Genetic selection designed to stabilize proteins uncovers a chaperone called Spy.

Quan S et al.

Nature Structural & Molecular Biology. 2011 Mar; 18(3):262-269

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PMID: 21317898

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Classifications

New Finding Technical Advance

Evaluations

Very Good ★ ★ 11 Mar 2011

Sophie Jackson

This is an important paper that has identified a new chaperone in the periplasm of Escherichia coli which has a completely novel structure unlike any known chaperones. By engineering a highly unstable mutant of the immunity protein Im7 into a loop region of the enzyme beta-lactamase, the authors have managed to develop an assay which links protein stability with antibiotic resistance. Using this construct, they have identified genes in E. coli which aid in the folding of unstable proteins and have thereby identified a hitherto unknown periplasmic chaperone which they call Spy. Using in vitro folding assays of a number of substrate proteins, they show that Spy is an ATP-independent chaperone. They also solve the crystal structure of Spy which has an unusual dimeric alpha-helical fold which forms a cradle-like structure. Spy is, therefore, the prototype of a new class of bacterial chaperone.

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Jackson S: Faculty Opinions Recommendation of [Quan S et al., Nat Struct Mol Biol 2011 18(3:262-269)]. In Faculty Opinions, 11 Mar 2011;

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Recommended 64.8

Score 64.8

❝ Relative citation ratio: **3.5**

★ Weighted sum of stars: **14.0**

↑ **Top 0.02%** in Structural Biology

6 Recommendations

1 ★★★ Exceptional

5 ★★ Very good

0 ★ Good

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Very Good ★★ 13 May 2011

Nikolay Dokholyan Srinivas Ramachandran

I find this article very interesting because of the ingenuity of the technique used to discover a new chaperone. Chaperones are molecular machines that inhibit protein aggregation and assist misfolded proteins to refold. Thus, chaperones are important components of a cells stress response, and chaperones were primarily discovered as proteins whose levels were substantially increased upon heat shock and other cellular stress. In this study, the authors propose a targeted approach to uncover new chaperones in Escherichia coli. They first engineer a fusion protein consisting of b-lactamase (essential for penicillin resistance) and Im7. Im7 is specifically destabilized by mutations, so that the fusion protein is a target of proteases (because a major fraction of the protein remains unfolded). Thus, the cell can survive under penicillin only if the fusion protein is somehow stabilized. The authors also make a similar construct with DsbA, which is responsible for cadmium resistance. With these constructs, they perform a genetic screen on E. coli, for genes that can rescue unfolded fusion proteins for survival under both penicillin and CdCl2. They find a 500-fold over-expressed protein, Spy, which through subsequent experiments is established as a chaperone that functions independent of ATP. Upon solving the crystal structure of Spy, they discover that Spy has a unique cradle shape that is not seen in any other chaperone of known structure. The exact mechanism adopted by Spy to assist misfolded proteins to refold is not clear, but this study has provided Spy crystal structure for further mechanistic studies. The utility of the authors approach to uncover new chaperones is summed up well by the opening sentence of their discussion: It may seem unusual that a chaperone as effective as Spy has not been studied in an organism as well-characterized as E. coli.

Classifications

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Very Good ★★ 10 Jun 2011

Ulrich Hartl Andreas Bracher

This paper reports the surprising discovery of a novel periplasmic chaperone of Escherichia coli named spheroplast protein y (Spy). Many researchers in the field may have assumed that all the proteins in the folding inventory of the E. coli periplasm have already been described. Spy has an intriguing open cradle structure, maximizing the interaction surface for partially folded client proteins. Understanding the control of protein folding in the bacterial periplasm is crucial, since many proteins within or at the outer boundary of the periplasm are involved in host-pathogen interactions and cell wall biosynthesis. After translation in the cytosol, these proteins are exported across the plasma membrane and then either fold in the periplasm or are funneled through to the bacterial outer membrane. Importantly, the periplasmic space lacks ATP as an energy source. Thus, alternative, nucleotide-independent chaperones are required, which might differ drastically from their cytosolic counterparts. Using an ingenious genetic screen for components of the periplasmic protein folding stress response in E. coli, the authors now uncovered the Spy gene product as a chaperone, which is massively up-regulated under these conditions. Spy encodes a 15.9kDa periplasmic protein forming anti-parallel dimers in the crystal structure, which is reported as well. Of note, the crystal structure of Spy was also independently solved by others, however, without in-depth functional analysis {1}. Interestingly, the Spy dimer has a peculiar open structure somewhat similar to a molecular hammock for unfolded proteins. The central region consists of a linear array of helices and strands, apparently having essentially no hydrophobic core. This structural feature maximizes the surface-volume ratio of Spy, potentially favoring protein-protein interactions with client proteins. Such an interaction with Spy might mask sticky surfaces on partially unfolded client proteins, which might otherwise trigger uncontrolled protein aggregation. The structure suggests that the central region of Spy may have sufficient plasticity to accommodate a variety of substrates or distinct conformational states of the same client protein. Hence, protection by Spy might prolong the time window of the client protein for exploring conformational space during protein folding. Interestingly, other ATP-independent chaperones such as the cytosolic trigger factor, periplasmic Skp, and prefoldin in archaea and eukaryotes seem to employ a similar principle, having tentacle-shaped coiled-coil extensions significantly increasing the surface-to-volume ratio of these chaperones in the case of prefoldin and Skp. Spy also displays superficial resemblance to the dimeric assembly chaperone RbcX, which binds the sticky C-terminal peptide of rubisco at its narrow central cleft. RbcX is, however, specific for Rubisco. It will be interesting to see which additional previously unrecognized, ATP-independent chaperones will be uncovered in bacteria that have no relation to classical chaperones. Selective expression of these might be useful in the production of recombinant proteins in bacterial hosts.

