The how and Y of cold shock

Daniel N Wilson & Knud H Nierhaus

© 2004 Nature Publishing Group http://www.nature.com/nsmb

A combined structural and biochemical analysis of an essential stress protein in *Escherichia coli* provides the first detailed insights into a bacterial translation mechanism designed to cope with cold shock conditions.

A cell from a higher eukaryote lives in homeostasis and is thus buffered against sudden changes in the environment. In contrast, a bacterial cell has to react immediately when exposed to unfavorable external conditions, through such responses as entering the stationary or sporulation phase. The major hurdle for bacterial cells is that translation, which consumes the lion's share of the total cellular energy, needs to be rapidly switched off, yet the translational apparatus must be preserved so that when the extracellular conditions improve, the ribosomes can be quickly reactivated for protein synthesis to take advantage of this situation. On page 1054 of this issue of *Nature Structural & Molecular Biology*, Vila-Sanjurjo *et al.*[1] reveal one pathway by which translation regulation occurs under conditions of cold shock.

Cold shock occurs when bacteria are exposed to sudden downshifts in temperature, and is characterized by the expression of a well-defined set of proteins, which include helicases, nucleases and a plethora of nucleic acid–binding proteins such as the so-called cold shock family of proteins (Csps)[2–4]. In contrast to stress conditions like heat shock, no cold-specific σ factor has so far been identified, suggesting that the cold shock response is predominantly post-transcriptional[2]. Consistent with this notion, many of the proteins upregulated in *E. coli* during cold shock are associated with the translational apparatus, such as the trigger factor, a ribosome-binding protein chaperone, CsdA, an ATP-dependent RNA helicase-like protein, RbfA, an RNA-binding protein implicated in ribosome assembly, and the translation initiation factors IF1 and IF3 (refs. 2–4).

The authors are at the Max Planck Institute for Molecular Genetics, Ihnestrasse 73, D14195 Berlin, Germany.
e-mail: nierhaus@molgen.mpg.de

The inhibition of translation owing to temperature decrements has been generally thought to result from the lowered kinetic and thermal constraints; however, the identification of a cold shock–induced ribosome-binding protein factor that inhibits translation suggests that a more active process may be operating[2]. In *E. coli*, this protein factor is encoded by the gene *yfi*A, and termed RaiA (ribosome-associated inhibitor) or protein Y (PY)[5,6]. The solution structures of PY[7,8] as well as of a PY homolog from *Haemophilus influenzae*[9] reveal the protein to have a globular domain comprising two α-helices and four β-sheets, followed by a flexible C-terminal tail. PY has been shown to bind to small (30S) ribosomal subunits and 70S ribosomes, which are stabilized against dissociation, but no binding to large (50S) ribosomal subunits has been detected[5]. During cold shock, or upon entry into stationary phase, PY associates with ribosomes[6,10], where it has been proposed to interfere with elongation by decreasing the accuracy of translation and preventing the binding of aminoacyl-tRNAs to the A site of the ribosome[6]. However, the exact mechanism by which PY regulates translation and protects the ribosomes during cold shock has remained unclear.

Cate and co-workers[1] have addressed this issue using a combined structural and functional approach: the relatively small size of PY (<15 kDa) allowed the complete protein to be soaked into preformed crystals of 70S *E. coli* ribosomes. The structure of the resulting complex was solved at a resolution of ~11 Å, and the known PY structure could be unequivocally fitted into the PY density. The binding site of PY is exclusively on the 30S

