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 December 1995, **2**:857–859

► Cancer Predisposition: **Ataxia-telangiectasia at the crossroads**

Stephen P Jackson (1995). *Curr. Biol.* **5**, 1210–1212.

Ataxia-telangiectasia (AT) is a human genetic disease with a wide variety of symptoms, including increased cancer risk, premature aging and neurological and immune defects. AT cells are radiation-sensitive, and lack radiation-induced cell cycle checkpoint arrests. The ATM (AT-mutated) gene has recently been cloned. Its protein product is large, and the carboxy-terminal 400 amino-acid residues are homologous to the catalytic domain of a subgroup of the phosphatidyl inositol 3-kinase family. This subgroup includes the checkpoint genes *rad3* (from *Saccharomyces cerevisiae*) and MEC1 (from *Schizosaccharomyces pombe*). The closest relative of ATM is the TEL1 gene from *S. cerevisiae*. *tel1* mutants have shortened telomeres, a feature common to AT cells that may explain the premature aging seen in AT. Another relative of ATM suggests that it may be, not a lipid kinase (lipid kinase activity has not been detected for any of the members of this subgroup), but a protein kinase. The catalytic subunit of DNA-dependent protein kinase (DNA-PK) falls into the ATM subgroup, and is active as a protein kinase only when bound to DNA ends or damaged DNA by its regulatory subunit. One potential substrate of ATM protein kinase activity is IκB, the negative regulatory subunit of the transcription factor, NF-κB; a truncated IκB that lacks carboxy-terminal phosphorylation sites rescues the radiosensitivity of AT cells. Incorrect NF-κB regulation could explain the immune system defects seen in AT patients. The tumor suppressor p53 is another possible downstream target of ATM, as AT cells fail to induce p53 in response to DNA damage, and DNA-PK can phosphorylate p53 directly *in vitro*.

1 November 1995, Dispatch, *Current Biology*

► Calcium Signalling: **Cracking I_{CRAC} in the eye**

Deborah L Bennett, Carl CH Petersen and Timothy R Cheek (1995). *Curr. Biol.* **5**, 1225–1228.

Many signal transduction pathways result in the generation of inositol 1,4,5-triphosphate ($InsP_3$), which binds to an intracellular receptor leading to the release of Ca^{2+} from intracellular stores. Depletion of these stores leads to the activation of Ca^{2+} channels in the plasma membrane, generating the calcium-release-activated current (I_{CRAC}). What is the channel that carries this current? The prime candidates have come from the study of phototransduction in *Drosophila*. Invertebrate photoreceptors respond to light with a rise in $InsP_3$ levels, causing Ca^{2+} entry and depolarization. Genetic experiments identified the transient receptor potential (*trp*) gene product as a potential light activated Ca^{2+} channel. The cloned gene has some similarity to voltage-sensitive Ca^{2+} channels, but lacks a residue involved in sensing voltage. The *trp* mutant still shows some transient current, which may be mediated by a related channel, TRPL, that is rapidly activated by $InsP_3$. After Ca^{2+} store depletion (due to $InsP_3$ signalling), the TRP channel provides the sustained portion of the Ca^{2+} conductance. TRP has recently been shown to reside in the plasma membrane adjacent to the internal Ca^{2+} stores in *Drosophila* photoreceptors, suggesting that it may directly interact with the $InsP_3$ receptor. Physiological studies in other cells suggest that this activation parallels the induction of I_{CRAC}, indicating that no diffusible messenger is required.

1 November 1995, Dispatch, *Current Biology*

► Chromatin: **Nucleosome assembly during DNA replication**

Torsten Krude (1995). *Curr. Biol.* **5**, 1232–1234.

During S phase, not only is the entire genome replicated, but the underlying chromatin structure also has to be duplicated. The existing histones from the parental nucleosomes are transferred directly, and passively, onto the replicated DNA. The assembly of new nucleosomes, however, requires chromatin assembly factors. Chromatin assembly factor 1 (CAF-1) mediates the first step of the assembly reaction — the deposition of histones H3 and H4 onto DNA. This reaction is dependent on ongoing DNA replication, whereas the subsequent association of H2A/H2B dimers to form a complete nucleosome does not require CAF-1 or DNA replication. The two larger subunits of CAF-1, p150 and p60, have recently been cloned. They interact physically with each other, and p150 probably interacts with histones through its highly negatively charged central portion. CAF-1 is present at replication foci, and biochemical results suggest an indirect association with single-stranded DNA, but it will take more work to determine whether CAF-1 interacts with the DNA replication apparatus.

1 November 1995, Dispatch, *Current Biology*

► Protein Structure: **Why have six-fold symmetry?**

Zvi Kelman, Jeff Finklestein and Mike O'Donnell (1995). *Curr. Biol.* **5**, 1139–1142.

