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 July 1995, **2**:497–499

▶ HIV-1 Virology: **Simply MArvelous nuclear transport**
David S Goldfarb (1995). *Curr. Biol.* **5**, 570–572.

The matrix proteins (MAs) of both simple and complex retroviruses are anchored in the inner leaflet of the viral membrane by amino-terminal myristoyl groups, yet MA of HIV-1 is involved in the process by which the viral preintegration complex is imported into the nucleus during infection. As the HIV-1 preintegration complex is membrane-free, these two facts are difficult to reconcile. MA contains a nuclear localization signal (NLS), however, which is capable of targeting microinjected reporter proteins to the nuclei of tissue culture cells. Viruses with mutations of the MA NLS can be propagated in dividing cells, where nuclear envelope breakdown during the mitotic cycle presumably allows access to the host genome, but they cannot propagate in cell-cycle-arrested cells. Recent studies have shown that a subset of MA molecules that contain phosphoserine and phosphotyrosine are imported into nuclei during infection. Mutagenesis studies revealed a tyrosine residue whose phosphorylation is required for the formation of a nucleus-seeking HIV-1 preinitiation complex. The nuclear transport of HIV-1 may be a viable target for future vaccines and anti-HIV-1 drugs.
1 June 1995, Dispatch, *Current Biology*

▶ Cytokine-Processing Enzymes: **Stopping the cuts**
CA Dinarillo and NH Margolis (1995). *Curr. Biol.* **5**, 587–589.

Cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) are potent mediators of inflammation. Blocking the ability of IL-1 or TNF to trigger their respective receptors has had some success as an anti-inflammatory strategy. The clinical use of the natural IL-1 receptor antagonist (IL-1Ra), soluble IL-1 receptors, antibodies to TNF or soluble TNF receptors is, however, limited by the need to deliver such proteins by injection. The development of orally active drugs to reduce the production of IL-1 and/or TNF thus remains a highly desirable goal. Although cyclooxygenase inhibitors and corticosteroids are excellent anti-inflammatory drugs, their side effects are often unacceptable. A major breakthrough came with the discovery of IL-1β converting enzyme (ICE), an intracellular cysteine protease which seems to be the sole enzyme for cleaving the precursor to the IL-1β isotype at the site that releases the mature form of IL-1β. Substrate-based inhibitors of ICE have been used to prevent cleavage of the IL-1β precursor and thus release of mature IL-1β from human blood and animal monocytes. The putative TNFα converting enzyme (TACE) seems to fall into the general class of zinc-binding metalloproteinases. *In vitro*, metalloproteinase inhibitors and zinc chelators suppress the processing of TNFα from human blood monocytes and murine macrophages without affecting the release of IL-1β or a number of other cytokines. The expectation is that inhibitors of ICE or TACE will enter clinical medicine, as have inhibitors of angiotensin converting enzyme (ACE) and the human immunodeficiency virus-1 (HIV-1) protease.
1 June 1995, Dispatch, *Current Biology*

▶ RNA Editing: **An I for editing**
Brenda L Bass (1995). *Curr. Biol.* **5**, 598–600.

The discovery of RNA editing almost a decade ago brought a new twist to the familiar tale of how genetic information flows from DNA to protein. Most examples of RNA editing have been found in organelle-encoded RNAs. Of the few examples of edited nuclear-encoded RNAs that have been reported, the case of glutamate receptor (gluR) mRNA editing has shed new light on how such editing can be accomplished and given new life to a previously characterized enzyme known as double-stranded RNA (dsRNA) adenosine deaminase (dsRAD), which deaminates adenosines within dsRNA to produce inosines. Three recent reports have demonstrated that gluR transcripts synthesized *in vitro* can be correctly edited when incubated with mammalian nuclear extracts. This *in vitro* editing involves the conversion of adenosines to inosines and requires the dsRNA structure predicted to surround the editing sites, consistent, among other evidence, with the action of dsRAD in the editing process. The presence of dsRAD in several different organisms, as well as its presence in cells that do not express gluR genes, suggests that other dsRAD substrates exist, and raises the question of whether editing is the primary function it subserves. Clearly, it is an interesting time for studies of both gluR RNA editing and dsRAD, but it is also time for greater caution when assuming that a nuclear cDNA sequence corresponds to that of its gene.
1 June 1995, Dispatch, *Current Biology*

▶ Integrin–Ligand Binding: **Do integrins use a 'MIDAS touch' to grasp an Asp?**
JM Bergelson and ME Hemler (1995). *Curr. Biol.* **5**, 615–617.

