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Striking Similarities between the Regulatory Mechanisms Governing Yeast Mating-Type Genes and Mammalian Major Histocompatibility Complex Genes

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Expression of a mammalian major histocompatibility complex (MHC) class I gene is in part regulated by a silencer DNA sequence element which binds a complex of silencer factors. This negative regulatory system is shown to be strikingly similar to the yeast α2 mating-type repression system. A moderate DNA sequence homology exists between the MHC class I silencer DNA element and the yeast α2 operator. Mammalian silencer factors specifically bind to the yeast α2 operator DNA and also specifically interact with a yeast α2-binding protein. Furthermore, the α2 operator functions as a silencer element in mammalian cells when placed upstream of a MHC class I promoter.

Mechanisms of transcriptional activation have been conserved in evolution, as evidenced by the homology between a number of mammalian and yeast transcription factors: the mammalian transcription factor c-Jun and the yeast factor GCN-4 (27), and the mammalian c-*fos* serum response factor (SRF) and the yeast factor MCM-1 (10, 21). Indeed, interchange of DNA-binding sites and activation domains of yeast and mammalian factors can yield functional hybrid molecules (26). The instances in which such homology has been demonstrated have thus far been limited to transcriptional activators. Negative regulation of gene expression, which plays a major role in both prokaryotic and yeast gene regulation, is less well characterized in higher eukaryotes. We have recently demonstrated that major histocompatibility complex (MHC) class I genes are negatively regulated (7, 28) and have now used this system to assess the extent to which negative regulation is analogous in yeast and mammalian cells.

MHC class I molecules are cell surface glycoproteins that trigger and mediate immune responses by functioning as receptors for peptide antigens (3, 18). These molecules are expressed on nearly all somatic tissues, although their levels of expression vary considerably among tissues (25). Because the transplantation antigens present foreign peptide antigen to immune lymphocytes, the mechanisms regulating the expression of their genes have been widely examined (25). The overall structural and regulatory organizations of MHC class I genes from a variety of species bear a striking resemblance to one another. A series of regulatory elements, including a constitutive enhancer (enhancer A) and an interferon response element, is located within 200 bp upstream of the transcriptional promoter (4, 12, 17, 19, 24). Further upstream, a negative regulatory element has been found associated with a class I gene, PD1, encoding a transplantation antigen in the pig (7, 28). This silencer element, which maps between 700 and 800 bp upstream of the promoter, consists of a discontinuous binding site to which a silencer factor binds; the silencer factor consists of a complex of at least two proteins, neither of which binds DNA independently (Fig. 1) (28). Levels of class I gene expression are inversely correlated with the cellular concentration of this silencer factor (28).

Negative regulation of genes has been well established in the yeast *Saccaromyces cerevisiae*, in which **a**-type haploid-specific gene expression is regulated by a protein repressor, α2 (11). α2 is expressed only in α mating-type cells and specifically represses the battery of **a**-type haploid-specific genes expressed in **a** mating-type cells. The α2 repressor protein binds to its substrate DNA as a homodimer (23). The DNA-binding site for the α2 protein, or operator, consists of two stretches of about 10 bp separated by a 10-bp DNA segment to which a constitutive transcription factor, MCM-1 (PRTF), binds (Fig. 1) (1, 2, 15, 22, 23). Although α2 binds directly to its operators, its affinity is much greater in the presence of MCM-1 (15, 16).

In studying the PD1 silencer element, we noted a striking similarity in its overall organization to that of the α2 operator; in addition, a moderate sequence homology between the yeast and mammalian elements was noted. We have investigated the relationship between these two negative regulatory systems and now report that they are strikingly similar, reflecting parallel mechanisms of negative regulation in yeast and mammalian cells.

MATERIALS AND METHODS

Plasmid constructions, DNA probes, and oligonucleotides. The pN(-38) construct contains the chloramphenicol acetyltransferase (CAT) reporter gene downstream of the PD1 promoter and −502 bp of 5′ flanking sequence; it does not include the silencer element (7). A subclone (254-17) containing the 100-bp silencer region was generated by blunt-end ligation of a 254-bp *Dde*I DNA fragment into the *Sma*I site of pUC18. A 152-bp DNA fragment containing the 140-bp PD1 DNA segment spanning the entire PD1 silencer was isolated by digestion of 254-17 with *Ava*II-*Kpn*I. All oligonucleotides were synthesized on an Applied Biosystems model 381A DNA synthesizer. Complementary strands were annealed and purified on Elutip-d columns (Schleicher

