GENETIC PROPERTIES OF MUTATIONS AT THE *PEP4* LOCUS IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Yeast cells that inherit mutations at the *PEP4* locus exhibit a pronounced phenotypic lag in the expression of the mutant phenotype imparted by these mutations. This lag appears to extend to all of the enzymes that are affected by the *pep4-3* mutation. For at least two of the enzymatic activities, phenotypic lag shows mitotic cosegregation. Phenotypic lag is found for meiotic progeny and for mitotic segregants from heterokaryons. The phenotypic lag in the expression of the carboxypeptidase Y deficiency is abolished by nonsense mutations in either *PRC1*, the structural gene for carboxypeptidase Y, or *PRB1*, the structural gene for proteinase B. Models to explain these observations are proposed.

MUTATIONS in the *PEP4* locus of *Saccharomyces cerevisiae* are recessive and pleiotropic. Extracts prepared from *pep4-3* mutant cells are 90–95% deficient in the levels of at least five vacuolar hydrolases, namely carboxypeptidase Y, proteinase A, proteinase B, RNase(s) and the repressible, nonspecific alkaline phosphatase (JONES 1977; JONES *et al.* 1981; ZUBENKO 1981; JONES, ZUBENKO and PARKER 1982). Diploids homozygous for the *pep4-3* mutation show only 30% of the wild-type levels of protein degradation when exposed to a sporulation regimen and do not undergo meiosis or sporulation (ZUBENKO and JONES 1981). Recently, KANEKO, TOH-E and OSHIMA (1982) have shown that *pho9* mutations are allelic to the *pep4-3* mutation. They report that the nonspecific alkaline phosphatase isozyme is virtually absent from extracts of *pep4-3* and *pho9* mutants and that diploids homozygous for the mutation do not undergo commitment to intragenic recombination.

When a strain bearing a *pep4* mutation is outcrossed and the resulting heterozygous diploid sporulated, the *pep4* mutation segregates 2:2 in the resulting meiotic tetrads (JONES 1977; JONES *et al.* 1981; ZUBENKO 1981; JONES, ZUBENKO and PARKER 1982). However, the meiotic spore clones that inherit the *pep4* mutation exhibit a pronounced delay in the expression of the mutant phenotype imparted by the *pep4* mutation. This delay in expression can last longer than 20 generations in some cases. After 20 generations, the cytoplasm of the initial meiotic product has been diluted by a factor of more than 10^6. It was difficult to understand how the wild-type (Pep^+) phenotype could persist following a

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dilution of this magnitude. We report here the results of a series of experiments designed to provide some insight into this phenomenon.

MATERIALS AND METHODS

Sources of materials: Yeast extract, Bactopeptone, glucose and sucrose were purchased from Difco Laboratories. Nutrients used were purchased from Sigma Chemical Company, as were *N*-acetylphenylalanine β-naphthyl ester, α-naphthyl phosphate, sodium dodecyl sulfate and Tris-hydrochloride. Inorganic salts, glycerol, citric acid and sodium citrate were obtained from J. T. Baker Company. Hide powder azure (HPA) was from Calbiochem. Electrophoresis supplies were purchased from Acufine and Bio Rad. Glusulase was from Endo Laboratories. Fast black K salt was obtained from Roboz Surgical Instrument Company and fast garnet GBC was purchased from Roboz and Sigma. Agar was purchased from Sigma, Difco and Calbiochem.

Strains: The parent strain of the *pep* mutants was a *trp1* derivative of X2180-1B (α *gal2*). The *pep4* and *prc1* mutants were isolated after EMS mutagenesis and were identified by screening for colonies that had a reduced ability to cleave the carboxypeptidase Y (CPY) substrate, *N*-acetyl-phenylalanine β-naphthyl ester (APE), present in agar overlays (JONES 1977). The cleavage product, β-naphthol, was subsequently detected by coupling to the diazonium salt fast garnet GBC. The insoluble red dye which results precipitates at the site of enzyme activity. Because of the insolubility one can detect sectored colonies if they are present. Colonies that possess CPY activity stain red, whereas those that lack CPY activity remain yellow. This overlay test is referred to as an APE test. The *prc1-407* mutation is an amber mutation (M. SAFTNER, personal communication) that lies in the structural gene for carboxypeptidase Y (WOLF and WEISER 1977; HEMMINGS *et al.* 1981). A strain bearing a *prc1* mutation, isolated by WOLF and FINK (1975), was provided to us by G. FINK to allow allelism tests. The *prb1-1122* mutation is an ochre mutation that lies in the structural gene for proteinase B (ZUBENKO, MITCHELL and JONES 1979; ZUBENKO, MITCHELL and JONES 1980). Crosses were made within an isogenic series of strains. A strain bearing a *kar1* mutation was obtained from G. FINK. Other gene symbols are denoted as follows: **a** and α, mating type alleles; *leu1* and *trp1*, requirements for leucine and tryptophan, respectively; *gal2*, inability to ferment galactose; [KIL-K] and [KIL-O], the presence and absence of the killer plasmid, respectively; *kar1*, karyogamy defective (CONDE and FINK 1976).

