Elsewhere in Biology

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

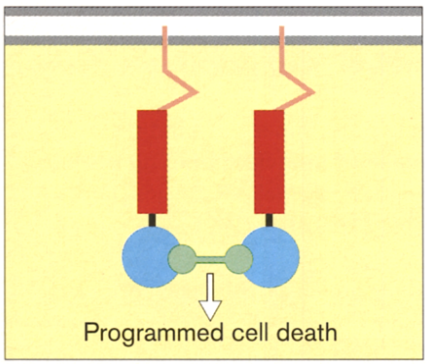
A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chemistry & Biology August 1996, **3**:689–692

 ISSN 1074-5521

☐ **Functional analysis of Fas signaling *in vivo* using synthetic inducers of dimerization.** David M. Spencer, Peter L. Belshaw, Lei Chen, Steffan N. Ho, Filippo Randazzo, Gerald R. Crabtree and Stuart L. Schreiber (1996). *Curr. Biol.* **6**, 839–847.

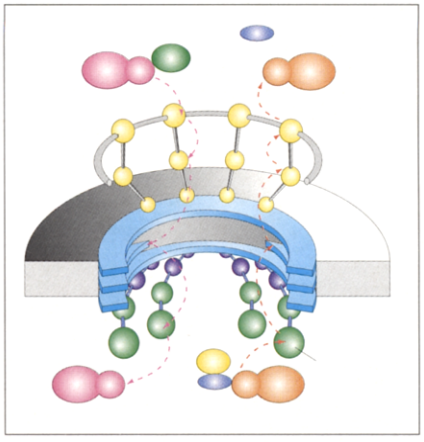
Genetic abnormalities in the Fas receptor or its trimeric ligand, FasL, result in massive T-cell proliferation and a lupus-like autoimmune syndrome, syndromes that are attributed to the absence of Fas-mediated programmed cell death or apoptosis. Fas does not, however, appear to be required for the negative selection of T cells in the thymus. Fas is activated when multimerized by its ligand, and here the synthetic dimerizer FK1012 is used to study the effects of dimerizing a modified Fas that contains an FK1012 binding site. Dimerization is sufficient to trigger apoptosis, with double-positive ($CD4^+$ $CD8^+$) T cells being particularly sensitive to Fas-mediated apoptosis in transgenic mice. This system should allow the function of Fas to be analysed in a tissue-specific and temporally controlled fashion.



1 July 1996, Research Paper, *Current Biology*

☐ **HIV Rev uses a conserved cellular protein export pathway for the nucleocytoplasmic transport of viral RNAs.** Christian C. Fritz and Michael R. Green (1996). *Curr. Biol.* **6**, 848–854.

The structural proteins of human immunodeficiency virus type 1 (HIV-1) are encoded by intron-containing mRNAs that normally are retained in the nucleus until a viral protein, Rev, induces their accumulation in the cytoplasm. This works shows that the Rev effector domain, which acts as a nuclear export signal (NES) by interacting with the human protein, hRIP, can be replaced by NESs from two cellular proteins, PKI (an inhibitor of protein kinase A) and IκB. It is well known that IκB can downregulate NFκB by masking its nuclear localization sequence, but the discovery of the NES on IκB suggests that it can also lead to the export of NFκB

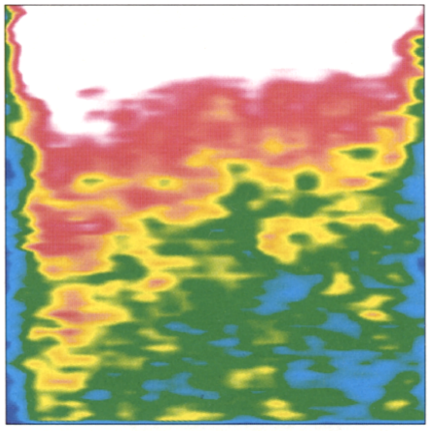


from the nucleus. All three NESs bind to hRIP and several nuclear pore proteins. The interactions with the two nuclear pore proteins on the nucleoplasmic side are stronger than with hRIP, while the interactions with a pore protein on the cytoplasmic side are stronger still. Sequential interactions with these proteins may therefore help drive the transfer of the protein that is being exported from hRIP, to the pore, and then through the pore to the cytoplasm. Finally, the human NESs function in yeast, suggesting that this pathway is highly conserved.

