Elsewhere in Biology

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

Current Biology Ltd is launching 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 first issue, on Combinatorial Chemistry, will be published in June, and is edited by Kevin T Chapman, Gerald F Joyce and W Clark Still.

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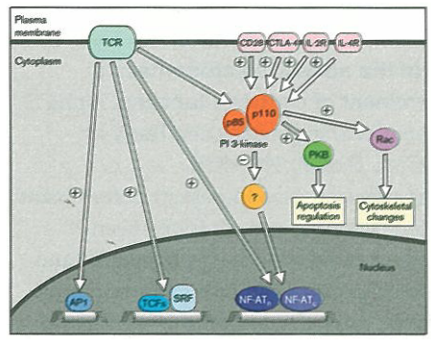
A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chemistry & Biology May 1997, 4:403–407

☐ **A negative role for phosphoinositide 3-kinase in T-cell antigen receptor function.** Karin Reif, Susan Lucas and Doreen Cantrell (1997). *Curr. Biol.* **7**, 285–293.

During T-cell activation, there is a balance between positive and negative regulatory mechanisms to determine the specificity and magnitude of an immune response. Phosphoinositide 3-kinase (PI 3-kinase) is activated during T-cell activation by a diverse set of receptors, including the

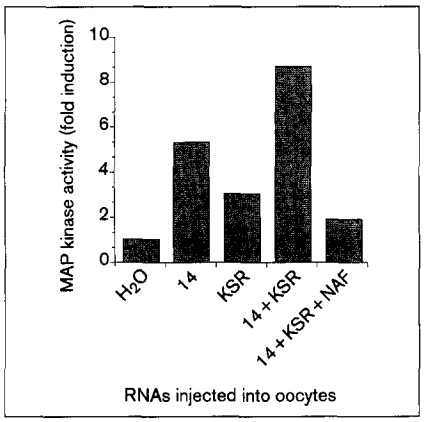


T-cell antigen receptor (TCR) and the costimulatory receptor CD28. PI 3-kinase can thus dynamically influence the outcome of the immune reactions at various stages. In this study, the authors investigated the importance of PI 3-kinase in TCR-directed T-cell activation using activated or inhibitory versions of PI 3-kinase. Certain aspects of TCR responses such as the induction of transcriptional activity of AP1 and serum response factor were not affected by expression of the mutant forms of

PI 3-kinase. PI 3-kinase was found to profoundly influence the transactivation capacity of 'nuclear factor of activated T cells' (NF-AT) elicited by the TCR, however. The data presented in this paper reveal a previously unrecognized function for PI 3-kinase as a selective negative regulator of TCR responses.
4 April 1997*, Research Paper, *Current Biology*

☐ **The protein kinase KSR interacts with 14-3-3 protein and Raf.** Heming Xing, Kerry Kornfeld and Anthony J Muslin (1997). *Curr. Biol.* **7**, 294–300.

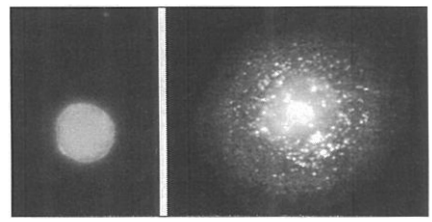
The Ras signaling pathway is involved in the regulation of cell differentiation, movement and proliferation. KSR (kinase suppressor of Ras) was recently discovered and studies suggest that it mediates Ras signaling in invertebrates. The function of vertebrate KSR is not well characterized biochemically or biologically. The authors examined the physiological role of KSR in vertebrate signal transduction using *Xenopus laevis* oocytes. Overexpression of KSR, in combination with over-expression of the intracellular dimeric protein 14-3-3, induced *Xenopus* oocyte meiotic maturation and cdc2 kinase activation; the effect of KSR and 14-3-3 on oocyte maturation was blocked by co-expression of dominant-negative Raf-1. KSR can bind to 14-3-3 and form a complex with Raf kinase both *in vitro* and in cultured cells. Cell fractionation studies revealed that KSR formed a complex with 14-3-3 in both the membrane and cytoplasmic fractions of cell lysates; but KSR only formed a complex with Raf-1 in the membrane fraction. These results suggest that KSR, 14-3-3 and Raf form an oligomeric signaling complex and that KSR positively regulates the Ras signaling pathway in vertebrate organisms.



