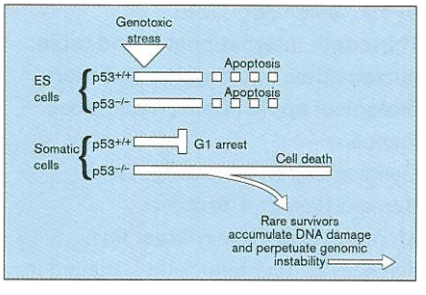
Elsewhere in biology

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

Chemistry & Biology March 1998, 5:R68–R72

☐ **ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage.**
Mirit I Aladjem, Benjamin T Spike, Luo Wei Rodewald, Thomas J Hope, Martina Klemm, Rudolf Jaenisch and Geoffrey M Wahl (1998). *Curr. Biol.* **8**, 145–155.

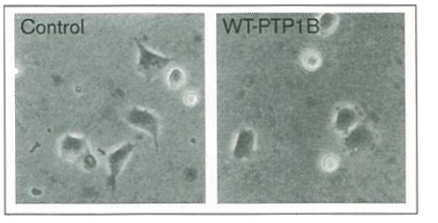
Embryonic stem (ES) cells can contribute precursors to all adult cell lineages. Consequently, damage to ES cell genomes may cause serious developmental malfunctions. In somatic cells, cell-cycle checkpoints limit DNA damage by preventing DNA replication under conditions that may produce chromosomal aberrations. The tumor



suppressor p53 is involved in such checkpoint controls and is also required to avoid a high rate of embryonic malformations. The authors have characterized the cell-cycle and DNA-damage responses of ES cells to elucidate the mechanisms that prevent accumulation or transmission of damaged genomes during development. ES cells derived from wild-type mice did not undergo cell-cycle arrest in response to DNA damage or nucleotide depletion, although they synthesized abundant quantities of p53. The p53 protein in ES cells was cytoplasmic and translocated inefficiently to the nucleus upon nucleotide depletion. Expression of high levels of active p53 from an adenovirus vector could not trigger cell cycle arrest. Instead, ES cells that sustained DNA damage underwent p53-independent apoptosis. The antimetabolite-induced p53-dependent arrest response was restored in ES cells upon differentiation. Cell-cycle regulatory pathways in early embryos differ significantly from those in differentiated somatic cells. In undifferentiated ES cells, p53 checkpoint pathways are compromised by factors that affect the nuclear localization of p53 and by the loss of downstream factors that are necessary to induce cell-cycle arrest. A p53-independent programmed cell death pathway is effectively employed to prevent cells with damaged genomes from contributing to the developing organism. The p53-mediated checkpoint controls become important when differentiation occurs.
12 January 1998, Research Paper, *Current Biology*.

☐ **Protein tyrosine phosphatase 1B negatively regulates integrin signaling.**
Feng Liu, Mary Ann Sells and Jonathan Chernoff (1998). *Curr. Biol.* **8**, 173–176.

Protein tyrosine phosphatase (PTP) 1B has long been known to regulate cell proliferation negatively, but the mechanism by which this inhibition occurs is poorly defined. The authors have shown previously that PTP1B binds to, and dephosphorylates, $p130^{Cas}$ (Crk-associated substrate), a protein that is thought to play a role in integrin signaling. In this report, the authors present evidence that PTP1B interferes specifically with cell-adhesion-stimulated, but not growth-factor-stimulated, signaling pathways. In rat fibroblasts that overexpress PTP1B, the activation of mitogen-activated protein (MAP) kinase by growth factors was not affected, but activation by cell adhesion was markedly impaired. The inhibition of adhesion-dependent MAP kinase activation by PTP1B required an intact