Media: The compositions of YEPD broth, minimal medium, and low phosphate minimal medium are given in JONES, ZUBENKO and PARKER (1982). Complete synthetic medium was minimal medium to which the following supplements were added to 1 liter of medium: adenine 20 mg, arginine 20 mg, histidine 20 mg, leucine 60 mg, lysine 50 mg, methionine 40 mg, phenylalanine 20 mg, serine 50 mg, threonine 200 mg, tryptophan 40 mg, tyrosine 20 mg and uracil 10 mg. Omission medium was complete synthetic medium that lacked one of these supplements. In YEPG agar, 5% glycerol replaced the dextrose in YEPD. Agar was added to a final concentration of 15 g/liter (Difco), 14 g/liter (Sigma) or 13.5 g/liter (Calbiochem) when solid medium was desired.

Genetic methods: The *pep4* and *prc1* mutations were scored by the ability of cells to cleave the APE in agar overlays (JONES 1977). When *pep4* and *prc1* mutations segregated in the same cross, they were distinguished by complementation tests. The *prb1* mutation was scored by the use of HPA-containing agar overlays as described by ZUBENKO, MITCHELL and JONES (1979). The proteinase B deficiency that results from the *pep4* mutations is not complete enough to give a negative HPA test and, therefore, it was not necessary to use complementation tests to distinguish the segregations of the *pep4* and *prb1* mutations. Presence of the killer plasmid was determined according to SOMERS and BEVAN (1969). Procedures for routine sporulation, dissection and scoring of nutritional markers have been described by MORTIMER and HAWTHORNE (1969).

Preparation of crude extracts: Cells were harvested from liquid culture and extracts prepared as described in JONES, ZUBENKO and PARKER (1982).

Native gel electrophoresis: Electrophoresis and staining of the gels for nonspecific alkaline phosphatase was carried out as described in JONES, ZUBENKO and PARKER (1982).

RESULTS

The *pep4-3* mutation segregates 2:2 in meiotic tetrads from a heterozygous diploid (JONES *et al.* 1981; ZUBENKO 1981; JONES, ZUBENKO and PARKER 1982).

However, when one initially tests the spore clones for carboxypeptidase Y activity, the two wild-type clones are red as expected, but the clones of *pep4-3* genotype are red, pink or sectored red and yellow (Figure 1). The mutant phenotype is yellow. A large number of generations elapse between the spore and the APE test. For the test shown in Figure 1, spores were allowed to form colonies of about 10^6 cells. The spore colony was streaked to YEPD medium and allowed to grow for 2 days. Replicas were made and allowed to grow for 3 days at which time the APE test was performed. Although it is difficult to estimate the number of generations that took place during growth of the colony streaks and the replica, about 20 generations took place during growth of the spore clone. Upon restreaking such mixed clones and retesting the isolated colonies for CPY activity, one recovers red, pink and yellow colonies again (Figure 2), even if the subcloning is performed on streaks of the replica. The segregation eventually obtained for the *pep4-3* mutation is shown in Figure 3. The tetrads in Figure 3 are the same as those in Figure 1. The unstable Pep^+ phenotype is maintained through at least three successive subclonings (about 60 generations), if the colony chosen for subcloning manifests CPY activity by the overlay test. Stable clones possessing CPY activity are not recovered from