Integrins are cell-surface glycoproteins — each composed of an α and β subunit — that mediate cell interactions with a variety of ligands. Such ligands include extracellular matrix proteins, counter-receptors on other cells, plasma proteins and microbial pathogens. The recent determination of the crystal structure of the ligand-binding domain within the α^M subunit of the leukocyte integrin CR3 and the partial structure of an integrin ligand, the vascular cell-adhesion molecule VCAM-1, has revealed a potential mode of interaction between integrins and their ligands. It is proposed that the coordination of a magnesium ion by five oxygenated amino acids of the integrin domain, organized in what is called a 'metal ion-dependent adhesion site' (MIDAS) motif, is completed by the contribution of a sixth coordination site provided by a critical acidic residue (usually an aspartate) in the ligand. Divalent cations are essential for integrin interactions with almost all ligands, and mutagenesis of amino acids that comprise the MIDAS motif is known to prevent ligand binding. The eventual development of selective therapeutic agents that block integrin–ligand interactions will depend on understanding how specific integrins fit their particular ligands.
1 June 1995, Dispatch, *Current Biology*

▶ Apoptosis: **A sticky business**
Georg Häcker and David L Vaux (1995). *Curr. Biol.* **5**, 622–624.

A remarkably large and diverse array of agents can provoke cells to kill themselves by apoptosis. In a significant proportion of instances, this response can be blocked by expression of the *bcl-2* gene. Yet despite the identification of several proteins that have similar structures, and a number of proteins that interact with Bcl-2, we still do not know how the Bcl-2 protein actually works. The now extensive Bcl-2-like family, which also includes three viral genes, has given rise to Bcl-2 homology domains (BH) 1 and 2;

mutagenesis and yeast two-hybrid experiments show that family members can form homodimers and heterodimers via binding of these domains to each other. A tantalizing result from the yeast two-hybrid screens is the interaction of the nuclear membrane protein lamin a/c with a *bcl-2* homolog, a finding which may have significance as nuclear changes are an early feature of apoptosis. The tangle of Bcl-2-like and interacting proteins should not be surprising, given that these proteins control the fate of the cell.
1 June 1995, Dispatch, *Current Biology*

▸ Knockouts: **Targeting the mouse genome: a compendium of knockouts (part I)**
EP Brandon, RL Idzerda and GS McKnight (1995). *Curr. Biol.* **5**, 625–634.
It has been about six years since it was first possible to mutate a specific mouse gene by homologous recombination in embryonic stem cells and then transfer that mutation into a developing mouse. The most common goal of this technique has been to inactivate the targeted gene and to observe the phenotypic effects of the 'knockout' on the mouse. The authors provide a compendium of the hundreds of gene-targeted mutations that have been published, with the hope that it will become the foundation for an active database that will keep track of this burgeoning field. 327 independently derived mutants are included, although the duplication of knockouts reduces the number to 263 fundamentally different protein disruptions (or genetic modifications). Certain types of proteins seem to have been preferentially studied so far, for example, those involved in immune responses or embryonic development. A common early misconception — that null mutants often have no observable effects — is dispelled by the compendium, which shows that only a dozen or so of the mutants are apparently normal. This collection of mutant animals provides an important resource for the future of biomedical research and a vital counterpart to the human and mouse genome sequencing projects. Parts II and III of this table will be published in the July and August issues of *Current Biology*. A smaller table of double mutants will also appear in the August issue.
1 June 1995, Dispatch, *Current Biology*

