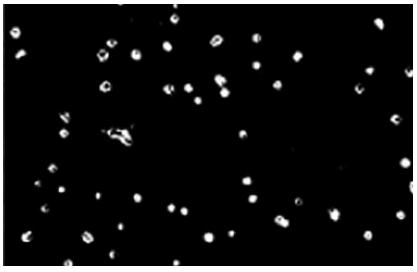
Elsewhere in biology

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**:R147–R151

☐ **Regulation of N-cadherin-mediated adhesion by the p35–Cdk5 kinase.**
Young T Kwon, Amitabh Gupta, Ying Zhou, Margareta Nikolic and Li-Huei Tsai (2000). *Curr. Biol.* **10**, 363–372.

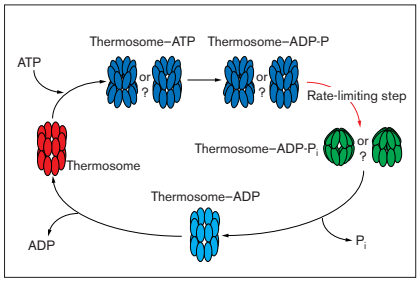
The p35–Cdk5 kinase has been implicated in a variety of functions in the central nervous system, including axon outgrowth, axon guidance, and neuronal migration during cortical development. In *p35*$^{-/-}$ mice, embryonic cortical neurons are unable to migrate past their predecessors, leading to an inversion of cortical layers in the adult cortex. The



authors identify molecules important for p35–Cdk5-dependent function in the cortex, by screening for p35-interacting proteins using the two-hybrid system. They identify a novel interaction between p35 and the versatile cell adhesion signaling molecule β-catenin. The association between p35–Cdk5 and an N-cadherin adhesion complex in cortical neurons and the modulation of N-cadherin-mediated aggregation by p35–Cdk5 suggest that the p35–Cdk5 kinase is involved in the regulation of N-cadherin-mediated adhesion in cortical neurons.
16 March 2000, Research Paper, *Current Biology*.

☐ **Conformational rearrangements of an archaeal chaperonin upon ATPase cycling.**
Irina Gutsche, Jörg Holzinger, Manfred Rößle, Hermann Heumann, Wolfgang Baumeister and Roland P May (2000). *Curr. Biol.* **10**, 405–408.

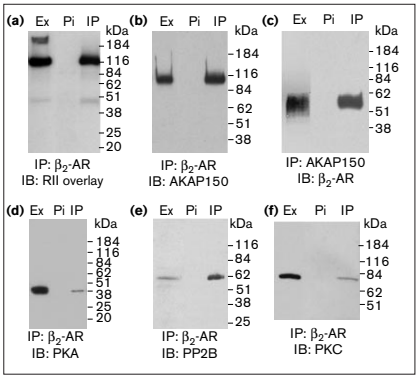
Chaperonins are double-ring protein assemblies with a central cavity that provides a sequestered environment for *in vivo* protein folding. The reaction cycle is thought to consist of a nucleotide-regulated alternation between an open substrate-acceptor state and a closed folding-active state. The cavity of ATP-charged group I



chaperonins, such as *Escherichia coli* GroEL, is sealed off by a co-chaperonin, whereas group II chaperonins, such as the archaeal thermosome and eukaryotic TRiC/CCT, have a built-in lid. Here, the conformation of the thermosome is analyzed at each step of the ATPase cycle, using small-angle neutron scattering. The apo-chaperonin is open in solution, and ATP binding induces its further expansion. Closure apparently occurs during ATP hydrolysis, and represents the rate-limiting step of the cycle. The allosteric regulation of group II chaperonins appears different from that of group I chaperonins.
24 March 2000, Brief Communication, *Current Biology*.

☐ **Assembly of an A kinase-anchoring protein–β_2-adrenergic receptor complex facilitates receptor phosphorylation and signaling.**
Iain DC Fraser, Mei Cong, Jihee Kim, Emily N Rollins, Yehia Daaka, Robert J Lefkowitz and John D Scott (2000). *Curr. Biol.* **10**, 409–412.