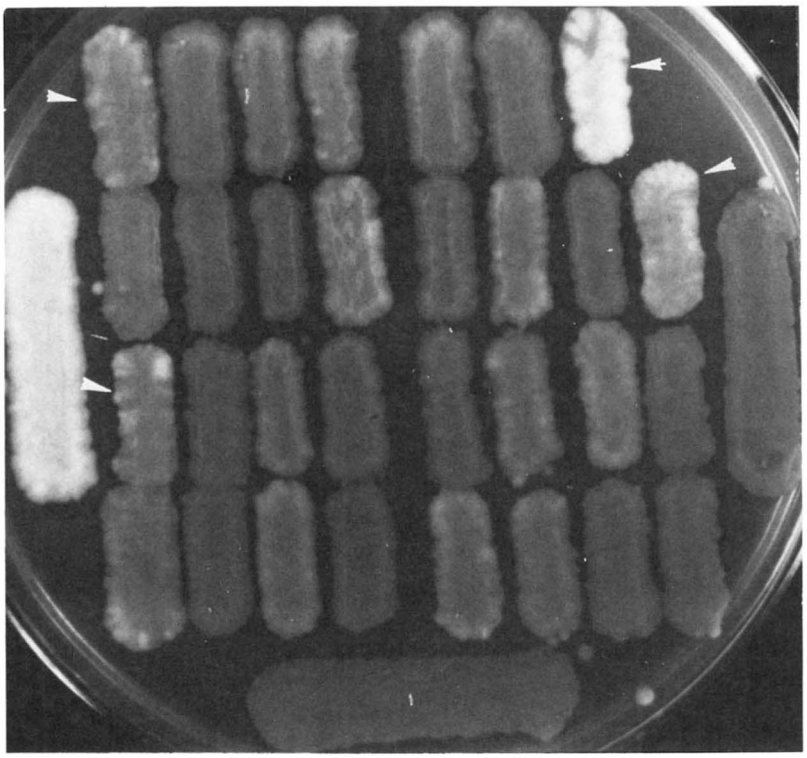

FIGURE 1.—Expression of the *pep4-3* mutation in meiotic segregants of a *pep4-3* heterozygote. Streaks of the *pep4-3* mutant and the wild type are on the left and right, respectively. Horizontal groups of four (or three in one case) are members of one tetrad. Cultures were tested for CPY activity (JONES 1977). Mutant streaks are yellow; wild type are red. Examples of obviously sectoring clones are indicated by arrows.

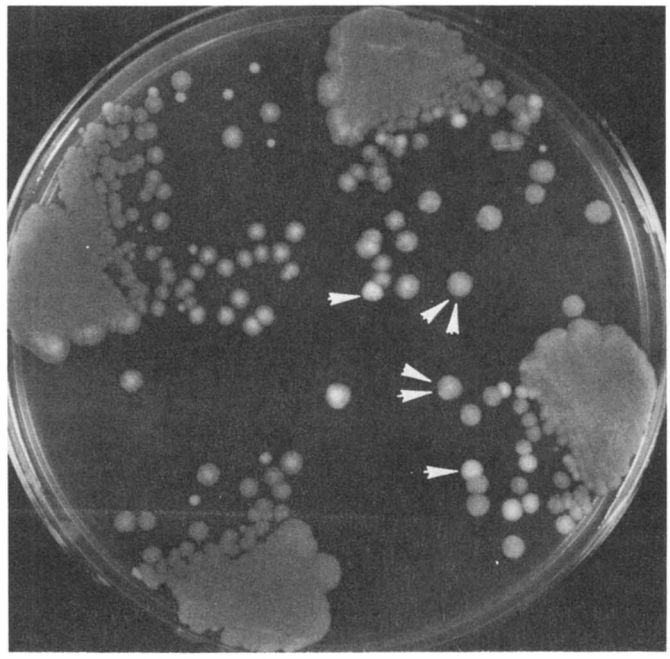

FIGURE 2.—Expression of the *pep4-3* mutation in colonies derived from spore clones. Spore clones (about 10^6 cells) from a tetrad derived from a *pep4-3*/+ diploid were streaked for single colonies. The resultant colonies (about 10^6 cells) were tested for CPY activity. Clockwise from top left the genotypes of the strains proved to be; +, *pep4-3*, *pep4-3*, +. Single arrows point to unstained (CPY^-) colonies; double arrows to stained (CPY^+) colonies.

such mixed clones. The last observation excludes postmeiotic segregation as the explanation for this behavior.

The successful expression of the mutant phenotype is not related exclusively to the passage of time but requires cell division. If *pep4-3*-bearing meiotic clones in the process of becoming mutant in phenotype are refrigerated at 4°, portions of these clones retain the Pep^+ phenotype indefinitely.

Because the *pep4-3* mutation results in a deficiency for at least five vacuolar hydrolases, we determined whether the delay in expression of the mutant phenotype was peculiar to CPY or whether it extended to include any of the other hydrolases as well. We selected the nonspecific alkaline phosphatase for examination. In addition to determining whether nonspecific alkaline phosphatase activity persists through many generations in the mitotic progeny of meiotic clones of *pep4-3* genotype, we also looked to see whether there was mitotic cosegregation of the CPY and alkaline phosphatase activities among the mitotic progeny.

