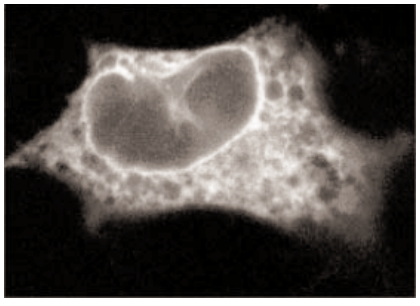
# Elsewhere in biology

**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** 2000, **7**:R77–R80

☐ **Head–head/tail–tail relative orientation of the pore-forming domains of the heterodimeric ABC transporter TAP.**
Jan C Vos, Eric AJ Reits, Eldine Wojcik-Jacobs, Jacques Neefjes (1999). *Curr.* Biol. **10**, 1–7.

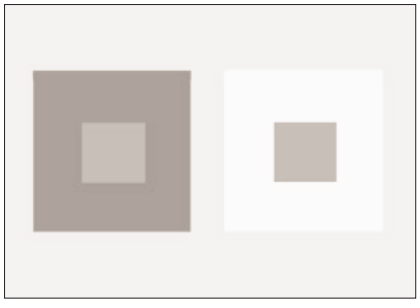
The transporter associated with antigen processing (TAP) is a heterodimeric member of the large family of ABC transporters. The study of interactions between the subunits TAP1 and TAP2 can reveal the relative orientation of the transmembrane segments, which form a



translocation pore for peptides. This is essential for understanding the architecture of TAP and other ABC transporters. The amino-terminal six transmembrane segments (TMs) of human TAP1, TAP1(1–6), and the amino-terminal five TMs of TAP2, TAP2(1–5), are thought to constitute the pore of TAP. Two new approaches are used to define dimer interactions. Dimerization of the pore-forming transmembrane domains of TAP1 (TM1–6) with its TAP2 counterpart (TM1–5) prevents the post-translational translocation of TM6 of TAP1 and results in a complex with reduced mobility within the endoplasmic reticulum membrane compared with the free subunit. The pore-forming domains of TAP are aligned in a head–head/tail–tail orientation. This positions the following peptide-binding segments of the two TAP subunits to one side of the pore.
10 December 1999, Research Paper, *Current Biology*.

☐ **Microscopic properties of elementary $Ca^{2+}$ release sites in non-excitable cells.**
David Thomas, Peter Lipp, Stephen C Tovey, Michael J Berridge, Wenhong Li, Roger Y Tsien, Martin D Bootman (1999). *Curr. Biol.* **10**, 8–15.

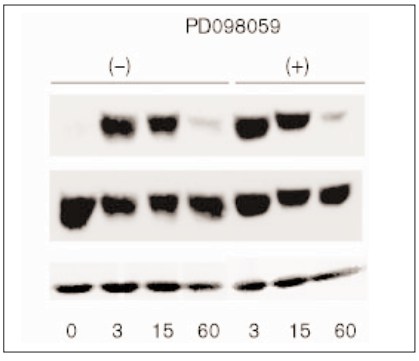
Elementary $Ca^{2+}$ signals, such as '$Ca^{2+}$ puffs', that arise from the activation of clusters of inositol 1,4,5,-trisphosphate ($InsP_3$) receptors are the building blocks for local and global $Ca^{2+}$ signalling. One or a few $Ca^{2+}$ puff sites within agonist-stimulated cells act as 'pacemakers' to initiate global $Ca^{2+}$ waves. The factors that distinguish these pacemaker $Ca^{2+}$ puff sites from the other $Ca^{2+}$ release sites that simply participate in $Ca^{2+}$ wave propagation are unknown. The spatiotemporal properties of $Ca^{2+}$ puffs were investigated using confocal microscopy of fluo3-loaded HeLa cells. The same pacemaker $Ca^{2+}$ puff sites were activated during stimulation of cells with different agonists. The



majority of agonist-stimulated pacemaker $Ca^{2+}$ puffs originated in a perinuclear location. A similar perinuclear distribution of $Ca^{2+}$ puff sites was also observed when $InsP_3$ receptors were stimulated directly with thimerosal or membrane-permeant $InsP_3$ esters. The pacemaker $Ca^{2+}$ puff sites that initiate $Ca^{2+}$ responses are temporally and spatially stable within cells. These $Ca^{2+}$ release sites are distinguished from their neighbours by an intrinsically higher $InsP_3$ sensitivity.
14 December 1999, Research Paper, *Current Biology*.

☐ **Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR.**
Kiyotaka Yokogami, Shinichiro Wakisaka, Joseph Avruch, Steven A Reeves (1999). *Curr. Biol.* **10**, 47–50.