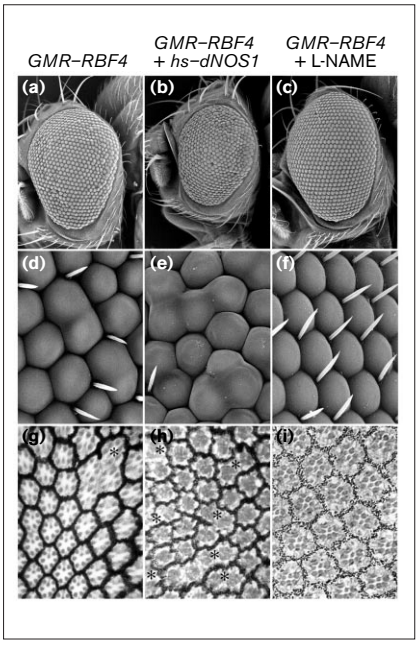
Phosphorylation of G-protein-coupled receptors by second-messenger-stimulated kinases is essential in the process of receptor desensitization. Phosphorylation of the β_2-adrenergic receptor (β_2-AR) by protein kinase A (PKA), in addition to uncoupling adenylate cyclase activation, is obligatory for receptor-mediated activation of mitogen-activated protein kinase (MAP kinase) cascades. Although mechanisms for linking G-protein-coupled receptor kinases to the activated receptor are well established, analogous mechanisms for targeting second messenger kinases to β_2-AR at the plasma membrane have not been



elucidated. Functional studies show that PKA anchoring is required to enhance β_2-AR phosphorylation and to facilitate downstream activation of the MAP kinase pathway. This defines a role for the A-kinase-anchoring protein AKAP79/150 in the recruitment of second-messenger-regulated signaling enzymes to a G-protein-coupled receptor.
24 March 2000, Brief Communication, *Current Biology*.

☐ **Nitric oxide interacts with the retinoblastoma pathway to control eye development in *Drosophila*.**
Boris Kuzin, Michael Regulski, Yuri Stasiv, Vladimir Scheinker, Tim Tully and Grigori Enikolopov (2000). *Curr. Biol.* **10**, 459–462.

Animal organ development requires that tissue patterning and



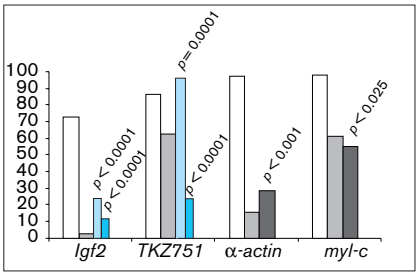
differentiation is tightly coordinated with cell multiplication and cell-cycle progression. In imaginal discs of developing *Drosophila* larvae, cell-cycle progression is controlled by a modified version of the well-characterized mammalian retinoblastoma (Rb) pathway, which integrates signals from multiple effectors ranging from growth factors and receptors to small signaling molecules. Nitric oxide, a multi-functional second messenger, can reversibly suppress DNA synthesis and cell division. In developing flies, the antiproliferative action of nitric oxide is essential for regulating the balance between cell proliferation and differentiation. The mechanisms of the antiproliferative activity of nitric oxide in developing organisms are not known, however, and the authors address this. Manipulation of endogenous or transgenic nitric oxide synthase activity during imaginal disc development can enhance or suppress the effects of the Rb-like protein RBF and the transcription factor E2F on development of the eye. The results suggest a role for nitric oxide in the developing imaginal eye disc through interaction with the Rb pathway.

7 April 2000, Brief Communication, *Current Biology*.

☐ **Active demethylation of the paternal genome in the mouse zygote.**

J Oswald, S Engemann, N Lane, W Mayer, A Olek, R Fundele, W Dean, W Reik and J Walter (2000). *Curr. Biol.* **10**, 475–478.

