C-terminal Trimerization, but Not N-terminal Trimerization, of the Reovirus Cell Attachment Protein Is a Posttranslational and Hsp70/ATP-dependent Process

(Received for publication, November 30, 1995)

Gustavo Leone§, Matthew C. Coffey§, Ross Gilmore, Roy Duncan, Lloyd Maybaum, and Patrick W. K. Lee

From the Department of Microbiology and Infectious Diseases, University of Calgary, Health Sciences Centre, Calgary, Alberta, Canada T2N 4N1

The C-terminal globular head of the lollipop-shaped α1 protein of reovirus is responsible for interaction with the host cell receptor. Like the N-terminal fibrous tail, it has its own trimerization domain. Whereas N-terminal trimerization (formation of a triple α-helical coiled coil) occurs at the level of polymers (i.e. cotranslationally) and is ATP-independent, C-terminal trimerization is a posttranslational event that requires ATP. Coprecipitation experiments using anti-Hsp70 antibodies and truncated α1 proteins synthesized in vitro revealed that only regions downstream of the N-terminal α-helical coiled coil were associated with Hsp70. Hsp70 was also found to be associated with nascent α1 chains on polysomes as well as with immature postribosomal α1 trimers (hydralike intermediates with assembled N termini and unassembled C termini). These latter structures were true intermediates in the α1 biogenetic pathway since they could be chased into mature α1 trimers with the release of Hsp70. Thus, unlike N-terminal trimerization, C-terminal trimerization is Hsp70- and ATP-dependent. The involvement of two mechanistically distinct oligomerization events for the same molecule, one cotranslational and one posttranslational, may represent a common approach to the generation of oligomeric proteins in the cytosol.

There is now increasing evidence that the folding and assembly of proteins in vivo are mediated by other proteins known as molecular chaperones (1–4). In eukaryotes, some of these proteins were initially identified as stress or heat-shock proteins since their expression in cells is inducible by a variety of cellular stresses including heat (5, 6). It is now known that they are also constitutively expressed and play essential roles in promoting the correct folding and assembly of newly synthesized proteins in the cytosol (7–9) as well as in the transport of proteins across membranes of mitochondria (10), chloroplasts (11), and endoplasmic reticulum (12, 13). A number of chaperone families have been described, including Hsp90 (HtpG in prokaryotes) (14, 15), Hsp70 (DnaK in prokaryotes) (4, 16), Hsp60 (GroEL in prokaryotes) (17–19), Hsp40 (DnaJ in prokaryotes) (9–16), and Hsp10 (GroES in prokaryotes) (17–20), of which members of the Hsp70 and Hsp60 families are the best studied. Hsp70 proteins function as monomers or dimers, whereas Hsp60 proteins adopt a characteristic double toroid structure, with each toroid possessing seven identical subunits. These two chaperones are believed to function as a relay team in the processing of nascent or unfolded polypeptides, which involves a series of ATP-dependent binding and release events, with the eventual generation of the correctly folded and assembled products (16). Recent evidence suggests that Hsp70 preferentially binds to short extended peptides possessing alternating hydrophobic residues (21), a finding consistent with the view that chaperones interact with exposed hydrophobic regions of unfolded proteins that are otherwise buried in native proteins. The relative lack of more stringent recognition requirements apparently contributes to the promiscuous nature of substrate binding seen with all classes of chaperones.

Considerable information on chaperone function has been obtained from in vitro refolding experiments using purified components. However, the extent to which observations from these studies can be extrapolated to reflect the actual sequence of events that direct nascent proteins to their native conformations is unclear. Experiments involving the direct analysis of nascent chains are therefore necessary in order to reveal the actual mechanisms of chaperone function. In this regard, Beckmann et al. (7) were the first to demonstrate that nascent polypeptides in the mammalian cytosol associate with Hsp70 and postulated that this may represent a requirement of subsequent folding and/or assembly of polypeptides. More recently, Frydman et al. (9) used in vitro translated firefly luciferase to demonstrate the highly organized chaperone machinery involved in the successful folding of this protein.

