The role of structural disorder in the function of RNA and protein chaperones

PETER TOMPA[1] AND PETER CSERMELY*

Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest; and *Department of Medical Chemistry, Semmelweis University, Budapest, Hungary

ABSTRACT **Chaperones are highly sophisticated protein machines that assist the folding of RNA molecules or other proteins. Their function is generally thought to require a fine-tuned and highly conserved structure: despite the recent recognition of the widespread occurrence of structural disorder in the proteome, this structural trait has never been generally considered in molecular chaperones. In this review we give evidence for the prevalence of functional regions without a well-defined 3-D structure in RNA and protein chaperones. By considering a variety of individual examples, we suggest that the structurally disordered chaperone regions either function as molecular recognition elements that act as solubilizers or locally loosen the structure of the kinetically trapped folding intermediate via transient binding to facilitate its conformational search. The importance of structural disorder is underlined by a predictor of natural disordered regions, which shows an extremely high proportion of such regions, unparalleled in any other protein class, within RNA chaperones: 54.2% of their residues fall into disordered regions and 40% fall within disordered regions longer than 30 consecutive residues. Structural disorder also prevails in protein chaperones, for which frequency values are 36.7% and 15%, respectively. In keeping with these and other details, a novel "entropy transfer" model is presented to account for the mechanistic role of structural disorder in chaperone function.—Tompa, P., Csermely, P. The role of structural disorder in the function of RNA and protein chaperones. *FASEB J.* 18, 1169–1175 (2004)**

Key Words: chaperone evolution · chaperone function · entropy transfer · intrinsically unstructured protein

Intrinsically disordered/unstructured proteins and protein domains exist and function without a well-defined 3-dimensional fold (1–4). Although these proteins defy the classical structure-function paradigm, they are not exceptions that violate the rule: they are rather common in living organisms (4–6) and fulfill essential cellular functions (1, 3, 4). The prevalence of intrinsic disorder has been determined in different functional classes of proteins, and the highest level was found in regulatory, cell signaling, and cancer-associated proteins (7). Disorder and flexibility have never been comprehensively assessed in chaperones, although sporadic data suggest their presence and functional importance in this protein class.

Chaperones assist RNA molecules or other proteins in reaching their functional 3-dimensional structure. Often, folding of long polypeptide or polynucleotide chains is impeded by kinetic traps (i.e., local energy minima), which are stable enough to halt the folding process for a physiologically significant amount of time. Furthermore, such partially folded or misfolded intermediates tend to undergo irreversible aggregation, which poses a particular danger to the organism. This folding problem can be overcome by the aid of specific chaperone proteins that prevent aggregation and unfold the intermediate to offer another chance for a folding attempt (8, 9). Chaperone function is structurally and mechanistically rather demanding, as chaperones have to assist the folding of a wide range of unrelated RNAs/proteins in the crowded intracellular milieu. No wonder the appearance of such highly sophisticated protein machines was a critical early evolutionary invention (10).

The intriguing aspect of disorder in chaperone function and evolution is highlighted by a recent suggestion that the first proteins to come into being were RNA chaperones (11). It is generally accepted that the biosphere once was dominated by organisms in which RNA was used for information storage and catalysis (12). Because RNA is especially prone to misfolding (13, 14), simple proteins with chaperone activity must have conferred a significant selective advantage in a world so highly dependent on RNA function. As the first proteins must have been short and unfolded polypeptides (15), the presence of disordered sequences in protein chaperones (16–19) and the recent discovery of fully disordered chaperone-like proteins (20–22) and structural disorder in RNA chaperones (23–27) underline the possibility of the critical early contribution, and the likely contemporary significance, of structural disorder in chaperone function.

In this paper, the experimental evidence for the occurrence and functional role of structural disorder in RNA and protein chaperones is reviewed. We show that

[1] Correspondence: Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, 1518 Budapest, P.O. Box 7, Hungary. E-mail: tompa@enzim.hu

doi: 10.1096/fj.04-1584rev

disordered segments occur in a wide range of chaperones and such regions are often indispensable in functional terms. The general occurrence of structural disorder is further demonstrated by the newly developed predictor for protein disorder (PONDR) algorithm (see the legend of **Table 1**): the frequency of disorganized structure is higher among RNA chaperones than in any other protein class, and is also very high for protein chaperones. The model of entropy transfer for disorder in chaperone function is suggested to contain all the individual examples and the very high frequency of structural disorder observed.

STRUCTURAL DISORDER IN RNA CHAPERONES

It has been noted that structural flexibility is common in RNA/protein recognition processes, as such interactions almost invariably involve conformational changes in the structure of the RNA, the protein, or both (1, 28, 29). The most prominent examples for such behavior on the part of proteins are various ribosomal proteins (1, 30), the transcriptional antitermination protein N of bacteriophage λ (31), the nuclear La protein (32), and the HIV-1 REV protein (33); other examples can be found in the reviews cited above.

These cases, however, are not necessarily relevant with respect to chaperone function, as not all RNA binding proteins are RNA chaperones; they fall into two distinct categories: those of ligands, which stabilize RNA fold via specific binding; and chaperones, which bind folding intermediate(s) of cognate RNA in a somewhat nonspecific manner (14, 32, 34, 35). Classification of individual proteins is not always straightforward; there are certain cases, however, where structural disorder is found in a protein with a clear-cut RNA chaperone function.