DNA methylation is essential for the control of many biological mechanisms in mammals. Mammalian development is accompanied by two major waves of genome-wide demethylation and remethylation: one during germ-cell development and the other after fertilisation. Most studies have suggested that the genome-wide demethylation observed after



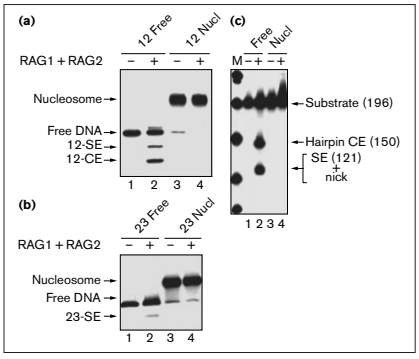
fertilisation occurs passively, by the lack of maintenance methylation following DNA replication and cell division, although one study has reported that replication-independent demethylation might also occur during early embryogenesis. The authors report that genes that are highly methylated in sperm are rapidly demethylated in the zygote only hours after fertilisation, before the first round of DNA replication commences. By contrast, the oocyte-derived maternal alleles are unaffected by this reprogramming. Whereas paternally derived sequences are exposed to putative active demethylases in the oocyte cytoplasm, maternally derived sequences are protected from this reaction. These results have important implications for the establishment of biparental genetic totipotency after fertilisation, the establishment and maintenance of genomic imprinting, and the reprogramming of somatic cells during cloning.

7 April 2000, Brief Communication, *Current Biology*.

☐ **Stimulation of V(D)J recombination by histone acetylation.**

Fraser McBlane and Joan Boyes (2000). *Curr. Biol.* **10**, 483–486.

V(D)J recombination assembles functional immunoglobulin and T cell receptor genes from individual gene segments. A common recombination mechanism, initiated by the proteins RAG1 and RAG2 at conserved recombination signal sequences (RSSs), operates at all rearranging loci. It has been proposed that the key regulator of the reaction is 'accessibility' of the RSS within chromatin. Recently, the packaging of RSSs into nucleosomes was shown to inhibit initiation of V(D)J recombination. Nevertheless, the tight tissue specificity of regulation cannot be explained by nucleosome-mediated repression alone because a significant fraction of RSSs would be predicted to lie in linker regions between nucleosomes. Some aspect of the regulation of the recombination reaction must therefore rely on the disruption of higher-order chromatin structure.

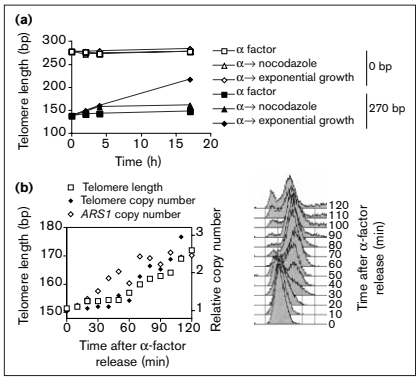


Histone acetylation directly stimulates the recombination reaction *in vivo* in the correct cell- and stage-specific manner. Neither expression of RAG genes nor activity of RAG proteins was increased by acetylation. Histone acetylation failed to overcome nucleosome-mediated repression of RSS recognition and cleavage *in vitro*. The data suggest a role for histone acetylation in stimulating recombination *in vivo* through disruption of higher-order chromatin structures.

7 April 2000, Brief Communication, *Current Biology*.

☐ **Cell cycle restriction of telomere elongation.**
Stéphane Marcand, Vanessa Brevet, Carl Mann and Eric Gilson (2000). *Curr. Biol.* **10**, 487–490.

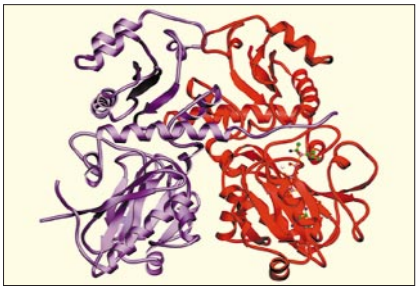
Telomere elongation by telomerase balances the progressive shortening of chromosome ends resulting from the succession of replication cycles. Telomerase activity is regulated *in vivo* at its site of action by the telomere itself. In yeast and human cells, the mean telomere length is maintained at a constant value through a *cis*-inhibition of telomerase by factors specifically bound to the telomeric DNA. The authors test the link between telomere dynamics and cell-cycle progression in the