The in vitro translation system has also been used extensively in our laboratory to reveal the mechanisms of folding and oligomerization of the reovirus cell attachment protein α1, a trimeric protein located at the 12 vertices of the icosahedral virion (22–26). The α1 trimer is highly asymmetric, with a N-terminal fibrous tail that is anchored to the virion and a C-terminal globular head that interacts with the cell receptor (27–33). These two structurally distinct domains are separated by a short protease-sensitive region (32, 34). Evidence from in vitro translation studies has revealed that these two domains are generated by independent trimerization events (26), with N-terminal trimerization preceding C-terminal trimerization. The core of the N-terminal trimerization domain is the N-terminal one-third of the protein, which is highly α-helical and contains an extended heptad repeat of hydrophobic residues (35, 36), endowing this region with the intrinsic propensity to form a triple coiled coil. During α1 biogenesis, N-terminal assembly takes place cotranslationally (i.e. on the polysome),...
Trimerization of Reovirus Cell Attachment Protein

Involving neighboring nascent chains on those ribosomes that have traversed past the midpoint of the S1 mRNA encoding α1. Interestingly, this process is intrinsically ATP-independent, suggesting the lack of chaperone involvement.

Although the C terminus possesses its own independent trimerization domain, its assembly into a globular head requires the prior trimerization of the N terminus (26), which presumably serves to bring the three C termini into close proximity to each other for interaction. Also, unlike N-terminal trimerization, which can tolerate relatively large alterations (26, 38), C-terminal trimerization is under stringent control, which requires that the C-terminal halves of all three subunits be intact (26, 32, 39). The global nature of this trimerization event is compatible with a posttranslational assembly mechanism, as opposed to a cotranslational one as seen with N-terminal trimerization. A pertinent question would be whether the two trimerization processes also differ in terms of ATP and chaperone involvement.

In the present study, we demonstrate that trimerization of the C-terminal globular head indeed occurs posttranslationally, and is an ATP-dependent process. In addition, we find that nascent α1 chains and immature, but not mature, α1 trimers are transiently associated with Hsp70 and that this association is strictly limited to regions downstream of the N-terminal α-helical coiled coil. We conclude that distinct Hsp70/ATP-dependent and -independent oligomerization and folding domains can coexist within the same protein.

MATERIALS AND METHODS

In Vitro Transcription—The plasmids encoding the full-length and various truncated α1 products have been described previously (26, 32). Transcripts were generated in vitro using Sp6 RNA polymerase (Pharmacia Biotech Inc.). Typically, the 50-μl reaction contained 1 x reaction buffer (40 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl (Promega), 1 mM ATP, CTP, and UTP, 0.1 mM GTP, 0.5 mM cap analog, 6 mM diithothreitol, 10 mg/ml bovine serum albumin, 1.0 unit/μl RNAguard (Pharmacia), 5 μg of linearized DNA template, and 15 units of Sp6 RNA polymerase/μg of DNA. The reactions were incubated for 30 min at 37 °C, at which time GTP was added to a final concentration of 1.0 mM and incubated for an additional 45 min. The DNA templates were digested by the addition of 0.15 μg of RNase-free DNaseI (Life Technologies, Inc.) and 1.0 unit/μl RNAguard, followed by a 15-min incubation at 37 °C. The reactions were diluted with 10 μl Tris-HCl (pH 7.5) and 1 μl EDTA, extracted with phenol, phenol-chloroform, and chloroform, and ethanol-precipitated in the presence of 2.5 mM ammonium acetate. The pellets were washed, resuspended in sterile water, and stored in small aliquots at −70 °C until needed for translation. The resulting mRNA was homogeneous in size, and yields were approximately 10–15 μg/reaction.

