ELSEWHERE IN BIOLOGY

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

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

Chemistry & Biology November 1995, **2**:771–773

► Laue Crystallography: **It's show time**
Gregory K Farber (1995). *Curr. Biol.* **5**, 1088–1090.

To understand how an enzyme catalyzes a chemical reaction, enzymologists would like to a have a series of structures representing all the important points of the reaction. Such a molecular movie would give one a new type of enzyme mechanism — the structural mechanism. X-ray crystallography is the obvious tool to use to determine the structural mechanism of an enzyme, but monochromatic data collection takes hours, far longer than the milliseconds enzymes typically take to catalyze a reaction. In the time required to collect the data, the enzyme cycles through many catalytic events, and the structure one sees is that of the enzyme with an empty active site (the unbound enzyme has a lower free energy than any intermediate). Such problems can now be overcome in two ways. By altering substrate and product concentrations in the crystal lattice, one can sometimes cause one intermediate to have a lower free energy than all the others, and thus obtain its structure. It is now also possible to obtain complete data sets on a millisecond timescale by using the polychromatic Laue method of data collection at a synchrotron X ray source. These methods, combined with site-directed mutagenesis, have recently been used to produce an almost complete view of how isocitrate dehydrogenase works.
1 October 1995, Dispatch, *Current Biology*

► DNA-Mismatch Repair: **The intricacies of eukaryotic spell-checking**
Thomas A Kunkel (1995). *Curr. Biol.* **5**, 1091–1094.

The rare errors left behind by the cell's replication machinery are later corrected by the spell-checking machinery, which undertakes post-replication DNA-mismatch repair. Interest in this process in human cells was greatly stimulated two years ago by studies suggesting that inactivation of mismatch repair may be the initial event leading to certain types of cancers. A flurry of reports in the last few months suggest that the mismatch-repair system is more complex than was previously thought, and that its multiple gene products participate in a variety of fundamentally important DNA transactions. It appears that eukaryotic mismatch-repair genes, much like their bacterial counterparts, safeguard the genome from promiscuous genetic exchange between DNA strands with less than perfect homology. Other evidence suggests that the mismatch-repair system may participate in control of the cell cycle at the G2 'checkpoint' that prevents cell-cycle progression in response to treatment with the base analog 6-thioguanine or other DNA-damaging agents.
1 October 1995, Dispatch, *Current Biology*

► Protein–Protein Interactions: **Putting the pieces together**
Ben Bax and Harren Jhoti (1995). *Curr. Biol.* **5**, 1119–1121.

The effector region of the Ras signalling molecule is known to be involved in interactions with downstream effector molecules such as the serine/threonine protein kinase Raf and phosphatidylinositol 3-kinase (PI 3-kinase). The mechanism by which Ras activates Raf involves direct association between the two proteins, mediated by Raf's amino-terminal Ras-binding domain (RBD). The recently determined crystal structure of the Raf RBD bound to the Ras homolog Rap1A provides a structural basis for understanding the interaction between Ras and Raf, and may also provide a view of how members of the highly conserved 14-3-3 family of proteins might bind to and regulate Raf. The recently determined structures of the τ and ζ isoforms of 14-3-3 show a channel or clamp in the dimer whose inner surface is highly conserved amongst different isoforms, suggesting a binding site for a conserved protein module. The fact that 14-3-3 proteins can form heterodimers suggests that such heterodimers might bring two different signalling molecules together, and suggests a possible mechanistic connection between the way Ras and 14-3-3 proteins activate Raf: Raf may be turned on by a combination of Ras and kinases such as Src or Lck, or by a 14-3-3 heterodimer plus PKC, a kinase known to be activated by 14-3-3 proteins. The idea that a 14-3-3 heterodimer brings together PKC and Raf, allowing PKC to phosphorylate and activate Raf, is attractive but unproven, and the *in vivo* functions of 14-3-3 proteins are not yet clear.
1 October 1995, Dispatch, *Current Biology*

► Cell Cycle: **The NIMA kinase joins forces with Cdc2**
Andrew M Fry and Erich A Nigg (1995). *Curr. Biol.* **5**, 1122–1125.

