Elsewhere in biology

Current Biology Ltd has launched a new journal entitled *Current Opinion in Chemical Biology*, which will be of particular interest to readers of *Chemistry & Biology*. The editors of the journal are Donald Hilvert and Steven V Ley. Volume 2 Issue 2 covers Bio-inorganic chemistry (edited by Andrew J Thomson and Harry B Gray) and is published in April.

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

Chemistry & Biology April 1998, 5:R92–R96

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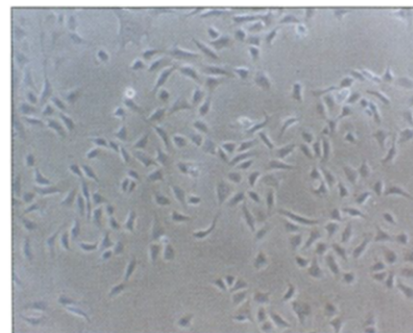
☐ **Overexpression of the transmembrane tyrosinephosphatase LAR activates the caspase pathway and induces apoptosis.**
Liang-Ping Weng, Junying Yuan and Qiang Yu (1998). *Curr. Biol.* **8**, 247–256.

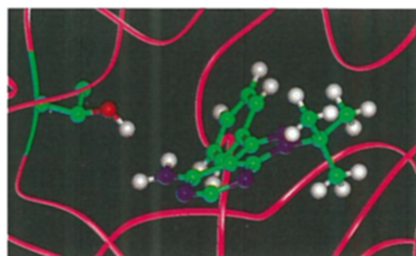
The protein tyrosine phosphatase family comprises transmembrane receptor-like and cytosolic forms. Although the exact biological functions of these enzymes are largely unknown, they are believed to counter-balance the effects of protein tyrosine kinases. The authors previously identified and characterized a mammalian transmembrane protein tyrosine phosphatase, called LAR (leukocyte common antigen related gene), whose expression is often associated with proliferating epithelial cells or epithelial progenitor cells. This study investigates the potential role of LAR in the regulation of cell growth and death in mammals. The authors overexpressed in mammalian cells or in culture either the full-length wild-type LAR or a truncation mutant containing only the extracellular domain of the molecule, and found that whereas the truncated LAR could be readily overexpressed in various cell lines, cells overexpressing the wild-type LAR were negatively selected. Using an inducible expression system, the authors demonstrated that overexpression of the wild-type LAR, but not the truncated LAR, activated the caspase pathway directly and induced p53-independent apoptosis. The data suggest that LAR might regulate cellular signals essential for cell survival. Overproduction of LAR may tilt the balance between the tyrosine phosphorylation and dephosphorylation of



proteins whose activities are critical for cell survival, and therefore lead to cell death. In addition, the observations that overexpression of LAR induces cell death without affecting cell adhesion suggest that LAR may activate the caspase pathway and induce cell death directly. This work is the first example of the involvement of a receptor-like protein tyrosine phosphatase in cell-death control and provides the basis for searching for molecules and mechanisms linking signal transduction by protein tyrosine phosphorylation to the caspase-mediated cell-death pathway.
10 February 1998, Research Paper, *Current Biology*

☐ **Design of allele-specific inhibitors to probe protein kinase signaling.**
Anthony C Bishop, Kavita Shah, Yi Liu, Laurie Witucki, Chi-yun Kung and Kevan M Shokat (1998). *Curr. Biol.* **8**, 257–266.

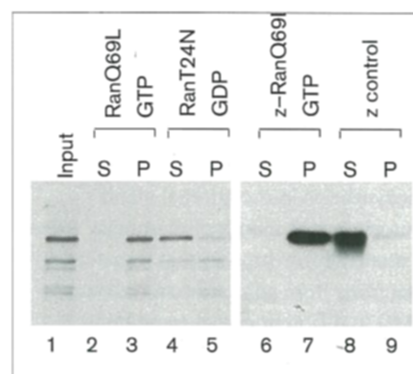
Deconvoluting protein kinase signaling pathways using conventional genetic and biochemical approaches has been difficult because of the overwhelming number of closely related kinases. If



