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 January 1996, **3**:67–70

▶ Appetite Control: **Does leptin lighten the problem of obesity?**

J Denis McGarry (1995). *Curr. Biol.* **5**, 1342–1344.

Is leptin the cure for post-Christmas blues? The product of the mouse *obese* gene, leptin is secreted by fat cells, and its administration not only corrects the overeating of the *obese* mutant mouse, but also accelerates the rate of metabolism and suppresses fat synthesis. Leptin treatment of the *obese* mutant mouse normalizes the elevated level of neuropeptide Y mRNA in the hypothalamus, a proposed site of action of leptin. Thus leptin appears to be the long sought satiety factor — a signal from fat cells to the rest of the body that sufficient fat is present. This signal probably feeds back in a loop involving insulin production by the pancreas. Disruption of elements of this loop may lead to obesity in humans.

1 December 1995, Dispatch, *Current Biology*

▶ Somatic Hypermutation: **Another piece in the hypermutation puzzle**

DB Winter and PJ Gearhart (1995). *Curr. Biol.* **5**, 1345–1346.

The increased strength of B cell responses to repeated antigenic challenge relies on a form of accelerated, somatic evolution — hypermutation of the rearranged immunoglobulin variable genes, followed by selection for cells producing antibodies with the highest affinities. The mutations are randomly distributed over a 2-kb region that includes part of the promoter, the V(D)J coding region and a downstream intron of the immunoglobulin gene. An enhancer in this intron and one 3' of the constant (C) region are required for significant hypermutation to occur, but the V(D)J sequences and the promoter are not. These sequences can be replaced by *gpt*, *neo* or β-globin sequences, and the β-globin promoter, respectively. These surprising results are the first step in localizing the *cis* DNA sequences necessary for operation of the hypermutation machinery.

1 December 1995, Dispatch, *Current Biology*

▶ Plant Photomorphogenesis: **A green light for cryptochrome research**

Gary Whitelam (1995). *Curr. Biol.* **5**, 1351–1353.

Plants react in many ways to different intensities and frequencies of illumination, such as by bending or by inhibiting axis elongation. As the blue-light photoreceptors responsible for receiving this information have proved so elusive, they have been named cryptochromes. CRY1 is the first cryptochrome to be identified. It was cloned following the isolation of an insertion mutation in the *HY4* gene of *Arabidopsis thaliana* that results in a failure of the normal inhibition of hypocotyl elongation in response to blue light. The first ~500 residues of CRY1 are strikingly similar in sequence to the apoproteins of type I microbial DNA photolyases. These enzymes use the energy of a photon of light to catalyze the repair of a pyrimidine dimer. CRY1 contains the same two chromophores as the photolyases, methenyltetrahydrofolate and $FADH_2$, but shows no photolyase activity. Overexpression of CRY1 in transgenic tobacco plants results in hypersensitivity to the inhibitory effects of blue, UV-A and green light. By analogy to the reaction mechanism of photolyases, CRY1 may initiate signal transduction by electron transfer — an entirely novel mechanism.

1 December 1995, Dispatch, *Current Biology*

▶ Motile Systems: **Tubulin-based motility races ahead**

Edward H Egelman (1995). *Curr. Biol.* **5**, 1354–1356.

Understanding biological motility — how macromolecules convert chemical energy to the production of force — is a primary goal of biophysics. Although the elucidation of the structures of actin and myosin seemed to be the first step in this direction, these structures have not been as informative as was intially hoped. It seems that the first full story may come instead from motor proteins that move along microtubules. A structure of tubulin at a resolution of 6.5 Å has been obtained, and structures for ncd and kinesin, two motor proteins that move in opposite directions on microtubules, are expected soon. Meanwhile, electron microscopy has been used to observe the interactions between tubulin and the motors. Both ncd and kinesin interact with, and track along, the ridge of a single microtubule protofilament. Part of the kinesin molecule has also been observed to rotate relative to the rest of the molecule when its associated nucleotide is lost. This rotation may represent the power stroke of the motor, whose energy is derived from ATP hydrolysis.

1 December 1995, Dispatch, *Current Biology*

▶ Left–Right Asymmetry: **The embryo's one-sided genes**

T King and NA Brown (1995). *Curr. Biol.* **5**, 1364–1366.

