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 September 1995, **2**:627–629

► Light-Harvesting Complex: **Rings of light**
CN Hunter (1995). *Curr. Biol.* **5**, 826–828.

In photosynthesis, light energy absorbed by chlorophyll pigments is converted into a photochemical form by reaction centres and ultimately is used to convert carbon dioxide into carbohydrate. In order to ensure that sufficient energy is available to drive these biosynthetic processes, reaction centres are surrounded with a many-fold excess of pigments, each held in a specific position by proteins. Such a combination of pigments and proteins is referred to as a light-harvesting complex. The recently reported atomic structure of a bacterial light-harvesting complex has significantly advanced our understanding of the process by which such pigment–proteins absorb light energy and transmit it with high speed and efficiency to the reaction centre. The structure reveals a circular arrangement of the pigments, allowing efficient capture of energy despite the inherently random nature of the incident radiation. The subsequent absorption and transfer of light energy can thus be reduced to a two-dimensional process involving transfer around the ring and along a level plane to the reaction centre.
1 August 1995, Dispatch, *Current Biology*

► Proteolysis: **The proteasome: a protein-degrading organelle?**
David M Rubin and Daniel Finley (1995). *Curr. Biol.* **5**, 854–858.

Of the many known proteolytic mechanisms, only the ubiquitin–proteasome pathway has evolved a tagging step. Recent studies, including the determination of the crystal structure of the proteasome core, suggest an explanation. The 26S protease is a two megadalton complex that degrades ubiquitinated proteins. Ubiquitin and ATP act in different ways to promote the passage of substrates through a 'molecular sieve', at the distal end of which is the proteolytic core, the 20S proteasome. The tight shell of the proteasome forms a compartmentalized space analogous to the lumen of a cytoplasmic organelle, and the apparent requirement for proteins to be unfolded before entering the proteasome is similar to the unfolding that is required for passage through the protein-conducting channels of the endoplasmic reticulum and mitochondria. It appears that substrates follow an ordered pathway through the 26S protease, interacting in a stepwise fashion with a ubiquitin-binding site, an unfolding site that includes ATPases, the protein-translocating channel and finally the peptidase sites. By holding the substrate in place, the ubiquitin tag may serve to increase the local concentration of substrate in the vicinity of the 26S ATPases, and thus to drive the unfolding reaction. The increased understanding of the proteasome, despite its unique nature, promises to provide general insights into the regulation of protein targeting, folding and translocation.
(See also Goldberg *et al.*, *Chemistry & Biology* **2** 503–508)
1 August 1995, Dispatch, *Current Biology*

► T-Cell Activation: **Two for T**
Sylvie Guerder and Richard A Flavell (1995). *Curr. Biol.* **5**, 866–868.

The interaction of the T-cell receptor with its natural ligand, a peptide presented by a major histocompatibility complex (MHC) molecule, is not sufficient to trigger full activation of resting T cells. It is now well established that two signals are required to activate T cells — one is the antigen-specific signal transmitted by the T-cell receptor, the other a 'costimulatory' signal that is provided by interactions between pairs of molecules, one expressed on the surface of the T cell and the other on the surface of the antigen-presenting cell. Interaction between the molecules CD28 on the T cell and B7-1 (CD80) or B7-2 (CD86) on the antigen-presenting cell at least partially fulfills the requirement for a second, costimulatory signal in T-cell activation. Recent reports, however, indicate that these costimulatory molecules may have a hitherto unsuspected role in the differential activation of the two main classes of helper T cells — known as Th1 and Th2 cells — and may even inhibit T-cell activation in some circumstances. Three different models have been proposed to account for the differential effects of B7-1 and B7-2 on the development of experimental allergic encephalomyelitis (EAE) and spontaneous autoimmune diabetes: the two types of B7 molecule may generate different signals in target T cells, antigen-presenting cells, or B cells.
1 August 1995, Dispatch, *Current Biology*

► Photosynthesis: **Regulation by redox signalling**
JF Allen, K Alexciev and G Håkansson (1995). *Curr. Biol.* **5**, 869–872.

The primary event of photosynthesis is light-driven electron transfer — a redox reaction — that sets in motion a chain of electron transfers upon which all life ultimately depends. The number of chlorophyll molecules that harvest light for each photosynthetic reaction centre is variable. This variability provides a 'gain control' that is turned up to increase photosynthetic efficiency in dim light, and down to prevent destructive redox chemistry in bright light. Increasing evidence from a range of systems suggests that photosynthesis is self-regulating, through molecular redox signalling. Recent work with the photosynthetic bacterium *Rhodobacter capsulatus* shows that redox control over photosynthesis genes is exerted by two new members of the growing family of two-component regulatory systems: RegA is a 'response regulator', phosphorylated on aspartate by transfer of a phosphate from its partner, RegB, a 'sensor kinase' which is autophosphorylated on a histidine residue under anaerobic conditions. Other work with higher plants suggests that redox signalling is involved in the translation and transcription of chloroplast genes, which should be no surprise if chloroplasts are, as is thought, the descendants of bacteria. It also appears that a redox signal is transmitted from the chloroplast to the nucleus, and that the pathway involves protein kinases and phosphatases. These findings may provide general insights into the roles of molecular redox signalling in cell regulation.
1 August 1995, Dispatch, *Current Biology*

► **Targeting the mouse genome: a compendium of knockouts (part III)**
EP Brandon, RL Idzerda and GS McKnight (1995). *Curr. Biol.* **5**, 873–881.

