Elsewhere in Biology

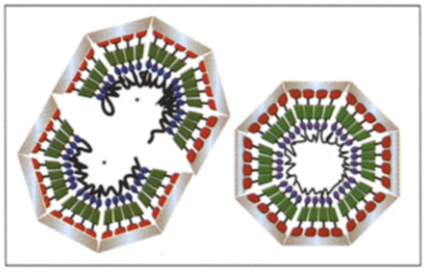
Chosen and summarized by the staff of *Chemistry & Biology*

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

Chemistry & Biology November 1997, **4**:881–884

☐ **Cryo-electron microscopy reveals ordered domains in the immature HIV-1 particle.** Stephen D Fuller, Thomas Wilk, Brent E Gowen, Hans-Georg Kräusslich and Volker M Vogt (1997). *Curr. Biol.* **7**, 729–738.

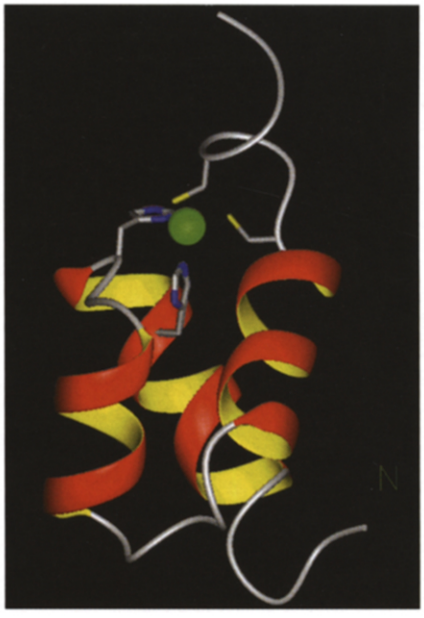
Human immunodeficiency virus type 1 (HIV-1) is the causative agent of AIDS and the subject of intense study. The immature HIV-1 particle is traditionally described as having a well ordered, icosahedral structure made up of uncleaved Gag protein surrounded by a lipid bilayer containing envelope proteins.



Expression of the Gag protein in eukaryotic cells leads to the budding of membranous virus-like particles (VLPs). In this paper, the authors used cryo-electron microscopy of VLPs from insect cells and lightly fixed, immature HIV-1 particles from human lymphocytes to determine their organization. Both types of particle were heterogeneous in size (1200-2600 Å diameter). Immature HIV-1 particles and VLPs both have a multi-sector structure characterized not by an icosahedral organization but by local order in which the structures of the matrix and capsid regions of Gag change upon cleavage. The authors propose a model in which lateral interactions between Gag protein molecules yield arrays that are organized into sectors for budding by RNA.
1 September 1997, Research Paper, *Current Biology**

☐ **The solution structure of the amino-terminal HHCC domain of HIV-2 integrase: a three-helix bundle stabilized by zinc.** Astrid PAM Eijkelenboom, Fusinita MI van den Ent, Arnold Vos, Jurgen F Doreleijers, Karl Hård, Thomas D Tullius, Ronald HA Plasterk, Robert Kaptein and Rolf Boelens (1997). *Curr Biol.* **7**, 739–746.

Integrase mediates a crucial step in the life cycle of the human immunodeficiency virus (HIV). The enzyme cleaves the viral DNA ends in a sequence-dependent manner and couples the newly generated hydroxyl groups to phosphates in the target DNA. Three domains have been identified in HIV integrase: an amino-terminal domain, a central catalytic core and a carboxy-terminal DNA-binding domain. The amino-terminal region is the only domain with unknown structure thus far. This domain, which is known to bind zinc, contains a HHCC motif that is conserved in retroviral integrases. Although the exact function of this domain is unknown, it is required for cleavage and integration. In this paper, the three-dimensional structure of the amino-terminal domain of HIV-2 integrase has been determined using two-dimensional and three-dimensional nuclear magnetic resonance data. The structure consists of three helices and a helical turn; the helices form a three-helix bundle that is stabilized by a zinc-binding unit. The helical arrangement is similar to that found in the DNA-binding domains of the trp repressor, the prd paired domain and Tc3A transposase. This structure shows no similarity with any of the known zinc-finger structures. The strictly conserved residues of the HHCC motif of retroviral integrases are involved in metal coordination, whereas many other well conserved hydrophobic residues are part of the protein core.