1 July 1996, Research Paper, *Current Biology*

☐ **Subcellular Ca^{2+} signals underlying waves and graded responses in HeLa cells.** Martin D. Bootman and Michael J. Berridge (1996). *Curr. Biol.* **6**, 855–865.

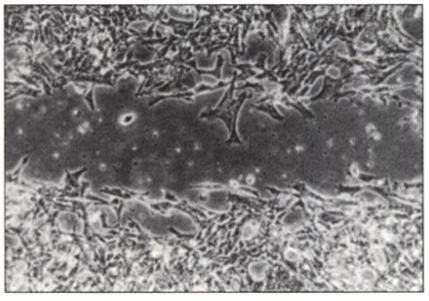
Many agonist-evoked intracellular signals show complex patterns of Ca^{2+} spikes, waves and puffs. In large cells such as oocytes and myocytes the Ca^{2+} appears to be released from several functionally discrete sites, but the architecture of the intracellular Ca^{2+} pool in smaller cells is unknown. Estimates of the size and distribution of the Ca^{2+} puffs in Xenopus cells suggest that cells of a more normal volume might only have the capacity for one Ca^{2+} release unit. But here the authors show that subcellular Ca^{2+} signals can be detected during the response of single Fura-2-loaded HeLa cells to histamine. These signals are particularly obvious in the 'pacemaker' phase of the Ca^{2+} response, the slow increase in calcium concentration that precedes each calcium spike. As the intracellular Ca^{2+} pool is made up of many discrete units, the cell has a considerable degree of flexibility in the size and spatial localization of its Ca^{2+} response to stimuli.



1 July 1996, Research Paper, *Current Biology*

☐ **CD44 phosphorylation regulates melanoma cell and fibroblast migration on, but not attachment to, a hyaluronan substrate.** David Peck and Clare M. Isacke (1996). *Curr. Biol.* **6**, 884–890.

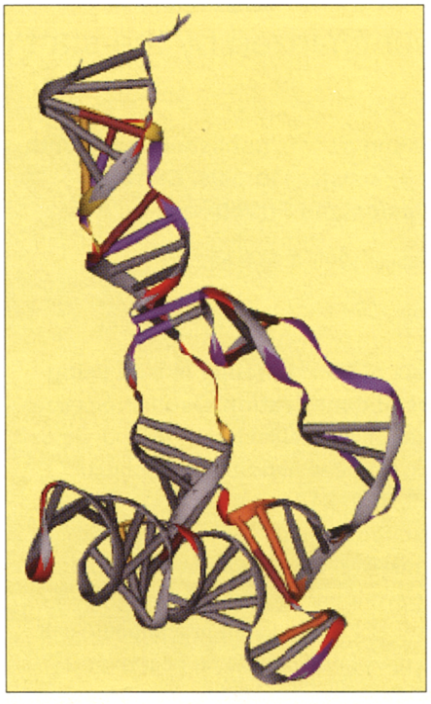
CD44 is a transmembrane receptor for the extracellular matrix glycosaminoglycan hyaluronan. The interaction of CD44 with its ligand is important in cell adhesion and migration in tumor progression, embryonic tissue morphogenesis and inflammation. CD44 is often up-regulated in metastatic cancer



cells, and its ability to bind to hyaluronan is strictly regulated. The receptor is known to be phosphorylated on serine residues in the cytoplasmic domain, yet mutant CD44 proteins in which the relevant serines have been mutated allow efficient binding to hyaluronan when expressed in T lymphoma cell lines; it has been suggested that phosphorylation may instead control CD44-dependent migration. Here, the authors use two adherent cell lines in which CD44 expression is low or absent to examine the effects of mutation at specific sites on adhesion and migration. They find that, although wild-type CD44 supports both adhesion to and migration on a hyaluronan-coated substrate, CD44 phosphorylation mutants only support adhesion, not migration. The phosphorylation of the serine residues may thus affect the interaction between the cytoplasmic tail of CD44 and elements of the cytoskeleton that are required for migration.
1 July 1996, Research Paper, *Current Biology*

