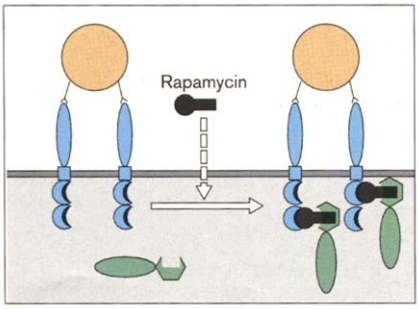
Elsewhere in biology

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 June 1999, 6:R182–R185

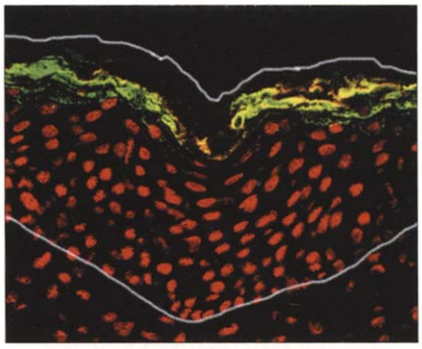
☐ **Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation.**
Flavia Castellano, Philippe Montcourrier, Jean-Claude Guillemot, Edith Gouin, Laura Machesky, Pascale Cossart and Philippe Chavrier (1999). *Curr. Biol.* **9**, 351–360.

Cdc42, a GTP-binding protein of the Rho family, controls actin cytoskeletal organization and helps to generate actin-based protruding structures, such as filopodia. *In vitro*, Cdc42 regulates actin polymerization by facilitating the creation of free barbed ends (the more rapidly growing ends of actin filaments) and subsequent elongation at these ends. The Wiskott–Aldrich syndrome protein, WASP, which has a pleckstrin-homology domain and a Cdc42/Rac-binding motif, has been implicated in cell signaling and cytoskeleton reorganization. The authors have investigated the consequences of local recruitment of activated Cdc42 or WASP to the plasma membrane.They used an activated Cdc42 protein that could be recruited to an engineered membrane receptor by adding rapamycin as a bridge and added antibody-coupled beads to aggregate these receptors. The authors demonstrate that the local recruitment of activated Cdc42 or its downstream effector, WASP, to a membrane receptor in whole cells is sufficient to trigger actin polymerization that results in the formation of membrane protrusions. The data suggest that Cdc42-induced actin-based protrusions result from the local and serial recruitment of cytoskeletal proteins including zyxin, VASP and ezrin.
22 March 1999, Research Paper, *Current Biology*.



☐ **Caspase activation in the terminal differentiation of human epidermal keratinocytes.**
Miguel Weil, Martin C Raff and Vania MM Braga (1999). *Curr. Biol.* **9**, 361–364.

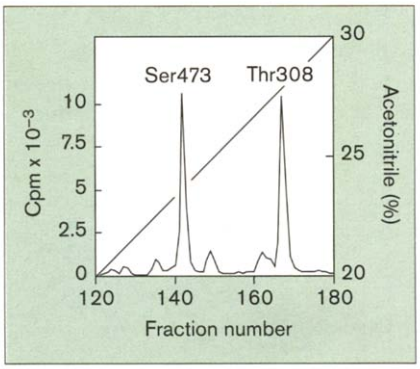
The epidermis is a multilayered squamous epithelium in which dividing basal cells withdraw from the cell cycle and progressively differentiate as they are displaced towards the skin surface. The cells eventually lose their nucleus and other organelles to become



flattened squames that are finally shed from the surface as bags of cross-linked keratin filaments enclosed in a cornified envelope. Although keratinocytes can undergo apoptosis when stimulated by a variety of agents, it is not known whether their normal differentiation programme uses any components of the apoptotic biochemical machinery to produce the cornified cell. Apoptosis involves an intracellular proteolytic cascade, mainly mediated by members of the caspase family of cysteine proteases, which cleave one another and various key intracellular target proteins to kill the cell. Here, the authors show for the first time that caspases are activated during normal human keratinocyte differentiation and that this activation is apparently required for the normal loss of the nucleus.
29 March 1999, Brief Communication, *Current Biology*.

