Role of Protein Kinase CK2 Phosphorylation in the Molecular Chaperone Activity of Nucleolar Protein B23*

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Protein B23 is a multifunctional nucleolar protein whose molecular chaperone activity is proposed to play a role in ribosome assembly. Previous studies (Szébeni, A., and Olson, M. O. J. (1999) Protein Sci. 8, 905–912) showed that protein B23 has several characteristics typical of molecular chaperones, including anti-aggregation activity, promoting the renaturation of denatured proteins, and preferential binding to denatured substrates. However, until now there has been no proposed mechanism for release of a bound substrate. Protein B23 can be phosphorylated by protein kinase CK2 (CK2) in a segment required for chaperone activity. The presence of bound substrate enhanced the rate of CK2 phosphorylation of protein B23 by 2–3-fold, and this enhancement was dependent on a nonpolar region in its N-terminal domain. Formation of a complex between B23 and chaperone test substrates (rhodanese or citrate synthase) was inhibited by CK2 phosphorylation. Furthermore, CK2 phosphorylation of a previously formed B23-substrate complex promoted its dissociation. The dissociation of complexes between B23 and the human immunodeficiency virus-Rev protein required both CK2 phosphorylation and competition with a Rev nuclear localization signal peptide, suggesting that Rev binds B23 at two separate sites. These studies suggest that unlike many molecular chaperones, which directly hydrolyze ATP, substrate release by protein B23 is dependent on its phosphorylation by CK2.

The nucleolus is the subcellular domain for the assembly of ribosomal subunits in which the initial building blocks are preribosomal RNA, 5 S rRNA, and ribosomal and nonribosomal proteins (1). This is a complex, multistep process that takes place in an environment that is likely to contain high concentrations of macromolecules. Although the concentrations in the various compartments of the nucleolus are not known, they are likely to be at least as high as and probably much higher than the estimated overall cellular macromolecular concentration of 200–300 mg/ml (2). At these concentrations proteins are faced with the potential problems of denaturation, aggregation, and immobilization as they participate in ribosome biogenesis. In addition to the macromolecular crowding effect, the process of incorporation of proteins into preribosomal complexes could expose hydrophobic surfaces and so further increase the probability for aggregation. Such a tendency to aggregate is usually relieved by the action of a variety of molecular chaperones (3), and it seems likely that specialized molecular chaperones exist in the nucleolus to aid in ribosome assembly. In fact, recent proteomic analyses of nucleoli identified 14 potential molecular chaperones in HeLa cell nucleoli (4). These could be important in preventing aggregation of proteins in the nucleolus.

To date, one nucleolar protein has been shown to have a general molecular chaperone activity by several criteria. Szébeni and Olson (5) tested a nucleolar nonribosomal protein called B23 (also called NO38, nucleophosmin/NPM, or numatrin) for chaperone activity using several protein substrate types typically used to test proteins for chaperone activity. Protein B23 was found to prevent aggregation of the HIV-1 Rev protein and also to suppress the aggregation of several other proteins during thermal denaturation. In addition, B23 preserves enzyme activities under high temperature conditions and actually promotes the renaturation and restoration of activities of enzymes previously denatured with guanidine-HCl. Finally, protein B23 preferentially binds denatured substrates; this is accompanied by an increase in exposure of the hydrophobic regions of the proteins in the B23-substrate complexes. A histone chaperone activity has also been proposed for protein B23, based on the observation that it binds to histones and mediates the formation of nucleosomes (6).

Because protein B23 is predominantly localized in nucleolus, it is believed to serve as a factor in ribosome biogenesis. Protein B23 binds RNA and DNA (7) and interacts with other nucleolar proteins, including nucleolin (8), protein P120 (9), and the HIV-1 Rev protein (10, 11). It has intrinsic endoribonuclease activity (12), and its capacity to preferentially cleave in the ITS2 region of pre-rRNA suggests that it could participate in late stages of ribosome biogenesis (13). In addition, protein B23 seems to have multiple functions outside of the nucleolus. The ability of the protein to shuttle between the nucleolus and cytoplasm (14) and to bind nuclear localization signal (NLS)-containing peptides (15) suggests a role in nuclear import. In support of this latter notion, protein B23 has been shown to stimulate the import of NLS-containing proteins into isolated nuclei (16), and it has been proposed that this ability is related to its molecular chaperone activity (5). The protein also stimulates the activity of DNA polymerase α (17), and it may serve a factor in DNA recombination through its capacity to promote annealing of complementary strands of DNA (18). Finally, pro-

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‡The abbreviations used are: HIV, human immunodeficiency virus; CK2, protein kinase CK2 (also known as casein kinase II); NLS, nuclear localization signal; MES, 4-morpholineethanesulfonic acid; HPLC, high performance liquid chromatography.
tein B23 seems to control the duplication of centrosomes in mammalian cells (19). Taken together, these observations have led researchers to conclude that B23 is a multifunctional protein with roles both in and outside of the nucleolus.