☐ **Comparative analysis of tertiary structure elements in signal recognition particle RNA.** Christian Zwieb, Florian Müller and Niels Larsen (1996). *Folding & Design* **1**, 315–324.

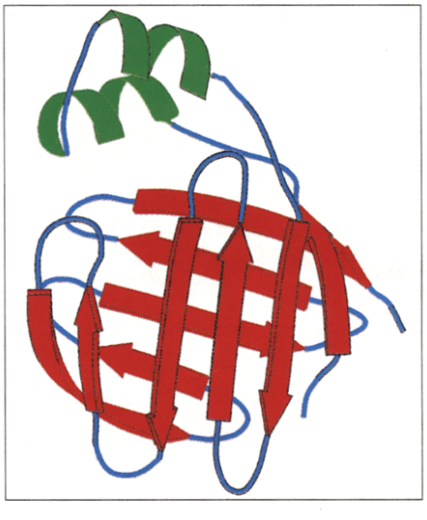
The signal recognition particle (SRP) is a ribonucleoprotein complex that associates with ribosomes to promote co-translational translocation of proteins across biological membranes. The human SRP is composed of a 301-nucleotide RNA and six associated proteins. In a previous study, a comparison of the sequences of a large number of bacterial, archaeal, and eukaryotic SRP RNAs revealed common secondary structural elements. Here, the predicted secondary structures from more than 70 SRP RNA sequences are compared, leading to a model of the three-dimensional structure of the human SRP RNA. The model is refined using data from previous studies of enzymatic and chemical modification, electron microscopy and site-directed mutagenesis of the RNA. Overall, the RNA is in the shape of a dumbbell, comprising a large domain and a small domain. It contains single-stranded interhelical and intrahelical RNA loops, a pseudoknot in the small SRP domain and a tertiary interaction in the large domain. The structure may help explain the binding properties of two



proteins that bind to the RNA. Whereas most SRP proteins interact directly with the RNA, the association of SRP54 is dependent on the binding of SRP19 to the RNA. Analysis of the three-dimensional model suggests that it is only after binding of SRP19 to the RNA that a 'knuckle' is formed in helix 8, thus generating a binding site for SRP54. This model should allow the interactions of other SRP components with the RNA and each other to be analysed.
22 Jul 1996*, Research Paper, *Folding & Design*

☐ **Flexibility is a likely determinant of binding specificity in the case of ileal lipid binding protein.** Christian Lücke, Fengli Zhang, Heinz Rüterjans, James A Hamilton and James C Sacchettini (1996). *Structure* **4**, 785–800.

The family of lipid-binding proteins (LBPs) includes a large number of fatty acid binding proteins (FABPs) but only two proteins (ileal lipid binding protein, ILBP, and liver fatty acid binding protein) that can bind both fatty acids and bile acids. Bile acid transport is medically and pharmacologically



important, but poorly understood. Here the authors determine the solution structure of ILBP with and without bound lipids. The tertiary structure of ILBP is similar to that of other LBPs, but appears to be unusually flexible, with a relatively weak hydrogen-bonding network.

