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SACCHAROMYCES CEREVISIAE KILLER EXPRESSION MUTANT KEX2 HAS ALTERED SECRETORY PROTEINS AND GLYCOPROTEINS

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The extracellular proteins and glycoproteins of a yeast mutant kex2-15 defective in killer toxin expression were separated by one and two dimensional polyacrylamide gel electrophoresis. Many mutant extracellular proteins and glycoproteins show both altered electrophoretic mobility and isoelectric points when compared with the parent strain. Altered proteins and glycoproteins from kex2-15 were identified with their parental counterparts by peptide mapping. The observed alterations co-segregated with the kex2 nuclear mutation in genetic crosses.

The mechanism of protein secretion is understood in outline only (1,2). This complex process involves membrane recognition and transfer events and in many cases processing of secretory proteins (3). Mutations affecting proteins involved in protein secretion should further our understanding of this process. We wish to report a mutant kex2-15 in the yeast Saccharomyces cerevisiae that appears to be defective in a secretion-related event. The mutation is in a nuclear gene kex2, and leads to a pleiotropic phenotype that includes loss of killer toxin expression, inability to produce α-factor pheromone, failure to undergo spore maturation (4) and poor growth on complex media (our unpublished observations). Here we report that many extracellular proteins and glycoproteins from kex2-15 show altered mobility on SDS polyacrylamide gel electrophoresis and altered isoelectric points, when compared with the parent strain A364A. These alterations show that secretion in kex2 mutants is abnormal, probably at the level of protein processing.

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Abbreviation: SDS, sodium dodecylsulphate

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MATERIALS AND METHODS

Yeast strains were grown and extracellular protein concentrates obtained as described (5). Asci were dissected using the open plate method of Cox and Bevan (6).

SDS polyacrylamide gel electrophoresis was performed by the method of Laemmli (7), linear 8-18% gradient gels were used and were stained with Coomassie Brilliant Blue. For preparative separations, a Savant apparatus was used with an 8% running and 3% stacking gel. Fractions were concentrated using dialysis tubing and Aquacide IIA. Two-dimensional polyacrylamide gel separations were made using the technique of O'Farrell (8). The isoelectric focusing gradient was pH 3.1-5.6 and the acrylamide gel was a linear 8-18% gradient.

Peptide mapping of proteins and glycoproteins was by the method of Cleveland (9) using V8 protease (Miles) at 25 µg/ml at 37°C for 30 min. Digests were electrophoresed on a 15% acrylamide gel and stained with Coomassie Brilliant Blue.

RESULTS

Many extracellular proteins and glycoproteins from kex2-15 showed reduced mobility on SDS polyacrylamide gel electrophoresis (Fig. 1) compared to A364A. In addition there was very much less killer toxin protein in kex2-15 than in the parent (5). The alteration in mobility of extracellular proteins and glycoproteins from kex2-15 co-segregated with

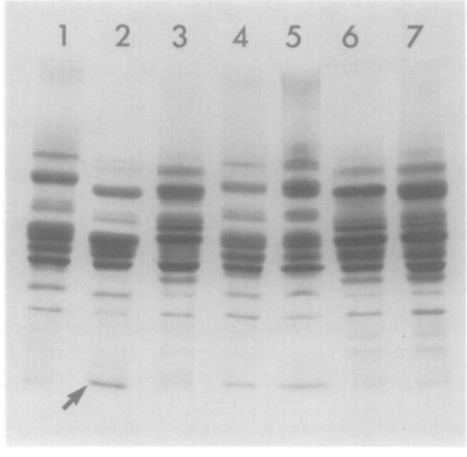

Fig. 1.

Co-segregation of altered electrophoretic mobility of extracellular proteins and glycoproteins with the kex2 phenotype.

The gel shows a tetrad derived from the cross kex2-15 x A8207NK, both parents, and the A364A strain from which kex2-15 was derived. Lane 1, A8207NK; lane 2, A364A; lane 3, kex2-15; lanes 4 and 5, the two killer spore clones from the tetrad; lanes 6 and 7 the two kex2 (non-killer) spore clones from the tetrad. Killer toxin is indicated by the arrow.

the kex2 mutation in genetic crosses. The mutant kex2-15 was mated with sensitive strain A8207NK. The resulting killer diploid was sporulated, tetrads dissected, and spores analysed for the killer phenotype and for extracellular proteins and glycoproteins. The co-segregation of the altered pattern of mobility with kex2 is shown in Fig. 1 (lanes 4-7). This co-segregation was found in 6 tetrads from the above cross and in a further 6 tetrads from crosses with kex2-15 and three other sensitive yeast strains. Although in the cross shown in Fig. 1 the kex2 altered mobility and cosegregation is clear, some variations appeared in the patterns of extracellular proteins and glycoproteins from the A364A parent and the A8207NK strain. Some of these variations also segregated in the cross. The alteration in mobility of extracellular proteins and glycoproteins was also found in a kex2 non-complementing killer expression mutant isolated from killer strain K12. A second unlinked gene affecting killer expression (kex1) is known (10). A mutant in kex1 had normal extracellular proteins and glycoproteins, the absence of the killer toxin protein band was the only alteration detected.