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Protein Sci 2010 Nov; 11(19):2252-9

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Hartl U and Bracher A: Faculty Opinions Recommendation of [Quan S et al., Nat Struct Mol Biol 2011 18(3:262-269)]. In Faculty Opinions, 10 Jun 2011;

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Very Good ★★ 11 Jul 2011

Tim Clausen Linn Gazda

Using a "fold-it or die" screen to select for bacteria with enhanced protein folding capacities, Bardwell and co-worker identified the Spy protein as a novel chaperone operating in the periplasm of Escherichia coli. We found this study fascinating because it provides a remarkably comprehensive characterization of a novel type of chaperone. Spy appears to constitute a molecular invisible coat protecting misfolded proteins from degradation and promoting their refolding. In a previous work, the authors reported a genetic screen they designed beta-lactamase sandwich fusion proteins to directly link protein folding with antibiotic resistance to isolate novel protein folding factors in E. coli {1}. Here, they report the first factor originating from this screen, which is the periplasmic protein Spy. In selected mutants, Spy is massively overproduced and drastically increased the stability of the unstable fusion protein, and thus also of beta-lactamase. Subsequent in vitro analysis revealed that the Spy chaperone is able to prevent the aggregation of several model substrates and to promote their refolding. The authors furthermore discuss the possible role of Spy being responsible for the protection of proteins from tannin-induced protein aggregation and inactivation, thus linking Spy function directly with bacterial pathogenicity. Finally, the crystal structure of Spy is presented, revealing a new chaperone fold. The protein forms a flat dimer that is exclusively built by alpha-helices, featuring hydrophobic binding patches on both of its concave and convex sides. Systematic labeling of surface residues implicated that Spy interacts with client proteins via large surface areas and may coat aggregation-prone regions of misfolded periplasmic proteins, thereby shielding them from degradation. It will be interesting to see the molecular mechanism of how Spy fulfills its chaperone function without using ATP and whether corresponding principles are conserved in other ATP-dependent chaperones spying on misfolded proteins.

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Mol Cell 2009 Dec 11; 5(36):861-71

PMID: 20005848

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Clausen T and Gazda L: Faculty Opinions Recommendation of [Quan S et al., Nat Struct Mol Biol 2011 18(3:262-269)]. In Faculty Opinions, 11 Jul 2011;

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Exceptional ★ ★ ★ 26 Jul 2011

 Mark Rose

This is a truly exceptional paper for several reasons. First, the authors have identified a major new ATP-independent protein chaperone in the Escherichia coli periplasm. Second, it is an amazingly complete story. The authors began with a remarkably clever genetic selection, then ran a complete course of demonstrating its chaperone activity in vitro, through to solving its crystal structure. The structure of Spy is elegant and highly evocative, forming a sort of a flexible wrapper for the unfolded stretches of the proteins, protecting them from aggregation. Still mysterious is how the protein disengages from the final folded protein in the absence of ATP. Finally, it is a model of clarity. I think this paper would serve as an excellent teaching tool for how a modern molecular biology project is carried on through multiple stages.

Classifications

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Very Good ★ ★ 13 Sep 2011

 Matthew Bogyo  Aimee Shen

We very much agree with the other reviewers that Quan et al. have developed an elegant method for identifying periplasmic chaperone proteins in bacteria. Their discovery of Spy, a new class of bacterial chaperone that acts independent of ATP yet also functions as a foldase, raises the possibility that similar chaperones may be discovered in eukaryotic systems. Spy appears to have a novel mechanism of action that will no doubt yield novel insights into chaperone and foldase function.

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