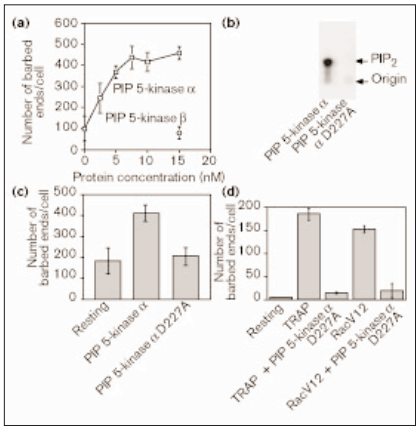
Elsewhere in biology

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**:R99–R102

☐ **Type Iα phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly.**
Kimberley F Tolias, John H Hartwig, Hisamitsu Ishihara, Yoshikazu Shibasaki, Lewis C Cantley and Christopher L Carpenter (2000). *Curr. Biol.* **10**, 153–156.

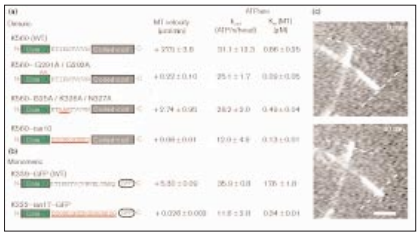
Actin polymerization is essential for a variety of cellular processes including movement, cell division and shape change. The induction of actin polymerization requires the generation of free actin filament barbed ends, which results from the severing or uncapping of pre-existing actin



filaments, or *de novo* nucleation, initiated by the Arp2/3 complex. Although little is known about the signaling pathways that regulate actin assembly, small GTPases of the Rho family appear to be required. In thrombin-stimulated platelets, the Rho family GTPase Rac1 induces actin polymerization by stimulating the uncapping of actin filament barbed ends. The mechanism by which Rac regulates uncapping is unclear, however. The authors previously demonstrated that Rac interacts with a type I phosphatidylinositol-4-phosphate 5-kinase (PIP 5-kinase) in a GTP-independent manner. Because PIP 5-kinases synthesize phosphatidyl-inositol-4,5-bisphosphate ($PI(4,5)P_2$), a lipid that dissociates capping proteins from the barbed ends of actin filaments, they are good candidates for mediating the effects of Rac on actin assembly. Here, the authors demonstrate that PIP 5-kinase α is a critical mediator of thrombin- and Rac-dependent actin assembly.
28 January 2000, Brief Communication, *Current Biology*.

☐ **Role of the kinesin neck linker and catalytic core in microtubule-based motility.**
Ryan B Case, Sarah Rice Cynthia L Hart, Bernice Ly and Ronald D Vale (2000). *Curr. Biol.* **10**, 157–160.

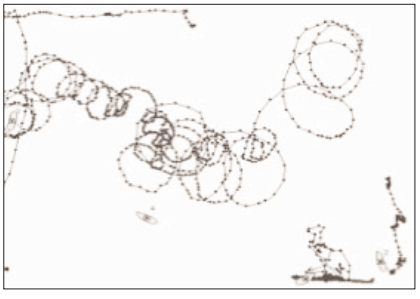
Kinesin motor proteins execute a variety of intracellular microtubule-based transport functions. Kinesin motor domains contain a highly conserved catalytic core, followed by a neck region, which is conserved within subfamilies and has been implicated in controlling the direction of motion along a microtubule. The authors have used mutational analysis to determine the functions of the catalytic core and the ~15 amino acid 'neck linker' (a



sequence contained within the neck region) of human conventional kinesin. Replacement of the neck linker with a designed random coil resulted in a 200–500-fold decrease in microtubule velocity. The catalytic core of kinesin alone displayed microtubule-stimulated ATPase activity, nucleotide-dependent microtubule binding, and very slow plus-end-directed motor activity. On the basis of these results, the authors propose that the catalytic core is sufficient for allosteric regulation of microtubule binding and ATPase activity and that the kinesin neck linker functions as a mechanical amplifier for motion. Because the neck linker undergoes a nucleotide-dependent conformational change, this region might act in an analogous fashion to the myosin converter, which amplifies small conformational changes in the myosin catalytic core.
28 January 2000, Brief Communication, *Current Biology*.

☐ **Dopamine modulates acute responses to cocaine, nicotine and ethanol in *Drosophila*.**
Roland J Bainton, Linus T-Y Tsai, Carol M Singh, Monica S Moore, Wendi S Neckameyer and Ulrike Heberlein (2000). *Curr. Biol.* **10**, 187–194.