5 September 1997 , Research Paper, *Current Biology*

☐ **p53 activity is essential for normal development in *Xenopus*.** John B Wallingford, Daniel W Seufert, Valerie C Virta and Peter D Vize (1997). *Curr. Biol.* **7**, 747–757.

The tumor suppressor p53 plays a key role in regulating the cell cycle and apoptosis in differentiated cells. Mutant mice lacking functional p53 develop normally but die from multiple neoplasms shortly after birth. There have been hints that p53 is involved in morphogenesis, but given the relatively normal development of p53 null mice, the significance of these data has been difficult to evaluate. To examine the role of p53 in vertebrate development, the authors have determined the results of blocking its activity in embryos of the frog *Xenopus laevis*. Two different methods have been used to block p53 protein activity in developing *Xenopus* embryos — ectopic expression of dominant-negative forms of human p53 and ectopic expression of the p53 negative regulator, *Xenopus* dm-2.

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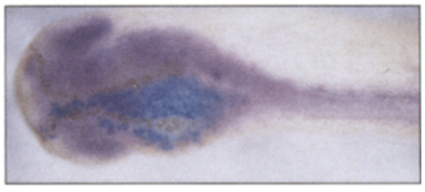
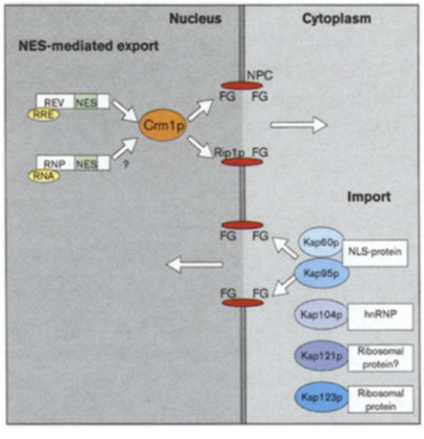
In both instances, inhibition of p53 activity blocked the ability of *Xenopus* early blastomeres to undergo differentiation and resulted in the formation of large cellular masses reminiscent of tumors. Inhibiting p53 function results in an early block to differentiation. Although it is possible that mutant human p53 proteins have a dominant gain-of-function or neomorphic activity in *Xenopus*, and that this is responsible for the development of tumors, most of the evidence indicates that this is not the case. Whatever the basis of the block to differentiation, these results indicate that *Xenopus* embryos are a sensitive system in which to explore the role of p53 in normal development and in developmental tumors.



5 September 1997, Research Paper, *Current Biology*

☐ **The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export.** Megan Neville, Francoise Stutz, Linda Lee, Laura I Davis and Michael Rosbash (1997). *Curr. Biol.* **7**, 767–775.

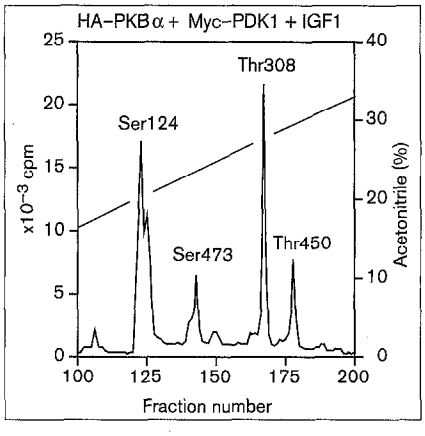
The human immunodeficiency virus (HIV-1) uses the viral protein Rev to regulate gene expression by promoting the export of unspliced and partially spliced viral transcripts. Rev has been shown to function in a variety of organisms, including *Saccharomyces cerevisiae*. The export activity of Rev depends on a nuclear export signal (NES), which is believed to interact with the nuclear pore complex to carry out its export function. Crm1p is a member of the importin-beta protein family, other members of which are known to be directly involved in nuclear import. Crm1p has recently been shown to contribute to nuclear export in vertebrate systems. Here, the authors have studied this mechanism of nuclear to cytoplasmic transport. Viable mis-sense mutations in the *CRM1* gene substantially reduced or eliminated the biological activity of Rev in *S. cerevisiae*, providing strong evidence that Crm1p also contributes to transport of Rev NES-containing proteins and ribonucleoproteins in this organism. Crm1p also interacted with nuclear pore proteins. The results indicate that Crm1p interacts with the Rev NES and nuclear pore proteins during delivery of cargo to the nuclear pore complex.



