Elsewhere in Biology

Chosen and summarized by the staff of *Chemistry & Biology*

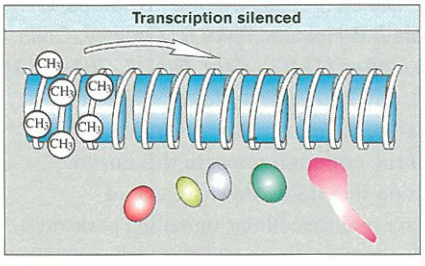
A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chemistry & Biology April 1997, **4**:323–327

 ISSN 1074-5521

☐ **DNA methylation directs a time-dependent repression of transcription initiation.** Stefan U Kass, Nicoletta Landsberger and Alan P Wolffe (1997). *Curr. Biol.* **7**, 157–165.

The regulation of DNA methylation is required for differential expression of imprinted genes during vertebrate development. Studies that monitored the activity of the Herpes simplex virus (HSV) thymidine kinase (*tk*) gene after injection into rodent cells have suggested that assembly of chromatin influences the methylation-dependent repression of gene activity. The authors examine the mechanism of methylation-dependent HSV *tk* gene regulation by direct determination of nucleoprotein organization during the establishment of a transcriptionally silenced state after the microinjection of templates with defined methylation states into *Xenopus* oocyte nuclei. The transcriptional silencing that was conferred by a methylated DNA segment was not immediate, because methylated templates were initially assembled into active transcription complexes. The eventual loss of DNase I hypersensitive sites and inhibition of transcription at the HSV *tk* promoter only occurred after several hours. Flanking methylated vector DNA silenced the adjacent unmethylated HSV *tk* promoter, indicative of a dominant transmissible repression originating from a center of methylation. The resulting repressive nucleoprotein structure silenced transcription in the presence of activators that are able to overcome repression of transcription by nucleosomes. Silencing of transcription by DNA methylation is therefore achieved at the level of transcription initiation and involves the removal of transcriptional machinery from active templates. This transcriptional repression can occur by indirect mechanisms involving the time-dependent assembly of repressive nucleoprotein complexes, which are able to inhibit transcription more effectively than nucleosomes alone.



3 February 1997*, Research Paper, *Current Biology*

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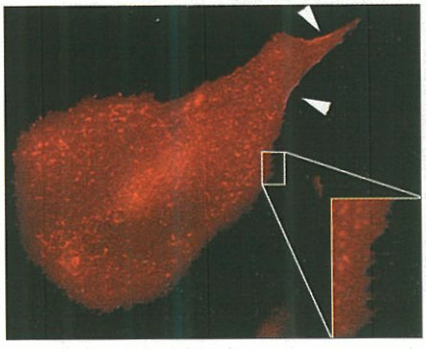
☐ **Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization.** William C Colley, Tsung-Chang Sung, Richard Roll, John Jenco, Scott M Hammond, Yelena Altshuller, Dafna Bar-Sagi, Andrew J Morris and Michael A Frohman (1997). *Curr. Biol.* **7**, 191–201.

The activation of phospholipase D (PLD) is an important but poorly understood component of receptor-mediated signal transduction responses and regulated secretion. The authors have previously reported the cloning of the human gene encoding PLD1; this enzyme has low basal activity and is activated by protein kinase C and the small GTP-binding proteins, ADP-ribosylation factor (ARF), Rho, Rac and Cdc42. Biochemical and cell biological studies suggest, however, that additional and distinct PLD activities exist in cells. Sung *et al.* have cloned the gene for a second PLD family member and characterized the protein product, which appears to be regulated differently from PLD1: PLD2 is constitutively active and may be modulated *in vivo* by inhibition. Unexpectedly, PLD2 localizes primarily to the plasma membrane, in contrast to PLD1 which localizes solely to peri-nuclear regions, where PLD activity has been shown to promote ARF-mediated coated-vesicle formation. PLD2 provokes cortical reorganization and undergoes redistribution in serum-stimulated cells, suggesting that it may be involved in signal-induced cytoskeletal regulation and/or endocytosis. These findings suggest that regulated secretion and morphological reorganization, the two most frequently proposed biological roles for PLD, are likely to be effected separately by PLD1 and PLD2.

