Paper Alert

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A selection of interesting papers that were published in the month before our press date in major journals most likely to report significant results in structural biology, protein and RNA folding.

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Structure 2000, 8:R163–R168

Crystal structure of T7 gene 4 ring helicase indicates a mechanism for sequential hydrolysis of nucleotides.


The authors have determined the crystal structure of an active, hexameric fragment of the gene 4 helicase from bacteriophage T7. The structure reveals how subunit contacts stabilize the hexamer. Deviation from expected sixfold symmetry of the hexamer indicates that the structure is of an intermediate on the catalytic pathway. The structural consequences of the asymmetry suggest a ‘binding change’ mechanism to explain how cooperative binding and hydrolysis of nucleotides are coupled to conformational changes in the ring that most likely accompany duplex unwinding. The structure of a complex with a nonhydrolyzable ATP analog provides additional evidence for this hypothesis, with only four of the six possible nucleotide-binding sites being occupied in this conformation of the hexamer.

9 June 2000, Cell


Rad50 catalytic domain (Rad50cd) from Pyrococcus furiosus displays ATPase activity plus ATP-controlled dimerization and DNA-binding activities. The authors present Rad50cd crystal structures that identify probable protein and DNA interfaces. The structures reveal an ABC-ATPase fold, linking Rad50 molecular mechanisms to ABC transporters, including P glycoprotein and cystic fibrosis transmembrane conductance regulator. Binding of ATP γ-phosphates to conserved signature motifs in two opposing Rad50cd molecules promotes dimerization that probably couples ATP hydrolysis to dimer dissociation and DNA release. These results, validated by mutations, suggest unified molecular mechanisms for ATP-driven cooperativity and allosteric control of ABC-ATPases in double-strand break repair, membrane transport, and chromosome condensation by structural maintenance of chromosomes (SMC) proteins.

23 June 2000, Cell

Crystal structure of the λ repressor C-terminal domain provides a model for cooperative operator binding.


The cI repressor of bacteriophage λ is a classic example of a protein that binds to its operator sites cooperatively. The C-terminal domain of the repressor mediates dimerization as well as a dimer–dimer interaction that results in the cooperative binding of two repressor dimers to adjacent operator sites. The authors present the crystal structure of the λ repressor C-terminal domain determined by multiwavelength anomalous diffraction. Interactions that mediate cooperativity are captured in the crystal, where two dimers associate about a twofold axis of symmetry. Based on the structure and previous genetic and biochemical data, they present a model for the cooperative binding of two λ repressor dimers at adjacent operator sites.

23 June 2000, Cell

Structural basis for recognition of the translocated intimin receptor (Tir) by intimin from enteropathogenic Escherichia coli.


Intimin is a bacterial adhesion molecule involved in the attachment of Escherichia coli to mammalian host cells. Intimin binds to the translocated intimin receptor (Tir), which is exported by the bacteria and integrated into the host cell plasma membrane. The authors localized the Tir-binding region of intimin to the C-terminal 190 amino acids (Int190) and determined its solution structure, which comprises an immunoglobulin domain that is intimately coupled to a novel C-type lectin domain. The structure is similar to the integrin-binding domain of the Yersinia invasin and C-type lectin families. NMR titration and mutagenesis data identified the binding site for Tir, which is located at the extremity of the Int190 moiety.

1 June 2000, EMBO J

SH3 domain recognition of a proline-independent tyrosine-based RKxxYxxY motif in immune cell adaptor SKAP55.


Src-homology 3 (SH3) domains recognize a PXXP core motif preceded or followed by positively charged residue(s). The
authors report SH3 domain binding to a novel proline-independent motif in the immune cell adaptor SKAP55, which is comprised of two N-terminal lysine and arginine residues followed by two tyrosines (i.e. RKxxYxxY). Domains capable of binding to class I proline motifs bound to the motif, whereas the class II domains failed to bind. Two-dimensional NMR analysis of the peptide-bound FYN-SH3 domain showed overlap with the binding site of a proline-rich peptide on the charged surface of the SH3 domain. Expression of the RKG/DYAS peptide potently inhibited TcRζ/CD3-mediated NF-AT transcription in T cells. These findings extend the repertoire of SH3 domain binding motifs to include a tyrosine-based motif. 