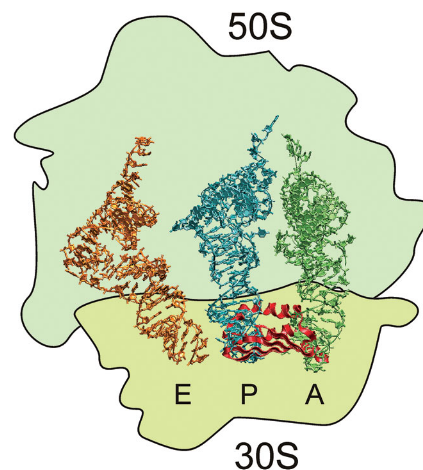

Figure 1 The binding site of protein Y on the ribosome overlaps with A- and P-site tRNA. The relative position of protein Y (red) to A- (green), P- (blue) and E-site (orange) tRNA is shown on the background of a schematic representation of a 70S ribosome, composed of 30S and 50S subunits as indicated.

subunit of the 70S ribosome, where it substantially overlaps with the known binding positions of the anticodon stems of A- and P-site tRNAs (**Fig. 1**). Footprinting and binding assays complemented this localization by showing competition between PY and tRNA binding at both A and P sites of the ribosome. PY protects the universally conserved bases G926 and C1400, which are diagnostic bases for the presence of a bound anticodon region of a P-tRNA[11], whereas the enhanced reactivity of A1493, an A-site residue involved in decoding[12], indicates that PY chases tRNA from the A site. PY inhibited binding of deacylated tRNA to the P site and was slightly more effective at the cold-shock temperature of 16 °C as compared with 37 °C. This trend was

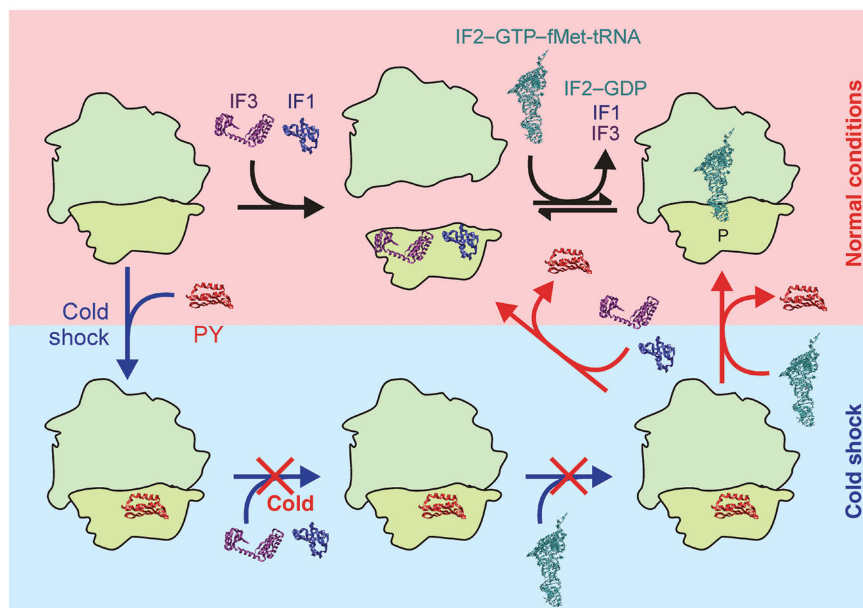

Figure 2 Scheme for protein Y–mediated regulation under cold-shock conditions. Under normal growth conditions (top panel, shaded red), run-off 70S ribosomes from translation are dissociated by the combined action of IF1 (blue) and IF3 (purple). In the presence of IF2, the ternary complex containing the initiator tRNA, IF2–GTP–fMet-tRNA, is delivered to the ribosome. Subunit joining leads to dissociation of the initiation factors, leaving the initiator tRNA at the P site (P_i complex). A decrease in temperature produces a cold shock (blue arrow) as seen in the bottom panel (shaded blue), which leads to upregulation in expression of cold-shock proteins such as protein Y (PY, red). PY binds to run-off 70S ribosomes at a position overlapping the A and P sites (see **Fig. 1**), preventing the binding of the initiation factors IF1 and IF3 and therefore the dissociation of the 70S ribosomes into its subunits. PY also prevents binding of mRNA and tRNA to the 70S ribosome, thus preventing translation initiation. When temperatures return to normal, PY has a lower affinity for the ribosome than tRNA and initiation factors, allowing ribosomes to follow the alternative pathway into active translation (red arrows).

