Elsewhere in Biology

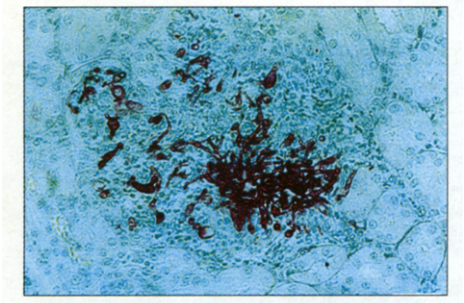
Chosen and summarized by the staff of *Chemistry & Biology*

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

Chemistry & Biology August 1997, 4:633–636

☐ **Virulence and hyphal formation of *Candida albicans* require the Ste20p-like protein kinase CaCla4p.** Ekkehard Leberer, Karl Ziegelbauer, Axel Schmidt, Doreen Harcus, Daniel Dignard, Josée Ash, Lyne Johnson and David Y Thomas (1997). *Curr. Biol.* **7**, 539–546.

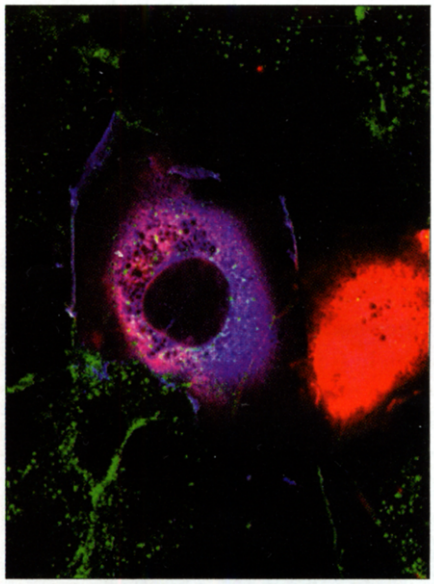
The pathogenic fungus *Candida albicans*, which causes various forms of candidiasis, can change its mode of growth from a unicellular budding yeast to a filamentous form. Extensive filamentous growth leads to the formation of hyphae with branches and lateral buds. Hyphae have been observed to adhere to and invade host tissues more readily than the yeast form, suggesting that filamentous growth may contribute to the virulence of this major human pathogen. In *Saccharomyces cerevisiae*, two members of the Ste20p serine/threonine protein kinase family, Ste20p and Cla4p, have been shown to have an essential role in budding. In this paper, the role of *C. albicans* CaCla4p, a homolog of Cla4p, in mediating morphological transitions and promoting fungal infections was investigated. Deletion of both alleles of *CaCLA4* in *C. albicans* caused defects in hyphal formation *in vitro* and also *in vivo* in a mouse model for systemic candidiasis. The gene deletions reduced colonization of the kidneys in infected mice and suppressed *C. albicans* virulence in the mouse model, indicating that the function of the CaCla4p protein kinase is essential for virulence and morphological switching of *C. albicans* in a mouse model. Hyphal formation of *C. albicans* mediated by CaCla4p may contribute to the pathogenicity of this fungus, suggesting that regulators of morphological switching may be targets for antifungal drugs.



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

☐ **Inhibition of receptor-mediated endocytosis by the amphiphysin SH3 domain.** Patrick Wigge, Yvonne Vallis and Harvey T McMahon (1997). *Curr. Biol.* **7**, 554–560.

The uptake of membrane at the cell surface by clathrin-mediated endocytosis is important for many cellular processes. Adaptor complexes bind at the cell membrane to receptors that are destined to be internalized. The protein clathrin also binds to the adaptor complexes, forming a structural scaffold for the budding vesicle, but the GTP-binding protein dynamin is required for completion of the process. How dynamin is recruited to clathrin-coated pits remains unclear. Dynamin contains several proline-rich clusters that bind to Src homology 3 (SH3) domains, and amphiphysin, a protein containing an SH3 domain, interacts with dynamin *in vitro*. The authors examined the role of amphiphysin in receptor-mediated endocytosis *in vivo*. Confocal immunofluorescence revealed that COS-7 fibroblasts transfected with the amphiphysin SH3 domain showed a potent blockade in receptor-mediated endocytosis, as measured using a transferrin-uptake-assay. To test whether the cellular target of amphiphysin is dynamin, COS-7 cells were cotransfected with both dynamin and the amphiphysin SH3 domain; here, transferrin uptake was efficiently rescued. The SH3 domains of some other proteins did not affect endocytosis. The results suggest that the SH3 domain of amphiphysin recruits dynamin to coated pits *in vivo*, probably via plasma membrane adaptor complexes. Amphiphysin might be a key player in dynamin recruitment in all cells undergoing receptor-mediated endocytosis.



