

human PNK with the consensus FHA domain (NCBI, CDD pfam 00498.5). Identical residues are boxed, and those residues most highly conserved in FHA domains are underlined. FHA residues underlined in red are those that directly contact the phosphopeptide [10]. The β sheet structure of FHA domains, as previously defined for Rad53 FHA1 [10], is also indicated.

is required to locate relatively rare, but potentially lethal, chromosomal breaks. This may explain why mammalian PNK interacts with XRCC1, a scaffold protein that helps recruit repair enzymes at sites of single-strand breakage [8]. The need to interact with other proteins may also explain why eukaryotic PNK is larger than T4 PNK. Indeed, a careful analysis of the extended amino terminus of mammalian PNK identifies a putative phosphopeptide binding FHA domain (see Figure, panel D). Thus, future structural studies should illuminate not only how the core enzymatic domains of different PNK polypeptides are adapted to their respective substrates, but also how these versatile enzymes are recruited to the appropriate place at the appropriate time.

Keith W. Caldecott
Genome Damage and Stability Centre
University of Sussex
Falmer
Brighton BN1 9RQ
United Kingdom

Organization of Protein Domains in T4 and Human PNK

(A) Organization of the kinase (red box) and phosphatase (blue box) domains of a T4 PNK polypeptide.

(B) Organization of a T4 PNK tetramer. The interfaces between the kinase domains and phosphatase domains that form the tetramer are indicated by overlapping red and blue boxes, respectively. The kinase and phosphatase domains proposed to cooperate to repair opposite ends of the same polynucleotide molecule are indicated by small circles of the same color. Note that the domains that cooperate in this manner are provided by different monomers and that there are potentially four such cooperating pairs per tetramer.

(C) Comparison of the domain structure of T4 and human PNK polypeptides.

(D) Alignment of the putative FHA domain of

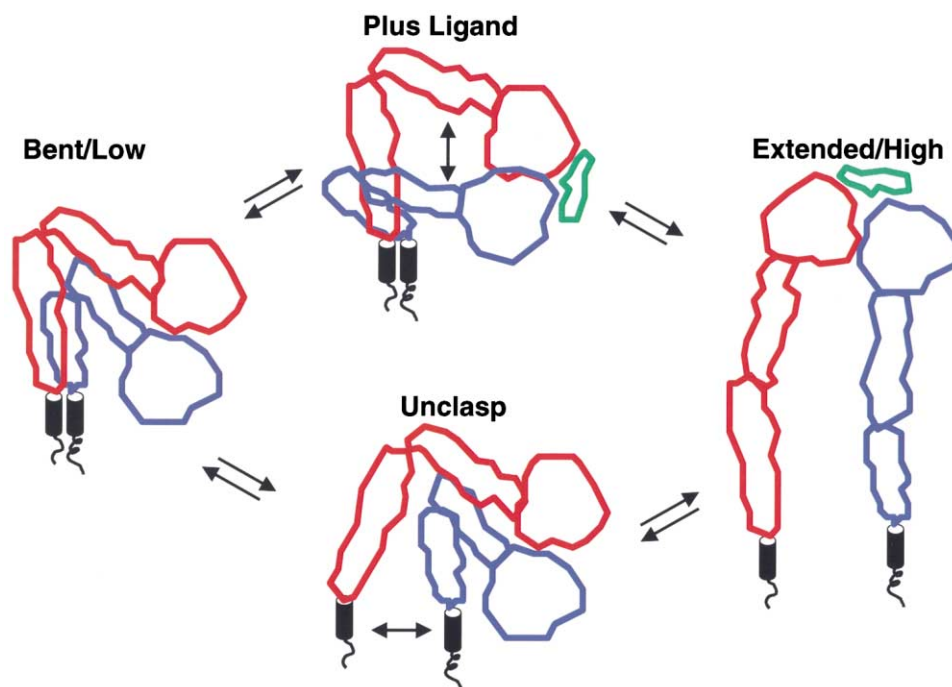
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New Light on the Integrin Switch

Recent structures of the integrin ectodomain and the cytoplasmic tail complex suggest a model for both “inside-out” and “outside-in” integrin signalling, whereby ligand binding and unclasping the cytoplasmic tails can shift integrin to a high-affinity form.