Meiotic spore clones from *pep4-3*/+ heterozygotes were streaked for single colonies, and the colonies were tested for CPY activity using the APE test. Two mitotic segregants exhibiting CPY activity (red) in the APE test and one colony lacking CPY activity (completely yellow) in the APE test were chosen from each of three meiotic spore clones of *pep4-3* genotype and were used to inoculate

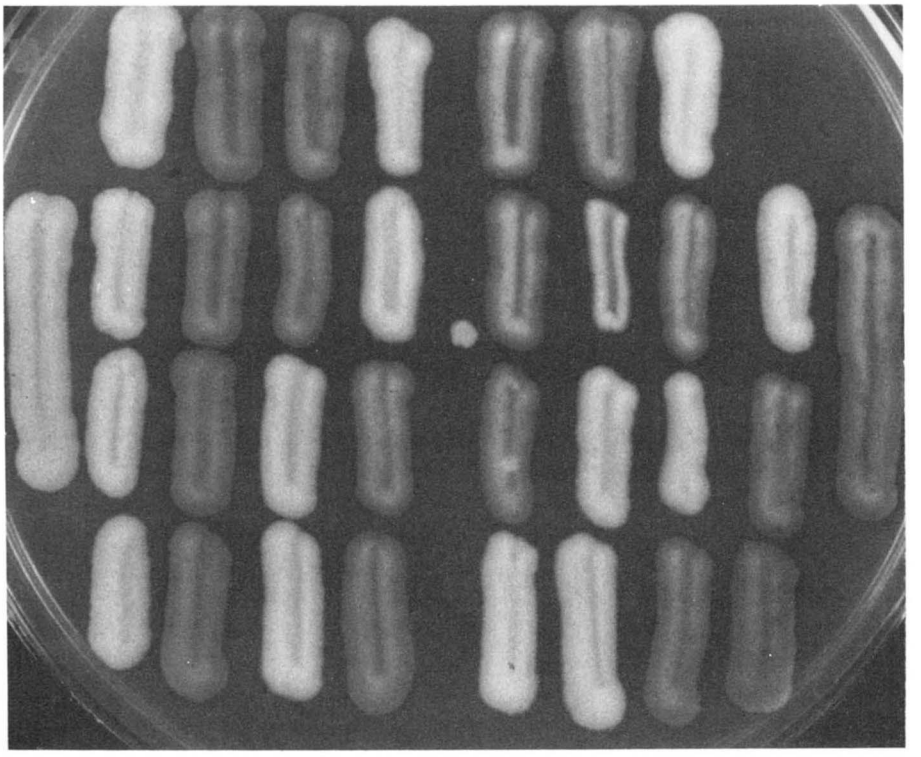

FIGURE 3.—Segregation of the *pep4-3* mutation in tetrads derived from a *pep4-3/+* diploid. After mitotic segregation pure clones exhibiting the mutant phenotype could be obtained from two of the four spore clones of each tetrad shown in Figure 1. Masters were remade from pure stable clones for these tetrads and were retested for CPY activity. Streaks of the mutant and wild type are on the left and right, respectively. Horizontal groups of four are members of one tetrad. Segregation is 2:2 for the *pep4* mutation. Colonies that showed sectors in Figure 1 prove to be of *pep4-3* genotype.

flasks of low phosphate synthetic medium containing the appropriate amino acid supplements at high cell density. The cultures were grown for 52 hr at 30°, cells were harvested, and extracts were prepared from each. Samples of these extracts were subjected to native gel electrophoresis, and the resulting gel was strained for α-napthylphosphatase (nonspecific alkaline phosphatase only) activity. The stained gel is shown in Figure 4. A sample from an extract made from wild-type cells was run in the first lane at half the protein concentration as that used for the other lanes. Samples of extract made from the CPY-containing mitotic segregants from spore clones 3B, 6A and 16C were run in lanes 2 and 3, 5 and 6, and 8 and 9, respectively. The extracts from mitotic segregants lacking CPY activity from spore clones 3B, 6A and 16C were run in lanes 4, 7 and 10, respectively. Clearly nonspecific alkaline phosphatase activity persists in the mitotic progeny clones of *pep4-3* genotype for many generations. Moreover, there is mitotic cosegregation of the CPY and the nonspecific alkaline phosphatase activities, for derivative mitotic clones either retain both activities or neither. Similar results were obtained for proteinase B activity (data not

