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 May 1996, 3:409–411

☐ **Vitamin A-deficient quail embryos have half a hindbrain and other neural defects.** Malcolm Maden, Emily Gale, Igor Kostetskii and Maija Zile (1996). *Curr. Biol.* **6**, 417–426.

Retinoic acid (RA), a derivative of vitamin A, has been implicated as a morphogen because of its ability to induce a duplication of regenerating limbs and to establish a secondary axis in embryos. Data on the localization of RA have suggested that it functions in the central nervous system (CNS), and during gastrulation in the establishment of the anterioposterior axis. Here the authors explore the functions of RA by withholding vitamin A from the diet of quail and examining the development of their eggs. As well as the cardiovascular defects described previously in vitamin A-deficient chicks and adults, the embryos lack the posterior rhombomeres of the hindbrain (although the dorsoventral patterning appears normal). This is suprising given that the level of RA is highest at the anterior of the brain, but may be explained by an abnormality in mesoderm specification at gastrulation. Neural crest cells in the embryos develop and begin migrating, but the migration is incomplete, ending in widespread cell death. The neural tube is less well developed and no neurons extend neurites outside of it. Finally, there is a similar number of somites in the RA-deficient embryos, but they are smaller than usual. This suggests that RA may function in the control of the overall size of the body axis.

1 Apr 1996, Research Paper, *Current Biology*

☐ **Calcium-mediated apoptosis in a plant hypersensitive disease resistance response.** Alex Levine, Roger I. Pennell, Maria E. Alvarez, Robert Palmer and Chris Lamb (1996). *Curr. Biol.* **6**, 427–437.

Plants fend off avirulent pathogens by activating a battery of defenses. One rapid response is the generation of H_2O_2, a substrate for the oxidative cross-linking of structural proteins (a process that toughens the cell walls), as well as a diffusible inducer of cellular protectant genes and a trigger of cell death. Here the authors use ionophores and channel blockers to show that H_2O_2 stimulates an influx of Ca^{2+} that is both necessary and sufficient to induce an active cell death process. The dying cells exhibit the morphological features and the large (~50 kb) fragments of nuclear DNA that are characteristic of apoptosis in animals. As Ca^{2+} is a common trigger of apoptosis in animal cells, the processes in animals and plants may be related. The cell wall precludes plant cell migration, and the hypersensitive response, which is cell-autonomous, may thus represent an unspecialized, possibly primitive eukaryotic defense system, contrasting with the deployment in mammals of specialized migratory cells such as neutrophils.

1 Apr 1996, Research Paper, *Current Biology*

☐ **Multiple forms of an inositol polyphosphate 5-phosphatase form signaling complexes with Shc and Grb2.** W.M. Kavanaugh, D.A. Pot, S.M. Chin, M. Deuter-Reinhard, A.B. Jefferson, F.A. Norris, F.R. Masiarz, L.S. Cousens, P.W. Majerus and L.T. Williams (1996). *Curr. Biol.* **6**, 438–445.

Shc and Grb2 form a complex when the SH2 domain of Grb2 binds a phosphotyrosine on Shc in response to growth factor stimulation or oncogenic transformation. The complex is thought to transmit mitogenic signals from receptor and non-receptor tyrosine kinases to Ras. This paper describes additional members of the complex. The SH3 domain of Grb2, which is already known to bind the Ras nucleotide-exchange factor Sos, also mediates binding to the 110-kDa protein SIP-110. SIP-130 and SIP-145, produced by differential RNA splicing from the same gene that encodes SIP-110, bind to the phosphotyrosine binding (PTB) domain of Shc, and themselves contain SH2 domains that may bind other proteins. All three new proteins are inositol polyphosphate 5-phosphatases, and their preference for inositol substrates phosphorylated at the 3-position suggests that signaling through Shc, Grb2 and phosphatidylinositol-3 kinase are linked.

1 Apr 1996, Research Paper, *Current Biology*

☐ **A mechanism of Bud1p GTPase action suggested by mutational analysis and immunolocalization.** Merrilyn Michelitch and John Chant (1996). *Curr. Biol.* **6**, 446–454.

Three genes from the budding yeast *Saccharomyces cerevisiae*, BUD1, BUD2 and BUD5, are necessary for both the axial pattern of budding (in haploid cells) and the bipolar pattern (in diploids); in their absence budding occurs in a random pattern. Bud1p is a Ras-related GTPase, and Bud2p and Bud5p act as its GTPase-activating protein and GDP–GTP exchange factor, respectively. The authors find that Bud1p is membrane-bound in four mutant situations that affect GTP/GDP binding and hydrolysis by Bud1p, eliminating the possibility that this system mirrors that of the small GTPases involved in secretion, which cycle on and off the membrane as they proceed through a cycle of GDP/GTP exchange. Bud1p also does not appear to resemble Ras, a GTPase that is constitutively membrane associated but, when GTP-bound, recruits one of its downstream effectors to the membrane. The postulated Bud1p effectors are constitutively membrane associated, suggesting that the nucleation of these and other

polarity establishment factors may be dependent on localized GTPase cycling of Bud1p.
1 Apr 1996, Research Paper, *Current Biology*

