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ROAM Mutations Causing Increased Expression of Yeast Genes: Their Activation by Signals Directed Toward Conjugation Functions and Their Formation by Insertion of Tyl Repetitive Elements.

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Mechanisms available to eukaryotic organisms for the coordinate regulation of gene expression are being examined by genetic and biochemical characterization of an unusual mutation, CYC7-H2, which causes overproduction of iso-2-cytochrome c in the yeast Saccharomyces cerevisiae. The CYC7-H2 mutation causes approximately a twenty fold overproduction of iso-2-cytochrome c (Sherman et al. 1978) in haploid strains but only a one to four fold overproduction in MATa/MATα diploid strains (Rothstein and Sherman 1980). This regulation of overproduction has been characterized as a response to signals controlling conjugation in yeast. Furthermore, the abnormal controlling region has been identified as an insertion of a transposable and reiterated Tyl element adjacent to the structural gene (Errede et al. 1980). Therefore, we suggest that Tyl elements occur adjacent to some of the genes required for conjugation and thus normally function to control expression of this process. The suggested role of the Tyl element may represent a general mechanism of coordinate regulation in eukaryotes.

The CYC7-H2 mutation is closely related to other regulatory mutations occurring at the cargA, cargB and DUR1,2 loci which are the structural genes for arginase (Wiame 1971; Dubois et al. 1978), ornithine transaminase (Wiame 1971; Dubois et al. 1978; Deschamps and Wiame 1979) and urea amidolyase (Lemoine et al. 1978), respectively. Similar to the CYC7-H2 mutation, the mutations designated $cargA^{+}O^{h}$ (Dubois et al. 1978), $cargB^{+}O^{h}$ (Deschamps and Wiame 1979) and $durO^{h}$ (Lemoine et al. 1978) cause constitutive production of their respective gene products at much lower levels in MATa/MATα diploid strains than in the corresponding haploid strains. A consistent relationship between conjugation competence and the level of overproduction in all four mutants has been established (Errede et al. 1980). This correlation has suggested that overproduction in all four mutants is a response to signals normally controlling conjugation in yeast. Therefore, we have referred collectively to the CYC7-H2, $cargA^{+}O^{h}$, $cargB^{+}O^{h}$ and $durO^{h}$ mutant alleles by the acronym ROAM (regulated overproducing alleles

responding to mating signals). Observations characterizing the regulation of overproduction in the CYC7-H2 mutant are presented with the additional and parallel observations for the O^h mutants. Together these results provide a demonstration of the specificity and equivalence of regulatory control exhibited by ROAM mutants.

Mutants Overproducing Iso-2-cytochrome c

The total complement of cytochrome c in yeast normally consists of 95% iso-1-cytochrome c which is encoded by the CYC1 gene (Sherman et al. 1974) and 5% iso-2-cytochrome c which is encoded by the CYC7 gene (Downie et al. 1977a). Although either iso-cytochrome c can carry out the electron transport reactions necessary for utilization of lactate, a certain minimum amount is required for growth on medium containing lactate as the sole carbon and energy source. Therefore, mutants overproducing iso-2-cytochrome c have been conveniently isolated on lactate medium by reverting cyc1 strains which completely lack iso-1-cytochrome c but contain the normal low amount of iso-2-cytochrome c. Both intragenic and extragenic revertants can be obtained from cyc1 strains by this selection. However, if the cyc1 allele is not revertable or suppressible, the revertants contain increased amounts of exclusively iso-2-cytochrome c. Overproduction of iso-2-cytochrome c can be due to mutations at the CYC7 locus or to mutations at any number of loci unlinked to CYC7. The mutations at the CYC7 locus are dominant and involve gross chromosomal alterations while mutations at the unlinked loci are recessive, cause pleiotrophic effects and may not be directly involved in the regulation or biosynthesis of solely iso-2-cytochrome c. We have partially characterized the extended alterations in the three mutants designated CYC7-H1, CYC7-H2 and CYC7-H3, which contain twenty to thirty times the normal amount of iso-2-cytochrome c. The CYC7-H1 mutation is a reciprocal translocation which fuses an abnormal controlling region adjacent to the CYC7 gene (Sherman and Helms 1978). The

CYC7-H3 mutation is a deletion of a segment immediately adjacent to the CYC7 locus thus creating a new regulatory region (McKnight et al. in preparation). The CYC7-H2 mutation, which is the main topic of this paper, is an insertion of a Ty1 element at the CYC7 locus but outside of the translated portion of the gene.

Other Overproducing Mutants

Mutants causing overproduction of diverse gene products have been uncovered in a wide range of systems by various investigations. In particular, Wiame and his colleagues have systematically isolated and characterized regulatory mutations of arginine catabolism and urea utilization. Numerous $cargB^{+}O^{-}$ and $cargB^{+}O^{h}$ mutants constitutive for high amounts of ornithine transaminase have been isolated from $argR^{-}$ strains. They were selected on the basis of efficient growth on medium containing ornithine as the principle nitrogen source (Dubois et al. 1978). These $argR^{-}$ $cargB^{+}O^{-}$ strains utilize ornithine but not arginine; $cargA^{+}O^{-}$ and $cargA^{+}O^{h}$ mutants constitutive for arginase were selected from $argR^{-}$ $cargB^{+}O^{-}$ strains on medium containing arginine as the principal nitrogen source (Dubois et al. 1978). Using strains which grow slowly or do not grow on allantoin and urea, Lemoine et al. (1978) were able to isolate faster growing mutants, $durO^{h}$, with high constitutivity of urea amidolyase. The mutations causing overproduction were shown to be within or closely linked to the loci determining the respective gene products.

ROAM Mutants: Overproduction Diminished by MATa/MATα

The ROAM mutations, CYC7-H2, $cargA^{+}O^{h}$, $cargB^{+}O^{h}$ and $durO^{h}$ that overproduce iso-2-cytochrome c, arginase, ornithine transaminase and urea amidolyase, respectively, are distinct from other regulatory mutations occurring at these loci. Results summarized from independent genetic studies are presented in Table 1 to illustrate the distinction. The level of overproduction in ROAM

Table 1

mutants is substantially lower in MATa/MATα diploid strains homozygous for the ROAM allele than in the corresponding haploid strains. However, there is no difference in the level of overproduction observed in haploid or diploid strains containing the CYC7-H1, CYC7-H3, cargA^{+}O^{-} or cargB^{+}O^{-} mutations. Results in Table 1 also show that equivalent amounts of the corresponding gene products are produced in ROAM mutant haploid strains and MATa/MATa or MATα/MATα diploid strains. Therefore, the response of ROAM mutants to the MATa/MATα diploid condition is not a result of the ploidy but rather is due to the constitution of the mating type locus. In addition to the ROAM mutants that we describe, A. Toh-E (personal communication) has uncovered certain mutations at the PHOE locus that cause constitutive production of acid phosphatase in MATa and MATα diploid strains but not in MATa/MATα diploid strains. This observation suggests that mutations at yet another locus may belong to the ROAM class.

