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 September 1999, 6:R265–R268

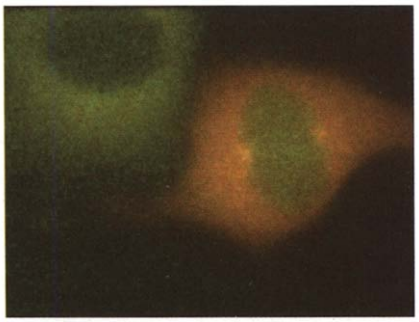
☐ **Comparison of H^+-ATPase and Ca^{2+}-ATPase suggests that a large conformational change initiates P-type ion pump reaction cycles.**
David L Stokes, Manfred Auer, Peijun Zhang and Werner Kühlbrandt (1999). *Curr. Biol.* **9**, 672–679.

Members of the P-type family of ion pumps are responsible for maintaining the ionic homeostasis of cells. Structures of two distantly related members of this family — Ca^{2+}-ATPase from rabbit sarcoplasmic reticulum and H^+-ATPase from *Neurospora crassa* — have recently been solved. They are thought to use similar mechanisms to generate ATP-dependent ion gradients across a variety

of cellular membranes. The authors have undertaken a detailed comparison of the two structure to describe their similarities and differences and how these relate to their mechanism of active transport. Their first important finding was that the arrangement of ten transmembrane helices was remarkably similar in the two molecules, strongly supporting the notion that the pumps use the same basic mechanism to transport their respective ions. The cytoplasmic regions of the two molecules were very different, however. The authors propose that these two crystal structures represent different intermediates in the transport cycle, distinguished by whether cations are bound to their transport sites. They propose that the corresponding conformational change has two components. The authors present a rough model for this important conformational change, which relays the effects of cation binding within the membrane-spanning domain to the nucleotide-binding site, thus initiating the transport cycle.
16 June 1999, Research Paper, *Current Biology*.

☐ **Translocation of cyclin B1 to the nucleus at prophase requires a phosphorylation-dependent nuclear import signal.**
Anja Hagting, Mark Jackman, Karen Simpson and Jonathon Pines (1999). *Curr. Biol.* **9**, 680–689.

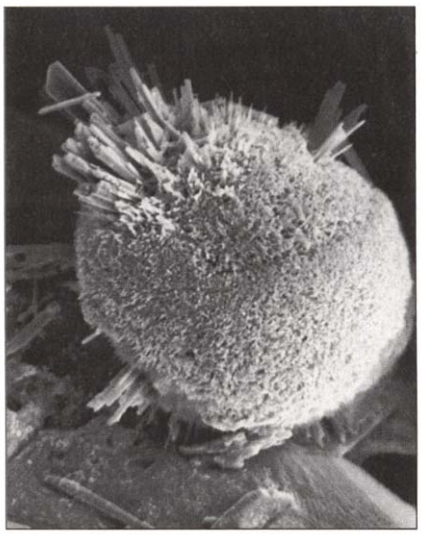
At M phase, cyclin B1 is phosphorylated in the cytoplasmic retention sequence (CRS), which is required for nuclear export. During interphase, cyclin B1 shuttles between the nucleus and the cytoplasm because constitutive nuclear import is counteracted by rapid nuclear export. In M phase, cyclin B moves rapidly into the nucleus coincident with its phosphorylation, an overall movement that might be caused simply by a decrease in its nuclear export. Whether CRS phosphorylation is required for cyclin B1 translocation in mitosis and whether a reduction in



nuclear export is sufficient to explain its rapid relocalisation are two questions that have remained unanswered. The authors have used two forms of green fluorescent protein to analyse simultaneously the translocation of wild-type cyclin B1 and a phosphorylation mutant of cyclin B1 in mitosis, and correlated this with an *in vitro* nuclear import assay. The authors show that phosphorylation of human cyclin B1 is required for its rapid translocation to the nucleus towards the end of prophase. Phosphorylation enhances cyclin B1 nuclear import by creating a nuclear import signal. The phosphorylation of the CRS is therefore a critical step in the control of mitosis.
16 June 1999, Research Paper, *Current Biology*.

☐ **Lead mineral transformation by fungi.**
Jacqueline A Sayer, Janet D Cotter-Howells, Conor Watson, Stephen Hillier and Geoffrey M Gadd (1999). *Curr. Biol.* **9**, 691–694.