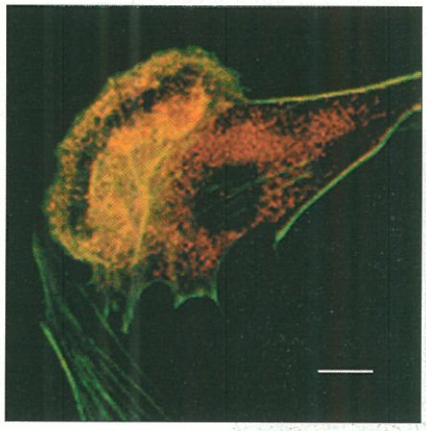


18 February 1997, Research Paper, *Current Biology*

☐ **Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells.** Mary Ann Sells, Ulla G Knaus, Shubha Bagrodia, Diane M Ambrose, Gary M Bokoch and Jonathan Chernoff (1997). *Curr. Biol.* **7**, 202–210.

The Rho family GTPases Cdc42, Rac1 and RhoA regulate the reorganization of the actin cytoskeleton induced by extracellular signals such as growth factors. In mammalian cells, Cdc42 regulates the formation of filopodia, Rac regulates lamellipodia formation and membrane ruffling, and RhoA regulates the formation of stress fibers. Recently, the serine/threonine protein kinase Pak was isolated by virtue of its

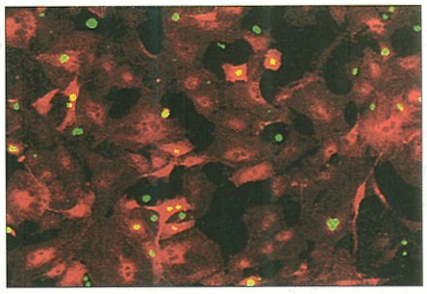
ability to bind to activated Cdc42 and Rac1. Upon binding, Pak autophosphorylates, thereby increasing its catalytic activity towards exogenous substrates. This kinase is therefore a candidate effector for the changes in cell shape induced by growth factors. The authors report that the microinjection of activated Pak1 protein into quiescent Swiss 3T3 cells induces the rapid formation of polarized filopodia and membrane ruffles. The prolonged overexpression of Pak1 amino-terminal mutants that are unable to bind Cdc42 or Rac1 results in the accumulation of filamentous actin in large, polarized membrane ruffles and the formation of vinculin-containing focal complexes within these structures. This phenotype resembles that seen in motile fibroblasts. The amino-terminal Pak1 mutant displays enhanced binding to the adaptor protein Nck, which contains three Src-homology 3 (SH3) domains. Mutation of a proline residue within a conserved SH3-binding region at the amino terminus of Pak1 interferes with SH3-protein binding and alters the effects of Pak1 on the cytoskeleton. These results indicate that Pak1, acting through a protein that contains an SH3 domain, regulates the structure of the actin cytoskeleton in mammalian cells, and may serve as an effector for Cdc42 and/or Rac1 in promoting cell motility.



18 February 1997, Research Paper, *Current Biology*

☐ **Ras signalling is required for inactivation of the tumour suppressor pRb cell-cycle control protein.** Sibylle Mittnacht, Hugh Paterson, Michael F Olson and Christopher J Marshall (1997). *Curr. Biol.* **7**, 219–221.

Ras proteins act as molecular switches, responding to signals by entering the active GTP-bound, rather than the inactive GDP-bound, state. Ras signalling has been shown to be required for growth factors to stimulate DNA synthesis, but the link between Ras and the cell-cycle machinery has not been clear. Regulation of the phosphorylation state of the retinoblastoma protein (pRb), the product of the tumour suppressor gene *Rb*, is a key event in the progression of cells from G1 phase into S phase. In growth-arrested or early G1 cells, pRb is hypophosphorylated and binds to transcription factors of the E2F family. These pRb–E2F complexes act to suppress gene transcription required for entry into DNA synthesis either by preventing E2F from stimulating transcription or by actively repressing transcription. During G1, cyclin-dependent kinases (CDKs) become activated and phosphorylate pRb at multiple sites, leading to the dissolution of pRb–E2F complexes and activation of gene transcription.