15 June 2000, EMBO Journal


Mutations in the genes encoding the interacting proteins AML1 and CBFβ are the most common genetic abnormalities in acute leukaemia, and congenital mutations in the related AML3 gene are associated with disorders of osteogenesis. The crystal structure of the complex between the AML1 Runt domain and CBFβ demonstrates that point mutations associated with cleidocranial dysplasia map to the conserved heterodimer interface, suggesting a role for CBFβ in osteogenesis, and reveals a potential protein interaction platform composed of conserved negatively charged residues on the surface of CBFβ. 

15 June 2000, EMBO Journal


The Tup1–Ssn6 co-repressor complex regulates the expression of several sets of genes, including genes that specify mating type in the yeast Saccharomyces cerevisiae. Repression of mating-type genes occurs when Tup1–Ssn6 is brought to the DNA by the Matt2 DNA-binding protein and assembled upstream of a- and haploid-specific genes. The crystal structure of the C-terminal domain of Tup1 reveals a seven-bladed β propeller with an N-terminal subdomain that is anchored to the side of the propeller and extends the β-sheet of one of the blades. Point mutations in Tup1 that specifically affect the Tup1–Matt2 interaction cluster on one surface of the propeller. 

15 June 2000, EMBO Journal


The authors have extended the resolution of the crystal structure of human bactericidal/permeability-increasing protein (BPI) to 1.7 Å. BPI has two domains with the same fold, but with little sequence similarity. To understand the similarity in structure of the two domains, they compare the corresponding residue positions in the two domains using 3D–1D profiles. A 3D–1D profile is a string formed by assigning each position in the three-dimensional structure to one of 18 environment classes. The environment classes are defined by the local secondary structure, the area of the residue that is buried from solvent, and the fraction of the area buried by polar atoms. A structural alignment between the two BPI domains was used to compare the 3D–1D environments of structurally equivalent positions. Greater than 31% of the aligned positions have conserved 3D–1D environments, but only 13% have conserved residue identities. Analysis of the 3D–1D environmentally conserved positions helps to identify pairs of residues likely to be important in conserving the fold, regardless of the residue similarity. The authors found examples of 3D–1D environmentally conserved positions with dissimilar residues which nevertheless play similar structural roles. To generalize their findings, the authors analysed four other proteins with similar structures yet dissimilar sequences. Together, these examples show that aligned pairs of dissimilar residues often share similar structural roles, stabilizing dissimilar sequences in the same fold. 

16 June 2000, Journal of Molecular Biology


The DNA in the core of spores of Bacillus species is saturated with a group of small, acid-soluble proteins (SASP). During spore germination, SASP’s are rapidly degraded to amino acids and this degradation is initiated by a sequence-specific protease called germination protease (GPR). The crystal structure of GPR in its zymogen form from Bacillus megaterium adopts a novel fold. The asymmetric unit contains two $P_{46}$ monomers and the functional tetramer is a dimer of dimers, with an ~9 Å channel in the center of the tetramer. 

1 June 2000, Journal of Molecular Biology


Transposition requires a coordinated series of DNA breakage and joining reactions. The Tn7 transposase contains two proteins: Tn5A, which carries out DNA breakage at the 5’ ends of the transposon, and Tn5B, a member of the retroviral integrase superfamily, which carries out breakage and joining at the 3’ ends of the transposon. The authors report the structure of Tn5A. Surprisingly, the Tn5A fold is that of a type II restriction endonuclease, suggesting that transposition
utilizes a restriction-enzyme-like mechanism for 5′-end strand breakage.

June 2000, Molecular Cell


TRADD is a multifunctional signaling adaptor protein that is recruited to tumor necrosis factor receptor (TNFR1) upon ligand binding. The C terminus of TRADD comprises the ‘death domain’ that is responsible for association of TNFR1 and other death-domain-containing proteins such as FADD and RIP. The N-terminal domain (N-TRADD) promotes the recruitment of TRAF2 to TNFR1 by binding to the C terminus of TRAF2, leading to the activation of JNK/AP1 and NF-κB. The solution structure of N-TRADD was determined, revealing a novel protein fold. A combination of NMR, surface plasmon resonance, and mutagenesis experiments was used to help identify the site of interaction of N-TRADD with C-TRAF2, providing a framework for future attempts to selectively inhibit the TNF signaling pathways.