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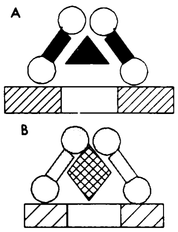

FIG. 1. Common organizational features of PD1 and yeast α2 silencer complexes. The PD1 silencer complex (A) consists of a discontinuous DNA-binding site (shaded boxes) associated with a complex of at least two distinct proteins (28) (Fig. 2). Neither protein alone is able to bind DNA stably. The yeast α2 silencer complex (B) also consists of a discontinuous DNA-binding site (shaded boxes) associated with two distinct proteins, α2 and MCM-1 (11). The MCM-1 moiety binds to the core region of the DNA sequence element. Although both MCM-1 and α2 bind DNA separately, they bind more stably in combination (16). The yeast and mammalian DNA sequences are weakly homologous (Fig. 2).

& Schuell). The Ste6 oligonucleotide used in the competition studies was the 32-bp sequence illustrated in Fig. 2; in the direct binding assay (see Fig. 4), the Ste6 sequence was contained in an 86-bp fragment with flanking pUC18 polylinker sequences (16). In the gel shift competition studies, relatively high molar excesses of all oligonucleotides were required because the cold competitors were short oligonucleotides (32 bp or less), whereas the labeled probe was a longer (140-bp) DNA segment. For functional studies, the 140-bp PD1 DNA fragment and the 32-bp Ste6 oligonucleotide were subcloned separately in the *Nde*I site of pN(-38).

Cell transfections. The COS-1 cell line was maintained in Dulbecco's modified Eagle's medium (Mediatek, Reston, Va.) supplemented with 10% (vol/vol) fetal calf serum (Biofluids, Rockville, Md.). COS-1 cells were transfected by using the mammalian transfection kit (Stratagene) based on the $CaPO_4$ method. CAT activity in cell extracts from transfected cells was determined as described previously (8).

Cellular extracts. COS cell extracts were made by a modification of the method of Dignam et al. (5). Briefly, cells were harvested by EDTA (GIBCO, Grand Island, N.Y.) treatment, washed in cold phosphate-buffered saline and pelleted into Eppendorf microcentrifuge tubes. The pellet was resuspended in 1 cell volume of Dignam buffer C (5). NaCl concentration was adjusted to 0.42 M, and cells were lysed by repeated cycles of freezing and thawing. After brief vortexing, the extract was centrifuged at 100,000 × *g* at 4°C for 20 min. The supernant was recovered, aliquoted, and stored at −70°C.

Yeast extracts were prepared by a modification of the procedure of Keleher et al. (15) as follows. α and **a** yeast cells were grown in YEPD medium to an optical density at 600 nm of 1. Cells were harvested by centrifugation, weighed, and resuspended at 0.2 g/ml of buffer A′ (100 mM Tris [pH 8.0], 1 mM EDTA, 10 mM β-mercaptoethanol, 20% glycerol, 1 mM phenylmethylsulfonyl fluoride, 200 mM NaCl, 2 mM benzamidine). Glass beads (0.5 g; 0.45 to 0.5 mm in diameter) were added, and the slurry was vortexed for 1 min and then cooled on ice for 1 min; this procedure was repeated 10 times. Lysate was checked microscopically to ensure complete cell lysis and then centrifuged at 12,000 × *g* for 40 min at 4°C. The supernatant was recovered and aliquoted for storage at −70°C.

Protein concentrations were determined by using the Micro BCA protein assay kit (Pierce, Rockford, Ill.).

Yeast α2 and MCM-1 proteins. Yeast α2 was purified by affinity chromatography by the method of Keleher et al. (15) and was a kind gift of Drew Vershon. MCM-1 was purified according to the method of Passmore et al. (22) and was a kind gift of Bik Tye.

