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# Deletions, Duplications and Transpositions of the COR Segment that Encompasses the Structural Gene of Yeast Iso-1-cytochrome *c*

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## DISCLAIMER

Running head:

COR SEGMENT OF YEAST

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Rearrangements of chromosomal segments generally are rare events that appear to occur more-or-less randomly throughout the genomes of all organisms. However, occasionally consistent chromosomal alterations of specific segments are observed to occur at relatively high frequencies. We have recently found that a specific chromosomal segment, in certain but not all laboratory strains of Saccharomyces cerevisiae, is deleted and transposed at high frequencies. This segment, denoted COR, encompasses the three closely linked loci CYC1, OSM1 and RAD7 which control iso-1-cytochrome c, osmotic sensitivity and UV-sensitivity, respectively. Two types of apparently normal laboratory strains of yeast designated COR1 and COR2, were uncovered after the examination of the frequencies and types of mutations causing either deficiencies or overproduction of iso-1-cytochrome c; in contrast to COR1 strains which give predominantly point mutations causing deficiencies of iso-1-cytochrome c, COR2 strains give rise to deletions and transpositions of the COR segment. We have undertaken a systematic investigation of the physical structure and genetic properties of the COR region and of the aberrations arising in COR2 strains.

## Cloning of the COR1 Region

The physical structure of the CYC1 locus and adjacent regions are being investigated with recombinant DNA procedures. The studies were initiated after the analysis of extensive numbers of altered iso-1-cytochromes c from frameshift revertants led to the determination of a unique sequence of 44 nucleotides at one end of the translated region of the gene (Sherman and Stewart 1973; Stewart and Sherman 1974). It was found that DNA restriction fragments containing the CYC1 gene could be identified with a 15 base long synthetic deoxyribonucleotide (Szostak et al. 1977) that was complementary to a portion of this region of known sequence. Fragments prepared by partial digestion of COR1 DNA with EcoRI were ligated into the bacteriophage λgtWES and the resulting bank was screened with the probe by the procedure of Szostak et al. (1979). A 9 kb COR1 fragment,

denoted a-h in Figure 1, was obtained after screening approximately 8500 plaques; this fragment was shown to contain the entire CYC1 locus and adjacent regions. Figure 1 Defined probes were prepared by cleaving the cloned fragment with various restriction endonucleases and by transferring the smaller segments to the plasmid pBR322. Montgomery et al. (1978) also cloned the CYC1 gene using a similar procedure with a different synthetic probe.

More extensive regions adjacent to the CYC1 locus were cloned by integrating a hybrid plasmid into the COR1 region and then excising the plasmid along with new chromosomal material (Fig. 2). A plasmid consisting of the Escherichia coli plasmid pBR322 that contained the URA3 yeast gene and the COR1 segment g-h, was used to transform a COR1 yeast strain that contained the ura3-52 mutation. Figure 2 Genetic and physical analysis of one of the transformants established that the plasmid integrated into the corresponding chromosomal segment g-h near the CYC1 locus, probably by homologous recombination. Genomic DNA from the transformant was partially digested with the restriction endonuclease EcoRI, ligated and subsequently used to transform an E. coli strain for the $amp^r$ marker. Plasmids prepared by less extensive digestion were found to contain larger segments of new chromosomal DNA. One of the plasmids, pAB40, was used to determine the EcoRI and HindIII cleavage sites in the COR1 region and to prepare probes for investigating the structure of the COR2 region and of aberrations arising in COR2 strains (Figure 1). The restriction map, presented in Figure 1, spans a 12 kb region that probably encompasses all three of the loci, CYC1, OSM1 and RAD7. A moderately reiterated segment, within COR1 fragment n-p, at the distal end of the cloned region was revealed by its hybridization to a multitude of restriction fragments from genomic DNA. This reiterated segment probably corresponds to a portion of a Ty1 element (Cameron et al. 1979). There is also a less reiterated sequence within the fragment e-n, as seen when a XhoI derived fragment, j-n, is

used as a probe to genomic DNA. This segment, whose approximate location is represented by the arrow in fragment j-h, is present in three or four copies per genome.

