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 October 1995, **2**:697–699

► Cell Cycle: **The only way out of mitosis**
Michael Glotzer (1995). *Curr. Biol.* **5**, 970–972.

The progression of the cell cycle through mitosis is governed by the activation and inactivation of cyclin-dependent kinase 1 (Cdk1, also known as $p34^{cdc2}$), whose activity oscillates during the cell cycle because its activating subunit, cyclin B, is stable in interphase and unstable in mitosis. Cyclin stability, in turn, is under control of the ubiquitin protein-degradation system, in which proteins are linked to a chain of ubiquitin subunits, marking them for degradation by the proteasome. It has recently been shown that the multiprotein anaphase-promoting complex, or APC, ubiquitinates cyclin B. This complex contains the products of the cell-cycle regulatory genes *CDC16*, *CDC23*, *CDC27* and additional factors; it has also been shown to promote the separation of sister chromatids at anaphase, and probably ubiquitinate other substrates as well as cyclins. The APC is itself regulated indirectly by cyclin B. Further study of these and other aspects of the APC will help to elucidate the multiple ways in which the complex is regulated to ensure high-fidelity cell division.
1 September 1995, Dispatch, *Current Biology*

► Protein Kinase C: **Seeing two domains**
Alexandra C Newton (1995). *Curr. Biol.* **5**, 973–976.

The protein kinase C family of enzymes are important in the transduction of signals that promote lipid hydrolysis to produce second messengers. To qualify as a protein kinase C a protein must have both a lipid regulatory region and a kinase domain. The lipid regulatory domain binds to the lipid messenger diacylglycerol; this results in the removal of an autoinhibitory pseudosubstrate domain from the active site, so that the protein becomes catalytically active, and also causes a dramatic increase in the enzyme's affinity for membranes, resulting in its relocation to the plasma membrane. The regulatory region can be further subdivided into the C1 domain, which binds diacylglycerol or its functional analogs, the phorbol esters, and the C2 domain, which binds calcium. Recent reports have revealed how ligand interactions in the regulatory half of protein kinase C affect the location of the protein. The cocrystal structure of the C1 domain and phorbol ester indicates that phorbol ester binding could increase the protein's membrane affinity by creating a contiguous surface of hydrophobic residues. The crystal structure of the C2 domain of synaptotagmin shows how binding of Ca^{2+} may cause an allosteric change which causes bulky aromatic residues to stick out and serve as effective membrane anchors.
1 September 1995, Dispatch, *Current Biology*

► Natural Killer Cells: **Right-side-up and up-side-down NK-cell receptors**
Wayne M Yokoyama (1995). *Curr. Biol.* **5**, 982–985.

Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells both kill target cells and produce cytokines that regulate acquired immune responses. These cells appear to share an immediate common progenitor cell and similar methods of cell killing, but recognize different receptors and thus have distinct criteria for recognizing target cells. Every CTL displays a unique antigen-specific T-cell receptor that recognizes foreign peptides presented by major histocompatibility complex (MHC) class I molecules on target cells. NK cells, on the other hand, do not express the T-cell receptor and do not require MHC class I molecules on targets cells to kill them. In fact, MHC class I molecules inhibit NK-cell activation, suggesting that NK cells survey tissues for aberrant or absent expression of these molecules. Two groups have now used different strategies to clone cDNAs encoding two human NK-cell receptors for MHC class I molecules, termed p58 and NKAT. The receptors are closely related, and are members of the immunoglobulin superfamily. They are very different from the apparent functional homolog in mouse, however; the human protein is oriented with the carboxyl terminus in the cytoplasm, whereas the mouse protein has the reverse orientation and has homology to the C-type lectins. This suggests that mouse and man may have evolved structurally distinct molecules to serve identical functions. It remains possible, however, that corresponding homologs of the human receptors in the mouse and *vice versa* have not yet been identified.
1 September 1995, Dispatch, *Current Biology*

► Intracellular Signalling: **New directions for phosphatidylinositol transfer**
Tom Martin (1995). *Curr. Biol.* **5**, 990–992.

