The Role of the Carrier Protein and Disulfide Formation in the Folding of β-Lactamase Fusion Proteins in the Endoplasmic Reticulum of Yeast*

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We have studied the relationship between folding and secretion competence of hsp150-β-lactamase fusion proteins in Saccharomyces cerevisiae. hsp150 is a secretory protein of yeast, and β-lactamase was chosen, since its folding can be monitored by assaying its enzymatic activity. The hsp150 pre-pro-protein consists of a signal peptide, subunit I, a repetitive region, and a unique C terminus. Fusion of β-lactamase to the C terminus of hsp150 produced Cla-bla protein, which was secretion competent but inactive. The Pst-bla protein, where β-lactamase was fused to subunit I, was also inactive and mostly secreted, but part of it remained in the per-Golgi compartment. When β-lactamase was fused to the C terminus of the repetitive region, the fusion protein (Kpn-bla) was translocated to the endoplasmic reticulum, acquired disulfide bonds, and adopted an enzymatically active conformation. Kpn-bla was secreted to the medium without decrease of specific activity or retention in the cell. Folding of Kpn-bla to an active and transport-competent form was required co-translationally, disulfide formation, since treatment of cells with dithiothreitol resulted in endoplasmic reticulum-retained inactive Kpn-bla. When dithiothreitol was removed, Kpn-bla resumed transport competence but remained inactive. Reduction of prefolded Kpn-bla did not inhibit enzymatic activity or transport. The repetitive hsp150 carrier may have use in heterologous protein production by conferring secretion competence to foreign proteins in yeast.

Correct folding is a prerequisite for intracellular transport of proteins in mammalian cells. ER proteins like BiP have been suggested to assist the folding process and to exert quality control by binding to immature and malfolded proteins (1-5). Folding studies of proteins in the ER of yeast have lagged behind, although yeast offers a genetic approach to characterize relevant proteins. Moreover, heterologous proteins are usually not secreted in yeast without a carrier polypeptide (6). Thus, the understanding of protein folding and acquisition of a secretion-competent conformation in Saccharomyces cerevisiae is important for the development of production systems to secrete correctly folded heterologous proteins to the culture medium of yeast. The relationship of folding and transport competence has been studied in mammalian cells by altering the presence of relevant proteins. Moreover, heterologous proteins are usually not secreted in yeast without a carrier polypeptide (6).

MATERIALS AND METHODS

Strains and Media—The S. cerevisiae strains H1 (SEY2101a, MATa, ade2-101 ura3-52 leu2-3,112 sad1-29 gal1), sec18 (mby12-60, Matb sec18-1 trp1-289 leu2-3,112 ura3-3 his5), sec63 (RSY153, Matu sec63-1 leu2-3,112 ura3-32), and the transformants H337 (H1, Cla-bla), H335 (H1, Kpn-bla), H393 (sec18, Kpn-bla), H396 (H1, Pat-bla), H421 (sec18, Pat-bla), and H422 (sec63, Pat-bla), were grown at 24 °C in YPD medium. Escherichia coli strains XL-1 Blue (Stratagene) and DH5a (Life Technologies, Inc.) were grown in LB medium supplemented with ampicillin (100 μg/ml) or tetracycline (12.5 μg/ml).

Construction of the HSP150-bla Fusion Genes and the Integration Vector—From the genomic clone of HSF150 (11), a SalI-ClaI DNA fragment, which contains the HSP150 gene devoid of its four last codons, plus approximately 2 kb of upstream sequence, was cloned between the SalI and ClaI sites of Bluescript II SK- (Stratagene). The 450 bp ADCl terminator from pAAHS (12) was ligated downstream from HSF150, between the HindIII and BamHI sites. The KpnI site of the vector was removed by digesting approximately 100 bp of the plasmid with Bal31 nuclease starting from the SalI site, and a BamHI linker was added. The bla gene without its signal sequence was synthesized with polymerase chain reaction using Pfua polymerase (Stratagene), and pUC8 as template. The 3' primer A (5'-GCAAACAAAATTCAATCTGCAGACATGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTTGTTG
staining with antiserum diluted 1:1000, using the ECL™ Western blotting reagents (Amersham), according to manufacturer’s instructions.

