Elsewhere in Biology

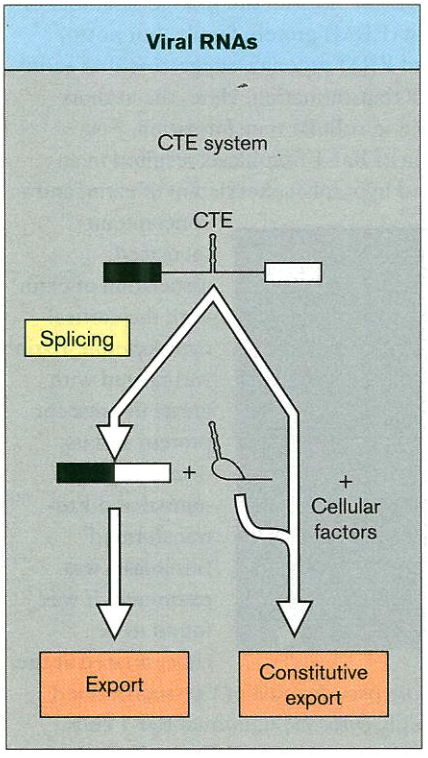
Chosen and summarized by the staff of *Chemistry & Biology*

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

Chemistry & Biology September 1997, **4**:705–709

☐ **The simian retrovirus-1 constitutive transport element, unlike the HIV-1 RRE, uses factors required for cellular mRNA export.** Claudio Saavedra, Barbara Felber and Elisa Izaurralde (1997). *Curr. Biol.* **7**, 619–628.

Cellular pre-mRNAs are usually retained in the nucleus. After removal of the intron, the RNA is exported to the cytoplasm. In contrast, unspliced retroviral genomic RNA is exported to the cytoplasm. In complex retroviruses, such as human immunodeficiency virus-1 (HIV-1), nuclear export is accomplished by the interaction of a virally encoded protein, Rev, with a *cis*-acting RNA element, the Rev-responsive element (RRE). In type D retroviruses, such as the simian retrovirus type 1 (SRV-1), however, genomic RNA is exported by cellular factor(s) that interact with a conserved *cis*-acting RNA element, the constitutive transport element (CTE). The authors found that the CTE was exported in a specific and saturable fashion from *Xenopus* oocyte nuclei. They show that the CTE promotes the export of intron-containing RNAs by a pathway distinct from that used by the Rev–RRE system. Furthermore, they show that the CTE seems to interact with evolutionarily conserved factors that are essential for cellular mRNA export.



25 July 1997, Research Paper, *Current Biology**

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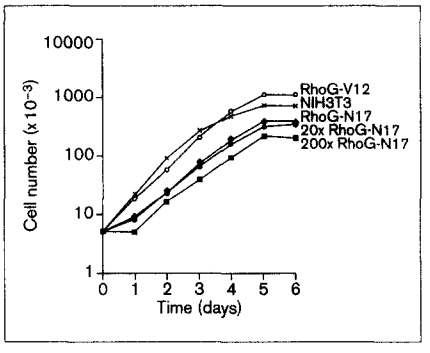
☐ **The small GTPases Cdc42Hs, Rac1 and RhoG delineate Raf-independent pathways that cooperate to transform NIH3T3 cells.** Pierre Roux, Cècile Gauthier-Rouviére, Sandrine Doucet-Brutin and Philippe Fort (1997). *Curr. Biol.* **7**, 629–637.

In mammalian cells, the expression of oncogenic Ras proteins has been shown to activate mitogenesis, as well as cause extensive changes in cell morphology. Ras-mediated transformation has also been shown to activate multiple signalling pathways, including those involving mitogen-activated protein kinases and the small GTPase Rho. Members of the Rho family affect cell morphology by controlling the formation of actin-dependent structures such as filopodia, lamellipodia, ruffles and stress fibers. In addition, Rho GTPases are involved in progression through the G1 phase of the cell cycle, and Rac1 and RhoA have recently been directly implicated in the morphogenic and mitogenic responses to transformation by oncogenic Ras. To examine the crosstalk between Ras and Rho proteins, the authors investigated the effects on focus-forming activity and cell growth of the Rho-family members Cdc42Hs, Rac1 and RhoG by expressing constitutively active or dominant-negative forms in NIH3T3 cells. All three GTPases were required for cell transformation mediated by Ras but not by constitutively active Raf. The selective activation of each GTPase was not sufficient to induce the formation of foci; only the coordinated activation of all three GTPases elicited a high focus-forming activity. The authors conclude that Ras-mediated transformation induces extensive changes in cell morphology which require the activity of members of the Rho family of GTPases.