Vertebrates have symmetrical bodies but asymmetrical visceral organs. Commitment to left or right appears to arise relatively late in development, and the first asymmetric patterns of gene expression have now been detected, at the appropriate stage, in the primitive streak of the chick. Four developmental genes were found to be expressed asymmetrically. Ectopic expression experiments have given rise to a model in which the initial asymmetric expression of an (unidentified) activin increases the expression of the activin type IIa receptor gene on the right side. This represses *Sonic hedgehog* (*Shh*) expression, restricting it to the left side, where it induces *nodal-related 1* expression. *HNF3β*, a transcription factor related to the *Drosophila forkhead* product, may be involved in the maintenance of *Shh* expression. This model presumes that the observed expression asymmetries are relevant to the future body plan. Expression asymmetry of *Shh*, *nodal* or *HNF3b* has not been detected in the mouse and zebrafish, however, and a knockout mutation of the type IIa activin receptor in mice shows no defects in left–right asymmetry. Perhaps different organisms use different molecules to specify left–right asymmetry. Further studies are necessary to address this, and to answer the larger question of how the initial asymmetry is established.

1 December 1995, Dispatch, *Current Biology*

▶ Cell–Cell Signaling: **The ins and outs of receptor tyrosine phosphatases**

Sarah J Fashena and Kai Zinn (1995). *Curr. Biol.* **5**, 1367–1369.

The correct balance of tyrosine phosphorylation is maintained by the opposing actions of tyrosine kinases and phosphatases.

Recent results have identified proteins that interact with receptor tyrosine phosphatases and have implicated these phosphatases in adhesion and cell signaling. The receptor tyrosine phosphatase RPTPβ has an extracellular carbonic anhydrase-like domain that binds the neuronal ligand contactin, a member of the immunoglobulin superfamily. This leads to adherence and differentiation of the contactin-expressing cells, and subsequently, to neurite extension. A soluble form of the RPTPβ extracellular domain can, however, block adhesion of glia and neurons mediated by N-CAM and Ng-CAM. As Ng-CAM and contactin may be in a complex, the outcome of their interaction with the various forms of RPTPβ is hard to predict. Another receptor tyrosine phosphatase, PTPμ, is a homophilic adhesion molecule that associates with a complex containing cadherins and α- and β-catenins. Cadherins appear to be a substrate for PTPμ, and the absence of PTPμ would presumably lead to increased phosphorylation of the cadherins, which is known to weaken intercellular adhesion at adherens junctions.
1 December 1995, Dispatch, *Current Biology*

▶ **The core of the mammalian centriole contains γ-tubulin**
Stephen D Fuller, Brent E Gowen, Sigrid Reinsch, Alan Sawyer, Brigitte Buendia, Roger Wepf and Eric Karsenti (1995). *Curr. Biol.* **5**, 1384–1393.
The centrosome is the organelle that nucleates microtubules to form a cytoplasmic cytoskeleton and the mitotic spindle. Microtubules, which are composed of alternating subunits of α- and β-tubulin, originate from the fuzzy pericentriolar material. This material surrounds the centrioles, the barrel of nine triplets of microtubules that lies at the center of the centrosome. The conserved protein γ-tubulin, which has previously been localized to the pericentriolar material, is a strong candidate for the protein that nucleates microtubule formation. It does not enter microtubules formed from α- and β-tubulin, but in its absence, no microtubules are formed. In this study, post-sectioning fixation is incorporated into an immunogold localization protocol for γ-tubulin, allowing a more detailed analysis of γ-tubulin localization. Foci of γ-tubulin are observed in the pericentriolar material, often near the termini of microtubules. A novel site of localization of γ-tubulin is a structure within the centriolar barrel, in a region from which α-tubulin is excluded. It is also seen in a structure that links duplicated centrioles. These localization patterns suggest that γ-tubulin may act as a template for growth of the microtubules that make up the centriole, in addition to its established role in the nucleation of astral microtubules.
1 December 1995, Research Paper, *Current Biology*