In Vitro Translation and Chase—Capped S1-specific mRNAs were translated in vitro in rabbit reticulocyte lysates according to the manufacturer’s specifications (Promega). Typically, the reaction mixture contained 50–100 ng of RNA and 20 μCi of [35S]methionine (DuPont NEN) in a total volume of 25 μl. Reactions were incubated at 37 °C for various durations indicated and then analyzed directly by SDS-PAGE.

The supernatants, which contained no translation activity, were then subjected to ultracentrifugation at 4 °C to pellet the polysomes. The supernatants, which contained no translation activity, were then incubated at 37 °C for various durations (chase). Aliquots from both the pulse and chase samples were incubated in SDS-containing sample buffer at either 4 °C (at this temperature both N- and C-terminal trimers were stable), or 37 °C (at this temperature the N-terminal trimer, but not the C-terminal trimer, was stable). SDS-PAGE was carried out at 4 °C.

RESULTS

Assembly of Trimeric C-terminal Head Occurs Posttranslationally—Previously, we used an in vitro translation system to demonstrate that α1 trimerization is initiated cotranslationally at the N terminus by the formation of a three-stranded coiled coil and that this is necessary for the subsequent assembly of the C termini into a trimeric and functional head (26). Since stable trimer formation at the N terminus occurs only after the release of nascent chains from polysomes (as an unstable complex), assembly of the C termini into a trimeric head would be predicted to occur posttranslationally. To verify this prediction, a pulse-chase experiment was performed in which α1 was translated in rabbit reticulocyte lysate for 10 min, by which time a significant population of α1 had trimerized at the N terminus but not at the C terminus (hydra-like folding intermediate) (23, 26). Polysomes were then removed by ultracentrifugation, and the supernatant was further chased at 37 °C. Chase samples were subjected to SDS-PAGE (Fig. 1) under conditions that allowed detection of the trimeric state of both the N and C terminus (4 °C) or the trimeric state of the N

FIG. 1. Posttranslational C-terminal trimerization of protein α1. Full-length reovirus S1 transcripts were translated in rabbit reticulocyte lysate in the presence of [35S]methionine for 10 min (pulse) and then subjected to ultracentrifugation at 4 °C to pellet the polysomes. The supernatants, which contained no translation activity, were then incubated at 37 °C for various durations (chase). Aliquots from both the pulse and chase samples were incubated in SDS-containing sample buffer at either 4 °C (at this temperature both N- and C-terminal trimers were stable), or 37 °C (at this temperature the N-terminal trimer, but not the C-terminal trimer, was stable). SDS-PAGE was carried out at 4 °C.

Man SW 28.1 rotor, and the gradient was fractioned and analyzed for absorbance at 254 nm.

Immunoprecipitation—The anti-Hsp70 antibody was originally obtained from Dr. W. Welch (University of California, San Francisco) and was raised against a 20-amino acid synthetic peptide derived from the C-terminal region of the constitutive form of Hsp70 (His73) (40). This antibody was subsequently raised in our own laboratory. The monoclonal anti-α1 antibody G5 was obtained from the late Dr. B. N. Fields (Harvard Medical School) (41) and shown to interact with a C-terminal conformational epitope of α1 (25, 42). [35S]Methionine-labeled in vitro translation lysates were immunoprecipitated with the above antibodies using a modified immunoprecipitation procedure described previously (24).

SDS-PAGE—Discontinuous SDS-PAGE was performed using the protocol of Laemmli (43). Depending on the size of α1 products analyzed, 7.5%, 10%, or 12.5% polyacrylamide gels were used. Samples were incubated in protein sample buffer (final concentration 50 mM Tris (pH 6.8), 1% SDS, 2% β-mercaptoethanol, 10% glycerol, and 0.01% bromphenol blue) for 30 min at either 37 °C or 4 °C, or, alternatively, boiled for 5 min prior to SDS-PAGE. The different SDS-PAGE conditions were used in order to differentiate between N-terminal trimerization and C-terminal trimerization. Under dissociating conditions where samples were boiled for 5 min prior to SDS-PAGE (carried out at room temperature), both N- and C-terminal trimers dissociated and migrated as monomers. Under non-dissociating conditions, samples were either incubated for 30 min at 37 °C, at which temperature only the trimeric N-terminal domain is stable, or incubated at 4 °C, at which temperature both the N- and C-terminal trimeric domains are stable (SDS-PAGE was carried out at 4 °C in both cases).

