# Elsewhere in biology

**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*.**

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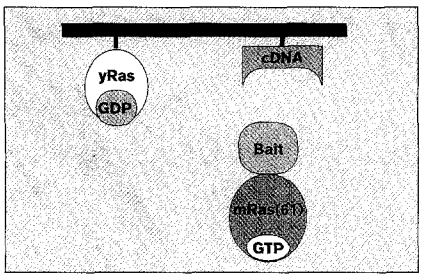
☐ **The Ras recruitment system, a novel approach to the study of protein–protein interactions.**
Yehoshua C Broder, Sigal Katz and Ami Aronheim (1998). *Curr. Biol.* **8**, 1121–1124.

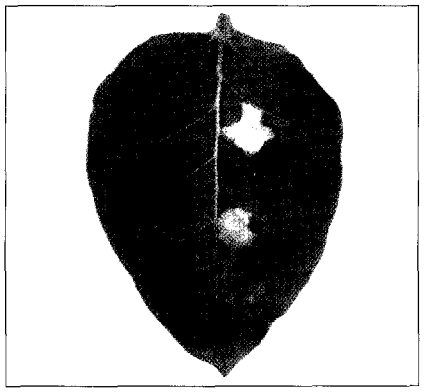
The yeast two-hybrid system represents one of the most efficient approaches currently available for identifying and characterizing protein–protein interactions. Although very powerful, this procedure has several problems and inherent limitations. A new system, the Sos recruitment system (SRS), was developed recently based on a different readout from that of the two-hybrid system. SRS overcomes several of the limitations of the two-hybrid system and thus serves as an attractive alternative for studying protein–protein interactions between known and novel proteins. Because the authors encountered a number of problems using SRS, they developed an improved protein recruitment system, designated the Ras recruitment system (RRS), based on the absolute requirement that Ras be localized to the plasma membrane for its function. Ras membrane localization and activation can be achieved through interaction between two hybrid proteins.




The the effectiveness of the novel RRS system has been demonstrated using five different known protein–protein interactions; two previously unknown protein–protein interactions were identified through a library screening protocol. The RRS system significantly extends the usefulness of the previously described SRS system and overcomes several of its limitations.
28 September 1998, Brief Communication, *Current Biology*.

☐ **Caspases and programmed cell death in the hypersensitive response of plants to pathogens.**
Olga del Pozo and Eric Lam (1998). *Curr. Biol.* **8**, 1129–1132.

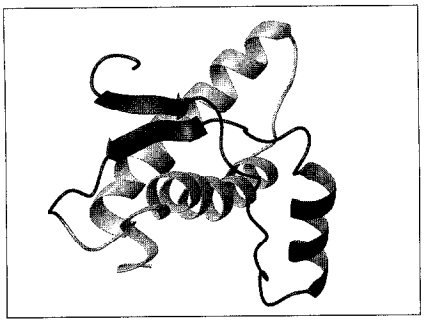
The hypersensitive response (HR) is induced by certain plant pathogens and involves programmed cell death (PCD) to restrict the spread of pathogens from the infection site. Concurrent with the induction of cell death, the host activates a defense response. The cell death associated with the HR in several plant–pathogen systems has



morphological similarities to animal apoptosis, which suggests that cell death mechanisms in plants and animals might share common components that lead to similar cellular events. Caspases are conserved cysteine proteases that regulate animal PCD; caspase activity or an involvement of caspases in cell death has yet to be reported in plants. Here, the participation of caspases in HR cell death is investigated. Caspase-specific peptide inhibitors, Ac-YVAD-CMK and Ac-DEVD-CHO, abolished bacteria-induced plant PCD but did not significantly affect the induction of other aspects of HR, such as the expression of defense genes. This result confirmed the previous model that cell death can be uncoupled from defense gene activation during HR. Caspase-like proteolytic activity was detected in tobacco tissues that were developing HR following infection with tobacco mosaic virus. The results provide evidence for the presence of caspase-like plant protease(s) that participate in HR cell death.
28 September 1998, Brief Communication, *Current Biology*.

