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Mechanism of transcription regulation by conditional co-operativity

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Much of the knowledge we have about regulation of transcription in prokaryotes comes from two particularly well-studied systems: the Lac repressor-lac operon [1] and the λ-repressor and λ-Cro for the control of the lysogenic/lytic cycles of λ-phage [2]. In both cases, the repressors are well-folded species lacking appreciable structural disorder. Eukaryotic transcription factors on the other hand are characterized by frequently possessing intrinsically disordered (ID) segments or domains [3]. Intrinsic disorder however, can be detected in the genomes of prokaryotes as well but its functional relevance is less well understood [4], [5].

Here we present a novel mechanism of prokaryotic transcription regulation that crucially depends on the intrinsically disordered nature of the transcription factor. In toxin-antitoxin modules autoregulation of the operon happens through the antitoxin protein, which is DNA binding protein and is entirely or in part intrinsically unstructured. The DNA repressor activity is modulated by the well-folded toxin protein. A C-terminal ID domain of the antitoxin binds to the toxin in two distinct conformations to two binding sites. These binding events trigger a disorder-to-order transition in the N-terminal domain of the antitoxin, thus increasing its affinity for the operator DNA and illustrating for the first time allostery between two distinct disordered protein domains [6]. In addition, excess of toxin will drive a switch to a different toxin-antitoxin complex which is no longer able to bind to the operator DNA. This functioning of the toxin as co- or de-repressor depending on the ratio between toxin and antitoxin protein is known as conditional co-operativity and can now be explained by a switch between a high and a low affinity binding mode [6], [7].

Transcription regulation by conditional co-operativity is intimately linked to regulation of protein activity [7]. Indeed, the same allosteric interactions between toxin and antitoxin function to control gene repression and toxin activity such as gyrase poisono-ing in the case of the *ccd* TA module. Furthermore, the difference in affinity of the two antitoxin binding sites on the toxin serve to fine-tune these tow levels of regulation with respect to each other.

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Keywords: macromolecular, transcription, complex

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Cryo-EM of helical polymers: New insights into evolutionary divergence

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Unless a helical polymer has exactly 2, 3, 4 or 6 subunits per turn, it cannot be crystallized so that every subunit is in an equivalent environment. That is why so few crystal structures exist for protein polymers, which are highly abundant in biology, and why electron cryo-microscopy has emerged as one of the most useful tools for studying these filaments. However, cryo-EM studies of most protein polymers do not directly lead to high resolution structures due to the substantial degree of disorder and variability that is present. We have developed a number of methods for surmounting these problems, and the applications of these methods to a range of polymers, from bacterial pili to recombination filaments, have suggested some general principles, such as the lability of quaternary structure [1]. Our studies of eukaryotic actin [2] and prokaryotic actin-like proteins [3], [4] raise new insights into why actin has been so exquisitely conserved over large evolutionary distances (e.g., there are no amino acid changes between chickens and humans in the muscle isoform), but why the prokaryotic actin homologs have diverged considerably. We suggest that large networks of allosteric interactions within the actin filament have placed selective pressure on most, if not all, buried residues, and that these allosteric relations are responsible for the remarkable properties of cooperativity within the actin filament [5]. We show that F-actin does not exist in a single state, and that actin-binding proteins have the ability to impose large conformational changes on the actin subunits in the filament. Many of these insights are only possible due to the complementarity between x-ray crystallography, which has generated many atomic structures for the actin subunit, and cryo-EM, which can reconstruct at increasingly high resolutions the structure of the filament.

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Keywords: helical polymers, electron cryo-microscopy, three-dimensional reconstruction

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What makes homologous small GTPases specific? A combined X-ray, SAXS & NMR study

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How do highly homologous protein isoforms achieve exquisitely specific functions in cells? The small GTPases Arf1 and Arf6 are central regulators of almost every aspect of cellular traffic. They are highly similar: they have over 60% sequence identity, and structural studies have shown that the surfaces they use to interact with regulators and effectors are essentially identical in sequence and structure. Yet, they have non-overlapping functions in cells. Arf1 is a major regulator of vesicular traffic at the Golgi, while Arf6 is restricted to the plasma membrane where it acts at the crossroads of trafficking and cytoskeletal functions. Consistent with their cellular specificities,

Arf1 and Arf6 also have distinctive biochemical properties *in vitro*, for which no straightforward structural explanation has been put forward.