budding yeast *Saccharomyces cerevisiae*. They observed that telomere elongation is linked to the succession of cell divisions. In cells progressing synchronously through the cell cycle, telomere elongation coincided with the time of telomere replication. On a minichromosome, a replication defect partially suppressed telomere elongation, suggesting a coupling between *in vivo* telomerase activity and conventional DNA replication.
7 April 2000, Brief Communication, *Current Biology*.

☐ **Enzymes of vancomycin resistance: the structure of D-alanine–D-lactate ligase of naturally resistant *Leuconostoc mesenteroides*.**
Alexandre P Kuzin, Tao Sun, Jodi Jorczak-Baillass, Vicki L Healy, Christopher T Walsh and James R Knox (2000). *Structure* **8**, 463–470.

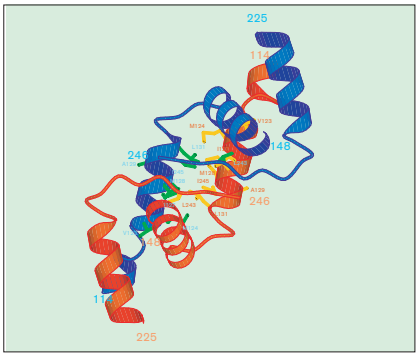
The bacterial cell wall and the enzymes that synthesize it are targets of glycopeptide antibiotics (vancomycins and teicoplanins) and β-lactams (penicillins and cephalosporins). Biosynthesis of cell-wall peptidoglycan requires a cross-linking of peptidyl moieties on adjacent glycan strands. The D-alanine–D-alanine transpeptidase, which catalyzes this cross-linking, is the target of β-lactam antibiotics. Glycopeptides do not inhibit an enzyme, but bind directly to D-alanine–D-alanine and prevent subsequent cross-linking by the transpeptidase. Resistance to vancomycin in enterococcal pathogens has been traced to altered ligases producing D-alanine–D-lactate rather than D-alanine–D-alanine. The structure of a D-alanine–D-lactate ligase has been determined. Comparison of this D-alanine–D-lactate ligase with the known structure of DdlB D-alanine–D-alanine ligase, a wild-type enzyme that does not provide vancomycin resistance, reveals alterations in the size and hydrophobicity of the site for D-lactate



binding (subsite 2). Structural differences at subsite 2 of the D-alanine–D-lactate ligase help explain a substrate specificity shift (D-alanine to D-lactate) leading to remodeled cell wall peptidoglycan and vancomycin resistance in gram-positive pathogens.
18 April 2000, Research Paper, *Structure*.

☐ **The structure of the N-terminal actin-binding domain of human dystrophin and how mutations in this domain may cause Duchenne or Becker muscular dystrophy.**
Fiona LM Norwood, Andrew J Sutherland-Smith, Nicholas H Keep and John Kendrick-Jones (2000). *Structure* **8**, 481–492.

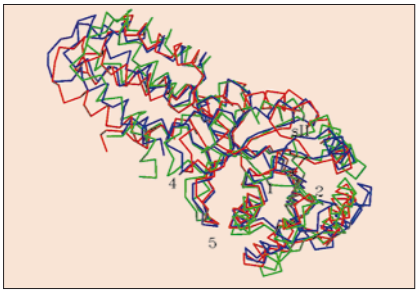
Dystrophin is a large multidomain protein component of skeletal muscle cells. Its amino-terminal domain binds to F-actin and its carboxyl terminus binds to the dystrophin-associated glycoprotein complex in the membrane. Dystrophin is thought to serve as a link from the actin-based cytoskeleton of the muscle cell through the plasma membrane to the extracellular matrix. Pathogenic mutations in dystrophin result in Duchenne or Becker muscular dystrophy. The crystal structure of the dystrophin actin-binding domain (ABD) has been determined. The structure is an antiparallel dimer of two ABDs, each comprising two calponin homology (CH) domains that are linked by a central α helix. The dystrophin ABD structure reveals a previously uncharacterised



arrangement of the CH domains within the ABD. This observation has implications for the mechanism of actin binding by dystrophin and related proteins. Examining the position of three pathogenic missense mutations within the structure suggests that they exert their effects through misfolding of the ABD, rather than through disruption of the binding to F-actin.
25 April 2000, Research Paper, *Structure*.