9 July 1997, Research Paper, *Current Biology*

☐ **Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV.** Susan E Critchlow, Richard P Bowater and Stephen P Jackson (1997). *Curr. Biol.* **7**, 588–598.

A DNA double-strand break, induced by ionising radiation, is one of the most dangerous forms of damage that can befall a cell. Cells have evolved systems to recognize and repair this damage and one of these repair mechanisms is end-joining. Mammalian cells deficient in the XRCC4 DNA repair protein are impaired in DNA double-strand break repair and are

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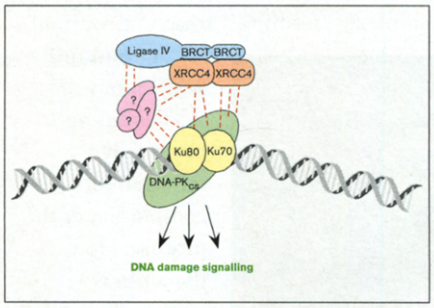
consequently hypersensitive to ionising radiation. These cells are also defective in site-specific V(D)J recombination, a process that generates the diversity of antigen receptor genes in the developing immune system. These features are shared

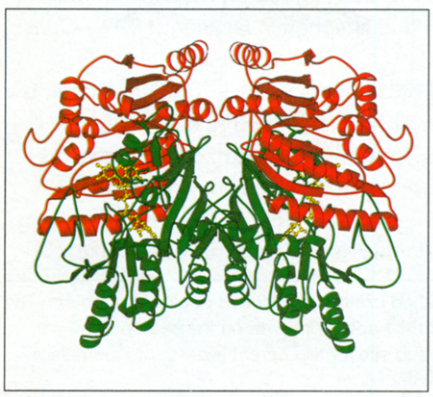


by cells lacking components of the DNA-dependent protein kinase (DNA-PK). The authors found that XRCC4 is a nuclear phosphoprotein and was an effective substrate *in vitro* for DNA-PK. Human XRCC4 was shown to associate extremely tightly with the recently identified human DNA ligase IV. XRCC4 was shown to interact with ligase IV via the unique carboxy-terminal ligase IV extension that comprises two tandem BRCT (BRCA1 carboxyl terminus) homology motifs, which are also found in other DNA repair-associated factors and in the breast cancer susceptibility protein BRCA1. These findings provide a function for the carboxy-terminal region of ligase IV and suggest that BRCT domains of other proteins may mediate contacts between DNA repair components.The results also implicate mammalian ligase IV in V(D)J recombination and the repair of radiation-induced DNA damage, and provide a model for the potentiation of these processes by XRCC4.

15 July 1997, Research Paper, *Current Biology*

☐ **Crystal structures and inhibitor binding in the octameric flavoenzyme vanillyl-alcohol oxidase: the shape of the active-site cavity controls substrate specificity.** Andrea Mattevi, Marco W Fraaije, Andrea Mozzarelli, Luca Olivi, Alessandro Coda and Willem JH van Berkel (1997). *Structure* **5**, 907–920.

Lignin degradation leads to the formation of a broad spectrum of aromatic molecules that can be used by various fungal microorganisms as their sole source of carbon. When grown on phenolic compounds, *Penicillium simplicissimum* induces the strong expression of a flavin-containing vanillyl-alcohol oxidase (VAO). The enzyme catalyzes the oxidation of a vast array of substrates, ranging from aromatic amines to 4-alkylphenols.



VAO is a member of a novel class of widely distributed oxidoreductases, which use flavin adenine dinucleotide (FAD) as a cofactor covalently bound to the protein. The paper describes the crystal structure of VAO in the native state and in complexes with four inhibitors. The structure of VAO complexed with the inhibitor 4-(1-heptenyl)phenol shows that the catalytic cavity is completely filled by the inhibitor, explaining why alkylphenols bearing aliphatic substituents longer than seven carbon atoms do not bind to the enzyme. The shape of the active-site cavity controls substrate specificity by providing a 'size exclusion mechanism'. Inside the cavity, the substrate aromatic ring is positioned at an angle of 18° to the flavin ring. This arrangement is ideally suited for a hydride transfer reaction, which is further facilitated by substrate deprotonation. Burying the substrate beneath the protein surface is a recurrent strategy, common to many flavoenzymes that effect substrate oxidation or reduction via hydride transfer.