June 2000, Molecular Cell


Target cell lysis is regulated by natural killer (NK) cell receptors that recognize class I MHC molecules. The crystal structure of the human immunoglobulin-like NK cell receptor KIR2DL2 in complex with its class I ligand HLA-Cw3 and peptide reveals that KIR binds in a nearly orthogonal orientation across the α1 and α2 helices of Cw3 and directly contacts positions 7 and 8 of the peptide. The receptor footprint on HLA overlaps with, but is distinct from, that of the T-cell receptor. A hydrogen bond between Lys44 of KIR2DL2 and Asn80 of Cw3 confers the allotype specificity.

1 June 2000, Nature


Calcium ATPase is a member of the P-type ATPases that transport ions across the membrane against a concentration gradient. The crystal structure of the calcium ATPase of skeletal muscle sarcoplasmic reticulum (SERCA1a) reveals two calcium ions bound in the transmembrane domain, which comprises ten α-helices. The cytoplasmic region consists of three well-separated domains, with the phosphorylation site in the central catalytic domain and the adenosine-binding site on another domain. Comparison with a low-resolution electron-density map of the enzyme in the absence of calcium and with biochemical data suggests that large domain movements take place during active transport.

8 June 2000, Nature


The 70 kDa heat shock proteins (the Hsp70 family) assist refolding of their substrates through ATP-controlled binding. The authors analyzed mutants of DnaK, an Hsp70 homolog, altered in key residues of its substrate-binding domain. Substrate binding occurs by a dynamic mechanism involving a hydrophobic pocket for a single residue that is crucial for affinity, a two-layered closing device involving independent action of an α-helical lid and an arch, and a superimposed allosteric mechanism of ATP-controlled opening of the substrate-binding cavity that operates largely through a β-structured subdomain. Correlative evidence from mutational analysis suggests that the ADP and ATP states of DnaK differ in the frequency of the conformational changes in the α-helical lid and β-domain that cause opening of the substrate-binding cavity. The affinity for substrates, as defined by this mechanism, determines the efficiency of DnaJ-mediated and ATP-hydrolysis-mediated locking-in of substrates and chaperone activity of DnaK.

7 May 2000, Nature Structural Biology


The basis of the chemiosmotic theory is that energy from light or respiration is used to generate a transmembrane proton gradient. This is largely achieved by membrane-spanning enzymes known as ‘proton pumps’. In Azotobacter vinelandii ferredoxin I, reduction of a buried iron–sulphur cluster draws in a solvent proton, whereas re-oxidation is ‘gated’ by proton release to the solvent. Studies of this ‘proton-transferring module’ by fast-scan protein film voltammetry, high-resolution crystallography, site-directed mutagenesis and molecular dynamics, reveal that proton transfer depends on the position and pK of a single amino acid. The proton is delivered through the protein matrix by rapid penetrative excursions of the sidechain carboxylate of a surface residue (Asp15), the pK of which shifts in response to the electrostatic charge on the iron–sulphur cluster.

15 June 2000, Nature


Diverse molecules, from small antibacterial drugs to large protein toxins, are exported directly across both cell
membranes of Gram-negative bacteria. This export is brought about by the reversible interaction of substrate-specific inner-membrane proteins with an outer-membrane protein of the TolC family, thus bypassing the intervening periplasm. The crystal structure of TolC from Escherichia coli reveals that three TolC protomers assemble to form a continuous, solvent-accessible conduit—a ‘channel-tunnel’ over 140 Å long that spans both the outer membrane and periplasmic space. The periplasmic end of the tunnel is sealed by sets of coiled helices. The structure suggests a general mechanism for the action of bacterial efflux pumps.

