Elsewhere in Biology

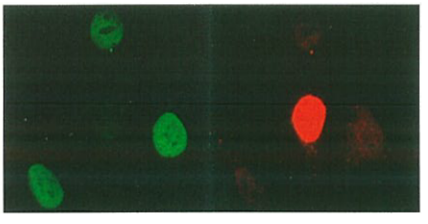
Chosen and summarized by the staff of *Chemistry & Biology*

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

Chemistry & Biology June 1997, **4**:475–479

☐ **Cell growth inhibition by the Mad/Max complex through recruitment of histone deacetylase activity.** Anette Sommer, Stefanie Hilfenhaus, Annette Menkel, Elisabeth Kremmer, Christian Seiser, Peter Loidl and Bernhard Lüscher (1997). *Curr. Biol.* **7**, 357–365.

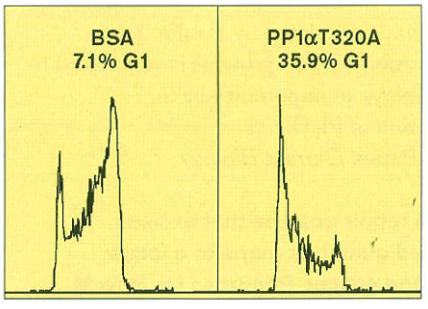
Chromatin structure is a crucial determinant in the regulation of gene expression; both the positioning and properties of nucleosomes influence promoter-specific transcription. The acetylation of core histones has been suggested to alter the properties of nucleosomes and affect the access of DNA-binding transcriptional regulators to promoters. A recently identified mammalian histone deacetylase (HD1) shows homology to the yeast Rpd3 protein, which together with Sin3 affects the transcription of several genes. Mammalian Sin3 proteins interact with the Mad components of the Myc/Max/Mad network of cell growth regulators. Mad/Max complexes may recruit mammalian Rpd3-like enzymes. The authors report the identification of a tetrameric complex composed of Max, Mad1, Sin3B and HD1. The histone deacetylase activity of this complex can be blocked by trichostatin A and sodium butyrate, which inhibit S-phase progression of cells in culture. The recruitment of a histone deacetylase by sequence-specific DNA-binding proteins provides a mechanism by which the state of acetylation of histones in nucleosomes and hence the activity of specific promoters can be influenced.



1 May 1997, Research Paper, *Current Biology**

☐ **Constitutively active protein phosphatase 1α causes Rb-dependent G1 arrest in human cancer cells.** Norbert Berndt, Mariam Dohadwala and Cathy WY Liu (1997). *Curr. Biol.* **7**, 375–396.

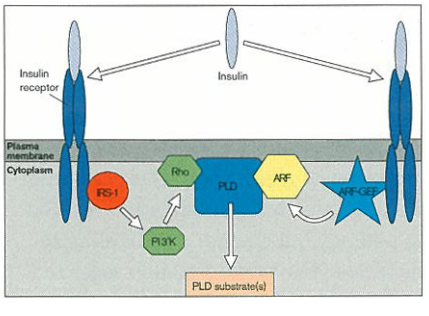
The retinoblastoma protein (Rb) needs to be phosphorylated by cyclin-dependent kinases (CDKs) before mammalian cells can enter the DNA replication (S) phase of the cell cycle. Protein phosphatase 1 (PP1) activates Rb and is itself a target for inhibitory phosphorylation by CDKs *in vitro*. Using electrotransfer of recombinant protein into Rb-positive and Rb-negative cells, the authors have compared the effects of a wild-type PP1 catalytic subunit, PP1α, and a constitutively active mutant of this subunit (PP1αT320A) on G1 progression, proliferation rates, and cell viability. In Rb-positive cells, PP1αT320A, but not PP1α, caused cell cycle arrest in late G1, which was associated with a lack of Rb phosphorylation. In Rb-negative cells, neither phosphatase caused any change in cell cycle progression. Excess PP1α caused increased cell death in both Rb-positive and Rb-negative cells, however. The difference between the effects of wild-type and mutant forms of PP1α suggests that PP1α has the potential to arrest cell growth in G1, unless it is inactivated by periodic phosphorylation at Thr320, presumably by CDKs that regulate passage through the G1–S cell cycle transition. The results suggest that PP1α requires functional Rb to induce growth arrest, and that possibly another pool of PP1α induces cell death. This identifies PP1 as a potential target for therapeutic anti-proliferative strategies.



