The Small Heat-shock Protein IbpB from Escherichia coli Stabilizes Stress-denatured Proteins for Subsequent Refolding by a Multichaperone Network*

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The role of small heat-shock proteins in Escherichia coli is still enigmatic. We show here that the small heat-shock protein IbpB is a molecular chaperone that assists the refolding of denatured proteins in the presence of other chaperones. IbpB oligomers bind and stabilize heat-denatured malate dehydrogenase (MDH) and urea-denatured lactate dehydrogenase and thus prevent the irreversible aggregation of these proteins during stress. While IbpB-stabilized proteins alone do not refold spontaneously, they are specifically delivered to the DnaK/DnaJ/GrpE (KJE) chaperonin system where they refold in a strict ATPase-dependent manner. Although GroEL/GroES (LS) chaperonins do not interact directly with IbpB-released proteins, LS accelerate the rate of KJE-mediated refolding of IbpB-released MDH, and to a lesser extent lactate dehydrogenase, by rapidly processing KJE-released early intermediates. Kinetic and gel-filtration analysis showed that denatured MDH preferentially transfers from IbpB to KJE, then from KJE to LS, and then forms a active enzyme. IbpB thus stabilizes aggregation-prone folding intermediates during stress and, as an integral part of a cooperative multichaperone network, is involved in the active refolding of stress-denatured proteins.

The small heat-shock proteins (sHSPs) belong to a ubiquitous family of low molecular mass (15–30 kDa), stress-induced proteins in prokaryotes and eukaryotes. Whereas various sHSPs share weak sequence homologies (1, 2), many sHSPs assemble into large globular complexes, whose oligomeric structures may vary depending on the degree of subunit phosphorylation or the concentration of ions (3–5). The overexpression of sHSPs in plant, yeast, and in mammalian cells correlates with increased levels of thermal resistance (6–9). In vitro, sHSPs specifically recognize, bind, and prevent the aggregation of non-native proteins during stress (3–5, 10), suggesting that similarly to GroEL/GroES (LS) and Hsp70 (11, 12), sHSPs can serve as an efficient binding reservoir for unstable protein-folding intermediates during stress. However, at variance with other molecular chaperones, such as Hsp70, Hsp60, Hsp104, and Hsp90 (for a review, see Ref. 13), small HSPs do not hydrolyze ATP and do not display a specific ability to promote the correct refolding of the bound stabilized proteins (3–5).

The small heat shock proteins IbpA and IbpB from Escherichia coli are two sequence-related 14- and 16-kDa proteins, respectively, co-transcribed during stress by the bacterial heat-shock transcription factor σ32 (14). IbpA and IbpB share a low sequence homology in their C-terminal region with sHSPs from yeast, plants, and mammals, including αB-crystallins (14–19, 2). Furthermore, they seem to be distantly related to other bacterial chaperones such as PapD and Caf1M. Based on the resolved x-ray structure of the PapD chaperone and on sequence homology, a model has been proposed where the three-dimensional structure of IbpB resembles that of immunoglobulins (20).

In E. coli, IbpA and IbpB are found associated with endogenous proteins that aggregate intracellularly during heat-shock (21) and with non-native recombinant proteins in inclusion bodies (14). They are implicated in the solubilization of protein aggregates after stress (21). In vitro, IbpB is a large globular complex, whose oligomeric state may vary at different temperatures and ionic concentrations.2 Similarly to other small HSPs, light-scattering measurements indicate that IbpB can specifically bind thermally or chemically denatured proteins such as MDH, LDH, and citrate synthase and thus prevent their irreversible aggregation (not shown).

We show here that IbpB can bind and stabilize denatured proteins, and furthermore, deliver them to the DnaK/DnaJ/GrpE (KJE) chaperonin system for subsequent active ATP-dependent refolding. KJE-mediated refolding of IbpB-stabilized proteins can be further activated by LS chaperonins, demonstrating cooperation between the different chaperone systems. IbpB can therefore function as a primordial protein-binding element of a multichaperone network, involved in stabilizing and active refolding of stress-denatured proteins.