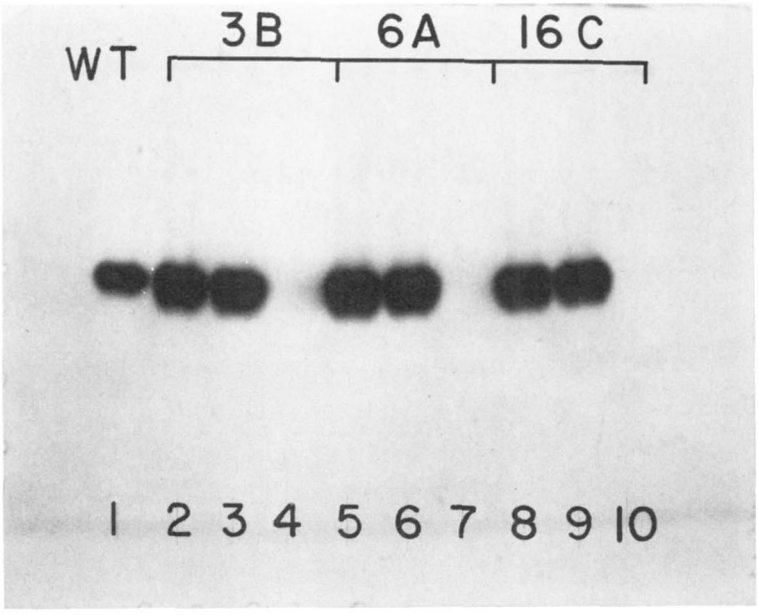

FIGURE 4.—Persistence of nonspecific alkaline phosphatase in mitotic segregants derived from meiotic spore clones of *pep4-3* genotype and mitotic cosegregation of absence or presence of nonspecific alkaline phosphatase activity and CPY activity in these same descendants. Extract made from a wild-type strain is in lane 1. Extracts were made from two CPY^+ mitotic segregants and one CPY^- (unsectored) mitotic segregant derived from each of three *pep4-3*-bearing meiotic segregants, 3B, 6A and 16C, obtained from a *pep4-3* heterozygote (see Figure 1 JONES, ZUBENKO and PARKER), and were loaded in lanes 2–4, 5–7 and 8–10, respectively. See text for further details.

shown). Therefore, the delay in the expression of the mutant phenotype imparted by the *pep4-3* mutation seems to extend to several, and perhaps all, of the enzymes whose levels are affected by the *pep4-3* mutation.

Diploids that are homozygous for the *pep4-3* mutation and that exhibit the multiple hydrolase deficiencies characteristic of the mutant phenotype neither undergo meiosis nor sporulate when subjected to a sporulation regimen (ZUBENKO and JONES 1981; KANEKO, TOH-E and OSHIMA 1982). However, if haploid meiotic products bearing the *pep4-3* mutation are crossed before the Pep^+ (CPY^+) phenotype is lost and CPY^+ diploids, homozygous for *pep4-3*, are isolated, such diploids are competent to undergo meiosis and sporulation. The meiotic products of such diploids all bear the *pep4-3* mutation and show the same delayed expression of the mutant phenotype that was characteristic of the two parental strains. Therefore, it seems likely that all of the components of the mutant phenotype imparted by the *pep4-3* mutation show mitotic cosegregation during the growth of strains that inherit the *pep4-3* mutation.

We have considered two broad explanations for this particular genetic behavior: that there is an autonomous genetic determinant showing cytoplasmic inheritance or that this is an extreme case of phenotypic lag. To account for these observations in terms of an autonomous cytoplasmic genetic element, one must postulate (for the case of CPY) that the determinant is essential for the

expression of CPY activity in the *pep4-3* genotype, that the element is dependent on the wild-type *PEP4* allele for normal replication, and that the determinant underreplicates in strains bearing the *pep4-3* allele. [One cannot postulate that the element is sufficient for CPY expression and depends on *PEP4* for maintenance, because the structural gene for CPY, *PRC1*, shows Mendelian inheritance (WOLF and FINK 1975; WOLF and WEISER 1977), mutations in *PRC1* produce an absolute deficiency for carboxypeptidase Y, and the expression of the *prc1* phenotype shows no delay in meiotic spore clones (WOLF and FINK 1975; G. S. ZUBENKO, F. J. PARK and E. W. JONES, unpublished observations).] Similar stipulations about the nature of the determinant as it concerns proteinase B and nonspecific alkaline phosphatase would have to be made and similar restrictions imposed. By the hypothesis of cytoplasmic inheritance, the stable mutant clones, which are recovered upon restreaking, have lost the cytoplasmic determinant. To test this hypothesis, we isolated Pep^+ revertants of stable *pep4-3* mutant reisolates, backcrossed them to stable *pep4-3* mutant reisolates and determined whether sectored clones were found among the meiotic progeny. If a cytoplasmic determinant were involved, both the revertant and the mutant would lack the determinant and normal 2:2 segregation, with no sectoring, would be expected (Figure 5). In fact, all meiotic progeny clones of *pep4-3* genotype resulting from this cross showed the sectored (mixed) phenotype when tested for CPY activity. These results make it unlikely that the sectored phenotype is a reflection of classical cytoplasmic inheritance. The remaining explanation is that the delayed expression of the mutant phenotype is an extreme case of phenotypic lag.