15 July 1997, Research Paper, *Structure*

☐ **A binary complex of the catalytic subunit of cAMP-dependent protein kinase and adenosine further defines conformational flexibility.** Narendra Narayana, Sarah Cox, Nguyen-huu Xuong, Lynn F Ten Eyck and Susan S Taylor (1997). *Structure* **5**, 921–935.

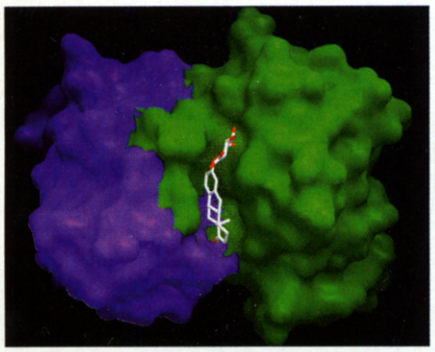
cAMP-dependent protein kinase (cAPK), a ubiquitous protein in eukaryotic cells, is one of the simplest members of the protein kinase family. It was the first protein kinase to be crystallized and continues to serve as a biochemical and structural prototype for this family of enzymes. The crystal structure of the catalytic subunit of mouse recombinant cAPK

(rC) complexed with adenosine was solved (2.6 Å resolution, R factor of 21.9%). This is the first structure of the rC subunit that lacks a bound inhibitor or substrate peptide. The structure comprises two lobes (large and small) which contain a number of conserved loops. The binary complex of rC and adenosine adopts an 'intermediate' conformation relative to the previously described 'closed' and 'open' conformations of other rC complexes. Based on a comparison of these structures, the induced fit that is necessary for catalysis and closing of the active-site cleft appears to be confined to the small lobe, as in the absence of the peptide the conformation of the large lobe, including the peptide-docking surface, does not change. Three specific components contribute to the closing of the cleft: rotation of the small lobe; movement of the carboxy-terminal tail; and closing of the so-called glycine-rich loop. There is no induced fit in the large lobe to accommodate the peptide and the closing of the cleft. A portion of the carboxy-terminal tail, residues 315–334, serves as a gate for the entry or exit of the nucleotide into the hydrophobic active-site cleft.

15 July 1997, Research Paper, *Structure*

☐ **Antibody fragment Fv4155 bound to two closely related steroid hormones: the structural basis of fine specificity.** Chi H Trinh, Sandra D Hemmington, Martine E Verhoeyen and Simon EV Phillips (1997). *Structure* **5**, 937–948.

The concentration of steroid glucuronides in serial samples of early morning urine (EMU) can be used to predict the fertile period in the female menstrual cycle. The monoclonal antibody 4155 has been used as a convenient means of measuring the concentration of steroid glucuronides in EMU, as it specifically recognises the steroid hormone estrone