The classical example is the A1 protein of heteronuclear ribonucleoprotein (hnRNP), which effectively promotes renaturation of complementary nucleic acid strands. This protein has an unstructured, Gly-rich carboxyl-terminal domain that is involved in maximal renaturation activity and facilitates assembly of the RNP complex (23). This observation has led to the general

TABLE 1. *Predicted disorder in RNA and protein chaperones*[a]

RNA chaperones				Protein chaperones			
	Accession #	Length	Disorder (%)		Accession #	Length	Disorder (%)
hnRNP A1	P09651	371	35.04	IbpA (Ec)	P29209	137	36.5
hnRNP C1/C2	P07910	306	55.23	IbpB (Ec)	P29210	142	30.3
hnRNP U	Q00839	824	46.97	DnaK (Ec)	P04475	637	40.4
SF2	Q07955	247	45.34	GroEL (Ec)	P06139	547	45.5
U2AF65	P26368	475	53.26	HtpG (Ec)	P10413	624	23.7
PSF	P23246	707	72.7	ClpB (Ec)	P03815	857	47.5
FUS	P35637	526	72.62	Hsp12 (Sc)	P22943	109	45.9
Yralp (Sc)	Q12159	226	73.89	Hsp26 (Sc)	P15992	213	26.3
gBP21 (Tb)	P90629	206	23.3	Hsp60 (Sc)	P19882	572	43.5
Nucleolin	P19338	706	56.37	Hsp70 (Sc)	P39987	644	33.9
p50	Q28618	324	87.35	Hsp82 (Sc)	P02829	709	24.8
FMRP	Q06787	632	43.04	Hsp104 (Sc)	P31539	908	36.7
eIF4B	P23588	611	76.6	Hsp20	P02511	175	34.3
Ribosomal S12 (Ec)	P02367	123	60.98	Hsp22	Q9UJY1	196	36.2
Xlrbpa (Xl)	Q91836	298	24.5	Hsp27	P04792	205	55.6
NCp7 (HIV)	Q9PY17	71	47.89	Hsp60	P10809	573	36.3
NCp9 (Sc)	gb_M34549	59	91.53	Hsp70	P08107	641	28.1
I factor (Dm)	gb_AAA70221	426	35.92	Hsp90	P08238	723	31.5
ORFIpL1 (Mm)	gb_AAA67726	357	47.62	NACP	P37840	140	37.1
TYA1 (Sc)	gb_AAA66937	440	56.36	Casein (Mm)	P19228	313	40.6
delta Ag (HDV)	P25989	214	82.71				
DnaX (Ec)	P06710	643	40.28				
StpA (Ec)	P30017	134	73.13				
CspA (Ec)	P15277	69	21.74				
La Ag	P05455	408	33.33				
Prion protein	P04156	253	50.99				
DdRBP1 (Dd)	Q94467	291	54.98				

[a] RNA and protein chaperones were selected as given in the text and are listed with their Swiss-Prot or GenBank (gb) accession number. Their disorder was predicted by using the predictor of natural disordered regions (PONDR) algorithm (4–6) available via the Internet. Access to PONDR was provided by Molecular Kinetics (P.O. Box 2475 CS, Pullman, WA 99165-2475, USA; E-mail: mkinetics@turbonet.com) under license from the WSU Research Foundation. PONDR is ©1999 by the WSU Research Foundation, all rights reserved. The percentage of disorder gives the total amount of residues predicted to be in a structurally disordered region irrespective of the length of the given region. For distribution of disordered regions of various lengths, see Fig. 1. Proteins are from *H. sapiens* (not indicated), *E. coli* (Ec), *D. discoideum* (Dd), *D. melanogaster* (Dm), *M. musculus* (Mm), *S. cerevisiae* (Sc), *T. brucei* (Tb), *X. laevis* (Xl), or of viral origin, i.e. from human immunodeficiency virus-1 (HIV) or hepatitis delta virus (HDV).

concept that unstructured, sticky and relatively nonspecific appendages accelerate association reactions such as nucleic acid renaturation and macromolecular assembly (36).

Other convincing examples can be found in HIV and the distantly related yeast Ty3 retrotranposon, which encode for a reverse transcriptase and a nucleocapsid protein (NCp7/9). The nucleocapsid protein has two zinc finger motifs and unfolded amino- and carboxyl-terminal segments that participate in facilitating strand transfer reactions during reverse transcription (24, 25). Chaperone effects have been described for ribosomal L5 (27) and S12 (37) proteins, which assist folding of rRNA by a mutual induced fit mechanism. Another example is the prion protein, for which an NCp7-like chaperone function has been demonstrated recently and mapped into the amino-terminal disordered domain (26).