☐ **PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2.**
Anudharan Balendran, Antonio Casamayor, Maria Deak, Andrew Paterson, Piers Gaffney, Richard Currie, C Peter Downes and Dario R Alessi (1999). *Curr. Biol.* **9**, 393–404.

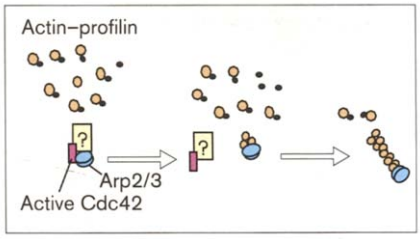
Protein kinase B (PKB) is activated by phosphorylation of Thr308 and of Ser473. Thr308 is phosphorylated by the 3-phosphoinositide-dependent protein kinase-1 (PDK1) but the identity of the kinase that phosphorylates Ser473 (provisionally termed PDK2) is unknown. In this paper, the authors show that the kinase domain of PDK1 interacts with a region of protein kinase C-related kinase-2 (PRK2), termed the PDK1-interacting fragment (PIF). PIF is situated carboxy-terminal to the kinase domain of PRK2, and contains a consensus motif for phosphorylation by PDK2 similar to that found in PKBα, except that the residue equivalent to Ser473 is aspartic acid. Mutation of any of the conserved residues in the PDK2 motif of PIF prevented interaction of PIF with PDK1. Interaction of PDK1 with PIF, or with a synthetic peptide encompassing the PDK2 consensus sequence of PIF, converted PDK1 from an enzyme that could phosphorylate only Thr308 of PKBα to one that phosphorylates both Thr308 and Ser473 of PKBα in a manner dependent on phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P_3). The authors have partially purified a kinase from brain extract that phosphorylates



Ser473 of PKBα in a PtdIns(3,4,5)P_3-dependent manner and that is immunoprecipitated with PDK1 antibodies. PDK1 and PDK2 might be the same enzyme, the substrate specificity and activity of PDK1 being regulated through its interaction with another protein(s). PRK2 is a probable substrate for PDK1.
8 April 1999, Research Paper, *Current Biology*.

☐ **Rho-family GTPases require the Arp2/3 complex to stimulate actin polymerization in *Acanthamoeba* extracts.**
R Dyche Mullins and Thomas D Pollard (1999). *Curr. Biol.* **9**, 405–415.

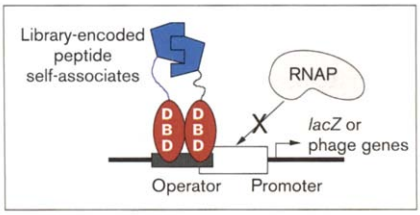
Actin filaments polymerize *in vivo* primarily from their fast-growing barbed ends. In cells and extracts, the nonhydrolyzable GTP analogue GTPγS and Rho-family GTPases, including Cdc42, stimulate barbed-end actin polymerization, but the mechanism responsible for the initiation of



polymerization is unknown. There are three formal possibilities for how free barbed ends may be generated in response to cellular signals: uncapping of existing filaments; severing of existing filaments; or *de novo* nucleation. The Arp2/3 complex localizes to regions of dynamic actin polymerization, including the leading edges of motile cells and motile actin patches in yeast, and *in vitro* it nucleates the formation of actin filaments with free barbed ends. Here, the authors investigated actin polymerization in soluble extracts of *Acanthamoeba*. Addition of actin filaments with free barbed ends to *Acanthamoeba* extracts is sufficient to induce polymerization of endogenous actin. Addition of activated Cdc42 or activation of Rho-family GTPases in these extracts by GTPγS stimulated barbed-end polymerization, whereas immunodepletion of Arp2 or sequestration of Arp2 using solution-binding antibodies blocked Rho-family GTPase-induced actin polymerization. For this system, the authors conclude that the accessibility of free barbed ends regulates actin polymerization, that Rho-family GTPases stimulate polymerization catalytically by *de novo* nucleation of free barbed ends and that the primary nucleation factor in this pathway is the Arp2/3 complex.
8 April 1999, Research Paper, *Current Biology*.

