# Elsewhere in biology

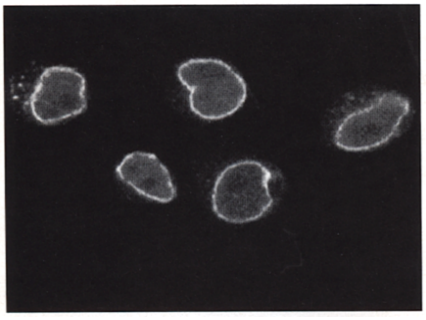
**A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology* and *Structure* with *Folding & Design*, chosen and summarized by the staff of *Chemistry & Biology*.**

**Chemistry & Biology** March 1999, 6:R87–R90



☐ **Receptor-mediated substrate translocation through the nuclear pore complex without nucleotide triphosphate hydrolysis.**
Ludwig Englmeier, Jean-Christophe Olivo, Iain W Mattaj (1998). *Curr. Biol.* **9**, 30–42.

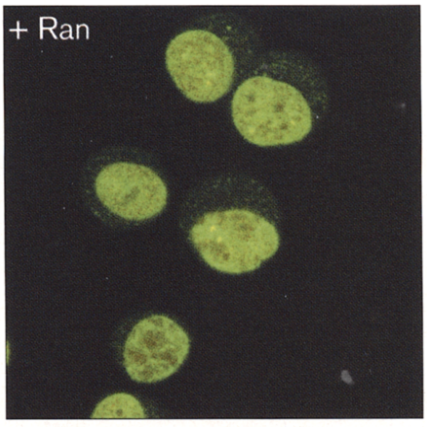
The transport of macromolecules between the nucleus and cytoplasm is an energy-dependent process. Substrates are translocated across the nuclear envelope through nuclear pore complexes (NPCs). Translocation requires nucleocytoplasmic transport receptors of the importin family, which interact both with the NPC and, either directly or via an adaptor, with the transport substrate. Although certain receptors have recently been shown to cross the NPC in an energy-independent manner, translocation of substrate–receptor complexes through the NPC has generally been regarded as an energy-requiring step. The authors describe an *in vitro* system that is based on permeabilised cells and supports nuclear export mediated by leucine-rich nuclear-export signals. In this system, export is dependent on exogenous



CRM1/Exportin1 — a nuclear-export receptor — the GTPase Ran and nucleotide triphosphates (NTPs), and is further stimulated by Ran-binding protein 1 (RanBP1) and nuclear transport factor 2 (NTF2). The author's data show that, contrary to expectation and prior conclusions, the translocation of substrate–receptor complexes across the NPC in either direction occurs in the absence of NTP hydrolysis and is thus energy independent. The energy needed to drive substrate transport against a concentration gradient is supplied at the step of receptor recycling in the cytoplasm.
18 December 1998, Research Paper, *Current Biology*.

☐ **The translocation of transportin–cargo complexes through nuclear pores is independent of both Ran and energy.**
Katharina Ribbeck, Ulrike Kutay, Efrosyni Paraskeva and Dirk Görlich (1999). *Curr. Biol.* **9**, 47–50.

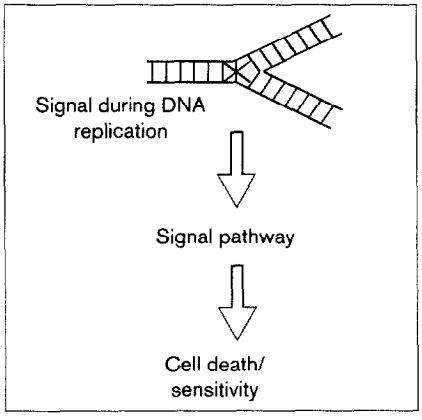
Active transport between nucleus and cytoplasm proceeds through nuclear pore complexes (NPCs) and is mediated largely by shuttling transport receptors that use direct RanGTP binding to coordinate loading and unloading of cargo. Import receptors such as importin β or transportin bind their substrates at low RanGTP levels in the cytoplasm and release them upon encountering RanGTP in the nucleus, where a high RanGTP concentration is predicted. This substrate release is, in the case of import by the importin α/β heterodimer, coupled directly to importin β release from the NPCs. If the importin β–RanGTP interaction is prevented, import intermediates arrest at the nuclear side of the NPCs. This arrest makes it difficult to probe directly the Ran and energy requirements of the actual translocation from the cytoplasmic to the nuclear side of the NPC, which immediately precedes substrate release. Here, the authors have shown that, in the case of transportin, dissociation of transportin–substrate complexes is uncoupled from transportin release from NPCs. This allowed the authors to dissect the requirements of translocation




