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 August 1995, **2**:563–565

▶ DNA Repair: **Nucleotide excision–repair in the test tube**

NGJ Jaspers and JHJ Hoeijmakers (1995). *Curr. Biol.* **5**, 700–702.

Cells have a number of different mechanisms for repairing the various types of damage done to their DNA by errors of internal metabolism or the action of external agents. Although many of these mechanisms are relatively simple and specialized for the repair of strictly defined types of DNA lesions, the eukaryotic nucleotide excision–repair (NER) pathway can recognize a broad spectrum of helix-distorting lesions, mostly generated by environmental DNA-damaging agents such as ultraviolet light. In eukaryotes, this versatility of recognition is produced by intricate interactions between many proteins, which mediate the excision and replacement of a single-strand patch substantially larger than the DNA region that is directly affected. The human inherited disorders xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy are caused by mutations, defining at least ten complementation groups that affect different proteins in the NER system. The proteins required have now been identified and classified into those required for lesion recognition, demarcation of the preincision patch, incision and gap filling and ligation. An estimated total of 25–30 proteins is required. The NER pathway has now been reconstituted *in vitro*, an achievement that should hasten the full enzymological characterization of this highly complex DNA-repair pathway.

1 July 1995, Dispatch, *Current Biology*

▶ Intracellular Regulation: **Rac and Bcr regulate phagocytic phoxes**

Anne J Ridley (1995). *Curr. Biol.* **5**, 710–712.

Small GTPases related to the oncoprotein Ras regulate a diverse array of intracellular processes, from signal transduction to vesicle trafficking. All act as molecular switches, interacting with different proteins according to whether they are bound to GTP or to GDP. Recently, rapid progress has been made in unravelling the involvement of Rac, a member of the Ras superfamily, in the activation and deactivation of the phox proteins that make up the NADPH oxidase of phagocytes. The NADPH oxidase produces superoxide anions in response to a number of inflammatory stimuli associated with microbial infection; superoxide is then released into phagosomes, killing engulfed bacteria. In resting neutrophils, the majority of Rac is cytosolic, and is complexed in its GDP-bound form with a protein known as RhoGDI. On activation, Rac–GDP dissociates from RhoGDI; an exchange factor then stimulates exchange of GDP for GTP in a reaction that may be affected by lipids, and Rac–GTP associates with the plasma membrane, where it activates the NADPH oxidase. The NADPH oxidase activity is presumably down-regulated by hydrolysis of the GTP bound to Rac. This reaction can be stimulated by Bcr, acting as a GTPase activating protein (GAP). Although the mechanism whereby Rac activates the NADPH oxidase remains to be elucidated, substantial progress has been made in characterizing the protein–protein interactions involved.

1 July 1995, Dispatch, *Current Biology*

▶ Intracellular Signalling: **Switching off signals**

JB Imboden and GA Koretsky (1995). *Curr. Biol.* **5**, 727–729.

Receptor-activated protein tyrosine kinases transduce signals by phosphorylating the receptor itself, which in turn recruits signalling molecules to the receptor, and by phosphorylating key components of signalling cascades. The central role of tyrosine phosphorylation suggests that protein tyrosine phosphatases might switch off signals by dephosphorylating critical molecules. A combination of genetic and biochemical approaches now indicates that, at least in the case of hematopoietic cells, this is indeed the case. Studies of PTP1C, a cytosolic protein tyrosine phosphatase which is expressed exclusively by hematopoietic cells and which contains two SH2 domains, have demonstrated the ability of this phosphatase to terminate proliferative signals delivered by the receptor for erythropoietin (EPO). The results suggest that the EPO-induced oligomerization of its receptor programs the termination of its own signalling by inducing phosphorylation of a tyrosine residue on the receptor, thus creating a binding site for the SH2 domains of PTP1C. It appears that recruitment of PTP1C to the receptor results in dephosphorylation (and presumably inactivation) of the non-receptor protein tyrosine kinase, Janus kinase 2 (JAK2), which is critical for EPO receptor signalling. Negative regulation by protein tyrosine phosphatases may well prove to be a common mechanism for switching off signal transduction by receptors that activate protein tyrosine kinases.

1 July 1995, Dispatch, *Current Biology*

▶ Steroid Hormones: **Membrane transporters of steroid hormones**

E Brad Thompson (1995). *Curr. Biol.* **5**, 730–732.

