Molecular mechanisms of signaling in fatty acid synthesis of Gram+ bacteria

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Context and aims

The molecular understanding of lipid synthesis regulation in Gram+ bacteria remains fragmentary. To unveil the mechanisms of signal transduction in two key pathways controlling lipid homeostasis in *Bacillus subtilis*, we have focused on two proteins that play a central role, DesK and FapR. This project is aimed at delivering molecular level understanding on: 1) how the trans-membrane His-kinase DesK is able to integrate the cold-shock input signal, to then transduce the information downstream, ultimately regulating membrane fatty acid desaturation; and, 2) the nature of the modifications induced by malonyl-CoA binding to FapR, triggering the release of this global transcriptional repressor from its DNA operator sites, inducing the cells to synthetize fatty acids.

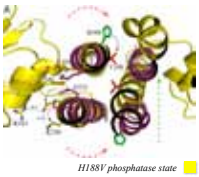
Results

A multidisciplinary approach combining single crystal X ray diffraction, structure-based mutagenesis, functional studies, complete thermodynamic characterizations and bioinformatics simulation methods, was followed in both protein systems. The *in silico* identification of compounds able to uncouple modulatory effects of molecules such as malonyl-CoA, guided the synthesis of candidate molecules that are being validated *in vitro* and *in vivo* as novel antibiotics.

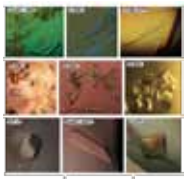
The thermosensor two-components system DesK-DesR

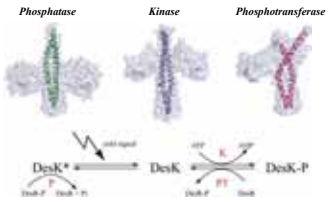
Overall, we have unveiled novel molecular details of the signal transduction mechanism in histidine kinases, an important group of trans-membrane proteins in bacteria. Their central helical structure plays a key role, alternative structural states allow toggling among the different functional configurations, which constitute the basis of output signaling.

A rotational shift and a shearing translation is observed in the central four-helix bundle, when comparing kinase vs phosphatase conformations



The central helical domain of DesK has a coiled-coil structure, with particular sequence features that allow for the 'cogwheel' rotary shift mechanism

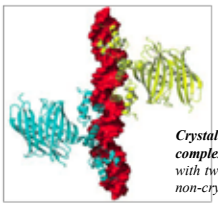




We have solved the crystal structures of the entire catalytic region of DesK in three conformational states. Each one can be ascribed to distinct functional roles of the enzyme: phosphatase-competent (DesK), kinase-competent (DesK) and phosphotransferase-competent (DesK-P). Each state was solved in at least two different crystal forms. Major conformational rearrangements of the central helical domain, correlate to modulations in the interdomain orientations. At high temperature the TM segments of DesK stabilize the central DHp domain in a conformation that promotes formation of an N-terminally extended coiled-coil, sequestering the ATP-binding domains (P state). Cold-shock (membrane thickness?) triggers helical rotations, changing the interaction surfaces and destabilizing the association between the two domains allowing for autophosphorylation (K state).*

FapR: a global repressor of the fatty acid synthase II (FAS II) regulon

The mode of action of malonyl-CoA as an inducer of the transcriptional network of fatty acid synthesis via FapR, has been unveiled at the molecular and atomic levels. The crystal structures of FapR at high resolution, alone and in complex with malonyl-CoA or DNA, revealed an equilibrium between a compact form and an open state, which regulates the spatial configuration of the DNA binding domains. *In silico* modelling has lead to a first series of candidate compounds, which should result in the identification of new families of active molecules (potential antibiotics).



***Crystal structure of FapR from the pathogen Staphylococcus aureus, in complex with DNA**. Surface representation of the DNA operator (in red) with two bound FapR homodimers (in cyan and yellow) looking down the non-crystallographic two-fold symmetry axis.*

***The transitional switch between the relaxed and tense states of the repressor FapR involves a significant rearrangement of the DNA-binding domains**. [Left panel] FapR in complex with malonyl-CoA (shown in stick representation). [Central panel] FapR in complex with DNA, in which the amphipatic linker helix from one protomer associates with the other's. [Right panel] Superposition of the two conformational states of the repressor illustrating the structural transition. The invariant effector-binding domain is shown in green, DBDs are yellow and orange for the apo/ligand-bound repressor, and cyan and blue for the DNA-bound form.*

Conclusions & Perspectives

The histidine kinase DesK has served as a model for understanding the mechanism of signal trasnduction in two-components systems, a mechanism that could be quite general among this important group of trans-membrane proteins in bacteria and plants.

Structural analysis of the entire membrane protein now seems feasible in view of the significant progress we have made in the production and purification of the active and regulatable protein in recombinant form. Solving the 3D structure of the complex DesK-DesR is one of the next steps, since crystallographic studies of *bona fide* complexes are still very scarce.

The mode of action of the inductor malonyl-CoA on the FAS II transcriptional network controlled by FapR repression, was unveiled at the atomic level. A series of crystal structures of FapR (in *B. subtilis* and *S. aureus*) allowed us to show the balance between a more compact form, where the DNA-binding domains are strongly bound to the core of the malonyl-CoA-fixing globular, and an open form, liberating the HTHs from the core to bind the operator DNA.

The crystallographic information was essential to guide the efforts of modeling and *in silico* screening on FapR. The experimental validation of a first series of lead molecules is underway and should lead to the identification of new families of compounds.

Publications

1. Albanesi D, Martín M, Trajtenberg F, Mansilla MC, Haouz A, Alzari PM, de Mendoza D, Buschiazzo A. Structural plasticity and catalysis regulation of a thermosensor histidine kinase. *Proc Natl Acad Sci USA* 2009 **106**:16185-90
2. Martinez MA, Zaballa ME, Schaeffer F, Bellinzoni M, Albanesi D, Schujman GE, Vila AJ, Alzari PM, de Mendoza D. A novel role of malonyl-ACP in lipid homeostasis. *Biochemistry* 2010 **49**:3161-7.
3. Martín M, Albanesi D, Alzari PM, de Mendoza D. Functional *in vitro* assembly of the integral membrane bacterial thermosensor DesK. *Protein Expr Purif* 2009 **66**:39-45.
4. Trajtenberg F, Grana M, Ruétalo N, Botti H, Buschiazzo A. Structural and enzymatic insights into the ATP-binding and autophosphorylation mechanism of a sensor histidine kinase. *J Biol Chem* 2010 **285**:24892-90.

Submitted / in preparation:

1. Albanesi D, Reh G, Schujman G, Debarbouille M, Schaeffer F, Guerin M, Buschiazzo A, de Mendoza D, Alzari PM. Essentiality of membrane lipid homeostasis in *Staphylococcus aureus*. 2011, submitted.
2. Martinez M, Albanesi D, de Mendoza D, Alzari, PM. Structural determinants of malonyl-CoA binding specificity in the FapR family of bacterial repressors. In preparation.
3. Reh G, Guerin M, Schujman G, de Mendoza D, Alzari PM, Schaeffer F. Thermodynamic studies of FapR binding to its specific DNA operators. In preparation.
4. Forman J, Merlet C, Buschiazzo A, Trajtenberg F, de Mendoza D, Nilges M. Targeted molecular dynamics of the histidine kinase DesK : signal transduction and helical rotations. In preparation.

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