☐ **Identification of a novel ubiquitin-conjugating enzyme involved in mitotic cyclin degradation.** Hongtao Yu, Randall W. King, Jan-Michael Peters and Marc W. Kirschner (1996). *Curr. Biol.* **6**, 455–466.
The regulated activation and inactivation of cyclin B proteolysis drives the early embryonic cell cycles of *Xenopus*. Proteolysis in *Xenopus* extracts is dependent on the ubiquitin-activating enzyme E1, at least two distinct E2 enzymes, and the cell-cycle-regulated E3 activity (variously called the cyclosome or anaphase-promoting complex (APC)). For this machinery to covalently attach ubiquitin moieties to cyclin, the cyclin must contain a specific degradation signal called the destruction box (D box). Neither of the E2 enzymes is cell-cycle regulated, and only one, UBC4, has been identified. This paper reports the identification of the other E2, UBCx, as a novel ubiquitin-conjugation enzyme, most closely related to yeast and human UBC2. (UBC2 cannot, however, substitute for UBCx.) Purified recombinant UBCx is sufficient to complement purified APC and E1 in D-box-dependent cyclin ubiquitination. UBCx and UBC4 are active in a similar concentration range and with similar kinetics. The pattern of the conjugates produced is influenced by the identity of the E2, indicating that these E2s may have distinct functions in cyclin degradation.
1 Apr 1996, Research Paper, *Current Biology*

☐ **Towards the complete structural characterization of a protein folding pathway: the structures of the denatured, transition and native states for the association/folding of two complementary fragments of cleaved chymotrypsin inhibitor 2. Direct evidence for a nucleation-condensation mechanism.** José L Neira, Ben Davis, Andreas G Ladurner, Ashley M Buckle, Gonzalo de Prat Gay and Alan R Fersht (1996). *Folding & Design* **1**, 189–208.
Single-module proteins, such as chymotrypsin inhibitor 2 (CI2), fold as a single cooperative unit. To solve its folding pathway, the authors characterize, under conditions that favor folding, its denatured and transition states and final folded structure. They use two cleaved fragments of the protein as the 'denatured state' and observe the folding process of wild-type and mutant enzymes as the fragments associate. The specific contacts in the transition state form a nucleus that extends from one fragment to the next, although the nucleus is only 'flickeringly' present in this state. This is direct evidence for the nucleation-condensation mechanism in which the nucleus is only weakly formed in the ground state and develops in the transition state.
19 Apr 1996*, Research Paper, *Folding & Design*

**Folding & Design* is published using a continuous publication system. The date given is the date that the paper appeared on the internet. You can access the World Wide Web site for all Current Biology Ltd. journals (including *Folding & Design*) via BioMedNet at http://BioMedNet.com.

☐ **X-ray structure of a hydroxamate inhibitor complex of stromelysin catalytic domain and its comparison with members of the zinc metalloproteinase superfamily.** V Dhanaraj, Q-Z Ye, LL Johnson, DJ Hupe, DF Ortwine, JB Dunbar, Jr, JR Rubin, A Pavlovsky, C Humblet and TL Blundell (1996). *Structure* **4**, 375–386.
The matrix metalloproteinases (MMPs), which include stromelysin, are a family of zinc-dependent endopeptidases important in matrix remodelling events during tissue morphogenesis and wound healing. They have been implicated in tissue degenerative diseases as diverse as arthritis and cancer. Stromelysin is an important drug target, as it is unique among the MMPs in its involvement in the regulation of the other members of the family (by activating their proenzymes) and its ability to degrade proteoglycans. The crystal structure of its catalytic domain, reported here, shows a deep specificity pocket which explains differences in inhibitor binding between the collagenases and stromelysin; such differences should facilitate the rational design of specific inhibitors. The binding of calcium ions by loops at the boundary of the active site provides a structural rationale for the importance of these cations for stability and activity. Major differences between the MMPs are clustered in the two regions forming the entrance to the active site and hence may be determinants of substrate selectivity.
15 Apr 1996, Research Paper, *Structure*

☐ **Structure of rat procathepsin B: model for inhibition of cysteine protease activity by the proregion.** Miroslaw Cygler, J Sivaraman, Pawel Grochulski, René Coulombe, Andrew C Storer and John S Mort (1996). *Structure* **4**, 405–416.
Cysteine proteases of the papain superfamily are important components of the lysosome and are involved in intracellular protein turnover, as well as the extracellular matrix protein degradation associated with many pathological conditions. Elucidation of the method by which the proregion of this class of proteases inhibits the parent protease may help in the design of more effective inhibitors. The structure of rat procathepsin B shows that inhibition occurs by blocking access to the active site. This is a common mechanism, but more unusual is that part of the proregion enters the substrate-binding cleft in a reverse orientation, and that the 62-residue proregion does not form a globular structure on its own, but folds along the surface of the mature cathepsin B. If inhibitors can be designed that also interact with these multiple sites on cathepsin B they should be potent and specific.
15 Apr 1996, Research Paper, *Structure*