1 Embo J. 1996. EMBO J. 15, 7027–7034.

The abbreviation used is: PAGE, polyacrylamide gel electrophoresis.
The addition of apyrase inhibited the translation. Samples from various chase periods were then incubated (chased) at 37 °C for 30 min prior to SDS-PAGE.

C-terminal Trimerization Is ATP-dependent—The occurrence of two separate trimerization events, one cotranslational and the other posttranslational, in the generation of two structurally distinct domains within the same protein has led to the interesting question of whether each of these two events has its own chaperoning requirements. We recently demonstrated that trimerization of the S1 N terminus is intrinsically ATP-independent, which in turn suggests that chaperones are not likely to be involved in this process. While it remains to be seen whether the lack of ATP involvement is universal for the generation of α-helical coiled coils, folding of the trimeric C-terminal globular head appears to be under more stringent control (26, 32, 39) and is therefore likely to be ATP/chaperone-mediated. To test this possibility, a pulse-chase experiment similar to the one described for Fig. 1 was performed except that apyrase, an ATPase, was added to the reactions after 10 min of translation. Samples from various chase periods were then analyzed by SDS-PAGE under nondissociating conditions (4 °C). As shown in Fig. 2, the addition of apyrase inhibited the hydra-like intermediate from being chased to the mature S1 form, whereas buffer (control) had no effect. Essentially the same observations were made using a nonhydrolyzable ATP analogue instead of apyrase (data not shown). These results demonstrate that assembly of the C terminus into a stable trimeric head occurs posttranslationally.

At 37 °C, the hydra-like folding intermediate (with an assembled N terminus and an unassembled C terminus) present in the supernatant could be chased into the mature, faster migrating, compact S1 form with both the N and C termini in the trimeric state (4 °C panel). This finding demonstrates that assembly of the C termini into a stable trimeric head occurs posttranslationally.

Regions on α1 Involved in Hsp70 Association—To delineate domains on α1 responsible for Hsp70 association, and thereby

Since the hydra-like folding intermediate represents the precursor to mature α1, and since C-terminal trimerization is ATP-dependent, it was of interest to determine whether this hydra-like folding intermediate might also be associated with Hsp70. We therefore translated α1 for 20 min, a time at which both the hydra-like folding intermediate and the mature form of α1 were present in the translation reaction, and examined their association with Hsp70 by communoprecipitation with the Hsp70-specific serum. Immunoprecipitates were subjected to SDS-PAGE under nondissociating conditions in order to resolve the various forms of α1 (Fig. 4). The results show that in addition to monomeric α1, the hydra-like folding intermediate was also associated with Hsp70. Importantly, the mature and functional form of α1 was not found to be in association with Hsp70. These results suggest that Hsp70 may be playing a dynamic role in α1 trimerization.