Phosphatidylinositol 4,5 bisphosphate (PIP_2) is the precursor of inositol 1,4,5-trisphosphate, the Ca^{2+}-mobilizing second messenger. PIP_2 itself also has regulatory roles in enzyme activation, cytoskeletal regulation and membrane trafficking; other phosphorylated inositides have been implicated as messengers in similar pathways. As there are several interlocking pathways of phosphatidylinositide phosphorylation, and as the regulatory roles of the phosphorylated lipids require them to be localized in specific areas of the cell, there must be cellular mechanisms for compartmentalizing the metabolism of these lipids and circumscribing their actions. A number of recent reports suggest that a cytosolic protein, phosphatidylinositol transfer protein (PI-TP), is important in these processes. PI-TP can exchange phosphatidylinositol (PI) and phophatidylcholine between lipid bilayers, and has recently been shown to present PI to a complex of enzymes that generates PIP_2 and includes PI 4-kinase and PIP 5-kinase. Selective presentation of PI to this or to other enzyme complexes may account for the segregated metabolism of phosphatidylinositides. There is also increasing evidence that PI-TP is involved in membrane fusion and fission. Recent studies have shown that PI-TP is required for the biogenesis of secretory vesicles *in vitro*, and it is possible that PIP_2 formation on a vesicle may trigger phospholipase-D-catalyzed changes in membrane composition, leading to fusion.
1 September 1995, Dispatch, *Current Biology*

► Protein Folding: **Prolyl isomerases join the fold**
Franz X Schmid (1995). *Curr. Biol.* **5**, 993–994.

Unfolded proteins contain a mixture of fast-folding and slow-folding species. Twenty years ago the 'proline hypothesis' was proposed to explain this phenomenon. Fast- and slow-folding molecules differ in the conformational state — *cis* or *trans* — of

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one or more Xaa–Pro peptide bonds. In the fast-folding molecules, the prolyl peptide bonds are in the same isomeric state as in the native protein, whereas in the slow-folding molecules, one or more prolyl peptide bonds are in the incorrect, non-native state. Prolyl isomerases catalyze the isomerization of these bonds. Cyclophilin, a prolyl isomerase, is the cytosolic binding protein for the immunosuppressive drug cyclosporin A (CsA). The role of cyclophilin in immunosuppression does not involve its prolyl isomerase activity, however; the cyclophilin–CsA complex binds to and inhibits the phosphatase calcineurin. But recently, two groups have provided evidence that the prolyl isomerase activity of cyclophilin may be involved in cellular protein folding; CsA decreases the rate of folding of a test protein in mitochondria by about five-fold. A non-immunosuppressive variant of CsA also retarded protein folding; in mutant cells that lack a functional cyclophilin, the folding rate is decreased, but is not further inhibited by CsA. Thus, cyclophilin seems to be directly important in the folding reaction, although it is still unclear why mutant cells that lack cyclophilins are almost perfectly viable.
1 September 1995, Dispatch, *Current Biology*

► Transcriptional Activation: **A holistic view of the complex**
Michael F Carey (1995). *Curr. Biol.* **5**, 1003–1005.

In prokaryotic cells the transcriptional apparatus exists as a holoenzyme comprising the RNA polymerase and ancillary factors. DNA-binding activator proteins stimulate transcription by contacting a surface of the holoenzyme and recruiting it to the DNA in a single step. Recent studies now indicate that the eukaryotic transcriptional apparatus in the yeast *Saccharomyces cerevisiae* may also exist as a multicomponent complex that is recruited to DNA by activator proteins. The traditional view of eukaryotic gene activation proposes that activators stimulate the stepwise assembly of a transcription complex, composed of RNA polymerase II and six general factors called TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH, which are conserved between yeast and humans. It now seems, however, that eukaryotic cells also contain a holoenzyme; a multiprotein complex was recently isolated from yeast cells that contains RNA polymerase II, TFIIB, TFIIF, TFIIH, two activators termed SUG1 and GAL11 and the 'suppressors of RNA polymerase B' (SRB) proteins. The functions of the SRB proteins are not yet understood. This holoenzyme can support transcription *in vitro* upon addition of only TFIIE and the TATA-box binding protein (TBP), a component of TFIID. Molecular biological studies have also recently shown that transcription can be activated in eukaryotes by tethering a single component of the holoenzyme, GAL11, to a DNA-binding domain. In eukaryotes, like prokaryotes, activators may stimulate transcription by binding to a DNA site and recruiting the RNA polymerase holoenzyme to the promoter in a single step.
1 September 1995, Dispatch, *Current Biology*

► **Re-creating the RNA world**
Ichiro Hirao and Andrew D Ellington (1995). *Curr. Biol.* **5**, 1017–1022.

The discovery of catalytic RNA has led to speculation that a prebiotic self-replicating system, the 'RNA world', may have existed in which RNA carried out biochemical reactions mirroring those found in modern life. The development of techniques for nucleic acid amplification has allowed models of the molecular evolution of nucleic acids to be recapitulated in a test tube. In this article, Hirao and Ellington review recent studies addressing three different aspects of the 'RNA world' hypothesis: that RNA catalysts can be functionally diverse, that the origin of the genetic code may lie in amino acid–RNA interactions, and that the evolution of the translation apparatus and other functional RNAs could have been guided by interactions with low molecular weight effectors. They conclude that the total number of potential RNA binding motifs and catalysts is massive, and therefore the molecular fossils, molecules that are believed to have survived from the 'RNA world' to the present, may just be 'frozen accidents of biology'.
1 September 1995, Review, *Current Biology*

► **Cooperative binding of Tn*3* resolvase monomers to a functionally asymmetric binding site**
David G Blake, Martin R Boocock, David J Sherratt and W Marshall Stark (1995). *Curr. Biol.* **5**, 1036–1046.