These examples and the consideration about a possible role of disordered RNA chaperones in early evolution prompted us to assess the general occurrence of structural disorder in the class of RNA chaperones. To this end, we took a recent, unbiased collection of 27 RNA chaperones (14) and predicted their internal disorder by the PONDR predictor (Table 1). Remarkably, the frequency of disorder in RNA chaperones is much higher than in any other protein class tested so far. In a recent paper, Dunker and colleagues predicted intrinsic disorder in different functional classes of proteins (7) and found the highest level in regulatory, cell signaling, and cancer-associated proteins. The percentage of RNA chaperones containing long disordered regions (**Fig. 1*A***) equals that of regulatory proteins; the percentage of disordered residues (Fig. 1*B*) is way above the value for regulatory, and thus for any other class of proteins examined (7). Most conspicuous—and perhaps functionally the most significant—is the difference for long continuous disordered segments: for example, less than 5% of the residues are found in disordered regions longer than 100 residues in regulatory proteins (7); the respective value is 18.5% for RNA chaperones. These remarkable numbers underline the exquisite importance of structural disorder in this protein class.

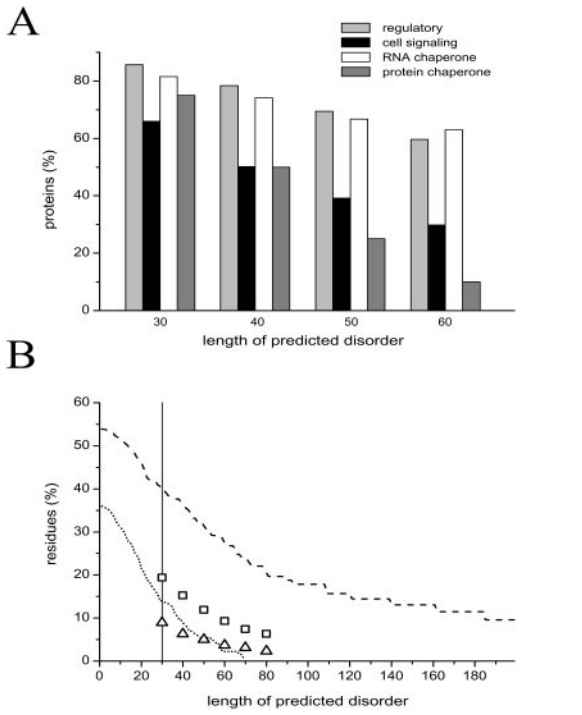

Figure 1. Range and distribution of predicted disorder in RNA and protein chaperones. *A)* Percentages of proteins with at least one uninterrupted disordered region of the indicated length in four datasets. Disorder in RNA and protein chaperones was predicted as given in the legend of Table 1. Data for regulatory and cell signaling proteins were taken from ref 7. *B)* Percentage of residues found in uninterrupted disordered regions predicted to be of, or above, the given length in the four datasets: RNA chaperones (dashed line), protein chaperones (dotted line), as well as regulatory (□), and cell signaling (△) proteins (from ref 7). A thin vertical line at 30 consecutive residues marks the length often considered as a threshold value for statistically and structurally significant disordered segments.

STRUCTURAL DISORDER IN PROTEIN CHAPERONES

The functional and mechanistic analogy of RNA chaperones with protein chaperones infers a significant amount of disorder in the latter class as well. In mechanistic terms, protein chaperones have been largely considered protein machines, which use the energy of ATP hydrolysis to get folding intermediates over the energy barrier of the folding trap (8, 9). Structural disorder, however, is unmistakable in these proteins, which points to the possible functional importance of this structural trait here as well.

Most apparent is the presence of structural disorder in two proteins recently recognized to have chaperone-like activity: α-synuclein (21, 22) and α-casein (20), which are considered to be disordered along their entire length (3). Disordered segments have been noted in cases of classical protein chaperones, which also have well-structured domain(s). Small heat-shock proteins (Hsps) such as α-crystallin (17, 38–40), Hsp16.9 (41) and Hsp25 (19) consist of a globular, ordered crystallin domain and disordered amino- and/or carboxyl-terminal tail(s) that sometimes undergo induced folding upon oligomerization and substrate binding. Disordered regions have also been described in the chaperonin family, as both the amino- and carboxyl-terminal segments of bacterial GroEL that project into the central, substrate binding cavity of the oligomeric structure are intrinsically unstructured (16, 42). p23, the co-chaperone of Hsp90, is noted for structural disorder. This protein is composed of an ordered amino-terminal part, which binds Hsp90 in an ATP-dependent manner, and a disordered carboxyl-terminal tail that does not contribute to Hsp90 binding, but is required for chaperone activity (18). Hsp90 itself contains a highly charged, disorganized

hinge region that is necessary for its function in higher eukaryotes (ref 43 and P. Csermely, unpublished data).

These examples suggest that structural disorder is present, and may be prevalent, in the class of protein chaperones. To test for this, we collected a representative set of protein chaperones, including the two newly recognized chaperone-like proteins α-synuclein and α-casein as well as the *Escherichia coli*, yeast, and human homologues of some sHsps, Hsp60, Hsp70, Hsp90, and Hsp105. Although their incidence of disorder predicted by PONDR (Table 1) falls short of that of RNA chaperones, it is still well within the range of regulatory and cell signaling proteins (Fig. 1*A, B*). An intriguing aspect of their disorder is that protein chaperones are often devoid of very long disordered regions but contain frequent, scattered short segments, which perhaps points to their spatially better organized, but very flexible and malleable, structure (44).