Gel mobility shift assays. Binding reactions were carried out in a volume of 20 μl for 30 min at room temperature. Typical reaction mixtures contained 1.5 fmol of [^{32}P]DNA, 3 μg of whole cell extract, and 3 μg of poly(dI-dC) in 10 mM Tris-Cl (pH 7.9)–1 mM MgCl–1 mM dithiothreitol–1 mM EDTA–5% glycerol–50 mM NaCl. DNA fragments were end labeled by T4 polynucleotide kinase. Where indicated, unlabeled competitor fragment or double-stranded oligonucleotide (ds-oligonucleotide) was also added to the reaction mixture prior to the addition of protein. The reaction mixtures were subjected to electrophoreses on 4% polyacryl-

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GAAATTACCATAATGATAGCATCCAAAATTATCTGAAAAGGTTATTAAAAATACATGTCCTACATGTGTGCGGG   PD1

                                                                       ds-oligonucleotide

               .G....................................GG                      S2

                                 CGCG..............................          S3

               ..G.GT...T..CC..ATT ....A....C.TG                             Ste6

               ---.GT...T..CC..ATT ....A....C.TG                             Ste6Del-1

       CGATGC.G...GG.T..G....  ...AGAAGC.GTTAG..C                             κ

       CGGTGGTGGGGAGTCCCCGTGTCC                                              Enh A
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FIG. 2. Homology of the PD1 silencer DNA sequence with the yeast a2 operator. The PD1 sequence shown represents 74 bases of the 140-bp *Ava*II-*Dde*I DNA fragment containing the silencer element. Underlined segments indicate the positions of the two silencer-binding sites (28). The other sequences represent different ds-oligonucleotides used in the competition studies: S2, a ds-oligonucleotide derived from the PD1 sequence spanning the silencer element; S3, a ds-oligonucleotide derived from PD1 which spans the 3′ silencer-binding site but does not compete for mammalian silencer complex; Ste6, yeast α2 operator sequence; Ste6Del, yeast α2 operator sequence with a three-nucleotide deletion within the α2 binding site; κ, octamer motif from the kappa light-chain enhancer; Enh A, sequence from PD1 derived from the promoter-proximal enhancer A. Sequences are aligned to maximize homology. Symbols: ., residues identical to the PD1 residue; −, deleted residues.

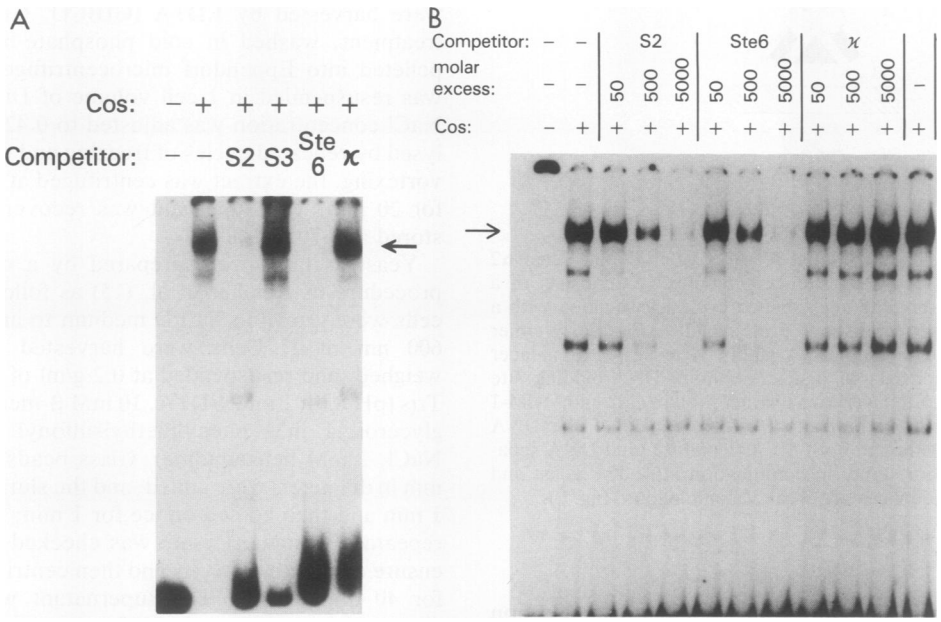

FIG. 3. Evidence that mammalian COS extracts form silencer complexes with the PD1 silencer DNA which are subject to specific competition for by yeast Ste6 operator ds-oligonucleotides. (A) COS extract was incubated with a 140-bp *Ava*II-*Dde*I DNA fragment which contains the silencer element of PD1. Cold ds-oligonucleotide competitors, as indicated, were added at 10^4 molar excess. Relatively high molar ratios are required because the cold competitor is a short ds-oligonucleotide and the labeled probe is a longer (140-bp) DNA segment capable of forming more stable complexes. (B) Competition of mammalian complexes by Ste6 titrates in parallel with the homologous S2 silencer ds-oligonucleotide. The PD1 silencer complex was formed as in panel A. Various molar excesses (between 50 and 5,000) of cold competitor were added to the binding reaction mixture. Arrows indicates positions of the major PD1 silencer complex. A second, lower band corresponds to a second form of the silencer complex. The third band represents an enhancer complex formed from sequences overlapping the silencer DNA sequences (see text for details). S2, specific competitor of silencer complexes; S3, sequence derived from PD1 which does not compete the complexes; Ste6, yeast Ste6 operator: κ, kappa light-chain enhancer sequence containing octamer sequence which binds Oct-1 and Oct-2. See Fig. 2 for sequences.

amide gels for 90 min at 160 V in 0.5× Tris-borate-EDTA. Gels were dried and autoradiographed.