7 April 1997, Research Paper, *Current Biology*

☐ **Lateral clustering of the adhesive ectodomain: a fundamental determinant of cadherin function.** Alpha S Yap, William M Brieher, Martin Pruschy and Barry M Gumbiner (1997). *Curr. Biol.* **7**, 308–315.

Cadherin-based cellular adhesion is an important determinant of tissue architecture in developing and adult organisms. Despite the involvement of cadherins in morphogenesis and development, very little is known about how cells determine and alter cadherin adhesive strength. In order to identify the specific mechanisms that modulate adhesion, the authors used both purified proteins and cells transfected with *Xenopus* C-cadherin mutant molecules. Using the FKBP–FK1012 protein oligomerization system, it was discovered that forced clustering, in cells, of cadherin mutants that lacked the cytoplasmic tail significantly increased cellular adhesive strength. Redistribution of the adhesive binding sites of cells into clusters can therefore influence adhesion independently of other protein interactions mediated by the cadherin cytoplasmic tail. Furthermore, cells transfected with full-length C-cadherin demonstrated dynamic changes in adhesion over time that correlated with clustering but not with changes in the surface expression of C-cadherin or in the composition of the cadherin–catenin complex. The cytoplasmic tail was, however, necessary for clustering of wild-type cadherin. These studies directly demonstrate a fundamental role for lateral clustering in cadherin function. The distribution of cadherin binding sites presented at the cell surface is an important mechanism which modulates cellular adhesion independently of cytoskeletal activity or signaling.



10 April 1997, Research Paper, *Current Biology*

☐ **Systematic identification of mitotic phosphoproteins.** P Todd Stukenberg, Kevin D Lustig, Thomas J McGarry, Randall W King, Jian Kuang and Marc W Kirschner (1997). *Curr. Biol.* **7**, 316–325.

The cell cycle is controlled by a family of cyclin-dependent kinases (CDKs). They are thought to initiate and coordinate cell division processes by sequentially phosphorylating key targets, but in most cases these substrates remain unidentified. The authors have used a screen that scores for phosphorylation of proteins to identify 20 mitotically phosphorylated proteins from *Xenopus* embryos, 15 of which have sequence similarity to other proteins. Of these proteins, five have previously been shown to be phosphorylated during mitosis, five are related to proteins postulated to have roles in mitosis, and nine are related to transcription factors . Of 16 substrates tested, 14 can be directly phosphorylated *in vitro* by the mitotic CDK, cyclin B–Cdc2, although three of these may be physiological substrates of other kinases activated during mitosis. Examination of this broad set of mitotic phosphoproteins has allowed the authors to draw three conclusions about how the activation of CDKs regulates cell-cycle events. First, Cdc2 itself appears to directly phosphorylate most of the mitotic phosphoproteins. Second, during mitosis most of the substrates are phosphorylated more than once and a number may be targets of multiple kinases, suggesting combinatorial regulation. Third, the large fraction of mitotic phosphoproteins that are presumptive transcription factors, two of which have been previously shown to dissociate from DNA during mitosis, suggests that an important function of mitotic phosphorylation

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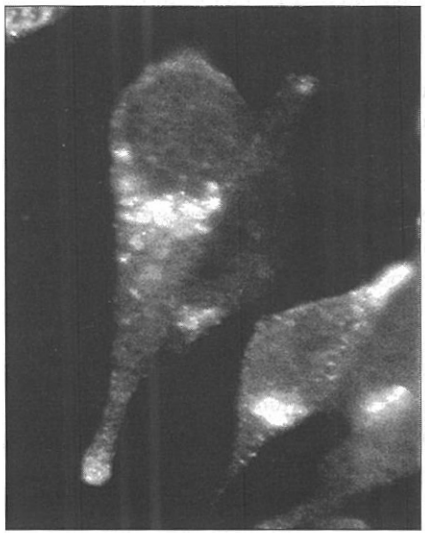
is to strip the chromatin of proteins associated with gene expression. Further characterization of these phosphoprotein substrates should provide new insights into their roles in mitosis and signal-transduction cascades.
17 April 1997, Research Paper, *Current Biology*

☐ **Targeting of leptin to the regulated secretory pathway in pituitary AtT-20 cells.** Raymond A Chavez and Hsiao-Ping H Moore (1997). *Curr. Biol.* **7**, 349–352.