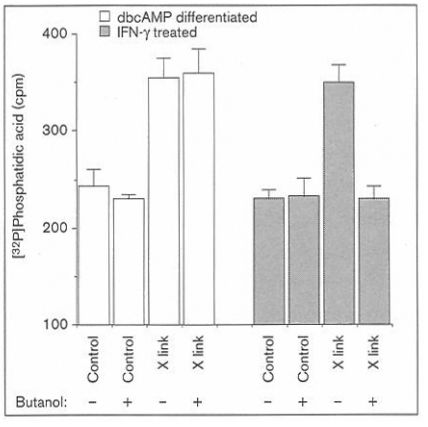


proline-rich region in the carboxyl terminus of PTP1B, a region the authors have shown to mediate binding to the Src-homology 3 (SH3) domain of $p130^{Cas}$. Overexpression of wild-type PTP1B, but not of a proline-to-alanine mutant form (PA-PTP1B) that is unable to bind or dephosphorylate $p130^{Cas}$, interfered with cell spreading, cytoskeletal architecture, and the formation of focal adhesion complexes. Cells overexpressing wild-type PTP1B also displayed markedly reduced migration in response to a fibronectin gradient, whereas cells expressing the PA-PTP1B mutant migrated normally. These data indicate that PTP1B exerts its inhibitory effects via proline-dependent interactions with one or more critical components of the adhesion-dependent signaling apparatus, and suggest that one of these components may be $p130^{Cas}$.
19 January 1998, Brief Communication, *Current Biology*.

☐ **A molecular switch changes the signalling pathway used by the FcγRI antibody receptor to mobilise calcium.**
Alirio Melendez, Floto R. Andres, Angus J Cameron, David J Gillooly, Margaret M Harnett and Janet M Allen (1998). *Curr. Biol.* **8**, 210–221.

Leukocytes express Fcγ receptors, which are specific for the constant region of immunoglobulin G. Aggregation of these receptors activates a repertoire of responses that can lead to targeted cell killing by antibody directed cellular cytotoxicity. The

nature of the myeloid response to Fcγ receptor aggregation is highly variable and depends on the maturation state of the cell, but little is known about the signalling mechanisms underlying this variability. The authors show that differentiation of a monocytic cell line, U937, to a more macrophage phenotype resulted in an absolute and fundamental switch in the nature of the

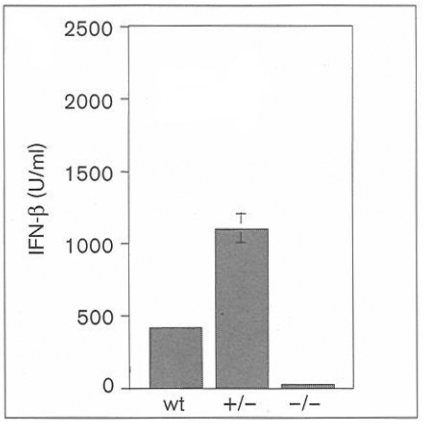


phospholipid signalling pathway recruited following Fcγ receptor aggregation. In cytokine-primed monocytes, aggregation of the high-affinity receptor FcγRI resulted in the activation of phospholipase D and sphingosine kinase, which in turn led to the transient release of stored calcium; these effects were mediated by the chain, an FcγRI accessory protein. In contrast, in cells differentiated to a more macrophage type, aggregation of FcγRI resulted in the FcγRIIa-mediated activation of phospholipase C, and the resulting calcium response was prolonged as calcium entry was stimulated. The switch in FcγRI signalling pathways upon monocyte differentiation is mediated by a switch in the accessory molecule recruited by FcγRI, which lacks its own intrinsic signal transduction motif. As many immune receptors have separate polypeptide chains for ligand binding and signal transduction (allowing a similar switch in signalling pathways), the mechanism described here is likely to be widely used.

28 January 1998, Research Paper, *Current Biology.*

☐ **Interferon-β is required for interferon-α production in mouse fibroblasts.**
Lena Erlandsson, Ralf Blumenthal, Maija-Leena Eloranta, Holger Engel, Gunnar Alm, Siegfried Weiss and Tomas Leanderson (1998). *Curr. Biol.* **8**, 223–226.