RESULTS

The α2 operator shares limited sequence homology with the MHC class I silencer. The MHC class I silencer element derived from the PD1 gene has been mapped to a 28-bp DNA segment centered around −730 bp upstream of transcription initiation (Fig. 2) (28); the element consists of two factor-binding sites separated by 10 bp (underlined in Fig. 2). Comparison of the PD1 silencer element with other known regulatory DNA sequence elements revealed no striking homologies (data not shown). However, a weak homology was found between the PD1 silencer and the yeast α2 operator, Ste6, which is a binding site for the α2 repressor (14, 15). Both sequence elements have discontinuous binding sites of 8 to 14 bp separated by 9 to 10 bp (23). When aligned to the PD1 silencer element, the Ste6 α2 operator shares 5 of 10 bases with the 5′ binding site of the PD1 silencer and 5 of 8 bases with the 3′ binding site (Fig. 2). A similar level of homology is found in the DNA region separating the repressor-binding sites, leading to an overall 59.4% homology.

Competition of PD1 silencer complexes by Ste6 oligonucleotides. Gel mobility shift assays demonstrated that the weak sequence homology between the MHC class I silencer and the α2 operator is sufficient to promote the binding of related *trans*-acting factors. Mammalian COS extracts generate two specific complexes with a 140-bp *Ava*II-*Dde*I DNA fragment containing the PD1 silencer element (Fig. 3). Both complexes result from specific interactions with the 28-bp silencer element and are subject to competition by a homologous oligonucleotide, S2 (Fig. 3).

To assess the ability of the yeast Ste6 operator DNA to bind the *trans*-acting factors associated with the PD1 silencer element, a Ste6 ds-oligonucleotide was used as a cold competitor in a gel shift assay. The Ste6 ds-oligonucleotide was capable of competing with the PD1 silencer element, inhibiting the formation of the mammalian silencer complex (Fig. 3A). In contrast, an irrelevant ds-oligonucleotide derived from DNA sequences outside the PD1 silencer element did not compete (S3; Fig. 3A). A ds-oligonucleotide derived from the kappa light-chain enhancer, containing an octamer motif which binds homeobox proteins, also did not compete. Thus, the observed Ste6 competition is not due to a fortuitous binding of homeodomain proteins (9, 13). Furthermore, inhibition of the mammalian silencer complex by the Ste6 ds-oligonucleotide does not depend on the presence of MCM-1 protein-binding sites, since a ds-oligonucleotide which binds only MCM-1 protein (TTTATATTTTCCTAA TTAGGAAAATAT) does not inhibit (data not shown). These data indicate that the competition of the PD1 silencer by the Ste6 ds-oligonucleotide is specific and requires the α2-binding site.

The relative abilities of the homologous S2 and yeast Ste6 ds-oligonucleotides to compete against the PD1 silencer complexes were determined by titrating various amounts of each competitor into the binding reaction mixture (Fig. 3B). The two ds-oligonucleotides compete against complex formation over comparable concentration ranges. Thus, the