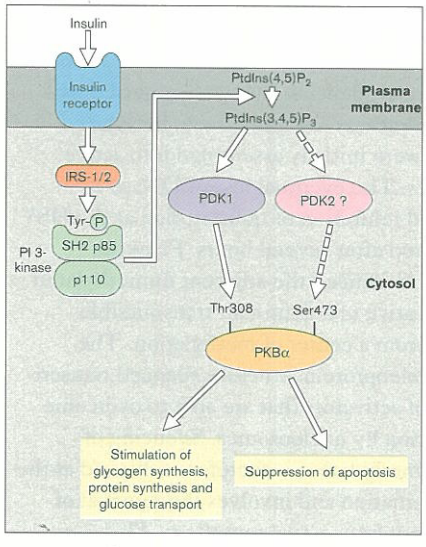


The authors have tested the hypothesis that Ras signalling is required for the inactivation of pRb. A neutralizing antibody directed against p21Ras was microinjected into cells derived from mutant mouse embryos that lack Rb or CDK inhibitors (CDKIs). Cells without pRb or the p16 CDKI were partially resistant to the inhibitory effects of the anti-Ras antibody. DNA synthesis in some tumour cell lines was completely resistant to the anti-Ras injection. The observations indicate that p21Ras is required for pRb inactivation but also has other functions in cell-cycle progression.

1 March 1997, Research Paper, *Current Biology*

☐ **Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα.** Dario R Alessi, Stephen R James, C Peter Downes, Andrew B Holmes, Piers RJ Gaffney, Colin B Reese and Philip Cohen (1997). *Curr. Biol.* **7**, 261–269.

Protein kinase B (PKB), also known as c-Akt, is activated rapidly when mammalian cells are stimulated with insulin and growth factors. Much of the current interest in this enzyme stems from the observation that it lies 'downstream' of phosphoinositide 3-kinase on intracellular signalling pathways.

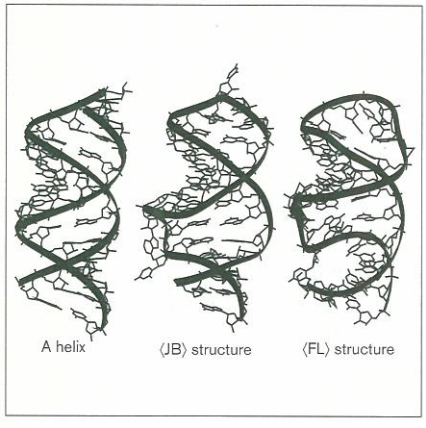


The authors recently showed that insulin or insulin-like growth factor 1 induce the phosphorylation of PKB at two residues, Thr308 and Ser473. The phosphorylation of both residues is required for maximal activation of PKB, but the kinases that phosphorylate PKB have been unknown. The authors have purified a protein kinase from rabbit skeletal muscle extracts which phosphorylates PKBα at Thr308 and increases its activity over 30-fold. The kinase was tested in the presence of several inositol phospholipids and found that only low micromolar concentrations of the D enantiomers of either phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P_3) or PtdIns(3,4)P_2 were effective in potently activating the kinase, which has been named PtdIns(3,4,5)P_3-dependent protein kinase-1 (PDK1). None of the inositol phospholipids tested

activated or inhibited PKBα or induced its phosphorylation under the conditions used. PDK1 activity was not affected by wortmannin, indicating that it is not likely to be a member of the phosphoinositide 3-kinase family. PDK1 is likely to be one of the protein kinases that mediate the activation of PKB by insulin and growth factors. PDK1 may, therefore, be very important in mediating many of the actions of the second messenger(s) PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2.
19 March 1997, Research Paper, *Current Biology*

☐ **Predicting RNA structures: the model of the RNA element binding Rev meets the NMR structure.** Fabrice Leclerc, Jayashree Srinivasan and Robert Cedergren (1997). *Fold. Des.* **2**, 141–147.