## Comparison of COR1 and COR2 Physical Structures

The physical structure of COR regions from various strains was investigated by digesting genomic DNA with either or both EcoRI and HindIII restriction endonucleases, separating the fragments by agarose gel electrophoresis and then transferring the fragments to nitrocellulose sheets (Southern 1975). Homologous sequences were identified by hybridization to plasmids containing defined segments of the COR1 region. The analysis of the restriction fragments, summarized in Figure 1 indicates that the COR1 and COR2 regions have one extensive difference as well as one minor difference. The major difference appears to be an extra segment in the COR2 region b-e.

The larger HindIII fragment b-g from the COR2 region compared to the smaller analogous fragment from the COR1 region is most easily explained by the presence of an extra 7 kb fragment in the COR2 region. In addition, a less extensive difference between the COR1 and COR2 regions is observed in fragment i-k. Since the COR2 HindIII fragment g-k is smaller than the corresponding COR1 fragment and the overlapping EcoRI fragment f-h is the same size in both COR types, the approximately 300 base pairs that are absent in COR2 must lie within COR2 segment h-k.

Strains used in our laboratory could be assigned as having either a COR1 or a COR2 region. Standard strains, exemplified by D311-3A, that were used for detailed genetic analysis of the CYC1 locus, contain COR1 regions. Strains giving rise to higher frequencies of COR deletions were found to have COR2 regions, including the DEL1 strain SL356-4A described by Liebman et al. (1979) and the strains described below.

## COR2 Strains Give Rise to cyc1-Δ Deletions

In studies requiring the selection of cyc1 mutants from a variety of strains by the chlorolactate procedure, it was noted that certain strains spontaneously produced unusually high frequencies of cyc1 mutants. Furthermore, the vast majority of the cyc1 mutants arising from these strains contain deletions of the CYC1, OSM1 and RAD7 loci. These mutants, having deletions of the COR segment, are denoted as cyc1-Δ.

Two genetic studies were undertaken with two different series of strains; in the first study, Liebman et al. (1979) examined meiotic segregants from pedigrees initiating with strain D609-2A; this study examined meiotic derivatives from strain D-923. Because of the difference in the rates of formation of cyc1-Δ deletions, initially it was believed that the two series of strains might be different. However, the examination of segregants from crosses of representatives of these two series indicates, in fact, that they may be identical and that the variation in mutation rates may reflect genetic differences extrinsic to the COR region. Strains from both studies were shown to contain COR2 regions that could not be distinguished from each other by restriction mapping.

Genetic analysis demonstrated that the formation of cyc1-Δ deletions is associated with the COR2 region. Test on meiotic segregants from COR1 × COR2 crosses established that cyc1-Δ deletions arise spontaneously at high frequencies from COR2 strains but not from COR1 strains (Liebman et al. 1979; Stiles et al. in preparation). COR1 and COR2 segregants were conveniently distinguished by using CYC1-166-B COR1 and CYC1+ COR2 strains. The CYC1-166-B allele codes for an altered iso-1-cytochrome c which has altered spectral properties such that it can easily be distinguished from the wild type protein. On one hand, over 98% of the cyc1 mutants derived from 49 different CYC1+ COR2 strains contained cyc1-Δ deletions; on the other hand, more than 97% of the cyc1 mutants derived

from 38 different CYC1-166-B COR1 or CYC1+ COR1 strains contained point mutations or at least lacked extended deletions of the cyc1-Δ type (Liebman et al. 1979; Stiles et al. in preparation).

The extent of over 100 independently derived deletions from COR2 strains was determined by tests for OSM1 and RAD7 deficiencies and by tests of recombination with cyc1 point mutants. These genetic tests revealed that all of the cyc1-Δ deletions encompassed the entire CYC1 locus and at least portions of the OSM1 and RAD7 loci (Liebman et al. 1979; Stiles et al. in preparation). Furthermore, there was no evidence that any of the cyc1-Δ deletions extended into the SUP4 locus, which is closely linked to the CYC1-OSM1-RAD7 gene cluster (Liebman et al. 1979).