Among the most compelling problems in structural biology are those for which it is completely mysterious how a particular molecule accomplishes its function. Until recently, integrins posed just such a problem. Integrins are heterodimeric cell surface receptors with a large extracellular region, a single transmembrane region for each subunit, and typically small (20–70 amino acids) cytoplasmic tails. Integrins bind to other cell surface receptors and components of the extracellular matrix to mediate cell adhesion and signaling events in a wide variety of physiological processes [1, 2]. Electron micrographs of integrins reveal a globular head region of



A Model for Integrin Activation

The resting, or low-affinity, integrin resembles the bent structure observed in crystals by Xiong et al. (2000). Binding ligand alters the head group structure, which perturbs interdomain interactions, leading to destabilization of the compact structure and a shift to an extended high-affinity structure. Unclasp of the cytoplasmic tail regions also destabilizes the interdomain interactions in the bent structure and leads to a shift to the extended high-affinity structure. Although space precludes further discussion, Takagi et al. observe an intermediate structure in electron micrographs. (This figure was adapted from Takagi et al.)

~80 × 140 Å that contains the ligand binding site coupled to two flexible “stalk” regions 120–150 Å in length. These stalks consist of the C-terminal portions of the subunits and terminate in the transmembrane and cytoplasmic regions.

The mysterious feature of integrin behavior that has piqued the interest of many is their ability to regulate their affinity for ligand. Integrins increase their affinity for ligand after exposure to several different stimuli, including the ligand itself, activating antibodies, and manganese ions (outside-in signaling). Perhaps more curiously, the affinity of integrins for ligands also increases after activation of the integrin-bearing cell through stimulation of other cell receptors (inside-out signaling). This “molecular switch” phenomenon underlies many dynamic physiological processes, including the movement of leukocytes to sites of inflammation and the adherence of platelets to developing blood clots. What has proven so difficult to understand about integrin affinity modulation is how events inside the cell are able to influence the ligand binding site, which sits at the end of the long and apparently quite flexible stalk regions, and vice versa.

Coupled with the recent landmark determination of the crystal structure of an integrin ectodomain [3, 4], two new papers appearing in *Cell* go a long way toward supplying a satisfying molecular model of how integrins work [5, 6]. The crystal structure of the $\alpha_v\beta_3$ integrin ectodomain proved surprising not only because such a seemingly flexible molecule formed well-ordered crys-

tals, but also because it revealed a bent integrin with its head folded back among the stalks, like a gymnast limbering up for competition. Extensive contacts are seen between both the head and stalk regions and between the stalks themselves. This bent conformation initially seemed a product of the crystal environment, but Takagi et al. now provide striking evidence that this bent integrin corresponds to a physiological low-affinity state.

These authors examined negatively stained ectodomains of the $\alpha_v\beta_3$ integrin by electron microscopy and observed a bent structure in excellent agreement with a 2D projection of the crystal structure. However, these “bent” images were the predominant form only in the presence of calcium ions, which are known to stabilize the low-affinity form of integrins. When either a ligand mimetic peptide or manganese ions, both known to stimulate high-affinity states of integrins, were present prior to staining, the integrins shifted to a predominantly extended conformation. Takagi et al. liken this transition to the opening of a switchblade. These results strongly suggested that the bent form represented a low-affinity conformation and that the extended form represented a high-affinity conformation, and this correlation was indeed observed when ligand binding was assayed by surface plasmon resonance with the same integrin preparations that were used for electron microscopy.