The functional role of the mating type locus essentially is to control the expression of two phenotypes that are normally exclusive of one another; MATa/MATα diploid strains are capable of meiosis but not conjugation while MATa and MATα haploid strains as well as diploid strains homozygous for either MAT allele are capable of conjugation but not meiosis. In order to relate MAT locus control of ROAM expression to one or the other phenotype, the level of overproduction in each mutant was determined in diploid strains with the unusual ability to both mate and sporulate (Errede et al. 1980). Diploid strains with either the mata-1/MATα (Kasir and Simchin 1976) or matα1,2/MATα (Strathern et al. 1979) genotype are capable of mating with MATa cells but these strains are defective in sporulation ability. Incorporation of the dominant SAD1 allele in the genotype of either diploid strain restores sporulation ability without preventing mating ability (Hopper and MacKay 1980; Kassir and Herskowitz 1980). The amount of gene product corresponding to each ROAM mutation in both mata-1/MATαSAD1 and matα1,2/MATαSAD1 was equivalent

to the high amounts generally found in strains with only mating competence (Errede et al. 1980). Thus, overproduction is not limited by sporulation capability. In addition, expression of the CYC7-H2 mutation was assessed in diploid strains that neither mate nor sporulate. This diploid phenotype is conveniently obtained by isolating ρ^- derivatives from standard MATa/MATα diploid strains. The ρ^- derivatives cannot sporulate because they are respiratory deficient. Equivalent and low amounts of iso-2-cytochrome c were observed in the ρ^+ MATa/MATα strains and their ρ^- derivatives. Together, these results domonstrate there is at least no superficial correspondence between the inability to express the overproducing phenotype and the ability to express sporulation. By contrast, there is a consistent correlation between conjugation ability and overproduction in ROAM mutants. Although these observations do not eliminate any number of possible regulatory relationships accounting for MATa/MATα control of conjugation and meiosis, they do suggest that overproduction is caused by signals required for conjugation competence.

ROAM Overproduction Is Diminished by Certain ste Mutations

The proposed mechanism for the diminution of overproduction in MATa/MATα strains suggests that certain mutations preventing the formation of the mating signal would concomitantly prevent conjugation in haploid cells and overproduction in ROAM mutants. Therefore, we are systematically investigating mating mutations for their possible effect on the level of iso-2-cytochrome c in CYC7-H2 strains. The results for the mutations that we have tested are summarized in Table 2. In contrast to ste mutations, certain mutations at the mating type locus (MAT) do not prevent mating; the mata-1 (Kassir and Simchen 1976) and the matα1,2 (Strathern et al. 1979) mutants are able to mate with MATα cells, The ste mutations are classified in Table 2 on the basis of the cell type in which the non-mating phenotype is expressed. The relative levels

Table 2

represent the average amounts of iso-2-cytochrome c in all segregants with the specified genotype derived from each cross. We have found genetic variation causes differences in the amount of iso-2-cytochrome c among strains containing identical CYC7 markers. These variations have a particularly pronounced effect on the amount of iso-2-cytochrome c in CYC7-H2 strains. Therefore, the effect on CYC7-H2 expression is assessed on the basis of the amounts of iso-2-cytochrome c in numerous segregants that come from the same pedigree and that either contain or lack the ste or mat mutation. As shown by the results in Table 2, the amount of iso-2-cytochrome c in ste7 CYC7-H2, ste11 CYC7-H2 and ste12 CYC7-H2 strains that cannot mate is appreciably lower than the amount in the corresponding control strains. We have previously shown that the ste7 mutation has no effect on the amount of iso-2-cytochrome c in CYC7-H1 and CYC7-H3 (Errede et al. 1980). Thus, certain ste mutations act with a high degree of specificity on certain allelic mutations of the CYC7 locus. However, the mat mutations and other ste mutations such as ste5 have no discernible effect on the amount of iso-2-cytochrome c. In addition, parallel experiments have shown that O^h strains with the ste7 mutation have amounts of their respective gene products equivalent to the depressed amounts in each of the O^h MATa/MATα diploid strains (Errede et al. 1980). Also in keeping with the results from the CYC7-H2 mutants (Table 2), the mata-1 and matα1,2 mutations have no effect on the expression of any of the O^h alleles nor does the ste5 mutation have an effect on the expression of the $cargA^+O^h$ mutation (Errede et al. 1980). These results establish that the diminution of ROAM expression is specific for mutation of only certain ste loci. Therefore, the observed overproduction in ROAM mutants is not merely related to the mating competent phenotype but rather is a response to a specific regulatory signal. Moreover, all ROAM mutants apparently respond to the same signal.

ROAM Mutations Occur Adjacent to Structural Genes and Are cis-Dominant

Genetic characterization of the ROAM mutations has been presented in previous publications (Sherman and Helms 1978; Sherman et al. 1978; Wiame 1971; Lemoine et al. 1978). The normal spore viability and the Mendelian segregation observed for each ROAM mutation indicate that they behave as single site mutations at the structural gene. Moreover, the normal amino acid sequence of iso-2-cytochrome c in the CYC7-H2 mutant and the normal catalytic activity of the gene products of the O^h mutants indicates that the mutations occur in a regulatory region which is outside of the translated portion of each gene. In fact, these two distinct regions of the CYC7 locus were defined in studies characterizing the cyc7-H2-1 mutation derived from a CYC7-H2 strain. The cyc7-H2-1 mutant lacking iso-2-cytochrome c was caused by a second-site mutation in the translated region of the gene corresponding to tryptophan 68 in the protein (Sherman et al. 1978).

ROAM mutations are cis-dominant and trans-recessive. High amounts of ROAM gene products observed in MATa/MATa or MATα/MATα diploid strains which are heterozygous for the corresponding ROAM allele established that each of these regulatory mutations is dominant (see references cited in Table 1). In addition, we have constructed diploid strains that demonstrate overproduction of iso-2-cytochrome c occurs when the dominant CYC7-H2 regulatory mutation is in the cis-configuration with respect to the CYC7 structural region but not when it is in the trans-configuration. The cyc7-H2-1 mutant was used for these studies because the second-site mutation within the translated region of the gene prevents expression of the regulatory mutation in the cis-configuration. Diploid strains with the mata-1/MATα and matα1,2/MATα genotypes were used for these tests to circumvent the MATa/MATα effect on CYC7-H2 expression (Errede et al. 1980). Overproduction of iso-2-cytochrome c in CYC7+ mata-1/CYC7-H2 MATα and in CYC7+ matα1,2/CYC7-H2 MATα strains was equivalent to the level found by Rothstein and Sherman (1980) for

MATa/MATa diploid strains heterozygous at the CYC7 locus. By contrast, CYC7+ mata-1/cyc7-H2-1 MATα and CYC7+ matα1,2/cyc7-H2-1 MATα diploid strains have amounts of iso-2-cytochrome c lower than diploid strains homozygous for the normal CYC7+ allele. Thus the regulatory region of the cyc7-H2-1 allele cannot cause overproduction when it is trans to the normal structural region of the CYC7+ gene and we conclude the CYC7-H2 mutation is cis-dominant. The cis-dominance of the $dur O^h$ (Lemoines et al. 1978) and $cargA^+O^h$ (Dubois et al. 1978) mutations was also rigorously established by analogous experiments using diploid strains homozygous for either allele at MAT to circumvent the MATa/MATα effect on expression of the O^h mutations. In the former study the $(dur1\text{-}1)O^h$ allele was employed in combination with DUR1,2 allele to demonstrate that the O^h mutation does not act in trans; the latter study employed a $cargA^-O^h$ allele in combination with the $cargA^+O^+$ allele for this demonstration.

Physical Structure of the Cloned CYC7-H2 Gene

CYC7-H2 contains a Ty1 insertion.

Recombinant DNA procedures were used to demonstrate that the overproduction of iso-2-cytochrome c in the CYC7-H2 mutant was caused by an alteration in the vicinity of the CYC7 locus, a result that was anticipated from the genetic properties. Initial experiments compared the sizes of the DNA fragments from HindIII digests of CYC7-H2 and CYC7+ genomic DNA that hybridize to the probe pAB32 containing the CYC7+ structural gene. The labeled CYC7+ probe hybridized to a single 3.5 kb fragment from the CYC7+ genomic DNA and a single 8.9 kb fragment from the CYC7-H2 genomic DNA, thereby suggesting that the mutant gene contains a gross alteration involving an extended region at or near the CYC7 locus (Errede et al. 1980). Subsequent restriction endonuclease mapping of a cloned CYC7-H2 fragment demonstrated that a segment of DNA approximately 5.5 kb in size was inserted, and that the insertion altered the XhoI site but not the PstI site (Errede et al. 1980) normally situated, respectively, 140 and approximately 260 bp in front of the AUG initiation codon (Montgomery et al. 1979).