☐ **The crystal structure of the conserved GTPase of SRP54 from the archaeon *Acidianus ambivalens* and its comparison with related structures suggests a model for the SRP–SRP receptor complex.**
Guillermo Montoya, Kai te Kaat, Ralph Moll, Günter Schäfer and Irmgard Sinning (2000). *Structure* **8**, 515–526.

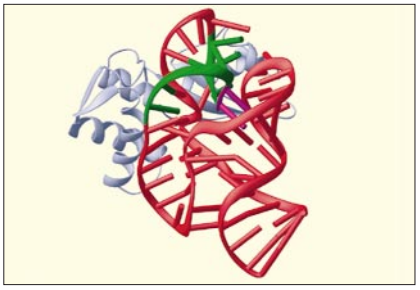
Protein targeting to the endoplasmic reticulum in eukaryotes and to the cell membrane in prokaryotes is mediated by the signal recognition particle (SRP) and its receptor (SR). Both contain conserved GTPase domains in the signal-peptide-binding proteins (SRP54 and Ffh) and the SR proteins (SRα and FtsY). These GTPases are involved in the regulation of protein targeting. Most studies have focused on the SRP machinery of mammals and bacteria, leaving the SRP system of archaea less well understood. The crystal structure of the conserved GTPase (NG-Ffh) from the thermophilic archaeon *Acidianus ambivalens* and of the Thr112→Ala mutant, which is inactive in GTP hydrolysis, has been determined. It allows a detailed comparison with related structures from *Escherichia coli* and thermophilic bacteria. In particular, differences in the conserved consensus regions for nucleotide binding and the subdomain interfaces are observed, which



provide information about the regulation of the GTPase. The overall structure of SRP-GTPases is well conserved between bacteria and archaea, which indicates strong similarities in the regulation of the SRP-targeting pathway. Surprisingly, structure comparisons identified a homodimeric ATP-binding protein as the closest relative. A heterodimer model for the SRP–SR interaction is presented.
2 May 2000, Research Paper, *Structure*.

☐ **Crystal structure of the Ffh and EF-G binding sites in the conserved domain IV of *Escherichia coli* 4.5S RNA.**
Luca Jovine, Tobias Hainzl, Chris Oubridge, William G Scott, Jade Li, Titia K Sixma, Alan Wonacott, Tadeusz Skarzynski and Kiyoshi Nagai (2000). *Structure* **8**, 527–540.

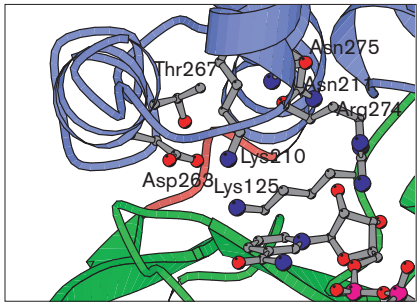
Bacterial signal recognition particle (SRP), consisting of 4.5S RNA and Ffh protein, plays an essential role in targeting signal-peptide-containing proteins to the secretory apparatus in the cell membrane. The 4.5S RNA increases the affinity of Ffh for signal peptides and is essential for the interaction between SRP and its receptor, protein FtsY. The 4.5S RNA also interacts with elongation factor G (EF-G) in the ribosome and this interaction is required for efficient translation. The crystal structure of a 4.5S RNA fragment containing binding sites for both Ffh and EF-G has been determined. The structure can be



regarded as two double helical rods hinged by the asymmetric loop that protrudes from one strand. The decanucleotide structure in the 4.5S RNA and the ribosomal protein L11–RNA complex crystals suggests how 4.5S RNA and 23S rRNA might interact with EF-G and function in translating ribosomes.
2 May 2000, Research Paper, *Structure*.