cell-permeable inhibitors of individual kinases could be designed, the role of each kinase could be systematically assessed The authors have devised an approach combining chemistry and genetics to develop the first highly specific cell-permeable inhibitor of the oncogenic tyrosine kinase v-Src. A functionally silent active-site mutation was made in v-Src to distinguish it from all other cellular kinases. A tight-binding cell-permeable inhibitor of this mutant kinase that does not inhibit wild-type kinases was designed and synthesized. *In vitro* and whole-cell assays established the unique specificity of the mutant v-Src-inhibitor pair. The inhibitor reversed cell transformation by the engineered but not the 'wild type' v-Src, establishing that changes in cellular signaling can be attributed to specific inhibition of the engineered kinase. The generality of the method was tested by engineering another tyrosine kinase, Fyn, to contain the corresponding active-site mutation to the one in v-Src. The same compound that inhibited mutant v-Src could also potently inhibit the engineered Fyn kinase. Allele-specific cell-permeable inhibitors of individual Src family kinases can be rapidly developed in an approach that should be applicable to all kinases. This approach will be useful for the deconvolution of kinase-mediated cellular pathways and for validating novel kinases as good targets for drug discovery both *in vitro* and *in vivo*.
11 February 1998, Research Paper, *Current Biology*

☐ **Identification of a nuclear export receptor for tRNA.**
Gert-Jan Arts, Maarten Fornerod and Iain W Mattaj (1998). *Curr. Biol.* **8**, 305–314.

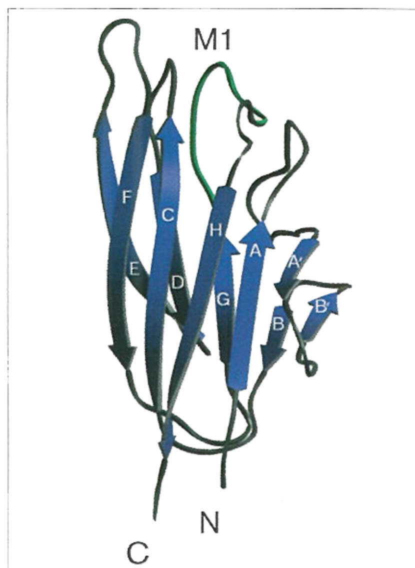
Transport of macromolecules between the nucleus and cytoplasm of eukaryotic cells is mediated by nuclear import and export receptors. The receptors identified to date are members of a



family of Ran GTPase-binding proteins whose founding member is importin-β. Interaction between these receptors and their cargo is regulated by the GTP-bound form of Ran. Export complexes form and import complexes disassemble on binding of RanGTP to the receptor. Yeast Los1p is a member of the importin-β family with a poorly defined role in tRNA production. A human member of the importin-β family that is distantly related to Los1p (21% identity) has been characterized. The protein shuttled between the nucleus and cytoplasm and interacts with tRNA in a RanGTP-dependent manner. Injection of the protein into the nuclei of *Xenopus* oocytes resulted in a specific stimulation of the export of tRNA from the nucleus and in relief of the competitive inhibition of tRNA export caused by the introduction of saturating amounts of nuclear tRNA. The human protein has the functional properties expected of a transport receptor that mediates export of tRNA from the nucleus was named the protein Exportin(tRNA).
11 February 1998, Research Paper, *Current Biology*

☐ **The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor.**
Lawrence Shapiro and Philipp E Scherer (1998). *Curr. Biol* **8**, 335–338.

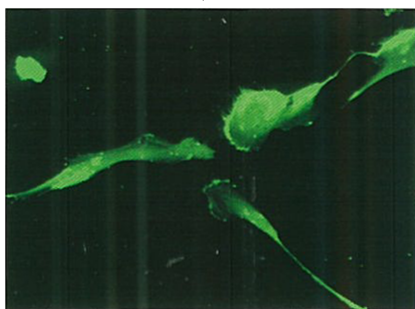
ACRP30 — adipocyte complement-related protein of 30 kDa or AdipoQ — is an abundant serum protein, secreted exclusively from fat cells, that is implicated in energy homeostasis and obesity. ACRP30 is a close homologue of the complement protein C1q, which is involved in the recognition of microbial surfaces and antibody–antigen complexes in the classical pathway of complement. The authors have determined the crystal structure of a homotrimeric fragment from ACRP30. The structure reveals an unexpected homology to the tumor necrosis factor (TNF) family. Identical folding topologies, key residue conservations, and similarity of trimer interfaces and intron positions firmly establish an evolutionary link between the TNF and C1q families. The authors suggest that TNFs — which control many aspects of inflammation, adaptive immunity, apoptosis and energy homeostasis — arose by divergence from a primordial



recognition molecule of the innate immune system. The evolutionary connection between C1q-like proteins and TNFs illuminates the shared functions of these two important groups of proteins.
2 March 1998, Brief Communication, *Current Biology*

☐ **Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts.**
Beth B McConnell, Maria Starborg, Sharon Brookes and Gordon Peters (1998). *Curr. Biol.* **8**, 351–354.