Hsp70-α1 Complexes Represent True Folding Intermediates—It was important to rigorously prove whether or not these Hsp70-α1 complexes do indeed represent legitimate folding intermediates in the α1 folding pathway. To this end, a pulse-chase experiment was carried out whereby α1 was translated for 7 min in the presence of [35S]methionine and subsequently chased with excess unlabeled methionine, followed by sequential immunoprecipitations (Fig. 5A). Chase samples were initially immunoprecipitated with either the Hsp70-specific serum or an anti-α1 monoclonal antibody, G5 (41), which recognizes a conformational epitope present only in mature α1 trimers (25, 26). The supernatants from the initial immunoprecipitations were in turn reciprocally immunoprecipitated with mAbG5 or the anti-Hsp70 serum, respectively. The results demonstrate that with time, the amount of α1 coprecipitated by anti-Hsp70 decreased, whereas that precipitated by mAbG5 increased. Subsequent SDS-PAGE under nondissociating conditions confirmed that the anti-Hsp70 immunoprecipitates contained exclusively monomeric α1 and hydra-like intermediates (data not shown). When these immunoprecipitates were resuspended in fresh RRL followed by incubation at 37 °C, a conversion of the hydra-like intermediate to the mature compact form was observed (Fig. 5B). As expected, this maturation process involves the ATP-dependent release of α1 from the Hsp70-α1 complex (data not shown). Collectively, these results provide strong evidence that the Hsp70-nascent α1 complex and the Hsp70-hydra-like intermediate complex are transient structures that represent true folding intermediates in the pathway leading to the generation of mature α1 trimer.

Regions on α1 Involved in Hsp70 Association—To delineate domains on α1 responsible for Hsp70 association, and thereby

**Fig. 2.** Effect of ATP deprivation on C-terminal trimerization of protein α1. Full-length S1 transcripts were translated for 10 min and subjected to ultracentrifugation to pellet the polysomes. The supernatant was then incubated (chased) at 37 °C for various durations indicated either in the presence (+) or absence (−) of apyrase (40 units/ml). Chase samples were incubated in protein sample buffer at 4 °C for 30 min prior to SDS-PAGE.

**Fig. 3.** Association of Hsp70 with polysome-bound α1 chains. Full-length S1 transcripts were translated in vitro for 10 min, and the reaction mixture was subjected to centrifugation through a 10–45% sucrose gradient as described under "Materials and Methods." Peak polysomal fractions were pooled (lane 1), concentrated, and immunoprecipitated with the Hsp70-specific serum (lane 2) or with the preimmune serum (lane 3). All samples were boiled prior to SDS-PAGE.
perhaps shed light on the Hsp70 mode of action, we constructed various C-terminally truncated and internal deletion mutants and probed for their interaction with Hsp70 using the same coimmunoprecipitation approach as above. The results are shown in Fig. 6A. As observed for ribosome-bound chains, the N-terminal onethird of (d294), which represents essentially the entire α-helical coiled coil region, did not interact with Hsp70. However, the addition of 40–100 amino acids downstream of this region allowed α1 to interact with Hsp70, albeit with low affinity. The further addition of downstream sequences provided α1 with maximal capacity to interact with Hsp70, and these downstream sequences, when expressed separately, were also able to efficiently interact with Hsp70 (Fig. 6B). These findings suggest that multiple Hsp70 binding sites are present in the C-terminal two-thirds of α1 and are compatible with the above demonstration that C-terminal trimerization is an ATP-dependent process. By the same token, the lack of Hsp70 association at the N-terminal one-third of α1 also correlates with the ability of this region, when expressed free of downstream sequences, to trimerize in an ATP-independent manner.1

FIG. 4. Association of Hsp70 with the α1 hydra-like intermediate. Apyrase was added to a 20-min α1 translation reaction, which was then diluted and microcentrifuged. The supernatant (lane 1) was immunoprecipitated with the Hsp70-specific serum (lane 2) or with the preimmune serum (lane 3). Immunoprecipitates were incubated in protein sample buffer at 4 °C prior to SDS-PAGE (also carried out at 4 °C) to allow for the detection of both the hydra-like intermediate and the mature trimer.

FIG. 5. Chase of Hsp70-associated α1 to mature α1. A, full-length S1 transcripts were translated in the presence of [35S]methionine for 7 min and subsequently chased with excess (16 mM) unlabeled methionine. At various times indicated (with the first time point being the time of addition of unlabeled methionine), chase samples (expression) were subjected to sequential immunoprecipitation: anti-Hsp70 serum followed by mAb G5 (a) or mAb G5 followed by anti-Hsp70 serum (b). All the immunoprecipitates were boiled in protein sample buffer prior to SDS-PAGE. B, full-length S1 transcripts were translated for 11 min in the presence of [35S]methionine. The reaction mixture was then immunoprecipitated with the anti-Hsp70 serum. After repeated washes in a buffer containing 150 mM NaCl and 25 mM Tris (pH 7.4), the immunoprecipitates were resuspended in fresh rabbit reticulocyte lysate and incubated at 37 °C. At the times indicated, aliquots were taken from the suspension and analyzed by SDS-PAGE under nondenaturing conditions (4 °C preincubation).

