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Binding protein BiP is required for translocation of secretory proteins into the endoplasmic reticulum in *Saccharomyces cerevisiae*

(70-kDa heat shock protein/conditional expression/invertase/α factor/secretion)

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ABSTRACT **The endoplasmic reticulum of mammalian cells contains a heat shock protein of ≈70 kDa (hsp70) termed binding protein BiP that is thought to promote the folding and subunit assembly of newly synthesized proteins. To study BiP function, we placed the BiP-encoding gene from *Saccharomyces cerevisiae* under the control of a regulated promoter and examined the effects of BiP depletion. Reduction of BiP protein to about 15% of normal levels led to a profound reduction in secretion of α factor and invertase. At the same time, unglycosylated precursors of these proteins accumulated intracellularly. The predominant form of the invertase precursor had undergone signal sequence cleavage but accumulated as a soluble species in the cytosol. In contrast, the α-factor precursor was exclusively in the signal-uncleaved form. It sedimented with microsomal membranes and was exposed at the cytoplasmic face in a protease-resistant form. These findings suggest that, in yeast, BiP function is required for translocation of soluble proteins into the endoplasmic reticulum at a stage beyond the initial nascent chain–membrane association.**

Binding protein BiP is the single endoplasmic reticulum (ER)-localized member of the hsp70 family of heat shock proteins of ≈70 kDa. In mammalian cells, BiP associates transiently with a number of newly synthesized secretory or membrane proteins and permanently with certain unassembled protein subunits or misfolded proteins that fail to leave the ER. BiP thus appears to recognize nonnative structures. It is thought that BiP may function to solubilize protein aggregates or to prevent their formation, or it may directly promote the proper folding and assembly of exocytotic proteins. Alternatively, BiP may function to retain proteins in the ER until proper folding or assembly has been completed (see refs. 1 and 2, and references therein).

BiP, along with cytoplasmic hsp70, has been shown to possess a peptide binding site that exhibits marked sequence preferences. Consistent with a catalytic function, both proteins bind peptide *in vitro* in an ATP-independent reaction and then release the peptide in a reaction involving ATP hydrolysis (3). An emerging view of hsp70 action is that these proteins bind to certain sites that are exposed on nascent or otherwise non-native polypeptides. In the case of misfolded, denatured, or aggregated proteins, hsp70, through cycles of binding and release, may effect repair by promoting unfolding and subsequent refolding. For nascent chains, the interaction may simply prevent rapid aggregation, thereby allowing time for productive folding or subunit assembly events to occur. hsp70 could also influence folding/assembly pathways by maintaining portions of the polypeptide in certain configurations (2). Such a model predicts that nascent chains in the cytosol or during translocation into the ER would interact extensively with hsp70 proteins. As yet, attempts to document such associations have been unsuccessful (4).

We and others have cloned a homologue of the BiP gene from the yeast *Saccharomyces cerevisiae* and have shown that it is an essential gene (5–7). We refer to this gene as *KAR2* in keeping with the original designation of this genetic locus, but we retain BiP as the name of the gene product. To further study BiP function, we placed the *KAR2* gene under the control of a repressible promoter. We found that BiP depletion leads to a block in secretion of both α factor and invertase, an effect that could be traced back to a defect in translocation of these proteins across the ER membrane.

MATERIALS AND METHODS

Strains and Microbial Techniques. Rich medium for yeast cells was YPD (2% glucose) or YPG (2% galactose) (19). Minimal medium was SD or SG (Wickerham's containing 2% glucose or galactose; 19). *S. cerevisiae* strains used were: HMSF176 (**a** *mal gal2 sec18-1*) (Yeast Genetic Stock Center, University of California, Berkeley), RDM15-5B (α *leu2-3, -112 ade2 ura3-52 pep4-3 sec61-2*) (8), RDM43-9C (α *his4 ura3-52 Δpep4::URA3 sec62-1*) (9), PBY405A (**a** *his4 leu2 ura3 suc2-Δ9*) (10), W3031B-H (α *can1-100 leu2-3,-112 trp1-1 ura3-1 ade2-1*) (5), DWY-1 (**a**/α *can1-100/can1-100 leu2-3, -112/leu2-3,-112 trp1-1/TRP1 his3-11/HIS3 ura3-1/ura3-1 ade2-1/ade2-1 kar2::LEU2/KAR2*) (5).