Activation of Arf proteins is a multi-step event, which involves the recruitment of Arf-GDP from the cytosol to membranes, followed by GDP/GTP exchange. We show that a truncated Arf6 mutant, a cytosolic construct that mimics membrane-bound Arf6-GDP, is partially unfolded in the crystal compared to full-length Arf6. This unusual conformation is the major species in solution, as shown by synchrotron SAXS analysis [1]. In contrast, the equivalent Arf1 mutant is essentially identical to full-length Arf1-GDP, as shown by NMR analysis. Taken together, these experiments suggest that the structural routes for the activation of Arf1 and Arf6 diverge at the step where GDP-bound Arf is recruited to membranes prior to nucleotide exchange [2]. These differences may account for the biochemical differences between Arf1 and Arf6, and yield their functional specificities.

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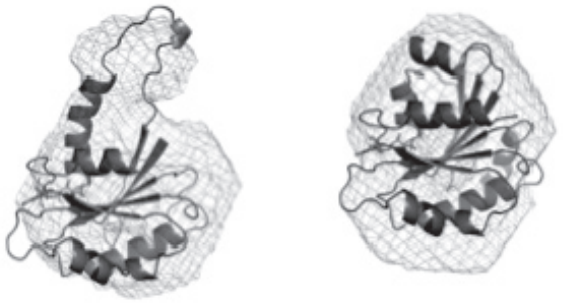

The figure shows the remarkable fit of the unfolded region as seen in the Δ13Arf6-GDP crystal with a protrusion in the 3D envelope calculated *ab initio* from SAXS data in solution (left). The fit of full-length Arf6 with the SAXS envelope is shown on the right panel.

Keywords: small GTPases, X-ray crystallography, SAXS

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Structural and energetic mechanisms of cooperative autoinhibition and activation of vav1

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Vav proteins are guanine nucleotide exchange factors (GEFs) for Rho family GTPases. They control processes including T cell activation, phagocytosis, and migration of normal and transformed cells. We report the structure and biophysical and cellular analyses of the five-domain autoinhibitory element of Vav1. The catalytic Dbl homology (DH) domain of Vav1 is controlled by two energetically coupled processes. The DH active site is directly, but weakly, inhibited by a helix from the adjacent Acidic domain. This core interaction is strengthened 10-fold by contacts of the calponin homology (CH) domain with the Acidic, pleckstrin homology, and DH domains. This construction enables efficient, stepwise relief of autoinhibition: initial phosphorylation events disrupt the modulatory CH contacts, facilitating phosphorylation of the inhibitory helix and consequent GEF activation. Our findings illustrate how the opposing requirements of strong suppression of activity and rapid kinetics of activation can be achieved in multidomain systems.

Here we report the crystal structure of the five-domain regulatory element of Vav1, CADPZ. The structure shows that the CH domain and the N terminus of the Acidic element bind to each other and to a platform formed by the PH domain and a C-terminal extension of the DH domain. Nuclear magnetic resonance (NMR), biochemical, and cell biological analyses show that these interactions suppress GEF activity by modulating the core helix-DH equilibrium, shifting it toward the inhibited state by approximately 10-fold, likely by restraining the inhibitory helix to the DH domain. Phosphorylation of Tyr142 and Tyr160 relieves the modulatory interactions, making Tyr174 more accessible to kinases, suggesting a sequential activation mechanism for full-length Vav1. The layered construction of Vav1 provides a means of achieving strong suppression of activity while still maintaining a route to rapid activation, features that are probably general among multidomain systems in biology.

The NMR approach we developed here provides a means to directly measure the populations of different states across regulatory equilibria and has allowed us to quantitatively characterize the energetic landscape of Vav1. This in turn has established coupled equilibria as a major mechanism of interdomain cooperativity in this system. Application of these methods to other systems should reveal how widespread cooperative inhibition through coupled equilibria is in multidomain proteins.

Keywords: inhibition, activation, cooperativity

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The Effect of Ligand-Stabilized Oligomerization on Rio1 Kinase Activity

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The Rio1 kinases belong to a family of atypical protein kinases for which there is little information about molecular function [1]. Rio1 kinases are essential and involved in ribosome biogenesis and cell cycle progression [2, 3]. We have identified a small molecule antibiotic, toyocamycin, which binds tightly to Rio1 in its ATP-binding pocket, confirmed by crystallographic studies. However, determination of the steady state kinetic parameters showed that toyocamycin inhibits Rio1 via mixed inhibition, and ATP inhibits Rio1 above micromolar concentrations. This lead to the hypothesis that Rio1 accesses multiple oligomeric states that impact its catalytic activity, which allows the inhibitor to influence activity via allosteric effects. In order to address this, we analyzed the oligomeric states of Rio1 in both the autophosphorylated and unphosphorylated forms, and in the absence and presence of toyocamycin and ATP. Sedimentation equilibrium analysis shows that Rio1 forms higher order oligomers, including dimer, trimer and tetramer, under all conditions except when autophosphorylated and unliganded. Analysis of crystal packing