Molecular chaperones are a diverse group of proteins that share the common general function of modulating protein conformation (20). They appear to operate through repeated cycles of binding partially unfolded substrates, release of the bound substrate, and folding after release until the correct conformation is achieved (21). Typically, substrate release by chaperones is coupled to ATP hydrolysis (20). Like the molecular chaperones in general, protein B23 preferentially binds denatured substrates (5). However, protein B23 does not directly hydrolyze ATP and, until now, a mechanism for release of substrate had not been found.

Protein B23 has been shown to be phosphorylated in vitro (22) and in vivo (23) by protein kinase CK2 (CK2). Furthermore, the CK2 sites are located in a segment of the molecule that is essential for chaperone activity (24). Therefore, we considered the possibility that phosphorylation of protein B23 by CK2 could promote the release of bound substrates. Here we report a possible mechanism for the dissociation of a substrate from protein B23. Phosphorylation of the B23-substrate complex by CK2 alters the interaction of B23 with denatured proteins and promotes their release from the complex. In this mechanism, the release of the chaperone substrate appears to be indirectly dependent on hydrolysis of ATP. Because CK2 and protein phosphatases are present in the nucleus and nucleolus, cycles of phosphorylation and dephosphorylation could control the binding and release of substrate proteins in vivo. This mechanism has not previously been observed in other molecular chaperones.

EXPERIMENTAL PROCEDURES

Proteins and Peptides—Recombinant rat proteins B23.1 and B23.2 used in these studies were produced in *Escherichia coli* and purified essentially as previously described (11) except that the respective cDNAs were inserted into the pET 11c vector (Novagen) for expression. Recombinant deletion mutants (ΔC35, ΔN35, ΔN90, and ΔN119) were expressed using the Qiagen pQE-30 vector and purified as described by Hingorani et al. (24). The mutant protein B23, where the CK2 phosphorylation site was changed to alanine (S125A), was prepared using the SOEing technique as previously described (15) and expressed in the *E. coli* strain 47, reverse order). The recombinant protein was labeled with 125I by guest on July 23, 2018

**RESULTS**

**Binding of Denatured Substrate Enhances the Rate of Phosphorylation of Protein B23 by CK2**—The major phosphorylation site of rat protein B23 by CK2 (serine 125) is located in the first highly acidic segment of the protein (22). Another potential phosphorylation site (threonine 185) is found in the second highly acidic segment. These sites are part of the region essential for maximal chaperone activity (24) and their phosphorylation could be altered by the presence of bound substrate. Therefore, we examined the effect of bound, heat-denatured proteins to protein B23 on its phosphorylation by CK2.

Rhodanese, which was previously used as a substrate to test chaperone activity, was incubated at an equimolar ratio with protein B23 for 20 min at 65 °C to form a complex. Incorporation of 32P from [γ-32P]ATP into the complex catalyzed by CK2 was monitored in the linear phase of the reaction (Fig. 1A). The presence of denatured rhodanese in the complex accelerated the rate of incorporation—5-fold compared with the heated control without rhodanese. Because heat treatment of protein B23 alone did not enhance the rate of incorporation of 32P, the binding of the substrate must be responsible for the increased rate of phosphorylation. In control samples, in which MgCl2 was omitted from the otherwise complete reaction mixture, there was no significant incorporation of 32P in either the heated or unheated sample. This indicated that non-specific adsorption of labeled ATP was not responsible for the enhanced rate of phosphorylation.

Similar experiments were performed using two other proteins, citrate synthase (heated at 50 °C) and the HIV-1 Rev protein (heated to 30 °C). The results were generally very similar to those obtained with rhodanese, with 2–3-fold stimulation of the rate of phosphorylation (Fig. 1, B and C).