Neuropoietic cytokines such as ciliary neurotrophic factor (CNTF) can activate several signaling pathways in parallel, including those involving Janus




kinase (JAK)–signal transducers and activators of transcription (STATs), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI 3-kinase) and mammalian target of rapamycin (mTOR)–p70 S6 kinase. Crosstalk occurs between these pathways, because studies have shown that STAT3 requires phosphorylation on tyrosine and serine residues by independent protein kinase activities for maximal activation of target gene transcription. Members of the JAK/Tyk family of tyrosine kinases mediate phosphorylation of STAT3 at Tyr705 during CNTF signaling but the kinase responsible for phosphorylation at STAT3 Tyr727 appears to depend on both the extracellular stimulus and the cellular context. The authors investigate the kinase activity responsible for

phosphorylation of STAT3 on Ser727 in CNTF-stimulated neuroblastoma cells. They found that CNTF-induced phosphorylation of Ser727 was inhibited by the mTOR inhibitor rapamycin, but not by inhibitors of MAPK and protein kinase C (PKC) activation. A STAT3 peptide was efficiently phosphorylated on Ser727 in a CNTF-dependent manner by mTOR, but not by a kinase-inactive mTOR mutant or by p70 S6 kinase. The ability of mTOR to contribute to activation of STAT3 extends the function of mTOR in mammalian cells to include transcriptional regulation.
17 December 1999, Brief Communications, *Current Biology*.

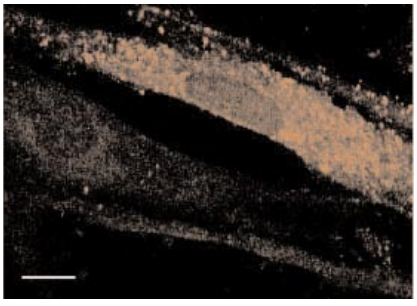
☐ **Differential codes for free $Ca^{2+}$–calmodulin signals in nucleus and cytosol.**
Mary N Teruel, Wen Chen, Anthony Persechini, Tobias Meyer (2000). *Curr. Biol.* **10**, 86–94.

Many targets of calcium signaling pathways are activated or inhibited by binding the $Ca^{2+}$-liganded form of calmodulin ($Ca^{2+}$–CaM). The authors test the hypothesis that local $Ca^{2+}$–CaM-regulated signaling processes can be selectively activated by local intracellular differences in free $Ca^{2+}$–CaM concentration. Energy-

transfer confocal microscopy of a fluorescent biosensor was used to measure the difference in the concentration of free $Ca^{2+}$–CaM between nucleus and cytoplasm. Subcellular differences in the distribution of $Ca^{2+}$–CaM-binding proteins can produce gradients of free $Ca^{2+}$–CaM concentration that result in a net translocation of CaM. This provides a mechanism for dynamically regulating local free $Ca^{2+}$–CaM concentrations, and thus the local activity of $Ca^{2+}$–CaM targets. Free $Ca^{2+}$–CaM signals in the nucleus remain low during brief or low-frequency calcium spikes, whereas high-frequency spikes or persistent increases in calcium cause translocation of CaM from the cytoplasm to the nucleus, resulting in similar concentrations of nuclear and cytosolic free $Ca^{2+}$–CaM.
11 January 2000, Research Paper, *Current Biology*.

☐ **Mobilization of late-endosomal cholesterol is inhibited by Rab guanine nucleotide dissociation inhibitor.**
Maarit Hölttä-Vuori, Juha Määttä, Oliver Ullrich, Esa Kuismanen, Elina Ikonen (2000). *Curr. Biol.* **10**, 95–98.

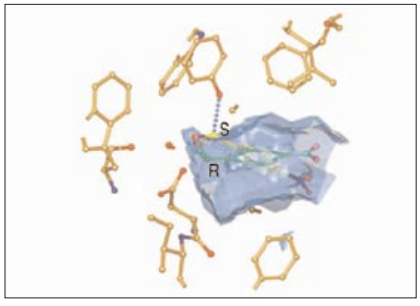
Cholesterol entering cells in low-density lipoproteins (LDL) through receptor-mediated endocytosis is transported to organelles of the late endocytic pathway for degradation of the lipoprotein particles. The fate of the free cholesterol released remains poorly understood. Recent observations suggest that late-endosomal cholesterol sequestration is regulated by the dynamics of lysobisphosphatidic acid (LBPA)-rich membranes. Genetic studies have pinpointed a protein, Niemann–Pick C-1 (NPC-1), required for the mobilization of late-endosomal/lysosomal cholesterol by an unknown mechanism. The authors report the



removal of accumulated cholesterol by overexpression of the NPC-1 protein in NPC-1-deficient fibroblasts from patients with Niemann–Pick disease, and in normal fibroblasts upon release of a progesterone-induced block of cholesterol transport. Late-endosomal/lysosomal cholesterol mobilization is specifically inhibited by microinjection of Rab GDP-dissociation inhibitor (Rab-GDI). A specific molecular component of the membrane-trafficking machinery is involved in cholesterol transport and the coupling of late-endosomal cholesterol egress to the trafficking of other lipid and protein cargo.
14 January 2000, Brief Communication, *Current Biology*.