EXPERIMENTAL PROCEDURES

Phage Complementation—Wild type and E. coli B mutant T850 (22) were used for in vivo complementation assays of T4 morphogenesis as described in Ref. 23. Plasmids pBF3-IbpA and pBF3-IbpB were from Dr. C. Hergersberg, Boehringer Mannheim GmbH, and pBE420 was from Invitrogen Inc.

Proteins—IbpB was purified as follows: late log phase E. coli cells, containing plasmid pBF3-IbpB in LB medium and chloramphenicol (50 μg/ml), were incubated for an additional 12 h at 37 °C in the presence of isopropyl-1-thio-D-galactopyranoside (100 μg/ml), collected, and resuspended in 50 mM Tris-Cl, pH 7.5, 150 mM KCl, 20 mM MgAc2 (Buffer A), containing 5 μg/ml leupeptin. Cells were disrupted five

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‡ The abbreviations used are: sHsp, small heat-shock protein(s); IbpB, inclusion body associated protein B; MDH, malate dehydrogenase; LDH, lactate dehydrogenase; S, GroES; L, GroEL; K, DnaK; J, DnaJ; E, GrpE.

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times at 25 °C in a French Press at 900 p.s.i. Soluble proteins in the 30,000 × g supernatant were incubated with 6% polyethylene glycol 6000 (Merek) for 30 min at 4 °C. The protein pellet from 30,000 × g was resuspended in buffer A, filtered through a 0.45-μm filter (Schleicher & Schuell), and separated by gel filtration (0.5 ml/min) on a semi-preparative Superose 6B column (Pharmacia) in buffer A. The fractions collected between 6.0 and 7.5 ml (above 2.106 daltons), which were highly enriched with soluble oligomeric IbpB, were applied to a resource-Q column (Pharmacia), equilibrated with buffer A. Elution was carried out with a linear gradient from 150 to 500 mM KCl in Buffer A. The fractions eluting at 230 ± 30 ml were collected and frozen as 34 ± 15 μM (protomers) (29). The apparent rates of protein refolding were expressed in terms of the individual protomers.

IbpB as Part of the E. coli Chaperone Network

RESULTS

IbpA and IbpB Can Suppress a Phage Growth groEL Mutation in E. coli—Wild type E. coli B cells are highly sensitive to T4 phages (23). In contrast, the mutant strain T850, which contains a chromosomal groEL with a R268C mutation, do not support T4 growth (Fig. 1). We found that phage growth was specifically restored in T850 cells containing plasmid-encoded ibpA or ibpB genes (Fig. 1), however, only in the presence of a higher phage titer (109 particles/ml) than in wild type cells.

1H Labeling—Native MDH was labeled with Na2B3H4, as described in Ref. 11. After purification by gel filtration (Superose 6B column, Pharmacia), 3H-labeled MDH was found to be as active as unlabeled MDH. During heat-denaturation and chaperone-assisted refolding, 3H-labeled MDH behaved indistinguishably from unlabeled MDH (not shown).

Chaperone Activity Assays—MDH was heat-denatured for 30 min at 47 °C as in Ref. 11 in the presence of various amounts of IbpB, DnaK, DnaJ, and/or GroEL, GroES as specified and subsequently refolded at 25 °C in the presence of supplemented DnaK/DnaJ/GroPE and/or GroEL/GroES (4, 1, 4, 6 μM, respectively) in 50 mM triethanolamine, pH 7.5, 20 mM MgAc2, 150 mM KCl, 5 mM dithiothreitol, 3 mM phosphoenol pyruvate, 7 μg/ml pyruvate kinase, and 2 mM ATP (buffered with KOH), as indicated.