The inverted repeat is a common feature of protein-binding sites in DNA. These sites vary considerably in length and the two-fold symmetry in the longer sites is rarely perfect; that is, the sequences of the two repeats do not match exactly. The reasons for this asymmetry are investigated using the 34 base-pair binding site II for the resolvase protein of transposon Tn*3*. This site contains inverted 12 base-pair motifs, which are not identical in sequence, separated by 10 base pairs. Two molecules of Tn*3* resolvase bind to this site. Using gel retardation studies, Blake *et al.* show that a monomer of Tn*3* resolvase first binds to the left-hand repeat, then a second monomer binds cooperatively to the right-hand repeat. Having one strong-binding recognition element might facilitate initial recognition of a site by its cognate protein, and increase the overall rate of formation of the dimer complex *in vivo*. The resolvase dimer–DNA complex is structurally asymmetric; this architecture may hold the DNA in an appropriate geometry for recombination to take place. The natural asymmetry of protein-binding sites may thus be essential to the structures and functions of the protein–DNA complexes.
1 September 1995, Research Paper, *Current Biology*

► **Transgenic expression of human acetylcholinesterase induces progressive cognitive deterioration in mice**
Rachel Beeri, Christian Andres, Efrat Lev-Lehman, Rina Timberg, Tamir Huberman, Moshe Shani and Hermona Soreq (1995). *Curr. Biol.* **5**, 1063–1071.

Progressive deterioration of memory and learning is a characteristic manifestation of Alzheimer's disease. This deterioration is notably associated with structural changes and subsequent cell death which occur, primarily, in acetylcholine (ACh)-producing neurons, progressively damaging cholinergic neurotransmission. Despite the advancement of the cholinergic theory of Alzheimer's disease and the development of cholinergic therapies, the question of whether imbalanced cholinergic neurotransmission can, by itself, contribute to the progressive decline in Alzheimer's disease has not been addressed. The authors report that the expression of the human ACh-hydrolyzing enzyme, acetylcholinesterase (AChE), in brain neurons of transgenic mice creates impairments in spatial learning and memory which appear shortly after early adulthood and become progressively more severe. In addition to displaying resistance to the hypothermic effects of the AChE inhibitor, paraoxon, the mice resisted muscarinic, nicotinic and serotonergic agonists, indicating that secondary pharmacological changes had occurred. The results suggest that upsetting cholinergic balance may contribute to the pathophysiology of Alzheimer's disease.
1 September 1995, Research Paper, *Current Biology*

► **Structures and mechanisms of glycosyl hydrolases**
Gideon Davies and Bernard Henrissat (1995). *Structure* **3**, 853–859.

Carbohydrates show wide stereochemical variation and can be assembled in many different fashions; living organisms take advantage of this diversity by using oligosaccharides and

polysaccharides for functions ranging from storage and structure to signalling. Selective hydrolysis of glycosidic bonds is therefore crucial for a number of important biological functions. The are presently 52 families of glycosyl hydrolases, and structures have been determined for at least one member of 22 different families (two are reported in this issue of *Structure*), providing insight into their mechanisms of action and the way in which selectivity is achieved. Many of the known structures have a triosephosphate isomerase (TIM) barrel fold.
15 September 1995, Minireview, *Structure*

See also: **Crystal structure of the catalytic domain of a bacterial cellulase belonging to family 5.** Valérie Ducros, Mirjam Czjzek, Anne Belaich, Christian Gaudin, Henri-Pierre Fierobe, Jean-Pierre Belaich, Gideon J Davies and Richard Haser (1995). *Structure* **3**, 939–949; and

The crystal structure of a cyanogenic β-glucosidase from white clover, a family 1 glycosyl hydrolase. T Barrett, CG Suresh, SP Tolley, EJ Dodson and MA Hughes (1995). *Structure* **3**, 951–960.