through the NPC, substrate release and transportin recycling. Surprisingly, translocation of transportin–substrate complexes into the nucleus requires neither Ran nor nucleoside triphosphates (NTPs). It is only nuclear RanGTP, not GTP hydrolysis, that is needed for dissociation of transportin–substrate complexes and for re-export of transportin to the cytoplasm. In addition, the authors provide evidence that at least one type of substrate can also complete NPC passage mediated by importin independently of Ran and energy.
4 January 1999, Brief Communication, *Current Biology*.

☐ **Dependence on RAD52 and RAD1 for anticancer drug resistance mediated by inactivation of mismatch repair genes.**
Stephen T Durant, Melanie M Morris, Maureen Illand, Helen J McKay, Carol McCormick, Gillian L Hirst, Rhona H Borts and Robert Brown (1999). *Curr. Biol.* **9**, 51–54.

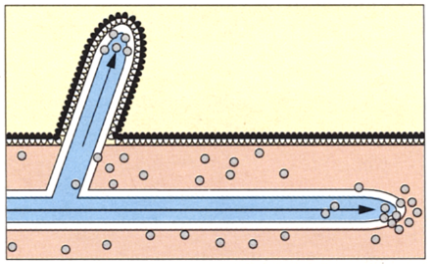
Mismatch repair (MMR) proteins repair mispaired DNA bases and have an important role in maintaining the integrity of the genome. Loss of MMR has been correlated with resistance to a variety of DNA-damaging agents, including many anticancer drugs. How loss of MMR leads to resistance is not understood, but is proposed to be due to loss of futile MMR activity and/or replication stalling. The authors report that inactivation of MMR genes (*MLH1*, *MLH2*, *MSH2*, *MSH3*, *MSH6*, but not PMS1) in isogenic strains of




*Saccharomyces cerevisiae* led to increased resistance to the anticancer drugs cisplatin, carboplatin and doxorubicin, but had no effect on sensitivity to ultraviolet C (UVC) radiation. Sensitivity to cisplatin and doxorubicin was increased in *mlh1* mutant strains when the *MLH1* gene was reintroduced, demonstrating a direct involvement of MMR proteins in sensitivity to these DNA-damaging agents. Inactivation of *MLH1*, *MLH2* or *MSH2* had no significant effect, however, on drug sensitivities in the *rad52* or *rad1* mutant strains that are defective in mitotic recombination and removing unpaired DNA single strands. The authors propose a model whereby MMR proteins, in addition to their role in DNA-damage recognition, decrease adduct tolerance during DNA replication by modulating the levels of recombination-dependent bypass. This hypothesis is supported by the finding that, in human ovarian tumour cells, loss of *hMLH1* correlated with acquisition of cisplatin resistance and increased cisplatin-induced sister chromatid exchange, both of which were reversed by restoration of *hMLH1* expression.
4 January 1999, Brief Communication, *Current Biology*.

❑ **How a fungus escapes the water to grow into the air.**
Han AB Wösten, Marie-Anne van Wetter, Luis G Lugones, Henny C van der Mei, Henk J Busscher and Joseph GH Wessels (1999). *Curr. Biol.* **9**, 85–88.

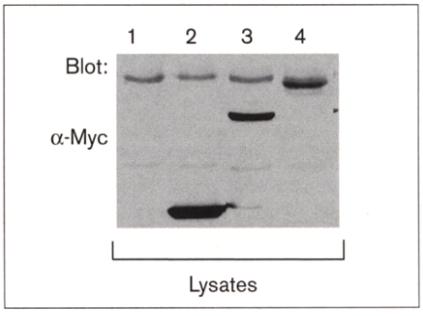
Fungi are well known to the casual observer for producing water-repelling aerial moulds and elaborate fruiting bodies such as mushrooms and polypores. Filamentous fungi colonize moist substrates (such as wood) and have to breach the water–air interface to grow into the air. Animals and plants breach this interface by mechanical force. Here, the authors show that a filamentous fungus such as *Schizophyllum commune* first has to reduce the water surface tension before its hyphae can escape the aqueous phase to form aerial structures such as aerial hyphae or fruiting bodies. The large drop in surface tension (from 72 to 24 mJ $m^{-2}$) results from self-assembly of a secreted hydrophobin (SC3) into a stable amphipathic protein film at the water–air interface. Other, but not all, surface-active molecules (that is, other class I hydrophobins and streptofactin from *Streptomyces tendae*)



can substitute for SC3 in the medium. This demonstrates that hydrophobins not only have a function at the hyphal surface but also at the medium–air interface, which explains why fungi secrete large amounts of hydrophobin into their aqueous surroundings.
18 January 1999, Brief Communication, *Current Biology*.