© 2004 Nature Publishing Group http://www.nature.com/nsmb

even more striking when the more physiological situation of initiator fMet-tRNA binding to the P site of programmed ribosomes was measured. At 37 °C, little effect on P-site occupation was observed in the presence or absence of PY, whereas at 16 °C, PY markedly reduced binding, especially in the absence of initiation factors (IFs). The stimulatory effect of IFs on fMet-tRNA binding is consistent with their *in vivo* role to promote binding of the initiator fMet-tRNA to the ribosome during initiation of protein synthesis. However, it should be noted that the initiation pathway generally progresses through a preinitiation complex involving only the 30S subunit, initiation factors and the fMet-tRNA. Because PY interacts exclusively with components of the 30S subunit, this suggests that a similar interplay among PY, IFs and fMet-tRNA would occur on the small subunit, although this remains to be confirmed. With respect to IF1, the partial overlap in binding site with that of PY could also contribute to the antagonistic effects that IFs have on PY activity.

Another function has been assigned to IF3, namely that after release of the completed polypeptide chain from the ribosome, IF3 has been proposed to dissociate the 70S ribosomes into subunits, recycling them for the next round of translation. This dissociative action of IF3 has been shown by Cate and coworkers[1] to be completely blocked by PY under near-*in vivo* conditions in the presence of polyamines. However, in the absence of polyamines, PY struggles to counteract the dissociative action promoted by IF3 alone, and barely influences the combined action of IF1 and IF3. This is another example of the potential dangers of inappropriate ionic conditions that can mask a physiological action, or even provoke artifacts.

Collectively, these results suggest the following scheme for PY-mediated regulation during cold-shock conditions (**Fig. 2**). Cold shock stimulates the synthesis of PY and other cold-shock proteins[4]. PY blocks the IF1- and IF3-dependent dissociation of run-off 70S ribosomes and stabilizes the 70S monosomes. Furthermore, PY prevents formation of initiation complexes at low temperatures by preventing the binding of mRNA and initiator fMet-tRNA to the ribosome. After the cold shock, when temperature levels return to 37 °C, the initiation of protein synthesis overcomes the PY inhibition, because at the elevated temperatures tRNA and IFs are more effective competitors with PY. Additionally, the concentration of PY is lower in exponentially growing cells than in cold-shocked[6] or stationary-phase cells[10], suggesting that PY expression is downregulated; this would also confer an advantage toward formation of the initiation complex.

Several other stress proteins in *E. coli* have been shown to interact with the ribosomes of stationary phase cells[10,13–15]. One of these, RMF (ribosome modulation factor), has been suggested to induce dimerization of 70S ribosomes[13,14], although the inactivation of the ribosomes may be due to probable binding of this factor within the active site for peptide bond formation on the large subunit[15], rather than the actual dimerization itself. Another factor, termed YhbH, although having ~40% identity to PY, was found to be associated exclusively with the dimerized 70S ribosomes, rather than 70S monosomes, suggesting that it may have a function distinct to PY during the stress response. Homologs for PY have also been identified in chloroplasts and in some cyanobacteria. The PY homolog in spinach was found to be present on chloroplast ribosomes and was termed ribosomal protein S22, then later reassigned as a plastid-specific ribosomal protein 1 (psrp1)[16]. The mature spinach chloroplast psrp1, when overexpressed in *E. coli*, was found to incorporate into 70S ribosomes and 30S subunits, but not into actively translating polysomes[17]. This may suggest that psrp1 is the chloroplast homolog of PY and not a ribosomal protein at all. However, it should be noted that the chloroplast and cyanobacterium PY homologs have considerably longer C-terminal extensions than PY and therefore may fulfill additional roles to that of PY. In this respect it is notable that a PY homolog in *Synechococcus* PCC 7002 termed lrtA (light repressed transcript) has been shown, as its name suggests, to be repressed by light—in other words, it is present under dark stress conditions where the protein synthesis machinery is less active[18]. Thus, the work of Vila-Sanjurjo *et al.*[1] adds further weight to the suggestion that PY may operate in several bacteria, chloroplasts and cyanobacteria under a variety of different stress conditions.