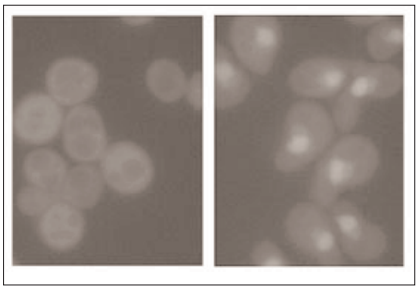
In mammals, drugs of abuse facilitate the release of the neurotransmitter and neuromodulator dopamine in specific regions of the brain involved in reward and motivation. This increase in synaptic dopamine levels is believed to act as a positive reinforcer and to mediate some of the acute responses to drugs. The mechanisms by which dopamine regulates acute drug responses and addiction remain unknown. The authors present evidence that dopamine plays a role in the responses of *Drosophila* to cocaine, nicotine or ethanol. A startle-induced negative geotaxis assay and a locomotor tracking system were used to measure the effect of psychostimulants on fly behavior. Using these assays, acute responses to cocaine and nicotine are



blunted by pharmacologically induced reductions in dopamine levels. Cocaine and nicotine showed a high degree of synergy in their effects, which is consistent with an action through convergent pathways. In addition, dopamine was found to be involved in the acute locomotor-activating effect, but not the sedating effect, of ethanol. The authors show that in *Drosophila*, as in mammals, dopaminergic pathways play a role in modulating specific behavioral responses to cocaine, nicotine or ethanol, suggesting that *Drosophila* can be used as a genetically tractable model system to study the mechanisms underlying behavioral responses to multiple drugs of abuse.
7 February 2000, Research Paper, *Current Biology*.

☐ **Clb/Cdc28 kinases promote nuclear export of the replication initiator proteins Mcm2–7.**
Van Q Nguyen, Carl Co, Kaoru Irie and Joachim J Li (2000). *Curr. Biol.* **10**, 195–206.

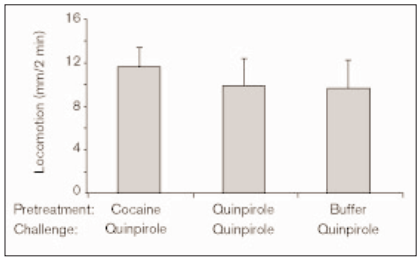
In the budding yeast *Saccharomyces cerevisiae*, initiation of DNA replication is limited to once per cell cycle by cyclin-dependent kinases of the Clb/Cdc28 family. These kinases prevent re-assembly of pre-replicative complexes (pre-RCs) at replication origins that have already initiated replication. This assembly involves the Cdc6-dependent loading of six minichromosome maintenance (Mcm) proteins, Mcm2–7, onto origins. How



Clb/Cdc28 kinases prevent pre-RC assembly is not understood. In living cells, the Mcm proteins were found to co-localize in a cell-cycle-regulated manner. Mcm2–4, 6 and 7 were concentrated in the nucleus in G1 phase, gradually exported to the cytoplasm during S phase, and excluded from the nucleus by G2 and M phase. Tagging any single Mcm protein with the SV40 nuclear localization signal made all Mcm proteins constitutively nuclear. Cells containing or lacking functional Cdc6 were examined for their ability to export a fusion protein between Mcm7 and the green fluorescent protein (Mcm7–GFP). The results suggest that Clb/Cdc28 kinases prevent pre-RC reassembly in part by promoting the net nuclear export of Mcm proteins. Mcm proteins may become refractory to this regulation when they load onto chromatin and must be dislodged by DNA replication before they can be exported. Such an arrangement could ensure that Mcm proteins complete their replication function before they are removed from the nucleus.
8 February 2000, Research Paper, *Current Biology*.

☐ **Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in *Drosophila melanogaster*.**
H Li, S Chaney, M Forte and J Hirsh (2000). *Curr. Biol.* **10**, 211–214.

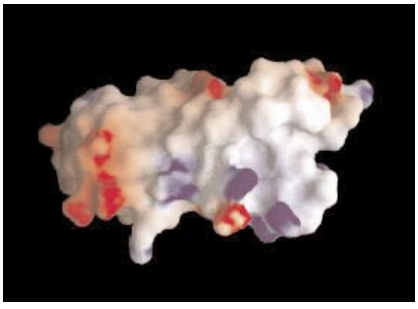
Sensitization to repeated doses of psychostimulants is thought to be an important component underlying the addictive process in humans. In all vertebrate animal models, including humans and fruit flies, sensitization is observed after repeated exposure to volatilized crack cocaine. In vertebrates, sensitization is thought to be initiated by processes that occur in brain regions that contain dopamine cell bodies. The authors show that modulated cell signaling in the *Drosophila* dopamine and serotonin neurons plays an essential role in cocaine sensitization. Targeted expression of either a stimulatory ($G\alpha_s$) or inhibitory ($G\alpha_i$) Gα subunit, or tetanus toxin light chain (TNT) in dopamine and serotonin neurons of living flies blocked behavioral sensitization to repeated cocaine exposures. These flies showed alterations in their initial cocaine



responsiveness that correlated with compensatory adaptations of postsynaptic receptor sensitivity. Finally, repeated drug stimulation of a nerve cord preparation that is postsynaptic to the brain amine cells failed to induce sensitization, further showing the importance of presynaptic modulation in sensitization.
11 February 2000, Brief Communication, *Current Biology*.

