Hygromycin B resistance as dominant selectable marker in yeast

Kevin R. Kaster, Stanley G. Burgett, and Thomas D. Ingolia

Lilly Research Laboratories, 307 E. McCarry Street, Indianapolis, Indiana 46285, USA

Summary. Saccharomyces cerevisiae is normally sensitive to the drug hygromycin B; a hygromycin B concentration of 200 μg/ml in agar plates is sufficient to completely inhibit growth. We constructed yeast-E. coli bifunctional plasmids which confer hygromycin B resistance to Saccharomyces cerevisiae. Promoters and amino terminal coding regions of a heat shock gene, a heat shock cognate gene, and the phosphoglycerate kinase gene from yeast were fused to a bacterial hygromycin B resistance gene. In all three cases, yeast cells containing plasmids with the hybrid hygromycin B resistance gene were resistant to high levels of the drug. Yeast cells containing these plasmids can also be directly selected after transformation by using hygromycin B. The intact bacterial hygromycin B resistance gene and the kanamycin resistance gene from Tn903 were also tested in yeast for their ability to confer resistance to hygromycin B and G418. The intact bacterial genes were not effective in conferring drug resistance to yeast cells.

Key words: Hygromycin B — Yeast — Plasmids

Introduction

Complementation of auxotrophic markers has been used in yeast transformations in order to select transformed cells. For example, the leu2 (Hinnen et al. 1978), ura3 (Struhl et al. 1979), his3 (Struhl 1982), his4 (Hinnen et al. 1979), and trp1 (Hitzeman et al. 1980) genes from Saccharomyces cerevisiae have been used as selectable markers, and presumably any auxotrophic marker could be used. However, complementation of an auxotrophic marker does require that the recipient cell contain the appropriate marker. This limitation can be a minor inconvenience, requiring only a genetic cross to introduce the appropriate marker into the desired background, or a major obstacle, for example, when one wants to introduce DNA into another species in which the required genetic lesions are not known. A plasmid-borne dominant selectable marker would be a valuable experimental tool in these cases. We therefore examined several fungicidal drugs and the corresponding inactivating genes from prokaryotes for their efficacy as selectable marker in yeast.

The aminoglycoside antibiotics G418 and hygromycin B were chosen because yeast cells are sensitive to G418 (Jimenez and Davies 1980), and hygromycin B (Gonzalez et al. 1978) and prokaryotic drug inactivation systems exist for each of these antibiotics. Both of these drugs disrupt ribosomal function and cause translational errors (Gonzalez et al. 1978; Davies and Smith 1978), and the drug resistance genes inactivate the drugs by covalent modification (Gonzalez et al. 1978; Davies and Smith 1978; Rao et al. 1983). Bacterial resistance to G418 can be conferred by several plasmid-encoded modifying enzymes (Davies and Smith 1978) including the aminoglycoside phosphotransferase encoded by Tn601 (903). In E. coli, a plasmid-borne gene encoding a hygromycin B phosphotransferase (Rao et al. 1983) confers resistance to hygromycin B. The DNA sequence of this gene has been determined and the protein coding region has been identified (Kaster et al. 1983). A nearly identical hygromycin B resistance gene has also been described by Gritz and Davies (Gritz and Davies 1983). Our previous results have shown that the first three amino acids of the hygromycin B phosphotransferase can be replaced by up to 15 amino acids from a foreign gene and still retain activity (Kaster et al. 1983).

In this paper we report the construction and characterization of plasmids containing a truncated hygromycin
B resistance gene fused to yeast genes, and demonstrate the effectiveness of these plasmids in conferring hygromycin B resistance to yeast cells.

Materials and methods

Materials: G418 was provided by Dr. Peter Daniels and the Schering Corporation. Hygromycin B was obtained from Eli Lilly and Company. BamHI and EcoRI linkers were provided by Dr. Rama Belagaje. Plasmids YG100PR1 and YG101HI were from Mike Slater and Elizabeth Craig. [Gamma-32P]-ATP was synthesized from 32P-phosphate and ADP (Johnson and Walseth 1979). Zymolyase 60,000 was obtained from Miles. All other biochemicals and enzymes were obtained from commercial sources.

Enzyme reactions. Restriction enzyme digestion of DNA was performed according to suppliers' recommendation. Protruding ends of restricted DNA molecules were removed, when necessary, by incubating up to 10 µg of DNA with about three units of DNA polymerase I large fragment in the presence of all four dNTPs at 300 µM concentration. Double stranded regions of DNA were shortened by digestion with Bal31 nuclease. For removing long (greater than 100 base pairs) stretches, about 10 µg of DNA were incubated at 37 °C with three units of Bal31 nuclease under conditions specified by the manufacturer. Aliquots were removed at different times after addition of enzyme, quenched with phenol, and analyzed on polyacrylamide gels to determine the extent of digestion. For removing shorter stretches of DNA, the units of enzyme per µg of DNA were reduced and the reaction carried out at 4 °C. Ligation of DNA molecules, including synthetic oligonucleotides, was accomplished using standard conditions (Scolic and Wensink 1981). When necessary, sequences of regions of plasmids were checked by the chemical cleavage method of Maxam and Gilbert (Maxam and Gilbert 1977).