All proteins examined thus far that can encircle DNA and translocate along the double helix have been found to have either true or pseudo six-fold symmetry. These include the β-subunit of *Escherichia coli* DNA polymerase III holoenzyme (two subunits, each with three domains) and the similar eukaryotic protein, proliferating cell nuclear antigen (PCNA), the processivity factor for DNA polymerase δ (three subunits, each with two domains). The authors argue that the total mass of protein necessary to encircle a cavity decreases sharply as the number of spherical subunits increases. A greater number of subunits also increases the difficulty of assembling a complete ring structure, however. This seems to have led to the fusing of subunits in multidomain proteins and also to the limitation of the number of subunits to six. The resultant subunit size is close to the observed average size for single domains in other proteins. Ring-shaped proteins with cavities larger than that necessary to accommodate DNA (proteasomes, chaperonins) have 7–9 fold symmetry, while those with smaller cavities (bacterial toxins) are pentamers.

1 November 1995, Dispatch, *Current Biology*

► Ion Channels: **A physiological function for polyamines?**

Ian D Forsythe (1995). *Curr. Biol.* **5**, 1248–1251.

The conductance of ion channels is typically gated by either voltage or ligands. Other molecules, such as Mg^{2+}, can modify

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the gating by blocking the open pore of the channel. This blockage will be voltage-dependent due to the charge on the Mg^{2+} — depolarization will attract an intracellular Mg^{2+} into the channel whereas hyperpolarization will repel it. This results in a channel that passes current preferentially in one direction, or a rectifier. Recently it has been realized that small, highly charged, aliphatic cations, such as the polyamines spermine and spermidine, may act as rectifiers. Washing membrane patches before patch clamp measurements of the K^+ channel IRK1 results in a decrease in the observed rectification, but this is restored by the addition of spermine to the internal face of the patch. A similar result is seen with a subtype of glutamate receptors; this rectification may shorten action potentials in neurons of auditory pathways. Previous studies of these receptors now need to be reexamined, as polyamines may have been lost in the previous experiments. We also now need to consider the possibility that the degree of rectification may be modified independently of channel expression.
1 November 1995, Dispatch, *Current Biology*

► **The nuclear envelope prevents reinitiation of replication by regulating the binding of MCM3 to chromatin in *Xenopus* extracts**
Mark A Madine, Chong-Yee Koon, Anthony D Mills, Christine Musahl and Ronald A Laskey (1995). *Curr. Biol.* **5**, 1270–1279.

A G2-phase nucleus will not re-replicate its DNA when placed in a pre-replicative (G1-phase) *Xenopus* extract. This control can be circumvented by making holes in the nuclear envelope and allowing the entry of a putative 'licensing factor'. It has been proposed that in a normal cell cycle the licensing factor gains access to the DNA only when the nuclear envelope is dissolved during mitosis, thus ensuring that DNA replication occurs only once per cell cycle. Some of the MCM proteins from budding yeast display characteristics expected of a licensing factor: they are localized to the nucleus during mitosis, disappear at the beginning of S phase, and mutations in their genes cause defects in the control of replication. A *Xenopus* homolog of MCM3 (XMCM3) is displaced from chromatin during replication and is necessary for the re-replication of permeabilized G2 nuclei, but not for the replication of permeabilized G1 nuclei. XMCM3 can freely diffuse into *Xenopus* nuclei, however. So why is permeabilization required for re-replication? In *Xenopus* extracts the nuclear envelope appears to restrict the entry not of XMCM3, but of a factor that promotes the binding of XMCM3 to chromatin. XMCM3 crosses intact nuclear membranes of G2-phase nuclei, but cannot then bind to chromatin unless the nuclear envelope is permeabilized. No molecular candidate has yet been identified for this loading factor.
1 November 1995, Research Paper, *Current Biology*

► **Chemoattractant-controlled accumulation of coronin at the leading edge of *Dictyostelium* cells monitored using a green fluorescent protein–coronin fusion protein**
Günther Gerisch, Richard Albrecht, Christina Heizer, Steve Hodgkinson and Markus Maniak (1995). *Curr. Biol.* **5**, 1280–1285.

The highly motile cells of *Dictyostelium* rapidly remodel their actin filament system when they change their direction of locomotion. Coronin is a cytoplasmic actin-associated protein required for rapid locomotion and cytokinesis that accumulates at the cortical sites of moving cells. A green fluorescent protein (GFP)–coronin fusion protein is used to visualize the redistribution of coronin during locomotion in real time in living cells. Coronin is reversibly recruited from the cytoplasm and is incorporated into the actin network of a nascent leading edge, where it participates in the reorganization of the cytoskeleton.
1 November 1995, Research Paper, *Current Biology*

► **The GTP-binding protein Rac does not couple PI 3-kinase to insulin-stimulated glucose transport in adipocytes**
J Marcusohn, SJ Isakoff, E Rose, M Symons and EY Skolnik (1995). *Curr. Biol.* **5**, 1296–1302.