Construction of a Yeast Strain That Conditionally Expresses BiP. A 2.4-kilobase (kb) *Dra* I–*Xho* I fragment containing the entire *KAR2* coding sequence, 47 base pairs (bp) of 5′ flanking region, and 0.45 kb of 3′ flanking sequence (5) was cloned into plasmid pYF466 (a gift of James Friesen, University of Toronto) adjacent to the *GAL10* promoter. The *GAL10-KAR2* fusion gene was excised and inserted into a centromere- and *URA3*-containing yeast shuttle vector. This plasmid was used to transform the diploid yeast strain DWY-1, which contained one wild-type *KAR2* allele and one allele that had been disrupted by insertion of the *LEU2* gene (5). Several Ura$^+$ transformants were sporulated, and spores were selected (on galactose plates) for leucine and uracil prototrophy. The strain used for subsequent studies (DWY-2GK) had the following genotype: α *can1-100 leu2-3,-112 ura3-1 ade2-1 kar2::LEU2* with *URA3* and the *GAL10-KAR2* fusion gene present on the plasmid.

Cell Radiolabeling and Immunoprecipitations. Metabolic radiolabeling and immunoprecipitation of secreted and intracellular forms of invertase were performed as described by Deshaies and Schekman (8). Invertase was analyzed by SDS/PAGE (10% gel).

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Abbreviations: BiP, binding protein; ER, endoplasmic reticulum; hsp70, heat shock protein(s) of ≈70 kDa.

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Radiolabeling of α factor was performed in the same manner as for invertase. The labeling medium was adjusted to contain 1.4% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride and was used for isolation of secreted α factor. To obtain intracellular α-factor precursor, spheroplasts were lysed by boiling 4 min in 1% sodium dodecyl sulfate (SDS), and the lysate was adjusted to 0.07% SDS/1.4% Triton X-100/1 mM phenylmethylsulfonyl fluoride. Medium and lysate samples were precleared with *Staphylococcus aureus* cells and then incubated for 3 hr on ice with 1–4 μl of rabbit anti-α factor antiserum (provided by Dennis Shields, Albert Einstein College of Medicine, Bronx, NY). Intracellular α-factor immune complexes were isolated as described by Waters *et al.* (11) with analysis by SDS/PAGE in 4 M urea (18% gel). Secreted α-factor immune complexes were isolated as described for invertase with analysis by standard SDS/PAGE (15% gel).

Subcellular Fractionation and Proteolysis of Cell Extracts. DWY-2GK cells incubated for 8–10 hr in YPD were derepressed for invertase synthesis as necessary and radiolabeled for 1–2 hr. Isolation of microsomal and cytosolic fractions and assessment of the sensitivity of accumulated prepro-α-factor to proteinase K digestion were performed as described (8). As an internal control for vesicle intactness, either radiolabeled *sec18-1* cells or tunicamycin-treated wild-type cells (W3031B-H) were pooled with DWY-2GK prior to spheroplast formation and homogenization. *sec18-1* cells accumulate core glycosylated invertase, and tunicamycin-treated W3031B-H cells accumulate unglycosylated pro-α-factor within microsomal vesicles. *sec18-1* cells were grown at room temperature in YPD, preincubated in minimal medium containing 0.1% glucose for 10 min at 38°C, and then radiolabeled at 38°C for 1 hr. W3031B-H cells were grown in YPD, preincubated in SD containing 20 μg of tunicamycin per ml for 30 min, and radiolabeled in the presence of tunicamycin for 1 hr.