22 June 2000, Nature


The authors have determined the solution structure of NusB, a transcription antitermination protein from Escherichia coli. The solution structure resembles that of Mycobacterium tuberculosis NusB determined by X-ray diffraction, but differs substantially from a solution structure of E. coli NusB reported earlier. The structure reveals a novel, all α-helical protein fold. NusB mutations that cause a loss of function or alter specificity for RNA targets are localized to surface residues and probably affect RNA–protein or protein–protein interactions. Residues that are highly conserved among homologs stabilize the protein core. (The crystal structure of NusB from Mycobacterium tuberculosis was reported in the same issue by Gopal et al., (2000). Nat. Struct. Biol. 7, 475–478.)

June 2000, Nature Structural Biology


Human Flt3 ligand (Flt3L) stimulates early hematopoiesis by activating a type III tyrosine kinase receptor on primitive bone marrow stem cells. The crystal structure of soluble Flt3L reveals that it is a homodimer of two short-chain α-helical bundles. Comparisons of structure–function relationships of Flt3L with the homologous hematopoietic cytokines macrophage colony stimulating factor (MCSF) and stem cell factor (SCF) suggest that they have a common receptor binding mode that is distinct from the paradigm derived from the complex of growth hormone with its receptor.

June 2000, Nature Structural Biology


Bone morphogenetic proteins (BMPs) belong to the large transforming growth factor–β (TGF–β) superfamily of multifunctional cytokines, BMP-2 can induce ectopic bone and cartilage formation in adult vertebrates and is involved in central steps in early embryonal development in animals. The authors report the crystal structure of human dimeric BMP-2 in complex with two high-affinity BMP receptor Iα extracellular domains (BRIαE). The receptor chains bind to the ‘wrist’ epitopes of the BMP-2 dimer and contact both BMP-2 monomers. No contacts exist between the receptor domains. The model reveals the structural basis for discrimination between type I and type II receptors and the variability of receptor-ligand interactions that is seen in BMP–TGF–β systems.

June 2000, Nature Structural Biology


Eukaryotic nuclei contain three different types of RNA polymerases (RNAPs), each consisting of 12–18 different subunits. The evolutionarily highly conserved RNAP subunit RPB5 is shared by all three enzymes and therefore represents a key structural/functional component. The authors present the crystal structure of the RPB5 subunit from Saccharomyces cerevisiae. The bipartite structure includes a eukaryote-specific N-terminal domain and a C-terminal domain resembling the archaeal RNAP subunit H. The experimentally mapped regions of RPB5 involved in interactions with transcription factor IIB and the hepatitis B virus transactivator protein X correspond to distinct and surface-exposed α-helical structures. (The solution structure of RPB5 from Methanobacterium thermoautotrophicum is reported in the same issue by Yee et al., (2000). Proc. Natl Acad. Sci. USA 97, 6311–6315.)

6 June 2000, Proceedings of the National Academy of Science USA


The RNA polymerase subunit RPB10 displays a high level of conservation across archaea and eukarya and is required for cell viability in yeast. Structure determination of this RNA polymerase subunit from Methanobacterium thermoautotrophicum reveals a topology, which the authors term a zinc-bundle, consisting of three α helices stabilized by a zinc ion. The metal ion is bound within an atypical sequence motif and serves to bridge an N-terminal loop with helix 3. This represents an example of two adjacent zinc-binding cysteine residues within an α-helix conformation. Conserved surface features of RPB10 include discrete regions of neutral, acidic and basic residues, the latter being located around the zinc-binding site.

6 June 2000, Proceedings of the National Academy of Science USA
Three-dimensional structures of functionally uncharacterized proteins may furnish insight into their functions. The potential benefits of three-dimensional structural information regarding such proteins are particularly obvious when the corresponding genes are conserved during evolution, implying an important function the classification of which cannot be inferred from their sequences. The authors determined the crystal structure of the *Bacillus subtilis* Maf protein, a representative of a family of proteins that has homologs in many of the completely sequenced genomes from archaea, prokaryotes and eukaryotes, but the function of which is unknown. The structure, in combination with multiple sequence alignment, reveals a putative active site. Phosphate ions present at this site and structural similarities between a portion of Maf and the anticondon-binding domains of several tRNA synthetases suggest that Maf may be a nucleic-acid-binding protein. The crystal structure of a Maf–nucleoside triphosphate complex provides support for this hypothesis and hints at di- or oligonucleotides with either 5′- or 3′-terminal phosphate groups as ligands or substrates of Maf.