Enzymatic Assays—The activity of MDH was measured as described in Ref. 27, at 25 °C in 150 mM potassium phosphate buffer, pH 7.5, 10 mM dithiothreitol, 0.5 mM oxaloacetate, and 0.28 mM NADH (Sigma). The activity of LDH was measured as described in Ref. 28 in 100 mM Tris, pH 7.5, 0.5 mM pyruvate, and 0.28 mM NADH. The time-dependent oxidation of NADH by MDH or LDH was monitored at 340 nm. Native MDH dimers remain stable and active in solutions above a concentration of 6 μM (protomers) (29). The apparent rates of protein refolding were calculated from the time-dependent changes in the enzymatic activity of chaperonin solutions containing more than 20 μM enzyme.

Gel Filtration—3H-Labeled MDH (0.25 μM) was heat-denatured in the presence of IbpB (4 μM), DnaK + DnaJ (4, 0.8 μM) or GroEL (4 μM), and incubated for 1 h at 25 °C with ATP and ATP-regeneration systems, in the absence or presence of KJE, KJEL, or L (Fig. 5). A Superose 6B gel filtration column (Pharmacia) (at 0.5 ml/min), run in the presence of the refolding buffer and 1 mM ATP was used for separation. 1H-Labeled samples were collected at the indicated elution volumes (Fig. 5), mixed with a 6-fold volume of Lumax®120, and counted.

ATP, a significant amount (10%) of native MDH was slowly recovered, at a slow rate of ~0.7 nmol min−1. This KJE-mediated refolding of IbpB-MDH was a specific reaction, as it did not occur when IbpB was absent during the heat denaturation (Fig. 2A and B), or when ATP or GrpE were absent during renaturation (not shown).

KJE-mediated Refolding of IbpB-MDH Is Activated by LS—When IbpB-MDH was supplemented with LS chaperonins and ATP, only insignificant amounts of MDH were reactivated (1.5%), in addition to the small amounts generated by spontaneous refolding (2.5% within 4–5 h, Fig. 2A). Remarkably, when KJE and LS chaperones and ATP were concomitantly added to IbpB-MDH after the heat shock, a major fraction (40%) of the MDH was actively recovered at an apparent rate 5.5 times higher (3.7 nmol min−1), than without LS (Fig. 2A). Interestingly, a significant amount (10%) of MDH that was heat-inactivated without IbpB was also recovered at a slow rate of 0.8 nmol min−1, but only in the presence of the two chaperone systems KJE and LS (Fig. 2B).

Three independent refolding rates and yields of MDH, on the presence of IbpB or phospho- (pB) or phospho-ibpB (pBpB) isoforms (1, 2, and 0.5 μM (squares) or both KJE and LS chaperones (diamonds) (4, 4, and 0.5 μM (triangles), or KJE chaperones (4, 4, and 0.5 μM (circles)) of ibpB and pBF3-IbpB, encoding the ipbA (3) or ipbB genes (4).
for a small heat-shock protein under stringent conditions.

The yields of the reaction depended on the concentration of IbpB present during the heat-denaturation of MDH (Fig. 3A). When denatured in the presence of a 20-fold molar excess of IbpB, up to 83% of the denatured MDH was recovered by KJE + LS after the stress. Half of the MDH molecules were recovered when denatured in the presence of a 5.7 molar excess of IbpB (Fig. 3A). The refolding rate of IbpB-MDH was half-maximal when the LS concentration (fixed 1:1 ratio between L and S, respectively), or when the KJE concentration (with a fixed molar ratio of 4:4:0.5) was half that of the LS (4:4 1/5), or when the KJE concentration (with a fixed molar ratio of 4:4:0.5) was half that of the LS (4:4 1/5).