18 September 1997, Research Paper, *Current Biology*

☐ **3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the *Drosophila* DSTPK61 kinase.** Dario R Alessi, Maria Deak, Antonio Casamayor, F Barry Caudwell, Nick Morrice, David G Norman, Piers Gaffney, Colin B Reese, Colin N MacDougall, Diane Harbison, Alan Ashworth and Mary Bownes (1997). *Curr. Biol.* **7**, 776–789.

The activation of protein kinase B (PKB, also known as c-Akt) is stimulated by insulin or growth factors and results from its phosphorylation at Thr308 and Ser473. The authors recently identified a protein kinase, termed PDK1, that phosphorylates PKB at Thr308 only in the presence of lipid vesicles containing phosphoinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) or phosphatidylinositol, 4-bisphosphate (PtdIns(3,4)P_2). Here, the authors have cloned and sequenced human PDK1. The 556-residue monomeric enzyme comprises a catalytic domain that is most similar to the PKA, PKB and PKC subfamily of protein kinases and a carboxy-terminal pleckstrin homology (PH) domain. The PDK1 gene is located on human chromosome 16p 13.3 and is expressed ubiquitously in human tissues.



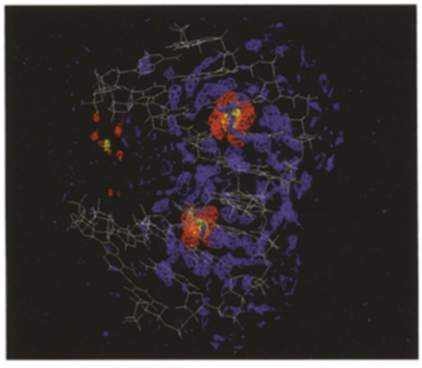
Human PDK1 is homologous to the *Drosophila* protein kinase DSTPK61, which has been implicated in the regulation of sex differentiation, oogenesis and spermatogenesis. Expressed PDK1 and DSTPK61 phosphorylated Thr308 of PKBα only in the presence of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2. Overerexpression of PDK1 in 293 cells activated PKBα and potentiated the IGF1-induced phosphorylation of PKBα at Thr308. Experiments in which the PH domains of either PDK1 or PKBα were deleted indicated that the binding of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 to PKBα is required for phosphorylation and activation by PDK1. IGF1 stimulation of 293 cells did not affect the activity or phosphorylation of PDK1. PDK1 is likely to mediate the activation of PKB by insulin or growth factors. DSTPK61 is a Drosophila homologue of PDK1. The effect of PtdIns(3,4,5)P_3/PtdIns(3,4)P_2 in the activation of PKBα is at least partly substrate directed.

18 September 1997, Research Paper, *Current Biology*

☐ **Insight into the stabilization of A-DNA by specific ion association: spontaneous B-DNA to A-DNA transitions observed in molecular dynamics simulations of d[ACCCGCGGGT]$_2$ in the presence of hexaamminecobalt(III).** Thomas E Cheatham III and Peter A Kollman (1997). *Structure* **5**, 1297–1312.