Steroid hormones are generally thought to diffuse into cells where they encounter, bind to, and activate their receptors, which are already in the nucleus or will enter it on activation. The recent discovery of a plasma membrane protein that exports steroids in yeast demonstrates that cells might alter the effectiveness of specific steroids by controlling the access of steroids to their receptors. The protein, named LEM1, is a member of the ABC (ATP-binding cassette) superfamily of transporter proteins, and selectively pumps dexamethasone and triamcinolone, but not deoxycorticosterone, from yeast cells. As a result of the action of LEM1, the effective potencies of these glucocorticoids are changed relative to their actual binding affinities for the glucocorticoid receptor. The discovery, taken together with the fact that ABC proteins are found across vast taxonomic and phylogenetic spreads, highlights the possibility that similar membrane-sorting systems in mammalian cells may modulate the access of steroids to their receptors.

1 July 1995, Dispatch, *Current Biology*

▶ **Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus**

Michael Karin and Tony Hunter (1995). *Curr. Biol.* **5**, 747–757.

Two general mechanisms have evolved for the rapid and accurate transmission of signals from cell-surface receptors to the nucleus, both involving protein phosphorylation. One mechanism depends on the regulated translocation of activated protein kinases from the cytoplasm to the nucleus, where they phosphorylate target transcription factors. In the second mechanism, transcription factors are kept in a latent state in the cytoplasm and are translocated into the nucleus upon activation. Instead of providing an exhaustive review of all the relevant trans-cytoplasmic signalling pathways and the affected transcription factors, this review restricts

 ISSN 1074-5521

its discussion to a small number of well developed experimental systems that illustrate the mechanisms of communication between the surface of a eukaryotic cell and its nucleus: the MAP kinase cascade, NF-κB activation and the Jak/Stat pathway.
1 July 1995, Review, *Current Biology*

► **Targeting the mouse genome: a compendium of knockouts (part II)**
EP Brandon, RL Idzerda and GS McKnight (1995). *Curr. Biol.* **5**, 758–765.
This is the second 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 foundation for an active database that will keep track of this burgeoning field. Part III and a smaller table of double mutants will be published in the August issue of *Current Biology*.
1 July 1995, *Current Biology*

► **The first characterization of a eubacterial proteasome: the 20S complex of *Rhodococcus***
Tomohiro Tamura, István Nagy, Andrei Lupas, Friedrich Lottspeich, Zdenka Cejka, Geert Schoofs, Keiji Tanake, René De Mot and Wolfgang Baumeister (1995). *Curr. Biol.* **5**, 766–774.
The ubiquitin pathway of protein degradation was originally believed to occur only in eukaryotes. Recently, it was also found in the archaebacterium *Thermoplasma acidophilum*. The 26S proteasome is the central protease of the ubiquitin-dependent protein degradation pathway and is essential for many cellular processes including the cell cycle, transcriptional regulation and antigen presentation (see also, Goldberg, *et al.*, this issue). The proteolytic core of the complex is formed by the 20S proteasome, a cylinder-shaped particle that in archaebacteria contains two different subunits (α and β) and in eukaryotes contains 14 different subunits (seven of the α-type and seven of the β-type). The authors report the discovery, purification and characterization of a 20S proteasome complex from a nocardioform actinomycete, *Rhodococcus* sp. strain NI86/21, that is closely related to *Mycobacterium*. Purified preparations reveal the existence of four subunits, two of the α-type and two of the β-type, for which the genes were cloned and sequenced. This discovery extends the occurrence of proteasomes to the third kingdom and shows that the proteasome is an ancestral particle of universal distribution.
1 July 1995, Research Paper, *Current Biology*

► **Phosphatidylinositol transfer protein dictates the rate of inositol trisphosphate production by promoting the synthesis of PIP_2**
Emer Cunningham, Geraint MH Thomas, Andrew Ball, Ian Hiles and Shamshad Cockcroft (1995). *Curr. Biol.* **5**, 775–783.
Phosphatidylinositol transfer protein (PI-TP), which can transfer phosphatidylinositol (PI) from one membrane compartment to another, is required in the inositol lipid signalling pathway. This pathway acts through phospholipase C–β (PLC–β), which is regulated by GTP-binding protein(s) in response to extracellular signals. PI-TP is required for efficient activation of this pathway, suggesting that the plasma membrane has a limited supply of substrate for PLC-β and that replenishment from internal membranes is necessary for continued inositol phosphate production. Thus, it would be expected that PI-TP would not affect the initial rate of inositol lipid hydrolysis, but would function in maintaining that rate. But when the authors examined the effect of both PI-TP and PLC-β on the rate of inositol phosphate production, they found that PI-TP strongly influenced the initial rate of inositol phosphate production. They conclude that PI-TP acts as a cofactor, interacting directly with the lipid kinases, and hypothesize that the preferred substrate for PLC is the lipid transported by PI-TP. In this way, PI-TP could be seen as a soluble vector for PI, both allowing provision of inositol lipids at remote sites (PI-transfer activity) and, perhaps, channeling PI directly into the inositol lipid kinase pathway (cofactor activity).
1 July 1995, Research Paper, *Current Biology*