The apparent refolding rate of heat-preformed KJE-MDH complexes was 8.6 times slower than the refolding rate of KJE-MDH, as illustrated in Fig. 3A. This difference was exploited to address, by kinetic measurements, the order of events during multichaperone IbpB/KJE/LS-mediated refolding reactions. The apparent refolding rates of heat-preformed IbpB-MDH complexes (Fig. 4A), as compared with heat-preformed KJ-MDH and GroEL-MDH complexes (Fig. 4B), in the presence of various co-chaperones added either immediately or 53 min after the heat shock.

Significant refolding of IbpB-MDH was observed only when supplemented after the heat shock with the three chaperones KJE and ATP, however, at a rate which was about half (Fig. 4A; Table I, 0.64 nm min⁻¹) that of GrpE-mediated refolding of KJ-MDH (Fig. 4A; Table I, 1.17 nm min⁻¹). When the addition of ATP and KJE or KJELs was delayed for 53 min after the heat shock, the refolding rate of IbpB-MDH was significantly lower (Table I, 0.28 and 1.15 nm min⁻¹, respectively), than without a delay (0.64 and 1.96 nm min⁻¹, respectively). This suggests that the IbpB-MDH is a loose complex from which aggregation-prone MDH can dissociate.

While LS and ATP alone did not directly assist the refolding of IbpB-MDH (Fig. 2A; Table I, 0.09 nm min⁻¹), the presence of LS chaperonins tripled the rate (and yields, not shown) of KJE-mediated refolding of IbpB-MDH (Fig. 4A, 1.96 nm min⁻¹). Noticeably, the extent of LS activation of the KJE-mediated refolding of IbpB-MDH was yet 4 times slower than that of E + LS-mediated refolding of KJ-MDH (Fig. 4B; Table I, 7.2 nm min⁻¹).

Remarkably, delaying the addition of E + LS to IbpB-MDH, which was incubated 53 min with ATP and KJE, increased the rate of refolding from 1.96 to 2.99 nm min⁻¹ (Fig. 4A; Table I). Likewise, delaying the addition of GroES to IbpB-MDH which was incubated 53 min with ATP and KJEL, more than doubled the refolding rates from 1.96 to 4.25 nm min⁻¹ (Fig. 4A; Table I). Thus, the rate-limiting dissociation of MDH from IbpB can overcome by a delay during which it is allowed to transfer on KJE and then on GroEL. Whereas GrpE-mediated refolding of KJ-MDH was inhibited 3-fold in the presence of added free (equimolar) IbpB (Table I, 1.17 → 0.4 nm min⁻¹), LS enhancement of KJE-MDH was not inhibited by added IbpB (Table I, 7.2 → 7.64 nm min⁻¹). This indicates that, at this late stage of refolding, GroEL has a much higher affinity to the protein than KJE and IbpB.

The refolding of urea-denatured LDH displayed a similar dependence on the various components of the IbpB/KJE/LS chaperone network. LDH is a thermostable enzyme, which therefore requires first to be denatured in 5 M urea (11) before being diluted into IbpB, LS, or KJ solutions and further incubated as MDH at 47 °C. Like MDH, LDH alone did not reactivate from the urea-heated denaturation, and no spontaneous refolding was observed even upon addition of KJE or ATP after the stress (not shown). Nor did urea-heat denatured LDH in the presence of IbpB (IbpB-LDH) refold after the stress in the presence of ATP alone, or with supplemented KJ or LS chaperones alone (not shown). Only in the presence of KJE or...
KJELS chaperones did IbpB-LDH actively refold in a strict ATP-dependent manner (Table II). KJE-mediated refolding of IbpB-LDH (0.73 nM min$^{-1}$) was slower than GrpE-mediated refolding of KJ-LDH (0.89 nM min$^{-1}$), and KJELS-mediated refolding of IbpB-LDH (0.93 nM min$^{-1}$) was slower than ELS-mediated refolding of KJ-LDH (1.27 nM min$^{-1}$), showing that the release of LDH from IbpB was rate-limiting. In contrast to MDH refolding, however, LS chaperonins did not triple, but only activated 1.3-fold, the rate of KJE-mediated refolding of IbpB-LDH (Table II, 0.96 nM min$^{-1}$), suggesting that LS chaperonins are not as important for LDH refolding, as for MDH refolding.