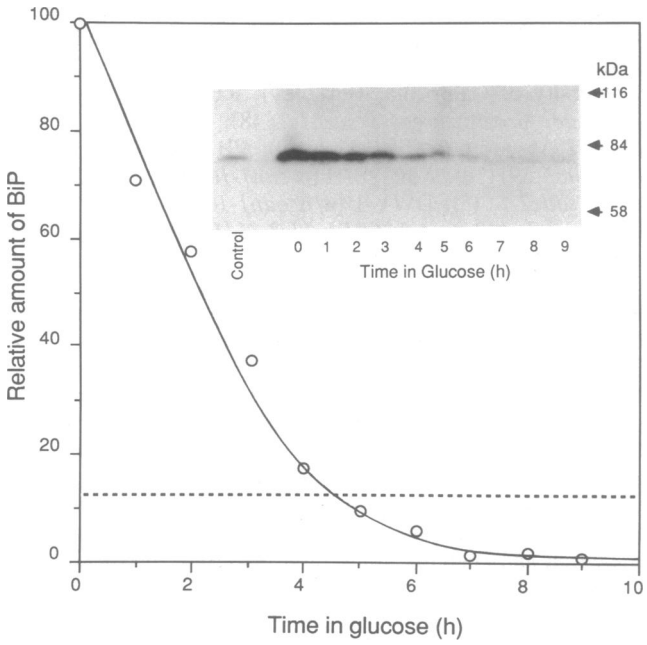

FIG. 1. Conditional expression of BiP protein. DWY-2GK cells were transferred to glucose-containing medium and incubated for the indicated times. Lysate equivalent to 8×10^7 cells at each time point was subjected to SDS/PAGE (7.5% gel). W3031B-H cells were used as a control for normal BiP level. Proteins were transferred to nitrocellulose and were probed with a rabbit antiserum raised against the N-terminal BiP peptide, CAADDVENYGTVI (provided by M.-J. Gething, University of Texas Southwest Medical Center, Dallas; ref. 7). (*Inset*) Bound antibodies were detected with ^{125}I-labeled protein A and visualized by autoradiography. Quantitative analysis of the autoradiogram was performed by densitometry. The amount of BiP detected in control cells is indicated by the horizontal dashed line. h, Hour(s).

RESULTS

Conditional Expression of Yeast BiP. We showed previously (5) that the BiP-encoding *KAR2* gene is essential. To examine the function of BiP in more detail, we developed a strain in which the expression of BiP is regulated by the glucose-repressible *GAL10* promoter (12). When grown in galactose, this strain overproduced BiP mRNA and BiP protein by about 5- and 10-fold, respectively, relative to haploid control cells. However, within 15 min of transfer to glucose, no BiP mRNA could be detected (not shown). A longer period was required to deplete cells of BiP protein. It dropped to the level present in control cells after 4.5 hr in glucose and to about 15% of control after 8–9 hr (Fig. 1). At later times (not shown), there was little further reduction in BiP level, most likely due to an arrest of cell growth.

BiP depletion resulted in growth arrest and loss of viability. Fig. 2*A* shows a lowered growth rate after 8 hr of incubation in glucose and complete arrest by 10 hr. Viability of cells grown in glucose did not drop significantly for 8 hr (Fig. 2*B*), but then decreased to ≈65% by 10 hr.

Effects of BiP Depletion on Protein Secretion. We examined the biogenesis of two yeast secretory proteins, invertase and α factor, in BiP-depleted cells. *S. cerevisiae* synthesizes two forms of invertase, a constitutively expressed cytoplasmic form (≈60 kDa) and a highly glycosylated secreted form that is regulated by glucose repression. Both are products of the *SUC2* gene, but the cytoplasmic form is synthesized without an amino-terminal signal sequence (13). α factor is a 13-amino acid mating pheromone that is secreted by cells of α mating type. It is initially synthesized as a 19-kDa precursor (prepro-