The type I interferons — interferon-α (IFN-α) and interferon-β (IFN-β) — are critical for protection against viruses during the acute stage of viral infection. Furthermore, type I interferons have been implicated as important mediators in the regulation of lymphocyte development, immune responses and the maintenance of immunological memory of cytotoxic T cells. The different IFN-α subtypes are encoded by 12 genes in the mouse whereas IFN-β is encoded for by only one gene. IFN-α and IFN-β have a high degree of sequence homology and are thought to interact with the same surface receptor on target cells. As an approach to analysing the different biological functions of IFN-α and IFN-β,

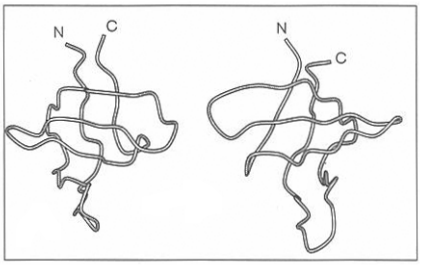


a mouse strain with an inactivated IFN-β gene has been generated. The authors report here that embryonic fibroblasts from such mice produce neither IFN-β nor IFN-α upon Sendai virus infection, whereas the production of IFN-α by leukocytes from the same strain of mice is intact. IFN-α production in embryonic fibroblasts from IFN-$\beta^{-/-}$ mice could be rescued by 'priming' the cells using exogenous IFN-β. These results imply a unique role for IFN-β in the induction of type I interferons in peripheral tissues.

2 February 1998, Brief Communication, *Current Biology.*

☐ **Reading protein sequences backwards.**
Emmanuel Lacroix, Ana Rosa Viguera and Luis Serrano (1998). *Fold. Des.* **3**, 79–85.

Reading a protein sequence backwards provides a new polypeptide that does not align with its parent sequence. The foldability of this new sequence is questionable. On one hand, structure prediction at low resolution using lattice simulations for such a protein provided a model close to the native parent fold or to a topological mirror image of it. On the other hand, there is no experimental evidence to tell whether such a retro

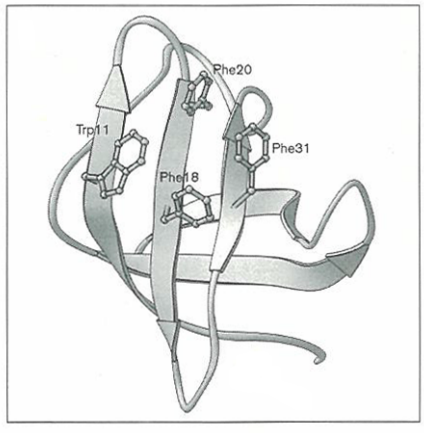


protein folds (and to which structure). The authors have analysed the possibility of a retro protein folding in two different ways. First, the retro sequence of the α-spectrin SH3 domain was modelled through distance geometry and molecular dynamics. This contradicted the plausibility of a mirror image of the native domain, whereas basic considerations opposed the likelihood of the native fold. Second, the authors obtained evidence that the retro sequences of the SH3 domain, as well as the B domain of Staphylococcal protein A and the B1 domain of Streptococcal protein G, are unfolded proteins, even though some propensities for the formation of secondary structures might remain. Retro proteins are no more similar to their parent sequences than any random sequence despite their common hydrophobic/hydrophilic pattern, global amino acid composition and possible tertiary contacts. Although simple folding models contribute to the global understanding of protein folding, they cannot yet be used to predict the structure of new proteins.

5 February 1998, Research Paper, *Folding & Design.*

☐ **Coupling protein stability and protein function in *Escherichia coli* CspA.**
Brian J Hillier, Hector M Rodriguez and Lydia M Gregoret (1998). *Fold. Des.* ***5*** , 87–93.