In contrast to the CYC7+ probe which only hybridized to a single genomic DNA fragment containing the CYC7 structural gene, the probe pAB35 containing the CYC7-H2 cloned DNA hybridized to a multiplicity of different size HindIII fragments from both the CYC7+ and CYC7-H2 genomic DNA (Errede et al. 1980). This observation indicated that the CYC7-H2 fragment contains a sequence that is reiterated throughout the yeast genome. Recently, Cameron et al. (1979) identified and characterized a family of dispersed repetitive elements in yeast which they designated Ty1. The patterns resulting from hybridization of HindIII genomic DNA fragments to the CYC7-H2 fragment and to a fragment containing the central portion of a Ty1 element were similar, suggesting that the reiterated sequence in the CYC7-H2 cloned DNA is homologous to a Ty1 sequence (Errede et al. 1980). Thus, restriction fragments generated from pAB35 were examined directly for homology to the Ty1 probe. Because the fragments of CYC7-H2 DNA containing the segment denoted c-d in Figure 1 hybridized to the Ty1 probe, it was concluded that at least some portion of the CYC7-H2 inserted segment is homologous to the central portion of a Ty1 element. (Fig. 1)

CYC7-H2 contains δ sequences.

The yeast reiterated segment, Ty1, typically consists of a 5.6 kb sequence that includes 0.25 kb segments of a sequence referred to as "δ" at each end of the element. The haploid genome of yeast contains approximately 35 copies of Ty1 elements and at least 100 copies of δ elements; some of the δ elements are not contiguous with Ty1 elements (Cameron et al. 1979). The hybridization of the abnormal segment in the CYC7-H2 DNA to the central portion of the Ty1 element, together with the comparable size of the inserted segment and typical Ty1 elements suggests that the abnormal region may be a complete Ty1 sequence. An investigation was undertaken to determine whether or not δ sequences are present in the terminal regions of the CYC7-H2 insertion segment because the presence of these elements would be taken as evidence that the inserted segment is, in fact, a complete Ty1

element. The ends of the insertion fall within the two restriction fragments denoted a-b and e-f in Figure 1. Radioactive probes were prepared from these two fragments (a-b and e-f) as well as to the 2.2 kb EcoRI fragment containing the CYC7+ gene and to the 1.6 kb SalI-EcoRI fragment containing the central portion of the Ty1 element. The patterns resulting from hybridization of these probes to the HindIII fragments from genomic DNA of the standard CYC7+ strain D311-3A and of the CYC7-H2 strain D901-2B are presented in Figure 2. As expected, genomic DNA fragments that hybridize to the Ty1 probe are all greater than 5 kb in size; the Ty1 family of reiterated sequences does not contain internal HindIII restriction endonuclease cleavage sites and the typical element is 5.6 kb in size (Cameron et al. 1979). The hybridization pattern of the genomic DNA digests probed with the end fragments a-b and e-f and with the entire CYC7-H2 fragment are similar to the Ty1 patterns in the region of genomic fragments greater than 5 kb. However, these end fragments and the CYC7-H2 fragment also hybridize to a number of genomic DNA fragments considerably smaller than 5 kb. Thus the ends of the CYC7-H2 insertion contain sequences that hybridize not only to the family of Ty1 elements but also to another family of reiterated sequences. These observations are consistent with the suggested presence of δ elements at the ends of the CYC7-H2 insertion. It is also evident in Figure 2 that the hybridization pattern from the CYC7-H2 fragment a-b is similar but not identical to the pattern from the fragment e-f. This result suggests that the reiterated termini are homologous but that they may not be completely identical.

Fig. 2

Additional evidence for the presence of δ sequences comes from the hybridization of the end fragments a-b and e-f to λ recombinants that contain δ elements. Plaques prepared from the following phages and transferred to nitrocellulose sheets were shown to hybridize to the end fragments: λS1 that contains δ elements but not Ty1 elements; λB1 that contains δ elements and a part of a Ty1 element (Cameron et al. 1979); λAB35 that contains the entire CYC7-H2 gene (Errede et al. 1980). However, no hybridization was observed with plaques prepared from the parent

phage, λgtλB and λ598.

Heteroduplex analysis of the cloned CYC7-H2 fragments.

Heteroduplex analysis was employed to confirm and further characterize the abnormal region in the cloned CYC7-H2 DNA. Heteroduplexes were formed between two recombinant phages, λAB25 and λAB35, that contained, respectively, the CYC7+ and CYC7-H2 HindIII fragments shown in Figure 1. Because the two yeast fragments are in opposite orientation with respect to the λ molecule, two heteroduplex structures were observed; in one configuration only the homologous λ sequences hybridized; in the other configuration, shown in Figure 3a, only the homologous yeast sequences hybridized. In the latter structure, the extra segment in the CYC7-H2 fragment formed a single stranded loop having a size and position expected from the restriction map. Furthermore, equivalent loop structures were observed when the λAB25 phage, containing the CYC7+ gene, was hybridized to the CYC7-H2 fragment prepared by HindIII digestion of the plasmid pAB35. None of the heteroduplexes revealed "hairpin" or "lollipop" structures which would be indicative of inverted repeat sequences.

Fig. 3

Additional heteroduplex experiments were performed with DNA from the isolated CYC7-H2 1.1 kb fragment a-b and the 2.1 kb fragment e-f (Figure 1). Electron micrographs revealed an unusual structure, shown in Figure 4, that consists of a single duplex eye and two duplex tails. The contour lengths of the eye and tail regions are consistent with a structure that would result from heteroduplex formation between homologous sequences in the fragment a-b and fragment e-f with subsequent homoduplex formation between the single stranded nonhomologous sequences of each fragment. Such a structure not only requires sequences in fragment a-b and fragment f-h that are homologous but also requires that these sequences have the same orientation. Estimates from the contour length of the putative heteroduplex region in this structure suggest that the homologous region in the two CYC7-H2 fragments is at least 570 bp. This region of homology is more extensive than

Fig. 4

expected from the presence of only a 250 bp δ element at each end of the inserted segment.

In summary, results from both hybridization and heteroduplex studies indicate the presence of homologous and reiterated sequences which are present in the same orientation in the CYC7-H2 fragment a-b and fragment e-f. These two fragments contain the distal and proximal end points of the inserted segment in the CYC7-H2 cloned DNA. In addition, these fragments hybridize to λB1 and λS1 DNA that contain yeast inserts having δ sequences. The results from these investigations *in toto* strongly suggest the inserted segment in the CYC7-H2 fragment is a complete Ty1 element including δ segments with the same orientation at each end.

Proposed Role for the Yeast Transposable Element in Causing ROAM Mutations

The properties of ROAM mutations suggest that increased expression of ROAM alleles is a specific response to signals required for conjugation competence. Importantly, all ROAM mutants appear to respond to the same signal suggesting that the mutation at each ROAM locus involves a similar, if not identical alteration. Molecular characterization of the CYC7-H2 mutant has localized the alteration to a site within a region 140 to about 260 bp in front of the translated portion of this gene. Moreover, these investigations have identified the alteration as an inserted segment belonging to the Ty1 family.

As described above, Cameron et al. (1979) identified and characterized the Ty1 family of dispersed repetitive elements in yeast. The typical element consists of a 5.6 kb sequence that includes 0.25 kb segments denoted δ at each end. Thus, the yeast element structurally resembles prokaryotic transposable elements (Kleckner 1977). The additional similarity of the transposable nature of the yeast element was demonstrated by the variable distribution of Ty1 elements among related strains as well as for the same strain after prolonged culture. This characteristic property of the Ty1 element, together with the well established

precedent from prokaryotic insertion mutations (Starlinger and Saedler 1976). naturally suggests that each ROAM mutation occurred by transposition of a Ty1 element adjacent to the translated portion of the affected structural gene.

