Elsewhere in Biology

Chosen and summarized by the staff of *Chemistry & Biology*

Current Biology Ltd has launched a new journal entitled *Current Opinion in Chemical Biology*, which will be of particular interest to readers of *Chemistry & Biology*. The editors of the journal are Donald Hilvert and Steven V Ley. The third issue, on mechanisms (edited by Steven J Benkovic and Christopher T Walsh) and analytical techniques (edited by Saul JB Tendler) is published in October.

Current Opinion in Chemical Biology
Mechanisms / Analytical techniques
Contents for October 1997 issue

A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chemistry & Biology October 1997, **4**:777–781

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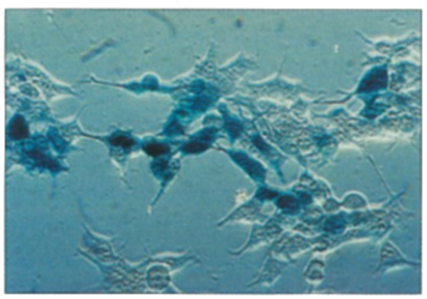
☐ **TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL.** Gavin R Screaton, Juthathip Mongkolsapaya, Xiao-Ning Xu, Alison E Cowper, Andrew J McMichael and John I Bell (1997). *Curr. Biol.* **7**, 693-696.

Members of a subset of the tumour necrosis factor (TNF) receptor family contain a conserved intracellular motif, the death domain. Engagement of these receptors by their respective ligands initiates a signalling cascade that rapidly leads to cell death by apoptosis. The authors have cloned a new member of this family, TRICK2, the TRAIL (TNF-related apoptosis-inducing ligand) receptor inducer of cell killing 2. TRICK2 is expressed in a number of cell types and to particularly high levels in lymphocytes and spleen. Two isoforms of the TRICK2 mRNA that differ by a 29 amino-acid extension to the extracellular domain were generated by alternative pre-mRNA splicing. Overexpression of TRICK2 rapidly induced apoptosis in 293T cells; this induction was dependent upon the presence of the death domain of TRICK2. Using a soluble molecule containing the TRICK2 extracellular domain, the authors showed that TRICK2 is a

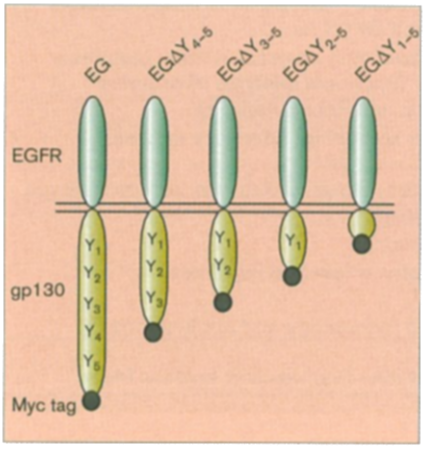


receptor for TRAIL/APO-2L and could inhibit TRAIL-induced killing of lymphocyte lines, such as the Jurkat T-cell line. TRAIL is upregulated upon

lymphocyte activation. TRAIL and its receptors might therefore provide another system for the regulation of lymphocyte selection and proliferation, as well as providing an additional weapon in the armoury of cytotoxic lymphocytes.
1 August 1997*, Brief Communication, *Current Biology*

☐ **The protein tyrosine phosphatase SHP-2 negatively regulates ciliary neurotrophic factor induction of gene expression.** Aviva Symes, Neil Stahl, Steven A Reeves, Thomas Farruggella, Tiziana Servidei, Tom Gearan, George Yancopoulos and J Stephen Fink (1997). *Curr. Biol.* **7**, 697-700.

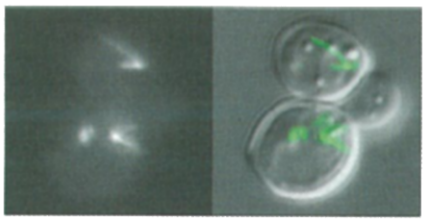
Ciliary neurotrophic factor, along with other neuropoietic cytokines, signals through the shared receptor subunit gp130, leading to the tyrosine phosphorylation of a number of substrates, including the transcription factors STAT1 and STAT3 and the protein tyrosine phosphatase SHP-2. SHP-2 is a positive regulatory molecule required for the activation of the mitogen-activated protein kinase pathway and the stimulation of gene expression in response to epidermal growth factor, insulin and platelet-derived growth factor stimulation. The authors have previously shown that cytokines that signal via the gp130 receptor subunit activate transcription of the vasoactive intestinal peptide (VIP) gene through a 180 base pair cytokine response element (CyRE). To characterize the role of SHP-2 in the regulation of gp130-stimulated gene expression, the regulation of the VIP CyRE in two systems that prevented ligand-dependent SHP-2 phosphorylation was studied. Inhibition of SHP-2 resulted in dramatic increases in gp130-dependent gene expression, through the VIP CyRE and more specifically through multimerized STAT-binding sites. These data suggest that SHP-2 has a negative role in gp130 signalling by modulating STAT-mediated transcriptional activation.