If some processing component is defective in kex2 then incubation of extracellular proteins and glycoproteins from kex2-15 with wild type cell extracts might allow processing to occur. Mixing experiments using [^{35}S]methionine-labelled extracellular proteins and glycoproteins from kex2 and unlabelled crude cell extracts from sonicated cells of A364A, failed to show any conversion of the kex2 pattern to that of A364A. To explore the extent of the kex2 phenotype, yeast plasma membrane proteins were examined. Two-dimensional gel electrophoresis of proteins from yeast cell ghosts (11) from kex2-15 and A364A were identical, suggesting that membrane protein assembly is kex2 independent.

To further characterize the kex2 defect, two-dimensional gel electrophoretic separations of extracellular proteins and glycoproteins from kex2-15 and A364A were made (Fig. 2). It can be seen that some kex2

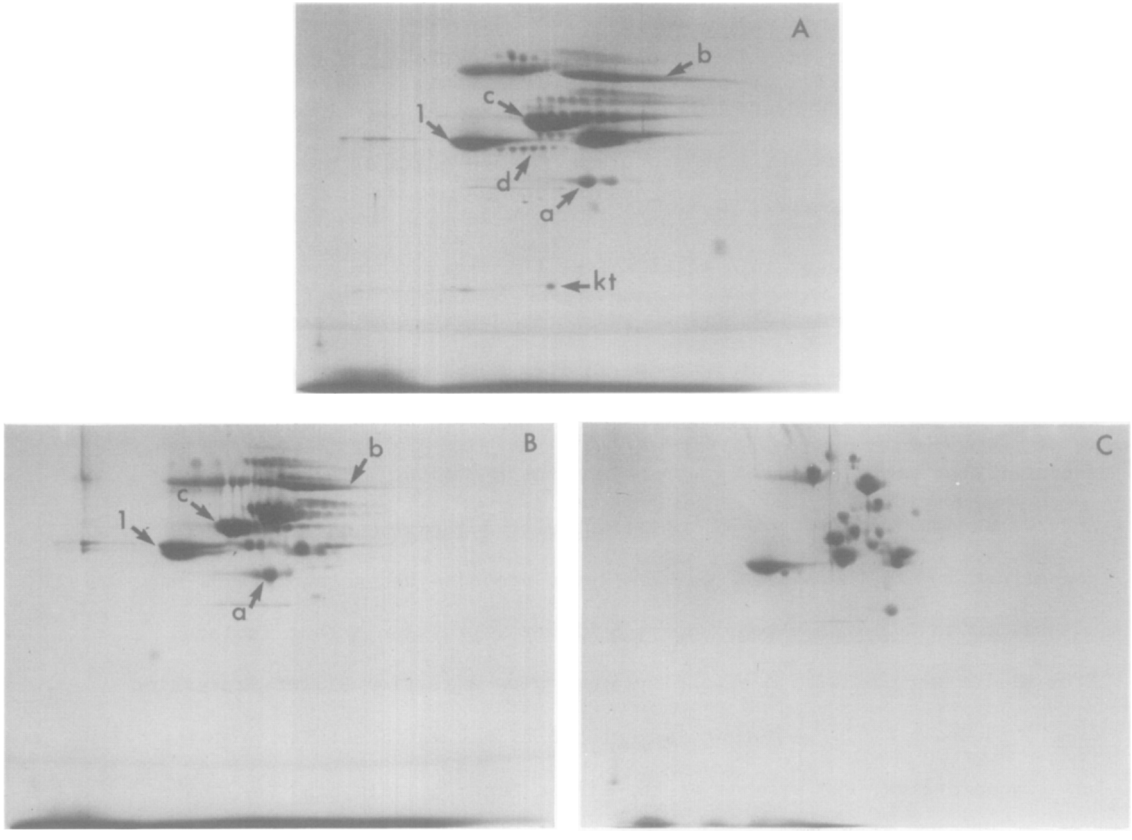

Fig. 2.

Two-dimensional polyacrylamide gel separation of A364A and kex2-15 extracellular proteins and glycoproteins.