Assay of β-Lactamase Activity—The β-lactamase activity was assayed using Nitrocefin (Glaxo), according to Ref. 16, but at room temperature. Culture medium samples were assayed after removal of the cells by centrifugation. For the determination of β-lactamase activity of whole cells, the pelleted cells were lysed and suspended in 100 mM potassium phosphate, pH 7, to the original sample volume. To determine the β-lactamase activity entrapped in the cell wall (Nitrocefin is non-penetrable), washed cells were suspended in 1 mM of potassium phosphate buffer containing 10 mM NaN₃, to the original volume. The intracellular activity was calculated by subtracting the cell wall activity from the whole cell activity. Secretory β-lactamase was not nonspecifically bound to the cells, as shown by assaying the β-lactamase activity of H1 cells incubated with Kpn-bla-containing growth medium.

In Vitro Translation—The Pst-bla construct was placed under the control of the lac promoter in PUC18, at the BamHI site. The plasmid was digested with PvuII, and a 1.9-kb fragment containing both the promoter and the fusion gene was isolated to serve as a template for in vitro transcription/translation, using the E. coli S30 Extract System for Linear Templates (Promega) according to manufacturer’s instructions.

Other Procedures—Northern analysis of total RNA was performed, as described (17). The stability of the transformants was studied by growing them to early, middle, and late logarithmic phase in YPD medium, followed by plating on YPD plates. The β-lactamase activity of 60 colonies from each plate was assayed by mixing them with Nitrocefin solution on microtiter plates; all produced β-lactamase activity and grew on SC-URA plates. SDS-PAGE was in reducing 8% gels unless otherwise stated. Non-reducing SDS-PAGE and in vivo protein reduction were performed as before (10). Precipitation was with 1.4% trichloroacetic acid for 1 h on ice. Zymolase 100T was from Seikagaku; cycloheximide and DTT were from Sigma. For scanning, an LKB Ultrascan XL laser densitometer was used.

RESULTS

Fusion of the β-Lactamase Gene to Fragments of the HSP150 Gene—The product of the HSP150 gene is composed of four domains: (i) the signal peptide, (ii) subunit I, (iii) the repetitive region (rr) of subunit II, and (iv) the C-terminal region of subunit II (Fig. 1A). The repetitive region consists of an 11-fold repeat of a 19-amino acid peptide. The signal peptide is cleaved off in the ER, and subunit I is detached from subunit II at a kex2 recognition site. The subunits remain noncovalently associated and are efficiently secreted to the culture medium (11). We wanted to study whether fusion of β-lactamase to fragments of hsp150 would allow folding of the β-lactamase portion to an enzymatically active conformation, and whether the fusion protein would be secreted to the growth medium. The E. coli TEM β-lactamase gene, bla, lacking its signal sequence, was fused to various portions of the HSP150 gene. β-Lactamase was chosen as a reporter, because its folding can be monitored by determining its enzymatic activity (18) and because it is not secreted in S. cerevisiae in its authentic form (19, 20). The largest construct, Cla-bla, lacked the last four codons of HSP150. Kpn-bla contained the signal sequence, and codons for SUI and the repetitive region.

Transcription of the Chimeric Genes—The chimeric genes were under the control of the heat-regulated HSP150 promoter (17). Two RNA samples were extracted: one from cells incubated at 24 °C (HS−) and the other from cells heat-shocked for 30 min at 37 °C (HS+). Northern analysis using an HSP150 probe (Fig. 1B, panel a) and a bla probe (panel b) showed that...
the transformants synthesized novel mRNA molecules, in addition to HSP150 mRNA of 1.6 kb. The chimeric mRNAs hybridized with the bla probe (panel b), and the HSP150 probe (panel a), but hybridization of Pst-bla mRNA with the HSP150 probe was not detectable (panel a, lanes P). The chimeric genes were heat-regulated like HSP150 (compare HS− to HS+). The amounts of the samples were controlled by hybridization with the actin gene ACT1, which is not heat-regulated (panel c).

Secretion of the hsp150-β-Lactamase Fusion Proteins—The expression and secretion of the fusion proteins were studied by labeling them with [35S]methionine/cysteine for 1 h at 37 °C, followed by a 20-min chase with cycloheximide to stop protein synthesis. The culture media and cell lysates were subjected to immunoprecipitation and SDS-PAGE analysis. Immunoprecipitation with anti-hsp150 detected from the culture medium of H337 cells (Cla-bla) hsp150 of 150 kDa, and a new protein of about 135 kDa (Fig. 2A, lane 1; m, medium). The new protein was also detected with anti-β-lactamase (lane 3), and was designated Cla-bla. Cla-bla was detected also in the cell lysate with both antisera (lanes 2 and 4; c, cell lysate). Preimmune serum recognized no proteins (lanes 5 and 6).