How accurate are the predictions of RNA three-dimensional structures? Assessing this accuracy requires the detailed comparison of the prediction with the experimentally determined structure. Previously, sequence variation in RNA aptamers that bind the Rev protein has been used to infer a three-dimensional model of the Rev-binding element RNA. Although much of this model was substantiated by subsequent experimental data, its validity remained to be determined by confronting it with the structure determined by NMR spectroscopy. A series of different criteria such as geometric parameters (root mean square deviation, interproton distances, torsion angles and puckering), helicoidal parameters (base pairing and base stacking) and stability considerations (conformational energies) have now been evaluated to identify common and distinguishing structural characteristics of the model and the NMR structure. The detailed comparison of the two structures reveals striking structural similarities at both the global and the local level that validate the RNA modeling approach used by the authors. Analysis of the structural differences and the precision of the model suggest that the limitations of the method are related to the amount of structural information available for modeling.



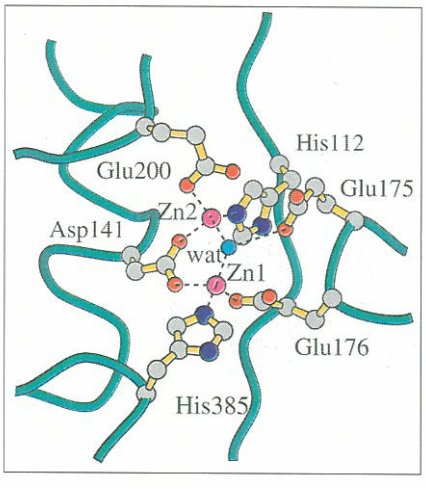
7 March 1997, Research Paper, *Folding & Design*

☐ **The future of protein secondary structure prediction accuracy.** Dmitrij Frishman and Patrick Argos (1997). *Fold. Des.* **2**, 159–162.

The accuracy of secondary structure prediction for a protein from a knowledge of its sequence has been significantly improved by ~7% to the 70–75% range by inclusion of information residing in sequences similar to the query sequence. The scientific literature has been inconsistent, if not negative, regarding chances for further improvement from the vast knowledge to be provided by genome sequencing efforts. By applying a prediction technique that is particularly sensitive to added sequence information to a standard set of query sequences with related primary structures taken from chronologically successive releases of the SWISS-PROT database, the authors show that prediction accuracy can be expected to reach 80–85% with a large tenfold increase in present sequence knowledge. Even with present prediction approaches, improvement in prediction accuracy can still be expected, albeit limited to no more than 10%.
11 March 1997, Research Paper, *Folding & Design*

☐ **Crystal structure of carboxypeptidase G2, a bacterial enzyme with applications in cancer therapy.** Siân Rowsell, Richard A Pauptit, Alec D Tucker, Roger G Melton, David M Blow and Peter Brick (1997). *Structure* **5**, 337–347.