▶ **A family of phosphoinositide 3-kinases in *Drosophila* identifies a new mediator of signal transduction**
Lindsay K MacDougall, Jan Domin and Michael D Waterfield (1995). *Curr. Biol.* **5**, 1404–1415.
Phosphoinositide 3-kinases (PI 3-kinases) are involved in signaling in multiple cellular processes. They phosphorylate three different phosphatidyl inositol lipids, but the products of these reactions are not substrates for any of the known phospholipase C enzymes, and thus may themselves act as second messengers. One form of PI 3-kinase is composed of a regulatory p85 subunit and a catalytic p110 subunit, and is activated by receptor tyrosine kinases. Another form is G-protein-linked, and a third is involved in protein trafficking. In this study, PCR is used to identify PI 3-kinases from *Drosophila*. Three enzymes are identified, one showing similarity to the tyrosine kinase-linked enzyme, and one to the enzymes involved in protein trafficking. The third enzyme (PI3K_68D) is novel, and contains an amino-terminal proline-rich sequence, which could bind to SH3 domains, and a carboxy-terminal C2 domain, which mediates weak, Ca^{2+}-independent binding to lipid. PI3K_68D has a distinct substrate specificity *in vitro*, and may define a novel signal transduction pathway.
1 December 1995, Research Paper, *Current Biology*

▶ **Cell lineage patterns and homeotic gene activity during *Antirrhinum* flower development**
Coral A Vincent, Rosemary Carpenter and Enrico S Coen (1995). *Curr. Biol.* **5**, 1449–1458.
Homeotic genes control the developmental fate of serially repeated units within organisms, such as insect parasegments and flower whorls. The definition of boundaries of expression of different homeotic genes, and therefore of segment boundaries, may rely on differential cell affinity. In plants, however, cells are unable to move relative to each other, and so cell sorting of this kind is not possible. Perhaps plant cells maintain plasticity until late in development to facilitate the definition of sharp borders of organ identity in the face of continued cell division. In this study, a temperature sensitive transposon is used to analyse clones of plant cells to determine the time at which organ identity is fixed. Before the emergence of organ primordia, cells in the floral meristem have not been allocated organ identity. Restrictions arise between whorls around the time that genes controlling organ identity are first expressed. A further lineage restriction appears slightly later, between the dorsal and ventral surfaces of the petal. Thus, identity is set relatively early in flower development. Boundaries may be maintained by the product of the *fimbriata* gene, as this gene is expressed at whorl junctions. The underlying mechanism may be oriented cell divisions near whorl junctions and reduced numbers of divisions at this site.
1 December 1995, Research Paper, *Current Biology*

▶ **How to make my blood boil**
Adrian Goldman (1995). *Structure* **3**, 1277–1279.
What special features of proteins from thermophiles make the proteins heat stable? Two recent papers compare the structures of two proteins from a thermophile with the structures of their heat-labile counterparts from garden-variety microbes. Both papers conclude that numerous ion-pairs, often arranged in large networks, explain the thermostability. To create the networks, some residues, often arginine, have to form multidentate interactions. These results agree with earlier studies, but clash with theoretical considerations of the energy of forming an ion pair, which indicate that two solvent-screened ions have to be desolvated, with at best an enthalpy change of around zero. The answer may lie in the formation of the networks, where an additional salt bridge involves the desolvation and immobilization of only one additional residue. It is clear from the recently-determined structure of Taq polymerase, however, that there is more than one way to stabilize a thermophilic protein. When compared to *Escherichia coli* DNA polymerase I, Taq polymerase has an increased hydrophobic core, increased interdomain interface area, and removal of unfavorable elecrostatic interactions. Structures of other proteins have suggested that the most important factor is a minimization of the surface-to-volume ratio, resulting in tight packing of the residues. With the determinants of thermostability being as subtle as this, it seems that at present it will be easier to transform proteins from being heat stable to heat labile than to do the reverse.
15 December 1995, Minireview, *Structure*

See also: **The structure of *Pyrococcus furiosus* glutamate dehydrogenase reveals a key role for ion-pair networks in maintaining enzyme stability at extreme temperatures.** KSP Yip, TJ Stillman, KL Britton, PJ Artymiuk, PJ Baker, SE Sedelnikova, PC Engel, A Pasquo, R Chiaraluce, V Consalvi, R Scandurra and DW Rice (1995). *Structure* **3**, 1147–1158; and

2.0 Å structure of indole-3-glycerol phosphate synthase from the hyperthermophile *Sulfolobus solfataricus*: possible determinants of protein stability. Michael Hennig, Beatrice Darimont, Reinhard Sterner, Kasper Kirschner and Johan N Jasonius (1995). *Structure* **3**, 1295–1306.