☐ **The 1.1 Å crystal structure of the neuronal acetylcholine receptor antagonist, α-conotoxin PnIA from *Conus pennaceus*.** Shu-Hong Hu, John Gehrmann, Luke W Guddat, Paul F Alewood, David J Craik and Jennifer L Martin (1996). *Structure* **4**, 417–423.
α-Conotoxins are peptide toxins, isolated from *Conus* snails, that block the nicotinic acetylcholine receptor (nAChR). The

16-residue peptides PnlA and PnlB from *Conus pennaceus* differ in function from most α-conotoxins by blocking the neuronal nAchR, rather than the skeletal muscle subtype. The structure of synthetic PnlA determined here by direct methods shows that the conformation is stabilised by two disulfide bridges that form the interior of the molecule, with all the other side chains oriented outwards. The compact architecture provides a rigid framework for presentation of chemical groups that are required for activity. There are distinct hydrophobic and polar surfaces, and the sole positive and negative charges are located at opposite ends of the molecule. These features may be important for the specific recognition of the neuronal subtype of the nAchR.
15 Apr 1996, Research Paper, *Structure*

☐ **Insights into substrate binding by D-2-ketoacid dehydrogenases from the structure of *Lactobacillus pentosus* D-lactate dehydrogenase.** Vincent S Stoll, Matthew S Kimber and Emil F Pai (1996). *Structure* **4**, 437–447.

D-lactate dehydrogenases (D-LDHs) and L-lactate dehydrogenases (L-LDHs) both catalyze the reversible reduction of pyruvate to generate lactate, differing only in the chirality of the product. They act at the last step of glycolysis under anaerobic conditions, allowing the regeneration of NAD^+. The enzyme mechanism of L-LDH is well understood, and it has been proposed that the key active site substituents of D-LDH are related to those of L-LDH by a simple mirror plane. Cloning of D-LDH revealed, however, that it is a member of a different protein family, raising the possibility that the two enzymes use different enzymatic mechanisms. The crystal structure of D-LDH reported here shows that no structural relationship exists between the L-LDH and D-LDH enzymes.The small size of the common substrate may explain why the two enzymes have converged upon the use of very similar residues for binding and catalysis, but the two enzymes probably use these residues in distinctly different ways.
15 Apr 1996, Research Paper, *Structure*

☐ **The evolution of an allosteric site in phosphorylase.** Virginia L Rath, Kai Lin, Peter K Hwang and Robert J Fletterick (1996). *Structure* **4**, 463–473.

Glycogen phosphorylases catalyze the phosphorylytic degradation of glycogen into glucose 1-phosphate, the substrate for glycolysis. Sequence analysis of this family of enzymes suggests that regulatory features were added as structural modules onto a conserved catalytic core to suit the needs of the cell type in which each individual enzyme is expressed. Here the structure of yeast phosphorylase *b* is further refined and compared with the known structure of muscle phosphorylase. This allows a detailed examination of the allosteric site that binds only the inhibitor glucose 6-phosphate (Glc-6-P) in the yeast enzyme but is regulated by both inhibitors (ATP and Glc-6-P) and activators (AMP) in the muscle enzyme. It appears that the Glc-6-P site of yeast phosphorylase evolved into an allosteric switch in muscle phosphorylase by altering the binding residues such that bound ligands contact different structural elements at the subunit interface. These elements are linked to their respective catalytic sites by a common route.
15 Apr 1996, Research Paper, *Structure*

☐ **Electrostatic control of GTP and GDP binding in the oncoprotein $p21^{ras}$.** I Muegge, T Schweins, R Langen and A Warshel (1996). *Structure* **4**, 475–489.

$p21^{ras}$ is an important molecular switch, crucial for the control of cell growth and proliferation. It is active when GTP is bound, and inactive when GDP or no nucleotide is bound. Exchange factors convert p21–GDP to p21–GTP by drastically reducing the affinity of both GDP and GTP for $p21^{ras}$. This accelerates the replacement of GDP by GTP because the concentration of GTP in the cell is so much higher than that of GDP. Crystal structures of $p21^{ras}$ in all three states have been solved, and here these structures are correlated with the binding affinities of GTP and GDP by calculating the relevant electrostatic energies. Such calculations can provide a road map to the location of 'hot' residues whose mutation are likely to change functional properties of the protein. Calculation of the effect of specific mutations on GTP and GDP binding are consistent with those observed. The protein main chain provides a major contribution to the binding energies of nucleotides and is probably very important in relaying the effects of guanine-nucleotide-exchange factor (GEF) action. Mutational analysis suggests one small region as the major GEF binding site, and computer simulations indicate that the effect of GEF is probably propagated to the β-phosphate-binding P-loop, reducing the interaction between the main-chain dipoles of the P-loop and the nucleotide. This explains the similar effect of GEFs on GDP and GTP binding, as both nucleotides contain a β-phosphate.
15 Apr 1996, Research Paper, *Structure*