8 May 1997, Research Paper, *Current Biology*

☐ **ARF proteins mediate insulin-dependent activation of phospholipase D.** Kuntala Shom, Chandrasekaran Vasudevan and Guillermo Romero (1997). *Curr. Biol.* **7**, 387–396.

ADP-ribosylation factors (ARFs) have been shown to activate phospholipase D (PLD) in the presence of GTP analogs *in vitro*. PLD is modulated by a number of growth factors, including insulin. The authors have tested the hypothesis that ARF proteins are involved specifically in insulin-induced activation of PLD. They found that in membranes obtained from HIRcB cells, a cell line derived from Rat-1 fibroblasts that overexpresses normal human insulin receptors, binding of the GTP analog GTPγS to purified bovine or recombinant ARF was enhanced in the presence of insulin. Membranes obtained



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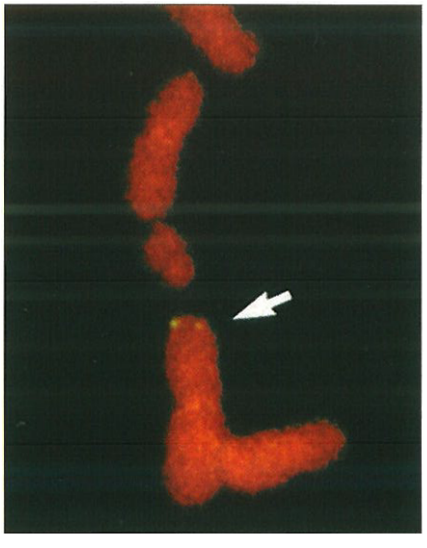
from cells that overexpressed a mutated nonfunctional insulin receptor failed to stimulate ARF activation. In permeabilized cells, insulin promoted the association of ARF proteins with membranes in the presence of GTPγS and activated PLD by a process that required GTPγS and ARF. Azido-γ[^{32}P]-GTP labelling of immunoprecipitated receptors revealed the presence of a unique 19 kDa band; ARF proteins are approximately this size. ARF proteins co-immunoprecipitated with the insulin receptor but this co-immunoprecipitated was inhibited by guanine nucleotides and stimulated by insulin. It was concluded that the activation of ARF proteins is stimulated by insulin and this process plays an important role in insulin-mediated regulation of PLD.
8 May 1997, Research Paper, *Current Biology*

☐ **A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer.** Rongzhen Lu, Huw M Nash and Gregory L Verdine (1997). *Curr. Biol.* **7**, 397–407.

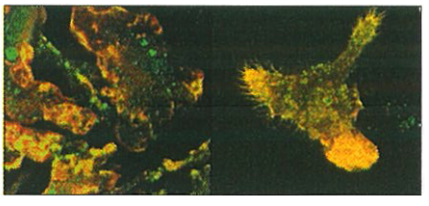
Guanine residues in the genome are vulnerable to attack by free radicals and reactive oxygen species. A major lesion thus produced, 8-oxoguanine (^{O}G), causes mutations by mispairing with adenine during replication. In bacteria and budding yeast, ^{O}G is removed from the genome by base-excision DNA repair (BER) enzymes. Although ^{O}G is known to be produced in and cleansed from mammalian genomes, the enzymes responsible for ^{O}G repair in these cells have remained elusive. The authors report the cloning and biochemical characterization of mammalian BER enzymes that specifically target ^{O}G residues in DNA. These DNA glycosylases, hOgg1 (human) and mOgg1 (murine), are homologous to each other and to yeast Ogg1. They also contain an active site motif that is characteristic of a superfamily of BER proteins. Both hOgg1 and mOgg1 exhibit exquisite selectivity for the base opposite ^{O}G in DNA, operating with high efficiency only on ^{O}G base-paired to cytosine. The proteins operate through a classical glycosylase/lyase catalytic mechanism; mutation of a catalytically essential lysine residue results in loss of catalytic potency but retention of binding to ^{O}G-containing oligonucleotides. The *hOGG1* gene is localized on the short arm of chromosome 3 (3p25/26) in a region commonly deleted in cancers. These results conclusively establish the existence and identity of an ^{O}G DNA glycosylase/lyase in human and murine cells. The observation that at least one allele of *hOGG1* is commonly deleted in cancer cells suggests that such cells may possess a reduced capacity to counter the mutagenic effects of reactive oxygen species, which could increase their overall genomic instability.



