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Mechanism of transcription regulation by conditional cooperativity

Remy Loris,^b Abel Garcia-Pino,^a Natalie de Jonge,^a ^a*Structural Biology Brussels, Vrije Universiteit Brussel (Belgium)*. ^b*Flanders Institute for Biotechnology (VIB), Brussels, (Belgium)*. E-mail: Remy.Loris@VIB-VUB.be

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 poisoning 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 two levels of regulation with respect to each other.

[1] M. Lewis, G. Chang, N.C. Horton, M.A. Kercher, H.C. Pace, M.A. Schumacher, R.G. Brennan, P. Lu, *Science* **1996**, *271*, 1247-1254. [2] R.A. Albright, B.W. Matthews, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3431-3436. [3] J. Liu, N.B. Perumal, C.J. Oldfield, E.W. Su, V.N. Uversky, A.K. Dunker, *Biochemistry* **2006**, *45*, 6873-6888. [4] A.K. Dunker, C.J. Brown, J.D. Lawson, L.M. Iakoucheva, Z. Obradovic, *Biochemistry* **2002**, *41*, 6573-6582. [5] P. Tompa, *Trends Biochem. Sci.* **2002**, *27*, 527-533. [6] A.Garcia-Pino, S. Balasubramanian, L.Wyns, E. Gazit, H. De Greve, R.D. Magnuson, D. Charlier, N. Van Nuland, R. Loris, *Cell* **2010**, *142*, 101-111. [7] N. De Jonge, A. Garcia-Pino, L. Buts, S. Haesaerts, D. Charlier, D., K. Zangger, L. Wyns, H. De Greve, R. Loris, **2009**. *Mol. Cell* **2009**, *35*, 154-163.

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

Edward H. Egelman, Vitold E. Galkin, Albina Orlova, *Department*

of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA (U.S.A.). E-mail: egelman@virginia.edu

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.

[1] E.H. Egelman, *Curr. Opin. Cell Biol.* **2010**, *22*, 68-74. [2] V.E. Galkin, A. Orlova, G.F. Schroder, E.H. Egelman, *Nat. Struct. Mol. Biol.* **2010**, *17*, 1318-1323. [3] A. Orlova, E.C. Garner, V.E. Galkin, J. Heuser, R.D. Mullins, E.H. Egelman, *Nat. Struct. Mol. Biol.* **2007**, *14*, 921-926. [4] V.E. Galkin, A. Orlova, C. Rivera, R.D. Mullins, E.H. Egelman, *Structure* **2009**, *17*, 1253-1264. [5] A. Orlova, E. Prochniewicz, E.H. Egelman, *J. Mol. Biol.* **1995**, *245*, 598-607.

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

Jacqueline Cherfils,^a Valérie Biou,^a Kaheina Aizel,^a Mahel Zeghouf,^a Vanessa Buosi,^b Aurélien Thureau,^b Eric Jacquet,^b Eric Guittet,^b Carine van Heijenoort,^b Pierre Roblin,^c Javier Perez,^c ^a*Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, (France)*. ^b*Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, (France)*. ^c*Synchrotron SOLEIL, Gif-sur-Yvette, (France)*. E-mail: cherfils@lebs.cnrs-gif.fr.

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,