In an astonishing experiment, Takagi et al. further confirmed this correlation of structure and function by introducing a disulfide bond into the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins

that locked them in the bent conformation. In the $\alpha_V\beta_3$ crystal structure, residues in the head region of the α subunit are adjacent to regions near the tip of the β subunit tail; in an extended structure, these sites would be greater than 100 Å apart. Mutation of sites in the α head and β tail to cysteine led to the formation of a disulfide bond in integrins when expressed on the cell surface. The presence of this disulfide bond was confirmed by the appearance of a covalent link between the α and β chains that disappeared in the presence of a reducing agent. Remarkably, cells expressing this mutant integrin failed to bind ligand, unless a reducing agent was added. These results indicate not only that the bent conformation exists on the surface of cells, but also that it is not competent to bind ligand with high affinity.

Vinogradova et al. approached the problem of integrin activation from the cytoplasmic side of the membrane. By manipulating expression conditions, they were able to determine the NMR structure of a previously elusive complex of the cytoplasmic tails of the $\alpha_{11b}\beta_3$ integrin subunits. This structure shows a small interaction interface between α -helical regions of the two subunits. Notably, residues that lead to activation of integrins when mutated appear at this interface, and the authors show that introduction of these mutations into the tail regions results in loss of the observed complex. Addition of a fragment of talin, a protein known to interact with cytoplasmic tail of the β subunit and to lead to integrin activation, also disrupts the cytoplasmic tail complex. These results are consistent with a model in which the interaction of the cytoplasmic domains preserves the integrin in an inactive state and disruption of this interaction promotes a transition to a higher affinity state.

A satisfying model for both inside-out and outside-in integrin signaling now emerges (see Figure). Future experiments will no doubt refine this model, but it seems clear that extensive, but weak, interdomain interactions maintain integrins in a compact state with low affinity for ligand. Since these interactions are coupled and involve both the head and tail regions, perturbations of either the head or tail destabilize the compact structure and favor a more extended structure with higher affinity for ligand. Thus, both ligand binding and unclasping the cytoplasmic tails are able to shift the integrin to a high-affinity form—in much the same way that one can pry an oyster open from either end.

Daniel J. Leahy

HHMI and

Department of Biophysics and Biophysical Chemistry
Johns Hopkins University School of Medicine
725 North Wolfe Street
Baltimore, Maryland 21205

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Structure, Vol. 10, September, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0969-2126(02)00837-7

Another Worm in Translation

Elongation factor EF-Tu is a key component in the translation step of protein synthesis, where it forms a complex with amino-acyl tRNA and delivers it to the ribosome. Until now, none of the known EF-Tu molecules have discriminated between the different species of tRNA, but now a new discovery sheds light on a curious EF-Tu homolog that binds just a single tRNA species.

The mitochondria constitute the cell's sustainable energy source. They even contain a bit of DNA of their own (mt-DNA), typically coding for a dozen proteins of the respiratory cycle, a couple of ribosomal rRNAs, and 22 tRNAs. However, most of the proteins needed for the household tasks of mitochondria are encoded by the cell's nucleus and produced in the cytosol. The RNA species encoded by mt-DNA have been reduced to a

bare minimum, and the secondary structures of mt-tRNAs are strange variations of the well-known cloverleaf motif, most of them lacking the T arm.

For several years, Kimitsuna Watanabe and his co-workers at the University of Tokyo have studied mitochondrial tRNAs and their participation in translation. For their studies, they have chosen the nematode worm *Caenorhabditis elegans*, a minimal organism of 959 somatic cells. With the 10,000 known nematode species, many parasitic to humans, the importance of studying these animals is evident.

Last year, Ohtsuki et al. reported the finding of a *C. elegans* mitochondrial EF-Tu possessing an unusual C-terminal extension of 57 residues [1]. This specialized EF-Tu was shown to bind efficiently to mt-tRNAs lacking the T arm, something that ordinary bacterial EF-Tu molecules cannot do. The study answered the long-standing question of whether a unique mitochondrial EF-Tu exists in nematode mitochondria. It had long been suspected that such an EF-Tu exists, since cytosolic EF-Tu could not bind the special T stem lacking tRNAs and here was the proof.

Earlier this year, Ohtsuki et al. [2] reported an NMR