The proposal accounting for the occurrence of ROAM mutations is further supported by the finding that another yeast mutation, his4-912, has been shown to contain an inserted Ty1 element adjacent to the HIS4 gene (D. Chaleff, S. Roeder and G. Fink, private communication). In contrast to the increased expression of ROAM mutations, the Ty1 insertion at the HIS4 locus inactivates expression of the gene. This distinction indicates that increased expression of an adjacent structural gene may require a particular orientation or position of the Ty1 element. Such may be the case, particularly if the δ sequences of the yeast element are acting as promoters with properties analogous to those demonstrated for IS-elements present as terminal repeat sequences in bacterial transposons (Kleckner 1977; Starlinger and Saedler 1976).

The occurrence and generality of insertion mutations extends to other eukaryotic systems. Indeed, the first evidence for the existence of transposable elements affecting gene expression has come from the well studied eukaryotic organisms maize (McClintock 1956) and Drosophila (Green 1977). Furthermore, several families of repetitive elements in Drosophila are remarkably similar to the Ty1 elements both in their physical structure and in their ability to transpose (Finnegan et al. 1977; Potter et al. 1979; Strobel et al. 1979). The role of two such elements, denoted copia and DM225, in unstable mutations at the white locus of Drosophila has recently been described (Green 1977; Rasmuson et al. 1980; Gehring and Paro 1980). In addition, the controlling elements in maize have been attributed to insertion mutations analogous to those caused by insertion sequences in bacteria (Nevers and Saedler 1977).

Hypothetical Model for Concomitant Regulation of Conjugation and Overproduction In ROAM Mutants

The genetic, molecular and regulatory properties of the ROAM mutants can be interpreted according to the model presented in Figure 5. The pertinent feature of the model is that Tyl elements normally occur adjacent to some of the genes required for conjugation and that these Tyl elements act as receptors for the positive regulatory determinant (PRD) specifically controlling the mating process. As we have proposed in the previous section, the ROAM mutants are the result of the insertion of Tyl elements and thereby become members of the hypothetical Tyl gene battery. This group of genes, then, is coordinately activated by the PRD controlling mating functions. (Fig. 5)

According to this hypothesis both conjugation and overproduction in ROAM mutants requires the presence of PRD. The formation or expression of PRD occurs in MAT$\underset{\sim}{a}$ and MATα haploid strains capable of mating. Inhibition of PRD occurs under conditions in which both of the codominant MAT alleles are expressed, such as in normal MAT$\underset{\sim}{a}$/MATα diploid strains incapable of mating. The inhibition of PRD also occurs in haploid strains incapable of mating because of mutations denoted by the general symbol steA in Figure 5. SteA mutations could either directly inactivate the PRD or could indirectly prevent its expression by establishing a regulatory condition equivalent to that of the MAT$\underset{\sim}{a}$/MATα diploid cell. The absence of PRD would result in a nonmating phenotype and in concomitant decreased expression of ROAM mutations. The results presented in Table 2 and summarized in Table 3 indicate that the ste4, ste7, ste11 and ste12 mutations are representative of the steA class. In addition, evidence characterizing the mar1-1 mutation (Klar and Fogel 1979) indicates that this mutation prevents conjugation because it allows expression of the normally silent $\underset{\sim}{a}$ gene and α gene information residing at the HML and HMR loci. Thus, mar1-1 represents the hypothetical steA mutations that indirectly inhibit PRD by mimicking the MAT$\underset{\sim}{a}$/MATα regulatory state in haploid cells. The expected result is shown in Table 2; the amount of iso-2-cytochrome c in the CYC7-H2 mutant is depressed in mar1-1 haploid strains. (Table 3)

Other mutations of the general type denoted steB and steC in Figure 5 inactivate mating capacity but not the PRD. The mutations such as matα1, ste3 and ste5 which prevent conjugation but not overproduction of iso-2-cytochrome c (Table 2 and Table 3) could represent either general class. Conversely, mutations of the ROAM gene, such as the cyc7-H2-1 mutation, would prevent overproduction but not conjugation. Thus, analysis of ste mutants illustrates the potential utility of the CYC7-H2 mutant in providing a basis for understanding the complex regulatory network controlling the mating competent cell type in yeast.

Implications of the Hypothetical Role of Ty1 in Coordinate Regulation of Gene Expression

The arrangement of repetitive sequence elements in eukaryotic genomes, together with their sequence length and repetition frequencies indicate that they do not resemble structural genes but indirectly suggest they must provide some genomic function (Davidson et al. 1977). More recent studies have shown that transcripts of certain repetitive sequence families are present at very different concentrations in the nuclear RNA of sea urchin embryos as compared with adult intestine cells (Scheller et al. 1978). In addition, physical studies of animal genomes have revealed repetitive sequences within regions containing developmentally regulated genes (Duncan et al. 1979; Lomedico et al. 1979; Dodgson et al. 1979; Jelinek et al. 1980; Shen and Maniatis 1980). These various lines of evidence have suggested elaborate models involving repetitive sequences for coordinate regulation of gene expression during cellular differentiation and development of eukaryotes (Davidson and Britten 1979). The results presented here provide direct evidence for the role of the yeast repetitive element, Ty1, in the regulation of ROAM mutant expression. In addition, the regulatory properties of these mutants suggests a requirement for the Ty1 element in coordinate regulation of genes determining mating functions in yeast. These observations suggest to us that a major function of repetitive DNA in all eukaryotes may be the regulation of gene expression.

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Table 1. Expression of Overproducing Mutations in Strains with Different Mating Type Locus Compositions.

Gene Product	Mutation	Relative amount of gene product[a]			Reference
		Haploid	Diploid[b]		
		*MAT*a or *MAT*α	*MAT*a / *MAT*a or *MAT*α / *MAT*α	*MAT*a / *MAT*α	
Iso-2-cytochrome *c*	*CYC7-H1*	30	—	30	Rothstein and Sherman 1980
	CYC7-H2	20	20	2	
	CYC7-H3	20	—	20	
Arginase	*cargA*$^{+}$*O*$^{-}$*-1*	20	19	22	Dubois et al. 1978
	cargA^{+}*O*^{h}*-1*	34	34	9	
Ornithine transaminase	*cargB*$^{+}$*O*$^{-}$*-1*	200	—	200	Deschamps and Wiame 1979
	cargB^{+}*O*^{h}*-1*	500	520	65	
Urea amidolyase	*durO*^{h}*-1*	275	220	20	Lemoine et al. 1978

[a]The amount of each gene product produced was determined according to methods given in the corresponding references. The amounts are relative to the amounts in strains with the wild type allele for each of the structural genes. One unit of the normal values corresponds to the following: iso-2-cytochrome *c*, 10-20 mg (kg dry wt)$^{-1}$ (Sherman et al. 1965; other numerous measurements); arginase, 7 μmoles of urea produced hr^{-1} (mg protein)$^{-1}$; ornithine transaminase, 0.02 μmoles of Δ pyrroline-5-carboxylic acid produced hr^{-1} (mg protein)$^{-1}$; urea amidolyase, 1 nmole CO_2 hr^{-1} (mg protein)$^{-1}$.

[b]The diploid strains used are homozygous for the designated mutant alleles.

Table 2. Average Amount of Iso-2-cytochrome *c* in *CYC7-H2* Segregants with and without Various Mating Type Mutations.