STRUCTURAL DISORDER IS INVOLVED IN CHAPERONE FUNCTION

The variety of individual examples and the range of predicted disorder suggest the importance of structural disorder in RNA and protein chaperones. Although functional significance does not necessarily follow from the prevalence of a given feature, in the case of structural disorder there is a great deal of direct evidence for its causal link with chaperone function in both classes.

In the case of RNA chaperones studied for this feature, chaperone function is found to map almost invariably into the disordered segment, as its deletion abolishes activity in most cases. This has been observed for the prion protein (26), hnRNPA1 (23), and NCp9 (25). The only exception might be the ribosomal protein L5, for which all substitutions affecting RNA binding clustered into the central, structured region; the consequence of deleting the amino- and carboxyl-terminal disordered regions has not been tested with this protein, though (27).

Structural disorder has been noted as a functional feature of protein chaperones. Two newly recognized chaperone-like proteins, α-synuclein and α-casein, are completely disordered, which indicates that the molecular mechanism of their chaperone function must be linked with their unstructured nature (20–22). Removal of the disordered segment by limited proteolysis often abolishes or markedly reduces chaperone activity in other instances, such as for p23 co-chaperone (18), α-crystallin (38), and Hsp25 (19). The diminution of chaperone activity by decreasing the flexibility of the disordered segment by a point mutation has been demonstrated for α-crystallin (17). A reduction in flexibility of the disordered segment upon direct contact with the partially folded or misfolded substrate protein has been shown by time-resolved fluorescence spectroscopy and NMR in GroEL (16) and α-crystallin (39). Intriguingly, in the case of α-crystallin, the disordered carboxyl-terminal extension remains disordered with certain substrates (39); a similar behavior has been witnessed with Hsp25 (19), indicating a subtle balance of these regions between structural order and disorder in the functional cycle of chaperones.

A MECHANISTIC MODEL OF THE ROLE OF DISORDER IN CHAPERONE ACTION

As seen, structural disorder abounds in chaperones and frequently is involved in chaperone function. In this section we will show that all the diverse mechanistic elements, along with functional information on other disordered/unstructured proteins, can be assembled into a coherent picture of how structural disorder subserves chaperone action. This model appears to apply to RNA and protein chaperones alike, capturing the essence of their mechanistically similar modes of action.

The first mechanistic component to be considered is that disordered proteins/regions often serve as elements that offer a unique versatility in the recognition process. As discussed in recent reviews (1, 3, 4), such regions can bind several different partners, they may enable an enhanced speed of interaction and they can uncouple specificity from binding strength. Chaperone action may benefit from each of these; in fact, disordered segments do serve as recognition elements in RNA chaperones [La protein (32), λN protein (31), prion protein (26), ribosomal L5 protein (27), HIV-1 REV protein (33), and others (1, 29)] and protein chaperones [α-synuclein (21, 22), α-casein (20), α-crystallin (39), and GroEL (42)]. Their capacity to adapt to many binding partners, termed binding promiscuity or one-to-many signaling (1, 3, 6, 45), may be of particular advantage, as chaperones need to bind a wide range of unrelated, misfolded substrates; this point has been emphasized in the case of the disordered carboxyl-terminal tail of GroEL (42). The enhanced speed of interaction enabled by disordered segments (3, 46) is of prime importance in preventing fast aggregation reactions, for example. Finally, uncoupling specificity from binding strength (3, 47, 48) may be of advantage for reversible, still selective, interactions with the partner.

Another key mechanistic element is that disordered segments, once bound directly or via a globular domain, provide a significant solubilizing effect, as demonstrated with protein chaperones such as α-synuclein (21, 22), α-casein (20), α-crystallin (17, 40), and Hsp25 (19); this may be important in preventing aggregation of their misfolded substrates. As aggregation usually results from the association of exposed hydrophobic surfaces, this effect may simply stem from the highly hydrophilic character of disordered segments (2, 3, 6). An additional factor may result from the entropic exclusion effect of disordered segments, which prevents molecules from approaching each other. Such a long-range repulsive force has been described for the

disordered regions of microtubule-associated proteins (49), neurofilaments (50), and nucleoporins (51), and its contribution has been explicitly stated in the chaperone function of Hsp25 (19).

An even more subtle contribution of disordered segments to chaperone function may stem from a combination of their capacity of rapid and transient binding of the substrate and the ensuing local ordering, which may "pay" the thermodynamic cost of local substrate unfolding. As kinetically trapped substrates become stuck in a local conformational energy minimum, chaperones assist folding by randomly disrupting the misformed bonds via repeated cycles of binding and release, allowing the substrate to resume search in the conformational space toward the global energy minimum. This usually is thought to require an energy-driven active process formulated in mechanistic models such as "iterative annealing" (8) or "chaperone percolator" (9). A disordered, nonspecific binding segment offers a less demanding and perhaps more ancient mechanism for this chaperoning principle, as the energy demand of local unfolding of the substrate may simply be covered by the binding of the chaperone.