☐ **Structure of *Aspergillus niger* epoxide hydrolase at 1.8 Å resolution: implications for the structure and function of the mammalian microsomal class of epoxide hydrolases.**
Jinyu Zou, B Martin Hallberg, Terese Bergfors, Franz Oesch, Michael Arand, Sherry L Mowbray, T Alwyn Jones (2000). *Structure* **8**, 111–122.

Epoxide hydrolases have important roles in the defense of cells against potentially harmful epoxides. Conversion of epoxides into less toxic and more easily excreted diols is a universally successful strategy. A number of microorganisms employ the same chemistry to process epoxides for use as carbon sources. The X-ray

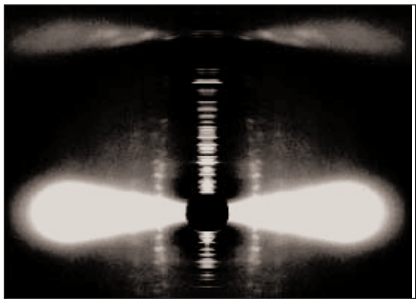



structure of the epoxide hydrolase from *Aspergillus niger* reveals a dimer consisting of two 44 kDa subunits in the asymmetric unit. Each subunit consists of an α/β hydrolase fold, and a primarily helical lid over the active site. The dimer interface includes lid–lid interactions as well as contributions from an amino-terminal meander. The active site contains a classical catalytic

triad, and two tyrosines and a glutamic acid residue that are likely to assist in catalysis. The *Aspergillus* enzyme provides the first structure of an epoxide hydrolase with strong relationships to the most important enzyme of human epoxide metabolism — the microsomal epoxide hydrolase. Differences in active-site residues, especially in components that assist in epoxide ring opening and hydrolysis of the enzyme–substrate intermediate, might explain why the fungal enzyme attains the greater speeds necessary for an effective metabolic enzyme. The amino-terminal domain that is characteristic of microsomal epoxide hydrolases corresponds to a meander that is critical for dimer formation in the *Aspergillus* enzyme.
25 January 2000, Research Paper, *Structure*.

☐ **The *in situ* conformation and axial location of the intermolecular cross-linked non-helical telopeptides of type I collagen.**
Joseph P Orgel, Tim J Wess, Andrew Miller (2000). *Structure* **8**, 137–142.

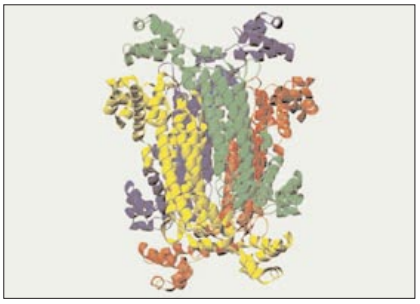
Type I collagen contains specific lysine and hydroxylysine residues that are important in the formation of intermolecular cross-links. These cross-links are required for the normal configuration and stability of the 67 nm axial repeat of collagen fibrils in the extracellular matrix. The major cross-linkage sites are believed to occur



between the non-helical terminal regions (telopeptides) and helical segments of adjacent collagen molecules. In this X-ray fibre diffraction study, the tissue has been maintained in the hydrated fibrillar state, whilst detailed structural information was obtained using highly collimated synchrotron radiation. The axially projected electron-density profile and the electron-density difference maps showed that both the amino- and carboxy-terminal telopeptides are contracted structures. This profile puts narrow constraints on the possible conformations of the carboxy-terminal telopeptide; the best fit to the electron-density profile is when the α 1 chains adopt a folded conformation with a sharp hairpin turn around residues 13 and 14 of the 25-residue telopeptide. The results reveal the location, parallel to the fibril axis, of the intermolecular cross-links in normal hydrated tissue. These cross-links are essential for the biological function of the tissue.
27 January 2000, Research Paper, *Structure*.

☐ **The structure of adenylosuccinate lyase, an enzyme with dual activity in the *de novo* purine biosynthetic pathway.**
Eric A Toth, Todd O Yeates (2000). *Structure* **8**, 163–174.