Class of mutation	Class	Mutant segregants		Control segregants	
		Pertinent genotype[b]	Average amount of[c] iso-2-cytochrome *c*	Pertinent genotype[b]	Average amount of[c] iso-2-cytochrome *c*
MAT:					
nonsporul.[d]	E375 & E376	*mat***a**-1	16	*MAT*α	16
α nonmater	E322	*mat*α1-2	20	*MAT***a**	22
α nonmater	E323	*mat*α1-5	20	*MAT***a**	20
nonsporul.[d]	E420	*mat*α1,2	15	*MAT*α	7
a nonmater	E168	*MAT***a** *ste2*	12	*MAT***a** *STE2+*	7
	E194	*MAT***a** *ste2 (ts)*	9	*MAT***a** *STE2+*	20
	E254	*MAT***a** *ste6 (ts)*	9	*MAT***a** *STE6+*	17.5
α nonmater	E347	*MAT*α *ste3*	17.5	*MAT*α *STE3+*	15
	E279	*MAT*α *ste13*	15	*MAT*α *STE13+*	19
a & α nonmater	E170	*ste4 (ts)*	12	*STE4+*	22
	E423	*ste4 (ts)*	8	*STE4+*	22
	E192	*ste5 (ts)*	11	*STE5+*	15
	E198	*ste5 (ts)*	17	*STE5+*	14
	E238	*ste7 (ts)*	1.5	*STE7+*	18
	E296	*ste8 (ts)*	9	*STE8+*	9
	E299	*ste9 (ts)*	14	*STE9+*	20
	E320	*ste11 (ts)*	2.5	*STE11+*	15
	E311	*ste12 (ts)*	2.5	*STE12+*	20
	E309	*MAT***a** *mar1-1*	5	*MAT***a** *MAR1+*	15

Table 2. (cont.)

[a]Crosses with either matα1-2 and matα1-5 mutants were made by selecting rare prototrophic diploid strains (MacKay and Manney 1974). Crosses involving mutations that are expressed in only a mating or only α mating strains were made using haploid strains of the alternate mating type in which the mutation has no observable phenotype. Crosses involving the ts mutations were made at the permissive temperature of 22°C which allows mating and not at the restrictive temperature of 35°C which prevents mating. Crosses involving the mar1-1 mutation were made with MATα mar1-1 hmra-1 HMLα haploid strains which are capable of mating with MATa strains due to the mutation at the HMR locus (Klar and Fogel 1979).

[b]Segregants were obtained and pertinent genotypes were identified using procedures described by Errede et al. (1980). The mating type mutations were derived from the following strains: 17-15 (mata-1) provided by Y. Kassir (Hebrew University); VC2 (matα1-2), VN33 (matα1-5), VAB2 (ste2 allele in E168), VZ4 (ste4 allele in E170) and VAC1 (ste5 allele in E198) provided by V. MacKay (Rutgers University); 381-11-1-50b (ste2 allele in E194), 381-11-1-2b (ste4 allele in E423), 381-11-1-10d (ste5 allele in E192), 381-11-1-26a (ste7), 797-1-3 (ste8), 762-4-3 (ste9), 381-11-1-6a (ste11) and 381-11-10a (ste12) provided by L. Hartwell (University of Washington); G54-17d (ste3) provided by G. Sprague and I. Herskowitz (University of Oregon); alf1-28 (mat 1,2), RSA21 (ste6) and A2S3 (ste13) provided by J. Rine and I. Herskowitz (University of Oregon).

[c]The strains used in this study contain one of several cyc1 alleles that prevent the formation of iso-1-cytochrome c (Sherman et al. 1974).

Table 2. (cont.)

Therefore, determination of the total cytochrome c concentration provides a direct measure of iso-2-cytochrome c. Strains were grown on solid medium (Sherman et al. 1974) at 35°C if they were derived from pedigrees containing ts mutations; all other strains were grown at 30°C. The amounts of cytochrome c were estimated by low-temperature (-190°C) spectroscopic examination of intact cells; the c_α band intensities were visually compared to standard strains containing known amounts of cytochrome c. The average value is determined from the amounts of cytochrome c in all segregants having the same pertinent genotype. One unit of iso-2-cytochrome c corresponds to 10-20 mg (kg dry wt)$^{-1}$, the amount in strains with the wild type CYC7+ allele (Sherman et al. 1965; and numerous other measurements.)

[d] mata-1/MATα and matα1,2/MATα diploid strains can mate with MATa cells and can not sporulate.

The results shown for the effect of the ste5 mutations and the ste7 mutations are summarized from results presented by Errede et al. (1980). Results shown for the effect of other mating type mutations are summarized from Errede et al. (in preparation).

Table 3. Classification of Mating Type Mutations.[a]

Phenotype	*ste*A	*ste*B or *ste*C	Unclear
α nonmater		*matα1-2*	
		matα1-5	
		ste3	
		ste13	
a nonmater			*ste2*
			ste6
a and α nonmater	*ste7*	*ste5*	*ste8*
	ste11		*ste9*
	ste12		
	ste4		
	mar1-1		

[a]The classification according to the model presented in Figure 5 is based on the results, presented in Table 2, in which steA mutations diminish the CYC7-H2 overproduction while steB and steC mutaions do not.

FIGURE LEGENDS

Figure 1. Restriction maps of the CYC7+ and CYC7-H2 genomic regions. Cleavage sites in the cloned segments containing the CYC7+ and the CYC7-H2 genes were determined with the following restriction endonucleases: HindIII (H); EcoRI (E); BamHI (B); PstI (P); XhoI (X); BglII (G); and SalI (S). The solid box depicts the translated portion of the CYC7 locus. The open bar in the CYC7-H2 sequence that is enclosed by the dotted lines designates the inserted Ty1 element. The two gaps indicated with dots in the CYC7-H2 fragment represent the junction region and contain sequences corresponding to either the normal CYC7 region or to the inserted segment. There may be an additional EcoRI site in the vicinity of the EcoRI site designated c. (Adapted from Errede et al. 1980.)

Figure 2. Restriction fragments from genomic DNA complementary to the CYC7+ gene, the central portion of a Ty1 element and the regions encompassing the Ty1 insertion junctions. Fragments from genomic DNA of the CYC7+ strain, D311-3A, and of the CYC7-H2 strain, D901-2B, were prepared by digestion with HindIII, were separated by electrophoresis in 0.8% agarose gels, transferred to nitrocellulose sheets and hybridized to nick-translated fragments corresponding to the following sequences described in Figure 1: the 2.2 kb EcoRI fragment containing the CYC7+ gene; the central portion of a Ty1 element equivalent to the CYC7-H2 fragment c-d; the CYC7-H2 fragment a-b; and the CYC7-H2 fragment e-f. The pattern of genomic fragments hybridizing to the probe a-b, the probe c-d, and the probe e-f indicates that genomic DNA contains more copies of reiterated sequences which are homologous to the teminal portions than to the central portions of the of the inserted segment. In addition, the restriction patterns suggest

that the fragments complementary to each of the end fragments a-b and e-f represent two families of reiterated sequences that are similar to each other but are not identical.

Figure 3. Heteroduplexes between CYC7+ and CYC7-H2 fragments. A micrograph (a) and tracing (b) of a heteroduplex between λAB25 and λAB35 are shown. The two bacteriophage λ strains contain, respectively, the CYC7+ and CYC7-H2 HindIII fragments represented in Figure 1. The λ portions of the molecule are unpaired because the CYC7+ and CYC7-H2 fragments are in opposite orientations. The single-stranded loop, indicated by the arrow, is the 5.6 kb Ty1 insertions of the CYC7-H2 fragment. The double stranded circular molecule in the upper right corner of (a) is a size standard ϕX174 RF DNA.

A micrograph (c) and tracing (d) of a heteroduplex between λAB25 and the CYC7-H2 HindIII restriction fragment from pAB35 are shown. The single stranded Ty1 insertion from the CYC7-H2 fragment, which is indicated by the arrow, and the single-stranded portions of the λ molecule can be seen.