Different genetic backgrounds can greatly modify the efficiency of detection of cyc1 mutants with the chlorolactate selection procedure. Also the rates of spontaneously arising cyc1-Δ deletions may differ with different COR2 strains. In some COR2 strains the rates were as high as $8 \times 10^{-6}$ cyc1-Δ deletions per cell per generation, whereas no cyc1-Δ deletions were detected in COR1 strains, even in experiments designed to reveal rates as low as $10^{-10}$ (Liebman et al. 1979).

The DNA segment lacking in the cyc1-Δ deletions was investigated with the cloned COR1 probes. We previously demonstrated with limited probes that three independently derived cyc1-Δ deletions were identical and lacked at least a 10 kb segment in the COR2 region (Stiles et al. in preparation). The extent of the deletion was further characterized with the cloned regions shown in Figure 1. These results demonstrate that the cyc1-Δ deletion starts within or near the COR2 segment c-d and extends to another site over 12 kb away in the vicinity of the moderately reiterated segment. However, it does not appear that the deletions extend into this reiterated segment. Hybridization of the COR1 probe j-n to restriction fragments from cyc1-Δ DNA indicates that the deletion contains at least part of the j-n sequence; the presence of altered restriction fragments indicate that one endpoint of the deletions is in this segment j-n. Since COR1 probe g-1 shows no homology to the cyc1-Δ strains, the endpoint of the deletion must be between sites 1 and n.

## COR2 Strains Give Rise to Mutants Overproducing Iso-1-cytochrome c

In addition to giving rise to high frequencies of cyc1-Δ deletions, COR2 strains give rise to mutants overproducing iso-1-cytochrome c. The cause of the overproduction appears to be due to increases in the number of COR segments which, in turn, results in an increased number of CYC1 genes.

Mutants overproducing iso-1-cytochrome c were obtained from a COR2/COR2 diploid strain that was irradiated with UV or X-rays; 22 mutants having higher than normal cytochrome c levels were obtained after screening approximately 458,000 colonies with a benzidine staining procedure specific for heme proteins, followed by low temperature spectroscopic examination of the more intensely staining colonies. Genetic analysis of nine of these mutants indicated that the overproduction can be ascribed to two general classes of mutations denoted CYC1-H or CYC1-T. Alterations confined to the vicinity of the normal CYC1 locus are defined as CYC1-H mutations. It is conceivable that CYC1-H mutations could involve a number of different types of aberrations including tandem duplications of the COR segment. Transpositions that include the CYC1 gene and that result in the insertion of genetic material remote from the normal CYC1 locus are denoted CYC1-T. One of the transpositions, CYC1-T1, has been subjected to an intense genetic and physical analysis.

## The CYC1-T1 Transposition

The parental diploid strain having a genotype CYC1+ COR2/CYC1+ COR2 gave rise to a mutant, D-995, that ultimately was deduced to have the genotype CYC1+ (COR2)/CYC1+ (COR2) CYC1-T1. Through a series of genetic manipulations, the CYC1-T1 transposition was coupled to a cyc1 mutation. Genetic analysis of cyc1 CYC1-T1 × cyc1 crosses showed that the CYC1-T1 transposition segregated 2:2 as a single Mendelian unit unlinked to the normal CYC1 locus on chromosome X. Pedigree analysis of crosses with various markers established that CYC1-T1 is located on the right arm of chromosome VII between the centromere and the marker rme1. The extent of the material in the CYC1-T1

transposition was examined genetically with crosses containing various recessive mutations on the right arm of chromosome X (Figure 3). The complementation of cyc1, osm1 and rad7 deficiencies and the lack of complementation of ilv3 and cdc8 deficiencies indicated that the CYC1-T1 transposition contains the CYC1+, OSM1+ and RAD7+ genes but not the flanking genes ILV3+ and CDC8+.