► **Switching to Rac and Rho**

Christoph Block and Alfred Wittinghofer (1995). *Structure* **3**, 1281–1284.

For a brief moment the world of signaling by small GTPases seemed neatly compartmentalized — Ras was the prototypic regulator of proliferation, the Rab family controlled intracellular vesicle transport and the Rho/Rac family directly regulated cytoskeletal reorganization. It now seems that both Rho and Rac also activate mitogen-activated protein kinase (MAPK) cascades distinct from, but analogous to, that used by Ras. Rac interacts with and activates a kinase called PAK, resulting ultimately in the activation of the MAPK, JNK, which phosphorylates a number of transcription factors including members of the Jun family. Less is known about the pathway downstream of Rho, although the kinase that interacts with Rho has been identified as PKN, and the Rho-mediated signal is ultimately targeted to the serum-response factor. The MAPK modules appear to form a well-ordered arrangement of parallel pathways, although there is a limited amount of crosstalk; activation from Ras to Rac to Rho has been documented at the top of the pathways. For full mitogenesis and morphological transformation more than one MAPK module has to be switched on.

15 December 1995, Minireview, *Structure*

► **The enigma of LIM domains**

Gordon N Gill (1995). *Structure* **3**, 1285–1289.

LIM domains are cysteine-rich protein motifs of ~55 residues found in proteins of diverse functions. The core structure of the one LIM domain to be studied in detail is predominantly an antiparallel β-sheet with two Zn^{2+}-binding modules located at either end of a hydrophobic core. The proteins that gave the domain its name (Lin-11, Isl-1 and Mec-3) all also contain homeodomains, but more recently discovered proteins have various enzymatic activities, or consist solely of two LIM domains. The LIM-only proteins provide a strong argument that LIM domains mediate protein–protein interactions. LIM domains have been shown to interact with other LIM domains and with tyrosine-containing tight turns in the insulin receptor. The interactions are specific for a particular LIM domain; LIM domains have been relatively well conserved in evolution. Various mouse proteins with LIM domains are important in development: *Lhx-1* is necessary for the development of head structures, kidneys and gonads, *Isl-1* is induced by Sonic hedgehog and necessary for motor neuron development, and *LMO2* encodes a LIM-only protein essential for erythroid development. LMO2 interacts with the basic helix-loop-helix (bHLH) transcription factor Tal 1; similarly the synergistic activation of the rat insulin I gene promoter by the LIM homeodomain protein lmx-1 and the bHLH E47 protein requires the LIMs of lmx-1. It should be possible to exploit the interactions mediated by LIM domains to link components in pathways in development, cytoskeletal structure, signaling, trafficking and growth control.

15 December 1995, Minireview, *Structure*

► **X-ray structure of human nucleoside diphosphate kinase B complexed with GDP at 2 Å resolution**

Solange Moréra, Marie-Lise Lacombe, Xu Yingwu, Gérard LeBras and Joël Janin (1995). *Structure* **3**, 1307–1314.

Nucleotide diphosphate kinase (NDP kinase) exchanges γ-phosphates between nucleoside tri- and diphosphates to provide precursors for DNA and RNA synthesis. Independently of its catalytic function, human NDP kinase B activates transcription of the *c-myc* oncogene by binding to the promoter DNA. How do these two functions coexist? The structure of human NDP kinase B has been solved and compared with the structures of DNA polymerase I and HIV reverse transcriptase. All three enzymes bind mono- and polynucleotides and have the βαββαβ fold. The proposed binding site for DNA is not accessible to such a large ligand in the NDP kinase structure, however. Binding to DNA is likely to involve the movement of a pair of very mobile α-helices to expose the top of a β sheet. The bound DNA would prevent substrate binding, and in any case the mobile helices carry residues involved both in substrate binding and catalysis. Thus functioning of the protein as a transcriptional activator necessarily switches it to a catalytically inactive state.