DISCUSSION

Studies on the structure/function relationships of the reovirus cell attachment protein α1 have led to a number of interesting revelations. Earlier sequence analysis suggests that the N-terminal portion of α1 is an α-helical coiled coil, while the C-terminal portion exists as a globular head (35). Such a prediction was subsequently confirmed by electron microscopic studies that showed purified α1 as a lollipop-shaped structure with a fibrous tail topped by a globular head (27, 28, 30). Similar structures have been found to project from the surfaces of virus particles, with the globular heads being most distal from the virions (28). That the globular head and the fibrous tail indeed represent the C- and N-terminal portions, respectively, of α1 has been confirmed by biophysical analysis of the two fragments (representing the C- and N-terminal halves) generated by trypsin digestion of purified α1 (23). It was further shown that the C-terminal portion of α1 harbors the conformation-dependent receptor binding domain (29, 32, 39, 44) and that the N-terminal portion possesses intrinsic digimerization and virion anchoring function (24, 27, 31, 33, 37). Biochemical and biophysical evidence suggests that intact protein α1, as well as the N- and C-terminal tryptic fragments, are all trimeric (23) and that trimerization of α1 is accompanied by extensive conformational changes necessary for its cell attachment function (25). Of particular significance was the subsequent demonstration that the N- and C-terminal halves of α1 each possesses its own trimerization domain (26), leading us to further characterize the two trimerization events in terms of
N-terminal trimerization of α1 has recently been found to be a cotranslational event that is intrinsically ATP-independent, suggesting the lack of chaperone involvement. This is not the likely case for C-terminal trimerization, which appears to be global and thus necessarily occurs posttranslationally. The global nature of this process is suggested by the following observations. First, the deletion of as few as four amino acids from the C terminus totally abrogates the cell binding function of α1 (32), as does the single substitution of certain conserved amino acids at the C-terminal half of α1 (39). In both cases, the N-terminal half of the protein remains intact (trimeric and protease-resistant), whereas the C-terminal half is grossly misfolded (unassembled and protease-sensitive). Second, α1 heterotrimers comprised of two wild-type subunits and a mutant subunit with deletion or substitution at the C terminus are found to be nonfunctional and manifest C-terminal misfolding (26). These observations have led to the prediction that trimerization of the C terminus can proceed only when the C termini of all three subunits are intact, and is accordingly a posttranslational and global event. That this is in fact the case is clearly demonstrated in the present study by following the fate of α1 intermediates in the postribosomal fractions. C-terminal trimerization therefore contrasts sharply with N-terminal trimerization in both temporality and stringency. The involvement of Hsp70 and possibly other chaperones leads to global trimerization and folding of the C terminus, generating mature α1 with the characteristic lollipop-shaped structure that migrates as an unretarded trimer in SDS-PAGE under nondissociating conditions.