☐ **Structure of the zinc-binding domain of *Bacillus stearothermophilus* DNA primase.**
Hu Pan and Dale B Wigley (2000). *Structure* **8**, 231–239.

DNA primases catalyse the synthesis of the short RNA primers required for DNA replication by DNA polymerases. Primases are composed of three functional domains: a zinc-binding domain that is responsible for template recognition, a polymerase domain and a domain that interacts with the replicative helicase, DnaB.The crystal structure of the zinc-binding domain of DNA primase from *Bacillus*

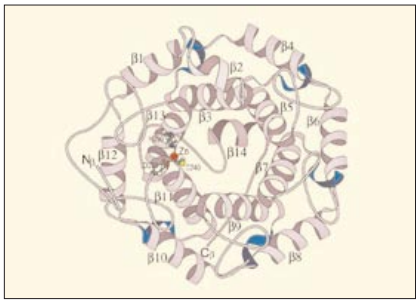


stearothermophilus has been determined. A model is discussed for the interaction of this domain with the single-stranded DNA template. The structure of the DNA primase zinc-binding domain confirms that the protein belongs to the

zinc ribbon subfamily. Structural comparison with other nucleic-acid-binding proteins suggests that the β sheet of primase is likely to be the DNA-binding surface, with conserved residues on this surface being involved in the binding and recognition of DNA.
15 February 2000, Research Paper, *Structure*.

☐ **Crystal structure of Rab geranylgeranyltransferase at 2.0 Å resolution.**
Hong Zhang, Miguel C Seabra and Johann Deisenhofer (2000). *Structure* **8**, 241–252.

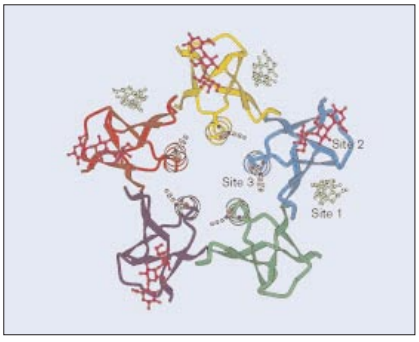
Rab geranylgeranyltransferase (RabGGT) catalyzes the addition of two geranylgeranyl groups to the carboxy-terminal cysteine residues of Rab proteins, which is crucial for membrane association and function of these proteins in intracellular vesicular trafficking. Unlike protein farnesyltransferase (FT) and type I geranylgeranyltransferase, which both prenylate monomeric small G proteins or short peptides, RabGGT can prenylate Rab only when Rab is in a complex with Rab escort protein (REP). The crystal structure of rat RabGGT reveals an assembly of four distinct structural modules. The β subunit forms



an α–α barrel that contains most of the residues in the active site. The α subunit consists of a helical domain, an immunoglobulin (Ig)-like domain, and a leucine-rich repeat (LRR) domain. The amino-terminal region of the α subunit binds to the active site in the β subunit; residue His2α directly coordinates a zinc ion. The prenyl-binding pocket of RabGGT is deeper than that in FT. LRR and Ig domains are often involved in protein–protein interactions; in RabGGT they might participate in the recognition and binding of REP. Binding of the amino-terminal peptide of the α subunit to the active site suggests an autoinhibition mechanism might contribute to the inability of RabGGT to recognize short peptides or Rab alone. Replacement of residues Trp102β and Tyr154β in FT by Ser48β and Leu99β, respectively, in RabGGT largely determine the different lipid-binding specificities of the two enzymes.
18 February 2000, Research Paper, *Structure*.

☐ **A mutant Shiga-like toxin IIe bound to its receptor Gb_3: structure of a group II Shiga-like toxin with altered binding specificity .**
Hong Ling, Navraj S Pannu, Amechand Boodhoo, Glen D Armstrong, Clifford G Clark, James L Brunton and Randy J Read (2000). *Structure* **8**, 253–264.