15 December 1995, Research Article, *Structure*

► **Two conformations of the integrin A-domain (I-domain): a pathway for activation?**

Jie-Oh Lee, Laurie Anne Bankston, M Amin Arnaout and Robert C Liddington (1995). *Structure* **3**, 1333–1340.

Integrins are membrane proteins that mediate adhesion to other cells and to components of the extracellular matrix. Adhesion is regulated by intracellular signals, which lead to conformational changes in the extracellular domains of these receptors. The nature of these conformational changes is unknown. The interaction of several integrins with their ligands is mediated by an I-domain, the structure of which has previously been solved with bound Mg^{2+}. This structure is now compared with one containing bound Mn^{2+}. The Mg^{2+}-bound structure appears to represent the active (ligand-binding) form of the domain, as one of the residues coordinating the metal is a glutamate side chain from another I-domain. This residue is probably acting as a ligand mimetic, as a key feature of all known integrin ligands is an acidic residue. The Mn^{2+}-bound structure may represent the inactive form of the domain, as there is no equivalent for the exogenous glutamate. Changes in coordination of the metal upon crystallization with Mn^{2+} rather than Mg^{2+} parallel those seen upon conversion of active (GTP-bound) $p21^{ras}$ to the inactive (GDP-bound) form. In both cases a bond from the metal to a threonine is lost, and a direct bond between the metal and an aspartate is gained. In addition, one ($p21^{ras}$) or two (the I-domain) previously-exposed hydrophobic residues are buried, probably leading to a more stable structure. For the integrins, this structure can be modified conformationally to give the active form either by ligand binding or crystallization in the presence of Mg^{2+}.

15 December 1995, Research Article, *Structure*

► **Crystal structure of an acetylcholinesterase–fasciculin complex: interaction of a three-fingered toxin from snake venom with its target**

Michael Harel, Gerard J Kleywegt, Raimond BG Ravelli, Israel Silman and Joel L Sussman (1995). *Structure* **3**, 1355–1366.

The α-neurotoxins from the venoms of kraits and cobras inhibit a diverse set of biological targets, yet have substantial

sequence and structural homology. All contain a core with four disulfide bridges, from which three loops, or 'fingers', protrude. Fasciculin-II (FAS-II), a 61-residue member of this family, is a powerful reversible inhibitor of acetylcholinesterase (AChE). This synaptic enzyme terminates transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter, acetylcholine (Ach). The active site of AChE is located near the bottom of a deep and narrow gorge, and the structure of FAS-II bound to AChE shows FAS-II sealing the entrance to this gorge. The interface between AChE and FAS-II consists largely of hydrophobic interactions, together with 11 hydrogen bonds and only 3 polar side-chain interactions. There are no salt links, despite the presence of a number of charged residues. There is remarkable surface complementarity between the molecules, involving a large contact area of ~2000 $Å^2$. In addition, the substantial dipole moments of AChE and FAS-II are aligned. Two aromatic residues of AChE make important contacts with FAS-II, but are absent from chicken and insect AChEs, perhaps explaining the large reduction in the affinity of these enzymes for FAS-II.

15 December 1995, Research Article, *Structure*

▶ **The crystal structures of the oligopeptide-binding protein OppA complexed with tripeptide and tetrapeptide ligands**

Jeremy RH Tame, Eleanor J Dodson, Garib Murshudov, Christopher F Higgins and Anthony J Wilkinson (1995). *Structure* **3**, 1395–1406.

The periplasmic oligopeptide-binding protein OppA functions as the receptor for peptide transport by binding to peptides of two to five amino-acid residues with high affinity, but little regard to sequence. How is this achieved? Toleration of sequence variability of peptide ligands is usually due to partial solvent exposure of the ligand, as is seen, for example, in the major histocompatibility complex proteins. In contrast, OppA accommodates the peptides in a large hydrated pocket, with strong hydrogen bonding between the protein and the main chain of the ligand. The peptides are in an extended conformation, forming β-sheet-like interactions with the protein. The charges at the end of the peptides are countered by oppositely-charged side chains from the protein, and different basic side chains on the protein form salt bridges with the carboxyl terminus of peptide ligands ranging from di- to pentapeptides.

15 December 1995, Research Article, *Structure*