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# Cloning of Genes That Complement Yeast Hexokinase and Glucokinase Mutants

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Genes complementing the glucose-negative fructose-negative *Saccharomyces cerevisiae* triple mutant strain (*hxk1 hxk2 glk1*), which lacks hexokinase PI, hexokinase PII, and glucokinase, were obtained from a pool of yeast DNA in the multicopy plasmid YEp13.

*Saccharomyces cerevisiae* has three glucose-phosphorylating enzymes, hexokinase PI (or A), hexokinase PII (or B), and glucokinase. *hxk1*, *hxk2*, and *glk1* are deficiency mutations for the three enzymes, respectively (9). The functions of the individual enzymes are not well understood. Any one of the three kinases is adequate for growth on glucose, and either of the two hexokinases is adequate for growth on fructose (4, 8). Hexokinase PII seems to predominate in growth on glucose (4), and a direct role for it in carbon catabolite repression has been proposed (3). We have obtained evidence implicating the kinases in glucose uptake (1).

We have recently reported use of a yeast DNA pool (a *Sau*3A partial digest from strain AB320 inserted in the *Bam*HI site of vector YEp13) for obtaining a number of glycolysis genes by complementation of the appropriate yeast mutants (5). We now report on complementation of *hxk1*, *hxk2*, and *glk1*. A mutant strain lacking the three kinases and carrying a *leu2* mutation, N517-6C (α *hxk1 hxk2 glk1*, *leu2*, *can1 cyh2 ade2*) was constructed from triple kinase mutant strain D308.3 (kindly supplied by P. K. Maitra) by appropriate crosses and screening of segregants for glucosamine resistance; the kinase mutations were confirmed by assay. Protoplasts of strain N517-6C were transformed with the pool in YEp13 with simultaneous selection for growth on glucose in a medium lacking leucine, as described previously (5). Of 20 colonies tested, 14 showed several percent or more cosegregation of the two selected markers after growth under nonselective conditions (i.e., leucine-auxotrophic glucose-negative segregants); these clones presumably carried the glucose-complementing gene on the vector with *LEU2*. (In the other six colonies, in which segregation was not observed, its frequency may have been less, integration may have occurred, or the growth on glucose might have been caused by reversion; those isolates were not studied further.) The 14 colonies were examined for phosphorylation of glucose and fructose in extracts. Hexokinase PI is known to have a $V_{max}$ with fructose about three times that with glucose, hexokinase PII has approximately equal activities with the two substrates, and glucokinase acts marginally, if at all, on fructose (8). Accordingly, the transformants were provisionally classified as hexokinase PI (2 of 14), hexokinase PII (10 of 14), and glucokinase (2 of 14).

Plasmid DNA was obtained from the clones (10) and used to transform *Escherichia coli* RR1 (1a), selecting ampicillin resistance. Plasmid DNA from the *E. coli* transformants was examined on agarose gels. The smallest plasmid in each of the three presumptive classes was chosen (pBW111-113); their restriction patterns (Fig. 1) show them to clearly differ. (*HXK1*, *HXK2*, and *GLK1* are unlinked [9].) The approximate insert sizes (YEp13 is 10.7 kilobases) were 5.3, 4.8, and 3.8 kilobases for plasmids pBW111, pBW112, and pBW113, respectively.

A new yeast mutant lacking the three kinases was prepared from strain DFY87 (*hxk1 hxk2*, analogous to DFY64 [2]) by selection of spontaneous resistance to 2-deoxyglucose (7), followed by backcrossing to the wild type, giving strain DFY437 (α *leu2 lys1 hxk1 hxk2 glk*). DFY437 was transformed independently (by L. Bisson) with the three plasmids (pBW111, pBW112, or pBW113), and extracts were chromatographed on hydroxylapatite (Fig. 2). The three clones gave single peaks of hexose phosphorylation, eluting at different phosphate concentrations and, like the crude extracts, showing fructose-to-glucose phosphorylation ratios characteristic of the three known kinases. Similar experiments were done with the plasmids in the original triple kinase mutant background and in a double ki-

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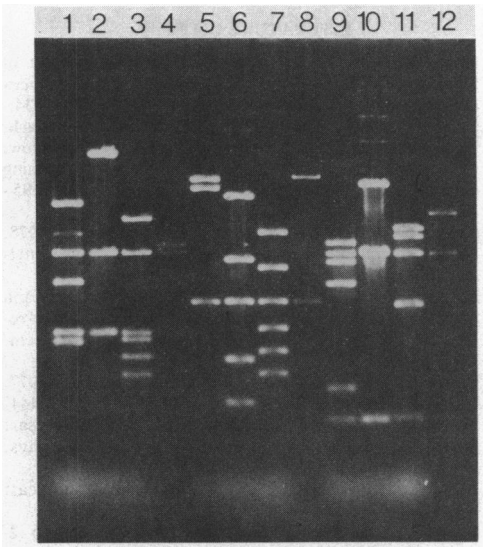
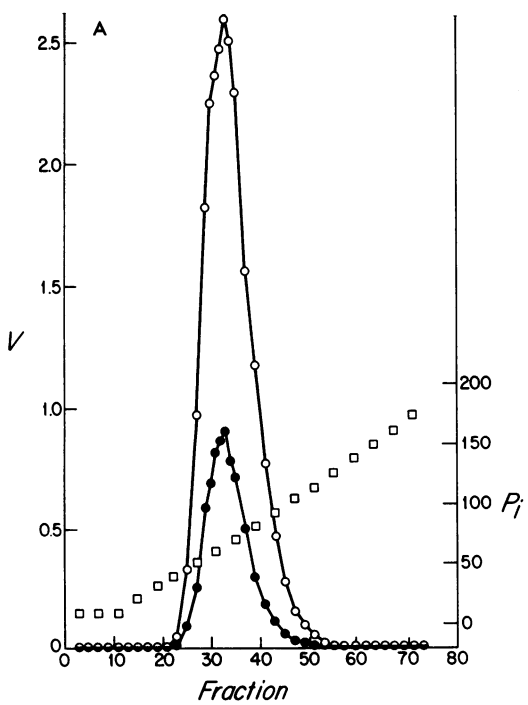