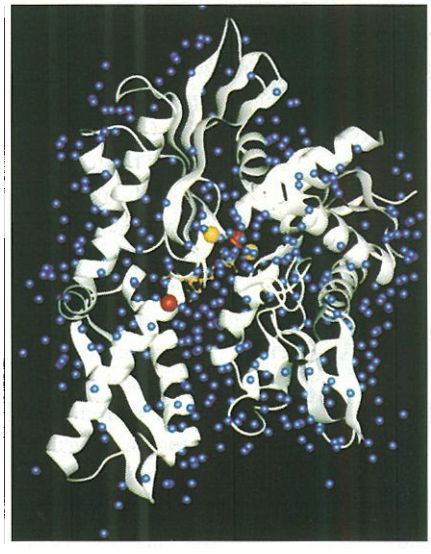
Carboxypeptidase G enzymes hydrolyze the carboxy-terminal glutamate moiety from folic acid and its analogs, such as methotrexate. Carboxypeptidase G_2 (CPG_2) is a dimeric zinc-dependent exopeptidase produced by *Pseudomonas* sp. strain RS-16. CPG_2 has applications in cancer therapy: following its administration as an immunoconjugate, in which CPG_2 is linked to an antibody to a tumour-specific antigen, it can enzymatically convert subsequently administered inactive prodrugs to cytotoxic drugs selectively at the tumour site. The crystal structure of CPG_2 was determined at 2.5 Å resolution. Each subunit of the molecular dimer consists of a larger catalytic domain containing two zinc ions at the active site and a separate, smaller domain that forms the dimer interface. The CPG_2 catalytic domain has structural homology with other zinc-dependent exopeptidases. The closest structural homology is with the aminopeptidase from *Aeromonas proteolytica*, where the similarity includes superposable zinc ligands but does not extend to the rest of the active-site residues, consistent with the different substrate specificities. The mechanism of peptide cleavage is likely to be very similar in these two enzymes. Most anticancer drugs have inadequate selectivity between normal and tumor cells. Therefore, the design of tumor-targeted drugs based on the structure of CPG_2 may allow the design of methotrexate-degrading enzymes with altered specificity.



15 March 1997, Research Paper, *Structure*

☐ **Human Hsp70 molecular chaperone binds two calcium ions within the ATPase domain.** M Sriram, J Osipiuk, BC Freeman, RI Morimoto and A Joachimiak (1997). *Structure* **5**, 403–414.

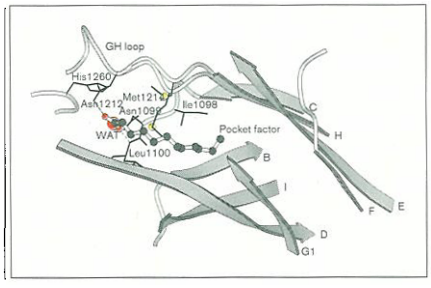
The 70 kDa heat shock protein (Hsp70) is a major molecular chaperone, promoting protein folding and participating in many cellular functions, in eukaryotes including humans. Hsp70 proteins are composed of two major domains. The amino-terminal ATPase domain binds to and hydrolyzes ATP, whereas the carboxy-terminal domain is required for polypeptide binding. Cooperation of both domains is needed for protein folding. The molecular chaperone activity and conformational switch are functionally linked with ATP hydrolysis. A high-resolution structure of the ATPase domain is required to provide an understanding of the mechanism of ATP hydrolysis and how it affects communication between carboxy-terminal and amino-terminal domains. The crystal structure of the human Hsp70 ATPase domain (hATPase) has been determined and refined at 1.84 Å, using synchrotron radiation at 120K. The structural similarity of hATPase and bovine ATPase and the sequence similarity within the Hsp70 chaperone family suggest a universal mechanism of ATP hydrolysis among all Hsp70 molecular chaperones. Two calcium ions have been found in the hATPase structure. One corresponds to the magnesium site in bATPase and appears to be important for ATP hydrolysis and *in vitro* phosphorylation. Local changes in protein structure that result from calcium binding may facilitate phosphorylation. A small, but significant, movement of metal ions and sidechains could position catalytically important threonine residues for phosphorylation. The second calcium site represents a new calcium-binding motif that can play a role in the stabilization of protein structure. The authors discuss how the information about catalytic events in the active site could be transmitted to the peptide-binding domain.



15 March 1997, Research Paper, *Structure*

☐ **The refined structure of human rhinovirus 16 at 2.15 Å resolution: implications for the viral life cycle.** Andrea T Hadfield, Wai-ming Lee, Rui Zhao, Marcos A Oliveira, Iwona Minor, Roland R Rueckert and Michael G Rossmann (1997). *Structure* **5**, 427–441.