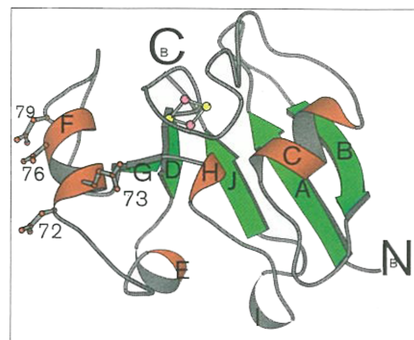
After a limited number of population doublings (PDs), cultures of normal mammalian diploid cells undergo an irreversible growth arrest known as replicative senescence. As well as contributing to cellular ageing, senescence is viewed as an important mechanism of tumour suppression by preventing the emergence of immortal



cell clones. Senescent cells have a number of characteristics that distinguish them from cycling or quiescent cells including elevated levels of two cyclin-dependent kinase (Cdk) inhibitors, $p^{16INK4a}$ and p^{21CIP1}. Here, the authors demonstrate that both of these Cdk inhibitors, as well as other members of their protein families (the INK4 and CIP/KIP families, respectively), induce several facets of the senescent phenotype when ectopically expressed in young human diploid fibroblasts. These include a reduced proliferative capacity, an altered size and shape, the presence of underphosphorylated retinoblastoma protein (pRb), increased expression of plasminogen activator inhibitor (PAI-1) and the appearance of senescence-associated ß-galactosidase (SA-ß-gal) activity. A 20 amino acid peptide from $p^{16INK4a}$ that inhibits Cdks active in the G1-phase of the cell cycle produces similar effects in a dose-dependent manner suggesting that, in primary fibroblasts, inhibition of G1-specific Cdk activity is sufficient to induce phenotypic changes that normally occur at the end of their finite lifespan.
2 March 1998, Brief Communication, *Current Biology*

☐ **New aspects of electron transfer revealed by the crystal structure of a truncated bovine adrenodoxin, Adx(4–108).**
Alexander Müller, Jürgen J Müller, Yves A Muller, Heike Uhlmann, Rita Bernhardt and Udo Heinemann (1998). *Structure* **6**, 269–280.

Adrenodoxin (Adx) is a [2Fe–2S] ferredoxin involved in steroid hormone biosynthesis in the adrenal gland mitochondrial matrix of mammals. Adx is a small soluble protein that transfers electrons from adrenodoxin reductase (AR) to different cytochrome P450 isoforms where they are consumed in hydroxylation reactions. The crystal structure of a truncated bovine adrenodoxin, Adx(4–108), was determined using multiple wavelength anomalous dispersion phasing techniques, making use of the iron atoms in the [2Fe–2S] cluster of the protein. The protein displays the compact (α+β) fold typical for [2Fe–2S] ferredoxins. The polypeptide chain is organized into a large core domain and a smaller interaction domain which comprises 35 residues, including all those previously determined to be involved in binding to AR and cytochrome P450. A small interdomain motion is observed as a structural difference between the two independent molecules in the asymmetric unit of the crystal. Charged residues of Adx(4–108) are clustered to yield a strikingly asymmetric electric potential of the protein molecule. The crystal structure of Adx(4–108) provides the first detailed description of a vertebrate [2Fe–2S] ferredoxin and serves to explain a large body of biochemical studies in terms of a three-dimensional structure. The structure suggests how a change in the redox state of the [2Fe–2S] cluster may be coupled to a domain motion of the protein. It seems likely that the clearly asymmetric

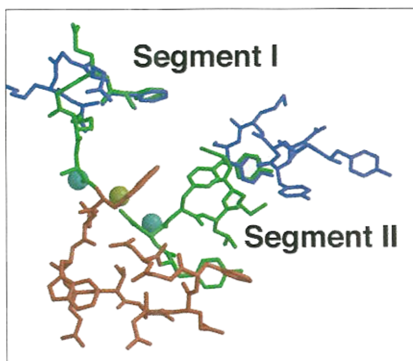


charge distribution on the surface of Adx(4–108) and the resulting strong molecular dipole are involved in electrostatic steering of the interactions with AR and cytochrome P450.
15 March 1998, Research Paper, *Structure*

☐ **Activation of *Bacillus licheniformis* α-amylase through a disorder order transition of the substrate-binding site mediated by a calcium–sodium–calcium metal triad.**
Mischa Machius, Nathalie Declerck, Robert Huber and Georg Wiegand (1998). *Structure* **6**, 281–292.