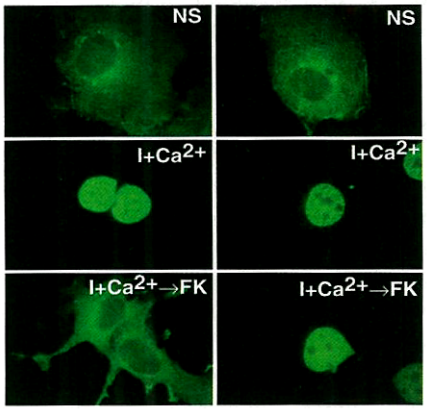


30 July 1997, Research Paper, *Current Biology*

☐ **Rapid targeting of nuclear proteins to the cytoplasm.** Juli D Klemm, Chan R Beals and Gerald R Crabtree (1997). *Curr. Biol.* **7**, 638–644.

The transcription factor NF-ATc is involved in the activation of many early immune response genes and is regulated by subcellular localization. NF-ATc translocates from the cytoplasm to the nucleus in response to a rise in intracellular calcium, and immediately returns to the cytoplasm when intracellular calcium levels fall. The rapid nuclear exit of NF-ATc, which suggests that NF-ATc contains a nuclear export signal (NES), is thought to be one mechanism by which

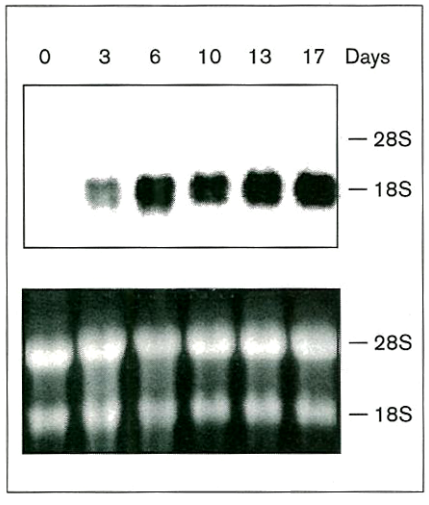
cells distinguish between sustained and transient calcium signals. To study the nuclear export of NF-ATc, the authors have developed a general, non-invasive assay for the identification and study of NESs. The NES is defined by its ability to translocate a protein from the nucleus to the cytoplasm when the two are tethered by a membrane-permeable ligand. The authors have identified an NES within NF-ATc that functions in concert with a glycogen synthase kinase-regulated process to direct the rapid nuclear exit of NF-ATc. The rapid nuclear export of NF-ATc via its NES and a glycogen synthase kinase-regulated event may be an important mechanism for insulating cells from transient spikes in intracellular calcium. This assay can be used as a general method for the inducible cytoplasmic export of nuclear proteins.



8 August 1997, Research Paper, *Current Biology*

☐ **TYMSTR, a putative chemokine receptor selectively expressed in activated T cells, exhibits HIV-1 coreceptor function.** M Loetscher, A Amara, E Oberlin, N Brass, DF Legler, P Loetscher, M D'Apuzzo, E Meese, D Rousset, J-L Virelizier, M Baggiolini, F Arenzana-Seisdedosx and B Moser (1997). *Curr. Biol.* **7**, 652–660.