The DNA heteroduplexes were formed and spread for electron microscopy using the formamide-Kleinschmidt procedure of Davis et al. (1971).

Figure 4. A heteroduplex between fragments containing the ends of the segment inserted into CYC7-H2. The figure shows a micrograph (a) and tracing (b) of a heteroduplex between the two restriction fragments denoted a-b and e-f in Figure 1; each fragment encompasses a different end of the Ty1 insertion. The double-stranded eye-structure indicates that the opposite ends of the inserted segment are homologous. The interpretation of the heteroduplex is consistant with the determinations of the fragment lengths corresponding to 1.1 and 2.1 kb whether measurements are made on

heteroduplex or homoduplex structures.

Figure 5. The model for the coordinate regulation of ROAM mutations and genes controlling conjugation functions. An undefined and hypothetical PRD (positive regulatory determinant) activates the transcription of genes that are contiguous with Ty1 elements (open rectangles). Although normally Ty1 elements are contiguous with genes that only control conjugation functions, Ty1 elements can be inserted adjacent to other genes, resulting in the so-called ROAM mutations. The PRD is lacking in MATa/MATα diploid strains and in haploid strains with steA mutations. The STEB and STEC genes are required for conjugation functions; haploid strains with mutations of these genes are unable to conjugate but the PRD is not affected and the ROAM gene product is still overproduced. (Modified from Errede et al. 1980.)

Fig. 1

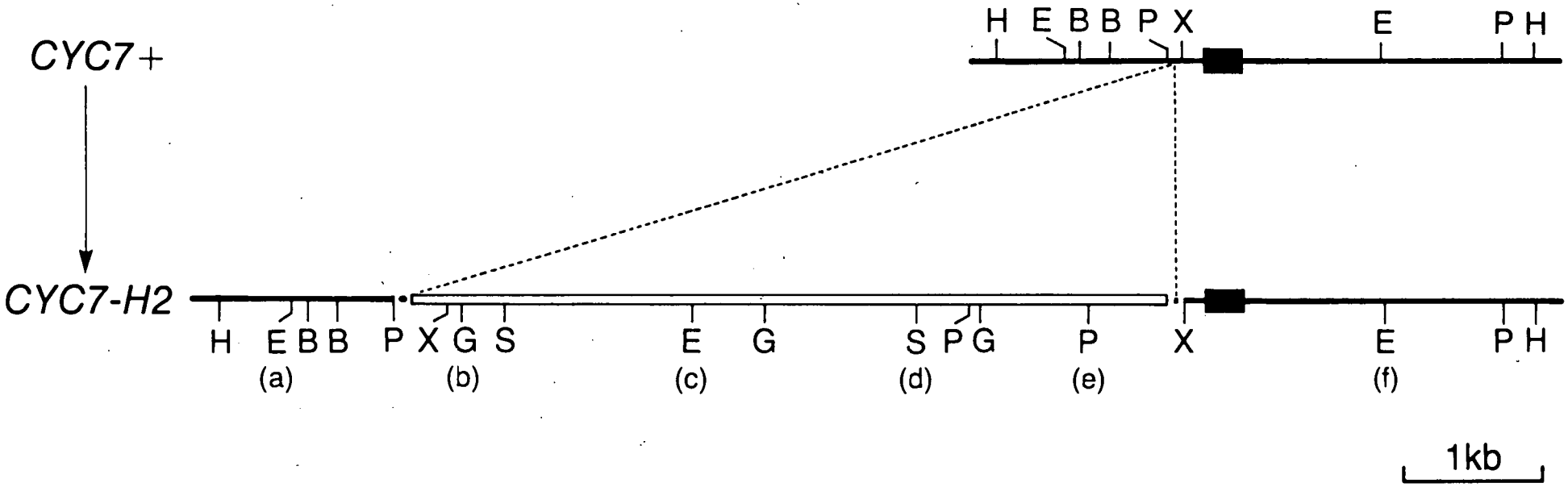
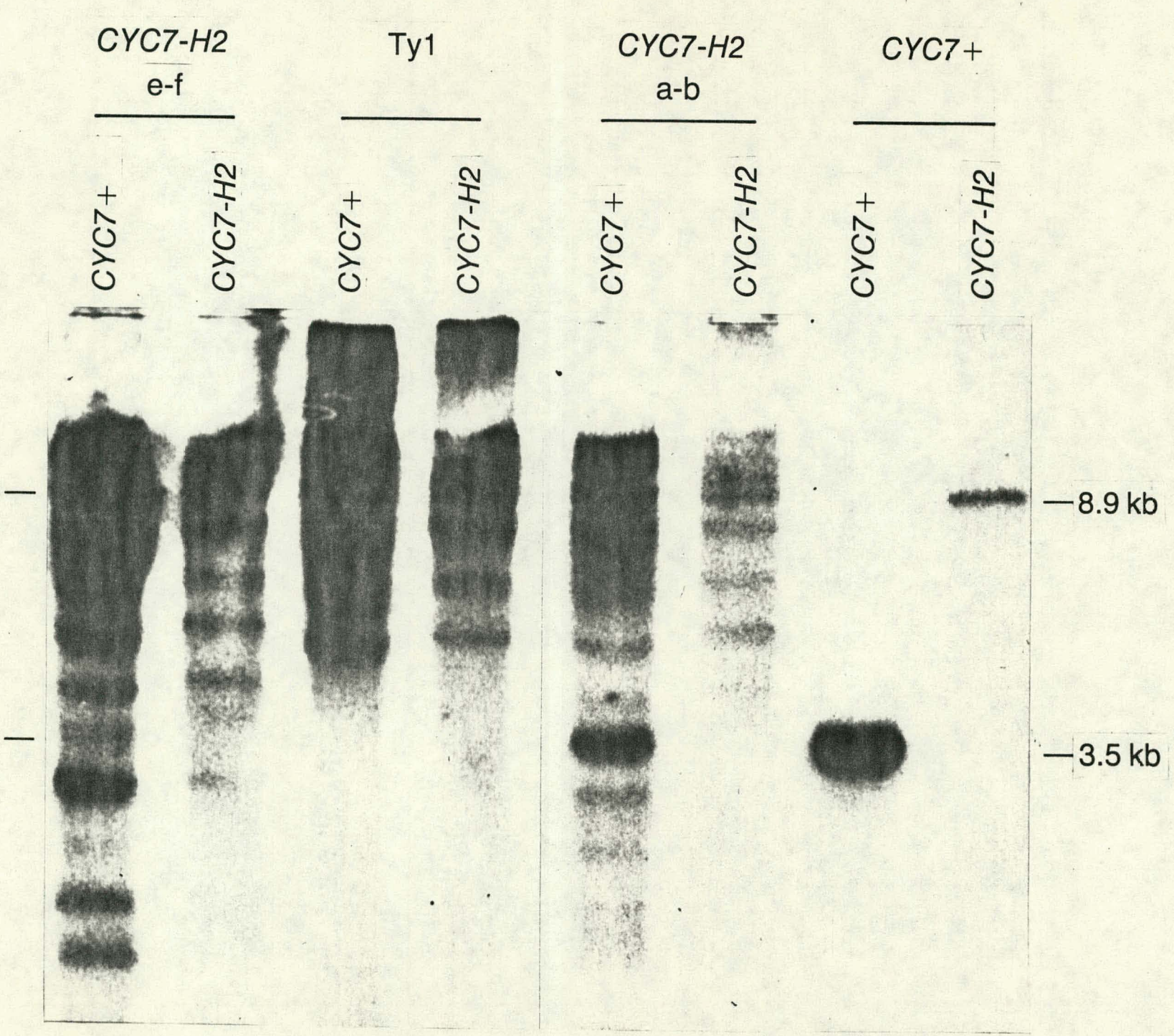