In each case the isoelectric focusing first dimension was from the left to the anode on the right. The molecular weight separation in the second dimension was from the top to the anode at the bottom. A, A364A; B, kex2-15; C, mnn1, mnn4. Spots indicated by arrows are referred to in the text, kt is killer toxin. A364A carries a mutation in the mnn1 gene coding for α-1$\rightarrow$3 mannosyl transferase (12); mnn4 carries a defect in mannosyl phosphate transferase.

species migrate more slowly in SDS polyacrylamide gel electrophoresis and focus at a higher pH on isoelectric focusing than in A364A. In our two-dimensional gel separations, most extracellular glycoproteins showed multiple spots which appeared independent of kex2 and to be caused by the presence of mannose phosphate in the outer mannan chains. A mutant deficient in mannosyl phosphate transferase (12) (obtained from C. Ballou) did not show these multiple spots (Fig. 2c).

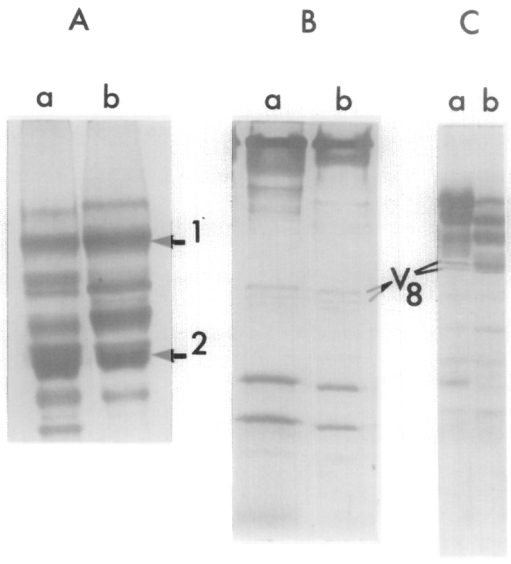

Fig. 3.

Peptide maps of kex2-15 and A364A extracellular proteins and glycoproteins.

A. Upper region of an 8-18% gel of a,(A364A) and b,(kex2-15) extracellular material showing position of bands 1 and 2 used for peptide mapping.
B. V_8 digest of band 1. a, (A364A), b, (kex2-15), undigested band 1 does not enter the gel (not shown).
C. V_8 digest of band 2. a, (kex2-15), b, (A364A).

Two-dimensional gel electrophoretic fingerprinting of extracellular proteins and glycoproteins is good evidence for spot identity in kex2-15 and A364A. In addition, peptide mapping (9) has shown that bands with altered mobility have peptides in common (Fig. 3). We have also peptide mapped the following spots cut from two-dimensional polyacrylamide gels of extracellular proteins and glycoproteins from kex2-15 and A364A (see Fig. 2); glycoproteins a, b, c and protein 1. In all cases kex2-15 and A364A spots have peptides in common. Protein 1 is apparently a protein as it does not stain with the periodic acid-Schiff procedure (13) and shows no charge heterogeneity.

The kex2 mutation alters both extracellular proteins and glycoproteins suggesting that kex2 mutants are deficient in some aspect of protein processing related to secretion. The nature of the defect remains unknown

and we can state no simple rule governing the altered extracellular proteins and glycoproteins from kex2. Many migrate more slowly and have an altered isoelectric point, consistent with the presence of an unprocessed piece of protein. However, some proteins and glycoproteins appear to be missing from the extracellular medium. These include the killer toxin protein and glycoprotein d (see Fig. 2). Killer toxin does not appear to be present as an unprocessed component in kex2-15. Using anti-killer toxin antibody (14) we have been unable to find cross reacting material either in [^{35}S] methionine labelled extracellular or intracellular extracts from kex2-15. For some proteins and glycoproteins the kex2 event may be a prerequisite to some second event necessary for secretion. However, in kex2-15 secretion of many altered species can clearly continue; this also appears to be the case with human proalbumin (15). The amount of extracellular protein and glycoprotein produced by kex2-15 was slightly higher than in A364A (ratio kex2-15/A364A, 1.44 $\pm$ 0.2 std deviation). The kex2 dependent alterations seen here are consistent with the loss of several functions previously observed (4); all of which probably have secretory proteins involved. Whether the altered kex2-15 extracellular proteins and glycoproteins seen here are biologically active remains unknown.

The use of the killer plasmid-coded toxin (Bostian, K.A., Hopper, J.E., Rogers, D.T. and Tipper, D.J. in preparation) to define nuclear gene mutants necessary for toxin expression may be a useful approach to study events in the processing of secretory proteins.

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