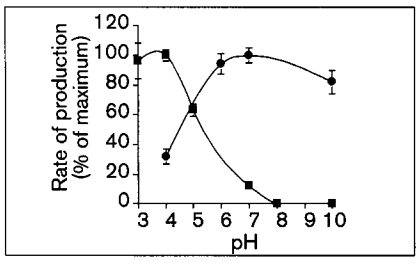
Pyromorphite ($Pb_5(PO_4)_3Cl$) is the most stable lead mineral under a wide range of geochemical conditions, and it can form in urban and industrially contaminated soils. The low solubility of this mineral could reduce the bioavailability of lead. In fact, several studies have advocated pyromorphite formation as a technique for cleaning up lead-contaminated land, adding phosphate if necessary. Many microorganisms can, however, make insoluble soil phosphate bioavailable, and the solubilisation of insoluble metal phosphates by free-living and symbiotic fungi has been reported. If pyromorphite can be solubilised by microbial phosphate-solubilising mechanisms, what would happen to the released lead? The authors have clearly demonstrated that pyromorphite can be solubilised by organic-acid-producing fungi (e.g. *Aspergillus niger*) and that plants grown with pyromorphite as sole phosphorus source take up both phosphorus and lead. They have also discovered the production of lead oxalate dihydrate by *A. niger* during pyromorphite transformation. These



mechanisms of lead solubilisation, or its immobilisation as a novel lead oxalate, have significant implications for metal mobility and transfer to other environmental compartments and organisms. The results also emphasise the importance of considering microbial processes when developing remediation techniques for toxic metals in soils.
21 June 1999, Brief Communication, *Current Biology*.

☐ **Nicotinic acid adenine dinucleotide phosphate triggers Ca^{2+} release from brain microsomes.**
Judit Bak, Peter White, György Tim´r, Ludwig Missiaen, Armando A Genazzani and Antony Galione (1999). *Curr. Biol.* **9**, 751–754.

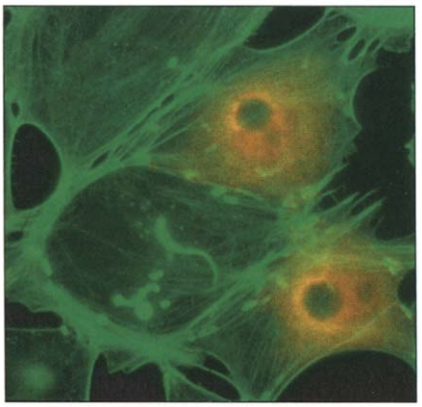
Mobilization of Ca^{2+} from intracellular stores is an important mechanism for generating cytoplasmic Ca^{2+} signals. Two families of intracellular Ca^{2+}-release channels — the inositol-1,4,5-trisphosphate (IP_3) receptors and the ryanodine receptors (RyRs) — have been described in mammalian tissues.



Recently, nicotinic acid adenine dinucleotide phosphate (NAADP), a molecule derived from $NADP^+$, has been shown to trigger Ca^{2+} release from intracellular stores in invertebrate eggs and pancreatic acinar cells. The nature of NAADP-induced Ca^{2+} release is unknown, however. Mammalian cells can synthesize and degrade NAADP, suggesting that NAADP-induced Ca^{2+} release may be widespread and thus contribute to the complexity of Ca^{2+} signalling. The authors show for the first time that NAADP stimulates Ca^{2+} release from rat brain microsomes through a mechanism distinct from those sensitive to IP_3 or cADPR, and has a remarkably similar pharmacology to the action of NAADP in sea urchin eggs. Membranes prepared from the same rat brain tissues are able to support the synthesis and degradation of NAADP. They suggest, therefore, that NAADP-mediated Ca^{2+} signalling could play an important role in neuronal Ca^{2+} signalling.
5 July 1999, Brief Communication, *Current Biology*.

☐ **The Arp2/3 complex is essential for the actin-based motility of *Listeria monocytogenes*.**
Robin C May, Margaret E Hall, Henry N Higgs, Thomas D Pollard, Trinad Chakraborty Juergen Wehland, Laura M Machesky and Antonio S Sechi (1999). *Curr. Biol.* **9**, 759–762.

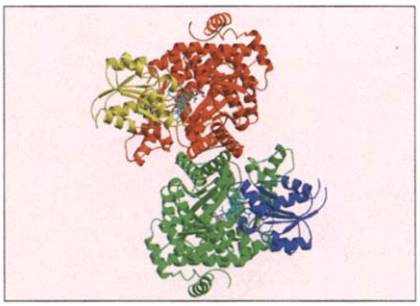
Actin polymerisation is thought to drive the movement of eukaryotic cells and some intracellular pathogens such as *Listeria monocytogenes*. The *Listeria* surface protein ActA synergises with recruited host proteins to induce actin polymerisation, propelling the bacterium through the host cytoplasm. The Arp2/3 complex is one recruited host factor; it is also believed to regulate actin dynamics in lamellipodia. The Arp2/3 complex promotes actin filament nucleation *in vitro*, which is further enhanced by ActA. The Arp2/3 complex also interacts with members of the Wiskott–Aldrich syndrome protein (WASP) family — Scar1 and WASP itself. The authors interfered with the



targeting of the Arp2/3 complex to *Listeria* using carboxy-terminal fragments of Scar1 that bind the Arp2/3 complex. From their studies, they conclude that the Arp2/3 complex is a host-cell factor essential for the actin-based motility of *L. monocytogenes*, suggesting that it plays an important role in regulating the actin cytoskeleton.
5 July 1999, Brief Communication, *Current Biology*.

