teorites' petrology and cosmic ray exposure ages suggest at least three sites of origin, and possibly six (8). Production of martian meteorites now seems embarrassingly easy. So why don't we have more, especially more highland rocks?

The newest martian meteorite is QUE94201, a fragment of basalt similar to the other martian basalts (9). It is uniquely depleted in "incompatible" elements, those that do not easily fit into common silicate minerals. This meteorite is twice or more as depleted as the other martian meteorites, and five times as depleted as comparable terrestrial basalts. The how and why of this geochemical anomaly are not yet known.

Even after years of intense study, the martian meteorites will likely continue as crucial resources. In effect, the meteorites are sample return missions, albeit from unknown parts of Mars. With the present interest in life on Mars, it seems likely that the martian meteorites will be studied intensely for traces of extraterrestrial organic compounds. More practically, the compositions of martian water (required for life) will be studied in the meteorites' alteration minerals. The meteorites will continue to provide geochemical and isotopic clues about the earliest events in the solar system: accretion, planet formation, core formation, and mantle differentiation. NASA is now studying a Mars sample return mission for 2005; given Mars's complexity, it seems unlikely that any returned sample would duplicate a martian meteorite. Rather, a returned sample would increase the value of the martian meteorites by providing a firm geological context by which to judge them.

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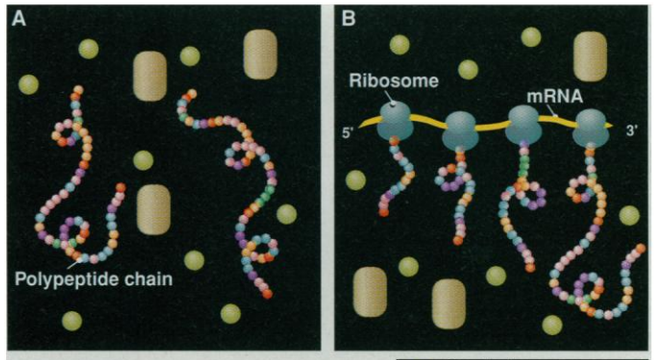
The "Bio" in Biochemistry: Protein Folding Inside and Outside the Cell

R. John Ellis

To understand how organisms work, biochemists first convert an organism into a soup. They then study these soups and the molecules purified from them to unravel the chemical mechanisms inside the living cell. The more chemically defined such in vitro systems become, the more sophisticated are the analytical techniques that can be applied to them. But as these systems become more defined, they differ more and more from whole cells. So, biochemists continually question the relevance of such in vitro systems to the living cell. As David Green pointed out many years ago, a clever engineer can make a vacuum cleaner from the wreck of an automobile, but this does not show that cars contain vacuum cleaners. The report by Frydman and Hartl in this issue (*1*) addresses this problem with respect to the role of molecular chaperones in assisting protein folding.



Will the real chaperone process please stand up. The two types of in vitro system used to study chaperone–polypeptide chain interactions yield different folding patterns. (**A**) Chemically defined system containing full-length polypeptide chains. (**B**) Crude protein-synthesizing extract, where several ribosomes move along each messenger RNA molecule, adding amino acids progressively to the growing polypeptide chain.

Molecular chaperones are a class of unrelated proteins that assist in the correct folding, association, and breakdown of certain other proteins but which then dissociate from these proteins before they perform their normal biological roles (*2*). Some, but not all, molecular chaperones are also stress proteins, because their help in assembling proteins is particularly required when organisms are subjected to environmental stresses that cause proteins to unfold. Classic experiments by Anfinsen (*3*), and subsequently many others, showed that pure proteins that have been unfolded by chemical denaturing agents will often refold spontaneously to their correct functional conformations on dilution or removal of the denaturing agent. Because no molecules other than the solvent were present, these experiments led to the important conclusion that all the steric information for polypeptide chains to refold correctly is contained within their primary structures. However, success in such refolding experiments is favored by protein concentrations much lower than those found inside cells. At concentrations similar to those of the cell, proteins often aggregate because partially folded chains can interact with one another incorrectly through transiently exposed hydrophobic areas (*4*). Misfolded chains can also arise when partially folded intermediate states become trapped and are unable to proceed toward functional conformations at suitable rates. How do cells tackle these problems?

Over the past decade, it has become clear that molecular chaperones combat both problems by binding transiently to interactive surfaces exposed during the folding process. These proteins are called chaperones because their binding prevents or reverses incorrect interactions, but does not have an actual positive effect—hence the aptness of the analogy to the human chaperone. Because it is not yet possible to study directly the binding of molecular chaperones to polypeptide chains in living cells, two types of in vitro system are being used.