Immunoprecipitation of the culture medium of H335 cells (Kpn-bla), [35S]labeled like the H337 cells above, revealed a new protein of about 135 kDa which was recognized by anti-hsp150 (Fig. 2B, lane 1) and anti-β-lactamase (lane 3). It was designated Kpn-bla. Very little of Kpn-bla-related proteins could be detected in the lysate (lanes 2 and 4), indicating that Kpn-bla was secreted efficiently to the growth medium.

When H395 cells (Pst-bla) were analyzed, anti-hsp150 detected only hsp150 (Fig. 2C, panel a, lanes 1 and 2), because the antiserum was raised against subunit II (see Fig. 1A). Anti-β-lactamase detected a 48-kDa protein from the culture medium (lane 3), and a 44-kDa protein from the cell lysate (lane 4). According to densitometric scanning, about 35% of Pst-bla (32 and 38% in two experiments) remained cell-associated, and the rest was secreted. The 44-kDa protein was the pre-Golgi form, since Pst-bla labeled at 37 °C in H421 cells migrated like a 42-kDa protein (Fig. 2C, panel b, lane 1). In the H421 strain (sec18), secretory proteins accumulate in the pre-Golgi compartment at 37 °C. When Pst-bla was arrested in the cytoplasm in the translation-deficient H422 cells (sec63), it migrated like a 38-kDa protein (lane 2), like the in vitro transcription/translation product of the Pst-bla gene (lane 3).

To compare the relative amounts of the three fusion proteins secreted to the culture medium, they were [35S]-labeled and subjected to immunoprecipitation and SDS-PAGE analysis in parallel. According to scanning of the bands, similar amounts of Cla-bla (10 Met, 6 Cys) and Kpn-bla (9 Met, 4 Cys) were detected in the media (Fig. 3A, lanes 1 and 2, open arrowhead), whereas the amount of Pst-bla (9 Met, 2 Cys) was roughly one-fourth of that of Cla-bla (lane 3, black arrowhead). This was confirmed by Western analysis of culture medium samples of strains H1, H337 (C), H335 (K) and H395 (P), using anti-β-lactamase (Fig. 3B, panel a) and anti-hsp150 (panel b). Thus, Kpn-bla was efficiently secreted to the culture medium, apparently without significant retardation in secretory organelles, or in the cell wall or periplasm. Cla-bla was transport-competent, part of it remaining cell-associated. Pst-bla was translocated to the ER, but only small amounts were secreted. Pst-bla was evidently harmful for the cells, since the generation time of strain H395 was 3 h, 25 min, whereas that of the other transformants and the parental strain was 2 h.

β-Lactamase Activity of the Fusion Proteins—To monitor the folding of the fusion proteins, their β-lactamase activities were measured. When strain H335 (Kpn-bla), was incubated at 24 °C, 0.31 unit/ml β-lactamase activity was in the culture medium after 3 h (Fig. 4C, triangles). Much less activity was found in the cell wall/periplasm (circles) and intracellularly (diamonds). When the incubation was performed at 37 °C, to enhance synthesis by heat shock, 1.62 units/ml β-lactamase activity was detected in the culture medium, 0.29 unit/ml in the cell wall, and only traces intracellularly (Fig. 4D). Very little β-lactamase activity could be detected at either temperature in the media or cells of strains H337 (Cla-bla) (Fig. 4, A and B) or H395 (Pst-bla) (Fig. 4, E and F).

To study whether the fusion proteins were enzymatically active in the ER, the Kpn-bla and Pst-bla constructs were expressed in a sec18 mutant (strains H393 and H421, respectively). When H393 (Kpn-bla) was incubated for 90 min at 37.5 °C, β-lactamase activity accumulated inside the cells (Fig.
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Then, and subjected to SDS-PAGE. The proteins were blotted and immunostained with anti-β-lactamase (panel a) or anti-hsp150 (panel b). Open arrowhead, Cla-bla and Kpn-bla; black arrowhead, Pst-bla; star, hsp150. Molecular size markers are on the left.