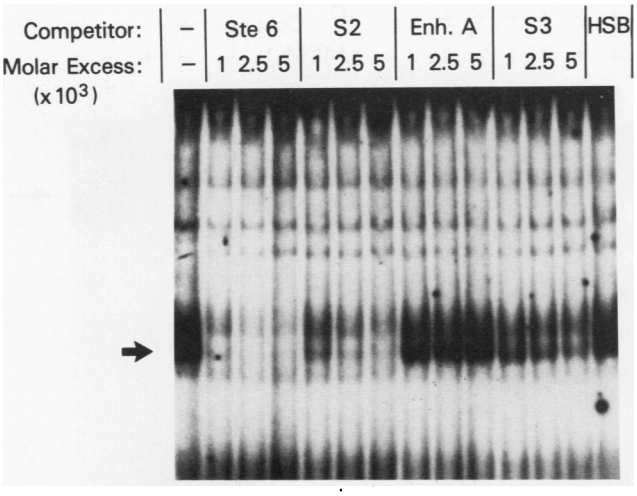

FIG. 4. Specific complexes formed between the PD1 silencer factor and the 32-bp yeast Ste6 operator. COS extract was incubated with the Ste6 operator ds-oligonucleotide in the presence or absence of various amounts of cold competitor ds-oligonucleotides. Ste6, α2-binding site ds-oligonucleotide; S2, PD1 silencer-specific ds-oligonucleotide; Enh A, enhancer A-specific ds-oligonucleotide; S3, PD1-derived, nonspecific ds-oligonucleotide; HSB, high-salt buffer, added to adjust the final salt concentration of the reaction mixture to that of 5×10^3 molar excess of unlabeled ds-oligonucleotide. The arrow indicates the position of the specific complex.

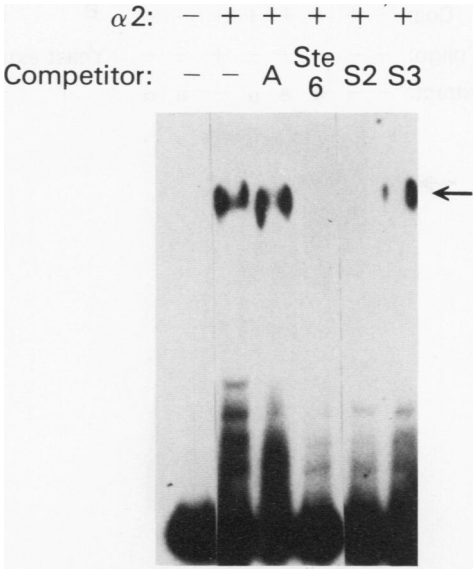

FIG. 5. Evidence that interaction of α2 with the Ste6 operator is specifically competed against by PD1 silencer ds-oligonucleotides. Purified recombinant α2 was complexed with an 81-bp Ste6 operator (arrow) in the presence or absence of a 10^4 molar excess of various cold ds-oligonucleotide competitors. A, enhancer A sequence; Ste6, 32-bp Ste6 operator sequence; S2, specific competitor of PD1 silencer complexes; S3, noncompetitor ds-oligonucleotide.

yeast Ste6 ds-oligonucleotide encoding the α2 operator is nearly as effective at competing against the mammalian silencer complex as is the homologous PD1-derived ds-oligonucleotide.

The PD1 silencer factor binds the α2 operator. The ability of the Ste6 α2 operator to compete against the PD1 silencer complex suggests that it is able to bind mammalian PD1 silencer factors. The specificity of this interaction (i.e., the extent to which other mammalian factors interact with the Ste6 operator) was next examined. When a Ste6 ds-oligonucleotide is incubated with COS extract, a major retarded band is generated (Fig. 4). This complex is specifically competed against by both the yeast (Ste6) and mammalian (S2) operators. It is not effectively competed against by the nonspecific ds-oligonucleotide S3 or the enhancer A ds-oligonucleotide. Since it has not proven possible to footprint the binding of the PD1 silencer to its cognate PD1 silencer element (28), no attempt was made to footprint its interaction with the α2 operator. From these experiments, we conclude that only a limited number of factors in the COS extract are capable of specifically binding to the yeast operator and that these factors also bind the PD1 silencer.

Competition of α2/Ste6 complexes by PD1 silencer ds-oligonucleotides. Purified recombinant yeast α2 protein forms complexes with the Ste6 ds-oligonucleotide in vitro which can be resolved by gel mobility shift assays (Fig. 5). The PD1 silencer ds-oligonucleotide, S2, competed against this yeast α2 complex, whereas irrelevant ds-oligonucleotides, such as S3 and enhancer A, were unable to compete (Fig. 5). Thus, a ds-oligonucleotide encoding the PD1 silencer element is able to disrupt the specific interaction of purified α2 protein with its homologous Ste6-binding site. Although the interaction of α2 protein with the PD1 silencer element is specific, it is weak; complexes between α2 protein and the PD1 silencer element are unstable and poorly visualized in gel shift assays (data not shown).