Since segments from the C-terminal half, when generated separately, were found to be independently associated with Hsp70 (Fig. 6), it is likely that a good portion of the C-terminal half of α1 nascent chains and intermediate forms is protected by Hsp70. This association persists as the α1 complex leaves the polysome. It is not known at present whether other chaperones such as Hsp40 and/or TRiC are involved prior to this release. However, results from our experiments (not shown) suggest that the release of Hsp70 from the α1 substrate requires an additional factor(s) present in the reticulocyte lysate. Recent evidence from pulse-chase experiments performed in our laboratory (not shown) indicates that with time, there is a decrease of Hsp70-α1 association, suggestive of an ordered sequence for the interaction of these two chaperones with α1. This involvement of Hsp40 in the α1 folding process may be a likely possibility since this chaperone has recently been shown to cooperate with Hsp70 and TRiC in promoting the folding of firefly luciferase in vitro (9). Whatever the nature of cochaperones, the almost exclusive interaction of Hsp70 with the α1 folding process is also a likely possibility since this chaperone has recently been shown to cooperate with Hsp70 and TRiC in promoting the folding of firefly luciferase in vitro (9).
intermediates. In this respect, the present identification of a transient association between Hsp70 and α1 intermediates represents an important step toward a testable model.

The overall scheme of protein α1 biogenesis is summarized in a schematic in Fig. 7. Commitment of the α1 N termini to trimerize occurs at the level of polypeptides (depicted as the cotranslational formation of a triple coiled coil, the most plausible scenario) and with no ATP or Hsp70 involvement. Binding of Hsp70 (and possibly other chaperones such as Hsp40 and TRiC) to sequences downstream of the N-terminal region prevents tightening of the coiled coil, and α1 leaves the polysome as an SDS-unstable trimeric complex. Subsequent ATP-dependent release of the bound chaperones leads to the second (C-terminal) trimerization event, which completes the α1 maturation process. Such a strategy of cotranslational followed by posttranslational oligomerization is highly efficient since it spares individual subunits the need to search for their partners in a soluble pool. It would be interesting to see whether other homooligomeric proteins in the cytosol follow a similar strategy of biogenesis.