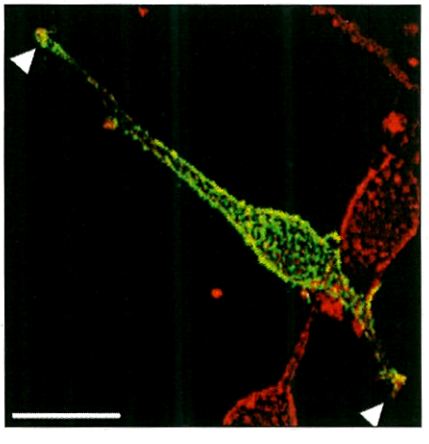
Chemokines bind to specific receptors and mediate leukocyte migration to sites of inflammation. Recently, some chemokine receptors, notably CXCR4 and CCR5, have been shown to be essential fusion factors on target cells for infection by human immunodeficiency virus (HIV); the chemokines bound by these receptors have also been shown to act as potent inhibitors of HIV infection. Here, the authors describe the isolation of a novel, putative chemokine receptor, termed TYMSTR (T-lymphocyte-expressed seven-transmembrane domain receptor). The TYMSTR gene is localized to human chromosome 3 and encodes a protein that has a high level of identity with chemokine receptors. TYMSTR mRNA was selectively expressed in interleukin-2-stimulated T lymphocytes. Cells co-expressing TYMSTR and human CD4 fused with cells expressing envelope glycoproteins of macrophage (M)-tropic HIV-1 as well as T-cell line (T)-tropic HIV-1 isolates. Addition of infectious, T-tropic HIV-1 particles to TYMSTR/CD4-expressing cells resulted in viral entry and proviral DNA formation. The findings demonstrate that TYMSTR, in combination with CD4, mediates HIV-1 fusion and entry and that it might function as HIV coreceptor at both early and late stages of infection.



11 August 1997, Research Paper, *Current Biology*

☐ **Essential functions of ezrin in maintenance of cell shape and lamellipodial extension in normal and transformed fibroblasts.** Richard F Lamb, Bradford W Ozanne, Christian Roy, Lynn McGarry, Christopher Stipp, Paul Mangeat and Daniel G Jay (1997). *Curr. Biol.* **7**, 682–688.

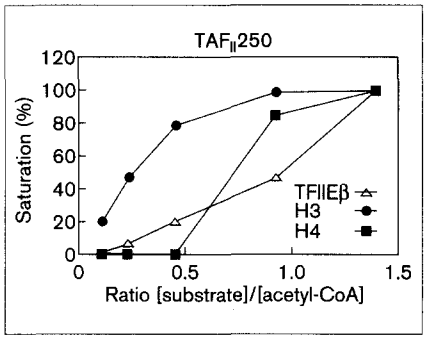
Changes in cell shape and motility are important manifestations of oncogenic transformation, but the mechanisms underlying these changes and key effector molecules in the cytoskeleton remain unknown. The Fos oncogene induces expression of ezrin, the founder member of the ezrin/radixin/moesin (ERM) protein family, but not expression of the related ERM proteins, suggesting that ezrin has a distinct role in cell transformation. Here, the authors examine the role of ezrin in cellular transformation. Fos-mediated transformation of Rat-1 fibroblasts resulted in an increased expression and hyperphosphorylation of ezrin, and a concomitant increased association of ezrin with the cortical cytoskeleton. Ezrin was tagged with green fluorescent protein and its distribution in normal and Fos-transformed fibroblasts was examined: it was found to be concentrated at the leading edge of extending pseudopodia of Fos-transformed Rat-1 cells, and was mainly cytosolic in normal Rat-1 cells. Functional ablation of ezrin by micro-CALI (chromophore-assisted laser inactivation) blocked plasma-membrane ruffling and motility of Fos-transformed fibroblasts. Ablation of ezrin in normal Rat-1 cells caused a marked collapse of the leading edge of the cell. Ezrin plays an important role in pseudopodial extension in Fos-transformed Rat-1 fibroblasts, and maintains cell shape in normal Rat-1 cells. The results suggest a critical role for ezrin in oncogenic transformation.



19 August 1997, Research Paper, *Current Biology*

☐ **Acetylation of general transcription factors by histone acetyltransferases.** Axel Imhof, Xiang-Jiao Yang, Vasily V Ogryzko, Yoshihiro Nakatani, Alan P Wolffe and Hui Ge (1997). *Curr. Biol.* **7**, 689–692.