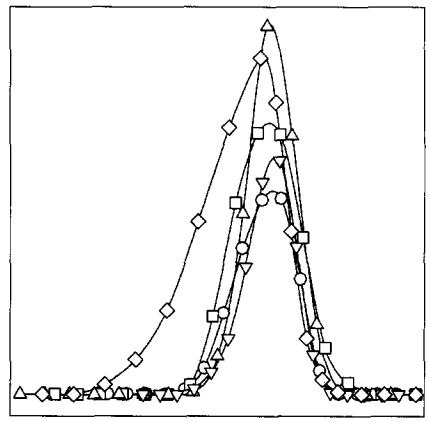
Leptin is a key regulator of fat homeostasis. It is the product of the *obese* gene and is secreted from fat storage cells but the secretory pathway is not well characterized. Several studies have implicated serum insulin levels in the upregulation of leptin gene expression but it is currently not known whether leptin levels are also subject to regulation at the level of secretion. Here, the authors used pituitary AtT-20 cells, which serve as a model system for both regulated and constitutive secretory pathways, to examine the intracellular targeting and secretion of leptin. Confocal immunofluorescence analysis of AtT-20 cells expressing an epitope-tagged human leptin (FLAG–leptin) demonstrated that FLAG–leptin colocalized with endogenous adrenocorticotrophic hormone (ACTH) at the tips of processes extended from these cells, where regulated secretory granules accumulate. FLAG–leptin secretion was increased in the presence of 8-Br-cAMP, which stimulates the secretion of ACTH. For FLAG–leptin, the calculated sorting index, a quantitative measure of the degree to which a given protein is targeted to the regulated secretory pathway, was similar to those of other regulated secretory proteins. These results demonstrate that FLAG–leptin behaves like a regulated protein in cells with a biosynthetic regulated secretory pathway, suggesting that the release of leptin from fat storage cells might follow a regulated pathway that is not yet understood.



1 May 1997, Brief Communication, *Current Biology*

☐ **Conformational transitions provoked by organic solvents in β-lactoglobulin: can a molten globule like intermediate be induced by the decrease in dielectric constant?** Vladimir N Uversky, Natalya V Narizhneva, Steffen Omar Kirschstein, Stefan Winter and Günter Löber (1997). *Fold. Des.* **2**, 163–172.

It is known that non-native states of protein molecules can exist in living cells and can be involved in a number of physiological processes. It has also been established that the membrane surface can be responsible for the partial denaturation of proteins due to negative charges on it. The local decrease in the effective dielectric constant of water near the organic surface has been suggested to be an additional driving force for protein denaturation in the membrane field, but data to confirm this suggestion were lacking. Conformational transitions induced in β-lactoglobulin by organic solvents were studied by ultra-violet circular dichroism, steady-state tryptophan fluorescence and fluorescence decay of 8-anilinonaphthalene-1-sulfonate (8-ANS). The existence of at least two noncoinciding cooperative transitions was established in all solvent systems studied. The first of these transitions describes the disruption of rigid tertiary structure in protein molecules, while the second reflects the formation of an expanded helical conformation typical of proteins in concentrated organic solvents. This means that the organic solvents provoke the formation of a denatured intermediate state with pronounced secondary structure and native-like compactness. We show that the positions of maxima in f_I versus dielectric constant dependence virtually coincide for all five solvent systems studied. The decrease in the dielectric constant of the solvent induces an equilibrium intermediate state in β-lactoglobulin. This state, being denatured, is relatively compact and has pronounced secondary structure and high affinity for the hydrophobic fluorescent probe 8-ANS, in other words it possesses all the properties of the molten globule intermediate state.