1 August 1997, Brief Communication, *Current Biology*

☐ **Imaging green fluorescent protein fusion proteins in *Saccharomyces cerevisiae*.** Sidney L Shaw, Elaine Yeh, Kerry Bloom and ED Salmon (1997). *Curr. Biol.* **7**, 701-704.

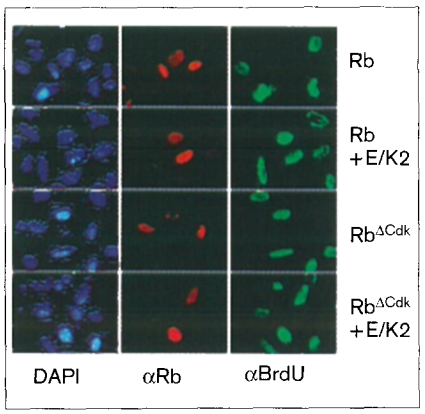
Tagging expressed proteins with the green fluorescent protein (GFP) from *Aequorea victoria* is a highly specific and sensitive technique for studying the intracellular dynamics of proteins and organelles. The authors have developed, as a probe, a fusion protein of the carboxyl terminus of dynein and GFP (dynein–GFP), which fluorescently labels the astral microtubules of the budding yeast *Saccharomyces cerevisiae*. This paper describes the modifications to the authors' multimode microscope imaging system, the acquisition of three-dimensional data sets and the computer processing methods that have been developed to obtain time-lapse recordings of fluorescent astral microtubule dynamics and nuclear movements over the complete duration of the yeast cell cycle. This required low excitation light intensity to prevent GFP photobleaching and phototoxicity, efficient light collection by the microscope optics, a cooled charge-coupled device camera with high quantum efficiency, and image reconstruction from serial optical sections through the 6 µm-wide yeast cell to see most or all of the astral molecules. Methods are also described for combining fluorescent images of the microtubules labelled with dynein–GFP with high resolution differential interference contrast images of nuclear and cellular morphology, and fluorescent images of the chromosomes stained with 4,6-diamidino-2-phenylindole.



1 August 1997, Brief Communication, *Current Biology*

☐ **S-Phase entry upon ectopic expression of G1 cyclin-dependent kinases in the absence of retinoblastoma protein phosphorylation.** Xiaohong Leng, Lisa Connell-Crowley, David Goodrich and J Wade Harper (1997). *Curr. Biol.* **7**, 709-712.

In mammalian cells, the retinoblastoma protein (Rb) is thought to negatively regulate progression through the G1 phase of the cell cycle by its association with the transcription factor E2F. Rb–E2F complexes suppress transcription of genes required for DNA synthesis, and the prevailing view is that phosphorylation of Rb by complexes of cyclin-dependent kinases (Cdks) and their regulatory cyclin subunits, and the subsequent release of active E2F, is required for S-phase entry. This view is based, in part, on the fact that ectopic expression of cyclin-Cdks leads to Rb phosphorylation and that this modification correlates with S-phase entry. In *Drosophila*,