▸ **Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells**
Rosario Rizzzuto, Marisa Brini, Paola Pizzo, Marta Murgia and Tullio Pozzan (1995). *Curr. Biol.* **5**, 635–642.
Each biological application of imaging techniques for visualizing physiological events in living cells depends on the availability of suitable probes for labeling the structures and/or for measuring the parameters of interest. Small fluorescent molecules often distribute non-exclusively to the compartment of interest, complicating the interpretation of results. But the cellular sorting of a polypeptide is strictly controlled by the targeting information included in its primary sequence or by other signals such as hormone binding. The authors modified the cDNA of the green fluorescent protein (GFP) of the jellyfish *Aequorea victoria*, which has recently been demonstrated to retain its fluorescent properties when recombinantly expressed in both prokaryotic and eukaryotic living cells, to include a mitochondrial targeting sequence. When transiently transfected into mammalian cells, their construct drove the expression of a strongly fluorescent GFP chimera which selectively localizes to the mitochondria. The targeted chimera allowed the visualization of mitochondrial movement in living cells, and, unlike dyes such as rhodamine, it revealed morphological changes induced in mitochondria by drugs that collapse the organelle membrane potential. By the addition of suitable targeting sequences, GFP could, in principle, be directed to virtually any subcellular compartment, making various cell structures visible *in vivo*. Furthermore, GFP could be fused to specific proteins, providing a tool for monitoring *in vivo* the sorting and intracellular fate of these proteins.
1 June 1995, Research Paper, *Current Biology*

▸ **Secretion of the amino-terminal fragment of the Hedgehog protein is necessary and sufficient for *hedgehog* signalling in *Drosophila***
MJ Fietz, A Jacinto, AM Taylor, C Alexandre and PW Ingham (1995). *Curr. Biol.* **5**, 643–650.
The *Drosophila* segment polarity gene *hedgehog* (*hh*) encodes a member of a family of secreted proteins that are involved in a variety of patterning processes in both vertebrates and invertebrates. Some of these processes depend upon short-range or contact-dependent interactions, whereas others seem to involve long-range signalling. The Hedgehog protein undergoes post-translational modification to yield three major species generated by cleavage of a signal peptide sequence and by the autoproteolysis of this secreted form into amino-terminal and carboxy-terminal peptides. The amino-terminal peptide appears closely associated with the cells in which it is expressed in the *Drosophila* embryo, whereas the carboxy-terminal species is more widely distributed across each segment. These findings have led to the suggestion that different modes of *hh* function may be mediated by these two different portions of the protein, the amino-terminal form mediating short-range effects and the carboxy-terminal peptide effecting long-range signalling. By mutating the *hh* coding region such that only the amino-terminal or carboxy-terminal half of the protein is secreted, the authors demonstrate that all of the signalling activity of Hedgehog resides in the amino-terminal portion of the protein, the secretion of which is essential for its function. They also find that although the range of Hedgehog is limited by the close association of the amino-terminal peptide with the cell surface, it can be extended by elevating the level of *hh* expression.
1 June 1995, Research Paper, *Current Biology*

▸ **Where freedom is given, liberties are taken**
Gerard J Kleywegt and T Alwyn Jones (1995). *Structure* **3**, 535–540.
Many people believe that the pictures of macromolecules they see published in journals are accurate down to the most intricate detail. But some aspects of protein crystallography require a subjective interpretation of the diffraction results that can lead to serious errors. Kleywegt and Jones argue that, overall, the crystallographic community is doing a poor job in its treatment of structures whose crystals diffract poorly. In the worst cases, even if there are no 'errors', biological results are being interpreted with a precision that is not warranted by the information contained in the diffraction data. Thus, they propose that it should be possible for anyone who is interested in doing so to be able to re-refine structures deposited at the Protein Data Bank using better protocols. This would require researchers to find all their old reflection data sets and send them to the PDB.
15 June 1995, Ways & Means, *Structure*