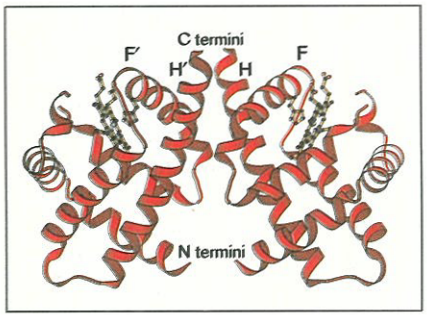


24 April 1997, Research Paper, *Folding & Design*

☐ **Unusual structure of the oxygen-binding site in the dimeric bacterial hemoglobin from *Vitreoscilla* sp.** Cataldo Tarricone, Alessandro Galizzi, Alessandro Coda, Paolo Ascenzi and Martino Bolognesi (1997). *Structure* **5**, 497–507.

The first hemoglobin identified in bacteria was isolated from *Vitreoscilla stercoraria* (VtHb) as a homodimeric species. The wild-type protein has been reported to display medium oxygen affinity and cooperative ligand-binding properties. Moreover, VtHb can support aerobic growth in *Escherichia coli* with impaired terminal oxidase function. This ability of VtHb to improve the growth properties of *E. coli* has important applications in fermentation technology, assisting the over-expression of recombinant proteins and antibiotics. Oxygen binding heme domains have been identified in chimeric proteins from bacteria and yeast, where they are covalently

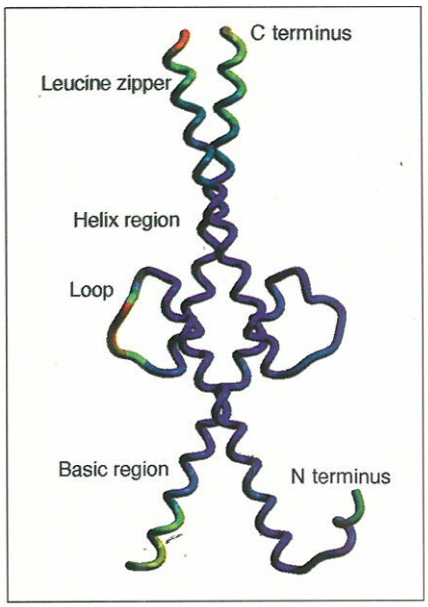
linked to FAD-binding and NAD(P)H-binding domains. In this paper, the authors describe the fold, the distal heme site structure and the quaternary assembly of a bacterial hemoglobin which does not bear the typical flavohemoglobin domain organization. The VtHb three-dimensional structure conforms to the well known globin fold. The polypeptide



chain disorder observed in the polypeptide sequence connecting α helices C and E indicates a potential site of interaction with the FAD/NADH reductase partner, in analogy with observations in the chimeric flavohemoglobin from *Alcaligenes eutrophus*. Binding of azide to the heme iron introduces substantial structural perturbations in the heme distal site residues, showing that previously undescribed molecular mechanisms of ligand stabilization are operative in VtHb. The quaternary assembly of homodimeric VtHb, not observed before within the globin family, is based on a molecular interface defined by α helices F and H of both subunits, the two heme iron atoms being 34 Å apart.
15 April 1997, Research Paper, *Structure*

☐ **The crystal structure of an intact human Max–DNA complex: new insights into mechanisms of transcriptional control.** P Brownlie, TA Ceska, M Lamers, C Romier, G Stier, H Teo and D Suck (1997). *Structure* **5**, 509–520.