Figure 3

The analysis of the amounts of iso-1-cytochrome c in CYC1-T1 strains suggested that the transposition was responsible for producing approximately two gene equivalents of iso-1-cytochrome c. Although the levels varied among different segregants having the same CYC1 genotypes, the amounts of iso-1-cytochrome c in cyc1 CYC1-T1 segregants were distinctly higher than the amounts in related CYC1+ segregants. However, it is difficult to precisely determine the relationship between the number of copies of the CYC1 locus and the amount of iso-1-cytochrome c because of the inability of yeast to produce over an upper limit of cytochrome c. More meaningful values were obtained by examining the amounts of iso-1-cytochrome c in triploid strains obtained by crossing CYC1+, cyc1 CYC1-T1 and CYC1+ CYC1-T1 haploid segregants to cyc1/cyc1 diploid strains. The levels of iso-1-cytochrome c observed is consistent with the interpretation that the CYC1-T1 transposition contains two copies of the CYC1 locus.

The physical structure of the CYC1-T1 transposition was characterized by hybridization of the COR1 probes to genomic DNA fragments generated by digestion with various restriction endonucleases. Because the CYC1-T1 transposition arose in a COR2 strain, analyses were carried out with CYC1+ (COR1) CYC1-T1 and cyc1-Δ CYC1-T1 strains. The restriction map, summarized in Figure 1, is consistent with the deduction obtained from the iso-1-cytochrome c levels that the CYC1-T1 transpositions contain two copies of the CYC1 gene. The pattern of restriction fragments indicate that the CYC1-T1 transposition is composed of two COR regions. Each of the two COR regions is equivalent or nearly equivalent to each other and to the region lacking in the cyc1-Δ deletions. It was found that both copies of

the COR region comprising the CYC1-T1 transposition, as well as the cyc1-Δ deletions, have end points that fall within the segment b-e unique to the COR2 region. Although the arrangement of the two COR regions in the CYC1-T1 transposition is unknown, the segregation of CYC1-T1 as a single Mendelian unit indicates that the two regions are adjacent or at least close to each other on chromosome VII.

## The COR Segment May be Flanked by Reiterated Sequences

A definitive model explaining the formation of deletions, duplications and transpositions of the COR segment will require a more complete physical description of the COR region. The characterization of certain crucial features of the COR2 structure requires the cloning of appropriate regions. This is currently in progress. However, we wish to point out that the unusual properties of the COR2 region can be attributed to reiterated sequences flanking the COR segment. Deletions, duplications and transpositions could arise by recombination between two distinct reiterated segments surrounding the COR region. Recombination between two reiterated segments on one strand, or between reiterated segments at different homologous sites on sister strands could lead directly to deletions. Similarly, duplications can occur as a direct consequence of recombination between sister strands, whereas circular chromosomal fragments can occur as a direct consequence of recombination between two reiterated segments on one strand. Transpositions would arise when a circular chromosomal fragment is integrated at a new site.

Deletions of regions flanked by repeat sequences already have been observed to occur at high rates in yeast. The introduction of yeast fragments along with foreign plasmid DNA by integrative transformation results in a structure in which the plasmid DNA is surrounded by duplicated segments (Hinnen et al. 1978; Struhl et al. 1979; Scherer and Davis 1979). These regions flanked by duplicated segments are lost at relatively high frequencies (Struhl et al. 1979; Scherer and Davis 1979).

Because one end of the cyc1-Δ deletions and one end of the CYC1-T1

transposition falls within the extra segment unique to the COR2 region, this extra segment at the proximal end of the COR region may, in fact be another copy of the slightly reiterated sequences present near the distal end of the COR region, as discussed above. While the properties of the COR2 strains and the available information on the physical structure are best explained by a COR segment flanked by two reiterated elements, the precise structure will be determined from the characterization of the COR2 clones.