☐ **A potential target enzyme for trypanocidal drugs revealed by the crystal structure of NAD-dependent glycerol-3-phosphate dehydrogenase from *Leishmania mexicana*.**
Stephen Suresh, Stewart Turley, Fred R Opperdoes, Paul AM Michels and Wim GJ Hol (2000). *Structure* **8**, 541–552.

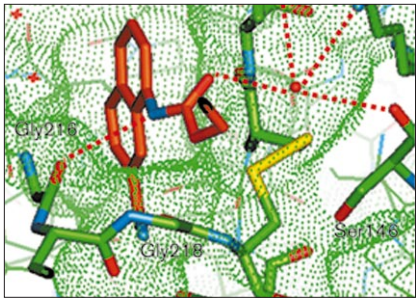
NAD-dependent glycerol-3-phosphate dehydrogenase (GPDH) catalyzes the interconversion of dihydroxyacetone phosphate and l-glycerol-3-phosphate. The enzyme has been characterized and cloned from a number of sources, but no three-dimensional structure has been determined for this enzyme. Although the use of this enzyme as a drug target against *Leishmania mexicana* is yet to be established, the critical role played by GPDH in the long slender bloodstream form of the related kinetoplastid *Trypanosoma brucei* makes it a viable drug target against sleeping sickness. The crystal structure of apo GPDH from *L. mexicana* was determined and used to solve the holo structure in complex with NADH. It is the first



structure of this enzyme from any source and, in view of the sequence identity of 63%, is a valid model for the *T. brucei* enzyme. The differences between the human and trypanosomal enzymes are extensive, with only 29% sequence identity between the parasite and host enzyme, and support the feasibility of exploiting the NADH-binding site to develop selective inhibitors against trypanosomal GPDH. The structure also offers a plausible explanation for the inhibition of the *T. brucei* enzyme by melarsen oxide, the active form of the trypanocidal drugs melarsoprol and cymelarsan.
3 May 2000, Research Paper, *Structure*.

☐ **Structure-directed discovery of potent non-peptidic inhibitors of human urokinase that access a novel binding subsite.**
Vicki L Nienaber, Donald Davidson, Rohinton Edalji, Vincent L Giranda, Vered Klinghofer, Jack Henkin, Peter Magdalinos, Robert Mantei, Sean Merrick, Jean M Severin, Richard A Smith, Kent Stewart, Karl Walter, Jieyi Wang, Michael Wendt, Moshe Weitzberg, Xumiao Zhao and Todd Rockway (2000). *Structure* **8**, 553–563.

Human urokinase-type plasminogen activator has been implicated in the regulation and control of basement membrane and interstitial protein degradation. Because of its role in tissue remodeling, urokinase is a central player in the disease progression of cancer, making it an attractive target for the design of an anticancer clinical agent. Few urokinase inhibitors have been described, which suggests that discovery of such a compound is in the early stages. A new human urokinase crystal form amenable to structure-based drug design has been used to discover potent urokinase inhibitors. On the basis of crystallographic data, 2-naphthamidine was chosen as the lead scaffold for structure-directed optimization. This co-crystal structure shows the compound binding at the primary specificity pocket



of the trypsin-like protease and at a novel binding subsite accessible from the 8-position of 2-napthamidine. This novel subsite was characterized and used to design two compounds with very different 8-substituents that inhibit urokinase with K_i values of 30–40 nM. Utilization of a novel subsite yielded two potent urokinase inhibitors even though this site has not been widely used in inhibitor optimization with other trypsin-like proteases, such as those reported for thrombin or factor Xa. The extensive binding pockets present at the substrate-binding groove of these other proteins are blocked by unique insertion loops in urokinase, thus necessitating the utilization of additional binding subsites. Successful implementation of this strategy and characterization of the novel site provides a significant step towards the discovery of an anticancer agent.

3 May 2000, Research Paper, *Structure*.