To ensure that the enhanced rate of phosphorylation was not because of incorporation of phosphate into nonspecific sites, phosphopeptide mapping studies were done on the phosphorylated protein mixtures. Protein B23 was phosphorylated with CK2 and [γ-32P]ATP for 60 min in the presence of rhodanese either before or after heating the mixture at 65 °C. Under the conditions of phosphorylation, CK2 alone did not incorporate significant label (Fig. 2A, lane 1) due to auto-phosphorylation. The labeling patterns of the heated and unheated, undigested B23-rhodanese mixtures treated with CK2 were virtually identical, with incorporation predominantly into the protein B23 band, with traces of label in degradation products or minor contaminants (Fig. 2A, lanes 2 and 4). There was no significant incorporation into rhodanese (location indicated), and there were no differences in labeling patterns between heated and unheated samples. When the samples were subjected to digestion with endoproteinase Arg-C only one major band was obtained in either case (lanes 3 and 5). The molecular mass of the phosphopeptide cleavage product of endoproteinase Arg-C was estimated to be ~5 kDa; this fits with the molecular mass of the
fragment between residues 102 and 142. Because this segment contains only one serine residue, the presence of this cleavage product supports the notion that only one serine residue is phosphorylated by CK2. To confirm that there were no additional sites labeled when the heat-treated B23-substrate complex was phosphorylated with CK2, similar experiments were done with tryptic digestion. In this case, the reverse phase HPLC elution profiles of the untreated and heat-treated samples were virtually identical, with only one peak of radioactivity (Fig. 2B). These studies indicate that the presence of the denatured substrate did not expose additional sites to nonspecific phosphorylation by CK2, and only serine 125 is phosphorylated by CK2 under these conditions (see Ref. 22).

Protein B23 has also been shown to be phosphorylated in the C-terminal region by a cdc2-type protein kinase (23). To determine whether the effect seen with CK2 in the presence of denatured proteins was specific or was due to phosphorylation in general, protein B23 was also incubated with a cdc2-type protein kinase. When the complex was phosphorylated by the cdc2-type kinase there was no significant increase in the rate of incorporation of radioactivity into protein B23 in the presence of any of the three denatured chaperone substrates mentioned above (data not shown). Thus, the effect of complex formation on the rate of phosphorylation is specific to the CK2 sites.

The N-terminal Region of Protein B23 Is Required for Stimulation of CK2 Phosphorylation by Denatured Chaperone Substrates—Protein B23 has several distinctive segments and
functional domains in its primary structure including a nonpolar N-terminal domain, a basic C-terminal region, and two highly acidic segments in the middle. Previous studies (24) showed that maximal molecular chaperone activity requires nonpolar and acidic segments in the N-terminal half of the polypeptide chain (see the schematic in Fig. 3A). To determine whether specific segments are also important for stimulation of phosphorylation of protein B23 by CK2, we tested this effect whether specific segments are also important for stimulation of CK2 phosphorylation by denatured chaperone (light gray).

Phosphorylation of Protein B23 by CK2 Promotes Dissociation of the B23-Substrate Complex—One of the characteristics of protein B23 that is typical of molecular chaperones is its preference for binding denatured substrates (5). The formation of the complex between denatured substrate and protein B23 seems to be largely dependent on hydrophobic interactions between the proteins. In this complex, the substrate is seemingly irreversibly bound to protein B23. To determine whether CK2 phosphorylation of protein B23 could facilitate dissociation of the substrate, we examined the unmodified and phosphorylated complexes by sedimentation analyses. Equimolar mixtures of 125I-labeled control or CK2-phosphorylated protein B23 and rhodanese were heated and layered onto sucrose gradients and subjected to ultracentrifugation. The gradients were fractionated, and enzyme activity and radioactivity were measured in each fraction (Fig. 4). When rhodanese and B23 were mixed at room temperature they sedimented as separate peaks (Fig. 4A), indicating a lack of interaction, as previously shown (5). Heating the mixture at 60 °C caused the proteins to sediment essentially as a single peak (Fig. 4B). However, when phosphorylated protein B23 and rhodanese were heated together at 60 °C the two proteins again sedimented as separate peaks (Fig. 4C). Similar results were obtained with a CK2-phosphorylated B23-citrate synthase complex analyzed under similar conditions (Fig. 4, D–F). In control experiments, mock phosphorylations of B23 were performed in the absence of CK2, ATP, or MgCl2 before heating with the substrate; under these conditions B23 and the substrate protein sedimented together (Fig. 4, G–I). This indicates that the phosphorylation reaction was essential for dissociation of the B23-substrate complex, which was not promoted simply by the presence of CK2, ATP, or MgCl2.