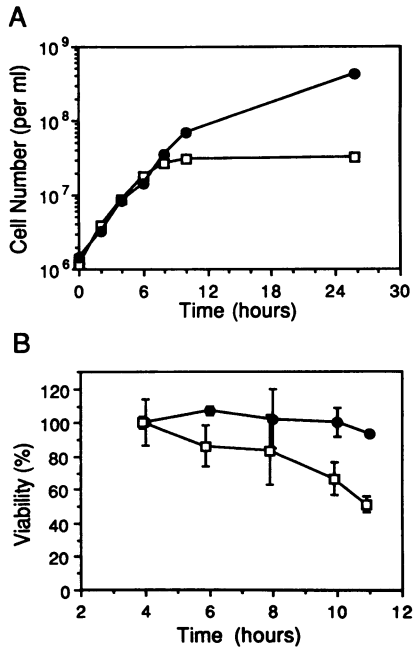

FIG. 2. Effects of BiP depletion on cell growth and viability. Cells (DWY-2GK) were initially suspended in YPG (2% galactose) (●) or YPD (2% glucose) (□). (*A*) Growth was followed by counting aliquots of cells at the times indicated. (*B*) At the indicated times, samples were dispersed by passage through a 25-gauge syringe needle and spread in duplicate on YPG plates. Colonies were counted, and percentage viabilities were expressed relative to the number of viable cells at the 4-hr time point. Error bars represent the range of the duplicate samples.

α-factor) that is converted to a 26-kDa form (pro-α-factor) following signal cleavage and N-linked glycosylation (14).

Cells that had been incubated for various durations in galactose- or glucose-containing medium were radiolabeled and subjected to immunoprecipitation to recover secreted and intracellular forms of α factor and invertase. Fig. 3*A* shows that highly glycosylated invertase ($M_r \approx 120$–200 kDa) was secreted normally by galactose-grown cells and by cells grown in glucose for up to 6.5 hr. However, by 8.5 hr in glucose, when BiP levels dropped to about 15% of normal, secretion appeared reduced. It decreased to trace levels by 10.5 hr and was undetectable thereafter. Similarly, secreted α factor (Fig. 3*B*) was readily detected after 6.5 hr in glucose, but it decreased to trace levels by 8.5 hr and disappeared by 10.5 hr of incubation.

Cell lysates were examined to determine if invertase and α-factor precursors were accumulating intracellularly. Whereas only trace amounts of the constitutive, cytoplasmic form of invertase could be detected in galactose-grown cells at all times (inv in Fig. 3*C*), there was a marked accumulation of a form that comigrated with this species in cells incubated for 8.5 hr in glucose. Since this time coincided with the onset of the secretion defect, the accumulated species most likely represents a precursor of secreted invertase that is unglycosylated and has undergone signal sequence cleavage. Upon continued incubation in glucose, a second form appeared that comigrated with invertase and accumulated in the translocation-defective mutant *sec61* (pinv), suggesting that it represents invertase with an uncleaved signal sequence (8). The signal-cleaved form of invertase predominated at all times examined. Neither of the two accumulated forms was glycosylated, since neither reacted with endoglycosidase H; both forms retained enzymatic activity (data not shown).

The results obtained for intracellular α-factor precursor are shown in Fig. 3*D*. A single form of α-factor precursor accumulated in glucose-treated cells beginning at 6.5 hr of incubation, somewhat sooner than observed for invertase. This form comigrated with the unglycosylated, signal sequence-containing prepro-α-factor accumulating in the translocation-defective mutant *sec62* (9). Thus, the secretion defect observed in BiP-depleted cells is accompanied by the intracellular accumulation of unglycosylated precursors that either possess (α factor and invertase) or lack (invertase) an intact signal sequence. This phenotype is not a consequence of growth arrest or cell death, since it first appeared prior to growth arrest and loss of cell viability (Fig. 2).

Subcellular Localization of Accumulated Secretory Proteins in BiP-Depleted Cells. The observed accumulation of precursors could be due to a block in translocation across the ER membrane or to defects in signal cleavage and glycosylation. To distinguish between these possibilities, we initially determined whether the accumulated precursors were present in microsomal vesicles or the cytosol.