A yeast factor interacts with the PD1 silencer factor. In vivo, yeast α2 binds its operator in association either with the constitutive transcriptional activator MCM-1 in mating type α cells (Fig. 1) or with **a**1 in diploid cells (6, 11). Similarly, the PD1 silencer complex requires the association of at least two factors (Fig. 1) (28). Although the results presented above indicate that heterologous protein-DNA interactions can occur, the possibility of interactions between yeast and mammalian factors has not been addressed. To test whether any of the mammalian factors required for complex formation with the PD1 silencer are capable of interacting with any yeast factors, extracts from COS cells were mixed with extracts from either α or **a** mating-type yeast cells. The mixtures were then incubated with the 140-bp PD1 silencer probe. Whereas COS extract alone generates the silencer complexes, the presence of yeast extract, either α or **a**, inhibits the formation of these complexes (Fig. 6A).

The yeast competitor factor is not mating-type specific, since both α and **a** yeast extracts are capable of competing against the silencer complex. Since neither yeast extract alone forms visible complexes with the silencer element (Fig. 6A), the competition of COS extract/PD1 silencer complex by yeast extract most likely results from protein-protein interactions between yeast and mammalian factors (Fig. 7). Further, the absence of silencer complexes is not the result of nonspecific proteolysis mediated by the yeast extracts, since the disappearance of the silencer complex is accompanied by the augmented appearance of another complex (faster-migrating band in Fig. 6A and B). This second complex has been characterized as an enhancer complex which originates from the association of COS factors with an enhancer sequence that overlaps the PD1 silencer (28).

The molecular nature of the yeast competitor was indirectly examined by the following experiment. Since extracts

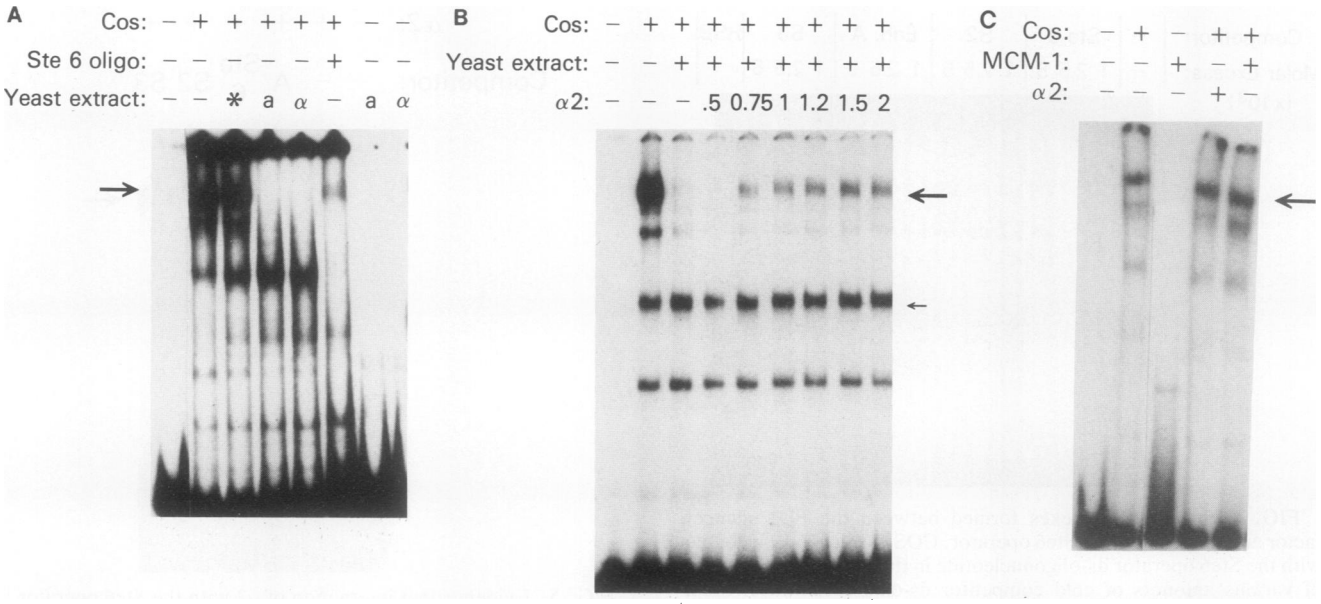

FIG. 6. (A and B) Binding of yeast factor to a component of the PD1 silencer factor. (A) COS and yeast extracts, either alone or in combination, were incubated with the 140-bp DNA segment containing the PD1 silencer element. *, yeast extraction buffer alone. (B) COS and yeast extracts, either alone or in combination, were incubated with the 140-bp DNA segment containing the PD1 silencer element. Increasing amounts (in microliters) of α2 protein, as indicated, were added to reaction mixtures containing both extracts. Long arrow, major silencer complex; short arrow, enhancer complex (see text for explanation). (C) Recombinant MCM-1 and α2 do not inhibit mammalian silencer complex. Either MCM-1 (0.5 ng/μl) or α2 (4.5 ng/μl) was incubated with COS extract and the 140-bp DNA segment containing the PD1 silencer.