☐ **Genetic selection of short peptides that support protein oligomerization *in vivo*.**
Zhiwen Zhang, Anne Murphy, James C Hu and Thomas Kodadek (1999). *Curr. Biol.* **9**, 417–420.

In protein engineering, controlling associations between designed proteins is most often carried out by fusing known, naturally occurring oligomerization modules, such as leucine zippers, to the proteins of interest. It is of considerable interest to design or discover new oligomerization domains that have novel binding specificities to expand the 'toolbox' of the protein engineer and also to eliminate associations of the designed proteins with endogenous factors. The authors report here a simple genetic selection scheme through which to search libraries for peptides that are able to mediate homodimerization or higher-order self-oligomerization of a protein *in vivo*. They found several peptides that support oligomerization of the repressor



DNA-binding domain in *Escherichia coli* cells, some of them as efficiently as the endogenous dimerization domain or the GCN4 leucine zipper. Many are very small, comprising as few as six residues. This study strongly supports the notion that peptide sequence space is rich in small peptides.
12 April 1999, Brief Communication, *Current Biology*.

☐ **Crystal structure of 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, a potential target for the development of novel antimicrobial agents.**
Bing Xiao, Genbin Shi, Xin Chen, Honggao Yan and Xinhua Ji (1999). *Structure* **7**, 489–496.

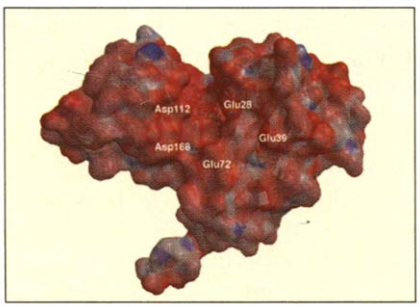
Folate cofactors are essential for life. Mammals derive folates from their diet, whereas most microorganisms must synthesize folates *de novo*. Enzymes of the folate pathway are therefore ideal targets for the development of antimicrobial agents. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes the transfer of pyrophosphate from ATP to 6-hydroxymethyl-7,8-dihydropterin

(HP), the first reaction in the folate biosynthetic pathway. The authors report the determination of the crystal structure of HPPK from *Escherichia coli*. The HPPK molecule has a novel three-layered αβα fold that creates a valley.

The active center of HPPK is located in the valley and the substrate-binding sites have been identified with the aid of NMR spectroscopy. The HP-binding site is located at one end of the valley, near Asn55, and is sandwiched between two aromatic sidechains, whereas the ATP-binding site is located at the other end of the valley. The adenine base of ATP is positioned near Leu111 and the ribose and the triphosphate extend across and reach the vicinity of HP. The HPPK structure provides a framework to elucidate structure–function relationships of the enzyme and to analyze mechanisms of pyrophosphoryl transfer. Furthermore, this work may prove useful in the structure-based design of new antimicrobial agents.
27 April 1999, Research Paper, *Structure*.

☐ **Crystal structure of an aminoglycoside 6′-*N*-acetyltransferase: defining the GCN5-related *N*-acetyltransferase superfamily fold.**
Leanne E Wybenga-Groot, Kari-ann Draker, Gerard D Wright and Albert M Berghuis (1999). *Structure* **7**, 497–507.

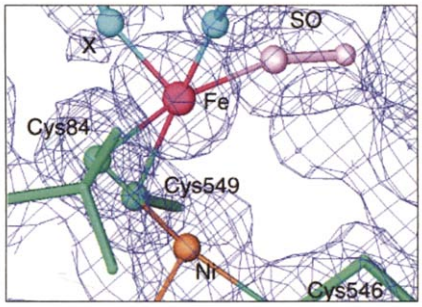
The predominant mechanism of antibiotic resistance employed by pathogenic bacteria against the clinically used aminoglycosides involves chemical modification of the drug. The reactions are catalyzed by enzymes that promote either the phosphorylation, adenylation or acetylation of aminoglycosides. Structural studies of these aminoglycoside-modifying enzymes may assist in the development of therapeutic agents that could circumvent antibiotic resistance. In addition, such studies may shed light on