Such an entropy transfer (i.e., the ordering of the chaperone with a concomitant unfolding of the substrate) has in fact been demonstrated for several RNA and protein chaperones. For example, a loss in flexibility upon substrate binding has been shown for the highly flexible apical domain region facing the internal cavity of GroEL (16) and the carboxyl-terminal tail of α-crystallin (39). On the substrate side, a transient elevation of interior flexibility by the chaperone has been observed many times, as in a group I intron by StpA (52), the TAR element (53–55), and $tRNA^{Lys}$ (56) by NCp7 during reverse transcription of HIV-1, mRNA by cold-shock protein (57), and both the RuBisCO protein (58) and carbonic anhydrase (59) by GroEL.

It should be kept in mind, however, that there are chaperones, such as nucleolin (48), which stabilize their substrates. In these cases increased binding energy may cover the entropy cost of the organization of both the chaperone and the substrate structure. Similarly, most RNA binding, nonchaperone proteins, such as Cyt-18 or many ribosomal proteins, form a higher affinity protein/RNA complex and induce the stabilization of the bound RNA (60, 61). Rescuing of the RNA from these complexes often needs bona fide RNA chaperones such as C19 (62) in the case of Cyt-18. To add to the complexity of the entropy transfer reactions, some RNAs can transiently unfold proteins behaving as protein chaperones, as demonstrated for rRNA (63, 64), and perhaps for the unidentified RNA catalysts of the conformational transition from normal to infectious prions (65).

Another favorable aspect of substrate unfolding assisted by a disordered chaperone is that despite their local unfolding, different segments/strands of the substrate are kept at a close range by the bound chaperone. This proximity limits the subsequent conformational search and speeds up the folding process, as

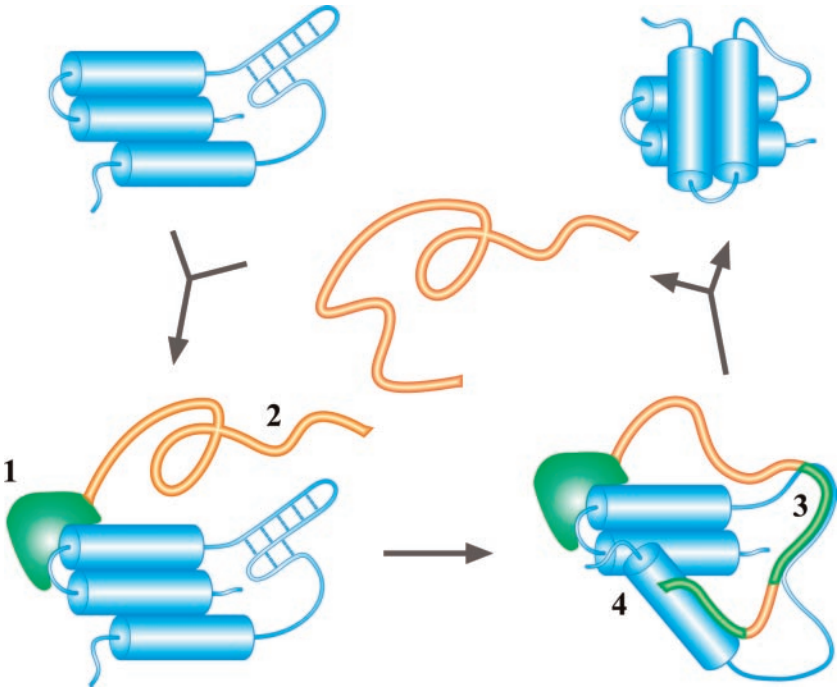

Figure 2. The entropy transfer model of the role of structural disorder in chaperone function. A highly schematic summary of the suggested mechanistic role of structural disorder in chaperone action. The model incorporates most of the mechanistic details described for the action of disordered proteins/regions in chaperones (see text); it makes no distinction between RNA and protein chaperones, as their critical mechanistic features are similar. Decisive elements are as follows. *1)* A fully disordered chaperone (orange) binds a partially misfolded substrate (RNA or protein, blue) in a relatively nonspecific manner. The recognition segment locally folds (illustrated with green) and anchors the chaperone to the substrate. Naturally, this binding element may already be folded prior to binding; for simplicity, this situation is not shown. *2)* A disordered appendage projects away from the substrate and provides a solubilizing effect due to its highly hydrophilic character and entropic exclusion of other molecules. This disordered segment thus prevents aggregation of the substrate. *3)* A disordered segment contacts the misfolded part of the substrate. As a result, part of the chaperone becomes ordered (green) whereas the substrate becomes disordered (i.e., locally unfolds due to the reciprocal entropy transfer process). Of course, this secondary binding need not be mechanistically separate from the primary binding event (1), as entropy transfer may already take place upon primary recognition of the substrate. *4)* In this state of increased flexibility, the substrate is facilitated in its search through conformational space toward the native conformation by keeping its unfolded segments in close proximity by the folded chaperone. The chaperone may contact and release the substrate several times in rapid succession until it finally releases the properly folded substrate to resume the catalytic cycle.

witnessed by an increase in DNA renaturation speed in the presence of the unstructured carboxyl-terminal domain of hnRNPA1 (23); this mechanism may apply to many renaturation and complexation reactions (36).