☐ **Glutamate mutase from *Clostridium cochlearium*: the structure of a coenzyme B_{12}-dependent enzyme provides new mechanistic insights.**
R Reitzer, K Gruber, G Jogl, UG Wagner, H Bothe, W Buckel and C Kratky (1999). *Structure* **7**, 891-902.

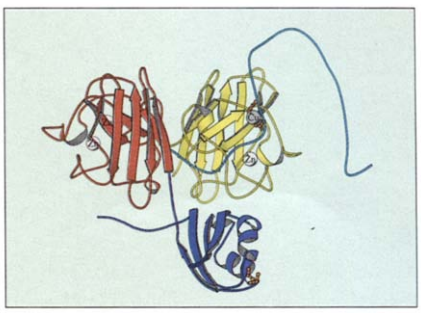
Glutamate mutase (Glm) equilibrates (*S*)-glutamate with (2*S*,3*S*)-3-methylaspartate. Catalysis proceeds with the homolytic cleavage of the organometallic bond of the cofactor to yield a 5′-desoxyadenosyl radical. This radical then abstracts a hydrogen atom from the protein-bound substrate to initiate the rearrangement reaction. Glm from *Clostridium cochlearium* is a heterotetrameric molecule consisting of two σ and two ε polypeptide chains. The authors have determined the crystal structures of inactive recombinant Glm reconstituted with either cyanocobalamin or methylcobalamin. The molecule shows close similarity to the structure of methylmalonyl CoA mutase (MCM). Each of the two independent B_{12} cofactor molecules is associated with a



substrate-binding site, which was found to be occupied by a (2*S*,3*S*)-tartrate ion. The tight binding of the tartrate ion conforms to the requirements of tight control of the reactive intermediates and suggests how the enzyme might use the substrate-binding energy to initiate cleavage of the cobalt–carbon bond. The cofactor does not appear to play a role during the radical rearrangement reaction.
7 July 1999, Research Paper, *Structure*.

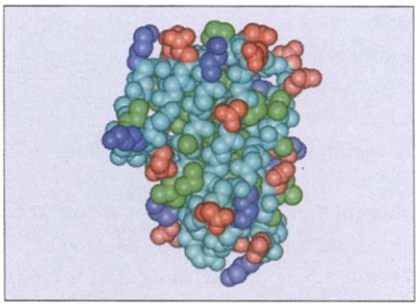
☐ **A model for the incorporation of metal from the copper chaperone CCS into Cu,Zn superoxide dismutase.**
Mattia Falconi, Mariacristina Iovino and Alessandro Desideri (1999). *Structure* **7**, 903–908.

Recent studies have identified the human copper chaperone CCS as the factor responsible for copper incorporation into superoxide dismutase (SOD). Dominantly inherited mutations in SOD are responsible for the fatal motor neuron disorder amyotrophic lateral sclerosis, and evidence to date suggests that the toxicity from mutant SOD results from aberrant chemistry (enhanced free radical generating activity) by the bound active copper ion. Determining the mechanism of copper transferral has been impeded by a lack of knowledge of the chaperone's structure. The three-dimensional structure of CCS was homology modelled using the periplasmic protein from the bacterial mercury-detoxification system and the structure of one subunit of the human SOD dimeric enzyme as templates. The authors propose a mechanism for the transfer of copper from CCS to SOD. On the basis of the three-dimensional model, a mechanism for the transfer of copper from CCS to SOD is proposed that accounts for electrostatic acceptor recognition, copper storage and copper-transfer properties. The proposed model identifies a path for copper transfer based on the presence of different metal sites characterized by sulphur ligands. Such a model should facilitate the development of strategies to interfere with copper incorporation in SOD, providing a possible way to prevent or arrest degeneration in amyotrophic lateral sclerosis.
12 July 1999, Research Paper, *Structure*.



☐ **An immunoglobulin-like fold in a major plant allergen: the solution structure of Phl p 2 from timothy grass pollen.**
S De Marino, MA Castiglione Morelli, F Fraternali, E Tamborini, G Musco, S Vrtala, C Dolecek, P Arosio, R Valenta and A Pastore (1999). *Structure* **7**, 943–952.