the development of antibiotic resistance and the evolution of different enzyme classes. The authors have determined the crystal structure of the aminoglycoside-modifying enzyme aminoglycoside 6′-*N*-acetyltransferase type Ii (AAC(6′)-Ii) in complex with the cofactor acetyl coenzyme A. The structure establishes that this acetyltransferase belongs to the GCN5-related *N*-acetyltransferase superfamily, which includes such enzymes as the histone acetyltransferases GCN5 and Hat1. Comparison of the AAC(6′)-Ii structure with the crystal structures of two other members of this superfamily, *Serratia marcescens* aminoglycoside 3-*N*-acetyltransferase and yeast histone acetyltransferase Hat1, reveals that of the 84 residues that are structurally similar, only three are conserved and none can be implicated as catalytic residues. Despite the negligible sequence identity, functional studies show that AAC(6′)-Ii possesses protein acetylation activity. AAC(6′)-Ii is therefore both a structural and functional homolog of the GCN5-related histone acetyltransferases.
28 April 1999, Research Paper, *Structure*.

☐ **Removal of the bridging ligand atom at the Ni–Fe active site of [NiFe] hydrogenase upon reduction with H_2, as revealed by X-ray structure analysis at 1.4 Å resolution.**
Yoshiki Higuchi, Hideaki Ogata, Kunio Miki, Noritake Yasuoka and Tatsuhiko Yagi (1999). *Structure* **7**, 549–556.

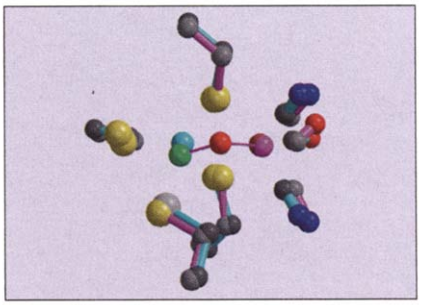
The active site of [NiFe] hydrogenase, a heterodimeric protein, is suggested to be a binuclear Ni–Fe complex that has three diatomic ligands to the Fe atom and three bridging ligands between the Fe and Ni atoms in the oxidized form of the enzyme. Two of the bridging ligands are thiolate sidechains of cysteinyl residues of the large subunit, but the third bridging ligand was assigned as a nonprotein monatomic sulfur species in *Desulfovibrio vulgaris* Miyazaki F hydrogenase. The X-ray crystal structure of the reduced form of *D. vulgaris* Miyazaki F [NiFe] hydrogenase has been solved. The overall structure is very similar to that of the oxidized form, with the exception that the third monatomic bridge



observed at the Ni–Fe site in the oxidized enzyme is absent, leaving this site unoccupied in the reduced form. The unusual ligand structure found in the oxidized form of *D. vulgaris* Miyazaki F [NiFe] hydrogenase was confirmed in the reduced form of the enzyme, with the exception that the electron density assigned to the monatomic sulfur bridge had almost disappeared. On the basis of this finding, as well as the observation that H_2S is liberated from the oxidized enzyme under an atmosphere of H_2 in the presence of its electron carrier, it was postulated that the monatomic sulfur bridge must be removed for the enzyme to be activated.
30 April 1999, Research Paper, *Structure*.

☐ **The crystal structure of a reduced [NiFeSe] hydrogenase provides an image of the activated catalytic center.**
E Garcin, X Vernede, EC Hatchikian, A Volbeda, M Frey and JC Fontecilla-Camps (1999). *Structure* **7**, 557–566.