The structural basis as to how metals regulate the functional state of a protein

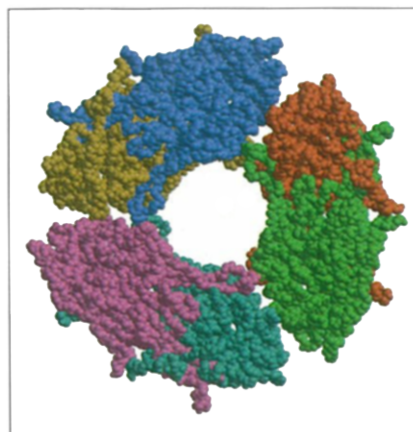
by altering or stabilizing its conformation has been characterized in relatively few cases because the metal-free form of the protein is often partially disordered and unsuitable for crystallographic analysis. This is not the case, however, for *Bacillus licheniformis* α-amylase (BLA) for which the structure of the metal-free form is available. BLA is a hyperthermostable enzyme widely used in biotechnology, for example in the breakdown of starch or as a component of detergents. The determination of the structure of BLA in



the metal-containing form, together with comparisons to the apo enzyme, will help in understanding the way in which metal ions can regulate enzyme activity. The authors report here the crystal structure of native, metal-containing BLA. The structure shows that the calcium-binding site, which is conserved in all α-amylases forms part of an unprecedented linear triadic metal array, with two calcium ions flanking a central sodium ion. A region around the metal triad comprising 21 residues exhibits a conformational change involving a helix unwinding and a disorder order transition compared to the structure of metal-free BLA. Another calcium ion, not previously observed in α-amylases, is located at the interface between domains A and C. The authors present a structural description of a major conformational rearrangement mediated by metal ions. The metal-induced disorder–order transition observed in BLA leads to the formation of the extended substrate-binding site and explains on a structural level the calcium dependency of α-amylases. Sequence comparisons indicate that the unique Ca–Na–Ca metal triad and the additional calcium ion located between domains A and C might be found exclusively in bacterial α-amylases, which show increased thermostability. The information presented here may help in the rational design of mutants with enhanced performance in biotechnological applications.
15 March 1998, Research Paper, *Structure*

☐ **Copper amine oxidase from *Hansenula polymorpha*: the crystal structure determined at 2.4 Å resolution reveals the active conformation.**
Rongbao Li, Judith P Klinman and F Scott Mathews (1998). *Structure* **6**, 293–308.

Copper-containing amine oxidases (CAOs) are widespread in nature. These enzymes oxidize primary amine substrates to the aldehyde product, reducing molecular oxygen to hydrogen peroxide in the process. CAOs contain one type 2 copper atom and topaquinone (TPQ), a modified tyrosine sidechain utilized as a redox cofactor.