▸ **Surprising leads for a cholera toxin receptor-binding antagonist: crystallographic studies of CTB mutants**
Ethan A Merritt, Steve Sarfaty, Teh-tsai Chang, Leslie M Palmer, Michael G Jobling, Randall K Holmes and Wim GJ Hol (1995). *Structure* **3**, 561–570.
Cholera toxin, the direct causative agent of cholera, is an AB_5 hexameric protein secreted by *Vibrio cholerae*. In the absence of the A subunit, the B pentamer of the toxin retains the ability to bind specifically to the cell-surface receptor, ganglioside G_{M1}. The authors report the crystal structure of a recombinant B pentamer containing a Gly33→Asp substitution known to abolish recep-

tor-binding ability and an Arg35→Asp substitution which results in deficient assembly of the AB_5 holotoxin; although assembly of the B_5 pentamer is not affected. The Gly33→Asp mutant structure proves to be informative, not only by explaining the receptor-binding properties of mutants reported previously at this site, but also by demonstrating an unexpected mode of pentamer–pentamer association in the crystal. This association arises from the interaction of residues 10–14 from a B subunit in one pentamer with the receptor-binding site on another pentamer. The Arg35→Asp mutant structure is isomorphous to that of the Gly33→Asp mutant, indicating that the pentamer–pentamer structure observed is not an artifact of the Gly33→Asp substitution. The effect of the Arg35→Asp mutation on holotoxin assembly appears to be due to a decrease in the rigidity of the B pentamer in solution rather than a specific change in conformation. These studies suggest that analogs to all or part of the pentapeptide corresponding to residues 10–14 of the B subunit may constitute lead compounds for the design of binding-site inhibitors effective against cholera and related enterotoxigenic diseases.
15 June 1995, Research Article, *Structure*

▸ **Structure of HIV-1 protease with KNI-272, a tight-binding transition-state analog containing allophenylnorstatine**

Eric T Baldwin, T Narayana Bhat, Sergei Gulnik, Beishan Liu, Igor A Topol, Yoshiaki Kiso, Tsutomu Mimoto, Hiroaki Mitsuya and John W Erickson (1995). *Structure* **3**, 581–590.

HIV-1 protease (HIV PR) is essential for the production of infectious virus particles and has become an important target for the design of antiviral agents for AIDS. The authors report the three-dimensional crystal sructure of HIV PR with the inhibitor KNI-272, which has potent antiviral activity and is undergoing clinical trials. They find that two bridging water molecules in the solvated structure of HIV PR are important in the interaction with KNI-272. In addition, they propose that the conformationally constrained P1 allophenylnorstatine–P1' thioproline linkage of KNI-272, which is favorably recognized in its low energy *trans* conformation, may relate to the high potency of KNI-272. The rigid core conformation may be detrimental, however, from the standpoint of drug resistance. The results suggest that single mutations in HIV PR can have large effects on KNI-272 binding. Nevertheless, the structure provides a framework for designing second-generation inhibitors targeted against drug-resistant HIV PR mutants.
15 June 1995, Research Article, *Structure*

▸ **Electrostatic analysis of TEM1 β-lactamase: effect of substrate binding, steep potential gradients and consequences of site-directed mutations**

Peter Swarén, Laurent Maveyraud, Valérie Guillet, Jean-Michel Masson, Lionel Mourey and Jean-Pierre Samama (1995). *Structure* **3**, 603–613.

Extensive use of antibiotics has caused bacteria to become resistant to these agents. The resistance is mainly mediated by the emergence of β-lactamase enzymes exhibiting extended substrate specificities and is spread by plasmid exchange. Although the amino acids essential for catalysis have been identified, their exact mode of action and interrelations have been a matter of debate. Given that enzyme catalysis involves acid–base chemistry and charge transfers, the authors address the mechanism of β-lactamase action by electrostatic analysis of the enzyme's refined structure. They propose that substrate binding triggers the acylation reaction because it raises the pK_a of the active site Lys73. The amine group of Lys73 can then abstract the Ser70 hydroxyl group proton and promote acylation. In the acyl–enzyme complex, the deacylating water is situated between the carboxylate group of Glu166, within the enzyme, and the ester-carbonyl carbon of the acyl–enzyme complex. A steep electrostatic potential gradient aids in polarizing the water molecule and directs the proton and hydroxyl groups. As this wild-type acylation pathway cannot operate in mutant proteins where protonation of Lys73 is imposed, an alternative and less catalytically efficient pathway in these mutants may proceed through activation of a water molecule by Glu166, with Lys73 contributing as a proton shuffle partner.
15 June 1995, Research Article, *Structure*