We would like to point out that these distinct elements of rapid and versatile binding, solubilization, local unfolding, and proximal positioning of segments of the misfolded substrate by the flexible/disordered region(s) of the chaperone can be assembled into a mechanistic model of chaperone action (**Fig. 2**). This novel model, in which protein disorder plays a fundamental mechanistic role, is suggested here to be denoted as the entropy transfer model of chaperone action. Although this model may not always apply in all its details, it probably captures important general aspects of the action of most, if not all, chaperones we know. FJ

The authors are grateful to Imre Csizmadia (Department of Chemistry, University of Toronto, Ontario, Canada) for pointing out the importance of entropy transfer in chaperone action. This work was supported by grants from the EU 6th Framework Program (FP6-506850), ICGEB (CRP/HUN99-02), Hungarian Science Foundation (OTKA-T32360, T37357), and the Hungarian Ministry of Social Welfare (ETT-32/03). P.T. acknowledges the support of the International Senior Research Fellowship GR067595 from the Wellcome Trust and a Bolyai János Scholarship.

REFERENCES

1. Wright, P. E., and Dyson, H. J. (1999) Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J. Mol. Biol.* **293,** 321–331
2. Uversky, V. N. (2002) Natively unfolded proteins: a point where biology waits for physics. *Protein Sci.* **11,** 739–756
3. Tompa, P. (2002) Intrinsically unstructured proteins. *Trends Biochem. Sci.* **27,** 527–533
4. Dunker, A. K., Brown, C. J., Lawson, J. D., Iakoucheva, L. M., and Obradovic, Z. (2002) Intrinsic Disorder and Protein Function. *Biochemistry* **41,** 6573–6582
5. Dunker, A. K., Obradovic, Z., Romero, P., Garner, E. C., and Brown, C. J. (2000) Intrinsic protein disorder in complete genomes. *Genome Inform. Ser. Workshop* **11,** 161–171
6. Dunker, A. K., Lawson, J. D., Brown, C. J., Romero, P., Oh, J. S., Oldfield, C. J., Campen, A. M., Ratliff, C. M., Hipps, K. W., Ausio, J., et al. (2001) Intrinsically disordered protein. *J. Mol. Graph. Model.* **19,** 26–59
7. Iakoucheva, L., Brown, C., Lawson, J., Obradovic, Z., and Dunker, A. (2002) Intrinsic disorder in cell-signaling and cancer-associated proteins. *J. Mol. Biol.* **323,** 573–584
8. Todd, M. J., Lorimer, G. H., and Thirumalai, D. (1996) Chaperonin-facilitated protein folding: optimization of rate and yield by an iterative annealing mechanism. *Proc. Natl. Acad. Sci. USA* **93,** 4030–4035
9. Csermely, P. (1999) Chaperone-percolator model: a possible molecular mechanism of Anfinsen-cage-type chaperones. *Bioessays* **21,** 959–965
10. Csermely, P. (1997) Proteins, RNAs and chaperones in enzyme evolution: a folding perspective. *Trends Biochem. Sci.* **22,** 147–149
11. Poole, A. M., Jeffares, D. C., and Penny, D. (1998) The path from the RNA world. *J. Mol. Evol.* **46,** 1–17
12. Gilbert, W. (1986) The RNA world. *Nature (London)* **319,** 618
13. Treiber, D. K., and Williamson, J. R. (2001) Beyond kinetic traps in RNA folding. *Curr. Opin. Struct. Biol.* **11,** 309–314
14. Cristofari, G., and Darlix, J. L. (2002) The ubiquitous nature of RNA chaperone proteins. *Prog. Nucleic Acid Res. Mol. Biol.* **72,** 223–268
15. Doi, N., and Yanagawa, H. (1998) Origins of globular structure in proteins. *FEBS Lett.* **430,** 150–153
16. Gorovits, B. M., and Horowitz, P. M. (1995) The molecular chaperonin cpn60 displays local flexibility that is reduced after binding with an unfolded protein. *J. Biol. Chem.* **270,** 13057–13062
17. Smulders, R., Carver, J. A., Lindner, R. A., van Boekel, M. A., Bloemendal, H., and de Jong, W. W. (1996) Immobilization of the C-terminal extension of bovine alphaA-crystallin reduces chaperone-like activity. *J. Biol. Chem.* **271,** 29060–29066