► **Mechanistic implications from the structure of a catalytic fragment of Moloney murine leukemia virus reverse transcriptase**
Millie M Georgiadis, Sven M Jessen, Craig M Ogata, Alice Telesnitsky, Stephen P Goff and Wayne A Hendrickson (1995). *Structure* **3**, 879–892.
Reverse transcriptase (RT) converts the single-stranded RNA genome of a retrovirus into a double-stranded DNA copy for integration into the host genome. The overall organization of HIV-1 RT is known from previously reported crystal structures, but these structures lacked the metal ion that is necessary for activity. The authors report a 1.8-Å resolution crystal structure of a catalytically active fragment of RT from Moloney murine leukemia virus (MMLV), and a 2.6-Å resolution structure of this fragment with Mn^{2+} coordinated in the polymerase active site. A comparison with the known structures of HIV-1 RT is given, providing insight into the effects of mutations in HIV-1 RT that confer resistance to drugs such as AZT. The structural model of MMLV RT offers new information about the structural basis for fidelity, processivity and discrimination between deoxyribose and ribose nucleotides, and about the mechanism of catalysis.
15 September 1995, Research Article, *Structure*

► **The structure of HIV-1 reverse transcriptase complexed with 9-chloro-TIBO: lessons for inhibitor design**
Jingshan Ren, Robert Esnouf, Andrew Hopkins, Carl Ross, Yvonne Jones, David Stammers and David Stuart (1995). *Structure* **3**, 915–926.
HIV-1 reverse transcriptase (RT) is a key target of therapies for AIDS. Structural studies of RT complexed with different non-nucleoside inhibitors that contain two hinged rings have indicated that these inhibitors bind to and distort the polymerase catalytic site, inactivating it. The TIBO family of compounds cannot be fitted into the 'two-ring model', however. Here, the structure of HIV-1 RT in complex with 9-chloro-TIBO has been determined at 2.6-Å resolution. The inhibitor binds to the active site of the enzyme, like the other non-nucleoside inhibitors, but is distorted upon binding so that it mimics the binding of inhibitors containing two hinged rings. Comparisons of the different RT–inhibitor complexes suggest modifications to the TIBO group of inhibitors that might enhance their binding and their therapeutic efficacy.
15 September 1995, Research Article, *Structure*

► **Common themes in redox chemistry emerge from the X-ray structure of oilseed rape (*Brassica napus*) enoyl acyl carrier protein reductase**
John B Rafferty, J William Simon, Clair Baldock, Peter J Artymiuk, Patrick J Baker, Antoine R Stuitje, Antoni R Slabas and David W Rice (1995). *Structure* **3**, 927–938.
Enoyl acyl carrier protein reductase (ENR) catalyzes the reduction of *trans*-Δ2-enoyl acyl carrier protein, an essential step in *de novo* fatty acid biosynthesis. This reaction requires NADH or NADPH; separate NADH-dependent and NADPH-dependent forms of the enzyme are found in bacteria and plants. The authors report the crystal structure of an NADH-dependent ENR enzyme from oilseed rape at 1.9-Å resolution. The subunits of the tetrameric enzyme have a topology highly reminiscent of a dinucleotide-binding fold. The structure shows a striking similarity to those of epimerases and short-chain alcohol dehydrogenases, in particular, 3α,20β-hydroxysteroid dehydrogenase. Both 3α,20β-hydroxysteroid dehydrogenase and ENR appear to use a lysine residue, in structurally equivalent positions, to stabilize the transition state, and both appear to use a tyrosine residue as a proton donor. The authors propose that, despite the very different chemistries of the reactions catalyzed by the two enzymes, their underlying mechanism is very similar.
15 September 1995, Research Article, *Structure*

► **The three-dimensional structure of 6-phospho-β-galactosidase from *Lactococcus lactis***
Christian Weismann, Gerald Beste, Wolfgang Hengstenberg and George E Schulz (1995). *Structure* **3**, 961–968.
The enzyme 6-phospho-β-galactosidase hydrolyzes phospholactose, which, in bacteria such as *Lactobacilli, Lactococci* and *Streptococcus,* is produced by a phosphoenolpyruvate-dependent phosphotransferase system that is part of the lactose degradation pathway. The crystal structure of the *Lactococcus lactis* enzyme is reported here to 2.3-Å resolution. It has a triosephosphate isomerase (TIM) barrel fold. The active site is clearly located; the two catalytic glutamates are in a cavity lined by tryptophans and tyrosines, which are both frequently observed at glycoside-binding sites. A bound sulfate group marks the position at which the phosphate group on the substrate would bind to the enzyme. Two loops apparently open to allow the substrate access to the active site, then close over the bound substrate. This is the first crystal structure of a family 1 glycosidase; it provides a basis on which to model all other family 1 members, which will help in elucidating the catalytic mechanisms of these enzymes. This enzyme also belongs to a superfamily of glycosidases sharing a TIM barrel fold, with catalytic glutamates or aspartates at the ends of the fourth and seventh strands of the β-barrel.
15 September 1995, Research Article, *Structure*