Phosphoinositide 3-kinase (PI 3-kinase) is involved in several cellular signaling pathways. Some of the biological effects of PI 3-kinase are mediated by the GTP-binding protein Rac, including actin reorganization, which leads to the formation of lamellipodia. Is Rac always part of the pathway activated by PI 3-kinase? This study examines the effect caused by the expression of various mutant Rac proteins in an insulin-sensitive adipocyte cell line. Expression of constitutively active Rac1 results in the constitutive formation of lamellipodia and constitutive activation of the cJun-N-terminal kinase (JNK). A dominant-inhibitory Rac1 inhibits the insulin-stimulated formation of lamellipodia. Neither the basal glucose uptake nor insulin-stimulated glucose uptake are affected by the expression of either mutant protein, however. Thus adipocytes use an effector distinct from Rac to couple the insulin receptor to glucose uptake. The data are consistent with a branched signal transduction pathway, with PI 3-kinase coupled to this unknown effector in addition to Rac.
1 November 1995, Research Paper, *Current Biology*

► **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.

The hyperthermophile *Pyrococcus furiosus* is one of the most thermostable organisms known, with an optimum growth temperature of 100 °C. The proteins from this organism display extreme thermostability. The structure of *P. furiosus* glutamate dehydrogenase, which is a homohexamer, has been determined at 2.2 Å resolution and compared with the structure of the same enzyme from the mesophile *Clostridium symbiosum*. The major difference between the two structures is that the hyperthermophilic enzyme has a striking series of ion-pair networks on the surface of the protein and buried at both interdomain and intersubunit interfaces. The formation of such networks may account for the stability of this enzyme in the face of high temperatures. These observations may provide insight into the question of what types of amino acid changes are required to improve the thermostability of proteins.
15 November 1995, Research Article, *Structure*

► ***Ab initio* determination of the crystal structure of cytochrome c_6 and comparison with plastocyanin**
C Frazao, CM Soares, MA Carrondo, E Pohl, Z Dauter, KS Wilson, M Hervas, JA Navorro, MA de la Rosa and GM Sheldrick (1995). *Structure* **3**, 1159–1169.

Electron transfer between cytochrome *f* and photosystem I (PSI) can be accomplished by either the heme-containing protein cytochrome c_6 or by the copper-containing protein plastocyanin. Higher plants use PSI only, whereas most green algae and cyanobacteria can use either. The authors report the structure of cytochrome c_6 from the green alga *Monoraphidium*

braunii. The high resolution of the X-ray data (1.2 Å) and the fact that one iron and three sulfur atoms were present made it possible to determine the structure *ab initio*, without the need for heavy atom derivatives. Potential electron pathways within the structure have been identified; it appears that cytochrome c_6 may use just one electron transfer site, close to the heme, in contrast to the two sites used by plastocyanin.
15 November 1995, Research Article, *Structure*

► **Crystal structure of a quinoenzyme: copper amine oxidase of *Escherichia coli* at 2 Å resolution**
MR Parsons, MA Convery, CM Wilmot, KDS Yadav, V Blakeley, AS Corner, SEV Phillips, MJ McPherson and PF Knowles (1995). *Structure* **3**, 1171–1184.

Enzymes that catalyze oxidoreduction reactions often contain amino acid residues, especially tryptophan or tyrosine, in unusual redox states. *Escherichia coli* copper amino oxidase (ECAO) contains a tyrosine residue (Tyr466) in the active site that is modified to 2,4,5-trihydroxyphenylalanine quinone (TPQ), a novel redox-active residue. The formation of TPQ from tyrosine is a self-processing reaction that is dependent on the presence of the active site copper ion. The crystal structure of ECAO reveals that the TPQ residue is not coordinated to the copper ion, but suggests a mechanism by which the metal may be involved in the conversion of tyrosine to TPQ. Although TPQ is known to bind substrate, the details of its role in catalysis remain to be elucidated. The crystal structure of an inactive form of ECAO was also determined. The only structural differences between the active and inactive forms are in the active site, where changes in copper-coordination geometry and in the position and interactions of TPQ are observed. In the inactive enzyme, Asp383, which may be the active-site base, no longer faces the 5'-position of the TPQ ring, perhaps preventing substrate binding or proton extraction by Asp383.
15 November 1995, Research Article, *Structure*

► **The three domains of a bacterial sialidase: a β-propeller, an immunoglobulin module and a galactose-binding jelly roll.**
Andrew Gaskell, Susan Crennell and Garry Taylor (1995). *Structure* **3**, 1197–1205.