Fig 2


CYC7-H2
e-f
Ty1
CYC7-H2
a-b
CYC7+
CYC7+
CYC7-H2
CYC7+
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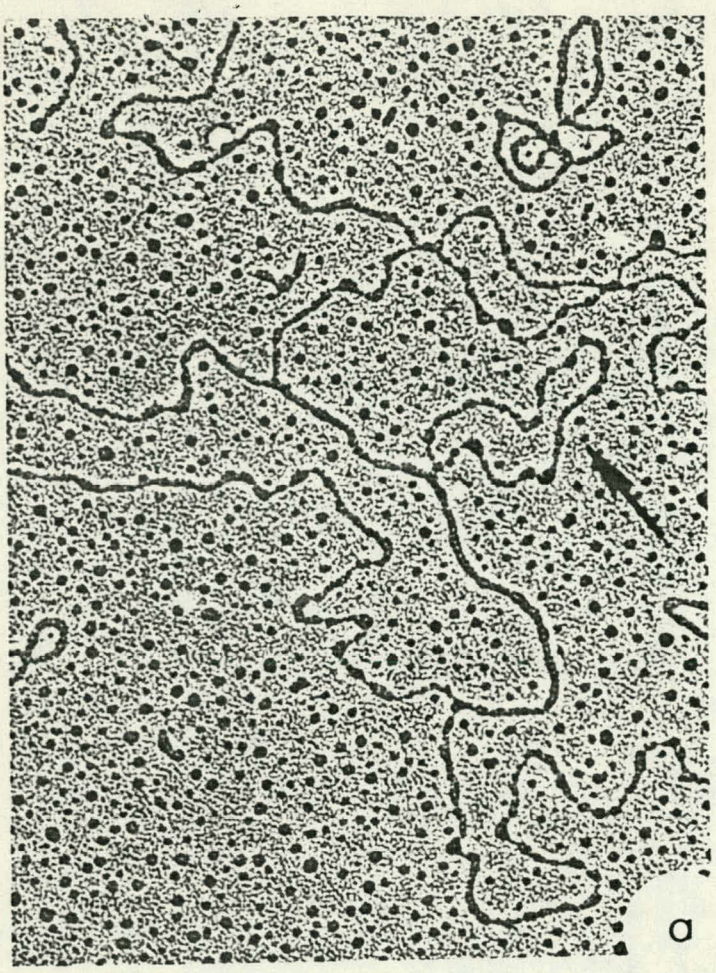
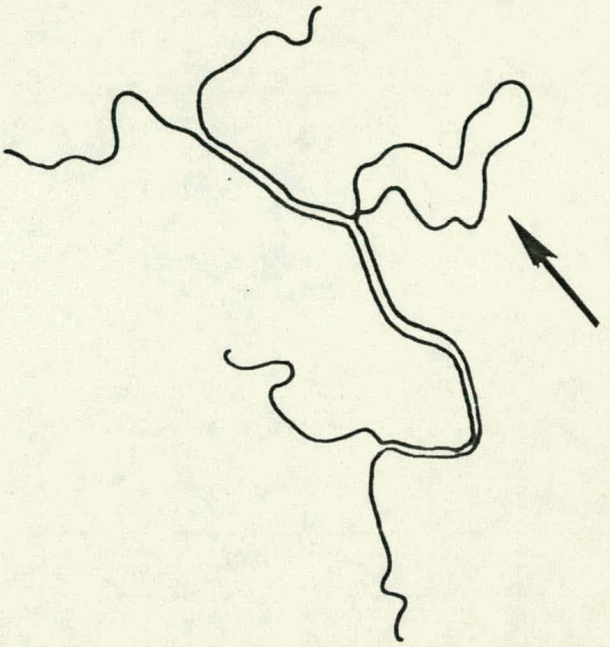
CYC7+
CYC7-H2
8.9 kb
3.5 kb



a



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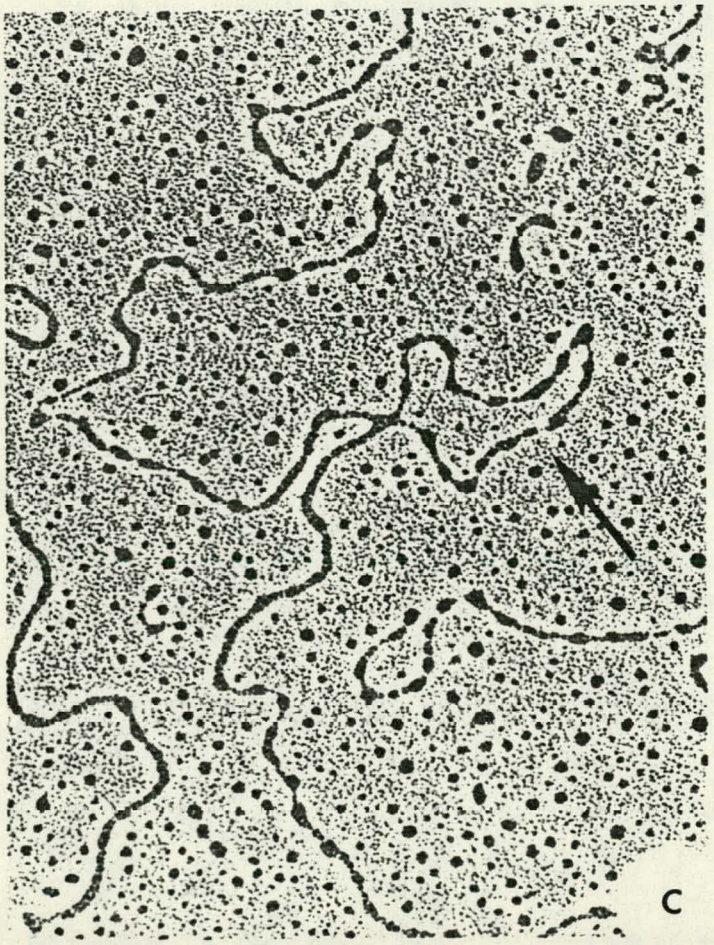
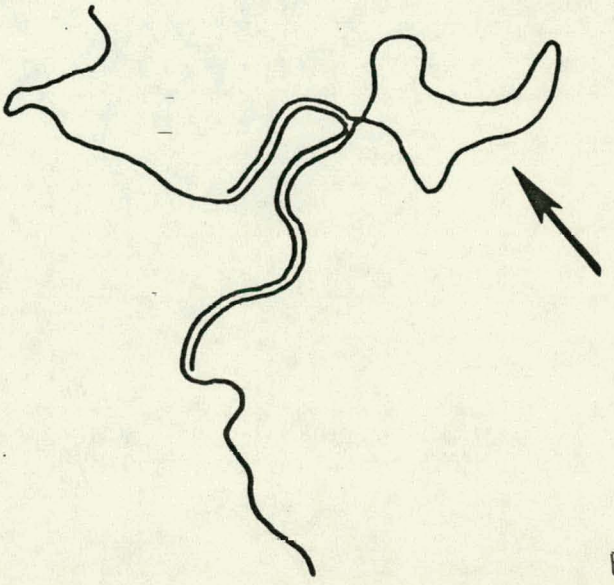