Duplex DNA is more than a simple information carrier. The sequence-dependent structure and its inherent deformability, in

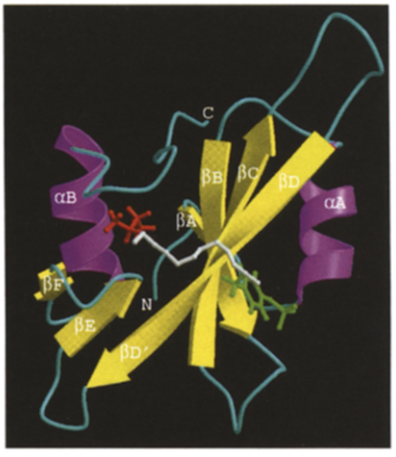
concert with the subtle modulating effects of the environment, play a crucial role in the regulation and packaging of DNA. Recent advances in force field and simulation methodologies allow molecular dynamics simulations to represent the specific effects of the environment. An understanding of the environmental dependence of DNA structure gives insight into how histones are able to package DNA, how various proteins are able to bind and modulate nucleic acid structure and will ultimately aid the design of molecules to package DNA for more effective gene therapy. Molecular dynamics simulations of d[ACCCGCGGGT]$_2$ in solution in the presence of hexaamminecobalt(III) [$Co(NH_3)_6^{3+}$] show stabilization of A-DNA and spontaneous B-DNA to A-DNA transitions. In the absence of $Co(NH_3)_6^{3+}$, A-DNA to B-DNA transitions are observed instead. In addition to their interaction with the guanines in the major groove, $Co(NH_3)_6^{3+}$ ions bridge opposing strands in the bend across the major groove, probably stabilizing A-DNA. The simulation methods and force fields have advanced to a sufficient level that some representation of the environment can be seen in nanosecond length molecular dynamics simulations. These simulations suggest that, in addition to the general explanation of A-DNA stabilization by dehydration, hydration and ion association in the major groove stabilize A-DNA.



15 October 1997, Research Paper, *Structure*

☐ **The SH2 domain from the tyrosine kinase Fyn in complex with a phosphotyrosyl peptide reveals insights into domain stability and binding specificity.** Terrence D Mulhern, Graeme L Shaw, Craig J Morton, Anthony J Day and Iain D Campbell (1997). *Structure* **5**, 1313–1324.

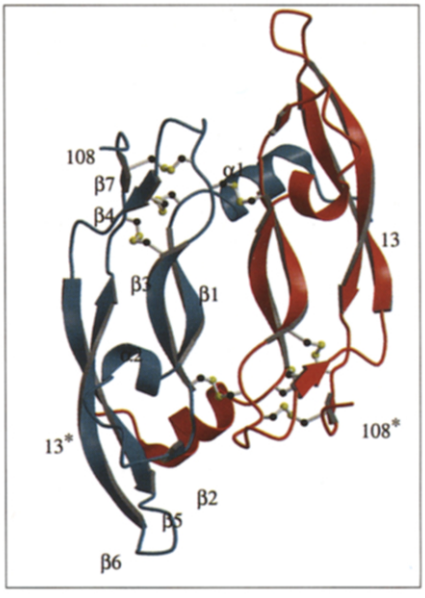
SH2 domains are found in a variety of signal transduction proteins; they bind phosphotyrosine-containing sequences, allowing them to recognize target molecules and regulate intramolecular kinase activity. Fyn is a member of the Src family of tyrosine kinases that are involved in signal transduction by association with a number of membrane receptors. The kinase activity of these signalling proteins is modulated by switching the binding mode of their SH2 and SH3 domains from intramolecular to intermolecular. The molecular basis of the signalling roles observed for different Src family members is still not well understood. The authors report the first structure of the Fyn SH2 domain, in complex with a phosphotyrosyl peptide (EPQpYEEIPIYL), determined by high resolution NMR spectroscopy. The overall structure of the complex is analogous to that of other SH2–peptide complexes. Noteworthy aspects of the structure are: the BG loop, which contacts the bound peptide, contains a type-I′ turn; a capping-box-like interaction is present at the amino-terminal end of helix αA; *cis–trans* isomerization of the ValβG1–ProβG2 peptide bond causes conformational heterogeneity of residues near the amino and carboxyl termini of the domain. Comparison of the Fyn SH2 domain structure with other structures of SH2 domains highlights several interesting features. Conservation of helix capping interactions among various SH2 domains is suggestive of a role in protein stabilisation. The presence of a type-I′ turn in the BG loop, which is dependent on the presence of a glycine residue at position BG3, is indicative of a binding pocket, characteristic of the Src family, SykC and Abl, rather than a binding groove found in PLC-γ1C, p85αN and Shc, for example.