18. Weikl, T., Abelmann, K., and Buchner, J. (1999) An unstructured C-terminal region of the Hsp90 co-chaperone p23 is important for its chaperone function. *J. Mol. Biol.* **293,** 685–691
19. Lindner, R. A., Carver, J. A., Ehrnsperger, M., Buchner, J., Esposito, G., Behlke, J., Lutsch, G., Kotlyarov, A., and Gaestel, M. (2000) Mouse Hsp25, a small shock protein. The role of its C-terminal extension in oligomerization and chaperone action. *Eur. J. Biochem.* **267,** 1923–1932
20. Bhattacharyya, J., and Das, K. P. (1999) Molecular chaperone-like properties of an unfolded protein, alpha(s)-casein. *J. Biol. Chem.* **274,** 15505–15509
21. Kim, T. D., Paik, S. R., and Yang, C. H. (2002) Structural and functional implications of C-terminal regions of alpha-synuclein. *Biochemistry* **41,** 13782–13790
22. Park, S. M., Jung, H. Y., Kim, T. D., Park, J. H., Yang, C. H., and Kim, J. (2002) Distinct roles of the N-terminal-binding domain and the C-terminal-solubilizing domain of alpha-synuclein, a molecular chaperone. *J. Biol. Chem.* **277,** 28512–28520
23. Pontius, B. W., and Berg, P. (1990) Renaturation of complementary DNA strands mediated by purified mammalian heterogeneous nuclear ribonucleoprotein A1 protein: implications for a mechanism for rapid molecular assembly. *Proc. Natl. Acad. Sci. USA* **87,** 8403–8407
24. Morellet, N., Jullian, N., De Rocquigny, H., Maigret, B., Darlix, J. L., and Roques, B. P. (1992) Determination of the structure of the nucleocapsid protein NCp7 from the human immunodeficiency virus type 1 by 1H NMR. *EMBO J.* **11,** 3059–3065
25. Cristofari, G., Gabus, C., Ficheux, D., Bona, M., Le Grice, S. F., and Darlix, J. L. (1999) Characterization of active reverse transcriptase and nucleoprotein complexes of the yeast retrotransposon Ty3 in vitro. *J. Biol. Chem.* **274,** 36643–36648
26. Gabus, C., Derrington, E., Leblanc, P., Chnaiderman, J., Dormont, D., Swietnicki, W., Morillas, M., Surewicz, W. K., Marc, D., Nandi, P., et al. (2001) The prion protein has RNA binding and chaperoning properties characteristic of nucleocapsid protein NCP7 of HIV-1. *J. Biol. Chem.* **276,** 19301–19309
27. DiNitto, J. P., and Huber, P. W. (2003) Mutual induced fit binding of *Xenopus* ribosomal protein L5 to 5S rRNA. *J. Mol. Biol.* **330,** 979–992
28. Williamson, J. R. (2000) Induced fit in RNA-protein recognition. *Nat. Struct. Biol.* **7,** 834–837
29. Leulliot, N., and Varani, G. (2001) Current topics in RNA-protein recognition: control of specificity and biological function through induced fit and conformational capture. *Biochemistry* **40,** 7947–7956
30. Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289,** 905–920
31. Mogridge, J., Legault, P., Li, J., Van Oene, M. D., Kay, L. E., and Greenblatt, J. (1998) Independent ligand-induced folding of the RNA-binding domain and two functionally distinct antitermination regions in the phage lambda N protein. *Mol. Cell* **1,** 265–275
32. Jacks, A., Babon, J., Kelly, G., Manolaridis, I., Cary, P. D., Curry, S., and Conte, M. R. (2003) Structure of the C-terminal domain of human La protein reveals a novel RNA recognition motif coupled to a helical nuclear retention element. *Structure (Cambridge)* **11,** 833–843
33. Battiste, J. L., Mao, H., Rao, N. S., Tan, R., Muhandiram, D. R., Kay, L. E., Frankel, A. D., and Williamson, J. R. (1996) Alpha helix-RNA major groove recognition in an HIV-1 rev peptide-RRE RNA complex. *Science* **273,** 1547–1551
34. Herschlag, D. (1995) RNA chaperones and the RNA folding problem. *J. Biol. Chem.* **270,** 20871–20874
35. Lorsch, J. R. (2002) RNA chaperones exist and DEAD box proteins get a life. *Cell* **109,** 797–800