☐ **Prion protein fragments spanning helix 1 and both strands of β sheet (residues 125–170) show evidence for predominantly helical propensity by CD and NMR.**
Gary J Sharman, Nigel Kenward, Huw E Williams, Michael Landon, R John Mayer and Mark S Searle (1998). *Fold. Des.* **3**, 313–320.

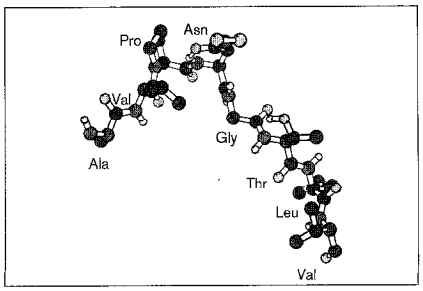
Transmissible spongiform encephalopathies are a group of neurodegenerative disorders of man and animals that are believed to be caused by an α-helical to β-sheet conformational change in the prion protein, PrP. Recently determined NMR structures of recombinant PrP (residues 121–231 and 90–231) have identified a short two-stranded anti-parallel β sheet in the normal cellular form of the protein ($PrP^C$). This β sheet has been suggested to be involved in seeding the conformational transition to the disease-associated form ($PrP^{Sc}$) via a partially unfolded intermediate state.The authors describe CD and NMR studies of three peptides (125–170, 142–170 and 156–170) that span the β-sheet and helix 1 region of PrP, forming a large part of the putative $PrP^{Sc}$–$PrP^C$ binding site that has been proposed to be important for self-seeding replication of $PrP^{Sc}$. The data suggest that all three peptides in water have predominantly helical propensities, which are enhanced in aqueous methanol (as judged by deviations from random-coil Hα chemical shifts and $^3J_{H\alpha-NH}$ values). Although the helical propensity is most marked in the region corresponding to helix 1 (144–154), it is



also apparent for residues spanning the two β-strand sequences. The authors have attempted to model the conformational properties of a partially unfolded state of PrP using peptide fragments spanning the region 125–170, and find no evidence in the sequence for any intrinsic conformational preference for the formation of extended β-like structure that might be involved in promoting the $PrP^{C}$–$PrP^{Sc}$ conformational transition.
8 July 1998, Research Paper, *Folding & Design.*

☐ **A structural role for glycosylation: lessons from the hp model.**
Daniel Hoffmann and Holger Flörkes (1998). *Fold. Des.* **3**, 337–343.

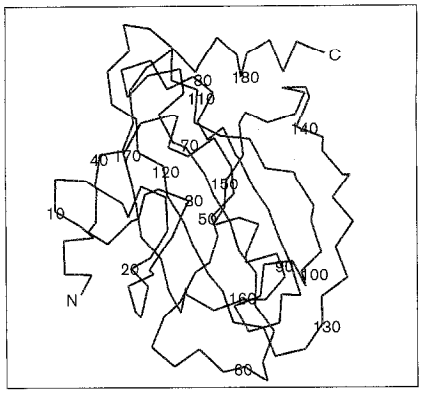
Protein glycosylation, the covalent attachment of carbohydrates, is very common, but in many cases the biological function of glycosylation is not well understood. Recently, fluorescence energy transfer experiments have shown that glycosylation can strongly change the global conformational distributions of peptides. The authors examine the physical mechanism behind this structural effect using a theoretical model. The framework of the 'hp model' is used to describe peptides and their glycosylated counterparts. Conformations are completely




enumerated and exact results are obtained for the effect of glycosylation. On glycosylation, the model peptides experience conformational changes similar to those seen in experiments. This effect is highly specific for the sequence of amino acids and also depends on the size of the glycan. Experimentally testable predictions are made for related peptides. Glycans can, by means of entropic contributions, modulate the free energy landscape of polypeptides and thereby specifically stabilize polypeptide conformations. With respect to glycoproteins, the results suggest that the loss of chain entropy during protein folding is partly balanced by an increase in carbohydrate entropy.
11 August, Research Paper, *Folding & Design.*

☐ **Function from structure? The crystal structure of human phosphatidylethanolamine-binding protein suggests a role in membrane signal transduction.**
Mark J Banfield, John J Barker, Anthony CF Perry and R Leo Brady (1998). *Structure* **6**, 1245–1254.