12 May 1997, Research Paper, *Current Biology*

☐ **CD45 regulates Src family member kinase activity associated with macrophage integrin-mediated adhesion.** Tamara Roach, Suzanne Slater, Michael Koval, Lynn White, Ellen Cahir McFarland, Meinoshin Okumura, Matthew Thomas and Eric Brown (1997). *Curr. Biol.* **7**, 408–417.

Adhesion of leukocytes to the extracellular matrix and to other cells is mediated by members of the integrin family of adhesion molecules. Tyrosine kinases from the Src kinase family are activated upon integrin-mediated adhesion. In lymphocytes, CD45 is a leukocyte-specific transmembrane protein tyrosine phosphatase that activates Src family kinases associated with B-cell and T-cell antigen receptor signaling by constitutive dephosphorylation of the inhibitory carboxy-terminal tyrosine phosphorylation site. In this paper, the authors show that CD45 is also involved in downregulating the kinase activity of Src family members during integrin-mediated adhesion in macrophages. They found that CD45 co-localized with β2 integrin and the Src family kinase p53/56^{lyn} to adhesion sites in bone-marrow-derived macrophages. CD45 dephosphorylates the Src kinases at a specific site, thereby reducing their activity; this modification is implicated in the control of integrin-mediated adhesion, as macrophages from CD45-deficient mice are unable to maintain integrin-mediated adhesion. These studies demonstrate that CD45 regulates Src family kinases and is required to maintain macrophage adhesion.

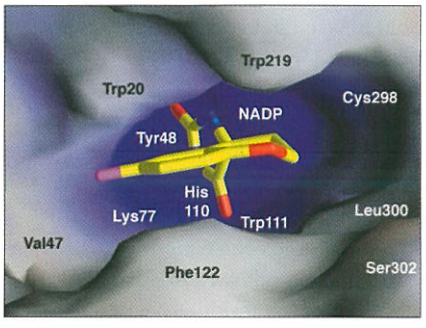


20 May 1997, Research Paper, *Current Biology*

☐ **A 'specificity' pocket inferred from the crystal structures of the complexes of aldose reductase with the pharmaceutically important inhibitors tolrestat and sorbinil.** A Urzhumtsev, F Tête-Favier, A Mitschler, J Barbanton, P Barth, L Urzhumtseva, J-F Biellmann, AD Podjarny and D Moras (1997). *Structure* **5**, 601–612.

Aldose reductase (AR) is an NADPH-dependent enzyme implicated in long-term diabetic complications. Buried at the bottom of a deep hydrophobic cleft, the NADPH coenzyme is surrounded by the conserved hydrophilic residues of the AR active site. The crystal structures of the porcine AR holoenzyme and its complexes with the inhibitors tolrestat and sorbinil have been solved; these structures are important as tolrestat and sorbinil are, pharmaceutically, the most well-studied AR inhibitors. The active site of the holoenzyme was