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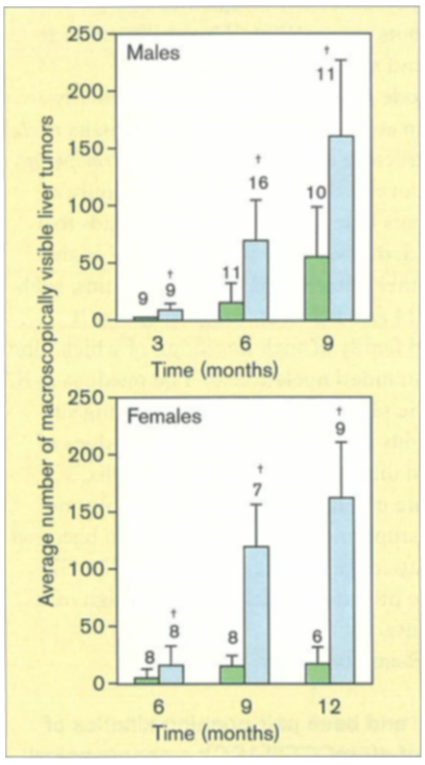
however, cyclin E expression can bypass a requirement for E2F, suggesting that cyclins may activate replication independently of the Rb/E2F pathway. The authors have examined whether Rb phosphorylation is a prerequisite for S-phase entry in Rb-deficient SAOS-2 osteosarcoma cells, using a commonly used cotransfection assay. The results of the study indicate that Rb phosphorylation is not essential for S-phase entry when G1 cyclin-Cdks are overexpressed, and that other substrates of these kinases can be rate-limiting for the G1 to S-phase transition.
1 August 1997, Brief Communication, *Current Biology*

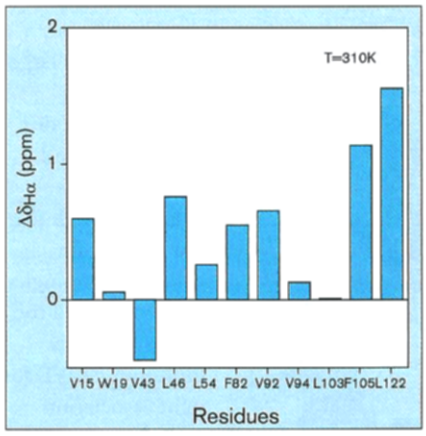
☐ **High incidence of spontaneous and chemically induced liver tumors in mice deficient for connexin32.**
Achim Temme, Albrecht Buchmann, Heinz-Dieter Gabriel, Eric Nelles, Michael Schwarz and Klaus Willecke (1997). *Curr. Biol.* **7**, 713-716.

Connexins are subunits of gap junction channels, which mediate the direct transfer of ions, second messenger molecules and other metabolites between contacting cells. Gap junctions are thought to be involved in tissue homeostasis, embryonic development and the control of cell proliferation. It has also been suggested that the loss of intercellular communication via gap junctions may contribute to multistage carcinogenesis. Transgenic mice that lack connexin32 (Cx32), the major gap junction protein expressed in hepatocytes, express lower levels of a second hepatic gap junction protein, Cx26, suggesting that Cx32 has a stabilizing effect on Cx26. Here, the authors report that male and female one-year-old mice deficient for Cx32 had 25-fold more and 8-fold more spontaneous liver tumours than wild-type mice, respectively. Incorporation of bromodeoxyuridine into the liver was higher for Cx32-deficient mice than for wild-type mice, suggesting that their hepatocyte proliferation rate was higher. Furthermore, intraperitoneal injection, two weeks after birth, of the carcinogen diethylnitrosamine led, after one year, both to more liver tumours in Cx32-deficient mice than in controls, and to accelerated tumour growth. Loss of Cx32 protein from hepatic gap junctions is therefore likely to cause enhanced clonal survival and expansion of mutated cells, which results in a higher susceptibility to hepatic tumours. The results show that functional gap junctions inhibit the development of spontaneous and chemically induced tumours in mouse liver.
1 August 1997, Brief Communication, *Current Biology*



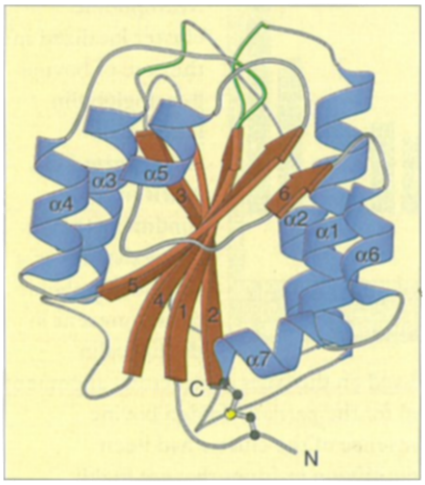
☐ **Identification of a conserved hydrophobic cluster in partially folded bovine β-lactoglobulin at pH 2.**
Laura Ragona, Francesca Pusterla, Lucia Zetta, Hugo L Monaco and Henriette Molinari (1997). *Fold. Des.* **2**, 281-290.