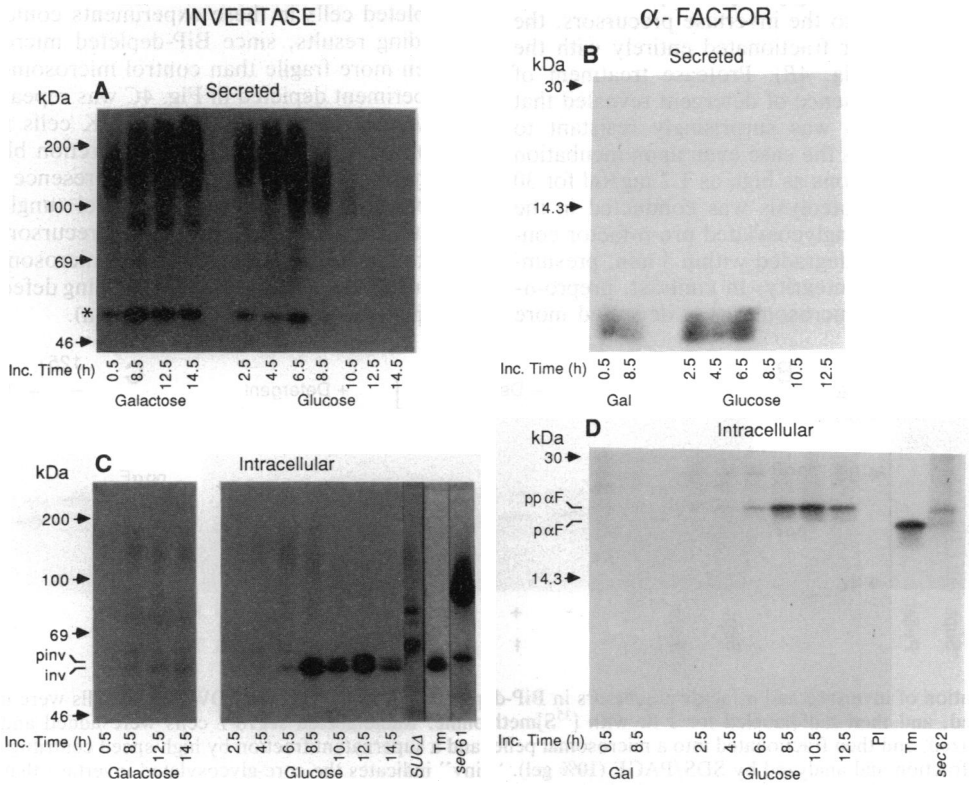

FIG. 3. Accumulation of invertase and α-factor precursors in BiP-depleted cells. DWY-2GK cells were incubated in YPG or YPD. At various incubation times (Inc. time in hours), aliquots were removed and either incubated for 30 min in minimal medium containing 0.1% glucose to derepress invertase synthesis or incubated in SG or SD for the same period (α-factor analysis). The cells were then radiolabeled with [^{35}S]methionine for 30 min. The incubation times shown represent the time elapsed prior to addition of the radiolabel. Media, periplasm, and spheroplast fractions were used for the immunoprecipitation of secreted α factor, secreted invertase, and intracellular forms of α factor and invertase, respectively. (*A*) Secreted invertase. The asterisk indicates a contaminating glycoprotein (endoglycosidase H sensitive) that is unrelated to invertase on the basis of its molecular weight and lack of heterogeneity. It may have been isolated because of low levels of anti-carbohydrate antibodies in the antiserum. (*B*) Secreted α factor. (*C*) Intracellular invertase. Lanes: *sec61*, invertase isolated from *sec61* cells derepressed and radiolabeled at the restrictive temperature [37°C; note that the translocation block is not complete at this temperature (9)]; Tm, invertase isolated from *sec61* cells derepressed and radiolabeled at 22°C in the presence of 20 μg/ml of tunicamycin per ml; SUCΔ, immunoprecipitate from a lysate of the invertase-deficient strain PBY405A. "inv" indicates the constitutive, cytoplasmic form of invertase; "pinv" indicates preinvertase. (*D*) Intracellular α-factor precursor. Lanes: sec62, α-factor precursor isolated from *sec62* cells radiolabeled at the restrictive temperature (38°C); Tm, α-factor precursor isolated from W3031B-H cells radiolabeled at 30°C in the presence of 20 μg of tunicamycin per ml; PI, preimmune control. "pαF" indicates unglycosylated pro-α-factor; "ppαF" indicates prepro-α-factor.