To confirm phosphorylation at the CK2 site (Ser-125) experiments similar to the ones above were performed using a mutant protein in which Ser-125 was converted to alanine (S125A). In these experiments there was no dissociation of the B23-substrate complex after treatment with CK2 either with rhodanese (Fig. 5C) or with citrate synthase (Fig. 5F). This result strengthens the case that phosphorylation of Ser-125 of protein B23 is essential for dissociation of the bound substrate. Taken together, the results of this series of experiments indicate that CK2-phosphorylated protein B23 has greatly reduced affinity for the denatured substrates.

Because the presence of a bound substrate enhances B23 phosphorylation and phosphorylated B23 has reduced affinity for the substrate, we considered the possibility that phosphorylation of a preformed complex could also promote its dissociation. To answer this question, experiments were performed in which an equimolar mixture of 125I-labeled protein B23 and rhodanese was heated as above, and the resulting complex was phosphorylated by CK2. Aliquots were taken for sedimentation analysis at various times after initiation of the phosphorylation reaction (Fig. 6). With each time point after the addition of CK2, there was an increase in the level of phosphorylation and a corresponding increase in the proportion of the two components dissociated from the complex. At the 5- and 15-min time points, ~0.1 and 0.7 mol of phosphate were incorporated per mol of B23, respectively. About 9 and 65% of the rhodanese was dissociated from the complex at the 5- and 15-min time points, respectively. When the complex was phosphorylated for 60 min, the molar ratio of phosphate to B23 was ~1.2, with greater than 95% dissociation of the complex (data not shown). Thus, phosphorylation of protein B23 by CK2 not only inhibits its ability to form a complex with a denatured substrate, but it also for the substrate-induced enhancement of CK2 phosphorylation of the protein.
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also is able to promote the dissociation of a preformed complex in a manner that is roughly proportional to the level of phosphorylation.

Studies using B23 mutants (Fig. 3) indicated that the nonpolar N-terminal end of the protein is required for chaperone activity and suggested that this region binds denatured protein substrates (24). To determine whether this segment is required for substrate binding, we performed the sedimentation analyses with the ΔN35, ΔN90, and ΔC35 deletion mutants. These experiments showed that removal of a short segment from the N-terminal end of the protein (ΔN35) had no effect on the binding of denatured substrate (Fig. 7B). As with the wild type protein, phosphorylation by CK2 promoted dissociation of the complex (Fig. 7C). Essentially the same set of results was obtained with the ΔC35 deletion mutant (data not shown). In contrast, the ΔN90 deletion mutant was unable to bind denatured rhodanese, which aggregated and lost its enzyme activity during thermal denaturation (Fig. 7E). As expected, the same result was seen with phosphorylated ΔN90 (Fig. 7F). This observation confirms that the N-terminal region protein B23 is essential for substrate binding as well as for the phosphorylation enhancement effect.

Additional Requirements for Dissociation of the HIV-1 Rev-B23 Complex—Earlier experiments (10, 11) showed that there is a strong affinity between nonphosphorylated protein B23 and the HIV Rev protein and that protein B23 also acts as a molecular chaperone toward Rev (5). To determine the effect of CK2 phosphorylation of protein B23 on its Rev binding properties we performed experiments similar to the ones above using phosphorylated and nonphosphorylated protein B23.

Sedimentation analyses were performed with equimolar mixtures of Rev and protein B23 where Rev was labeled with 