Grass pollen allergens are the most important and widespread elicitors of pollen allergy. One of the major plant allergens that millions of people worldwide are sensitized to is Phl p 2, a small protein from timothy grass pollen. Phl p 2 belongs to the large family of cross-reacting plant allergens classified as group 2/3. Recombinant Phl p 2 has been demonstrated to be immunologically equivalent to the natural protein. The three-dimensional structure of Phl p 2 consists of an all-β fold with nine antiparallel β strands that form a β sandwich. The Phl p 2 topology is that of an immunoglobulin-like fold with the addition of a carboxy-terminal strand, as found in the C2 domain superfamily. Knowledge of the

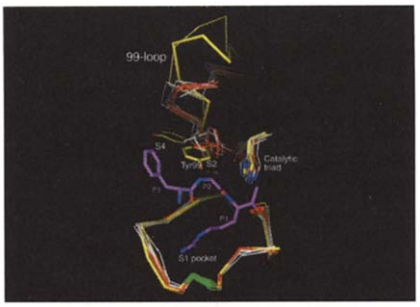


structure allows identification of a number of possible epitopes that could be specific for group 2/3 allergens and would make it possible to discriminate members of this family from the closely related group 1 grass pollen allergens. Knowledge of the Phl p 2 structure may assist the rational structure-based design of synthetic vaccines against grass pollen allergy.
15 July 1999, Research Paper, *Structure*.

☐ **Coagulation factor IXa: the relaxed conformation of Tyr99 blocks substrate binding.**
Karl-Peter Hopfner, Anja Lang, Annette Karcher, Katrin Sichler, Erhard Kopetzki, Hans Brandstetter, Robert Huber, Wolfram Bode and Richard A Engh (1999). *Structure* **7**, 989–996.

The serine proteinases of the coagulation cascade, together with cofactors, amplify initial procoagulant signals via a series of activation cleavages to produce thrombin. Thrombin stimulates platelets to aggregate, activates fibrinogen to stabilize the nascent clot and activates protein C to ultimately terminate the process. Of the proteinase coagulation factors, fIXa has been noted for its association with hemophilia; it is also uniquely inefficient against synthetic peptide substrates. Mutagenesis studies show that a loop of residues at the S2–S4 substrate-binding cleft (the 99-loop) contributes to the low efficiency. The structure of a recombinant two-domain construct of human fIXa in complex with *p*-aminobenzamidine shows that the Tyr99 sidechain adopts an atypical conformation in the absence of substrate

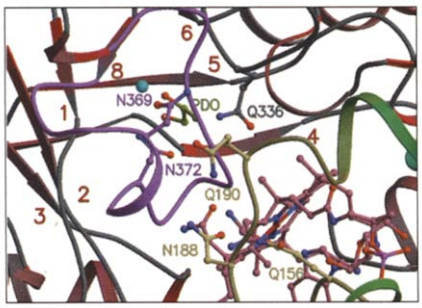
interactions. In this conformation, the hydroxyl group occupies the volume corresponding to the mainchain of a canonically bound substrate P2 residue. To accommodate substrate binding, Tyr99 must adopt a higher energy conformation that creates the S2 pocket and restricts the S4 pocket. Molecular recognition of substrates by fIXa seems



to be determined by the action of the 99-loop on Tyr99. This is in contrast to other coagulation enzymes where, in general, the chemical nature of residue 99 determines molecular recognition in S2 and S3–S4. This dominant role in substrate interaction suggests that the 99-loop may be rearranged in the physiological fX activation complex of fIXa, fVIIIa and fX.
21 July 1999, Research Paper, *Structure*.

☐ **A new mode of B_{12} binding and the direct participation of a potassium ion in enzyme catalysis: X-ray structure of diol dehydratase.**
Naoki Shibata, Jun Masuda, Takamasa Tobimatsu, Tetsuo Toraya, Kyoko Suto, Yukio Morimoto and Noritake Yasuoka (1999). *Structure* **7**, 997–1008.

Diol dehydratase catalyzes the adenosylcobalamin (coenzyme B_{12})-dependent conversion of 1,2-diols to the corresponding aldehydes. The reaction is initiated by homolytic cleavage of the cobalt–carbon bond of the coenzyme and proceeds by a radical mechanism. The enzyme is an $\alpha_2\beta_2\gamma_2$ heterooligomer and has an absolute requirement for a potassium ion for catalytic activity. The structure of a diol dehydratase–cyanocobalamin complex



was determined to help understand the enzyme mechanism. The structure provides the first crystallographic indication of the 'base-on' mode of cobalamin binding. An unusually long cobalt–base bond seems to favor homolytic cleavage of the cobalt–carbon bond and therefore to favor radical enzyme catalysis. Reactive radical intermediates can be protected from side reactions by spatial isolation inside the barrel. Direct interactions between the potassium ion and the two hydroxyl groups of the substrate strongly suggest direct participation of a potassium ion in enzyme catalysis.
28 July 1999, Research Paper, *Structure*.