54A, triangles), and very little was in the medium (closed circles). When cycloheximide was added (arrowhead), and the cells were shifted to 24 °C, the cell-associated activity decreased (closed triangles), while the activity in the medium increased, showing that the accumulated fusion protein was secreted. Thus, the β-lactamase portion of the Kpn-bla fusion protein folded in the ER to an active conformation, and the specific activity of the fusion protein did not change significantly after exit from the ER. In contrast, the Pst-bla molecules accumulating in the ER in strain H421 had very little enzymatic activity (Fig. 5B).

The Effect of Reduction of the Disulfides of Kpn-bla on Its Transport Competence—Next we wanted to manipulate the conformation of intracellular Kpn-bla by reducing its possible disulfides (10), to study the consequences on transport. H335 cells were metabolically labeled in the presence of DTT. Immunoprecipitations with anti-hsp150 (Fig. 6A, panel a) and anti-bla (panel b) showed that the fusion protein was not secreted (lanes 3). Cell-associated Kpn-bla was barely visible (lanes 4), probably due to heterogeneity of the glycanes. Nevertheless, it could be efficiently chased to the growth medium after the removal of DTT (lanes 5–12). Thus, under normal conditions, Kpn-bla acquired disulfides, which were reduced by DTT treatment, leading to reversible intracellular retention.

When Kpn-bla was synthesized in the presence of DTT, β-lactamase activity could not be detected in the culture medium (Fig. 6B, panel b, closed circles) or intracellularly (open circles). Removal of DTT (arrowhead) did not increase β-lactamase activity. In vitro reduction of secreted Kpn-bla did not activate the enzyme (not shown). Thus, synthesis under reducing conditions produced inactive Kpn-bla, and removal of DTT resulted in resumption of transport competence of the molecules. However, the reoxidized structure was different from the native one, since it had no enzymatic activity.

Kpn-bla was then allowed to fold in the absence of DTT, while retaining it at 37.5 °C in the ER in strain H393 (sec18) (Fig. 5A).

Part of the cells were incubated further at 24 °C in the presence of cycloheximide only, which led to secretion of the molecules, as described above (arrowhead, filled symbols). Addition of DTT with cycloheximide to part of the cells had no effect on the se-
creatin or enzymatic activity of Kpn-bla (open symbols). Native and in vivo reduced, ER-trapped Kpn-bla migrated differently in non-reducing SDS-PAGE, the latter comigrating with in vitro lysates (panel a) or anti-β-lactamase (panel b) and analyzed by SDS-PAGE. Small arrowhead, hsp150; large arrowhead, Kpn-bla. B, effect of DTT on β-lactamase activity of Kpn-bla. Strain H335 was incubated at 37 °C in the absence (panel a) or presence (panel b) of DTT. In panel b, the cells were washed after 2 h (arrowhead) and incubated with cycloheximide in the absence of DTT. β-Lactamase activities of the growth media (closed circles) and cell lysate (open circles) were plotted against incubation time.

Fig. 6. A, effect of DTT on secretion of Kpn-bla. Strain H335 (Kpn-bla) was pulse-labeled (p) for 1 h at 37 °C in the absence (lanes 1 and 2; DTT−), or presence of DTT (lanes 3 and 4; DTT+). Parallel cells, labeled in the presence of DTT, were washed and chased (c) at 37 °C with cycloheximide in the absence of DTT for 30 min (lanes 5 and 6), 60 min (lanes 7 and 8), 90 min (lanes 9 and 10), or 120 min (lanes 11 and 12). The growth media (m) and cell lysates (c) were subjected to immuno precipitation with anti-hsp150 (panel a) or anti-β-lactamase (panel b) and analyzed by SDS-PAGE. Small arrowhead, hsp150; large arrowhead, Kpn-bla. B, effect of DTT on β-lactamase activity of Kpn-bla. Strain H335 was incubated at 37 °C in the absence (panel a) or presence (panel b) of DTT. In panel b, the cells were washed after 2 h (arrowhead) and incubated with cycloheximide in the absence of DTT. β-Lactamase activities of the growth media (closed circles) and cell lysate (open circles) were plotted against incubation time.