Adenylosuccinate lyase is an enzyme that plays an important role in both cellular replication and metabolism through its action in the *de novo* purine biosynthetic pathway. Adenylosuccinate lyase is the only enzyme in this pathway to catalyze two separate reactions, enabling it to participate in the addition of a nitrogen at two different positions in adenosine monophosphate. Both reactions catalyzed by adenylosuccinate lyase involve the β-elimination of fumarate. Enzymes that catalyze this type of reaction belong to a superfamily, the members of which are homotetramers. Because adenylosuccinate lyase plays an integral part in maintaining proper cellular metabolism, mutations in the human enzyme can have severe clinical consequences, including mental retardation with autistic features. The crystal structure of adenylosuccinate lyase from *Thermotoga maritima* has been

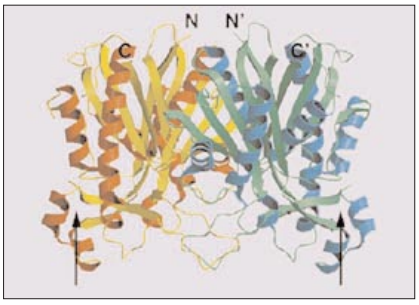


determined. The fold of the monomer is reminiscent of other members of the β-elimination superfamily. Its active tetrameric form shows striking differences in active-site architecture and cleft size, however. This first structure of an adenylosuccinate lyase reveals that, along with the catalytic base (His141) and the catalytic acid (His68), Gln212 and Asn270 might play a vital role in catalysis by properly orienting the succinyl moiety of the substrates. A model for the dual activity of adenylosuccinate lyase is proposed: a single 180° bond rotation must occur in the substrate between the first and second enzymatic reactions. Modeling of the pathogenic human S413P mutation indicates that the mutation destabilizes the enzyme by disrupting the carboxy-terminal extension.
31 January 2000, Research Paper, *Structure*.

☐ **The 1.8 Å crystal structure and active-site architecture of β-ketoacyl-acyl carrier protein synthase III (FabH) from *Escherichia coli*.**
Christopher Davies, Richard J Heath, Stephen W White, Charles O Rock (2000). *Structure* **8**, 185–195.

β-Ketoacyl-acyl carrier protein synthase III (FabH) initiates elongation in type II fatty acid synthase systems found in bacteria and plants. FabH is a ubiquitous component of the type II system and is positioned ideally in the pathway to control the production of fatty acids. The elucidation of the structure of FabH is important for the understanding of its regulation by feedback inhibition and its interaction with drugs. Although the structures of

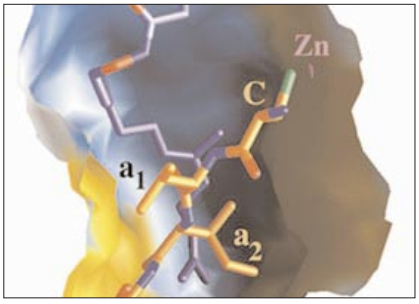
two related condensing enzymes are known, the roles of the active-site residues have not been tested experimentally. The crystal structure of FabH was determined. The active site (Cys112, His244 and Asn274) is formed




by the convergence of two α helices and is accessed through a narrow hydrophobic tunnel. Hydrogen-bonding networks that include two tightly bound water molecules fix the positions of His244 and Asn274, which are critical for the decarboxylation and condensation reactions. Surprisingly, the His244→Ala mutation does not affect the transacylation reaction, suggesting that His244 has only a minor influence on the nucleophilicity of Cys112. The histidine and asparagine active-site residues are both required for the decarboxylation step in the condensation reaction. The nucleophilicity of the active-site cysteine is enhanced by the α-helix dipole effect, and an oxyanion hole promotes the formation of the tetrahedral transition state.
1 February 2000, Research Paper, *Structure*.

☐ **The basis for K-Ras4B binding specificity to protein farnesyltransferase revealed by 2 Å resolution ternary complex structures.**
Stephen B Long, Patrick J Casey, Lorena S Beese (2000). *Structure* **8**, 209–222.

The protein farnesyltransferase (FTase) catalyzes addition of the hydrophobic farnesyl isoprenoid to a cysteine residue fourth from the carboxyl terminus of several protein acceptors that are essential for cellular signal transduction, such as Ras and Rho. This addition is necessary for the biological function of the modified proteins. The majority of Ras-related human cancers are associated with oncogenic variants of K-RasB, which is the highest affinity natural substrate of FTase. Inhibition of FTase causes regression of Ras-mediated tumors in animal models. The authors present four ternary complexes of rat FTase co-crystallized with farnesyl diphosphate analogs and K-Ras4B peptide substrates. These ternary complexes provide new insight into the molecular basis of peptide substrate specificity, and further define the roles of zinc and magnesium in the prenyltransferase reaction. Zinc is




essential for productive $Ca_1a_2X$ peptide binding, suggesting that the β-turn conformation identified in previous nuclear magnetic resonance (NMR) studies reflects a state in which the cysteine is not coordinated to the zinc ion. The structural information presented here should facilitate structure-based design and optimization of inhibitors of $Ca_1a_2X$ protein prenyltransferases.
2 February 2000, Research Paper, *Structure*.