6 June 2000, *Proceedings of the National Academy of Science USA*


One of the outstanding questions in protein folding concerns the degree of heterogeneity in the folding transition state ensemble: does a protein fold via a large multitude of diverse ‘pathways’ or are the elements of native structure assembled in a well-defined order? The authors build on previous point-mutagenesis studies of the src-homology (SH3) domain, there is a discrete order to structure assembly during folding.

20 June 2000, *Proceedings of the National Academy of Science USA*


Many annelids, including the earthworm *Lumbricus terrestris*, have giant cooperative respiratory proteins (molecular masses greater than 3.5 million Da) freely dissolved in the blood, rather than packaged in cells. These complexes, termed either erythrocruorins or hemoglobins, are assembled from many copies of both hemoglobin subunits and nonhemoglobin or ‘linker’ subunits. The authors present the crystal structure of *Lumbricus* erythrocruorin at 5.5 Å resolution, which reveals a remarkable hierarchical organization of 144 oxygen-binding hemoglobin subunits and 36 nonhemoglobin linker subunits. The hemoglobin chains arrange in novel dodecameric substructures. Twelve trimeric linker complexes project triple-stranded helical coiled-coil ‘spokes’ toward the center of the complex; interdigitation of these spokes appears crucial for stabilization. The resulting complex of linker chains forms a scaffold on which twelve hemoglobin dodecamers assemble.

20 June 2000, *Proceedings of the National Academy of Science USA*


Formation of a specific contact between two residues of a polypeptide chain is an important elementary process in protein folding. The authors describe a method for studying contact formation between tryptophan and cysteine based on measurements of the lifetime of the tryptophan triplet state. With tryptophan at one end of a flexible peptide and cysteine at the other, the triplet decay rate is identical to the rate of quenching by cysteine. This rate is also close to the diffusion-limited rate of contact formation. The length dependence of this end-to-end contact rate was studied in a series of Cys-(Ala-Gly-Gln)k-Trp peptides, with k varying from 1–6. The rate decreases from ~1/(40 ns) for k = 1 to ~1/(140 ns) for k = 6, approaching the length dependence expected for a random coil (n3/2) for the longest peptides.

20 June 2000, *Proceedings of the National Academy of Science USA*


The crystal structure of a membrane-associated glycosyltransferase involved in peptidoglycan biosynthesis is
reported. This enzyme, MurG, contains two α/β open sheet domains separated by a deep cleft. Structural analysis suggests that the C-terminal domain contains the UDP-GlcNAc-binding site whereas the N-terminal domain contains the acceptor binding site and probable membrane association site. The identification of a conserved structural motif involved in donor binding in different UDP-sugar transferases suggests that it may be possible to identify the residues that help determine donor specificity.

6 June 2000, Protein Science


Cyclic nucleotides are second messengers that are essential in vision, muscle contraction, neurotransmission, exocytosis, cell growth, and differentiation. These molecules are degraded by a family of enzymes known as phosphodiesterases, which serve a critical function by regulating the intracellular concentration of cyclic nucleotides. The authors have determined the three-dimensional structure of the catalytic domain of phosphodiesterase 4B2B to 1.77 Å resolution. The active site has been identified and contains a cluster of two metal atoms. The structure suggests the mechanism of action and basis for specificity and will provide a framework for structure-assisted drug design for members of the phosphodiesterase family.

9 June 2000, Science


Thymidylate kinase (TMPK) is a nucleoside monophosphate kinase that catalyzes the reversible phosphoryltransfer between ATP and TMP to yield ADP and TDP. Crystal structures of human TMPK in complex with TMP and ADP, TMP and the ATP analog AppNHp, TMP with ADP and the phosphoryl analog AlF3, TDP and ADP, and the bisubstrate analog TP5A were determined. The observed changes of nucleotide state and conformation and the corresponding protein structural changes are correlated with intermediates occurring along the reaction coordinate and show the sequence of events occurring during phosphate transfer.

30 May 2000, Structure


Phospholipase D (PLD) hydrolyzes the terminal phosphodiester bond of phospholipids to phosphatidic acid and a hydrophilic constituent. The first crystal structure of a