This is the third part of a three-part article, the purpose of which is to provide a compendium of the gene-targeted mutations that have been published, with the hope that it will become the

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foundation for an active database that will keep track of this burgeoning field.
1 August 1995, *Current Biology*

▶ **Only one of the two DNA-bound orientations of AP-1 found in solution cooperates with NFATp**
Lin Chen, Martha G Oakley, JN Mark Glover, Jugnu Jain, Peter B Dervan, Patrick G Hogan, Anjana Rao and Gregory L Verdine (1995). *Curr. Biol.* **5**, 882–889.
In response to mitogenic stimuli, the transcription factor AP-1 regulates gene expression both through solitary binding to independent recognition sites and, in cooperation with various heterologous transcription factors, through targeting to composite response elements. The two subunits that make up the AP-1 heterodimer, Fos and Jun, possess a homologous ~60 amino-acid motif known as the basic leucine zipper (bZip) domain, which contains residues responsible for DNA binding and dimerization. This degeneracy leaves the protein with no apparent way of orienting itself uniquely on DNA by differentially recognizing its two non-identical half-sites. The authors report the use of the affinity-cleaving technique to probe the orientation of AP-1 on its recognition sequence in solution, and to examine how this orientation is influenced through cooperative interactions with a heterologous DNA-binding protein, the 'nuclear factor of activated T cells' (NFATp). They demonstrate that the AP-1 bZip binds DNA as a mixture of two orientational isomers, but in the cooperative complex formed with NFATp, the AP-1 bZip adopts a single orientation. Because orientational isomers present markedly different structures to the transcriptional apparatus, it seems likely that orientation will exert an effect on the ability to activate transcription.
1 August 1995, Research Paper, *Current Biology*

▶ **Involvement of MAP kinase in insulin signalling revealed by non-invasive imaging of luciferase gene expression in single living cells**
Guy A Rutter, Michael RH White and Jeremy M Tavaré (1995). *Curr. Biol.* **5**, 890–899.
Using two well-characterized photoproteins, luciferase and aequorin, coupled with a microinjection approach, the authors have developed a method that allows highly quantitative and non-invasive monitoring of the activity of the growth-factor-regulated collagenase promoter. They found that insulin-stimulated collagenase promoter activity (assayed using luciferase as a reporter) is specifically blocked upon overexpression of either the mitogen-activated protein (MAP) kinase phosphatase CL100, or the dominant-negative mutant MAP kinase kinase, $MEK^{S217/221A}$. The transcriptional activity of a constitutive promoter was simultaneously monitored (using aequorin as a reporter) and found not to be altered by the presence of insulin, CL100 or $MEK^{S217/221A}$. The data strongly imply that MAP kinase is directly involved in insulin signalling. This method should have wide application to real-time studies of gene expression and its regulation.
1 August 1995, Research Paper, *Current Biology*

▶ **How does taxol stabilize microtubules?**
Isabelle Arnal and Richard H Wade (1995). *Curr. Biol.* **5**, 900–908.
Microtubules are ubiquitous components of the cytoskeleton in eukaryotic cells and are essential for cell division. They are hollow cylinders, ~24 nm in diameter, with a wall consisting of a lattice of tubulin heterodimers that are aligned head-to-tail to form protofilaments. The antimitotic agent taxol is an important new drug for the treatment of certain cancers. It blocks the cell cycle in its G1 or M phases by stabilizing the microtubule cytoskeleton against depolymerization. The authors used electron cryomicroscopy and image analysis to investigate the structure of microtubules assembled *in vitro*, and found that their fine structure was sensitive to the presence of taxol. The conformation of the microtubule lattice depended on whether the drug was added before or after assembly. The structure of preassembled microtubules changed only slightly when taxol was added; a larger change was observed when microtubules were assembled in the presence of the drug. In both cases, taxol-containing microtubules were stable over many days at, or below, room temperature. The larger structural modification produced when microtubules assemble in the presence of taxol might be related to a greater accessibility of the binding site before tubulin is incorporated into the microtubule surface lattice. (See also Wilson & Jordan, *Chemistry & Biology* **2** 569–573, this issue)
1 August 1995, Research Paper, *Current Biology*