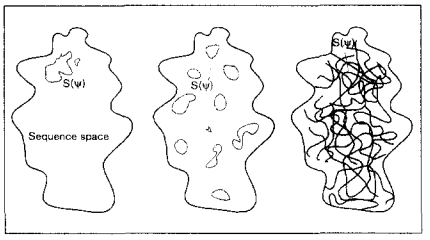
The acetylation of histones increases the accessibility of nucleosomal DNA to transcription factors, relieving transcriptional repression and correlating with the potential for transcriptional activity *in vivo*. The characterization of several novel histone acetyltransferases — including the human GCN5 homolog PCAF (p300/CBP-associated factor), the transcription coactivator p300/CBP, and $TAF_{II}250$ — has provided a potential explanation for the relationship between histone acetylation and transcriptional activation. In addition to histones, however, other components of the basal transcription machinery might be acetylated by these enzymes and directly affect transcription. The authors examine the acetylation of the basal transcriptional machinery for RNA polymerase II by PCAF, p300 and $TAF_{II}250$. All three acetyltransferases can direct the acetylation of TFIIEβ and TFIIF and a preferred site of acetylation was identified in TFIIEβ. Human TFIIE consists of two subunits, α (p56) and β (p34), which form a hetero-tetramer (α2β2) in solution. TFIIE enters the preinitiation complex after RNA polymerase II and TFIIF, suggesting that TFIIE may interact directly with RNA polymerase II and/or TFIIF. In addition, TFIIE can facilitate promoter melting either in the presence or absence of TFIIH and can stimulate TFIIH-dependent phosphorylation of the carboxy-terminal domain of RNA polymerase II.



19 August 1997, Brief Communication, *Current Biology*

☐ **Neutral networks in protein space: a computational study based on knowledge-based potentials of mean force.** Aderonke Babajide, Ivo L Hofacker, Manfred J Sippl and Peter F Stadler (1997). *Fold. Des.* **2**, 261–269.

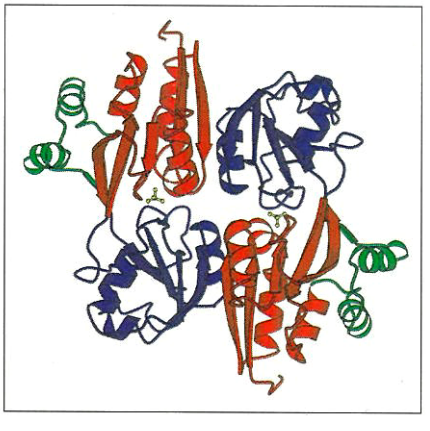
Many protein sequences, often unrelated, adopt similar folds. Sequences folding into the same shape thus form subsets of sequence space. The shape and the connectivity of these sets have implications for protein evolution and *de novo* design. This paper describes an investigation of the topology of these sets for some proteins with known three-dimensional structure using inverse folding techniques. It was found that sequences adopting a given fold do not cluster in sequence space and that there is no detectable sequence homology among them. Nevertheless, these sequences are connected in the sense that there exists a path such that every sequence can be reached from every other sequence while the fold remains unchanged. Similar results were found for some restricted amino acid alphabets (e.g. ADLG) and in other cases it seemed impossible to find sequences with native-like behavior (e.g. QLR). It appears that these findings are independent of the particular structure considered. Amino acid sequences folding into a common shape are distributed homogeneously in sequence space. Hence, the connectivity of the set of these sequences implies the existence of very long neutral paths on all examined protein structures. Regarding protein design, these results imply that sequences with more or less arbitrary chemical properties can be attached to a given structural framework, but the designability varies significantly among native structures. These features of protein sequence space are similar to what has been found for nucleic acids.



15 August 1997, Research Paper, *Folding & Design*

☐ **The structure of the cofactor-binding fragment of the LysR family member, CysB: a familiar fold with a surprising subunit arrangement.** Richard Tyrrell, Koen HG Verschueren, Eleanor J Dodson, Garib N Murshudov, Christine Addy and Anthony J Wilkinson (1997). *Structure* **5**, 1017–1032.