However not all properties can be simply explained by the suggested arrangements of the COR1 and COR2 regions. The finding that COR2/COR1 diploid strains can give rise to *cyc1-Δ* deletions in COR1 regions as well as COR2 (Liebman et al. 1979) is difficult to reconcile with the absence of a reiterated segment on the proximal end of the COR1 region.

We wish to point out that the characteristic properties of the COR2 region are observed in a wide range of organisms. An artificially constructed sequence, *Tn10-his-Tn10*, in *Salmonella typhimurium*, in which two *Tn10* transposable elements flank the *his* operon, has the arrangement and properties analogous to the COR2 region; the intervening *his* region was observed to be deleted, tandemly duplicated and transposed (Chumley and Roth 1980). Also, a chromosomal segment, TE encompassing at least the two contiguous genes $w^a$ and $rst^+$ of *Drosphilia melanogaster* was observed to be transposed from its normal site on the X chromosome to other sites on the X chromosome and to other chromosomes; in addition the high frequency of occurrence of $w^-$ $rst^-$ mutants suggests that the TE segment is frequently deleted (Ising and Ramel 1976). Recently a correlation was observed for the chromosomal positions of TE elements and for the sites of *in situ* hybridization of a cloned *copia* element (Gehring and Paro 1980). The *copia* element is a dispersed, transposable, and reiterated sequence (Potter et al. 1979; Strobell et al. 1979; Young and Hogness 1980). Gehring and Paro (1980) speculated that the TE element is composed of the $w^a$ and $rst^+$ genes flanked by

two reiterated copia sequences. Thus it appears as if deletions, tandem duplications and transpositions of extended segments may occur in all eukaryotic and prokaryotic organisms if the appropriate chromosomal arrangements are present.

Acknowledgments

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## References

Cameron, J. R., E. Y. Loh and R. Davis. 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. Cell 16:739.

Chumley, F. G. and J. R. Roth. 1980. Rearrangements of the bacterial chromosome using Tn10 as a region of homology. Genetics 94:1.

Gehring, W. J. and R. Paro. 1980. Isolation of a hybrid plasmid with homologous sequences to a transposing element of Drosophila melanogaster. Cell 19:897.

Hinnen, A., J. B. Hicks, and G. R. Fink. 1978. Transformation of yeast. Proc. Nat. Acad. Sci. 75:1929.

Ising, G. and C. Ramel. 1976. The behavior of a transposing element in Drosophila melanogaster. In (M. Ashburner and E. Novitski, eds.) The Genetics and Biology of Drosophila, Vol. 1b, p. 947. Academic Press, New York.

Lawrence, C. W., F. Sherman, M. Jackson and R. A. Gilmore. 1975. Mapping and gene conversion studies with the structural gene for iso-1-cytochrome c in yeast. Genetics 81:615.

Liebman, S. W., A. Singh and F. Sherman. 1979. A mutator affecting the region of the iso-1-cytochrome c gene in yeast. Genetics 92:783.

Montgomery, D. L., B. D. Hall, S. Gillam and M. Smith. 1978. Identification and isolation of the yeast cytochrome c gene. Cell 14:673.

Scherer, S. and R. W. Davis. 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. Proc. Nat. Acad. Sci. 76:4951.

Sherman, F. and J. W. Stewart. 1973. Mutations at the end of the iso-1-cytochrome c gene of yeast. In (J. K. Pollak and J. W. Lee, eds.) The Biochemistry of Gene Expression in Higher Organisms, p. 56. Australian and New Zealand Book Co. PTY. LTD., Sydney.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503.

Stewart, J. W. and F. Sherman. 1974. Yeast frameshift mutations identified by sequence changes in iso-1-cytochrome c. In (L. Prakash, F. Sherman, M. W. Miller, C. W. Lawrence and H. W. Taber, eds.). Molecular and Environmental Aspects of Mutagenesis, p. 102. C. C. Thomas Publ., Inc. Springfield.