▶ **$LMP2^+$ proteasomes are required for the presentation of specific antigens to cytotoxic T lymphocytes**
Catherine Sibille, Keith G Gould, Karen Willard-Gallo, Stuart Thompson, A Jennifer Rivett, Simon Powis, Geoffrey W Butcher and Patrick De Baetselier (1995). *Curr. Biol.* **5**, 923–930.
Major histocompatibility complex (MHC) class I molecules present short peptides generated by intracellular protein degradation to cytotoxic T lymphocytes (CTL). The multisubunit, non-lysosomal proteinases known as proteasomes have been implicated in the generation of these peptides. Two interferon-γ (IFN-γ)-inducible proteasome subunits, LMP2 and LMP7, are encoded within the MHC gene cluster in a region associated with antigen presentation. The incorporation of these LMP subunits into proteasomes may alter their activity so as to favor the generation of peptides able to bind to MHC class I molecules. The authors describe a T-cell lymphoma, termed SP3, that displays a novel selective defect in MHC class I-restricted presentation of influenza virus antigens. The defect is associated with a lack of LMP2 expression but a normal level of cell-surface MHC class I molecules. Antigen presentation and LMP2 expression are restored by IFN-γ, but the expression of an antisense LMP2 mRNA prevents this restoration. Moreover, the expression of this antisense mRNA in L929 fibroblast cells, which constitutively express LMP2 and have no presentation defect, reproduces the presentation defect of SP3 cells. LMP2-containing ($LMP2^+$) proteasomes are therefore required for the presentation of specific antigens to CTL.
1 August 1995, Research Paper, *Current Biology*

▶ **Hammerhead ribozyme structure: U-turn for RNA structural biology**
Jennifer A Doudna (1995). *Structure* **3**, 747–750.
RNA catalysts, or ribozymes, have intrigued evolutionary biologists and enzymologists since their initial discovery over a decade ago. How can a relatively simple polymer, constructed from only four nucleotide building blocks, provide the structural stability and active-site functional groups that are required of an enzyme? Two recently determined crystal structures of the hammerhead ribozyme, a small, self-cleaving RNA, provide the first atomic-resolution views of an RNA active site and its structural underpinnings. The structures suggest that the catalytic center may reside in a U-turn motif which was first seen in $tRNA^{Phe}$. This work advances our understanding of RNA architecture and may even facilitate ribozyme engineering for therapeutic purposes.
15 August 1995, Minireview, *Structure*

▶ **14-3-3 proteins: structure resolved, functions less clear**
Richard Marais and Chris Marshall (1995). *Structure* **3**, 751–753.
The 14-3-3 proteins are a family of highly conserved proteins found in lower and higher eukaryotic cells, to which a wide spectrum of functions has been ascribed, including roles in transcriptional regulation, cell-cycle control, and inhibition or activation of

protein kinase C. Last year a flurry of papers suggested that 14-3-3 proteins may be involved in intracellular signal transduction by associating with at least three proteins which regulate signaling: the middle T transforming protein of polyoma virus, the Raf-1 protein kinase and Bcr, a GTPase-activating protein (GAP) which activates Rac (a member of the Ras superfamily of small GTP-binding proteins). Although it has been difficult to define the precise biological function of 14-3-3 proteins, considerable progress has now been made in solving their structure. The crystal structures of 14-3-3 proteins reveal that they form dimers with a deep groove running along the length of the dimer. The groove is made up entirely of conserved residues, which suggests that it binds a conserved protein or structure, whereas the outer surface of the dimer, which contains the variable residues, may provide binding sites where the specific associations of individual isoforms may occur.
15 August 1995, Minireview, *Structure*

▶ **Unraveling transposition: γδ resolvase in complex with DNA**
Alfonso Mondragón (1995). *Structure* **3**, 755–758.

Transposons are mobile DNA elements that can migrate to different regions of the chromosome and that code for the proteins required to catalyze their own relocation. The γδ transposon belongs to the Tn3 family of replicative transposons, for which transposition is a two-step process. In the first step, fusion of the donor DNA molecule containing the transposon and the target DNA molecule creates a molecule in which the transposable element is duplicated. This is followed by a site-specific recombination event that 'resolves' the products, leaving a copy of the transposable element in the target molecule. The first step is catalyzed by transposase and the second is catalyzed by resolvase. The γδ resolvase requires the presence of two 114 base pair *res* sites, one in each copy of the transposon in the fused molecule, to resolve the products. The reaction involves the binding of multiple γδ resolvase dimers to the *res* sites to form a synaptosome, followed by strand exchange. The structure of γδ resolvase with a 34 base pair oligonucleotide reveals an intricate and novel complex of the protein with DNA and lays the foundations for understanding transposition at the atomic level. More information is needed, however, to discern the quaternary arrangement in the synaptosome and to define the mechanism of the strand exchange. As structures alone cannot show all the conformations that occur during the course of the reaction, the challenge for the future is to combine biochemical and structural data to understand how this type of protein does its work.
15 August 1995, Minireview, *Structure*

▶ **Crystal structure of the superantigen enterotoxin C2 from *Staphylococcus aureus* reveals a zinc-binding site**
Anastassios C Papageorgiou, K Ravi Acharya, Robert Shapiro, Edward F Passalacqua, Rossalyn D Brehm and Howard S Tranter (1995). *Structure* **3**, 769–779.