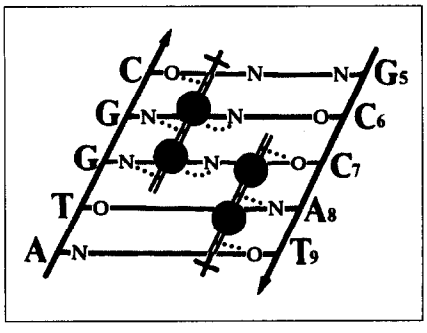
CysB is a tetrameric protein of identical subunits (M_r = 36,000) that controls the expression of genes associated with the biosynthesis of cysteine in bacteria. CysB is a member of the LysR family of prokaryotic transcriptional regulatory proteins which share sequence similarities over ~280 residues. The aims of this study were to explore the complex molecular biology and curious ligand binding properties of CysB and to provide structural insights into the LysR family of proteins. The crystal structure of a dimeric chymotryptic fragment of *Klebsiella aerogenes* CysB comprising residues 88–324, has been solved to 1.8 Å resolution. The protein comprises two α/β domains (I and II) connected by two short segments of polypeptide which, by analogy with the periplasmic solute-binding proteins, might be expected to serve as a hinge. This hinge would permit relative domain movements to take place as cofactor molecules are captured and then trapped in an enclosed cavity. A similar fold has also been observed in the cofactor-binding domain of Lac repressor, implying a structural relationship between the Lac repressor and LysR families of proteins. In



contrast to Lac repressor, in CysB the twofold axis of symmetry that relates the monomers in the dimer is perpendicular rather than parallel to the long axis of the cofactor-binding domain. This seems likely to place the DNA-binding domains at opposite extremes of the molecule possibly accounting for CysB's extended DNA footprints.
15 August 1997, Research Paper, *Structure*

☐ **Defining GC-specificity in the minor groove: side-by-side binding of the di-imidazole lexitropsin to C-A-T-G-G-C-C-A-T-G.** Mary L Kopka, David S Goodsell, Gye Won Han, Thang Kien Chiu, JW Lown and Richard E Dickerson (1997). *Structure* **5**, 1033–1046.

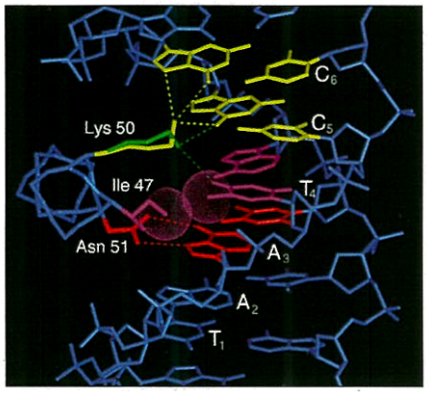
Polyamide drugs, such as netropsin, distamycin and their lexitropsin derivatives, can be inserted into a narrow B-DNA minor groove to form 1:1 complexes that can distinguish AT base pairs from GC, but cannot detect end-for-end base-pair reversals such as TA for AT. Hence, they can detect only half the information in the minor groove. In contrast, side-by-side binding of two drugs within a widened minor groove offers potential for greater discrimination, because each drug effectively reads one side of the groove, or one strand of the helix. Imidazole (Im) and pyrrole (Py) rings side-by-side read a GC base pair with the Im ring recognizing the guanine side.



But the reason for this specific G–Im association is unclear because the guanine NH_2 group sits in the center of the groove. A 2:1 drug:DNA complex that presents Im at both ends of a GC base pair should help unscramble the issue of imidazole reading specificity. The paper describes the determination of the crystal structure of a 2:1 complex of a di-imidazole lexitropsin (DIM), an analogue of distamycin, and a DNA decamer with the sequence C-A-T-G-G-C-C-A-T-G. Im and Py rings distinguish AT from GC base pairs because of steric factors involving the bulk of the guanine amine, and the ability of Im to form a hydrogen bond with the amine. Side-by-side Im and Py rings differentiate GC from CG base pairs because of tight steric contacts and sp^2 hybridization at the amine nitrogen atom, with the favored conformations being G/Im,Py/C and C/Py,Im/G. Discrimination between AT and TA base pairs may be possible using bulkier rings, such as thiazole to select the A end of the base pair. Thus side-by-side lexitropsins may permit binding selectively and specifically to a pre-chosen DNA sequence. This has the potential to be useful in vectoring a drug for a specific target in chemotherapy, probing genomic DNA to locate specific sequences, and, with the addition of molecular scissors, it becomes a useful tool for gene therapy.
15 August 1997, Research Paper, *Structure*

☐ **Engrailed (Gln50→Lys) homeodomain–DNA complex at 1.9 Å resolution: structural basis for enhanced affinity and altered specificity.** Lisa Tucker-Kellogg, Mark A Rould, Kristen A Chambers, Sarah E Ades, Robert T Sauer and Carl O Pabo (1997). *Structure* **5**, 1047–1054.