36. Pontius, B. W. (1993) Close encounters: why unstructured, polymeric domains can increase rates of specific macromolecular association. *Trends Biochem. Sci.* **18,** 181–186
37. Clodi, E., Semrad, K., and Schroeder, R. (1999) Assaying RNA chaperone activity in vivo using a novel RNA folding trap. *EMBO J.* **18,** 3776–3782
38. Andley, U. P., Mathur, S., Griest, T. A., and Petrash, J. M. (1996) Cloning, expression, and chaperone-like activity of human alphaA-crystallin. *J. Biol. Chem.* **271,** 31973–31980
39. Lindner, R. A., Kapur, A., Mariani, M., Titmuss, S. J., and Carver, J. A. (1998) Structural alterations of alpha-crystallin during its chaperone action. *Eur. J. Biochem.* **258,** 170–183
40. Pasta, S. Y., Raman, B., Ramakrishna, T., and Rao Ch, M. (2002) Role of the C-terminal extensions of alpha-crystallins. Swapping the C-terminal extension of alpha-crystallin to alphaB-crystallin results in enhanced chaperone activity. *J. Biol. Chem.* **277,** 45821–45828
41. van Montfort, R. L., Basha, E., Friedrich, K. L., Slingsby, C., and Vierling, E. (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. *Nat. Struct. Biol.* **8,** 1025–1030
42. Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D. C., Joachimiak, A., Horwich, A. L., and Sigler, P. B. (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. *Nature (London)* **371,** 578–586
43. Csermely, P., Schnaider, T., Soti, C., Prohaszka, Z., and Nardai, G. (1998) The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol. Ther.* **79,** 129–168
44. Fu, X., Li, W., Mao, Q., and Chang, Z. (2003) Disulfide bonds convert small heat shock protein Hsp16.3 from a chaperone to a non-chaperone: implications for the evolution of cysteine in molecular chaperones. *Biochem. Biophys. Res. Commun.* **308,** 627–635
45. Kriwacki, R. W., Hengst, L., Tennant, L., Reed, S. I., and Wright, P. E. (1996) Structural studies of p21Waf1/Cip1/Sdi1 in the free and Cdk2-bound state: conformational disorder mediates binding diversity. *Proc. Natl. Acad. Sci. USA* **93,** 11504–11509
46. Shoemaker, B. A., Portman, J. J., and Wolynes, P. G. (2000) Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. *Proc. Natl. Acad. Sci. USA* **97,** 8868–8873
47. Demchenko, A. P. (2001) Recognition between flexible protein molecules: induced and assisted folding. *J. Mol. Recognit.* **14,** 42–61
48. Dyson, H. J., and Wright, P. E. (2002) Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* **12,** 54–60
49. Mukhopadhyay, R., and Hoh, J. H. (2001) AFM force measurements on microtubule-associated proteins: the projection domain exerts a long-range repulsive force. *FEBS Lett.* **505,** 374–378
50. Brown, H. G., and Hoh, J. H. (1997) Entropic exclusion by neurofilament sidearms: a mechanism for maintaining interfilament spacing. *Biochemistry* **36,** 15035–15040
51. Rout, M. P., Aitchison, J. D., Suprapto, A., Hjertaas, K., Zhao, Y., and Chait, B. T. (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* **148,** 635–651
52. Waldsich, C., Grossberger, R., and Schroeder, R. (2002) RNA chaperone StpA loosens interactions of the tertiary structure in the td group I intron in vivo. *Genes Dev.* **16,** 2300–2312
53. Bernacchi, S., Stoylov, S., Piemont, E., Ficheux, D., Roques, B. P., Darlix, J. L., and Mely, Y. (2002) HIV-1 nucleocapsid protein activates transient melting of least stable parts of the secondary structure of TAR and its complementary sequence. *J. Mol. Biol.* **317,** 385–399
54. Urbaneja, M. A., Wu, M., Casas-Finet, J. R., and Karpel, R. L. (2002) HIV-1 nucleocapsid protein as a nucleic acid chaperone: spectroscopic study of its helix-destabilizing properties, structural binding specificity, and annealing activity. *J. Mol. Biol.* **318,** 749–764
55. Azoulay, J., Clamme, J. P., Darlix, J. L., Roques, B. P., and Mely, Y. (2003) Destabilization of the HIV-1 complementary sequence of TAR by the nucleocapsid protein through activation of conformational fluctuations. *J. Mol. Biol.* **326,** 691–700
56. Tisne, C., Roques, B. P., and Dardel, F. (2001) Heteronuclear NMR studies of the interaction of tRNA(Lys)3 with HIV-1 nucleocapsid protein. *J. Mol. Biol.* **306,** 443–454
57. Phadtare, S., Alsina, J., and Inouye, M. (1999) Cold-shock response and cold-shock proteins. *Curr. Opin. Microbiol.* **2,** 175–180
58. Rye, H. S., Burston, S. G., Fenton, W. A., Beechem, J. M., Xu, Z., Sigler, P. B., and Horwich, A. L. (1997) Distinct actions of *cis* and *trans* ATP within the double ring of the chaperonin GroEL. *Nature (London)* **388,** 792–798
59. Persson, M., Hammarstrom, P., Lindgren, M., Jonsson, B. H., Svensson, M., and Carlsson, U. (1999) EPR mapping of interactions between spin-labeled variants of human carbonic anhydrase II and GroEL: evidence for increased flexibility of the hydrophobic core by the interaction. *Biochemistry* **38,** 432–441
60. Weeks, K. M. (1997) Protein-facilitated RNA folding. *Curr. Opin. Struct. Biol.* **7,** 336–342
61. Semrad, K., and Schroeder, R. (1998) A ribosomal function is necessary for efficient splicing of the T4 phage thymidylate synthase intron in vivo. *Genes Dev.* **12,** 1327–1337
62. Mohr, S., Stryker, J. M., and Lambowitz, A. M. (2002) A DEAD-box protein functions as an ATP-dependent RNA chaperone in group I intron splicing. *Cell* **109,** 769–779
63. Sulijoadikusumo, I., Horikoshi, N., and Usheva, A. (2001) Another function for the mitochondrial ribosomal RNA: protein folding. *Biochemistry* **40,** 11559–11564
64. Hardesty, B., and Kramer, G. (2001) Folding of a nascent peptide on the ribosome. *Prog. Nucleic Acid Res. Mol. Biol.* **66,** 41–66
65. Deleault, N. R., Lucassen, R. W., and Supattapone, S. (2003) RNA molecules stimulate prion protein conversion. *Nature (London)* **425,** 717–720

Received for publication January 26, 2004.
Accepted for publication April 15, 2004.