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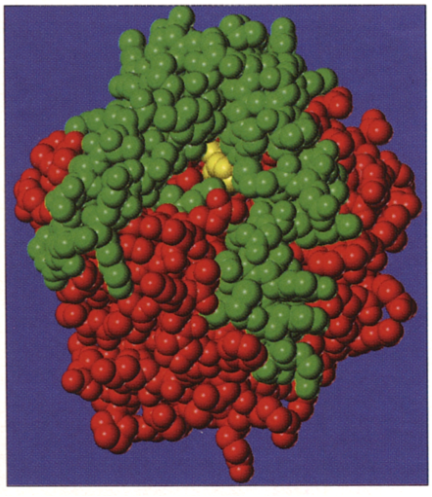
It is likely that this flexibility is important in allowing bile acids, which are larger and more rigid than fatty acids, to enter the central cavity of the protein.
15 July 1996, Research Paper, *Structure*

☐ **Mechanism of cyanogenesis: the crystal structure of hydroxynitrile lyase from *Hevea brasiliensis*.** UG Wagner, M Hasslacher, H Griengl, H Schwab and C Kratky (1996). *Structure* **4**, 811–822.

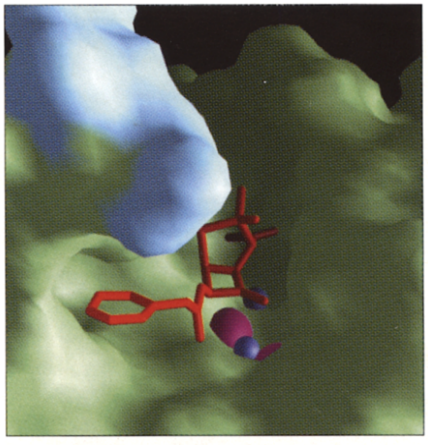
Over 3 000 species of plants, including important food crops such as cassava, generate HCN upon tissue damage as a defense against predation. Hydroxynitrilite lyase is one of the key enzymes in cyanogenesis, catalyzing the decomposition of an α-cyanohydrin to form HCN plus the corresponding aldehyde or ketone. These enzymes have also been exploited in industrial syntheses, as the reverse reaction can generate optically pure chiral cyanohydrins. The authors determined



the crystal structure of the enzyme to 1.9 Å resolution. The active site is deeply buried within the protein and connected to the outside by a narrow tunnel, and the catalytic triad is made up of Ser80, His235 and Asp207. The authors propose that catalysis should involve a tetrahedral hemiketal or hemiacetal intermediate formed by nucleophilic attack of Ser80 on the substrate, stabilized by an oxyanion hole. The SH group of Cys81 is probably involved in proton transfer between HCN and the hydroxynitrile OH. This mechanism is startlingly different from the corresponding uncatalyzed solution reaction.
15 July 1996, Research Paper, *Structure*

☐ **Crystal structure of the wide-spectrum binuclear zinc β-lactamase from *Bacteroides fragilis*.** Nestor O Concha, Beth A Rasmussen, Karen Bush and Osnat Herzberg (1996). *Structure* **4**, 823–836.

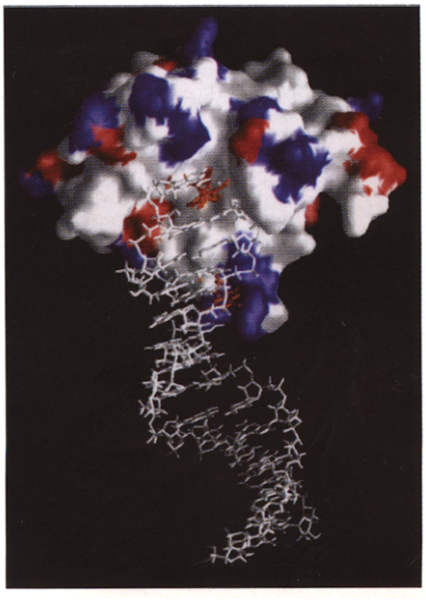
The metallo-β-lactamase from *Bacteroides fragilis* hydrolyzes a wide range of β-lactam antibiotics, and is not clinically susceptible to any known β-lactamase inhibitors. *B. fragilis* is associated with post-surgery hospital infections, and the gene encoding this enzyme can be transmitted to different pathogens via a plasmid. Inhibitors of this enzyme are therefore urgently needed. The authors have determined the crystal structure of the enzyme to 1.85 Å resolution. The active site contains a binuclear zinc center with one zinc tetrahedrally coordinated and the other in a trigonal bipyramidal coordination. The amino-acid residues that serve as ligands to



the two zincs are invariant in all metallo-β-lactamases that have been sequenced, with two exceptions. A water/hydroxide molecule serves as a ligand for both metals. The authors propose that the zinc cluster prepares an hydroxide for nucleophilic attack on the carbonyl carbon atom of the β-lactam. The presumed negatively-charged tetrahedral intermediate could be stabilized by the side-chain of the invariant Asn193 and the tetrahedral zinc.
15 July 1996, Research Paper, *Structure*