**Gel Filtration Analysis of Sequential MDH Transfer from IbpB to KJE and then to LS**

Gel filtration of chaperone complexes with bound heat-denatured [3H]-labeled MDH provided direct evidence for directional MDH transfer from IbpB to KJE, then to LS, during reactivation (Fig. 5). While native complexes of IbpB exceed 2$\times$10$^6$ daltons and consequently eluted near the void volume of the column (6–7.5 ml), individual native complexes of GroEL, KJ, or MDH, independently resolved at 11–13, 13.5–15.5, and 15.5–18 ml, respectively (arrows shown instead of profiles, Fig. 5). The elution profile of [3H]-MDH, which had been heat-denatured in the presence of IbpB alone and then incubated 1 h with ATP alone prior to injection, distributed in two peaks; one together with IbpB, and the other in the low molecular weight region corresponding to free MDH (Fig. 5, profile 1). The elution profile of heat-denatured IbpB-bound MDH, incubated 1 h with KJ and ATP but without GrpE, showed [3H] label associated to the broad KJ fraction, at the expense of the IbpB containing and the free MDH fractions (Fig. 5, profile 2). Thus, a significant amount of the non-native MDH refolding, however, LS chaperonins did not triple, but only activated 1.3-fold, the rate of KJE-mediated refolding of IbpB-LDH (Table II, 0.96 nM min$^{-1}$), suggesting that LS chaperonins are not as important for LDH refolding, as for MDH refolding.

**FIG. 4.** MDH refolding after heat denaturation with and without IbpB, KJE, and LS. MDH (0.25 μM) was heat denatured as described in the legend Fig. 2A, in the presence of IbpB (4 μM) (A) or GroEL (4 μM) or KJ (4 and 0.8 μM, respectively) (B). MDH refolding at 25 °C was initiated with 2 mM ATP, and GroEL (4 μM), KJ (4, 0.8 μM), GrpE (0.4 μM), or GroES (6 μM), supplemented either at t = 0 or t = 53 min as indicated. Before the first arrow, I, KJ, or L are present during the heat shock. After the first arrow, KJ, KJE, KJEL, and KJELS were added at the initiation of refolding (t = 0). After the second arrow, S, ELS, and E were added 53 min after initiation of refolding, as indicated.

**TABLE II**

| Chaperone present during heat shock | Chaperone and ATP added after heat shock, t = 0 | Apparent rate of refolding nM min$^{-1}$ |
|------------------------------------|-----------------------------------------------|----------------------------------------|
| IbpB ATP | 0.01 |
| IbpB ATP + KJE | 0.73 |
| IbpB ATP + KJELS | 0.96 |
| L ATP + S | 0.89 |
| KJ ATP + E | 1.27 |
| KJ ATP + ELS | 1.27 |

KJELS chaperones did IbpB-LDH actively refold in a strict ATP-dependent manner (Table II). KJE-mediated refolding of IbpB-LDH (0.73 nM min$^{-1}$) was slower than GrpE-mediated refolding of KJ-LDH (0.89 nM min$^{-1}$), and KJELS-mediated refolding of IbpB-LDH (0.93 nM min$^{-1}$) was slower than ELS-mediated refolding of KJ-LDH (1.27 nM min$^{-1}$), showing that the release of LDH from IbpB was rate-limiting. In contrast to