antibody to protein B23. The experiments showed that protein B23 binds the Rev protein both at 4 °C and at 30 °C (Fig. 8, B and C), indicating that the complex can be formed without heating. Furthermore, phosphorylation of protein B23 was not capable of dissociating the complex formed at either temperature (Fig. 8, D and E). This observation suggests that protein B23 has an additional binding site for the Rev protein. One of them could be a general binding site for denatured proteins as above and the other could be a site that interacts more or less specifically with the Rev protein. To test this hypothesis, additional experiments were performed in which a synthetic peptide based on the NLS of the Rev protein (Rev37–47; ARRNR-RRRWRRCY) was used as a competitor. It was reported earlier that the Rev37–47 sequence is necessary for nuclear localization of the Rev protein (27), and protein B23 binds the Rev37–47 NLS peptide with high affinity (15). In another series of experiments, equimolar amounts of the Rev protein were incubated at 4 and 30 °C with phosphorylated samples of protein B23 and nonphosphorylated controls. All of the incubation mixtures included a 10-fold molar excess of the Rev37–47 peptide. Fig. 6F shows that the Rev37–47 peptide was able to prevent formation of the Rev-B23 complex at 4 °C. However, competition with the Rev peptide was not able to prevent formation of the complex at 30 °C (Fig. 8G). When phosphorylated protein B23 was incubated with Rev in the presence of the Rev37–47 peptide either at 4 or at 30 °C the two proteins sedimented as separate peaks (Fig. 8, H and D). This indicated that both phosphorylation of protein B23 and the presence of the competing peptide were required to dissociate the complex formed at 30 °C. To determine whether the competition was due to a specific sequence and not simply caused by the charge of the peptide, another peptide with a sequence that was the reverse of that of the Rev NLS peptide was used in a similar experiment. In this case, the reverse NLS peptide was not
capable of dissociating the phosphorylated complex (Fig. 8J), indicating that the interaction has a relatively high degree of specificity. Thus, complete dissociation of the Rev-B23 complex requires phosphorylation of protein B23 as well as competing peptide. This supports the idea that there are two sites of interaction between Rev and protein B23, with two different modes of binding. One mode is through a generic, nonpolar substrate-binding site whose affinity is controlled by phosphorylation, and the other operates via a negatively charged site that interacts with the basic Rev NLS.

**DISCUSSION**

Protein B23 is a multifunctional protein that has many characteristics typically exhibited by molecular chaperones using *in vitro* assays (5). The current studies not only identify additional chaperone-like properties, but they also suggest that the protein B23-substrate interactions are regulated by a novel mechanism. First, protein B23 seems to detect the binding of a denatured substrate and respond by making sites for CK2 phosphorylation more accessible. Second, phosphorylation of B23 by CK2 promotes the dissociation of B23-substrate complexes. Finally, there seem to be two sites for binding at least one substrate, the HIV-1 Rev protein; that is, one site that recognizes nonpolar regions and another that interacts with basic segments of proteins. The former is regulated by phosphorylation, but what controls affinity for the latter has not been determined.

A key feature of molecular chaperones is that they are able to detect bound substrates and regulate their release through allosteric interactions. For example, in Hsp70 (28) and the Hsp100-Clp family (29), the binding of unfolded peptide increases the rate of ATP hydrolysis. In the case of the GroE molecular chaperones (3, 30), binding of a compact folding intermediate triggers a complex series of events including ATP binding, attachment of GroES, hydrolysis of the ATP, and...
eventual release of the substrate. With protein B23, the binding of substrate does not affect ATP hydrolysis directly, but it appears to enhance the accessibility of the CK2 phosphorylation sites of protein B23. The stimulation of CK2 phosphorylation by substrate binding has at least one other precedent in the nucleosome assembly factors NAP-1 and NAP-2; the addition of histones enhances CK2 phosphorylation of these proteins (31). In protein B23, the increased rate of phosphorylation is dependent on the presence of the nonpolar region in the N-terminal end, since the effect is not seen when that segment is deleted. Thus, the nonpolar region seems to communicate with the adjacent highly acidic segment, which contains the major CK2 phosphorylation site.

As indicated above, in most molecular chaperones the release of substrate is coupled with ATP hydrolysis, which is directly carried out by ATPase activity in the chaperone itself. Although protein B23 is capable of binding ATP (32), extensive studies in our laboratory have never detected any significant ATPase activity in the protein itself under any conditions.2 Thus, it is unlikely that the affinity of B23 for a substrate is regulated by direct nucleoside triphosphate hydrolysis. This observation prompted us to search for an alternative mechanism for dissociation of the complex. Because protein B23 is phosphorylated by CK2 (22, 23) and has been shown to be an interacting partner of the enzyme (33), it seemed reasonable to examine the possible effect of phosphorylation by this protein kinase on chaperone substrate binding. It was found that phosphorylation of protein B23 before the addition of denatured substrate inhibits the formation of a stable complex.