To test whether the phenotypic lag was restricted to meiotic progeny or would occur whenever mutant nuclei found themselves in wild-type cytoplasm, we performed a cytoduction experiment (CONDE and FINK 1976). A heterokaryon was formed by mating a strain of genotype α *pep4-3 trp1* [KIL-O] with one of genotype **a** *PEP4 kar1 leu1* [KIL-K]. Such heterokaryons were nonmating prototrophs and were Pep^+ and killers. Mitotic segregants that were tryptophan auxotrophs of α mating type which had become [KIL-K] (which ensures that the segregants were derived from heterokaryons) were obtained and tested for CPY activity using the APE test. All such clones showed the phenotypic lag characteristic of *pep4-3*-bearing meiotic segregants. We inferred from this result that phenotypic lag for expression of the *pep4-3* mutant phenotype will occur whenever a mutant nucleus, having been exposed to a wild-type cytoplasm, finds itself in a cell with is genotypically *pep4*.

To ensure that the red portions of the meiotic spore clones revealed by the APE test were indicative of cells that contained CPY activity and not some other esterase, we examined whether the *prc1-407* mutation, a nonsense mutation in the structural gene for CPY (HEMMINGS *et al.* 1981), would act to eliminate the lag in expression of the *pep4-3* mutation. A diploid heterozygous for both the *prc1-407* and *pep4-3* mutations was sporulated, and the meiotic products were examined by APE test. The results indicated that, as expected, a wild-type *PRC1* allele is required if the sectoring phenotype is to be observed (Table 1) and, therefore, that the ability to cleave APE in sectored colonies results from CPY activity. Moreover, the data indicate that *prc1-407* and *pep4-3* segregate

independently and are, hence, different genes. This finding is compatible with the observation that *prc1* and *pep4* mutations complement (JONES 1977).

An inactive 67,000-dalton precursor of CPY accumulates in cells that bear the *pep4-3* mutation (HEMMINGS *et al.* 1981). Although we had demonstrated that proteinase B is not required for the *in vivo* maturation of this precursor (HEMMINGS *et al.* 1981), HASILIK and TANNER (1978) had demonstrated that both proteinase B and trypsin could catalyze the conversion of the 67,000-dalton CPY precursor, *in vitro*, to a molecule of a molecular weight indistinguishable from that of mature CPY. We, therefore, attempted to activate the 67,000-dalton CPY precursor present in extracts made from cells of the *pep4-3* mutant by the addition of trypsin, *in vitro* (Table 2). Trypsin treatment results in the production of active CPY in extracts made from the *pep4-3* mutant at a specific activity at least 35% of that found in wild-type extracts treated similarly. Not unexpectedly, this treatment results in a reduction of the CPY activity in the extract made from the wild-type strain. Trypsin treatment of extracts made from the *prc1-407 pep4-3* doubly mutant strain does not result in the appearance of active CPY, as expected.