REFERENCES
1. Georgopoulos, C., and Welch, W. J. (1993) Annu. Rev. Cell Biol. 9, 601–634
2. Hendrick, J. P., and Hartl, F. U. (1993) Annu. Rev. Biochem. 62, 349–384
3. Ellis, R. J. (1994) Curr. Opin. Struct. Biol. 4, 117–122
4. Hartl, F. U., Hlodan, R., and Langer, T. (1994) Trends Biochem. Sci. 19, 20–25
5. Tissieres, A., Mitchell, H. K., and Tracy, U. M. (1974) J. Mol. Biol. 84, 389–398
6. Lindquist, S., and Craig, E. A. (1988) Annu. Rev. Genet. 22, 631–677.
7. Beckmann, R. P., Mizzen, L. A., and Welch, W. J. (1990) Science 248, 850–854
8. Nelson, R. J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M., and Craig, E. A. (1992) Cell 71, 97–105
9. Frydman, J., Nimmergut, E., Ohtsuka, K., and Hartl, F. U. (1994) Nature 370, 111–117
10. Stuart, R. A., Cyr, D. M., Craig, E. A., and Neupert, W. (1994) Trends Biochem. Sci. 19, 87–92
11. Soll, J., and Aisen, P. (1993) Physiol. Plant 87, 433–440
12. Chirico, W. J., Waters, M. G., and Blobel, G. (1988) Nature 332, 805–810
13. Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A., and Schekman, R. (1988) Nature 332, 800–805
14. Borkovich, K. A., Furely, F. W., Finkielstein, D. B., Taulin, J., and Lindquist, S. (1989) Mol. Cell Biol. 9, 3919–3930
15. Bardwell, J. C. A., and Craig, E. A. (1988) J. Bacteriol. 170, 2977–2983
16. Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M. K., and Hartl, F. U. (1992) Nature 356, 683–689
17. van Andel, S. M., Wootford, C., Van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. (1988) Nature 333, 330–334
18. Lewis, V. A., Hynes, G. M., Zheng, D., Saibil, H., and Willison, K. (1992) Nature 358, 249–252
19. Gao, Y., Thomas, J., Chow, R. L., Lee, G.-H., and Cowan, N. J. (1992) Cell 69, 1043–1050
20. Hartman, D. J., Hoogenraad, N. J., Condron, R., and Hoi, P. B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3394–3398
21. Blond-Elguindi, S., Cwirola, S. E., Dower, W. J., Lipshutz, R. J., Sprang, S. R., Sambrook, J. F., and Gething, M. H. (1993) Cell 75, 717–728
22. Lee, P. W. K., Hayes, E. C., and J d‘h, K. W. (1981) Virology 106, 156–163
23. Strong, J. E., Leone, G., Duncan, R., Sharma, R. K., and Lee, P. W. K. (1991) Virology 184, 23–32
24. Leone, G., Duncan, R., Mah, D. C. W., Price, A., Cashdollor, L. W., and Lee, P. W. K. (1991) Virology 182, 336–345
25. Leone, G., Mah, D. C. W., and Lee, P. W. K. (1991) Virology 182, 346–350
26. Leone, G., Maybaum, L., and Lee, P. W. K. (1992) Cell 71, 479–488
27. Banerjea, A. C., Brechling K. A., Ray, C. A., Erikson, H., Pickup, D. J., and Joklik, W. K. (1988) Virology 167, 601–612
28. Furlong, D. B., Nibert, M. L., and Fields, B. N. (1988) J. Virol. 62, 246–256
29. Yeung, M. C., Lim, D., Duncan, R., Shahrabadi, M. S., Cashdollor, L. W., and Lee, P. W. K. (1989) Virology 170, 62–70
30. Fraser, R. D. B., Furlong, D. B., Trus, B. L., Nibert, M. L., Fields, B. N., and Steven, A. C. (1990) J. Virol. 64, 2950–3000
31. Mah, D. C. W., Leone, G., jankoowski, J. M., and Lee, P. W. K. (1990) Virology 179, 95–103
32. Duncan, R., Horne, D., Strong, J. E., Leone, G., Pon, R. T., Yeung, M. C., and Lee, P. W. K. (1991) Virology 182, 810–819
33. Leone, G., Duncan, R., and Lee, P. W. K. (1991) Virology 184, 758–761
34. Duncan, R., and Lee, P. W. K. (1994) Virology 203, 149–152
35. Bassel-Duby, R., Jayasuriya, A., Chatterjee, D., Sonenberg, N., Maizel, J. V., J r., and Fields, B. N. (1985) Nature 315, 421–423
36. Nibert, M. L., Dermöody, T. S., and Fields, B. N. (1980) J. Virol. 46, 2976–2989
37. Banerjea, A. C., and Joklik, W. K. (1990) Virology 179, 460–462
38. Fernandez, J., Deng, E., Leone, G., and Lee, P. W. K. (1991) J. Biol. Chem. 269, 17043–17047
39. Turner, D. L., Duncan, R., and Lee, P. W. K. (1992) Virology 186, 219–227
40. Brown, C. R., Martin, R. L., Hansen, W. J., Beckmann, R. P., and Welch, W. J. (1993) J. Cell Biol. 120, 1101–1112
41. Burstein, S., Springer, D. R., and Fields, B. N. (1982) Virology 117, 146–155
42. Yeung, M. C., Guter, M. J., Tuler, S., A., Shahrabadi, M. S., and Lee, P. W. K. (1987) Virology 156, 377–385
43. Laemmli, U. K. (1970) Nature 227, 680–685
44. Nagata, S., Masri, S. A., Pon, R. T., and Lee, P. W. K. (1987) Virology 160, 162–168
C-terminal Trimerization, but Not N-terminal Trimerization, of the Reovirus Cell Attachment Protein Is a Posttranslational and Hsp70/ATP-dependent Process

Gustavo Leone, Matthew C. Coffey, Ross Gilmore, Roy Duncan, Lloyd Maybaum and Patrick W. K. Lee

J. Biol. Chem. 1996, 271:8466-8471.
doi: 10.1074/jbc.271.14.8466

Access the most updated version of this article at http://www.jbc.org/content/271/14/8466

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 44 references, 9 of which can be accessed free at http://www.jbc.org/content/271/14/8466.full.html#ref-list-1