Microsomal and cytosolic fractions were prepared from radiolabeled cells depleted of BiP for a period of 8 hr. As an internal control to assess release of soluble invertase from the ER lumen, *sec18* cells that accumulate glycosylated secretory proteins in the ER were radiolabeled at their restrictive temperature and then mixed with the BiP-depleted cells prior to homogenization. Virtually all of the invertase that accumulated in BiP-depleted cells was present in the supernatant (cytosolic) fraction (Fig. 4*A*). Less than 5% of the invertase that accumulated in *sec18* cells was released from within vesicles as a consequence of homogenization. Thus, in BiP-depleted cells, translocation of invertase into the ER is inhibited. The fact that invertase rather than preinvertase accumulated in these cells after 8 hr in glucose (Fig. 3*C*) indicates that some degree of translocation did occur for all invertase molecules. However, they clearly did not become stably associated with the ER membrane. This experiment was repeated in cells depleted of BiP for 10 hr, when both invertase and preinvertase accumulate (Fig. 3*C*). Preinvertase could not be recovered in this analysis, probably due to artifactual signal cleavage during homogenization. Consistent with the findings at 8 hr, signal-cleaved invertase was detected only in the supernatant fraction (data not shown).

A similar approach was used to localize the prepro-α-factor precursor in BiP-depleted cells except that the control for vesicle integrity was provided by tunicamycin-treated wild-type cells, which accumulate unglycosylated pro-α-factor in the ER lumen. In contrast to the invertase precursors, the accumulated prepro-α-factor fractionated entirely with the microsomal membranes (Fig. 4*B*). Protease treatment of these microsomes in the absence of detergent revealed that the prepro-α-factor species was surprisingly resistant to digestion (Fig. 4*B*). This was the case even upon incubation with protease at concentrations as high as 1.2 mg/ml for 30 min (not shown). When proteolysis was conducted in the presence of detergent, the unglycosylated pro-α-factor control was almost completely degraded within 3 min, presumably due to loss of vesicle integrity. In contrast, prepro-α-factor in the BiP-depleted microsomes was degraded more slowly, with 21% still intact after 6 min (Fig. 4*B*) and ≈10% intact after 15 min (not shown). This degree of resistance was not an inherent property of prepro-α-factor because previous studies on several translocation-defective strains have shown this precursor to be completely degraded in <4 min whether or not detergent is present (8, 9). These findings suggest that the accumulated prepro-α-factor in BiP-depleted cells may be in an aggregated form or in tight association with components of the microsomal membrane. Consequently, the sensitivity of this precursor to exogenous protease may not provide a reliable measure of its disposition in the membrane.

As an independent approach to this question, we asked if the accumulated prepro-α-factor is accessible to lactoperoxidase-catalyzed iodination. Since the iodination occurs via a reactive intermediate that diffuses from the enzyme (15), this technique can potentially radiolabel prepro-α-factor that may be sterically inaccessible to protease. The extent of labeling of the pro-α-factor control increased 4-fold upon addition of detergent, consistent with its luminal location (Fig. 4*C*). In contrast, the accumulated prepro-α-factor was readily accessible to radioiodination in the absence or presence of detergent. Taken together with the absence of signal cleavage and lack of core glycosylation, the data are most consistent with defective translocation of prepro-α-factor in BiP-depleted cells.

We were concerned that mixing control cells with BiP-depleted cells in these experiments could give rise to misleading results, since BiP-depleted microsomes may have been more fragile than control microsomes. Therefore, the experiment depicted in Fig. 4*C* was repeated, except that in place of control cells, DWY-2GK cells were treated with tunicamycin just prior to the secretion block and then BiP depletion was continued in the presence of the drug. This captured both the luminal control (unglycosylated pro-α-factor) and the prepro-α-factor precursor in the same cell. Subsequent iodination of these microsomes gave identical results to those in Fig. 4*C*, confirming defective translocation of prepro-α-factor (data not shown).