Struhl, K., D. T. Stinchomb, S. Scherer and R. W. Davis. 1979. High-frequency transformation of yeast: Autonomous replication of hybrid DNA molecules. Proc. Nat. Acad. Sci. 76:1035

Szostak, J. W., J. I. Stiles, C. P. Bahl and R. Wu. 1977. Specific binding of a synthetic oligodeoxyribonucleotide to yeast cytochrome c mRNA. Nature 265:61.

Szostak, J. W., J. I. Stiles, B-K. Tye, P. Chiu, F. Sherman and R. Wu. 1979. Hybridization with synthetic oligonucleotides. Methods Enzymol. 68:419.

Figure Legend

Figure 1. Restriction maps of COR1, COR2, cyc1-Δ and CYC1-T1

The arrangement of the restriction endonuclease cleavage sites was deduced by digesting genomic DNA from the various strains with either HindIII (H), EcoRI (E) or XhoI (X), separating the resulting fragments by agarose gel electrophoresis and, after transferring the fragments to nitrocellulose sheets, hybridizing to nick translated probes prepared from cloned segments of a COR1 strain as previously described (Stiles et al. 1980). The CYC1 locus, encompassing site f, is denoted by a solid box. The triangle between sites j and k in the COR1 map represents a 300 base pair segment found in COR1 but absent from COR2 and the COR2 derived transposition CYC1-T1. The underlined region, labeled Ty1, represents the position of a reiterated sequence described by Cameron et al. (1979). The arrow between sites m and n represents a slightly reiterated sequence; we hypothesize that another copy of this sequence may be in the extra material of the COR2 region b-e. The dashed lines indicate the approximate positions of this material. The dotted lines represent areas of ambiguity, the double lines non COR material detected in the CYC1-T1 transposition, and the blank area the material deleted in the cyc1-Δ deletions. The brackets in the CYC1-T1 map indicate that the end segments cannot be unambiguously assigned to either of the two COR segments present in the transposition.

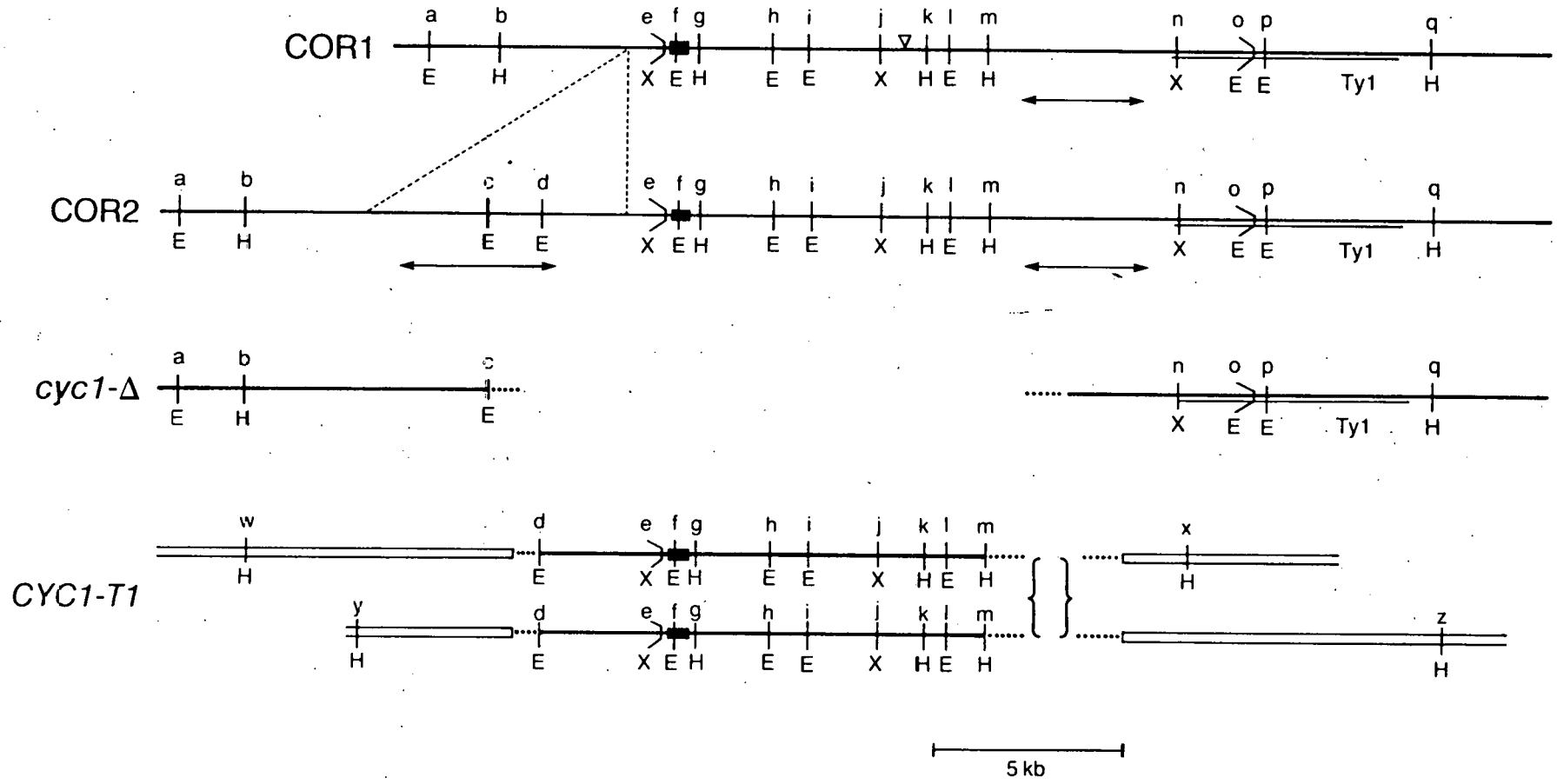
Figure 2. Cloning of extended regions of the COR segment