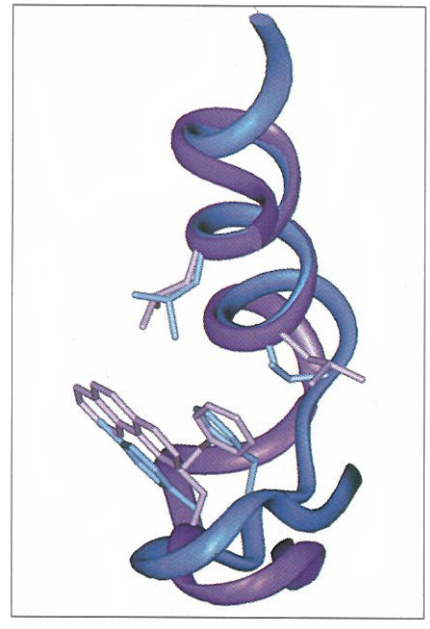
CspA is a small protein that binds single-stranded RNA and DNA. The binding site of CspA consists of a cluster of aromatic amino acids, which form an unusually large nonpolar patch on the surface of the protein. Because nonpolar residues are generally found in the interiors of proteins, this cluster may



have evolved to bind nucleic acids at the expense of protein stability. Three neighboring phenylalanines have been mutated singly and in combination to leucine and to serine. All mutations adversely affect DNA binding. Surprisingly, all mutations, and especially those to serine, are destabilizing. The aromatic cluster in CspA is required not only for protein function but also for protein stability. This result is pertinent to the design of β-sheet proteins and single-stranded nucleic acid binding proteins, whose binding mode is proposed to be of aromatic–aromatic intercalation.
5 February 1998, Research Paper, *Folding & Design*

☐ **Design and NMR analyses of compact, independently folded BBA motifs.**
Mary Struthers, Jennifer J Ottesen and Barbara Imperiali (1998). *Fold. Des.* **5**, 95–103.

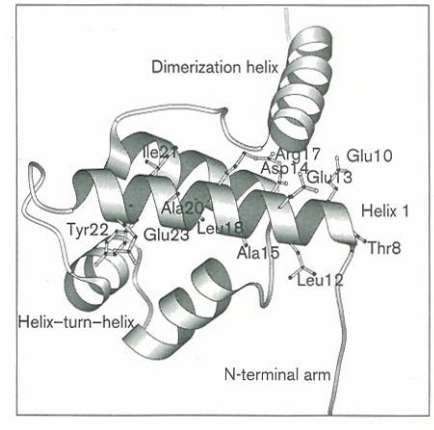
Small folded polypeptide motifs represent highly simplified systems for theoretical and experimental studies on protein structure and folding. The authors recently reported the design and characterization of a metal-ion-independent 23-residue peptide with a ββα structure (BBA1), based on the zinc finger domains. To understand better the determinants of structure for this small peptide, the authors investigated the conformational role of the synthetic residue 3-(1,10-phenan-throl-2-yl)-L-alanine (Fen) in BBA1. NMR analysis revealed that replacing the Fen residue of peptide BBA1 by either of the natural amino acids tyrosine (BBA2) or tryptophan (BBA3) resulted in conformational flexibility in the sheet and loop regions of the structure. This conformational ambiguity was



eliminated in peptides BBA4 and BBA5 by including charged residues on the exterior of the β hairpin designed to both select against the undesired fold and stabilize the desired structure. The evaluation of two additional peptides (BBA6 and BBA7) provided further insight into the specific involvement of the surface polar residues in the creation of well-defined structure in BBA4 and BBA5. The sequences of BBA5, BBA6 and BBA7 include only one non-standard amino acid (D-proline), which constrains a critical engineered type II' turn. Manipulation of residues on the exterior of small ββα motifs has led to the design of 23-residue polypeptides that adopt a defined tertiary structure in the absence of synthetic amino acids, increasing the availability and expanding the potential uses of the BBA motif. The importance of negative design concepts to the creation of structured polypeptides is also highlighted.
5 February 1998, Research Paper, *Folding & Design*

☐ **Tolerance of a protein helix to multiple alanine and valine substitutions.**
Lydia M Gregoret and Robert T Sauer (1998). *Fold Des.* **3**, 119–126.