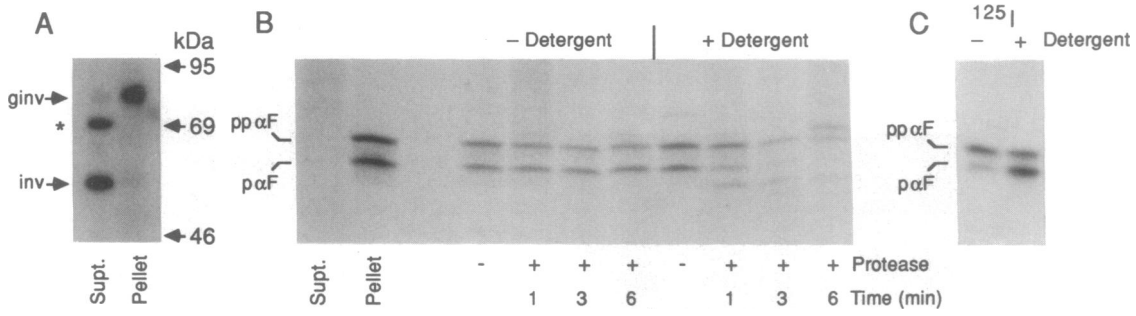

FIG. 4. Localization of invertase and α-factor precursors in BiP-depleted cells. (*A*) Invertase. DWY-2GK cells were incubated for 8 hr in glucose, derepressed, and then radiolabeled for 1 hr with [^{35}S]methionine. Radiolabeled *sec18-1* cells were added and spheroplasts were prepared, homogenized, and then fractionated into a microsomal pellet and a supernatant fraction by high-speed centrifugation. Invertase was isolated from each fraction and analyzed by SDS/PAGE (10% gel). "ginv" indicates the core-glycosylated invertase that accumulates in the ER of *sec18* cells; "inv" indicates invertase precursor that accumulates in DWY-2GK cells. A contaminating band that is also present in preimmune controls is shown by the asterisk. (*B*) α factor. DWY-2GK cells incubated in glucose for 9 hr were radiolabeled for 2 hr, and W3031B-H cells treated with tunicamycin were radiolabeled for 1 hr. The cells were mixed, and spheroplasts were prepared. The mixture was homogenized, and the cell-free lysate was separated into three portions: one was fractionated at high speed into a microsomal pellet (lane Pellet) and a supernatant fraction (lane Supt.) prior to α-factor isolation. Of the remaining two portions, one was adjusted with buffer (– detergent lanes) and the other adjusted to 0.1% Triton X-100 (+ detergent lanes). Equal aliquots of each portion were digested in the absence or presence of 0.3 mg of proteinase K per ml on ice for the periods shown. Reactions were terminated with trichloroacetic acid; precipitated proteins were solubilized and subjected to immunoprecipitation and analysis by SDS/PAGE in 4 M urea (18% gel). "ppαF" indicates prepro-α-factor; "pαF" indicates unglycosylated pro-α-factor. (*C*) ^{125}I radiolabeling of α-factor precursor. DWY-2GK cells were incubated in glucose medium and mixed with tunicamycin-treated W3031B-H cells as described in *B*. After spheroplast formation and homogenization, the cell-free lysate was sedimented onto a pad of 65% sucrose. The microsomal vesicles obtained from the interphase were divided into two aliquots, one of which was adjusted to 0.1% Triton X-100. Both aliquots were radioiodinated in a volume of 0.1 ml containing 3.4 units of lactoperoxidase, 5.8 units of glucose oxidase, and 0.5 mCi of Na^{125}I (1 Ci = 37 GBq) for 20 min on ice. The reaction was terminated with 0.1% NaN_3, and proteins were precipitated with trichloroacetic acid. Protein pellets were solubilized and subjected to immunoprecipitation and analysis by SDS/PAGE in 4 M urea (18% gel). Designations are as in *B*.

