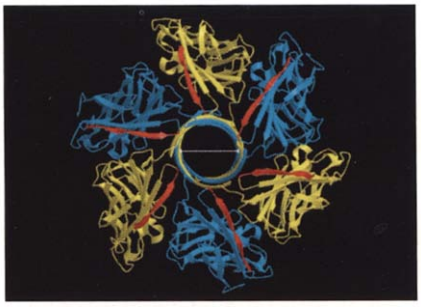
influences the orientation of one of the adjacent CRDs. The cleft between the three CRDs presents a large positively charged surface. The novel central packing of the tyrosine sidechain within the coiled-coil and the resulting asymmetric orientation of the CRDs has



unexpected functional implications. The positively charged surface might facilitate binding to negatively charged structures, such as lipopolysaccharides.
24 February 1999, Research Paper, *Structure*.

☐ **The structure of a *Staphylococcus aureus* leucocidin component (LukF-PV) reveals the fold of the water-soluble species of a family of transmembrane pore-forming toxins.**
Jean-Denis Pédelacq, Laurent Maveyraud, Gilles Prévost, Lamine Baba-Moussa, Ana González, Emmanuel Courcelle, William Shepard, Henri Monteil, Jean-Pierre Samama and Lionel Mourey (1999). *Structure* **7**, 277–288.

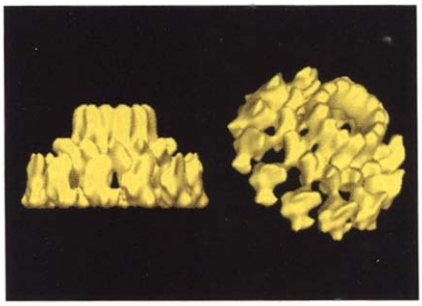
Leucocidins and γ-hemolysins are bi-component toxins secreted by *Staphylococcus aureus*. These toxins activate responses of specific cells and form lethal transmembrane pores. Their leucotoxic and hemolytic activities involve the sequential binding and the synergistic association of a class S and a class F component, which form hetero-oligomeric complexes. The components of each protein class are produced as nonassociated, water-soluble proteins that undergo conformational changes and oligomerization after recognition of their cell targets. The crystal structure of the monomeric water-soluble form of the F component of Panton–Valentine leucocidin (LukF-PV) has been solved by the multiwavelength anomalous dispersion (MAD) method. The core of this three-domain protein is similar to that of α-hemolysin, but significant differences occur in regions that might be involved in the mechanism of pore formation. The glycine-rich stem, which undergoes a major rearrangement in this process, forms an additional domain in LukF-PV. The fold of this domain is similar to that of the neurotoxins and cardiotoxins from snake venom. The structure analysis and a multiple sequence alignment of all toxic



components suggest that LukF-PV represents the fold of any water-soluble secreted protein in this family of transmembrane pore-forming toxins. The comparison of the structure of LukF-PV with that of α-hemolysin provides some insights into the mechanism of transmembrane pore formation for the bi-component toxins, which may diverge from that of the α-hemolysin heptamer.
26 February 1999, Research Paper, *Structure*.

☐ **The three-dimensional structure of a DNA translocating machine at 10 Å resolution.**
José María Valpuesta, José Jesús Fernández, José María Carazo and José L Carrascosa (1999). *Structure* **7**, 289–296.

Head–tail connectors are viral substructures that are very important in the viral morphogenetic cycle, having roles in the formation of the precursor capsid (prohead), DNA packaging, tail binding to the mature head and in the infection process. Structural information

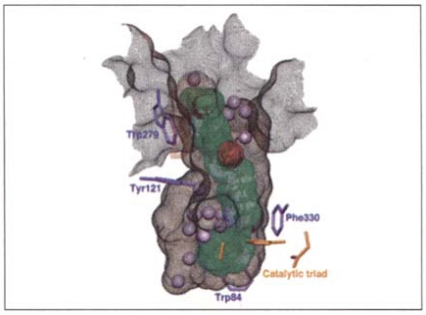


on the connector would, therefore, help us to understand how this structure is related to a multiplicity of functions. Recombinant bacteriophage ϕ29 connectors have been crystallized in two-dimensional aggregates, and an average projection image and a three-dimensional map have been obtained. The average projection image reveals a central mass surrounding a channel with 12 appendages protruding from the central mass. The three-dimensional map reveals a wide domain surrounded by 12 appendages that interact with the prohead vertex, and a narrow domain that interacts with the bacteriophage tail. At the junction of the two domains, 12 smaller appendages are visualized. A channel runs along the axis of the connector structure and is sufficiently wide to allow a double-stranded DNA molecule to pass through. The propeller-like structure of the ϕ29 connector strengthens the notion of the connector rotating during DNA packaging. The groove formed by the two lanes of large and small appendages might act as a rail to prevent the liberation of the connector from the prohead vertex during rotation.
26 February 1999, Research Paper, *Structure*.