The Cdc2 protein kinase is a cornerstone in our understanding of mitosis. This kinase, composed of the $p34^{cdc2}$ catalytic subunit and a B-type cyclin regulatory subunit, is the prototype for a family of cyclin-dependent kinases (CDKs) required for cell-cycle progression in all eukaryotes. Intriguingly, in the filamentous fungus *Aspergillus nidulans*, a second protein kinase, NIMA, is also required for mitotic progression. At first sight, NIMA appeared to lie on a pathway independent of Cdc2, but recent developments suggest that the two pathways are interlinked, with NIMA possibly being a substrate of the Cdc2 kinase. As is the case with cyclin B, a mutant of NIMA that is resistant to destruction by proteolysis causes mitotic arrest. The isolation of Nek (NIMA-related kinase) cDNAs from mammals and the characterization of human Nek2 suggest that Cdc2 and NIMA may also coordinately regulate mitosis in higher eukaryotes.
1 October 1995, Dispatch, *Current Biology*

► **Identification and partial characterization of a domain in CFTR that may bind cyclic nucleotides directly**
Stephen K Sullivan, Luis B Agellon and Rong Schick (1995). *Curr. Biol.* **5**, 1159–1167.

The cystic fibrosis transmembrane regulator (CFTR) is a chloride channel that is activated by cAMP-dependent phosphorylation. Its activity is also stimulated by cGMP-dependent protein kinase and protein kinase C. In a demonstration of a new pathway for the activation of CFTR, the authors compare the response to cAMP and to cGMP of CFTR expressed in *Xenopus* oocytes. They show that inhibition of protein kinase activity does not diminish the response to cGMP, and that a domain in the proximal portion of the third cytoplasmic loop resembles a class of cyclic-nucleotide-binding domains (cNBDs) related to that of the catabolite-gene activator protein (CAP). Moreover, two point mutations in this domain, predicted to disrupt direct

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contact with cGMP, alter the response to cGMP with no effect on the response to cAMP. The results support the idea that a site for direct cyclic-nucleotide activation has been identified on the CFTR, which may provide a useful target for pharmacological intervention in cystic fibrosis.
1 October 1995, Research Paper, *Current Biology*

► **Role of the regulatory domain of the EGF-receptor cytoplasmic tail in selective binding of the clathrin-associated complex AP-2**
Werner Boll, Andreas Gallusser and Tomas Kirchhausen (1995). *Curr. Biol.* **5**, 1168–1178.
Binding of the mitogenic epidermal growth factor (EGF) to the EGF receptor (EGF-R) leads to signaling events that stimulate cell growth. It also leads to the internalization of EGF-R in clathrin-coated pits and its subsequent lysosomal destruction, serving to down-regulate signaling. Clathrin-associated protein complexes (APs) link clathrin to the activated receptors, interacting with the cytoplasmic tails of the receptors and promoting the assembly of the clathrin lattice. What it is not known is how the specificity of this interaction is controlled. Internalization is abolished in EGF-R mutants that lack a cytoplasmic kinase domain, but mutation of the auto-phosphorylation sites in the EGF-R does not prevent endocytosis. This paper examines the association between the EGF-R and the AP present on the cytoplasmic membrane, AP-2, observing a specific association both *in vivo* and *in vitro* which is not seen using the AP from the trans-Golgi network, AP-1. The EGF-R–AP-2 association is dependent on the presence of the regulatory domain of the cytoplasmic tail of the receptor, but not on the kinase activity of the receptor. This suggests that the kinase activity of the receptor is required in endocytosis for a step other than the EGF-R–AP-2 association, perhaps the phosphorylation of a third, unidentified protein.
1 October 1995, Research Paper, *Current Biology*

► **The *chk1* pathway is required to prevent mitosis following cell-cycle arrest at 'start'**
Anthony M Carr, Mohammed Moudjou, Nicola J Bentley and Iain M Hagan (1995). *Curr. Biol.* **5**, 1179–1190.
The cell cycle can be viewed as a $p34^{cdc2}$ cycle, driven by the regular oscillations in the activity of this kinase. When the progression of downstream events such as DNA replication are perturbed, however, checkpoints can arrest the cell cycle until the error is corrected. The Chk1 protein kinase has been implicated in the arrest of the cell cycle after DNA damage, although it is not required for arrest when DNA synthesis is inhibited. In this paper a new mitotic control pathway is defined in *Schizosaccharomyces pombe* (the G1 checkpoint); Chk1 is found to be required to prevent cells arrested at 'start' (the point in G1 at which cells make the decision whether to enter the next cycle) from entering mitosis. Temperature-sensitive mutants in the G1 transcription factor encoded by the *cdc10* gene usually arrest at 'start', but double mutants that are also defective for *chk1* function cannot arrest. They undergo an aberrant mitosis without, apparently, entering S-phase, as their cell cycle progression cannot be blocked by a DNA synthesis inhibitor. The G1 checkpoint may monitor the pre-replication complexes known to be present in G1 phase, thus preventing the cell from entering mitosis before it has begun DNA replication.
1 October 1995, Research Paper, *Current Biology*