**FIG. 5.** Sequential MDH transfer from IbpB to KJE and then to LS chaperones by gel filtration analysis. Native [3H]-labeled MDH (0.25 μM) was heat denatured in the presence of IbpB (4 μM) as described in the legend to Fig. 2B. IbpB-bound MDH was then incubated 1 h at 25 °C with 1 mM ATP alone (track 1), ATP + KJ (4, 0.8 μM) (track 2), ATP + KJE + LS (track 3), or ATP + LS (4, 4 μM) chaperones (track 4). Samples were then separated in the presence of Buffer A and ATP on a Superose 6B column (Pharmacia, at 0.5 ml/min at room temperature). Arrows indicate the position of the native IbpB, GroEL, and KJ oligomers and of the native [3H]MDH enzyme.
MDH was transferred after the heat shock from IbpB and from the free state to the KJE chaperones. In contrast, when IbpB-MDH was incubated with ATP, KJ, and in addition with E + L (without S), the majority of the \(^{3}H\) label eluted associated to the GroEL fraction, at the expense of the IbpB-, KJ-, and free MDH-containing fractions (Fig. 5, profile 3). Thus, a significant amount of IbpB bound and free MDH, that was initially transferred to KJ, was further transferred to the GroEL.

When KJ chaperones were omitted and IbpB-MDH was incubated directly with GroEL and ATP, less \(^{3}H\) label co-eluted with the GroEL fraction, and correspondingly, more MDH eluted with the IbpB-containing and free MDH fractions (Fig. 5, profile 4). When S and ATP were subsequently added, MDH activity was fully recovered from the GroEL fractions from profile 3 (+KJE + L) but not from profile 4 (+L) (data not shown), showing that direct binding and IbpB-released MDH to GroEL is non-productive.

When, in a control experiment, \(^{3}H\)MDH (0.35 \(\mu\)M) was heat-denatured in the presence of an equimolar excess all three types of chaperones together (IbpB, KJ, and L, 10 \(\mu\)M each), gel filtration showed that over 90% of the \(^{3}H\) label co-eluted with GroEL (not shown). When GroEL was omitted during the denaturation, most of the \(^{3}H\) label co-eluted with KJ. Only when GroEL and KJ were both omitted during denaturation was a majority (60%) of the \(^{3}H\) label co-eluted with the IbpB (Fig. 5, track 1). This suggests that changes of affinity can define the sequence of transfer of folding intermediates between the various components of a chaperone network.

**DISCUSSION**

Whereas small Hsps share with other molecular chaperones the ability to recognize, bind, and prevent the aggregation of non-native proteins under stress, the classification of small Hsps as true chaperones remains an open question because of their poor performance at specifically promoting the correct refolding of the small Hsps-stabilized proteins. A first indication that small Hsps may collaborate with other chaperones in the refolding of bound denatured proteins came from the observation that the refolding of heat-denatured citrate synthase bound to mammalian Hsp25, can be activated by Hsp70, even without ATP (4).

We observed here that similarly to other small Hsps from eukaryotes (3–5, 10, 16), IbpB from *E. coli* can stabilize denatured proteins such as citrate synthase, \(\alpha\)-glucosidase, and MDH in a soluble non-aggregated form.2 Remarkably, whereas IbpB alone did not promote the active refolding of bound proteins after the stress, IbpB-bound MDH or IbpB-LDH were specifically reactivated by KJE chaperones and ATP, but not directly by LS chaperonins. Hence, the apparent rate of refolding of IbpB-MDH was 50 times higher in the presence of KJE + LS and the yield of the reaction was about one recovered MDH for 10 IbpB subunits present during denaturation. Similarly, ATP- and KJE-assisted refolding of IbpB-LDH was 70–90 times faster than without ATP or KJE.