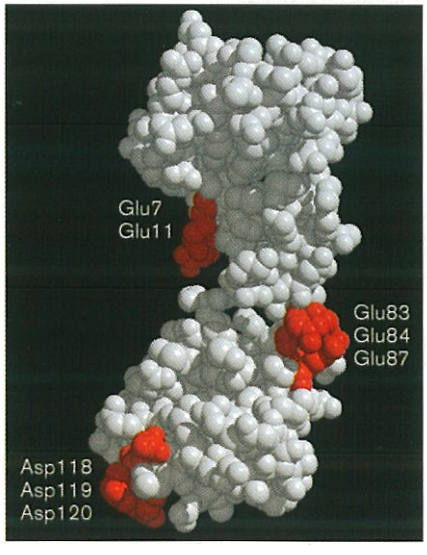
analyzed, and binding of the inhibitors was found to involve two contact zones in the active site: first, a recognition region for hydrogen-bond acceptors near the coenzyme, with three centers, including the anionic site; and second, a hydrophobic contact zone in the active-site cleft, which in the case of tolrestat includes the specificity pocket. The conformational change leading to the opening of the specificity pocket upon tolrestat binding is different to the one seen upon zopolrestat binding; this pocket binds inhibitors that are more effective against AR than against aldehyde reductase. Thus the active site of AR adapts itself to bind tightly to different inhibitors; this happens both upon binding to the inhibitor's hydrophilic heads, and at the hydrophobic and specificity pockets of AR, which can change their shape through different conformational changes of the same residues. This flexibility could explain the large variety of possible substrates of AR.



15 May 1997, Research Paper, *Structure*

☐ **The structure of a calmodulin mutant with a deletion in the central helix: implications for molecular recognition and protein binding.** Lydia Tabernero, Denise A Taylor, Ronald J Chandross, Mark FA VanBerkum, Anthony R Means, Florante A Quiocho and John S Sack (1997). *Structure* **5**, 613–622.

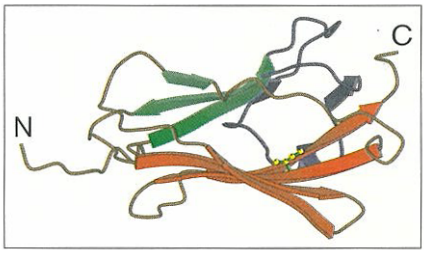
Calmodulin (CaM) is the major calcium-dependent regulator of a large variety of important intracellular processes in eukaryotes. The structure of CaM consists of two globular calcium-binding domains joined by a central 28-residue α helix. This linker helix is thought to act as a flexible tether and is crucial for the binding and activation of numerous target proteins. Although it is not known exactly how alterations of the central helix modulate the molecular recognition mechanism, the relative orientation of the globular domains seems to be of great importance. The structural analysis of central helix mutants may contribute to a better understanding of how changes in the conformation of CaM effect its function. The paper describes the determination of the crystal structure (at 1.8 Å resolution) of a calcium-saturated mutant of chicken CaM that lacks two residues in the central helix (Thr79 and Asp80). The main consequence of the mutation is a change in the relative orientation of the two globular calcium-binding domains, causing the hydrophobic patches in these domains to be closer and much less accessible to interact with the target enzymes. This may explain why this mutant of CaM shows a marked decrease in its ability to activate some enzymes while the mutation has little or no effect on its ability to activate others.



15 May 1997, Research Paper, *Structure*

☐ **A modulator of rho family G proteins, rhoGDI, binds these G proteins via an immunoglobulin-like domain and a flexible N-terminal arm.** Nicholas H Keep, Maria Barnes, Igor Barsukov, Ramin Badii, Lu-Yun Lian, Anthony W Segal, Peter CE Moody and Gordon CK Roberts (1997). *Structure* **5**, 623–633.