► **hSRY: molecular gender bender**
Janice Bramham and David G Norman (1995). *Structure* **3**, 631–633.
A new and unusual protein–DNA interaction has recently been found in the structure of a complex between the DNA-binding domain of hSRY, the protein encoded by the human testis-determining gene *SRY* (for sex-determining region Y), and a DNA octamer representing a specific target site on the Müllerian inhibiting substance (MIS) promoter. The product of the MIS gene is responsible for the regression of certain female sexual organ precursors in male embryos. The DNA-binding domain of hSRY is an example of a high mobility group (HMG) box, a motif initially identified in the non-histone nuclear protein HMG1, which has since been found in over 100 proteins. HMG-box-containing proteins have a range of functions, from DNA repair and recombination, to transcriptional activation, to a general role in DNA packaging. Central to all these functions seems to be the bending of DNA or the recognition of bent DNA. In agreement with previous predictions based on mutation analysis and NMR data, the recently determined structure of the hSRY-HMG/DNA complex shows Ile13 to be partially intercalated between two adenine bases in the specific recognition site. Mutation of this isoleucine is directly implicated in cases of human sex reversal and has been shown to have an essential role in sequence-specific DNA binding. The structure confirms and provides details of the involvement of Ile13 in the induction of DNA bending. The hSRY-HMG box appears to mould the DNA to its concave surface, producing a DNA conformation similar to that observed in the complex of the TATA-box-binding protein (TBP) with TATA-box DNA.
15 July 1995, Minireview, *Structure*

► **How Ras works: structure of a Rap–Raf complex**
Stephen R Sprang (1995). *Structure* **3**, 641–643.
The recent determination of the first three-dimensional structure of a complex between a member of the G-protein superfamily and its effector brings us closer to an understanding of Ras-mediated signal transduction. The structure is that of Rap bound to a non-hydrolyzable GTP analog, GppNHp, and in a complex with the Ras-binding domain (RBD) of Raf. The activation of the cytosolic serine/threonine-specific kinase c-Raf-1 by a receptor-linked tyrosine kinase requires that Raf must first be lured to the plasma membrane. This is the task of Ras, a small GTP hydrolase tethered by farnesylation to the inner leaflet of the plasma membrane. The active, GTP-bound form of Ras binds via a genetically defined effector-binding region (residues 32-40) to Raf. Activation by Ras is inhibited by Rap, which is a small GTP-binding protein and has an effector-binding region that is identical to that in Ras. Although GTP hydrolysis reduces the affinity between Ras and Raf by more than 1000-fold, the Rap–Raf binding surface does not encompass the nucleotide-binding site. Nevertheless, it is possible to relate, albeit indirectly, at least a subset of the conformational changes that accompany GDP/GTP exchange to the structure of the Rap–Raf complex. As Rap is a potent competitor of Ras, it is probable that the Rap–Raf complex will be found to recapitulate the essential features of the active Ras–Raf signaling species.
15 July 1995, Minireview, *Structure*

▶ **Viral envelope glycoproteins swing into action**

David Stuart and Patrice Gouet (1995). *Structure* **3**, 645–648.

The low-pH conformation of the influenza virus surface glycoprotein hemagglutinin (HA) provided a first insight into how massive conformational rearrangements of a metastable protein might drive key early events in the virus life cycle. Now comes the corresponding story for the major envelope glycoprotein (E) of a flavivirus, tick-borne encephalitis (TBE) virus, which is shown to contain an Ig-like domain. Although their structures are very different, the biological functions of the HA and the E proteins are very similar, and there is already an indication that the basic mechanism of one of these functions, membrane fusion, may be the same. The molecules appear to act as one-stroke motors: proteolysis of a surface protein upon leaving the host cell primes the system to function later, as the pH drops during entry to another cell. In the case of the influenza virus it is HA itself that is cleaved, in the case of TBE it is the precursor of another envelope protein M. In the precursor form, protein M interacts with protein E holding it in a dimer configuration. Cleavage of the precursor releases the dimer, which is vulnerable to conformational changes induced at low pH. The TBE dimer E is thought to become a trimer, at which point its fusion peptide is exposed and poised to initiate membrane fusion. Although the proteins and changes involved are distinct, the conceptual process is similar to that of the influenza system.