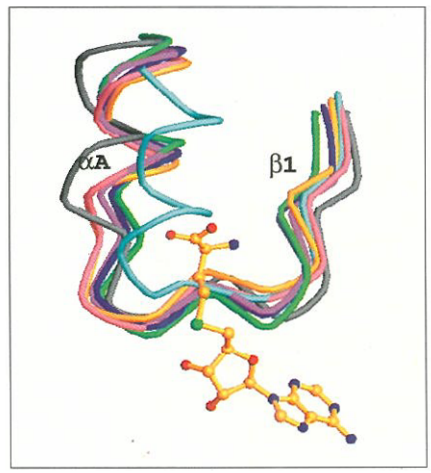
Max belongs to the basic helix–loop–helix leucine zipper (bHLHZ) family of transcription factors. Max is able to form homodimers and heterodimers with other members of this



family, which include Mad, Mxi1 and Myc; Myc is an oncoprotein implicated in cell proliferation, differentiation and apoptosis. The homodimers and heterodimers compete for a common DNA target site (the E box) and rearrangement amongst these dimer forms provides a complex system of transcriptional regulation. Max is also regulated by phosphorylation at a site preceding the basic region. The paper describes the first X-ray crystal structure of an intact bHLHZ protein bound to its target site. The structure of the intact human Max protein homodimer in complex with a 13-mer DNA duplex was determined to 2.8 Å resolution and refined to an R factor of 0.213. The structure confirms the importance of the HLH and leucine zipper motifs in dimerization as well as the mode of E box recognition which was previously analyzed by X-ray crystallography of shortened constructs. Disorder observed in the carboxy-terminal domain suggests that contacts with additional protein components of the transcription machinery are necessary for ordering the secondary structure. The tetramers seen in the crystal are consistent with the tendency of Max and other bHLHZ and HLH proteins to form higher order oligomers in solution and may play a role in DNA looping. The location of the two phosphorylation sites at Ser1 and Ser10 (the latter is the amino-cap of the basic helix) suggests how phosphorylation could disrupt DNA binding.
15 April 1997, Research Paper, *Structure*

☐ **Crystal structure of the chemotaxis receptor methyltransferase CheR suggests a conserved structural motif for binding *S*-adenosylmethionine.** Snezana Djordjevic and Ann M Stock (1997). *Structure* **5**, 545–558.

Flagellated bacteria swim towards favorable chemicals and away from deleterious ones. The sensing of chemoeffector gradients involves chemotaxis receptors, transmembrane proteins that detect stimuli through their periplasmic domains



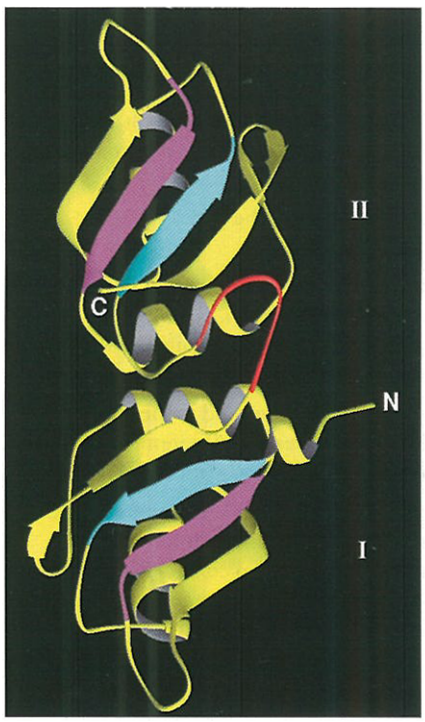
and transduce signals via their cytoplasmic domains to the downstream signaling components. Signaling outputs from chemotaxis receptors are influenced both by the binding of the chemoeffector ligand to the periplasmic domain and by methylation of specific glutamate residues on the cytoplasmic domain of the receptor. Methylation is catalyzed by CheR, an *S*-adenosyl-methionine-dependent methyltransferase. CheR forms a tight complex with the receptor by binding a region of the receptors that is distinct from the methylation site. CheR belongs to a broad class of enzymes involved in the methylation of a variety of substrates. Until now, no structure from the class of protein methyltransferases has been characterized. The CheR structure shares some structural similarities with small molecule DNA and RNA methyltransferases, despite a lack of

sequence similarity among them. In particular, there is significant structural preservation of the *S*-adenosylmethionine-binding clefts; the specific length and conformation of a loop in its α/β domain seems to be required for *S*-adenosylmethionine binding within these enzymes. Unique structural features of CheR, such as the β subdomain, are probably necessary for CheR's specific interaction with its substrates, the bacterial chemotaxis receptors.