In the first system, a pure protein is denatured by high concentrations of chemicals such as guanidinium chloride and then diluted into a buffer containing pure molecular chaperones. In the second system, a crude

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protein-synthesizing cell extract is made in which a single type of protein is synthesized from its messenger RNA in the presence of the molecular chaperones that occur naturally in that extract. The chemical denaturing system permits the use of much more sophisticated analytical techniques than the second because its chemical composition is both simple and defined, and it is thus favored by protein chemists. The second system is more similar to the in vivo situation because the polypeptide chain is synthesized vectorially in the extract as it is in the cell, and it is thus favored by cell biologists. In the chemical denaturing system, all parts of the polypeptide chain are simultaneously available for binding to the pure molecular chaperones, whereas in the second system, the polypeptide chain grows steadily in length as ribosomes traverse its messenger RNA and becomes progressively available for binding to whatever endogenous chaperones are present in the crude extract (see the figure).

Both systems have been used separately before, but Frydman and Hartl (*1*) have now directly compared the folding of the same proteins in the two systems. They also combined the two approaches—that is, chemically denatured full-length protein is diluted into a crude cell extract. Their results document that full-length, chemically denatured polypeptide chains and growing polypeptide chains interact differently with chaperones.

There are two contrasting models of chaperone-polypeptide interaction in the intact cell (*5*). One model suggests that chains that have folded incorrectly are unfolded by binding to chaperones after release from the ribosome; the proteins are then released in the unfolded state into the intracellular medium where they have another chance to fold correctly and, if they fail, may rebind the chaperones (*6*). This chaperone cycling model does not provide a means of avoiding aggregation of misfolded proteins; there is genetic evidence that, unless avoided, this is a hazard for some folding chains in vivo (*7*). The other model proposes that there is a selective and sequential binding of different kinds of chaperones to the polypeptide chain as it grows on the ribosome (*8*). Small chaperones of the heat shock protein 70–DnaJ–GrpE families bind first to the elongating chain and prevent it from folding prematurely, whereas large chaperones of the chaperonin family [called TRiC chaperones from the eukaryotic cytosol (*9*)] bind later to longer chains and provide them with a sequestered environment within which each complete chain can fold correctly without aggregating with other folding chains (*10*). In the latter model, the chain is released from the chaperones into the intracellular medium only after it has folded sufficiently for aggregation not to be a problem.

The results described in the new work (*1*) show that two polypeptides synthesized in a cell extract prepared from reticulocytes (actin and firefly luciferase) behave differently from full-length, chemically denatured chains of the same proteins refolding in the same extract, therefore supporting the second type of model—sequential binding to the growing chain. The inference is that chains of these proteins do not fold by cycling between chaperones and the free solution in the cell, but are released from chaperones into the intracellular medium only after they are committed to the correctly folded state.

Several questions remain to be resolved. If it occurs, the association of TRiC with growing chains should be demonstrable by pulse-chase immunoprecipitation experiments and visible in the electron microscope. What determines the sequential binding of different chaperones to particular polypeptide chains? The TRiC chaperone appears to be specific for a few proteins, especially actin and tubulin, so what about the folding of all the other chains in the eukaryotic cytosol? Perhaps chaperones bind in vivo only to polypeptides that are especially prone to aggregation. Establishing the relevance to the living cell of conclusions based on in vitro data is a continuing challenge to the ingenuity of biochemists.

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A New Turn (or Two) for Twist

Alan M. Michelson

Like scientists progressing through their careers, the cells of the embryo become more and more specialized. In an early embryonic stage of the fruit fly *Drosophila*, cells are assigned to particular fates characteristic of the primordial germ layers—the ectoderm, the mesoderm, and the endoderm. To form the mesoderm (*1*), a complex hierarchy of signals activates transcription of the gene *twist* (*twi*), which encodes a basic helix-loop-helix (bHLH) transcription factor required for early mesoderm formation (*2*). But then how is mesoderm subsequently partitioned into its more specialized derivatives? In a surprising finding reported in this issue of *Science*, Twi itself is reported to have a second function: participating in the choice between alternative mesodermal cell fates (*3*), in addition to its well-known role in specifying the early mesoderm. In a separate report, a vertebrate homolog of Twi (Mtwist) is also shown to be an important regulator of muscle differentiation, although it functions quite differently than its fly counterpart (*4*).

In *Drosophila*, Twi is initially expressed in the entire mesoderm, but later its expression becomes quite restricted (*2*, *5*). As the mesoderm separates into somatic and visceral components, the amount of Twi protein remains high in the somatic regions but is markedly reduced in visceral regions (see the figure). Subsequently, Twi is rapidly down-regulated in differentiating embryonic muscle cells but persists in the progenitors of the adult myoblasts that are specified during embryogenesis (*6*). In the new work, Baylies and Bate have elegantly addressed the functional significance of this modulation (*3*). The simplest hypothesis was that high levels of Twi would inhibit embryonic somatic muscle development. However, even with continuously high levels of Twi, embryonic muscles formed normally. An alternative possibility was that the relative amounts of Twi might distinguish among different mesodermal cell fates. Indeed, a high level of Twi suppressed heart and visceral muscle formation but was essential for proper somatic myogenesis. Furthermore, when ectopically expressed in the ectoderm, Twi repressed epidermal and nervous system differentiation while activating a myogenic program in these cells. So Twi is both essential for the establishment of mesodermal cell fate before gastrulation and is involved in subdividing the mesoderm later in development.

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