Nuclear magnetic resonance (NMR) studies of denatured states, both fully unfolded and partially folded, give insight into the conformations and interactions formed during folding. Although the complete structural characterization of partially folded proteins is a very difficult task, the identification of structured subsets, such as hydrophobic clusters, is of value in understanding the structural organization of such states. A well-defined hydrophobic cluster localized in the core of bovine β-lactoglobulin has been characterized by NMR in acidic conditions (pH 2). The existence of a small hydrophobic cluster present in the lipocalin protein family was assessed on the basis of structural alignment and NMR data obtained for the partially folded bovine β-lactoglobulin. The presence of the cluster had been predicted previously, identifying residues that are highly conserved in most members of the family. An NMR study conducted at pH 2, where the protein exhibits a very stable β-core together with disordered regions, revealed NOEs among sidechains of 11 hydrophobic residues centred around Trp19 and pointing towards the interior of the protein. This buried cluster was found to be unusually stable at pH 2, not only at room temperature but also at 323K. Furthermore, conserved hydrophobic residues pointing towards the surface of the protein define a hydrophobic surface patch located in a groove between the β strands and the α helix. The detected buried cluster is most likely to be important in β-lactoglobulin stability. The analysis of five structurally related proteins reveals that the same extended cluster is present in these structures. The authors propose that the buried cluster may represent the internal binding site as well as being important for stability and that the hydrophobic surface patch is involved in a second external binding site.
28 August 1997, Research Paper, *Folding & Design*



☐ **Crystal structure of the A3 domain of human von Willebrand factor: implications for collagen binding.** Eric G Huizinga, R Martijn van der Plas, Jan Kroon, Jan J Sixma and Piet Gros (1997). *Structure* **5**, 1147-1156.

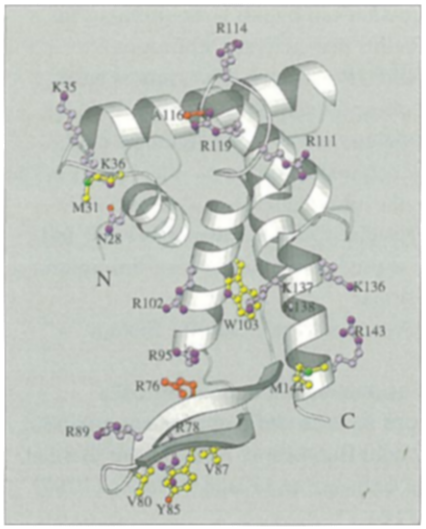
Bleeding from a damaged blood vessel is stopped (haemostasis) by the formation of a platelet plug. The multimeric plasma glycoprotein, von Willebrand factor (vWF), is essential for normal haemostasis, during which it anchors blood platelets to the damaged vessel wall under conditions of high shear stress, as is evident from the bleeding disorder which occurs when vWF is either absent or functionally abnormal. Binding of multimeric vWF to fibrillar collagen type I and/or III is a necessary prerequisite for it to function as a bridge between the connective tissue of the damaged blood vessel wall and its receptor on the platelet membrane, glycoprotein Ib. The A3 domain of vWF allows it to bind to collagen types I and III present in the perivascular connective tissue of the damaged vessel wall. The structure of the A3 domain suggests that adhesion to collagen is primarily achieved through interactions between negatively charged residues on A3 and positively charged residues on collagen. The absence of a pronounced binding groove precludes a large van der Waals surface interaction between A3 and collagen and is consistent with the low affinity for collagen of a single A3 domain and the requirement for multimeric vWF for tight association with collagen. The absence of bound metal ions upon soaking the crystal in $MgCl_2$ and vWF A3's conformational incompatibility for metal binding are consistent with the absence of a functional role for metal ion binding in A3, in contrast to the metal ion activation required for ligand binding by the homologous integrin I-type domains. The structure thus provides a structural basis for the understanding of the adhesion process of vWF to exposed collagen types I and III in haemostasis and a starting point for site-directed mutagenesis studies to test the importance of individual residues in vWF-A3 for collagen binding.



15 September 1997, Research Paper, *Structure*

☐ **The structure of ribosomal protein S7 at 1.9 Å resolution reveals a β-hairpin motif that binds double-stranded nucleic acids.** Brian T Wimberly, Stephen W White and V Ramakrishnan (1997). *Structure* **5**, 1187-1198.