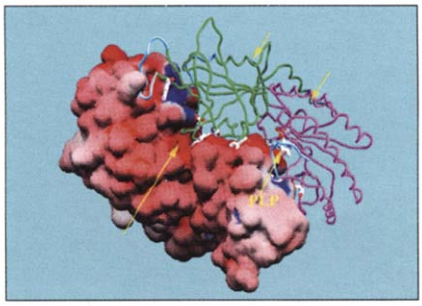
[NiFeSe] hydrogenases are generally heterodimeric metalloenzymes that contain three iron–sulfur clusters in their small subunit and a nickel–iron-containing active site in their large subunit that includes a selenocysteine (SeCys) ligand. The authors report here the X-ray structure of the periplasmic [NiFeSe] hydrogenase from *Desulfomicrobium baculatum* in its



reduced, active form, and compare it with the oxidized, as-prepared, *Desulfovibrio gigas* hydrogenase. The study reveals that the heterolytic cleavage of molecular hydrogen seems to be mediated by the nickel center and the selenocysteine residue. Besides modifying the catalytic properties of the enzyme, the selenium ligand might protect the nickel atom from oxidation. The putative oxo ligand is a signature of inactive 'unready' [NiFe] hydrogenases.
30 April 1999, Research Paper, *Structure*.

☐ **Structure of mammalian ornithine decarboxylase at 1.6 Å resolution: stereochemical implications of PLP-dependent amino acid decarboxylases.**
Andrew D Kern, Marcos A Oliveira, Philip Coffino and Marvin L Hackert (1999). *Structure* **7**, 567–582.

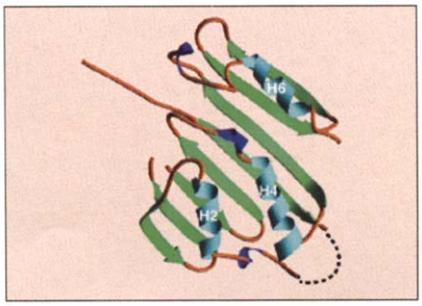
Pyridoxal-5′-phosphate (PLP) dependent enzymes catalyze a broad range of reactions, resulting in bond cleavage at Cα, Cβ or Cγ carbons of D and L amino acid substrates. Ornithine decarboxylase (ODC) is a PLP-dependent enzyme that controls a critical step in the biosynthesis of polyamines, small organic polycations whose controlled levels are essential for proper growth. ODC inhibitors may be useful therapeutic agents in the treatment of certain cancers and parasitic ailments such as African sleeping sickness. The authors have determined the structure of truncated mouse ODC (mODC′). The analysis of the mODC′ structure and its comparison with alanine racemase, together with modelling studies of the external aldimine intermediate, provide an insight into the stereochemical characteristics of PLP-dependent decarboxylation. The structure comparison reveals stereochemical differences from other PLP-dependent enzymes and the bacterial ODC. These characteristics may be exploited in the design of new inhibitors specific for eukaryotic and bacterial ODCs, and provide the basis for a detailed understanding of the



mechanism by which these enzymes regulate reaction specificity.
3 May 1999, Research Paper, *Structure*.

☐ **The crystal structure of human S-adenosylmethionine decarboxylase at 2.25 Å resolution reveals a novel fold.**
Jennifer L Ekstrom, Irimpan I Matthews, Bruce A Stanley, Anthony E Pegg and Steven E Ealick (1999). *Structure* **7**, 583–595.

S-Adenosylmethionine decarboxylase (AdoMetDC) is a critical regulatory enzyme of the polyamine synthetic pathway, and a well-studied drug target. The AdoMetDC decarboxylation reaction depends upon a pyruvoyl cofactor generated via an intramolecular proenzyme self-cleavage reaction. Both the proenzyme-processing and substrate-decarboxylation reactions are allosterically enhanced by putrescine.



The structure of human AdoMetDC has been determined. The quaternary structure of the mature AdoMetDC is an $(\alpha\beta)_2$ dimer, where α and β represent the products of the proenzyme self-cleavage reaction. The architecture of each (αβ) monomer is a novel four-layer α/β-sandwich fold, comprised of two antiparallel eight-stranded β sheets flanked by several α and 3_{10} helices. The structure and topology of AdoMetDC display internal symmetry, suggesting that this protein may be the product of an ancient gene duplication. The positions of conserved, functionally important residues suggest the location of the active site and a possible binding site for the effector molecule putrescine.
3 May 1999, Research Paper, *Structure*.