2 A. Szebeni and M. O. J. Olson, unpublished data.

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**Fig. 7.** Effect of phosphorylation of protein B23 deletion mutants on B23-substrate complex formation. Protein B23 deletion mutants ΔN35 (A–C) and ΔN90 (D–F) were labeled with 125I and phosphorylated with CK2. The phosphorylated and nonphosphorylated protein samples were incubated at 60 °C with equimolar concentrations of rhodanese and subjected to linear sucrose gradients as described in Fig. 4. For sedimentation analysis 0.65-ml fractions were collected. A and D, rhodanese and the B23 mutant mixed at room temperature. B and E, heat-treated rhodanese and B23 mutants. C and F, heat-treated mixture of phosphorylated B23 mutants and rhodanese. ○, rhodanese activity; □, B23 radioactivity.

**Fig. 8.** Sedimentation analyses of phosphorylated protein B23-Rev protein complex. An equimolar mixture of CK2-phosphorylated or nonphosphorylated protein B23 and 125I-labeled Rev protein were incubated (185 μg/ml) for 60 min at 4 or 30 °C in 10 mM sodium phosphate (pH 7.4), 140 mM NaCl, 200 mM KCl, and 0.05% (w/v) digitonin, applied to 5–30% linear sucrose gradients, and centrifuged in Beckman SW60 rotor for 18 h at 35,000 rpm. Fractions (0.35 ml) were collected, and the radioactivity of Rev protein was measured. Protein B23 concentrations were determined by an enzyme-linked immunosorbent assay using a monoclonal antibody. A, protein B23 and Rev protein subjected to sedimentation in two separate tubes. B, B23 and Rev incubated at 4 °C. C, B23 and Rev incubated at 30 °C. D, phosphorylated protein B23 and Rev incubated at 4 °C. E, phosphorylated protein B23 and Rev incubated at 30 °C. F, protein B23 and Rev incubated at 4 °C in the presence of the NLS peptide. G, protein B23 and Rev incubated at 30 °C in the presence of the NLS peptide. H, phosphorylated protein B23 and Rev incubated at 4 °C in the presence of the NLS peptide. I, phosphorylated protein B23 and Rev incubated at 30 °C in the presence of the NLS peptide. J, phosphorylated protein B23 and Rev incubated at 30 °C in the presence of the reverse NLS peptide. ○, Rev radioactivity; □, relative B23 concentration determined by enzyme-linked immunosorbent assay.
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Fig. 9. A model for the plausible involvement of phosphorylation of protein B23 in its molecular chaperone activity. Under the macromolecular crowding conditions of the nucleolus proteins would have a tendency to form aggregates when partially unfolded. Protein B23 could recognize and bind to an exposed surface of the protein and temporarily remove it from the aggregation pathway. Refolding of the substrate protein could be accomplished either in the complex or after its release. Phosphorylation of the complex by casein kinase II (abundant in the nucleolus) would reduce the affinity of B23 for a substrate and promote dissociation of the complex. After release of substrate, a phosphoprotein phosphatase (PPase) could dephosphorylate protein B23. The latter step would restore the affinity of B23 for partially unfolded substrates and allow it to participate in additional cycles of binding and release of substrates.

Of the abundance of protein B23 and the high concentration of proteins in the nucleolus, one could imagine that it would interact with a variety of nucleolar proteins, either ribosomal or nonribosomal proteins or both. To remove a partially unfolded protein from an aggregation pathway, protein B23 might recognize and bind to exposed surfaces on the protein (Fig. 9). The substrate protein would remain bound to B23 until it is phosphorylated by CK2. The latter enzyme is abundant in the nucleolus (34) and migrates to the nucleolus under heat shock conditions (35). Furthermore, CK2 is up-regulated during cell growth (36), in which nucleolar activity and protein concentration are also elevated. The latter conditions would increase the probability for proteins to be partially unfolded and to be bound by protein B23. The binding event could allow time for the substrate protein to properly fold either while associated with protein B23 or after its release. CK2 might then preferentially phosphorylate protein B23-substrate complexes to promote their dissociation. Once the substrate is released, protein B23 could then be dephosphorylated by phosphoprotein phosphatases, which are also present in the nucleolus (4, 37). The latter event would restore the potential for B23 to bind a partially unfolded substrate. This cycle could be repeated over and over to facilitate the ribosome assembly process. Whether protein B23 is a general chaperone as suggested by the current studies using generic chaperone substrates or whether it recognizes specific substrates in the nucleolus is currently under investigation.

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