The bacterial toxins secreted by *Staphylococcus aureus* and *Streptococcus pyogenes* produce a wide range of clinical conditions including food poisoning, toxic shock syndrome and scarlet fever. These molecules belong to a family of proteins, termed 'superantigens', that form complexes with class II MHC molecules without prior proteolytic processing. The interaction of the MHC–superantigen complex with T-cell receptors is much less specific than that of MHC–peptide complexes; as a consequence, superantigens stimulate a much larger number of T-cells than do other antigens. Although superantigens seem to act by a common mechanism, they vary in many of their specific interactions and biological properties. The authors report the crystal structure of *S. aureus* enterotoxin C2 (SEC2) at 2.0 Å resolution and compare it to the structures of two other superantigens, staphylococcal enterotoxin B (SEB) and toxic shock syndrome toxin-1 (TSST-1). They find that the SEC2 structure is similar to that of SEB, most notably in the regions corresponding to the binding sites for MHC molecules and T-cell receptors, but they also identify a striking feature not found in the other superantigen structures — the presence of a zinc ion coordinated to residues located in a solvent-exposed groove. The position of the zinc ion suggests that it may function as a bridge between SEC2 and MHC class II molecules.
15 August 1995, Research Article, *Structure*

▶ **Structure and function of a virally encoded fungal toxin from *Ustilago maydis*: a fungal and mammalian Ca^{2+} channel inhibitor**
Fei Gu, Anis Khimani, Stanley G Rane, William H Flurkey, Robert F Bozarth and Thomas J Smith (1995). *Structure* **3**, 805–814.

The P4 strain of the corn smut fungus, *Ustilago maydis*, secretes a fungal toxin, KP4, encoded by a fungal virus (UMV4) that persistently infects its cells. UMV4, unlike most non-fungal viruses, does not spread to uninfected cells by release into the extracellular milieu during its normal life cycle and is thus dependent upon host survival for replication. In symbiosis with the host fungus, UMV4 encodes KP4 to kill other competitive strains of *U. maydis*, thus promoting both host and virus survival. After determining the atomic structure of KP4 to 1.9 Å resolution, the authors found similarities between a highly basic protrusion from the main β-sheet of KP4 and the site on scorpion toxins that binds to (and alters) cation channels on excitable membranes. Further experiments demonstrated that KP4-sensitive cells can be rescued by the addition of Ca^{2+} or Mg^{2+} but not Na^{+} or K^{+}. The hypothesis that the toxic effects of KP4 are mediated by inhibition of cation channels was substantiated by experiments demonstrating that the toxin specifically inhibits voltage-gated Ca^{2+} channels in mammalian cells. The results suggest that certain properties of fungal Ca^{2+} channels are homologous to those in mammalian cells. Thus, KP4 may be a new tool for studying mammalian Ca^{2+} channels and current Ca^{2+} channel inhibitors may be useful lead compounds for new anti-fungal agents.
15 August 1995, Research Article, *Structure*

▶ **The 70S *Escherichia coli* ribosome at 23 Å resolution: fitting the ribosomal RNA**
Holger Stark, Florian Mueller, Elena V Orlova, Michael Schatz, Prakash Dube, Tarik Erdemir, Friederich Zemlin, Richard Brimacombe and Marin van Heel (1995). *Structure* **3**, 815–821.

The ribosome, the subcellular organelle responsible for protein synthesis, has been an evasive target for structural studies. The best available structures for the 70S *Escherichia coli* ribosome or its subunits, based on electron microscopical tilt experiments, are limited in resolution to 28–50 Å. The authors use an angular reconstitution approach, which exploits the random orientations of particles within a vitreous ice matrix, in conjunction with cryo-electron microscopy to yield a 23 Å map of the 70S ribosome. The structure elucidates many structural details, such as an extensive system of channels within the 50S subunit and an intersubunit gap ideally shaped to accommodate two transfer RNA molecules. The resolution achieved is sufficient to allow the preliminary fitting of some of the double-helical regions of an earlier three-dimensional model of the 16S rRNA into the 70S structure.
15 August 1995, Research Article, *Structure*