❑ **A *Drosophila* TNF-receptor-associated factor (TRAF) binds the Ste20 kinase Misshapen and activates Jun kinase.**
Hongzhi Liu, Yi-Chi Su, Elena Becker, Jessica Treisman and Edward Y Skolnik (1999). *Curr. Biol.* **9**, 101–104.

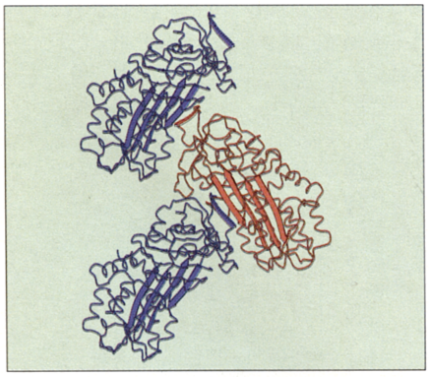
Two families of protein kinases that are closely related to Ste20 in their kinase domain have been identified — the p21-activated protein kinase (Pak) and SPS1 families. In contrast to Pak family members, SPS1 family members do not bind and are not activated by GTP-bound p21Rac and Cdc42. We recently placed a member of the SPS1 family, called Misshapen (Msn), genetically upstream of the c-Jun amino-terminal (JNK) mitogen-activated protein (MAP) kinase module in *Drosophila*. The failure to activate JNK in *Drosophila* leads to embryonic lethality due to the




failure of these embryos to stimulate dorsal closure. Msn probably functions as a MAP kinase kinase kinase kinase in *Drosophila*, activating the JNK pathway via an, as yet, undefined MAP kinase kinase kinase. The authors have identified a *Drosophila* TNF-receptor-associated factor, DTRAF1, by screening for Msn-interacting proteins using the yeast two-hybrid system. In contrast to the mammalian TRAFs that have been shown to activate JNK, DTRAF1 lacks an amino-terminal 'Ring-finger' domain, and overexpression of a truncated DTRAF1, consisting of only its TRAF domain, activates JNK. The authors also identified another DTRAF, DTRAF2, that contains an amino-terminal Ring-finger domain. Msn specifically binds the TRAF domain of DTRAF1 but not that of DTRAF2. In *Drosophila*, DTRAF1 is thus a good candidate for an upstream molecule that regulates the JNK pathway by interacting with, and activating, Msn. We have extended some of these observations to the mammalian homolog of Msn, Nck-interacting kinase (NIK), suggesting that TRAFs also play a critical role in regulating Ste20 kinases in mammals.
18 January 1999, Brief Communication, *Current Biology*.

☐ **The active conformation of plasminogen activator inhibitor 1, a target for drugs to control fibrinolysis and cell adhesion.**
Allan M Sharp, Penelope E Stein, Navraj S Pannu, Robin W Carrell, Mitchell B Berkenpas, David Ginsburg, Daniel A Lawrence and Randy J Read (1999). *Structure* **7**, 111–118.

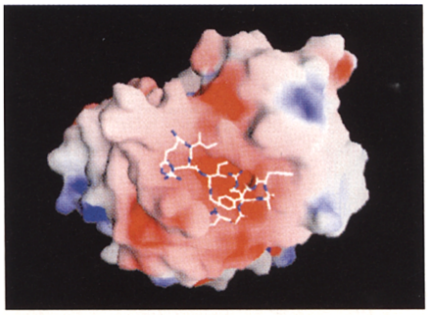
Plasminogen activator inhibitor 1 (PAI-1) is a serpin that plays a key role in the control of fibrinolysis through proteinase inhibition. PAI-1 also has a role in regulating cell-adhesion processes relevant to tissue remodeling and metastasis; this role is mediated by its binding to the adhesive glycoprotein vitronectin rather than by proteinase inhibition. Active PAI-1 is metastable and spontaneously transforms to an inactive latent conformation. Previous attempts to crystallize the active conformation of PAI-1 have failed. The authors have solved crystal structure of a stable quadruple mutant of PAI-1(Asn150→His, Lys154→Thr, Gln319→Leu, Met354→Ile) in its active conformation. The overall conformation resembles that seen for other active inhibitory serpins. The structure clarifies