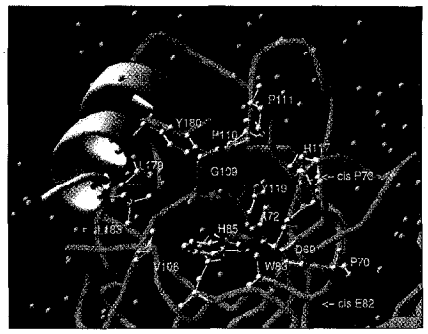
Proteins belonging to the phosphatidylethanolamine-binding protein (PEBP) family are highly conserved throughout nature and have no significant sequence homology with other known proteins. A variety of biological roles have been described previously for members of this family, including lipid binding, regulation of flowering plant stem architecture, and a function as a precursor protein of a bioactive brain neuropeptide. To date, no experimentally derived structural information has been available for this protein family. In an attempt to clarify the biological role of this unique protein family, the crystal structures of two forms of human (h)PEBP have been determined: one in the native state and one in complex with cacodylate revealing that hPEBP adopts a novel protein topology, dominated by the presence of a large central β sheet. This is expected to represent the archaetypal fold for this family of proteins. Two potential functional sites have been identified: a putative ligand-binding site




and a coupled cleavage site. hPEBP forms a dimer in the crystal with a distinctive dipole moment that could orient the oligomer for membrane binding. The ligand-binding site could accommodate the phosphate head groups of membrane lipids, allowing the protein to adhere to the inner leaf of bilipid membranes where it would be ideally positioned to relay signals from the membrane to the cytoplasm. The structure also suggests that ligand binding might lead to coordinated release of the amino-terminal region of the protein to form the hippocampal neurostimulatory peptide, which is known to be active in the development of the hippocampus. These studies are consistent with a primary biological role for hPEBP as a transducer of signals from the interior membrane surface.
15 October 1998, Research Paper, *Structure.*

☐ **Crystal structure of the phosphatidylethanolamine-binding protein from bovine brain: a novel structural class of phospholipid-binding proteins.**
Laurence Serre, Béatrice Vallée, Nicole Bureaud, Françoise Schoentgen and Charles Zelwers (1998). *Structure* **6**, 1255–1265.

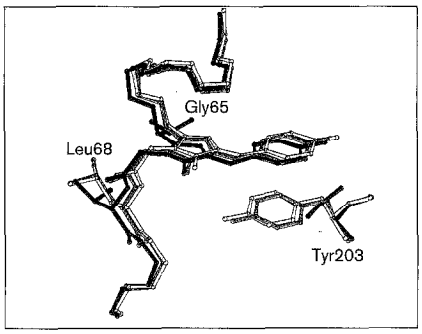
Phosphatidylethanolamine-binding protein (PEBP) is a basic protein found in numerous tissues from a wide range of species. The screening of gene and protein data banks defines a family of PEBP-related proteins that are present in a variety of organisms; PEBP binds to phosphatidylethanolamine and nucleotides *in vitro*, but its biological function *in vivo* is not yet known. The




expression of PEBP and related proteins seems to be correlated with development and cell morphogenesis, however. To obtain new insights into the PEBP family and its potential functions, the X-ray crystal structure of bovine brain PEBP has been solved. The structure has an α β fold and exhibits one nonprolyl *cis* peptide bond. Analysis of cavities within the structure and sequence alignments identified a putative ligand-binding site that corresponds to the binding site of the polar head group of phosphatidyl-ethanolamine. This study shows that PEBP is not related to the G-protein family nor to known lipid-binding proteins, and therefore defines a novel structural family of phospholipid-binding proteins. In PEBP, a small cavity close to the protein surface has a high affinity for anions and might be the binding site of the polar head group of phosphatidyl-ethanolamine.
15 October 1998, Research Paper, *Structure*.

☐ **Structural basis of spectral shifts in the yellow-emission variants of green fluorescent protein.**
Rebekka M Wachter, Marc-André Elsliger, Karen Kallio, George T Hanson and S James Remington (1998). *Structure* **6**, 1267–1277.