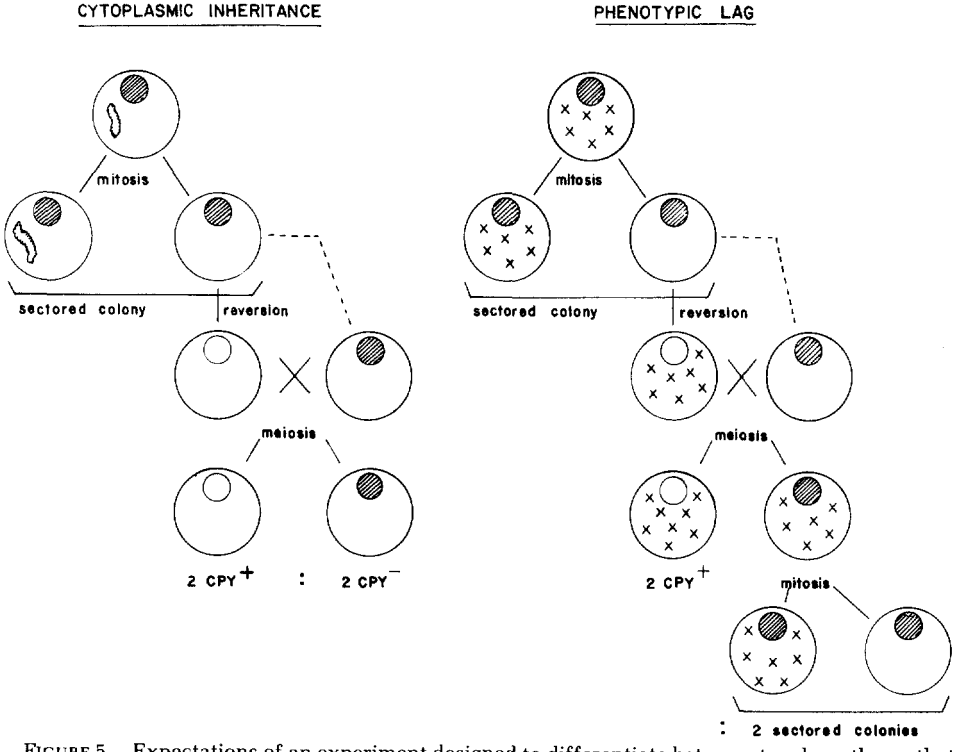

FIGURE 5.—Expectations of an experiment designed to differentiate between two hypotheses that could account for development of the sectoring phenotype of *pep4-3*-bearing meiotic products—cytoplasmic inheritance of a genetic determinant and extended phenotypic lag. Open circles are wild-type nuclei; hatched circles are mutant nuclei. Supercoils in the cytoplasm on the left indicate a genetic determinant in the cytoplasm. X's in the cytoplasm on the right indicate some gene product in the cytoplasm capable of generating CPY activity from CPY precursor. See text for discussion.

TABLE 1

Both prc1-407 *and* prb1-1122 *suppress the phenotypic lag of* pep4-3*-bearing segregants*

Genotypes of parent diploids	Genotypes of meiotic segregants	CPY phenotypes of meiotic segregants: Unsectored (CPY⁻):sectored
PRC1 *pep4-3* / *prc1-407* *PEP4*	*prc1-407 pep4-3*	12:1[a]
	PRC1 pep4-3	0:19
PRB1 *pep4-3* / *prb1-1122* *PEP4*	*prb1-1122 pep4-3*	15:0
	PRB1 pep4-3	0:20
PRC1 *PRB1* *pep4-3* / *prc1-407* *prb1-1122* *PEP4*	*prc1-407 PRB1 pep4-3*	9:0
	PRC1 prb1-1122 pep4-3	8:0
	prc1-407 prb1-1122 pep4-3	8:0
	PRC1 PRB1 pep4-3	0:9

[a] Greater than 50% of the sectored colony was of the mutant (CPY⁻) phenotype.

TABLE 2

Trypsin activation of the carboxypeptidase Y precursor in vitro[a]

Relevant genotype	Carboxypeptidase Y activity (U/mg × 10^3): − Trypsin	+ Trypsin
PRC1 PEP4	2.30	0.74
PRC1 pep4-3	0.02	0.266
prc1-407 pep4-3	0	0

[a] Extracts that had not been clarified by centrifugation were diluted with an equal volume of a 2-mg/ml solution of trypsin in 0.1 M Tris-HCl buffer, pH 7.6, and placed at 4° for 18 hr. These mixtures were then assayed for carboxypeptidase Y activity by the standard method except that 0.2 ml of a 20-mg/ml solution of soybean trypsin inhibitor in 0.1 M Tris-HCl, pH 7.6, was added to the reaction mixture before the initiation of the assay.

CABIB and his co-workers (CABIB and FARKAS 1971; ULANE and CABIB 1976) had shown that both trypsin and proteinase B can activate chitin synthetase zymogen. The finding that both trypsin and proteinase B can activate chitin synthetase zymogen, that both can catalyze cleavage of the CPY precursor to a molecule indistinguishable in size from mature CPY and that trypsin can activate the precursor to CPY, together suggested to us that, although proteinase B is not essential for the conversion of the CPY precursor to the mature form *in vivo*, it might, under certain circumstances, be sufficient to catalyze the conversion *in vivo*. We, therefore, investigated the possibility that mutations in the structural gene for proteinase B, *PRB1* (ZUBENKO, MITCHELL and JONES 1979), might eliminate the lag in expression of the *pep4-3* mutation. A diploid heterozygous for both *prb1-1122*, a nonsense mutation in the structural gene for proteinase B (ZUBENKO, MITCHELL and JONES 1980), and *pep4-3* was sporulated, and the sectoring patterns for spore clones of different genotypes were determined. The results appear in Table 1. These results show that the *prb1* mutation

eliminates the delay in expression of the *pep4-3* mutation. Hence, proteinase B activity is essential for the development of the phenotypic lag observed for *pep4-3*-bearing spore clones. The segregation data for this cross indicate that the *prb1-1122* and *pep4-3* alleles segregate independently and are, therefore, mutations in different genes.

The results of the final cross presented in Table 1 reinforce the conclusions of the two previous crosses. They also indicate that the *prc1-407* and *prb1-1122* alleles do not interfere with each other's ability to eliminate phenotypic lag.

DISCUSSION

The *pep4-3*-bearing spore clones that we have studied contain on the order of 10^6 cells. CPY activity persists in such spore clones and, indeed, can still be observed after several replica plating steps. It is difficult to imagine how the Pep^+ phenotype can be maintained by substantial numbers of cells after a dilution of the contents of the initial *pep4-3*-bearing spore by a factor of greater than 10^6.