☐ **Insights into specificity of cleavage and mechanism of cell entry from the crystal structure of the highly specific *Aspergillus* ribotoxin, restrictocin.** Xiaojing Yang and Keith Moffat (1996). *Structure* **4**, 837–852.

Restrictocin, a highly specific ribotoxin made by the fungus *Aspergillus restrictus*, cleaves a single phosphodiester bond in the 28S RNA of eukaryotic ribosomes, inhibiting protein synthesis. The sequence around this cleavage site is a binding site for elongation factors, and is conserved in all cytoplasmic ribosomes. The crystal structure of restrictocin was determined at 2.1 Å resolution and refined to 1.7 Å resolution using synchrotron Laue data. The protein is structurally homologous to ribonuclease T1. Large positively-charged peripheral loops near the active site construct a platform with a concave surface for RNA binding. Restrictocin appears to combine the catalytic components of T1 ribonucleases with the base-recognition components of Sa ribonuclease.

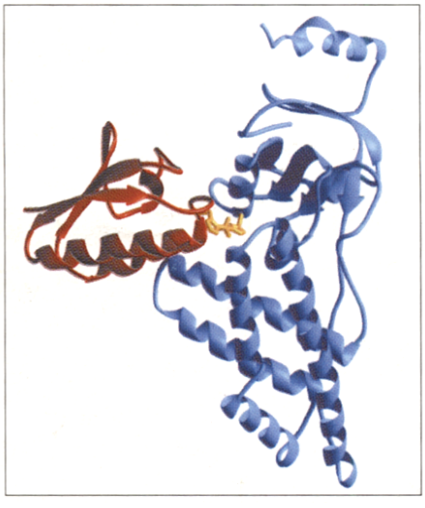


Modelling suggest that the tertiary structure of the substrate RNA is important in protein–RNA recognition, fitting closely into the concavity of the presumed binding site. The large 39-residue loop L3, which has similarities to loops found in lectin sugar-binding

domains, may be responsible for restrictocin's ability to cross cell membranes.
15 July 1996, Research Paper, *Structure*

☐ **The first step in sugar transport: crystal structure of the amino terminal domain of enzyme I of the *E. coli* PEP: sugar phosphotransferase system and a model of the phosphotransfer complex with HPr.** D-I Liao, E Silverton, Y-J Seok, BR Lee, A Peterkofsky and DR Davies (1996). *Structure* **4**, 861–872.

The bacterial phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS) transports exogenous hexose sugars through the membrane and tightly couples transport with phosphoryl transfer from PEP to the sugar via several phosphoprotein intermediates. The phosphate group is first transferred to enzyme I, second to the histidine-containing phosphocarrier protein HPr, and then to one of a number of sugar-specific enzymes II. The authors report the structure of



the amino-terminal half of enzyme I from *Escherichia coli* (EIN) at 2.5 Å resolution, and show that plausible models of complexes between EIN and HPr can be made without assuming major structural changes in either protein. One subdomain of EIN is topologically similar to the phospho-histidine domain of the enzyme pyruvate phosphate dikinase, which is phosphorylated by PEP on a histidyl residue but does not interact with HPr. It is therefore likely that features of this subdomain are important in the autophosphorylation of enzyme I. The other subdomain of EIN is not found in pyruvate phosphate dikinase; this subdomain is therefore more likely to be involved in phosphoryl transfer to HPr.
15 July 1996, Research Paper, *Structure*