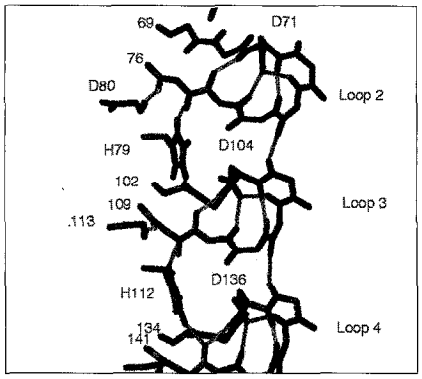
Because of its ability to spontaneously generate its own fluorophore, the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is used extensively as a fluorescent marker in molecular and cell biology. The yellow fluorescent proteins (YFPs) have the longest wavelength emissions of all GFP variants examined to date. This shift in the spectrum is the result of a T203Y substitution, (using single-letter-amino-acid code) a mutation rationally designed on the basis of the structure of GFP S65T. The authors have determined the crystal structures of YFP T203Y/S65G/V68L/S72A and YFP H148G. Both structures show clear electron density for nearly coplanar π–π stacking between Tyr203 and the chromophore. The chromophore has been displaced by nearly 1 Å in comparison to other available structures. Although the H148G mutation results in the generation of a solvent channel to the chromophore cavity, intense fluorescence is maintained. The chromophore in the intact protein can be titrated, and the two variants have $pK_a$ values of 7.0 (YFP) and 8.0 (YFP H148G). The observed red shift of the T203Y YFP variant is proposed to be mainly due to the additional polarizability of the π-stacked Tyr203. The altered location of the chromophore




suggests that the exact positions of nearby residues are not crucial for the chemistry of chromophore formation. The YFPs significantly extend the pH range over which GFPs could be employed as pH indicators in live cells.
15 October 1998, Research Paper, *Structure*.

☐ **Structure of human cyclin-dependent kinase inhibitor $p19^{INK4d}$: comparison to known ankyrin-repeat-containing structures and implications for the dysfunction of tumor suppressor $p16^{INK4a}$.**
Roland Baumgartner, Carlos Fernandez-Catalan, Astar Winoto, Robert Huber, Richard A Engh and Tad A Holaks (1998). *Structure* **6**, 1279–1290.

The four members of the INK4 gene family ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and

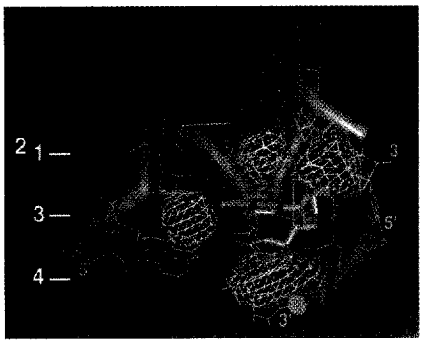



$p19^{INK4d}$) inhibit the closely related cyclin-dependent kinases CDK4 and CDK6 as part of the regulation of the $G_1 \rightarrow S$ transition in the cell-division cycle. Loss of INK4 function, particularly that of $p16^{INK4a}$, is found in 10–60% of human tumors, suggesting that broadly applicable anticancer therapies might be based on restoration of $p16^{INK4a}$ CDK inhibitory function. Although much less frequent, defects of $p19^{INK4d}$ have also been associated with some human cancers. The structures of some INK4 family members have begun to clarify the functional role of $p16^{INK4a}$. Here, the crystal structure of human $p19^{INK4d}$ has been determined. The fold of $p19^{INK4d}$ produces an oblong molecule comprising five approximately 32-residue ankyrin-like repeats. The architecture of the protein demonstrates the high structural similarity within the INK4 family. Comparisons to other ankyrin-repeat-containing proteins (GABPβ, 53BP2 and myotrophin) show similar structures with comparable hydrogen-bonding patterns and hydrophobic interactions. Such comparisons highlight the splayed β-loop geometry that is specific to INK4 inhibitors. This geometry is the result of a modified ankyrin structure in the second repeat. Among the INK4 inhibitors, the highest amino-acid sequence conservation is found in the helical stacks; this conservation creates a conserved β-loop geometry specific to INK4 inhibitors. Therefore, in addition to models which predict that the conserved helix α 6 is responsible for CDK inhibition, a binding mode whereby the loops of INK4 proteins bind to the CDKs should also be considered. A similar loop-based interaction is seen

in the complex formed between the ankyrin-repeat-containing protein GABPβ and GABPα. This mode of binding would be consistent with the observation that $p16^{INK4a}$ is sensitive to deleterious mutations found throughout this tumor suppressor protein; these mutations probably destabilize the three-dimensional structure.
15 October 1998, Research Paper, *Structure*.