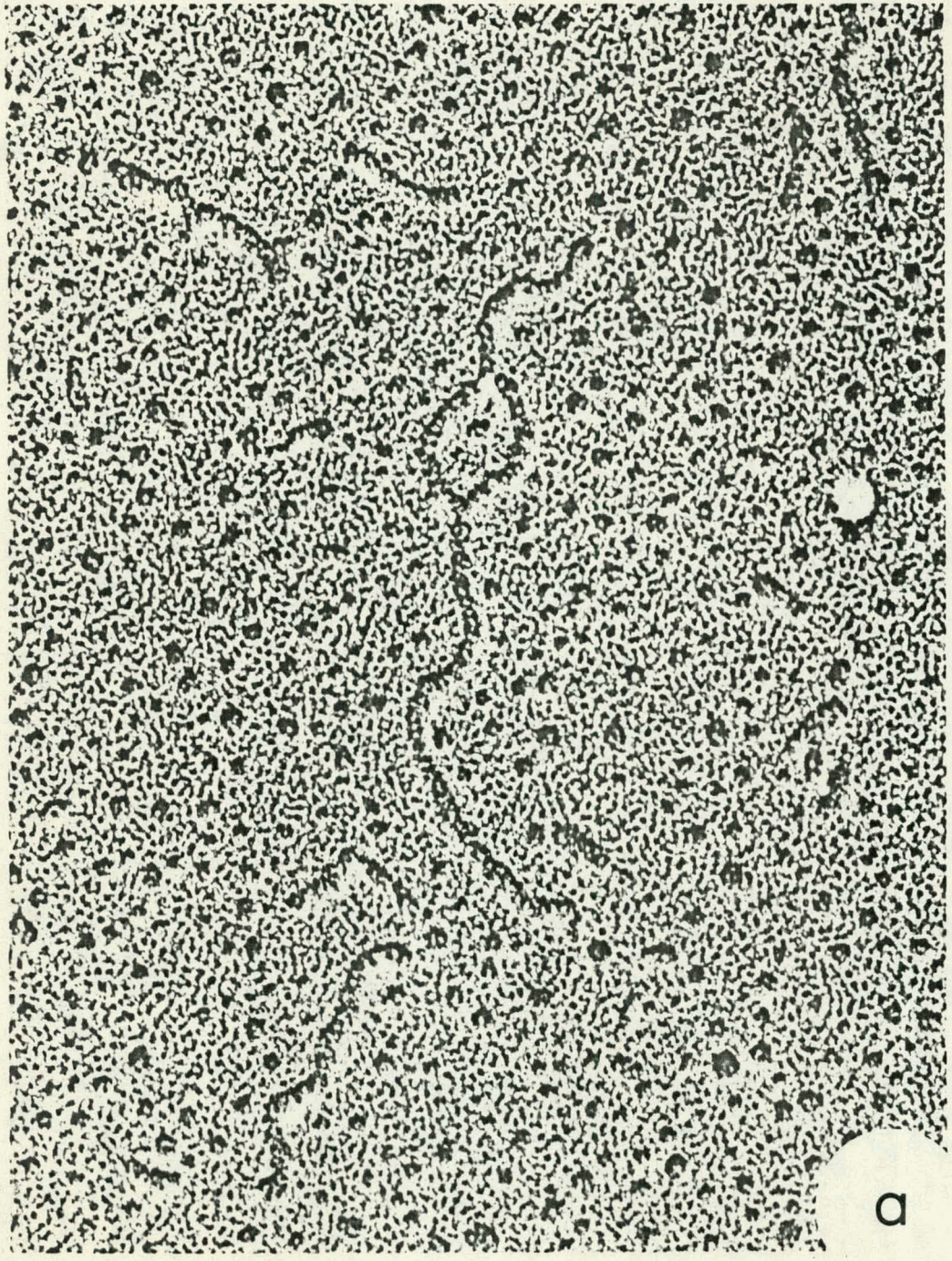
c



d

Fig 3

Fig 4



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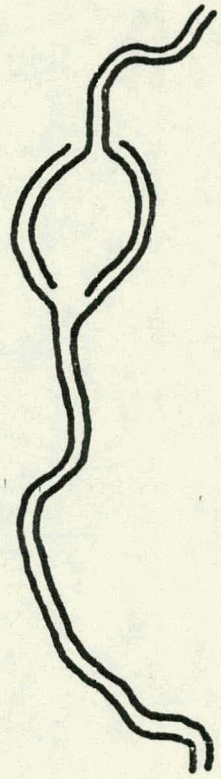

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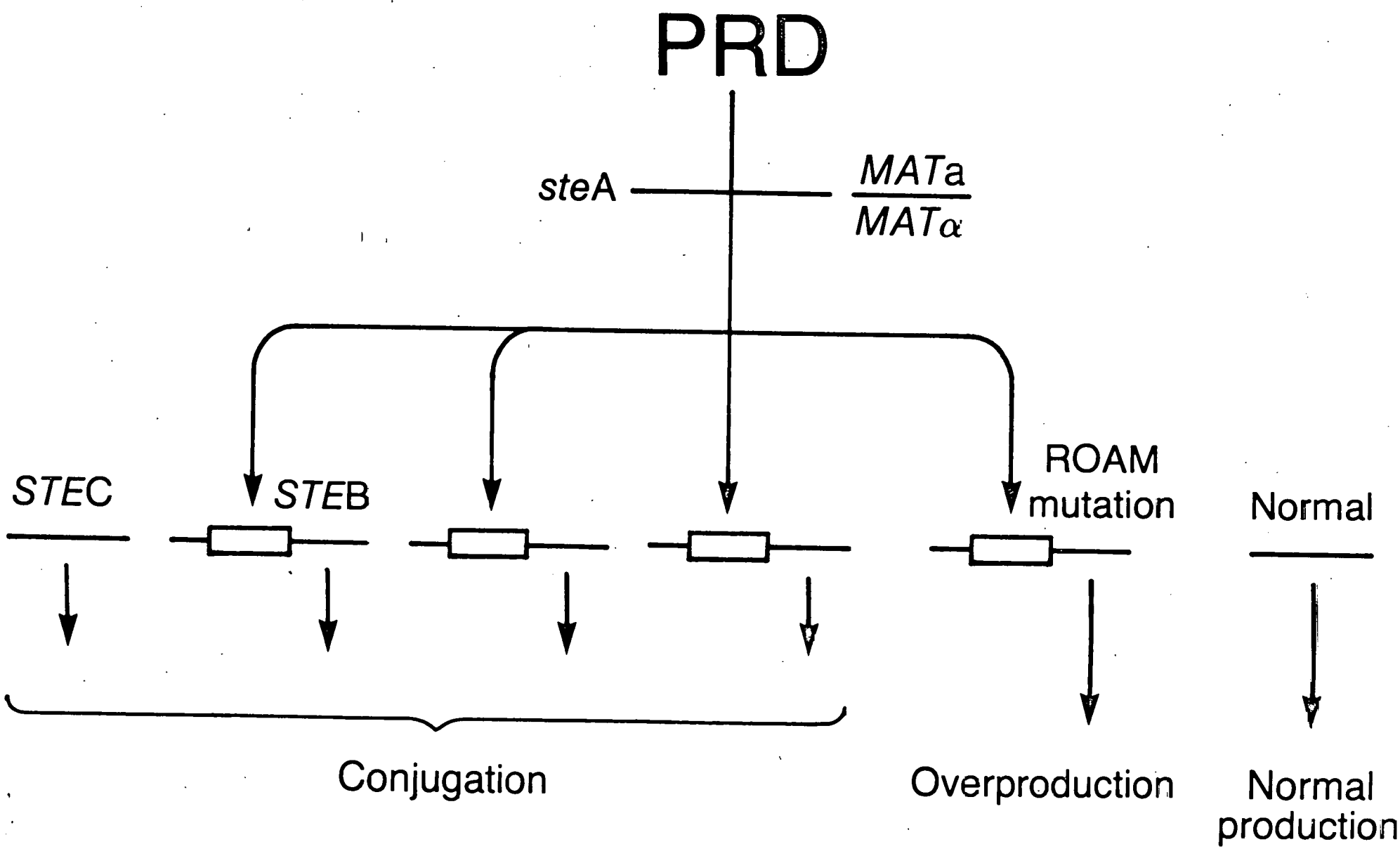
PRD
steA
MATa
MATα
STEC
STEB
ROAM
mutation
Normal
Conjugation
Overproduction
Normal
production