Protein stability is influenced by the intrinsic secondary structure propensities of the amino acids and by tertiary interactions, but which of these factors dominates is not known in most cases. Combinatorial mutagenesis has been used to examine the effects of substituting a good helix-forming residue (alanine) and a poor helix-forming residue (valine) at many positions in an α helix of a native protein, allowing the authors to average over many molecular environments and assess to what extent the results reflect intrinsic helical propensities or are masked by tertiary effects. Alanine or



valine residues were combinatorially substituted at 12 positions in α-helix 1 of λ repressor. Functional proteins were selected and sequenced to determine the degree to which each residue type was tolerated. On average, valine substitutions were accommodated

slightly less well than alanine substitutions. On a positional basis, however, valine was tolerated as well as alanine at the majority of sites. In fact, alanine was preferred over valine statistically significantly only at four sites. Studies of mutant protein and peptide stabilities suggest that tertiary interactions mask the intrinsic secondary structure propensity differences at most of the remaining residue positions in this α helix. At the majority of positions in α-helix 1 of the λ repressor, tertiary interactions with other parts of the protein can be viewed as an environmental 'buffer' that help to diminish the helix destabilizing effects of valine mutations and allow these mutations to be tolerated at frequencies similar to alanine mutations.
5 February 1998, Research Paper, *Folding & Design*

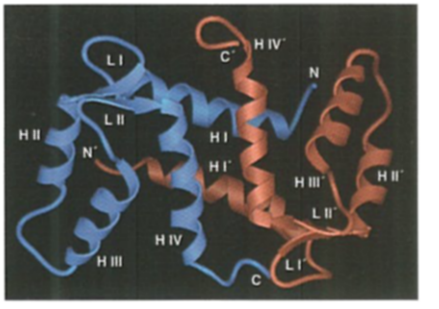
☐ **Crystal structure of $p14^{TCL1}$, an oncogene product involved in T-cell prolymphocytic leukemia, reveals a novel β-barrel topology.**
François Hoh, Yin-Shan Yang, Laurent Guignard, André Padilla, Marc-Henri Stern, Jean-Marc Lhoste and Herman van Tilbeurgh (1998). *Structure* **6**, 47–155.

Chromosome rearrangements are frequently involved in the generation of hematopoietic tumors. One type of T-cell leukemia, T-cell prolymphocytic leukemia, is consistently associated with chromosome rearrangements characterized by the juxtaposition of the

TCRA locus on chromosome 14q11 and either the T^{CL1} gene on 14q32.1 or the MTCP1 gene on Xq28. The T^{CL1} gene is preferentially expressed in cells of early lymphoid lineage; its product is a 14 kDa protein ($p14^{TCL1}$), expressed in the cytoplasm. $p14^{TCL1}$ has strong sequence similarity with one product of the MTCP1 gene, p13MTCP1 (41% identical and 61% similar). The functions of the TCL1 and MTCP1 genes are not known yet, and they have no sequence similarity to any other published sequence. To gain a more fundamental insight into the role of this particular class of oncogenes, the authors have determined the three-dimensional structure of $p14^{TCL1}$. The structure was solved by molecular replacement using the solution structure of $p13^{MTCP1}$, revealing $p14^{TCL1}$ to be an all-β protein consisting of an eight-stranded parallel β barrel with a novel topology. The barrel consists of two four-stranded β-meander motifs, related by a twofold axis and connected by a long loop. This internal pseudo-twofold symmetry was not expected on basis of the sequence alone, but structure-based sequence analysis of the two motifs shows that they are related. The structures of $p13^{MTCP1}$ and $p14^{TCL1}$ are very similar, diverging only in regions that are either flexible and/or involved in crystal packing. Structural similarities between $p14^{TCL1}$ and $p13^{MTCP1}$ suggest that their (unknown) function may be analogous. This is confirmed by the fact that these proteins are implicated in analogous diseases. Their structure does not show similarity to other oncoproteins of known structure, confirming their classification as a novel class of oncoproteins.
15 February 1998, Research Paper, *Structure*.