The kinetic and gel filtration data strongly suggested that MDH, but not necessarily LDH or glucose-6-phosphate dehydrogenase, was refolded in a sequential, multichaperone reaction. When native MDH was heat denatured in the presence of IbpB alone, an IbpB-MDH complex was formed which was in equilibrium after the heat shock with a free inactive MDH species. The IbpB-released MDH tended to convert into a kinetically trapped inactive species, unless allowed to bind KJE, but not LS chaperonins. The slow dissociation of MDH from the IbpB-MDH complex was the rate-limiting step. Delays in the addition of the various chaperone components showed that MDH was first transferred to KJE chaperones, where it accumulated, unless allowed to partially fold and then interact with LS chaperonins. In contrast with the IbpB-released species (MDH\(_{1}\)), which was characterized by a high affinity for the KJE chaperones and a low (non-productive) affinity for LS chaperonins, the KJE-released species (MDH\(_{2}\)) had a low affinity for IbpB and a high affinity for LS chaperonins. The increased affinity of IbpB-released MDH\(_{1}\) for KJE, and of KJE-released MDH\(_{2}\) for LS provides a preferential pathway for the sequential MDH refolding in the multichaperone network, as follows: IbpB-MDH\(_{1}\) + KJE + LS \(\rightarrow\) IbpB + KJE-MDH\(_{1}\) + LS \(\rightarrow\) IbpB + KJE + LS-MDH\(_{2}\) \(\rightarrow\) IbpB + KJE + LS + MDH\(_{1}\)nat.

Binding to IbpB during the heat shock was also a prerequisite for a strict KJE- and ATP-dependent refolding of urea/heat-denatured LDH. However, KJE-released LDH species proceeded to the native state almost as rapidly without as with LS chaperonins, suggesting that LS was optional. Interestingly, glucose-6-phosphate dehydrogenase displayed a yet different behavior. Fluorescence and light scattering indicated that heat-inactivated glucose-6-phosphate dehydrogenase was neither aggregated nor bind to IbpB or LS. Whereas denatured glucose-6-phosphate dehydrogenase did not refold spontaneously, it was specifically refolded by KJE + ATP, but not by IbpB and/or by LS + ATP (data not shown). Hence, IbpB can serve as a specific high capacity scavenger for aggregation-prone folding intermediates, but it does not necessarily interact with all types of denatured proteins.

It should be noted that the sequential IbpB \(\rightarrow\) KJE \(\rightarrow\) LS mediated refolding of MDH was observed only when MDH was heat denatured in the presence of IbpB alone. When saturative amounts of LS and KJE were also present during the denaturation, IbpB displayed the lowest affinity for denatured MDH. This implies that in the cell, various protein intermediates can bind and distribute according to the relative amounts, binding capacities of each of the chaperones present, and according to the specific affinity of the folding intermediates for the chaperones present during the stress.

We propose a model that describes the various protein-folding pathways that may exist in the multichaperon network (Fig. 6). Upon denaturation, native proteins form an unstable folding intermediate that can either aggregate irreversibly, refold spontaneously (pathway 1), or distribute between several components of the chaperone network. Whereas acid-denatured barnase refolds spontaneously without chaperones (31), heat-denatured MDH can bind all three components, de-
We demonstrated here involved in the re-solubilization of protein aggregates after stress (36). Similarly, IbpA/B proteins were found in heat shock (33–35). In protein granules of neurodegenerative diseases and after stress shock (11), this suppression is evidence that either IbpA or IbpB can replace, yet with low efficiency, some of the cellular functions carried on by the LS chaperonin.

There are major differences in the expression level of the various families of Hsps between organisms. While heat shock induces a number of distinct Hsp families in E. coli and yeast (39), Hsp70 is by far the major heat-induced protein in Drosophila (38), and small heat-shock proteins Hsp18.1 and Hsp17.7 are the major heat-induced proteins in higher plants (3, 5). We find that small heat-shock proteins, while possibly binding non-native proteins with relatively low affinity, may yet serve as a primary protein-binding matrix for subsequent refolding by a highly cooperative multichaperone machinery. Despite their high binding efficiency, LS chaperonins may not be present in sufficient amounts to bind all non-native protein during cellular stress (39). Hence, auxiliary chaperone systems such as sHsps may become essential in vivo for the stress response.