One hypothesis that might explain this phenomenon is that the *pep4-3* mutation reduces the level of the *PEP4* gene product to just below some critical threshold level necessary for maintenance of the Pep^+ phenotype. Many generations would then have to occur before a cell that inherited the *pep4-3* mutation would exhibit the mutant phenotype. This hypothesis does not explain how a *prb1* mutation abolishes the phenotypic lag. Moreover, we have found that a pronounced phenotypic lag occurs when cells inherit any one of four independently isolated *pep4* mutations. This hypothesis would require that all four *pep4* mutations reduce the level of the *PEP4* gene product to very similar levels, just below some critical threshold value. We cannot eliminate the possibility but consider it unlikely, especially since we have obtained preliminary evidence that *pep4-3* is a nonsense mutation.

Carboxypeptidase Y is synthesized as a larger 67,000-dalton precursor which is converted to the mature form by the scission of a peptide, most likely from the amino terminus of the precursor (HASILIK and TANNER 1976, 1978; HEMMINGS *et al.* 1981; ZUBENKO 1981). The function of the *PEP4* gene product is required for this maturation to occur and strains that bear the *pep4-3* mutation accumulate an inactive 67,000-dalton CPY precursor (HEMMINGS *et al.* 1981; JONES *et al.* 1981; ZUBENKO 1981). Since mutations in the *PEP4* gene result in a deficiency for at least five, and perhaps all, vacuolar hydrolases, we postulated that all of these enzymes are synthesized as inactive precursors and that the maturation of these precursors requires the function of the *PEP4* gene. This prediction has been confirmed for the case of proteinase A (G. S. ZUBENKO, F. J. PARK and E. W. JONES, unpublished results). The way in which the *PEP4* gene or its product are involved in the maturation process is not clear. Lesions in the *PEP4* gene could render the proteolysis machinery inactive or, alternatively, might prevent delivery of the hydrolase precursors into the intracellular compartment where the proteolysis machinery resides.

A model for the phenotypic lag that is consistent with previous observations and with the results reported here is presented in Figure 6. According to the model, a *PEP4* gene product-dependent process catalyzes the maturation of a

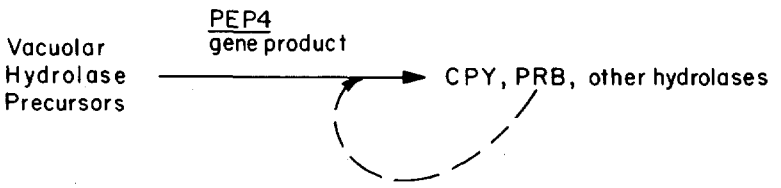

FIGURE 6.—Proposed basis of phenotypic lag. See text for discussion.

variety of inactive, vacuolar hydrolase precursors. The process cannot normally require proteinase B activity since *prb1* mutants have normal levels of proteinase A and carboxypeptidase Y (ZUBENKO, MITCHELL and JONES 1979). In the absence of the normal maturation mechanism, the proteinase B molecules present as a result of encapsulation within the spore could catalyze maturation of its own and the other hydrolase precursors. This would explain the persistence of the Pep^+ phenotype of the *pep4-3* mutant spore clones through successive generations. To account for the observation that the Pep^+ phenotype is unstable in these clones, one need only postulate that this auxiliary mechanism for the activation of proteinase B precursor occurs at a rate that is insufficient to ensure that all daughter cells receive some necessary threshold level of mature proteinase B. An inherent requirement of this model is that the proteinase B activity produced by this auxiliary mechanism must co-exist with the other hydrolase precursors in some common intracellular compartment. This compartment may be the vacuole, or it may be some other membranous compartment through which these proteins pass *en route* to the vacuole.

More complicated models can also be constructed to account for the observations. A minor variation would endow the proteinase B precursor with the capacity for autocatalysis. An additional variation would have proteinase B not contribute to the phenotypic lag by direct activation of the hydrolase precursors but instead act earlier to activate some other molecule.

We have considered a different class of model that would involve extrachromosomal elements, copied from a chromosomal DNA template, whose production and maintenance are dependent on the *PEP4* gene. Because the template resides in chromosomal DNA, genes present on such elements need not exhibit classical cytoplasmic inheritance. Although it is possible to construct such models to explain the phenotypic lag, it is difficult to incorporate the requirements for the *PRB1* gene function in a way that is consistent with the fact that *prb1* alleles segregate 2:2 in meiotic tetrads and show dosage effects on the levels of proteinase B activity in heterozygous strains (ZUBENKO, MITCHELL and JONES 1979).

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