1. Vila-Sanjurjo, A., Schuwirth, B.S., Hau, C.W. & Cate, J.H.D. *Nat. Struct. Mol. Biol.* **11**, 1054–1059 (2004).
2. Weber, M.H. & Marahiel, M.A. *Sci. Prog.* **86**, 9–75 (2003).
3. Inouye, M. & Phadtare, S. *Sci. STKE* **237**, pe26 (2004)
4. Gualerzi, C.O., Giuliodori, A.M. & Pon, C.L. *J. Mol. Biol.* **331**, 527–539 (2003).
5. Agafonov, D.E., Kolb, V.A., Nazimov, I.V. & Spirin, A.S. *Proc. Natl. Acad. Sci. USA* **96**, 12345–12349 (1999).

6. Agafonov, D.E., Kolb, V.A. & Spirin, A.S. *EMBO Rep.* **2**, 399–402 (2001).
7. Ye, K., Serganov, A., Hu, W., Garber, M. & Patel, D.J. *Eur. J. Biochem.* **269**, 5182–5191 (2002).
8. Rak, A., Kalinin, A., Shcherbakov, D. & Bayer, P. *Biochem. Biophys. Res. Commun.* **299**, 710–714 (2002).
9. Parsons, L., Eisenstein, E. & Orban, J. *Biochemistry* **40**, 10979–10986 (2001).
10. Maki, Y., Yoshida, H. & Wada, A. *Genes Cells* **5**, 965–974 (2000).
11. Moazed, D. & Noller, H.F. *Nature* **342**, 142–148 (1989).
12. Ogle, J.M. *et al. Science* **292**, 897–902 (2001).
13. Wada, A., Igarashi, K., Yoshimura, S., Aimoto, S. & Ishihama, A. *Biochem. Biophys. Res. Commun.* **214**, 410–417 (1995).
14. Yoshida, H. *et al. J. Biochem.* **132**, 983–989 (2002).
15. Yoshida, H., Yamamoto, H., Uchiumi, T. & Wada, A. *Genes Cells* **9**, 271–278 (2004).
16. Yamaguchi, K., von Knoblauch, K. & Subramanian, A.R. *J. Biol. Chem.* **275**, 28455–28465 (2000).
17. Bubunenko, M.G. & Subramanian, A.R. *J. Biol. Chem.* **269**, 18223–18231 (1994).
18. Tan, X., Varughese, M. & Widger, W. *J. Biol. Chem.* **269**, 20905–20912 (1994).

Another detour on the Toll road to the interferon antiviral response

John Hiscott

Recent characterization of two distinct signals leading to IRF-3 activation in response to double-stranded RNA recognition by Toll-like receptor 3 provides new mechanistic information on the regulatory events that link the detection of viral invasion by the cell with the development of the antiviral response.

© 2004 Nature Publishing Group http://www.nature.com/nsmb

Infection by viral or bacterial pathogens is one of the great stresses in the life of a cell. How the invading pathogen is initially recognized and how the host cell responds within the first minutes to hours can dictate the subsequent development of immune protection, with far-reaching implications for the pathogenesis of infectious, immune and allergic diseases. The innate immune response—a first line of defense against viral pathogens—is active long before adaptive responses such as neutralizing antibodies or cytotoxic T lymphocytes. An integral component of innate antiviral defense is the production of type 1 interferons (IFN), a family of antiviral cytokines composed of IFN-β and several IFN-α species[1,2]. These proteins induce a cascade of events through the activation of signaling mediated by the JAK-STAT pathway, resulting in the production of hundreds of proteins that function to limit viral replication and signal-adaptive immune responses[3].