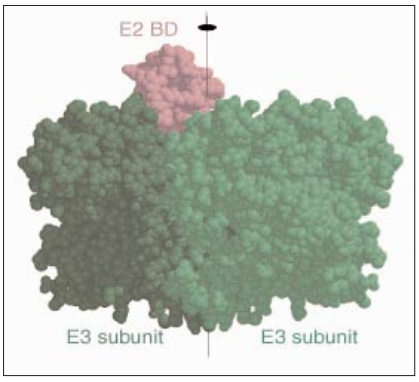
Shiga-like toxins (SLTs) are produced by the pathogenic strains of *Escherichia coli* that cause hemorrhagic colitis and hemolytic uremic syndrome. These diseases in humans are generally associated with group II family members (SLT-II and SLT-IIc), whereas SLT-IIe (pig edema toxin) is central to edema disease of swine. The pentameric B-subunit component of the majority of family members binds to the cell-surface glycolipid globotriaosyl ceramide (Gb_3), but globotetraosyl ceramide (Gb_4) is the preferred receptor for SLT-IIe. A double-mutant of the SLT-IIe B subunit that reverses two sequence differences from SLT-II (GT3; Gln65→Glu, Lys67→Gln, SLT-I numbering) has been shown to bind more strongly to Gb_3 than to Gb_4. The structure of the GT3 mutant B pentamer, both in native form and in complex with a Gb_3 analog, have been determined to understand the molecular basis of receptor binding and specificity. The structures confirm the previous observation of multiple binding sites on each SLT monomer. Analysis of the binding properties of mutants suggests that site 3 is a



secondary Gb_4-binding site. The two mutated residues are located appropriately to interact with the extra βGalNAc residue on Gb_4. Differences in the binding sites provide a molecular basis for understanding the tissue specificities and pathogenic mechanisms of members of the SLT family.
22 February 2000, Research Paper, *Structure*.

☐ **Crystal structure of human branched-chain α-ketoacid dehydrogenase and the molecular basis of multienzyme complex deficiency in maple syrup urine disease.**
Arnthor Ævarsson, Jacinta L Chuang, R Max Wynn, Stewart Turley, David T Chuang and Wim GJ Hol (2000). *Structure* **8**, 277–292.

Mutations in components of the extraordinarily large α-ketoacid dehydrogenase multienzyme complexes can lead to serious and often fatal disorders in humans, including maple syrup urine disease (MSUD). In order to obtain insight into the effect of mutations observed in MSUD patients, the authors determined the crystal structure of branched-chain α-ketoacid dehydrogenase (E1), the 170 kDa $\alpha_2\beta_2$ heterotetrameric E1b component of the branched-chain α-ketoacid dehydrogenase multienzyme complex. The structure of human E1b revealed essentially the full α and β polypeptide chains of the tightly packed heterotetramer, as well as the positions of two important potassium (K^+). One assists a loop that is close to the cofactor to adopt the proper conformation. The second is located in the β subunit near the interface with



the small carboxy-terminal domain of the α subunit. The known MSUD mutations affect the functioning of E1b by interfering with the cofactor and K^+ sites, the packing of hydrophobic cores, and the precise arrangement of residues at or near several subunit interfaces. The Tyr→Asn mutation at position 393-α occurs very frequently in the US population of Mennonites and is located in a unique extension of the human E1b a subunit, contacting the β′ subunit. Essentially all MSUD mutations in human E1b can be explained using this structure as a basis, with the severity of the mutations for the stability and function of the protein correlating well with the severity of the disease for the patients. The authors suggest that small molecules with high affinity for human E1b might alleviate effects of some of the milder forms of MSUD.
28 February 2000, Research Paper, *Structure*.

□ **Crystallographic analysis of the specific yet versatile recognition of distinct nuclear localization signals by karyopherin α**
Elena Conti and John Kuriyan (2000). *Structure* **8**, 329–338.

Karyopherin α (importin α) is an adaptor molecule that recognizes proteins containing nuclear localization signals (NLSs). The SV40 T antigen contains a prototypical NLS that is able to bind to karyopherin α, which consists of a short positively charged sequence motif. Distinct classes of NLSs (monopartite and bipartite) have been identified that are only partly conserved with respect to one another but are nevertheless recognized by the same receptor. Crystal structures of two peptide complexes of yeast karyopherin α (Kapα): one with a human c-*myc* NLS peptide, and one with a *Xenopus* nucleoplasmin NLS peptide are described here. Analysis of these structures reveals the determinants of specificity for the binding of a relatively hydrophobic monopartite NLS and of a bipartite NLS peptide. The peptides bind Kapα in its extended surface groove, which presents a modular array of tandem binding pockets for amino acid residues.

Monopartite and bipartite NLSs bind to a different number of amino-acid-binding pockets and make different interactions within them. The relatively hydrophobic monopartite c-*myc* NLS binds extensively at a few binding pockets in a similar manner to that of the SV40 T antigen NLS. In contrast, the bipartite nucleoplasmin NLS engages the whole array of pockets with individually more limited but overall more abundant interactions, which include the NLS two basic clusters and the backbone of its non-conserved linker region. Versatility in the specific recognition of NLSs relies on the modular and flexible structural framework of Kapα.
29 February 2000, Research Paper, *Structure*.