► **Functional significance of cytochrome *c* oxidase structure**
Robert A Scott (1995). *Structure* **3**, 981–986.
Cytochrome *c* oxidase is the third complex in the electron-transfer chain which generates the proton gradient necessary for oxidative phosphorylation. Phylogenetically, the three core subunits of cytochrome *c* oxidase appear to predate the evolution of oxygenic photosynthesis, presumably in a form that used an alternative to the present electron acceptor, molecular oxygen. The structure of this complex has been sought to help explain its many activities: electron transport, oxygen activation and proton pumping. The author reviews the first two structures of cytochrome *c* oxidase, obtained from *Paracoccus denitrificans* and cow. The structures agree with each other and, in many respects, with previous structure predictions made on the basis of hydropathy plots, mutagenesis and other structural probes. The number and length of transmembrane domains, the location of active sites coordinating the cytochromes and metal ligands, and two metal cofactor sites (for Zn^{2+} and Mg^{2+}) were all successfully predicted. In addition, the *P. denitrificans* structure identifies a number of residues which may aid in proton transfer by a series of minor, redox-linked conformational changes. Both structures reveal candidate residues which may function in electron transport by bridging the gaps between reaction centers. Confirmation of these proposed functions should be possible using standard techniques such as *in vitro* mutagenesis.
15 October 1995, Minireview, *Structure*

► **Evolutionary conservation in the hepatitis B virus core structure: comparison of human and duck cores**
John M Kenney, Carl-Henrik von Bonsdorff, Michael Nassal and Stephen D Fuller (1995). *Structure* **3**, 1009–1019.
Hepatitis B virus (HBV) and the related hepadnaviruses have two layers surrounding their DNA genome: an outer membrane composed of cellular lipids and viral envelope proteins, and an internal nucleocapsid (or core) assembled from dimers of a single core protein. The exterior of the core interacts with the envelope proteins in viral assembly, and its interior provides a suitable environment for reverse transcription of the RNA pre-genome. Previously, cryoelectron microscopy was used to determine the three-dimensional structure of HBV core particles. Here, the structures of various core particles formed from full length and truncated core proteins from HBV and the related duck virus, DHBV, are presented. Despite substantial variation in the length and sequence of the duck and human core proteins, the particles appear largely similar, with a characteristic icosahedral organization. The core particles formed by proteins expressed in *Escherichia coli* faithfully reproduce the native core structure even in the absence of the viral genome, confirming that proper assembly of the core is independent of genome packaging. The apparent sites of interaction between the core proteins and the genome are conserved between the duck and human viruses, but the projecting domains on the exterior of the core, which appear to be responsible for interactions between the core and envelope, vary.
15 October 1995, Research article, *Structure*

► **2 Å crystal structure of an extracellular fragment of human CD40 ligand**
Michael Karpusas, Yen-Ming Hsu, Jia-huai Wang, Jeff Thomson, Seth Lederman, Leonard Chess and David Thomas (1995). *Structure* **3**, 1031–1039.
The CD40 ligand (CD40L) is a member of the tumor necrosis factor (TNF) family of proteins and is transiently expressed on the surface of activated T cells. The binding of CD40L to CD40, which is expressed on the surface of B cells, provides an important pathway of cellular activation that is involved in essential immune system functions such as antibody isotype switching and establishing immunological memory. Several mutations of CD40L are known to cause a severe immunodeficiency, known as hyper-IgM syndrome (HIGMS), characterized by an inability

to produce immunoglobulins of the IgG, IgA and IgE isotypes. Inhibitors of CD40L signaling might have important therapeutic applications. The authors determined the crystal structure of a soluble extracellular fragment of human CD40L. Although the molecule forms a trimer similar to that found for other members of the TNF family and exhibits a similar overall fold, the structure shows considerable differences in several loops including those predicted to be involved in CD40 binding. The structure suggests that most of the HIGMS mutations affect the folding and stability of the molecule rather than the CD40-binding site directly. It is possible that single mutations in the CD40-binding site are insufficient to disrupt CD40–CD40L binding, and are therefore clinically undetectable.
15 October 1995, Research Article, *Structure*