The transcription factor interferon regulatory factor 3 (IRF-3) is a key player in this initial triggering of interferon gene transcription. After virus infection, latent IRF-3 is phosphorylated on clustered, C-terminal serine residues, leading to protein dimerization, translocation to the nucleus, association with coactivator molecules, binding to discrete DNA elements in the IFN-β and/or IFN-α promoters and the activation of gene transcription[4–7]. In the case of IFN-β, activation is accomplished in synergy with NF-κB and AP-1 family members[8]. The absence of IRF-3 or the closely related IRF-7 in murine knockout models results in markedly reduced IFN production in response to virus infection[9], thus supporting a pivotal role for the activation of IRF-3 and IRF-7 in the development of the cellular antiviral response.

On page 1060 of this issue of *Nature Structural & Molecular Biology*, Sarkar *et al.*[10] provide convincing evidence that, in response to double-stranded RNA (dsRNA)—long considered a product of virus replication and an early sentinel of virus infection—tyrosine phosphorylation of Toll-like receptor 3 (TLR-3) is required for dsRNA-dependent signaling, leading to IRF-3 activation. Recognition of dsRNA by TLR-3 results in the phosphorylation of two specific tyrosines (Tyr759 and Tyr858) within the cytoplasmic tail of TLR-3, recruitment of phosphatidylinositol-3 kinase (PI3K) to the receptor and the initiation of two distinct signaling pathways, mediated by PI3K and the noncanonical IKK-related kinases TANK-binding kinase (TBK-1) and IKK-ε (**Fig. 1**). Specifically, dsRNA activation of interferon-stimulated gene 56 (*ISG56*) was blocked under conditions that interfered with the PI3K pathway: treatment with inhibitors, expression of a catalytically inactive p110 subunit of PI3K or the use of a dominant-negative version of the downstream kinase Akt. Specific tyrosine point mutations abolished PI3K recruitment, indicating that the TLR-3–PI3K interaction was dependent on receptor phosphorylation.

The failure to activate PI3K in cells expressing TLR-3 mutants resulted in partial IRF-3 activation—phosphorylation, dimerization and nuclear translocation still occurred, but IRF-3 failed to associate with CBP coactivator or induce transcription. Despite incomplete activation, IRF-3 was phosphorylated on at least one of its important C-terminal residues, Ser396. Subsequent two-dimensional analysis revealed multiple IRF-3–phosphorylated forms in dsRNA-treated cells as compared with untreated cells. In contrast, dsRNA-activated, nuclear IRF-3 isolated from cells treated with a PI3K inhibitor or cells expressing the TLR-3 Y759F mutant had intermediate isoelectric points, demonstrating that incomplete activation was accompanied by incomplete phosphorylation. Together, the results emphasize a crucial role for the PI3K-Akt pathway in the functional activation of IRF-3 and downstream interferon antiviral immunity, following TLR-3 engagement by dsRNA.

The study by Sarkar *et al.*[10] clarifies a long-standing paradox in the IFN signaling field. Weaver *et al.*[6] originally recognized that inhibitors of tyrosine phosphorylation blocked dsRNA-dependent signaling and activation of IRF-3, even though the actual targets on IRF-3 were serine residues. The demonstration that tyrosine phosphorylation of cytoplasmic domain of TLR-3 is necessary for recruitment of PI3K to TLR-3 provides a probable explanation for the requirement for tyrosine phosphorylation.

The author is at the Lady Davis Institute for Medical Research-Jewish General Hospital, Departments of Microbiology & Immunology, Medicine and Oncology, McGill University, Montreal, Canada, H3T 1E2.
e-mail: john.hiscott@Mcgill.ca