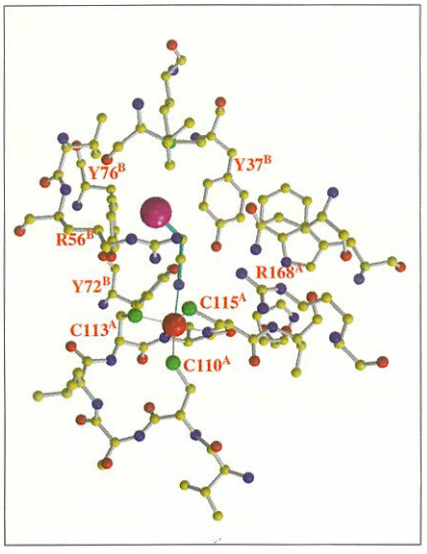
The rho family of small G proteins, including rho, rac and cdc42, are involved in many cellular processes, including cell transformation by ras and the organization of the actin cytoskeleton. In addition, rac is involved in the regulation of phagocyte NADPH oxidase. Guanine nucleotide dissociation inhibitors (GDIs) of the rhoGDI family bind to these G proteins and regulate their activity by preventing nucleotide dissociation and by controlling their interaction with membranes. The paper describes the structure of rhoGDI, determined by a combination of X-ray crystallography and nuclear magnetic resonance spectroscopy. The rhoGDI structure is notable for the existence of both a structured and a highly flexible domain, both of which appear to be required for the interaction with rac. The immunoglobulin-like fold of the structured domain is unusual for a cytoplasmic protein. The presence of equivalent cleavage sites in rhoGDI and the closely related D4/Ly-GDI (rhoGDI-2) suggest that proteolytic cleavage between the flexible and structured regions of rhoGDI may have a role in the regulation of the activity of members of this family. There is no detectable similarity between the structure of rhoGDI and the recently reported structure of rabGDI, which performs the same function as rhoGDI for the rab family of small G proteins.



15 May 1997, Research Paper, *Structure*

☐ **Crystal structure of nitrile hydratase reveals a novel iron centre in a novel fold.** Weijun Huang, Jia Jia, John Cummings, Mark Nelson, Gunter Schneider and Ylva Lindqvist (1997). *Structure* **5**, 691–699.

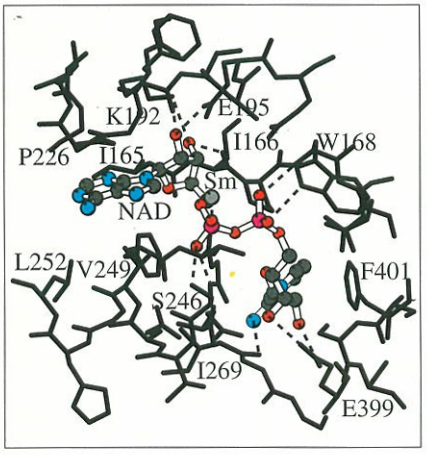
Nitrile hydratases are unusual metalloenzymes that catalyze the hydration of nitriles to their corresponding amides. They



are used as biocatalysts in acrylamide production, one of the few commercial scale bioprocesses, as well as in environmental remediation for the removal of nitriles from waste streams. Nitrile hydratases are composed of two subunits, α and β, and they contain one iron atom per αβ unit. This paper describes the determination of the crystal structure of photoactivated iron-containing nitrile hydratase from *Rhodococcus* species R312 (to 2.65 Å resolution) as a first step in the elucidation of its catalytic mechanism. The nitrile hydratase contains a novel iron centre with a structure not previously observed in proteins; it resembles a hybrid of the iron centres of heme and Fe–S proteins. The low-spin electronic configuration presumably results, in part, from two Fe–amide nitrogen bonds. The structure is consistent with the metal ion having a role as a Lewis acid in the catalytic reaction.
15 May 1997, Research Paper, *Structure*

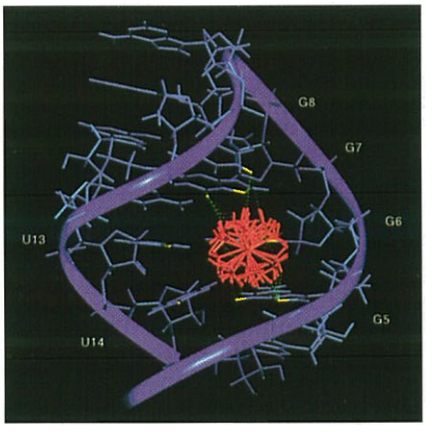
☐ **Structure of mitochondrial aldehyde dehydrogenase: the genetic component of ethanol aversion.** Curtis G Steinmetz, Peiguang Xie, Henry Weiner and Thomas D Hurley (1997). *Structure* **5**, 701–711.