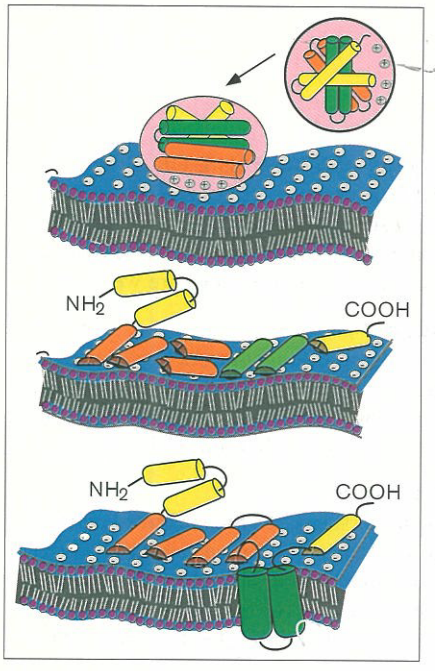
Rhinoviruses belong to the picornavirus family and are small, icosahedral, non-enveloped viruses containing one positive RNA strand. Human rhinovirus 16 (HRV16) belongs to the major receptor group of rhinoviruses, for which the cellular receptor is intercellular adhesion molecule-1. Rhinoviruses can be inhibited by a variety of capsid-binding compounds that inhibit uncoating and, in some cases, attachment. Some of these compounds have been through clinical trials including tests where HRV16 was used as a pathogen. The structure of native HRV16 has been refined to a resolution of 2.15 Å. The structure shows a number of new details relevant to viral cell entry, RNA packaging and properties of a hydrophobic pocket in viral coat proteins (VP1) that is known to bind antiviral compounds. The observation of a partially occupied hydrophobic pocket in HRV16 forms a missing link between HRV14, which is always observed with no pocket factor in the native form, and rhinovirus 1A and other picornaviruses which contain pocket factors. The pocket factor molecules probably regulate viral entry, uncoating and assembly. RNA bases stack against both a tryptophan and a phenylalanine residue on the internal surface of the viral capsid. Non-conservative mutations of the conserved tryptophan results in a non-viable virus. Picornavirus assembly is known to proceed via pentamers, therefore, the interaction of RNA with the conserved tryptophan residues across twofold axes between the pentamers may play a role in picornavirus assembly. The positioning of a cation on the icosahedral fivefold axes and the structure of the amino termini of VP4 and VP1 around these axes suggest a mechanism for the uncoating of rhinoviruses.



15 March 1997, Research Paper, *Structure*

☐ **A mechanism for toxin insertion into membranes is suggested by the crystal structure of the channel-forming domain of colicin.** Patricia Elkins, Amy Bunker, William A Cramer and Cynthia V Stauffacher (1997). *Structure* **5**, 443–458.

Channel-forming colicins, including colicin E1, are a subfamily of bacteriocins secreted by bacteria to kill sensitive strains of *Escherichia coli*. The toxin-like colicin E1 molecule must undergo a substantial structural transition from a soluble state, in which it binds the target cell, to a membrane-bound state. The mechanism by which colicins are imported into membranes is believed to have similarities to the import mechanisms of toxins such as diphtheria toxin and tetanus toxin. Therefore, details of the structural changes that accompany this conversion may be directly applicable to other channel-forming toxins, as well as to the mechanism by which proteins insert into or cross membranes. The structure of the 190-residue channel-forming domain of colicin E1 in its soluble form has been solved at 2.5 Å resolution. On the basis of the structure of the colicin E1 channel-forming domain, its



comparison with the structure of the colicin A domain and the known requirement for initial electrostatic and subsequent hydrophobic interactions, molecular details of the docking, unfolding and insertion of the channel-forming domain into the membrane are proposed. The model for docking and initial interaction with the membrane positions the hydrophobic hairpin 'anchor' approximately parallel to the membrane surface. Hydrophobic interactions in the docking layer may then be displaced by interactions with the membrane, spreading the helices on the surface and exposing the hydrophobic hairpin for insertion into the membrane.
15 March 1997, Research Paper, *Structure*