☐ **Exploration of metal-ion-binding sites in RNA folds by Brownian-dynamics simulations.**
Thomas Hermann and Eric Westhof (1998). *Structure* **6**, 1303–1314.

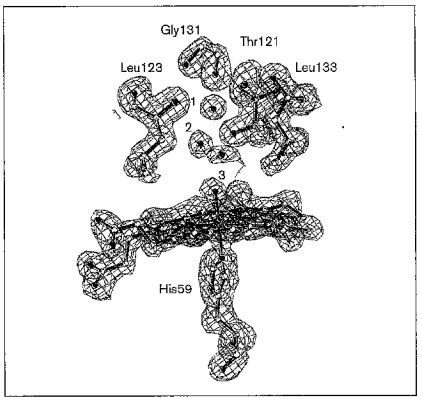
Metal ions participate in the three-dimensional folding of RNA and provide active centers in catalytic RNA molecules. The positions of metal ions are known for a few RNA structures determined by X-ray crystallography. In addition to the crystallographically identified sites, solution studies point to




many more metal-ion-binding sites around structured RNAs. Metal ions are also present in RNA structures determined by nuclear magnetic resonance (NMR) spectroscopy, but the positions of the ions are usually not revealed. A novel method for predicting metal-ion-binding sites in RNA folds has been successfully applied to a number of different RNA structures. The method is based on Brownian-dynamics simulations of cations diffusing under the influence of random Brownian motion within the electrostatic field generated by the static three-dimensional fold of an RNA molecule. In test runs, the crystallographic positions of $Mg^{2+}$ ions were reproduced with deviations between 0.3 and 2.7 Å for several RNA molecules for which X-ray structures are available. In addition to the crystallographically identified metal ions, more binding sites for cations were revealed: for example, tRNAs were shown to bind more than ten $Mg^{2+}$ ions in solution. The successful reproduction of experimentally observed metal-ion-binding sites demonstrates the efficiency of the prediction method. A promising application of the method is the prediction of cation-binding sites in RNA solution structures determined by NMR.
15 October 1998, Research Paper, *Structure*.

☐ **The crystal structure of nitrophorin 4 at 1.5 Å resolution: transport of nitric oxide by a lipocalin-based heme protein.**
John F Andersen, Andrzej Weichsel, Celia A Balfour, Donald E Champagne and William R Montfort (1998). *Structure* **6**, 1315–1327.

Nitrophorins are nitric oxide (NO) transport proteins from the saliva of blood-feeding insects that act as vasodilators and anti-platelet agents. *Rhodnius prolixus*, an insect that carries the trypanosome that causes Chagas' disease, releases four NO-loaded nitrophorins during blood feeding, whereupon the ligand is released into the bloodstream or surrounding tissue of the host. Histamine, a signaling molecule released by the host upon tissue damage, is tightly bound by the nitrophorins; this could facilitate the




release of NO and reduce inflammation in the host. Recombinant nitrophorin 4 (NP4) was expressed in *Escherichia coli*, reconstituted with heme, and found to bind NO and histamine in a manner similar to that of the natural protein. The crystal structure of NP4 revealed a lipocalin-like eight-stranded β barrel, with heme inserted into one end of the barrel. A detailed comparison with other lipocalins suggests that NP4 is closely related to the biliverdin-binding proteins from insects. The nitrophorins have a unique hemoprotein structure and are completely unlike the globins, the only other hemoproteins designed to transport dissolved gases. Compared with the recently described structure of NP1, the NP4 structure is considerably higher resolution, confirms the unusual placement of ionizable groups in the protein interior, and clarifies the solvent arrangement in the distal pocket. It also provides a striking example of structural homology where sequence homology is minimal.
15 October 1998, Research Paper, *Structure*.