DISCUSSION

Depletion of BiP to about 15% of normal level leads to a block in the translocation of secretory proteins that appears to occur at a relatively late stage. For most invertase molecules, the block is at a point where the polypeptide penetrates the lumenal side of the ER, at least far enough to permit signal cleavage. Surprisingly, invertase molecules are not stably associated with the membrane at this stage. Preinvertase molecules do appear after prolonged periods of BiP depletion and may arise due to jamming of translocation sites by precursors of other proteins that remain membrane-associated—e.g., prepro-α-factor. For the α-factor precursor, translocation arrest occurs exclusively prior to signal peptide cleavage. Prepro-α-factor sediments with microsomal membranes and is exposed on the cytoplasmic side of the membrane as judged by vectorial radioiodination. However, its protease resistance in the absence of detergent suggests that it may penetrate significantly into the membrane prior to arrest, perhaps forming complexes with other molecules within the membrane as well as at the cytosolic surface. That both invertase and prepro-α-factor appear to be arrested at a relatively late stage in translocation is consistent with the expectation that yeast BiP, like its mammalian counterpart, is localized to the ER lumen (1).

The translocation of α-factor and invertase precursors differed in threshold requirements for BiP. Translocation arrest of prepro-α-factor was first observed after BiP had been depleted to ≈40% of its normal level; at this point, invertase translocation and secretion were not impaired. Furthermore, even when translocation of invertase was arrested (≈15% of normal BiP level), it apparently penetrated the ER membrane to a greater extent than did prepro-α-factor, suggesting that translocation of the N-terminal portion of preinvertase has a minimal requirement for BiP function. Studies on the translocation-defective *sec* mutants have also shown that invertase is less severely affected than other secretory proteins including α-factor precursor (9, 16). It is notable that the invertase signal differs significantly from those of other secretory and vacuolar proteins (9).

How does BiP function to promote translocation? As suggested by Rothman (2), BiP may bind to certain sites on nascent polypeptides as they emerge into the ER lumen. This interaction may prevent aggregation, thereby allowing time for productive folding or subunit assembly events to occur. In this model, BiP depletion should result in aggregation of the nascent chain with other nascent proteins or with resident molecules of the ER near the translocation site, a situation that could prevent further translocation. The phenotype of accumulated prepro-α-factor is consistent with this view. Its N-terminal portion may partially penetrate to the ER lumen (at a point prior to signal cleavage) where, in the absence of BiP binding, it becomes complexed to other molecules and remains firmly membrane-associated. Invertase behavior is more difficult to explain. Its translocation has a less stringent requirement for BiP than prepro-α-factor, which may reflect higher affinity BiP binding sites, particularly within the signal sequence. However, upon arrest, the accumulated signal-cleaved species does not behave as a component of a large or insoluble aggregate and is readily released to the cytosol. An alternative possibility is that BiP acts indirectly by maintaining the activity of some component of the translocation machinery.

While this paper was under review, Vogel *et al.* reported that yeast cells harboring a temperature-sensitive mutation in the *KAR2* gene were defective in translocation of several soluble proteins across the ER membrane (17). In contrast to our results, all proteins accumulated exclusively in a signal-uncleaved form (including invertase) and were accessible to proteolytic digestion. A possible explanation for the different phenotypes is that, in our experiments, BiP could not be depleted beyond 10–15% of wild-type level. This level may support translocation of certain proteins, thereby preventing the complete jamming or inactivation of translocation sites that is thought to occur in the temperature-sensitive mutant (17). Thus, in the present study, translocation sites may not be severely limiting, and nascent chain can initiate translocation and penetrate the membrane to an extent that is dictated by their individual requirements for BiP function.

Recently, Dorner *et al.* (18) examined BiP function in CHO cells by using antisense BiP mRNA to reduce BiP protein to low levels. In contrast to our findings with yeast, no effects on cell growth or viability were observed. This may reflect differences in BiP function between yeast and mammals or, more simply, it may reflect that in CHO cells the threshold level of BiP below which essential processes are affected may be quite low compared with yeast.

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