The ribosome is the site of protein synthesis in all organisms. It is a large, two-subunit, complex of three RNAs and some



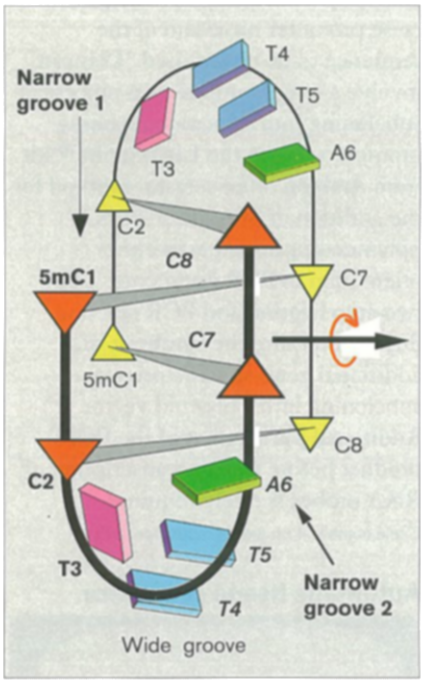
50 proteins. In the well-studied prokaryotic ribosome, the primary function of the ribosomal proteins appears to be the stabilization of the correct three-dimensional structure of ribosomal RNA. During ribosomal assembly, a few key ribosomal proteins bind to conserved regions of the ribosomal RNA and thereby initiate proper folding. S7 is the only such protein that interacts with the large 3′ domain of 16S RNA. The binding of S7 to this domain nucleates assembly of the functionally important 'head' region of the small (30S) subunit. In addition, it has been identified as a component of the decoding site, a crucial region of the ribosome that monitors codon–anticodon complementarity. Finally, bacterial S7 regulates its own synthesis by binding to its own mRNA. This ability of S7 to bind both messenger and ribosomal RNAs makes determination of its mode of RNA recognition particularly interesting. This and an accompanying paper (by Hosaka *et al.*,) describes the crystal structure of S7 from *Thermus thermophilus*. Structural similarity between S7 and the IHF/HU family of proteins strongly suggests that the β hairpin of S7 binds to a groove of double-stranded RNA. The β hairpin of S7 is also similar to those from other nucleic acid binding proteins, such as ribosomal protein L14 and BIV Tat, suggesting that it belongs to an extended family of such motifs, all of which bind to a groove of double-stranded nucleic acid. The residues in S7 loop 2 that belong to the second putative RNA-binding site may have a role analogous to the amino-terminal residues of IHF/HU, which grip an unbent portion of double helix. S7–RNA interactions are crucial for ribosome assembly and function, so specific disruption of such interactions in bacterial ribosomes may be an attractive antibacterial target. The S7 structure may therefore provide the basis for the design of novel antibacterial agents.

15 September 1997, Research Paper, *Structure*

☐ **Solution structure and base pair opening kinetics of the i-motif dimer of d(5mCCTTTACC): a noncanonical structure with possible roles in chromosome stability.** Sylvie Nonin, Anh Tuan Phan and Jean-Louis Leroy (1997). *Structure* **5**, 1231-1246.

Repetitive cytosine-rich DNA sequences have been identified in telomeres and centromeres of eukaryotic chromosomes. These sequences are important in maintaining chromosome stability during replication and may be involved in chromosome

pairing during meiosis. The C-rich repeats can fold into an 'i-motif' structure, in which two parallel-stranded duplexes with hemiprotonated C·C$^+$ pairs are intercalated. The discovery of the i-motif is relatively recent (1993) and it is therefore possible that there are i-motif-specific proteins and there is a biological function for the i-motif. Previous nuclear magnetic resonance (NMR) studies of naturally occurring repeats have produced poor NMR spectra. In this work, NMR spectroscopy of oligonucleotides, based on natural sequences, has shown that d(5′CCTTTACC) forms an i-motif dimer of symmetry-related and intercalated folded strands. The i-motif core includes intercalated interstrand C·C$^+$ pairs stacked in the order 2*·8 / 1·7* / 1*·7 / 2·8* (where one strand is distinguished by an asterisk and the numbers relate to the base positions within the repeat). The d(5′CCTTTTTCC) deoxyoligonucleotide, in which position 6 is occupied by a thymidine instead of an adenine, also forms a symmetric i-motif dimer. In this structure, however, the two TTTT loops are located on the same side of the i-motif core and the C·C$^+$ pairs are formed by equivalent cytidines stacked in the order 8*·8 / 1·1* / 7*·7 / 2·2*. Oligodeoxynucleotides containing two C-rich repeats can fold and dimerize into an i-motif. The change of folding topology resulting from the substitution of a single nucleoside emphasizes the influence of the loop residues on the i-motif structure formed by two folded strands.



15 September 1997, Research Paper, *Structure*