the molecular basis of the stabilizing mutations and the reduced affinity of PAI-1, on cleavage or in the latent form, for vitronectin. The infinite chain of linked molecules also suggests a new mechanism for the serpin polymerization associated with certain diseases. The results support the concept that the reactive center loop of an active serpin is flexible and has no defined conformation in the absence of intermolecular contacts. The determination of the structure of the active form constitutes an essential step for the rational design of PAI-1 inhibitors.
22 January 1999, Research Paper, *Structure*.

☐ **Dual conformations for the HIV-1 gp120 V3 loop in complexes with different neutralizing Fabs.**
RL Stanfield, E Cabezas, AC Satterthwait, EA Stura, AT Profy and IA Wilson (1999). *Structure* **7**, 131–142.

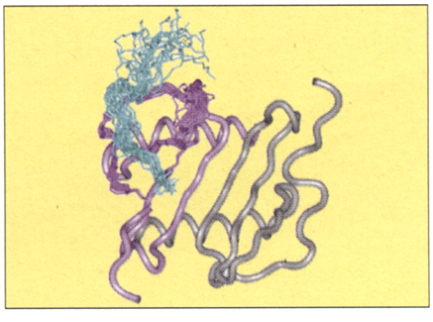
The third hypervariable (V3) loop of HIV-1 gp120 has been termed the principal neutralizing determinant of the virus and is involved in many aspects of virus infectivity. The V3 loop is required for viral entry into the cell via membrane fusion and is believed to interact with cell surface chemokine receptors on T cells and macrophages. Sequence changes in V3 can affect chemokine receptor usage, and can, therefore, modulate which types of cells are infected. Antibodies raised against peptides with V3 sequences can neutralize laboratory-adapted strains of the virus and inhibit syncytia formation. Fab fragments of these neutralizing antibodies in complex with V3 loop peptides have been studied by X-ray crystallography to determine the conformation of the V3 loop. The authors have determined three crystal structures of Fab 58.2, a broadly neutralizing antibody, in complex with one linear and two cyclic peptides, the amino-acid sequence of which comes from the MN isolate of the gp120 V3 loop. Although the peptide conformations are very similar for the linear and cyclic forms, they differ from that seen for the identical peptide bound to a different broadly neutralizing antibody, Fab 59.1, and for a similar peptide bound to the MN-specific Fab 50.1. The conformational difference in the peptide is localized around residues Gly–Pro–Gly–Arg, which are highly conserved in different HIV-1 isolates and are predicted to adopt a type II β turn. The V3 loop can adopt at least two different conformations for the



highly conserved Gly–Pro–Gly–Arg sequence at the tip of the loop. The HIV-1 V3 loop therefore has some inherent conformational flexibility that might relate to its biological function.
29 January 1999, Research Paper, *Structure*.

☐ **Structure of a CXC chemokine-receptor fragment in complex with interleukin-8.**
Nicholas J Skelton, Cliff Quan, Dorothea Reilly and Henry Lowman (1999). *Structure* **7**, 157–168.

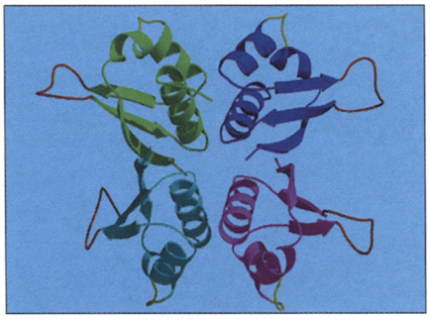
Interactions between CXC chemokines (e.g. interleukin-8, IL-8) and their receptors (e.g. CXCR-1) play a key role in host defense and disease by attracting and upregulating neutrophils to sites of inflammation. The transmembrane nature of the receptor impedes structure-based understanding of ligand interactions. Linear peptides based on the amino-terminal, extracellular portion of the receptor CXCR-1 do bind to IL-8, however, and inhibit the binding of IL-8 to the full-length receptor. The NMR solution structure of the complex formed between IL-8 and one such receptor-based peptide indicates that a cleft between a loop and a β hairpin constitute part of the receptor interaction surface on IL-8. Nine residues from the carboxyl terminus of



the receptor peptide (corresponding to Pro21–Pro29 of CXCR-1) occupy the cleft in an extended fashion. Intermolecular contacts are mostly hydrophobic and sidechain mediated. The results offer the first details at an atomic level of the interaction between a chemokine and its receptor. This first glimpse of how IL-8 binds to its receptor provides a foundation for the structure-based design of chemokine antagonists.
29 January 1999, Research Paper, *Structure*.