☐ **The three-dimensional structure of Ca^{2+}-bound calcyclin: implications for Ca^{2+}-signal transduction by S100 proteins.**
Mallika Sastry, Randal R Ketchum, Orlando Crescenzi, Christoph Weber, Michael J Lubienski, Hiroyoshi Hidaka and Walter J Chazin (1998). *Structure* **6**, 223–231.

Calcyclin is a member of the S100 subfamily of EF-hand Ca^{2+}-binding proteins. This protein has implied roles in the regulation of cell growth and division, and exhibits deregulated expression. The three-dimensional structure of Ca^{2+}-bound calcyclin was calculated with 1372 experimental constraints,and is represented by an ensemble of 20 structures that have a backbone root mean square deviation of 1.9 Å for the eight helices. Ca^{2+}-bound calcyclin has the same symmetric homodimeric fold as observed for the

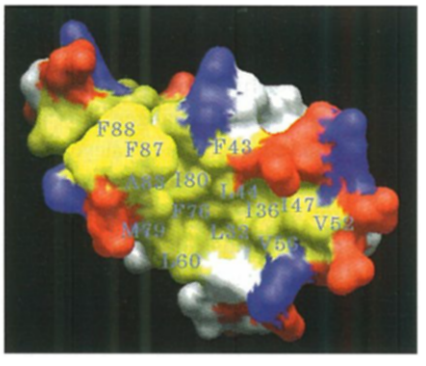


apo protein. The helical packing within the globular domains and the subunit interface also change little upon Ca^{2+} binding. A distinct homology was found between the Ca^{2+}-bound states of the calcyclin subunit and the monomeric S100 protein calbindin D9k. Only very modest Ca^{2+}-induced changes are observed in the structure of calcyclin, in sharp contrast to the domain-opening that occurs in calmodulin and related Ca^{2+}-sensor proteins. Thus, calcyclin, and by inference other members of the S100 family, must have a different mode for transducing Ca^{2+} signals and recognizing target proteins. This proposal raises significant questions concerning the purported roles of S100 proteins as Ca^{2+} sensors.
15 February 1998, Research Paper, *Structure*.

☐ **A novel mode of target recognition suggested by the 2.0 Å structure of holo S100B from bovine brain.**
Hiroyoshi Matsumura, Tomoo Shiba, Tsuyoshi Inoue, Shigeharu Harada and Yasushi Kai (1998). *Structure* **6**, 233–241.

S100B, a small acidic calcium-binding protein, is a member of the S100 protein family and is a multifunctional protein capable of binding several target

molecules, such as cytoskeletal proteins and protein kinases, in a calcium-dependent manner. S100B is a homodimer of S100β subunits (ββ) with a total of four calcium-binding motifs called EF hands. S100B is found abundantly in nervous tissue and has been implicated in Alzheimer's disease and Down's syndrome. Structural analysis of S100B in the calcium-bound state is required to gain a better understanding of the conformational changes that occur to S100B upon calcium binding and to elucidate the mode of recognition between S100B and its target molecules. The authors have determined the three-dimensional structure of holo S100B from bovine brain by X-ray diffraction. The dimeric S100B molecule is formed by non-covalent interactions between large hydrophobic surfaces on both S100ß subunits. There are two EF-hand motifs per S100β subunit, each of which binds one calcium ion. The authors observe, in



the calcium-bound structure, dramatic changes in the conformation of the terminal helices, from the compact structure in the apo form to a more extended form upon binding calcium. Following these changes, an exposed hydrophobic core, surrounded by many negatively charged residues, is revealed. Cys84 is positioned at an exposed surface of S100B, surrounded by hydrophobic residues, and could form a disulfide bond to τ protein, one of the known target molecules thought to interact with S100B in this way. The molecular structure of holo S100B suggests a novel mode of target recognition for the S100 family of calcium-binding proteins. Upon calcium binding, dramatic changes occur in the terminal helices of S100B, revealing a large hydrophobic surface, not observed in the apo form. It is through hydrophobic interactions and possibly a Cys84-mediated disulfide bond that S100B is thought to bind its target molecules.

15 February 1998, Research Paper, *Structure*.