FIG. 1. Electrophoresis of DNA. The three plasmids, pBW111-113, and the vector YEp13, were transformed into *E. coli* strain 294 *recA* (constructed by F. Daldal from strain 294 ($F^-$*pro thi endA hsdR*)), and the plasmid DNA was reisolated and purified on cesium chloride gradients. After treatment with restriction enzymes, electrophoresis was done in 0.7% agarose, and staining was done with ethidium bromide. Lanes 1 to 4, *Eco*RI-treated pBW112, pBW113, pBW111, and YEp13, respectively; lanes 5 to 8, *Bgl*II-treated pBW112, pBW113, PBW111, and YEp13 respectively; and lanes 9 to 12, *Pst*I-treated pBW112, pBW113, pBW111, and YEp13, respectively.



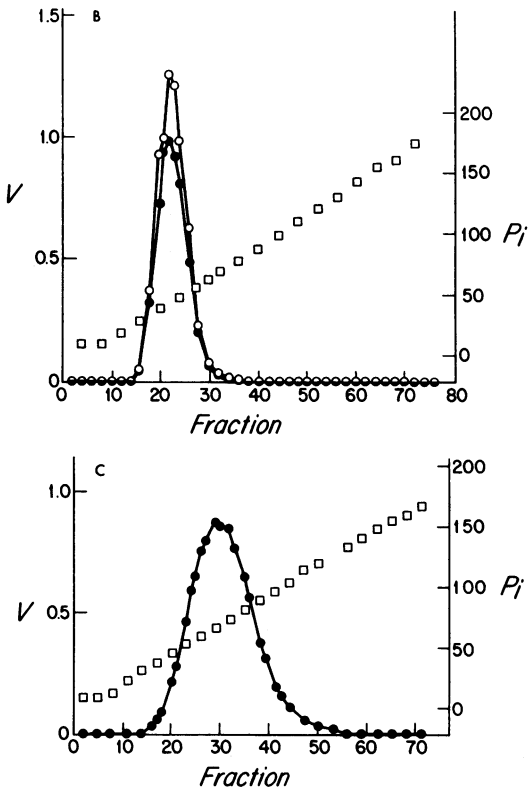




FIG. 2. Chromatography of kinases. Individual cultures of strain DFY437 carrying the three plasmids pBW111, pBW112, or pBW113 were grown in enriched medium containing 1% glucose (2). The cells were harvested at an absorbance at 580 nm of 5, washed twice with water, resuspended in 10 ml of buffer A (10 mM $KH_2PO_4$, 5 mM 2-mercaptoethanol, 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride [pH 7.0]) and treated in a French pressure cell at 20,000 lb/in$^2$. Debris was removed by centrifugation (15,000 × *g* for 10 min), protamine-$SO_4$ was slowly added to the supernatant to give 2 mg/ml, and the resulting precipitate was removed by a second centrifugation (12,000 × *g* for 10 min). As described previously (4), chromatography was done on hydroxylapatite (Bio-Gel HT; Bio-Rad Laboratories) mixed with cellulose (Whatman cellulose CF-1) to improve flow. A 10-U (μmol/min) amount of glucose-phosphorylating activity was added to 12-ml columns, and, after a wash with buffer A, a 200-ml linear gradient was applied (buffer A and buffer A containing 200 mM potassium phosphate [pH 7.0]) over 9 h. Fractions of ca. 2.5 ml were collected and assayed for glucose- or fructose-phosphorylating activity, with 5 mM substrates and a pH of 7.4 (2). Phosphate concentration was determined by conductivity. *V* is expressed in units per fraction. ●, Glucose phosphorylation; ○, fructose phosphorylation; □, millimolar concentration of inorganic phosphate ($P_i$). (A) DFY437(pBW111). (B) DFY437(pBW112). (C) DFY437(pBW113) (no fructose phosphorylation was observed). In the parental strain DFY437, neither glucose nor fructose phosphorylation was observed in the crude extract or after chromatography (data not shown).

nase mutant, and the plasmids were also introduced into a wild-type strain (data not shown). In all cases, the levels of activity, fructose-to-glucose phosphorylation ratios, and elution patterns or confirmed the assignment of hexokinase PI from pBW111, hexokinase PII from pBW112, and glucokinase from pBW113.

Of the three mutant loci, *hxk1*, *hxk2*, and *glk1*, at least the first two are the structural genes of the enzymes (6, 7). Enzyme levels in the clones ranged from 2 to 20 times that of the wild type; variability and uncertainty in these values reflect different degrees of plasmid segregation as well as difficulty in estimating individual levels of the kinases in the wild-type strain. Nonetheless, the high levels and complementation in two triple mutants of entirely different origin make it likely that complementation is not allele specific and that it is the structural genes themselves which have been cloned. In that case, pBW111 would carry *HXK1*, pBW112 would carry *HXK2*, and pBW113 might carry *GLK1*. Other possibilities seem less likely but are not excluded, e.g., nonallelic complementation by unknown other structural or regulatory genes.

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