Sialidases have been implicated in the pathogenesis of many diseases, but are also produced by many non-pathogenic bacteria. The sialidase from the soil bacterium *Micromonospora viridifaciens* is secreted in two forms, a 41-kDa form and a 68-kDa form, depending on the carbohydrate used to induce expression. The authors report the X-ray crystal structures of both forms of the enzyme, and complexes with an inhibitor and with galactose. The 41-kDa form shows the six-bladed β-propeller fold seen in the *Salmonella typhimurium* sialidase and influenza virus neuraminidase structures. The 68-kDa form has two extra domains, a linker with an immunoglobulin-like fold, and a domain that is homologous to the galactose-binding domain of a fungal galactose oxidase. This structure is reminiscent of the structure of the sialidase from *Vibrio cholerae*, which has a central β-propeller catalytic domain flanked by two lectin-like domains. This molecular architecture, combining carbohydrate-binding domains with catalytic domains, may be common to several members of the sialidase/neuraminidase family. The carbohydrate-binding domains are presumed to be important in targeting particular substrates, and may thus be attractive targets for therapeutic intervention.
15 November 1995, Research Article, *Structure*

► **Substrate binding and carboxylation by dethiobiotin synthetase — a kinetic and X-ray study**
Dmitriy Alexeev, Robert L Baxter, Otto Smekal and Lindsay Sawyer (1995). *Structure* **3**, 1207–1215.

The vitamin biotin is a ubiquitous prosthetic group of carboxylase and transcarboxylase enzymes. The penultimate step in biotin biosynthesis, catalyzed by dethiobiotin synthetase (DTBS) involves a unique ATP-dependent *N*-carboxylation of the erythro diamine of (7*R*, 8*S*) 7,8 diaminononanoic acid to form the *ureido* ring of dethiobiotin. The authors report the kinetics of enzyme–substrate association and substrate carboxylation in the presence and absence of Mg^{2+}, and the X-ray structures of the enzyme at several stages of the reaction. Mg^{2+} is required for catalysis and causes changes in the structure of the active site, optimizing the interactions between substrate and enzyme. The carboxylation site is identified as the N-7 amino group of the substrate, not the N-8 amino group previously suggested. The authors propose a catalytic mechanism for DTBS.
15 November 1995, Research Article, *Structure*

► **Crystal structure of the di-haem cytochrome *c* peroxidase from *Pseudomonas aeruginosa***
Vilmos Fülöp, Christopher J Ridout, Colin Greenwood and Janos Hádju (1995). *Structure* **3**, 1225–1233.

Cytochrome *c* peroxidase from *Pseudomonas aeruginosa* (PsCCP) is one of a new class of peroxidases that do not need to create a semi-stable free radical for catalysis. It contains two hemes with very different properties, one low-potential (where hydrogen peroxide is reduced), the other high-potential (which feeds electrons to the peroxidatic site from proteins such as cytochrome *c*). The authors report the crystal structure of the oxidized form of PsCCP to 2.4 Å, and show that the enzyme is organized into two domains, each containing a heme. The domain interface includes a newly discovered calcium-binding site with an unusual set of ligands, none of which are negatively charged. The calcium ion may stabilize the domain interface and/or modulate electron transfer between the two hemes. In the half-reduced, functional form of the enzyme the low-potential heme probably sheds one of its axial His ligands, His71. This is likely to trigger a reorganization of the active site.
15 November 1995, Research Article, *Structure*

► **The crystal structure of cyclin A**
NR Brown, MEM Noble, JA Endicott, EF Garman, S Wjakatsuki, E Mitchell, B Rasmussen, T Hunt and LN Johnson (1995). *Structure* **3**, 1235–1247.

Eukaryotic cell cycle progression is regulated by cyclin-dependent protein kinases (CDKs). The activity, subcellular localization and substrate specificity of CDKs is determined by cyclin association. All cyclins share a region of homology about 100 amino acids long, called the cyclin box. The authors have determined the structure of an active recombinant fragment of bovine cyclin A to 2.0 Å resolution. The cyclin box has an α-helical fold, comprising five α-helices; sequences that may form this fold are found in a wide range of regulatory molecules, such as the transcription factor TFIIB. Two clusters of exposed residues are conserved in all A, B and E cyclins; one has been shown to be involved in the association with CDK2, the other may be a second site of cyclin–target interactions. Comparison of this structure with the recently-reported structure of cyclin A complexed with CDK2 reveals that cyclin A does not undergo significant conformational changes on formation of the complex.
15 November 1995, Research Article, *Structure*