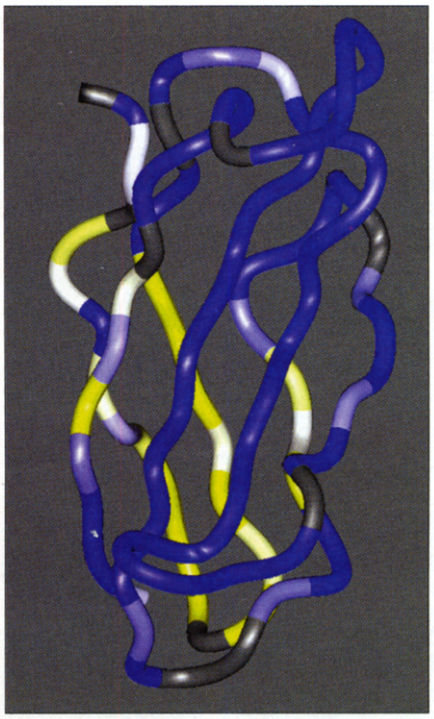


β-D-glucuronide (E3G), with very high affinity, and the closely related hormone estriol 3-(β-D-glucuronide) (El3G), with reduced affinity. Although 4115 binds these hormones with different affinities, EI3G differs from E3G only in the addition of a hydroxyl group and reduction of an adjacent carbonyl. To investigate the structural basis of this fine binding specificity, the crystal structures of the variable fragment (Fv) of 4155 in complex with each of these hormones was determined. In both complexes the binding of the glucuronic sugar, and rings A and B of the steroid, is specified by the shape of the narrow cleft. The difference in the binding affinity of Fv4155 for the two steroid hormones is accounted for by a subtle combination of a less favoured hydrogen-bond geometry, and a minor rearrangement of the water molecule network around the binding site, rather than changes in the Fv molecule. This emphasizes the importance of structured water molecules in biological recognition.

15 July 1997, Research Paper, *Structure*

☐ **Backbone dynamics of homologous fibronectin type III cell adhesion domains from fibronectin and tenascin.** Peter A Carr, Harold P Erickson and Arthur G Palmer III (1997). *Structure* **5**, 949–959.

Fibronectin type III domains are found as autonomously folded domains in a large variety of multidomain proteins, including extracellular matrix proteins. A subset of these domains employ an Arg–Gly–Asp (RGD) tripeptide motif to mediate contact with cell-surface receptors (integrins). This motif mediates protein–protein interactions in a diverse range of biological processes, such as in tissue development, wound healing and metastasis. The molecular basis for affinity and specificity of cell adhesion via type III domains has not been clearly established. The tenth type III domain from fibronectin (FNfn10) and the third type III domain from tenascin-C (TNfn3) have 27% sequence identity and share the same overall protein fold, but present the RGD motifs in



different structural contexts. The dynamical properties of the RGD motifs may affect the specificity and affinity of the FNfn10 and TNfn3 domains. The intramolecular dynamics of the protein backbones of FNfn10 and TNfn3 were studied by ^{15}N nuclear spin relaxation. A comparison of the structures of the FNfn10 and TNfn3 revealed several features related to their different dynamical properties. The larger amplitude motions of loops in FNfn10 are consistent with the hypothesis that flexibility of these regions facilitates induced-fit recognition of fibronectin by multiple receptors. Similarly, the more rigid loops of TNfn3 may reflect greater specificity for particular integrins. The correlations observed between structural features and dynamical properties of the homologous type III domains indicate the influence of hydrogen bonding and hydrophobic packing on dynamical fluctuations in proteins and serves as an example of the high information content of dynamics analyses by NMR.

15 July 1997, Research Paper, *Structure*

☐ **Structure of poliovirus type 2 Lansing complexed with antiviral agent SCH48973: comparison of the structural and biological properties of the three poliovirus serotypes.** Karen N Lentz, Allen D Smith, Sheila C Geisler, Stuart Cox, Peter Buontempo, Angela Skelton, Jason DeMartino, Edward Rozhon, Jerome Schwartz, V Girijavallabhan, John O'Connell and Edward Arnold (1997). *Structure* **5**, 961–978.

Polioviruses are human pathogens and the causative agents of poliomyelitis. Polioviruses are icosahedral single-stranded RNA viruses, which belong to the picornavirus family, and occur as three distinct serotypes. All three serotypes of poliovirus can infect primates, but only type 2 can infect mice. The crystal structures of a type 1 and a type 3 poliovirus are already known. Structural studies of poliovirus type 2 Lansing (PV2L) were initiated to try to enhance our understanding of the differences in host range specificity, antigenicity and receptor binding among the three serotypes of poliovirus. The crystal structure of the mouse neurovirulent PV2L complexed with a potent antiviral agent, SCH48973, was determined at

2.9 Å resolution. Structural differences among the three poliovirus serotypes occur primarily in the loop regions of the viral coat proteins (VPs). Some of the conformational changes required for infectivity and involved in the control of capsid stability and neurovirulence in mice may occur in the vicinity of the fivefold axis of the poliovirus, where there are significant structural differences among the three poliovirus serotypes in the surface exposed loops of VP1. A surface depression is located at the fivefold axis of PV2L that is not present in the other two poliovirus serotypes. The observed interaction of RNA with VP4 supports the observation that loss of VP4 ultimately leads to the loss of viral RNA. A model is proposed that suggests dual involvement of the virion fivefold and pseudo-threefold axes in receptor-mediated initiation of infection by picornaviruses.

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