15 July 1995, Minireview, *Structure*

▶ **The low ionic strength crystal structure of horse cytochrome *c* at 2.1 Å resolution and comparison with its high ionic strength counterpart**

R Sanishvili, KW Volz, EM Westbrook and E Margoliash (1995). *Structure* **3**, 707–716.

Cytochrome *c* is an integral part of the mitochondrial respiratory chain. It is confined to the intermembrane space of mitochondria and has the function of transferring electrons between its redox partners. Although solution studies of cytochrome *c* indicate that the conformation of the molecule is sensitive to the ionic strength of the medium, the crystal structures reported to date of cytochromes *c* from several species have been solved at the extremely high ionic strengths of near-saturated solutions of ammonium sulfate. The authors present the first crystal structure of ferri-cytochrome *c* solved at close to physiological ionic strength. In general, the structure has the same features as those determined earlier. There are some differences, however, in both backbone and side-chain conformations in areas that coincide with those observed by NMR and Raman spectroscopy to be sensitive to ionic strength. The authors report that at low ionic strength the residues Arg38, Tyr48 and Trp59 are further from the heme prosthetic group. The lengthening of these bonds may explain the increase in hydrogen/deuterium exchange observed at decreased ionic strength because a proton involved in a hydrogen bond is less available for exchange. Knowledge of these interactions may be important for understanding how the protein functions under physiological conditions. In addition, the authors analyze the properties of a γ-turn, present in all cytochromes *c* of known three-dimensional structure, leading them to propose a broadening of the classical definition of γ-turns.

15 July 1995, Research Article, *Structure*

▶ **Structural analysis of human alpha-class glutathione transferase A1-1 in the apo-form and in complexes with ethacrynic acid and its glutathione conjugate**

Alexander D Cameron, Irmgard Sinning, Guillaume L'Hermite, Birgit Olin, Philip G Board, Bengt Mannervik and T Alwyn Jones (1995). *Structure* **3**, 717–727.

Glutathione transferases (GSTs) help to protect the cell from potentially toxic hydrophobic alkylating agents by catalyzing their conjugation with the tripeptide glutathione. The glutathione conjugates are formed by attacks on the electrophilic groups of target compounds, and the increased water solubility of the adducts facilitates their removal from the cell. The authors report the structures of the human alpha-class GST A1-1, the major isoenzyme found in human liver, in the apo-form and in complexes with both ethacrynic acid (EA) and with the conjugate formed between EA and glutathione (EA–GSH). EA has been administered to cancer patients in attempts to increase the efficacy of alkylating cytostatic drugs. The rationale is that EA or EA–GSH would serve as GST inhibitors and overcome the resistance caused by inactivation of the anti-cancer drug by GST. The apo-structure is the first mammalian GST structure to be solved in the absence of a glutathione derivative. In the structure of the complex with EA, the substrate is revealed as binding in a non-productive mode, suggesting that the substrate will only form an active complex when glutathione is already bound. Thus, glutathione may be involved in the molecular recognition of the electrophilic substrate by GST and should be considered when designing drugs to inhibit GST.

15 July 1995, Research Article, *Structure*

▶ **Crystal structure of *Escherichia coli* pyruvate kinase type I: molecular basis of the allosteric transition**

Andrea Mattevi, Giovanna Valentini, Menico Rizzi, M Luisa Speranza, Martino Bolognesi and Alessandro Coda (1995). *Structure* **3**, 729–741.

Pyruvate kinase (PK) is a homotetrameric enzyme, which catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, coupled to the synthesis of one molecule of ATP. PEP and one or more allosteric effectors can control the activity of PK, which has a central role in cellular metabolism. In the first description of an atomic model for an allosteric PK, the authors report the structure of the unligated protein in the inactive T-state and compare it with the known structure of the muscle M1 isoenzyme, which is a non-allosteric PK and is thought to adopt an active conformation similar to the R-state. The comparison reveals striking differences between the two structures that suggest that allosteric control of PK is accomplished through remarkable domain and subunit rotations; all 12 domains of the functional tetramer modify their relative orientations. These differences probably reflect structural changes occurring during the R- to T-state transition. This illustrates how the modular nature of PK is instrumental to the allosteric regulation of its enzymatic activity, in agreement with the model of allosteric control proposed by Monod *et al.*, in which variations in the quaternary structure of an enzyme would be involved in its allosteric transition.

15 July 1995, Research Article, *Structure*