☐ **Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs.**
Gitay Kryger, Israel Silman and Joel L Sussman (1999). *Structure* **7**, 297–307.

Several cholinesterase inhibitors are either being utilized for symptomatic treatment of Alzheimer's disease or are in advanced clinical trials. E2020,

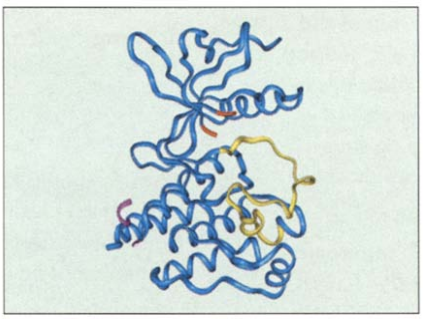
marketed as Aricept®, is a member of a large family of *N*-benzylpiperidine-based acetylcholinesterase (AChE) inhibitors developed, synthesized and evaluated by the Eisai Company in Japan. These inhibitors were designed on the basis of QSAR studies, prior to elucidation of the three-dimensional structure of *Torpedo californica* AChE (*Tc*AChE). E2020 significantly enhances performance in animal models of cholinergic hypofunction and has a high affinity for AChE, binding to both electric eel and mouse AChE in the nanomolar range. The authors' experimental structure of the E2020–*Tc*AChE complex pinpoints



specific interactions responsible for the high affinity and selectivity demonstrated previously. It shows that E2020 has a unique orientation along the active-site gorge, extending from the anionic subsite of the active site, at the bottom, to the peripheral anionic site, at the top, via aromatic stacking interactions with conserved aromatic amino acid residues. E2020 does not, however, interact directly with either the catalytic triad or the 'oxyanion hole', but only indirectly via solvent molecules. The authors' study shows that the design of E2020 took advantage of several important features of the active-site gorge of AChE to produce a drug with both high affinity for AChE and a high degree of selectivity for AChE versus butyrylcholinesterase (BChE). It also delineates voids within the gorge that are not occupied by E2020 and could provide sites for potential modification of E2020 to produce drugs with improved pharmacological profiles.
1 March 1999, Research Paper, *Structure*.

❑ **Crystal structure of the kinase domain of human vascular endothelial growth factor receptor 2: a key enzyme in angiogenesis.**
Michele A McTigue, John A Wickersham, Chris Pinko, Richard E Showalter, Camran V Parast, Anna Tempczyk-Russell, Michael R Gehring, Barbara Mroczkowski, Chen-Chen Kan, J Ernest Villafranca and Krzysztof Appelt (1999). *Structure* **7**, 319–330.

Angiogenesis is involved in tumor growth, macular degeneration, retinopathy and other diseases. Vascular endothelial growth factor (VEGF) stimulates angiogenesis by binding to specific receptors (VEGFRs) on the surface of vascular endothelial cells. VEGFRs are receptor tyrosine kinases that, like the platelet-derived growth factor receptors (PDGFRs), contain a large insert within the kinase domain. Here, the authors report the generation, kinetic characterization and crystal structure of the catalytic kinase domain



of VEGF receptor 2 (VEGFR2). This protein construct, which lacks 50 central residues of the 68-residue kinase insert domain (KID), has comparable kinase activity to constructs containing the entire KID. The crystal structure, determined in an unliganded phosphorylated state, reveals an overall fold and catalytic residue positions similar to those observed in other tyrosine-kinase structures. The kinase activation loop, autophosphorylated on Y1059 prior to crystallization, is mostly disordered; however, a portion of it occupies a position inhibitory to substrate binding. The ends of the KID form a β-like structure, not observed in other known tyrosine kinase structures, that packs near to the kinase C terminus. The majority of the VEGFR2 KID residues are not necessary for kinase activity. The unique structure observed for the ends of the KID may also occur in other PDGFR family members and may serve to properly orient the KID for signal transduction. This VEGFR2 kinase structure provides a target for design of selective anti-angiogenic therapeutic agents.
4 March 1999, Research Paper, *Structure*.