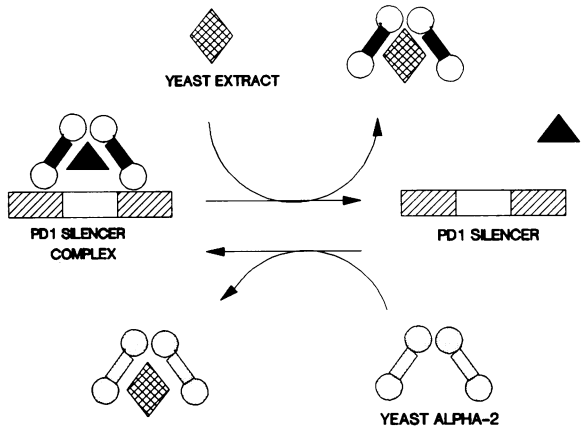

FIG. 7. Proposed mechanism for the inhibition of PD1 silencer complexes by yeast extracts and the relief of inhibition by purified α2 protein. In the absence of any yeast extract, the PD1 silencer complex, consisting of the DNA-binding site and a silencer factor composed of at least two protein components (⌐O, ▲), forms. In the presence of yeast extract, one of the components of the PD1 silencer factor forms a complex with the yeast factor (◆). This complex is no longer able to stably bind to the PD1 silencer DNA element; the isolated PD1 silencer factor component is also unable to bind DNA alone. This results in a loss of mammalian silencer complex. In the added presence of yeast α2 protein (⌐O), the yeast competitor factor preferentially forms complexes with the homologous yeast α2 protein, releasing the PD1 silencer factor component. This released factor component reassociates with the second component of the silencer factor, regenerating the PD1 silencer complex.

from both mating types contain the competitor, it cannot be α2, which is expressed only in α mating-type cells. Indeed, addition of purified α2 to the mammalian complex does not compete against it (Fig. 6C and data not shown). However, since the yeast and mammalian systems are so similar, we postulated that the yeast competitor is capable of interacting with yeast α2 (Fig. 7). To test this hypothesis, a titration experiment was performed in which purified recombinant α2 was added to mixtures of COS and yeast extracts (Fig. 6B). If an α2-binding yeast factor were responsible for binding to a COS factor and preventing it from forming complexes with the PD1 silencer, then the addition of α2 would compete against the yeast factor from the PD1 silencer factor and allow silencer complex formation (Fig. 7). Indeed, titration with increasing concentrations of α2 in the presence of yeast extract results in the reappearance of a complex indistinguishable from the PD1 silencer complex (Fig. 6B). This complex does not result from direct binding of α2 protein to the PD1 silencer element, since stable complexes between them are not observed in these gel shift assays. Rather, these results suggest that the α2 protein interacts with a yeast factor which in turn is capable of interacting with the PD1 silencer factor. The failure of increasing α2 protein concentrations to completely restore the PD1 silencer complex is due to the concomitant increase in salt concentration (from the α2 protein storage buffer) which destabilizes the PD1 silencer complexes, thereby counteracting the α2 protein titration. In summary, there appears to be a striking similarity between yeast and mammalian DNA-binding factors both in their DNA sequence recognition requirements and in their protein-protein interactions.

Because only a limited number of yeast proteins have been shown to bind α2, we attempted to identify the yeast factor that competed against the mammalian silencer complex.

TABLE 1. Evidence that the yeast α2 operator functions as a silencer element for the mammalian PD1 promoter when transfected into COS cells[a]

Construct	No. of insert copies	Relative activity (%)	
		Expt 1	Expt 2
pN(-38)X		100	100
pN(-38)Ste6-1	1	53.8 ± 8.5	31.4 ± 1.4
pN(-38)Ste6-2	2	40.5 ± 13.6	29.3 ± 1.4
pN(-38)Ste6Del-1	1	94.4 ± 20.7	ND
pN(-38)(140-1)	1	34.0 ± 3.2	34.4 ± 4.3

[a] The Ste6 ds-oligonucleotide was cloned as a monomer (Ste6-1) or dimer (Ste6-2) into the expression vector pN(-38)X, which contains the PD1 promoter upstream of the CAT gene. A mutated form of Ste6, Ste6Del-1, which contains a deletion of three nucleotides in one of the α2-binding sites (see Fig. 2), was cloned into the same vector. The 140-bp PD1 DNA fragment containing the silencer was also introduced into pN(-38)X. Each construct was transiently transfected into COS cells for 48 h; extracts were then assay for CAT activity as described in Materials and Methods. Results are expressed relative to those for the parental pN(-38)X vector. Each value represents the average ± standard error of the mean of three separate transfections. ND, not determined.