► **The solution structure of the Mu Ner protein reveals a helix-turn-helix DNA recognition motif**
Teresa E Strzelecka, G Marius Clore and Angela M Gronenborn (1995). *Structure* **3**, 1087–1095.
The Mu Ner protein is a 74-amino-acid, basic, DNA-binding protein found in phage Mu. It belongs to a class of proteins, the cro and repressor proteins, that regulate the switch from the lysogenic to the lytic state of the phage life cycle. Despite the functional similarity between Mu Ner and the cro proteins of other phages, the proteins share no significant sequence identity. The authors solved the three-dimensional solution structure of Mu Ner by three-dimensional and four-dimensional heteronuclear magnetic resonance spectroscopy. The structure consists of five α-helices, two of which comprise a helix-turn-helix (HTH) motif that is likely to be in contact with DNA. The cro proteins from phage λ and 434 also possess a HTH DNA recognition motif. Thus, functional similarity is coupled to structural similarity, lending support to the notion that phages Mu and λ may have a common ancestor. The Ner protein from phage D108 and the Nlp protein from *E. coli* are likely to have very similar tertiary structures due to high amino acid sequence identity with Mu Ner.
15 October 1995, Research Article, *Structure*

► **Crystal structure of thioredoxin-2 from *Anabaena***
Markku Saarinen, Florence K Gleason and Hans Eklund (1995). *Structure* **3**, 1097–1108.
Thioredoxins are ubiquitous reducing agents capable of regulating the activity of other enzymes by reducing disulfides. Thioredoxin-2 (Trx-2) is one of two thioredoxins found in the cyanobacterium, *Anabaena*. It is present at low levels, and is one of the most divergent thioredoxins, with 12 of 38 otherwise conserved residues altered. Despite these differences and the overall low level of homology, the structure of Trx-2 is highly similar to the previously determined structures of the *Escherichia coli* and human enzymes. Nearly all secondary structure relationships are conserved, and the conformation and disulfide geometry of the active-site disulfide ring is almost identical between the *E. coli* and *Anabaena* enzymes in spite of the differences in main-chain hydrogen bonding within this region. The tyrosine replacing the conserved aspartate near the active site occupies more space at the backside of the disulfide ring, and the alterations in structure induced by this change may have a significant effect on substrate specificity. The *Anabaena* structure shows a larger hydrophobic region near the active-site cysteines which may also affect enzyme–substrate interactions. Thus it appears that single amino acid substitutions around the protein interaction area account for the unusual enzymatic activities and substrate specificity of Trx-2.
15 October 1995, Research Article, *Structure*

► **Structure of β_2-bungarotoxin: potassium channel binding by Kunitz modules and targeted phospholipase action**
Peter D Kwong, Neil Q McDonald, Paul B Sigler and Wayne A Hendrickson (1995). *Structure* **3**, 1109–1119.
β-bungarotoxin is a heterodimeric neurotoxin consisting of a phospholipase subunit linked by a disulfide bond to a member of the Kunitz (pancreatic trypsin type) protease inhibitor superfamily. The toxin has no protease inhibitor capacity, however, and the Kunitz subunit serves instead to target the toxin to pre-synaptic membranes via its interaction with a subclass of voltage-sensitive K^+ channels. Once bound, the lipolytic action of the toxin permeabilizes and thus depolarizes the membrane. Here the authors solve the structure of the toxin at 2.45 Å. The channel binding region of the Kunitz subunit (identified by sequence comparison and solvent accessibility) is at the opposite end of the subunit from the loop typically involved in protease binding, and involves the only other exposed loop in the subunit. Recognition of the channel by the toxin may mimic the binding of some as yet poorly characterized endogenous neuropeptides, which are proposed to interact with the K^+ channels directly. The phospholipase subunit is highly specific; its low level of non-specific membrane binding may be due to a partially occluded, weakly hydrophobic active site. This low affinity of binding is overcome by the K^+ channel–Kunitz subunit interaction, which in turn leads to displacement of the occluding active-site residue of the phospholipase into the membrane where it acts as an anchor. The high specificity of the toxin, and the separation of the catalytic and localization functions in two distinct subunits, makes β-bungarotoxin an attractive candidate for modification to achieve the therapeutic destruction of infected cells and enveloped viruses.
15 October 1995, Research Article, *Structure*