A plasmid, pAB30, consisting of pBR322, the yeast URA3 gene and COR1 fragment g-h (Figure 1), was used to transform a COR1 strain containing the ura3-52 mutation. Transformants, selected as uracil prototrophs, were found to have the plasmid integrated into the homologous g-h segment of chromosomal DNA. Plasmids, which are shown as solid lines at the botton of the figure and which contain material adjacent to the site of integration were recovered by partial digestion with EcoRI followed by ligation and transformation of E. coli for the plasmid $amp^r$ marker.

Solid triangles represent EcoRI recognition sites, and open triangles certain HindIII sites.

Figure 3. Genetic map of normal and CYC1-T1 strains

The genetic map of the right arm of chromosome X (Lawrence et al. 1975) and the center portions of chromosome VII either in the normal configuration or containing the CYC1-T1 transposition (Stiles et al. 1980) are as shown.


COR1
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E H X E H E E X H E H X E E Ty1 H
COR2
a b c d e f g h i j k l m n o p q
E H E E X E H E E X H E H X E E Ty1 H
cyc1-Δ
a b c n o p q
E H E X E E Ty1 H
CYC1-T1
w d e f g h i j k l m x
H E X E H E E X H E H H
y d e f g h i j k l m z
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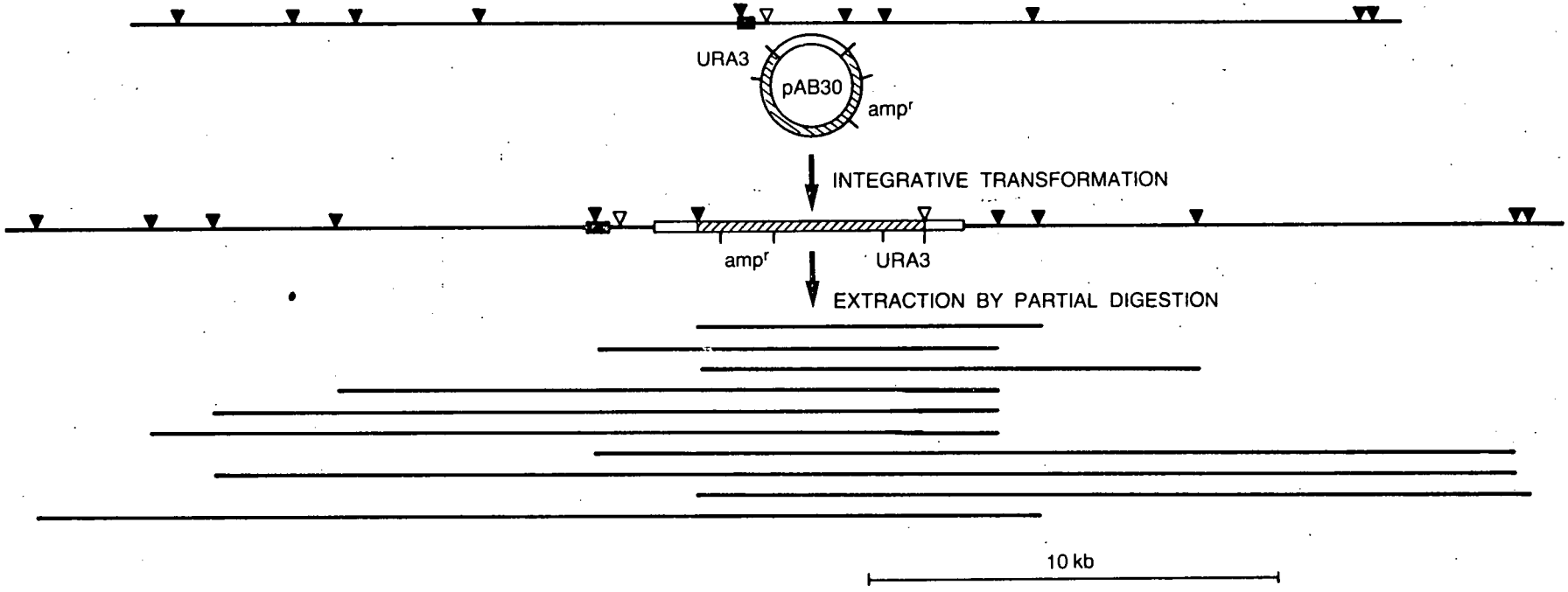
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pAB30
amp$^r$
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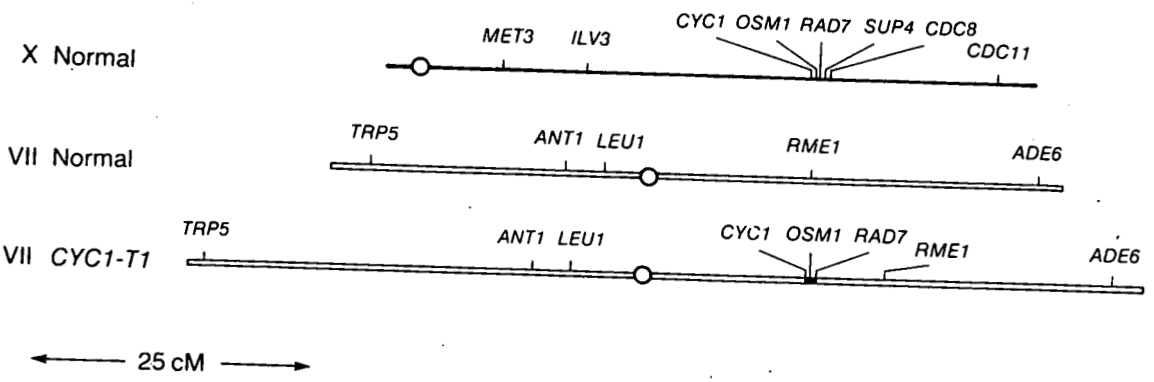
EXTRACTION BY PARTIAL DIGESTION
10 kb


X Normal
MET3
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CYC1
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RAD7
SUP4
CDC8
CDC11
VII Normal
TRP5
ANT1
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VII CYC1-T1
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ANT1
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CYC1
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RAD7
RME1
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25 cM