The homeodomain is one of the key DNA-binding motifs used in eukaryotic gene regulation, and occurs in many transcription factors that control differentiation and determine cell fate. Homeodomain–DNA interactions have been studied intensively both because of the intrinsic importance of the homeodomain and because the homeodomain has become a paradigm for the analysis of protein–DNA interactions. The residue at position 50 of many homeodomains appears to determine the differential DNA-binding specificity, helping to distinguish among binding sites of the form TAATNN. But the precise role(s) of residue 50 in the differential recognition of alternative sites has not been clear. The crystal structure of a complex containing the engrailed Gln50→Lys variant with its optimal binding site TAATCC (versus TAATTA for the wild-type protein) has been determined at 1.9 Å resolution. It was found that the structural context provided by the folding and docking of the engrailed homeodomain allows Lys50 to make remarkably favorable contacts. Although many different residues occur at position 50 in different homeodomains, and although numerous position 50 variants have been constructed, the most striking examples of altered specificity usually involve introducing or removing a lysine sidechain from position 50. This high-resolution structure also confirms the critical role of Asn51 in homeodomain–DNA recognition and further clarifies the roles of water molecules near residues 50 and 51.

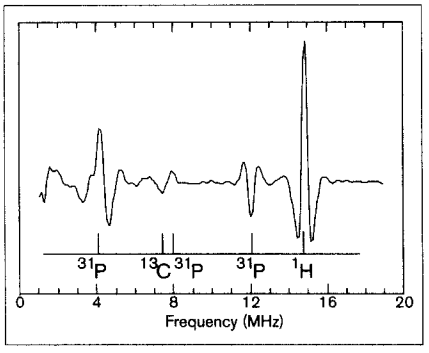


15 August 1997, Research Paper, *Structure*

☐ **The frozen solution structure of p21 ras determined by ESEEM spectroscopy reveals weak coordination of Thr35 to the active site metal ion.** Christian T Farrar, Christopher J Halkides and David J Singel (1997). *Structure* **5**, 1055–1066.

The G protein p21 ras is a molecular switch in the signal transduction pathway for cellular growth and differentiation. Hydrolysis of tightly bound GTP alters the conformation of p21, terminating the signal. The coordination of the p21 residue Thr35 to Mg^{2+} in its active site (observed in the crystal structure of p21 in complex with a GTP-analog GMPPNP, but not with GDP) has been proposed to drive the conformational change accompanying nucleotide substitution and may have a role in the GTP hydrolysis reaction itself. But previous

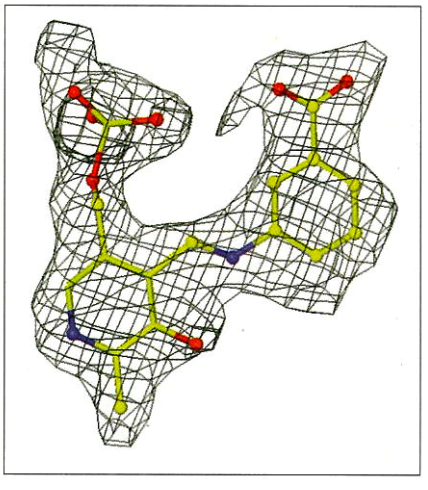
electron spin-echo envelope modulation (ESEEM) studies suggest that Thr35 only weakly coordinates the metal ion in the growth-active GTP-bound state of p21. The $^{13}C\beta$-Thr35 to Mn^{2+} distance found in the labeled p21·Mn^{2+}GMPPNP frozen solution structure is greater than that (3.16 Å) observed in the crystal structure. In contrast, the $^{15}N\varepsilon$-Lys16 to Mn^{2+} distance is in good agreement with the 5.1 Å crystal structure distance. Thus it appears that Thr35 only weakly coordinates the metal ion in frozen solution. Thr35 coordination of the metal ion is therefore unlikely to drive the conformational change between GTP-bound and GDP-bound states of p21. Thr35 may be essential for GTPase-activating protein (GAP)-stimulated GTP hydrolysis and/or signal transduction for other reasons.