We fused the TEM β-lactamase of E. coli, lacking its own signal sequence, to the C termini of various portions of the yeast secretory glycoprotein hsp150. The chimeric proteins were used to study the effect of the hsp150-carrier sequence on the folding of the β-lactamase portion and the effect of folding on the secretion competence of the fusion proteins. In the largest chimera, Cla-bla, hsp150 lacked its 4 C-terminal amino acids. In Kpn-bla, β-lactamase was fused to the repetitive region. The smallest fusion protein, Pst-bla, contained the signal peptide and two-thirds of subunit I (see Fig. 1A). The folding of the β-lactamase portion was monitored by assaying its enzymatic activity (18). The authentic structural bla gene produces a stable lactamase (19), but the secreted activity was 1/30 that of Kpn-bla. The HSPl50-bla fusion genes were transcribed similarly. Their products were translocated to the ER but differed in β-lactamase activity and secretion competence. Pst-bla did not gain an enzymatically active structure in the ER. Less Pst-bla was detected in the culture medium than Cla-bla or Kpn-bla, and one-third of it appeared to remain in the pre-Golgi compartment. The almost entire hspl50 fragment rendered Cla-bla secretion-competent, some remaining in the Golgi, periplasm, or cell wall. However, Cla-bla had virtually no β-lactamase activity. It seems that the incomplete subunit I and the C-terminal domain of hsp150 interfered somehow with the folding of the β-lactamase portion, causing malfolding.

In contrast to Pst-bla and Cla-bla, Kpn-bla adopted an enzymatically active conformation and acquired disulfide bonds in the ER. It was efficiently secreted to the culture medium, without decrease of specific activity, or retention in the secretory route or the cell wall/periplasm. Partial secretion of active, unstable β-lactamase has been achieved using the invertase signal sequence (21), but the secreted activity was 1/30 that of Kpn-bla.

We were able to distort the conformation of the fusion proteins by reducing their disulfides in vivo. When disulfide formation of Kpn-bla was prevented by DTT treatment of cells, the protein was inactive and retained in the ER. Removal of DTT allowed Kpn-bla to adopt a secretion-competent structure, which was different from the native form, since the enzymatic activity was not restored. DTT treatment distorted the folding of Kpn-bla severely only co-translationally. Once Kpn-bla was allowed to fold normally, subsequent reduction did not affect its enzymatic activity or exit from the pre-Golgi compartment. The role of protein folding in secretion in yeast has been observed before in the case of human α-amylase, where substitution of Cys14 abolished secretion and activity (22). In the case of yeast acid phosphatase, the removal of N-glycosylation sites caused malfolding and irreversible ER retention (23). Reduction of hsp150 and carboxypeptidase Y by DTT treatment resulted in their retention in the ER. This must have been due to cochaperone and not free sulphydryls per se, since sulphydryl-containing invertase was secreted normally in the presence of DTT (10). Thus, yeast clearly has a quality control apparatus, which monitors proteins and retains them in the ER, unless they display acceptable structural features. For foreign proteins, the transport-competent conformation does not need to be the biologically active structure, since Cla-bla, Pst-bla, and reoxidized Kpn-bla were se-
creted, even though they were inactive. Yeast proteins anticipated to be involved in folding of secretory proteins include BiP (Kar2p), protein disulfide isomerase (PDI), and the PDI homologs. Many heterologous proteins expressed in S. cerevisiae without a carrier sequence are not secreted. office, which so far has appeared to have general applicability, is the α-factor leader (6, 31–37). Pro-α-factor is a secretory protein of yeast. Biochemical and physiological properties are released from the leader in the Golgi and are secreted to the medium (38). It is not known how the carrier sequence confers secretion competence to the heterologous protein portion. Probably a functional carrier should form a complete domain to be recognized by the quality control machinery as properly folded and should not interfere severely with the folding of the heterologous protein portion. The repetitive region of the hsp150 is likely to form a structural entity and may have biotechnological potential in production of heterologous proteins to the yeast culture medium, since it confers secretion competence even to mammalian proteins.

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Fig. 7. Continuous secretion of Kpn-bla. A, strain H335 was grown at 24 °C to A600 = 1 (2.5 x 10^6 cells/ml) and divided in two. The cells were pelleted and resuspended in fresh YPD medium to the same density. Half of the suspension was grown at 24 °C (panel a), and the other half at 37 °C (panel b). The β-lactamase activities (units/ml; triangles) and the cell densities (A600; dots) are plotted versus time. B, strain H335 was grown at 30 °C. Samples (1 ml) were taken at the indicated cell densities, and the growth media were subjected to trichloroacetic acid precipitation, SDS-PAGE, and Coomassie Blue staining (lanes 1–7). Lane 8, growth medium of strain H1. Large arrowhead, Kpn-bla; small arrowhead, hsp150. Lane 9, 3.4 µg of BSA (arrow). Molecular size markers are on the left.

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