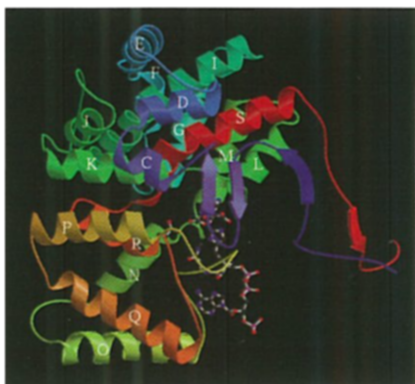


The methylamine oxidase from the yeast *Hansenula polymorpha* (HPAO) is an isoform of CAO with a preference for small aliphatic amine or phenethylamine substrates. The enzyme is dimeric with a subunit molecular weight of 78 kDa. Structural studies are directed at understanding the basis for cofactor biogenesis and catalytic efficiency. The X-ray crystal structure of HPAO has been solved using a combination of molecular replacement and single isomorphous replacement followed by refinement using sixfold symmetry averaging. The electron density at the catalytic site shows that the TPQ conformation corresponds to that of the active form of the enzyme. Two channels, one on either side of TPQ, are observed in the structure that provide access between the active site and the bulk solvent. The structure shows TPQ in a position poised for catalysis. This is the first active CAO structure to reveal this conformation and may help further our understanding of the catalytic mechanism. On the substrate side of TPQ a water-containing channel leading to the protein surface can serve as an entrance or exit for substrate and product. On the opposite side of TPQ there is direct access from the bulk solvent of the dimer interface by which molecular oxygen may enter and hydrogen peroxide depart. In addition, a network of conserved water molecules has been identified which may function in the catalytic mechanism.
15 March 1998, Research Paper, *Structure*

☐ **Structural adaptations of the cold-active citrate synthase from an Antarctic bacterium.**
Rupert JM Russell, Ursula Gerike, Michael J Danson, David W Hough and Garry L Taylor (1998). *Structure* **6**, 351–361.

The structural basis of adaptation of enzymes to low temperature is poorly understood. Dimeric citrate synthase has been used as a model enzyme to study the structural basis of thermostability, the structure of the enzyme from organisms living in habitats at 55°C and 100°C having previously been determined. The authors report in this paper the first crystal structure of a cold-active enzyme, citrate synthase, isolated from an Antarctic bacterium. In comparison with the same enzyme from a hyperthermophilic host, the cold-active enzyme has a much more accessible active site, an unusual electrostatic potential distribution and an increased relative flexibility of the small domain compared

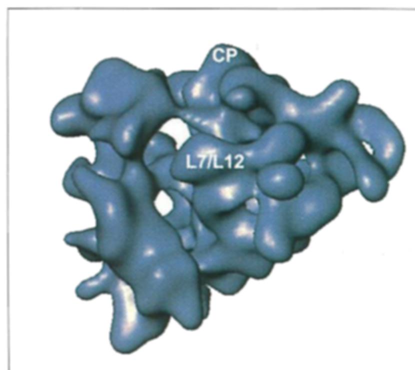
to the large domain. Several other features of the cold-active enzyme were also identified: reduced subunit interface interactions with no intersubunit ion-pair networks; loops of increased length carrying more charge and fewer proline residues; an increase in solvent-exposed hydrophobic residues; and an increase in intramolecular ion pairs. Enzymes from organisms living at extreme temperatures need to avoid hot or cold denaturation yet maintain sufficient structural



integrity to allow catalytic efficiency. For hyperthermophiles, thermal denaturation of the citrate synthase dimer appears to be resisted by complex networks of ion pairs at the dimer interface. For the cold-active citrate synthase, cold denaturation appears to be resisted by an increase in intramolecular ion pairs compared to the hyperthermophilic enzyme. Catalytic efficiency of the cold-active enzyme appears to be achieved by a more accessible active site and by an increase in the relative flexibility of the small domain compared to the large domain.
15 March 1998, Research Paper, *Structure*

☐ **The 80S rat liver ribosome at 25 Å resolution by electron cryomicroscopy and angular reconstitution.**
Prakash Dube, Martin Wieske, Holger Stark, Michael Schatz, Joachim Stahl, Friedrich Zemlin, Gudrun Lutsch and Marin van Heel (1998). *Structure* **6**, 389–399.

The ribosome is central to protein synthesis in all living organisms. Single-particle electron cryomicroscopy has recently led to the determination of



three-dimensional structures of bacterial ribosomes to ~20 Å, which have since revolutionised our understanding of ribosomal function. The structure the authors present here of the 80S rat liver ribosome leads the way to similar progress for mammalian ribosomes. Among the new details revealed by the 25 Å structure of the 80S rat liver ribosome are channels within the subunits, a large 'flat ribosomal surface' (FRS) on the outer surface of the large subunit and structural extensions of the mammalian compared to the bacterial ribosome. The main large subunit channel in both the bacterial and the mammalian species starts at the peptidyl transferase centre, below the central protuberance, and ends in the FRS, at the lower back of the large subunit. Structurally, the channels of both species can be directly superimposed. The mammalian structural extensions — none of which trespass the FRS — can be interpreted in terms of rRNA inserts and additional protein content over that of bacterial ribosomes. The main large subunit channel, which ends at the FRS, is the best candidate for the exit channel for proteins targeted for the endoplasmic reticulum.
15 March 1998, Research Paper, *Structure*