15 April 1997, Research Paper, *Structure*

☐ **Crystal structure of human UP1, the domain of hnRNP A1 that contains two RNA-recognition motifs.** Rui-Ming Xu, Lana Jokhan, Xiaodong Cheng, Akila Mayeda and Adrian R Krainer (1997). *Structure* **5**, 559–570.

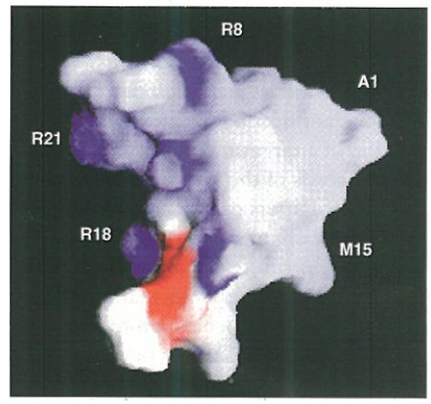
Heterogeneous nuclear ribonucleoprotein (hnRNP) A1 is one of the most abundant core proteins of hnRNP complexes in metazoan nuclei. It behaves as a global regulator of alternative pre-mRNA splicing by antagonizing the activities of several serine/arginine-rich splicing factors (SR proteins), resulting in the activation of distal alternative 5' splice sites and skipping of optional exons. Purified hnRNP A1 has nucleic acid annealing activity. The protein also shuttles continuously between the nucleus and the cytoplasm, a process mediated by signals within its carboxy-terminal glycine-rich domain. The amino-terminal region of human hnRNP A1, termed unwinding protein 1 (UP1), contains two RNA-recognition motifs (RRMs), RRM1 and RRM2. The crystal structure of UP1 has been determined to 1.9 Å resolution. This study shows how two RRMs are organized in a single polypeptide. The two independently folded RRMs in UP1 are held together in a fixed geometry, enabling the two RRMs to function as a single entity in binding RNA, and so explaining the synergy observed between the RRMs. The UP1 structure also suggests that residues which lie outside of the RRMs can make potentially important interactions with RNA. Understanding the structural elements by which hnRNP A1 interacts with RNA will have broad implications for studies of RNA processing.



15 April 1997, Research Paper, *Structure*

☐ **Solution structure of the sodium channel antagonist conotoxin GS: a new molecular caliper for probing sodium channel geometry.** Justine M Hill, Paul F Alewood and David J Craik (1997). *Structure* **5**, 571–583.

Voltage-dependent sodium channels are intrinsic membrane proteins that play an important role in fast communication in excitable cells. A short stretch of amino acids, the pore region, is the sole determinant of sodium selectivity and also forms the binding site for many channel blockers. Toxins that interact intimately with this region can be used as structural templates to deduce the spatial organization of the pore region of sodium channels. These models of pore structure are valuable for understanding the mechanisms of ion permeation, and may ultimately be useful for the rational design of drugs that could modify the function of ion channels in patients with ischaemic injury to the heart or brain. The venoms of *Conus* snails contain small, disulfide-rich inhibitors of voltage-dependent sodium channels. Conotoxin GS is a 34-residue polypeptide isolated from *Conus geographus* that interacts with the extracellular entrance of skeletal muscle sodium channels to prevent sodium ion conduction. Although conotoxin GS binds competitively with conotoxin GIIIA to the sodium channel surface, the two toxin types have little sequence identity with one another, and conotoxin GS has a four-loop structural framework rather than the characteristic three-loop conotoxin framework. The three-dimensional structure of conotoxin GS was determined using two-dimensional nuclear magnetic resonance spectroscopy and represents a valuable new structural probe for the pore region of voltage-dependent sodium channels. The distribution of amino acid sidechains in the structure creates several polar and charged patches, and comparison with the conotoxins provides a basis for determining the binding surface of the conotoxin GS polypeptide



15 April 1997, Research Paper, *Structure*