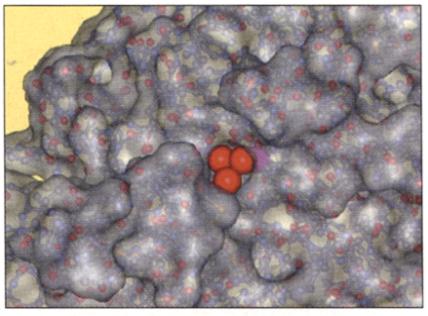
☐ **Crystal structures of Nova-1 and Nova-2 K-homology RNA-binding domains.**
Hal A Lewis, Hua Chen, Carme Edo, Ronald J Buckanovich, Yolanda YL Yang, Kiran Musunuru, Ru Zhong, Robert B Darnell and Stephen K Burley (1999). *Structure* **7**, 191–204.

Nova-1 and Nova-2 are related neuronal proteins that were initially cloned using antisera obtained from patients with the autoimmune neurological disease paraneoplastic opsoclonus-myoclonus ataxia (POMA). Both of these disease gene products contain three RNA-binding motifs known as K-homology or KH domains, and their RNA ligands have been identified via binding-site selection experiments. The KH motif structure has been determined previously using NMR spectroscopy, but not using X-ray crystallography. Although many proteins contain more than one KH domain, there is no published structural information regarding the behavior of such multimers. Here the first X-ray crystallographic structures of KH-domain-containing proteins, the third KH domains (KH3) of Nova-1 and Nova-2 are presented. These highly similar RNA-binding motifs form a compact protease-resistant domain resembling an open-faced sandwich, consisting of a three-stranded antiparallel β sheet topped by three α helices. In both Nova crystals, the lattice is composed of symmetric tetramers of KH3 domains that are created by two dimer interfaces. The most significant differences among the KH domains , from the previously determined NMR structures, involve changes in the positioning of one or more of the α helices with respect to the β sheet, particularly in the NMR structure of the KH1 domain of the Fragile X disease protein FMR-1. Loop regions in the KH domains are clearly visible in the crystal structure, revealing the conformation of the invariant Gly–X–X–Gly segment that is thought to participate in RNA-binding and of the variable region. The tetrameric arrangements of the Nova KH3 domains provide insights into how KH domains may interact with each other in proteins containing multiple KH motifs.
29 January 1999, Research Paper, *Structure*.



☐ **A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from *Bacillus pasteurii*: why urea hydrolysis costs two nickels.**
Stefano Benini, Wojciech R Rypniewski, Keith S Wilson, Silvia Miletti, Stefano Ciurli and Stefano Mangani (1999). *Structure* **7**, 205–216.

Urease catalyzes the hydrolysis of urea, the final step of organic nitrogen mineralization, using a bimetallic nickel centre. The role of the active-site metal ions and amino-acid residues has not been elucidated to date. Many pathologies are associated with the activity of ureolytic bacteria, and the efficiency of soil nitrogen fertilization with urea is severely decreased by urease activity. The development of urease inhibitors would therefore lead to a reduction of environmental pollution, to enhanced efficiency of nitrogen uptake



by plants and to improved therapeutic strategies for treatment of ureolytic bacterial infections. Structure-based design of urease inhibitors requires knowledge of the enzyme mechanism at the molecular level. The structures of native and inhibited urease from *Bacillus pasteurii* have been determined. The enzyme crystallized in the presence of phenylphosphorodiamidate contains the tetrahedral transition-state analogue diamidophosphoric acid, bound to the two nickel ions in an unprecedented mode. Comparison of the native and inhibited structures reveals two distinct conformations of the flap lining the active-site cavity. The mode of binding of the inhibitor, and a comparison between the native and inhibited urease structures, indicate a novel mechanism for enzymatic urea hydrolysis that reconciles the available structural and biochemical data.
1 February 1999, Research Paper, *Structure*.