15 October 1997, Research Paper, *Structure*

☐ **The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding.** Yves A Muller, Hans W Christinger, Bruce A Keyt and Abraham M de Vos (1997). *Structure* **5**, 1325–1338.

Vascular endothelial growth factor (VEGF) is an endothelial-cell-specific angiogenic and vasculogenic mitogen. VEGF also plays a role in pathogenic vascularization which is associated with a number of clinical disorders, including cancer and rheumatoid arthritis. The development of VEGF antagonists, which prevent the interaction of VEGF with its receptor, may be important for the treatment of such disorders. VEGF is a homodimeric member of the cystine knot growth factor superfamily. It binds to two different tyrosine kinase receptors, kinase domain receptor (KDR) and Fms-like tyrosine kinase 1 (Flt-1), and a



number of VEGF homologs are known with distinct patterns of specificity for these same receptors. The authors have determined the crystal structure of the receptor-binding domain of VEGF in a triclinic space group containing eight monomers in the asymmetric unit. Superposition of the eight copies of VEGF shows that the β sheet core regions of the monomers are very similar, with slightly greater differences in most loop regions. For one loop, the different copies represent different snapshots of a concerted motion; mutagenesis mapping shows that this loop is part of the receptor-binding site of VEGF. Mapping the receptor-binding determinants on a multiple sequence alignment of VEGF homologs suggests the differences in specificity towards KDR and Flt-1 may derive from both sequence variation and changes in the flexibility of binding loops. The structure can also be used to predict possible receptor-binding determinants for related cystine knot growth factors.
15 October 1997, Research Paper, *Structure*

☐ **The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain suggests a role for this complex in altered T cell receptor signaling.** Stefan Arold, Peet Franken, Marie-Paule Strub, Francois Hoh, Serge Benichou, Richard Benarous and Christian Dumas (1997). *Structure* **5**, 1361–1372.

Human immunodeficiency virus (HIV) Nef protein accelerates virulent progression of acquired immunodeficiency syndrome (AIDS) by its interaction with specific cellular proteins involved in signal transduction and host cell activation. Nef has been shown to bind specifically to a subset of the Src family of kinases. The structures of free Nef and Nef bound to Src homology region 3 (SH3) domain are important for the elucidation of how the affinity and specificity for the Src kinase family SH3 domains are achieved, and also for the development of potential drugs and vaccines against AIDS. The authors have determined the crystal structures of the conserved core of HIV-1 Nef protein alone and in complex with the wild-type SH3 domain of the $p59^{fyn}$ protein tyrosine kinase (Fyn). Comparison of the bound and unbound Nef structures revealed that a proline-rich motif (Pro–x–x–Pro), which is implicated in SH3 binding, is partially disordered in the absence of the binding partner; this motif only fully adopts a left-handed polyproline type II helix conformation upon complex formation with the Fyn SH3 domain. In addition, the structures show how an arginine residue of Nef interacts with Asp100 of the so-called RT loop within the Fyn SH3 domain, and triggers a hydrogen-bond rearrangement which allows the loop to adapt to complement the Nef surface. The three-dimensional structures support evidence that the Nef–Fyn complex forms *in vivo* and may have a crucial role in the T cell perturbating action of Nef by altering T cell receptor signaling. The structures suggest possible targets for the design of inhibitors which specifically block Nef–SH3 interactions.
15 October 1997, Research Paper, *Structure*