Construction of plasmids. The hygromycin resistance gene was obtained from pKC222 (Kaster et al. 1983). This plasmid was restricted with HphI and PstI, and the fragment containing the amino-terminal coding region was purified. The 3' protruding end left by the HphI enzyme was removed with E. coli DNA polymerase I large fragment (Klenow fragment) and BamHI linkers (5'-TGGATCCA) ligated. The fragment was restricted with BamHI and EcoRI and ligated into the BamHI and EcoRI sites of pBR322. This intermediate plasmid, called pT110, was cut with EcoRI, and the hygromycin resistance gene was reconstructed by inserting the small EcoRI fragment from pKC222 into pT112. The resulting plasmid, called pT112, contained the hygromycin resistance gene with a BamHI linker substituted for the BamHI linker. As shown in Fig. 1, the truncated hygromycin resistance gene on pT112 can be excised on a BamHI-BglII fragment.

The activator sequences from three yeast genes, a heat shock gene, a heat shock cognate gene, and the phosphoglycerate kinase gene, were modified to facilitate linkage to the hygromycin resistance gene. The heat shock gene is the yeast analogue to the major heat shock gene of Drosophila, hsp70, and is contained on pYG100 (Ingolia et al. 1982). Plasmid pYG100PR1, consisting of a PstI-EcoRI fragment from pYG100 containing the 5' end of the heat shock gene subcloned into pBR322, was used as the starting material, as shown in Fig. 2. This plasmid was digested with NcoI, which cuts within the coding information for amino acid 110 of the heat shock protein, digested with Bal31 nuclease, and ligated to EcoRI linkers. One isolate, called pT115, contained a linker inserted into the coding region for amino acid 5 of the heat shock protein. In order to place a BamHI site near the EcoRI site, the Xbal-BamHI fragment from pT115 was cloned into pNiIIAII (Nakamura and Inouye 1982) yielding pT116. In order to place a BglII site upstream of the activator sequence, the Xbal-BamHI fragment of pT116 was cloned into pBHI, a recombinant plasmid containing Drosophila DNA (Craig et al. 1979). The resulting plasmid, pT120, contains the activator sequence from the pYG100 heat shock gene on BglII-BamHI fragment. The sequence near the translation initiation site is also shown in Fig. 2. The reading frame at the BamHI site is the same as that used for the hygromycin resistance gene in pT112.

The activator sequence from a yeast heat shock cognate gene was also modified. The gene was contained in pYG101HI, a HindIII subclone into pBR322 obtained from Mike Slater and Elizabeth Craig. A 680bp ClaI-EcoRI fragment containing the 5' end of the gene was cloned into ClaI-EcoRI-cut pBR322 to yield pT117. This plasmid was cleaved with ClaI, which cuts at amino acid 10 of the coding region for the heat shock cognate gene, treated with Bal31 nuclease and ligated to BamHI linkers. One isolate, called pT1110, had a BamHI linker inserted after the nth codon of the protein coding region. Beginning with the translation initiation site, the DNA sequence of pT1110 includes 5'-ATG GCT GTA GGT GTT TTC CAA GGT GCT cgg gat cc -3', where the linker is depicted in small case letters. In order to introduce a BglII site upstream of the activator sequence, an EcoRI fragment from pYG100BH (Ingolia et al. 1982) was cloned into the EcoRI site of pT110 to yield pT118. The activator sequence from the heat shock cognate gene can be liberated on a BglII-BamHI fragments from pT118, and the reading frame at the BamHI site is the same as the reading frame at the BamHI site of the truncated resistance gene on pT112.

The phosphoglycerate kinase gene activator sequence was also adapted for use in gene fusions. The gene was isolated from a bank of yeast genome fragments cloned into lambda phage provided by Dr. John Woolford (Woolford and Rosbash 1981). The probe was a pBR322 derivative containing the Saccharomyces cerevisiae SUF16 gene, provided by Richard Gaber and Michael Culbertson. The SUF16 gene resides within about 5 kb of the phosphoglycerate kinase gene (Gaber and Culbertson 1982), so many recombinant phages should carry both genes. One such phage was identified, and a HindIII fragment containing the phosphoglycerate kinase gene and flanking regions (Hitzeman et al. 1982; Dobson et al. 1982) was cloned into pBR322 to yield pT141. A 958 bp HindIII-ClaI fragment containing the 5' end of the phosphoglycerate kinase gene was purified from pT141, and subsequently cut with MboII. The 5' extensions resulting from the MboII cleavage were filled in with DNA polymerase I large fragment and 8-mer BamHI linkers ligated to the molecules. A 240 bp fragment was purified on an acrylamide gel and ligated into BamHI-cut pUC8 (Vieira and Messing 1982). One isolate, called pT143, contained a BamHI fragment which comprised the 5' end of the phosphoglycerate kinase gene. The DNA sequence of pT143, beginning with the translation initiation site, was found to be 5'-Atg gat cc -3', where the linker sequence is depicted in small case letters. The BamHI site of pT143 is in phase with the BamHI site of the truncated hygromycin resistance gene on pT112.

The backbone for our yeast-E. coli bifunctional vectors was YEp24 (Botstein et al. 1978). The kanamycin resistance gene from Tn60/903 was incorporated into YEp24 by purifying a 1.7 kb PvuII fragment from pNG614 (a derivative of pNG18; Grindley and Joyce 1980) and ligating it into the sole SmaI