The single genetic factor most strongly correlated with reduced alcohol consumption and incidence of alcoholism is a naturally occurring variant of mitochondrial aldehyde dehydrogenase (ALDH2). This variant contains a glutamate to lysine substitution at position 487 (Glu487→Lys). The Glu487→Lys variant of ALDH2 is found in approximately 50% of the Asian population, and is associated with a phenotypic loss of ALDH2 activity in both heterozygotes and homozygotes. ALDH2-deficient individuals exhibit an averse response to ethanol consumption, which is probably caused by elevated levels of blood acetaldehyde. The prescription drug Antabuse® is widely used in the treatment of alcoholism, and it functions as an aversive agent by inhibiting ALDH2, which leads to increased blood acetaldehyde levels after ethanol consumption, like that seen in ALDH2-deficient individuals. But Antabuse® is a nonspecific alkylating agent and can therefore inhibit virtually any enzyme with an active-site cysteine residue, leading to undesirable side effects. Specific inhibitors of ALDH2 could be used in aversion therapy and would potentially be free of the negative side effects associated with currently available aversive agents. No known health problems have been observed in individuals with the inactive form of the ALDH2 enzyme. The X-ray structure of bovine ALDH2 has been solved to 2.65 Å in its free form and to 2.75 Å in a complex with NAD^+. Although there is a recognizable Rossmann-type fold, the coenzyme-binding region of ALDH2 binds NAD^+ in a manner not seen in other NAD^+-binding enzymes. The positions of the residues near the nicotinamide ring of NAD^+ suggest a chemical mechanism whereby Glu268 functions as a general base through a bound water molecule. The sidechain amide nitrogen of Asn169 and the peptide nitrogen of Cys302 are in position to stabilize the oxyanion present in the tetrahedral transition state prior to hydride transfer. The functional importance of residue Glu487 now appears to be due to indirect interactions of this residue with the substrate-binding site via Arg264 and Arg475.
15 May 1997, Research Paper, *Structure*



☐ **Solution structure of a metal-binding site in the major groove of RNA complexed with cobalt (III) hexammine.** Jeffrey S Kieft and Ignacio Tinoco Jr (1997). *Structure* **5**, 713–721.

Metal ions play two important roles within the structures of RNA: they stabilize the folding of structures and motifs and they take part in active-site chemistry. Despite the importance of these ions, the details of specific metal ion–RNA interactions are poorly understood. Cobalt (III) hexammine is similar in size, shape and binding surface to a magnesium ion and therefore provides a means to determine the interactions of this biologically important ion. Because the amine groups of cobalt (III) hexammine do not exchange with the solvent, as in the case of ligands on fully solvated magnesium, nuclear magnetic resonance can be used to determine the precise location of the metal ion. The paper describes the solution structure of the P5b stem–loop from a group I intron ribozyme bound to a cobalt (III) hexammine ion. The structure presents a picture of how tandem G·U base



pairs bind and position metal ions within the RNA major groove. The binding site is preformed in the absence of metal ions, and presents a negative pocket in the major groove with a variety of hydrogen-bond acceptors. Because G·U base pairs are such a common motif in RNA sequences, it is possible that this metal ion–RNA interaction is critical in forming large complex RNA structures, such as those found in the ribosome and self-splicing introns. This technique, using cobalt (III) hexammine as an analog for hexahydrated magnesium, may be applicable to other RNA sequences. Metal hexammines may prove to be useful general probes for locating RNA–metal ion binding sites in solution.

15 May 1997, Research Paper, *Structure*