15 August 1997, Research Paper, *Structure*

☐ **Human ornithine aminotransferase complexed with L-canaline and gabaculine: structural basis for substrate recognition.** Sapan A Shah, Betty W Shen and Axel T Brünger (1997). *Structure* **5**, 1067–1075.

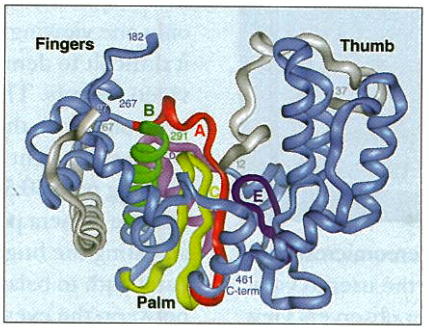
Ornithine aminotransferase (OAT) is a 45 kDa pyridoxal-5′-phosphate (PLP)-dependent enzyme that catalyzes the conversion of L-ornithine and 2-oxoglutarate to glutamate-D-semialdehyde and glutamic acid, respectively. In humans, loss of OAT function causes an accumulation of ornithine that results in gyrate atrophy of the choroid and retina, a disease that progressively leads to blindness. In an effort to learn more about the structural basis of this enzyme's function, the authors determined the X-ray structures of OAT in complex with two enzyme-activated suicide substrates: L-canaline, an ornithine analog, and gabaculine, an irreversible inhibitor of several related aminotransferases. Because L-canaline is virtually identical to ornithine, the structure presented here provides information on the specific residues in the active site that are involved in substrate recognition. The crystal structure of OAT in complex with gabaculine provides the first structural evidence that the potency of the inhibitor is due to energetically favorable aromatic interactions with residues in the active site. This aromatic-binding mode may be relevant to structure-based drug design efforts against other ω-aminotransferase targets, such as GABA aminotransferase.



15 August 1997, Research Paper, *Structure*

☐ **Structure of the RNA-dependent RNA polymerase of poliovirus.** Jeffrey L Hansen, Alexander M Long and Steve C Schultz (1997). *Structure* **5**, 1109–1122.

The central player in the replication of RNA viruses is the viral RNA-dependent RNA polymerase. The 53 kDa poliovirus polymerase, together with other viral and possibly host proteins, carries out viral RNA replication in the host cell cytoplasm. RNA-dependent RNA polymerases comprise a distinct category of polymerases that have limited sequence similarity to reverse transcriptases (RNA-dependent DNA polymerases) and perhaps also to DNA-dependent polymerases. Previously reported structures of RNA-dependent DNA polymerases, DNA-dependent DNA polymerases and a DNA-dependent RNA polymerase show that structural and evolutionary relationships exist between the different polymerase categories. The paper describes the determination of the structure of the RNA-dependent RNA polymerase of poliovirus at 2.6 Å resolution by X-ray crystallography. It has the same overall shape as other polymerases, commonly described by analogy to a right hand. The structures of the 'fingers' and 'thumb' subdomains of poliovirus polymerase differ from those of other polymerases, but the palm subdomain contains a core structure very similar to that of other polymerases. This conserved core structure is composed of four of the amino acid sequence motifs described for RNA-dependent polymerases. Structure-based alignments of these motifs has enabled previous sequence and structural alignments to be modified and extended so as to relate sequence conservation to function. Extensive regions of polymerase–polymerase interactions observed in the crystals suggest an unusual higher order structure that is thought to be important for polymerase function. As a first example of a structure of an RNA-dependent RNA polymerase, the results reported here provide for a better understanding of polymerase structure, function and evolution.



15 August 1997, Research Paper, *Structure*