Since the interaction of MCM-1 with α2 is the best characterized, we first tested its ability to compete against mammalian silencer complex formation. Recombinant MCM-1 did not affect the mammalian silencer complex when incubated COS extract and the 140-bp PD1 silencer fragment (Fig. 6C). That the yeast competitor was not MCM-1 was further indicated by the observation that yeast extracts derived from mutants in MCM-1, which contained significantly reduced MCM-1-binding activity, were as effective in competing against the mammalian complex as was the wild-type parent (data not shown). Thus, the yeast competitor of the mammalian complex is an α2-binding factor which appears to be distinct from MCM-1.

The α2 operator functions as a silencer element in association with the PD1 promoter. To test the functional significance of the relationship between the mammalian silencer complexes of PD1 and the α2 operator, a 32-bp ds-oligonucleotide containing the α2-binding sites (Ste6) (15) was introduced into the vector pN(-38)X, which contains the PD1 promoter directing expression of the reporter CAT gene (7). Approximately 500 bp of 5′ upstream sequences remain attached to the PD1 promoter in pN(-38); the PD1 silencer (located around −730 bp) has been removed from this vector. Constructs containing one or two copies of the Ste6 oligonucleotide were prepared.

The in vivo effect of the Ste6 ds-oligonucleotide on the PD1 promoter was tested by measuring levels of CAT activity in COS cells transiently transfected with each of the constructs. The presence of a single copy of the Ste6 operator reduces the level of CAT activity to approximately one-half that of the pN(-38) control (Table 1). This level of reduction is equivalent to that achieved by the homologous PD1 silencer [pN(-38) (140-1)] (Table 1). The silencing effect of the Ste6 oligonucleotide is specific, since a Ste6 sequence (Ste6Del-1) which is deleted for part of the α2-binding site is not effective as a silencer and other DNA fragments derived from PD1 5′ sequences do not reduce expression at all (28; Table 1).

DISCUSSION

The role of negative regulation in cell-type-specific gene expression is beginning to be appreciated in mammalian systems, although it has long been recognized in both bacterial and yeast systems. Negative regulation can be achieved by a variety of mechanisms: DNA-binding proteins that act directly on target elements, DNA-binding proteins that repress by physically blocking positive transcriptional factors (quenching), and proteins that repress by binding-positive transcriptional factors and prevent their binding to DNA (squelching) (20). In yeast cells, α2 binds directly to two DNA sequence elements flanking the binding site of the transcriptional activator MCM-1, leading to the proposal that α2 represses transcription by a quenching mechanism (15, 23). In mammalian cells, expression of the MHC class I gene PD1 is negatively regulated (7); the mechanism by which the negative regulation of PD1 is achieved is not yet known. However, a negative regulatory DNA sequence element has been identified which is recognized by a protein complex consisting of at least two distinct factors (28).

In this study, we have demonstrated that the molecular mechanisms mediating negative regulation of the yeast mating-type genes and mammalian histocompatibility genes are very similar. The yeast α2 operator consists of two distinct binding sites separated by 8 bp (11, 15, 23). The mammalian DNA sequence element shows a similar organization, with two binding sites separated by 10 bp (28). Although there is relatively weak DNA sequence homology between the two elements, the α2 operator is able to bind in vitro to mammalian factors which bind the PD1 silencer (Fig. 3 and 4) and to function in vivo as a silencer element (Table 1). Conversely, ds-oligonucleotides derived from the PD1 silencer DNA sequence element were able to specifically compete against binding of purified yeast α2 protein to the yeast Ste6 operator (Fig. 5). Furthermore, the mammalian and yeast factors are able to interact: the competition of mammalian silencer complexes by yeast extract indicates that a yeast factor binds to at least one of the components of the mammalian silencer factor (Fig. 7). Since yeast α2 protein relieves this competition of silencer complex by yeast extract, the yeast competitor must also bind to α2 (Fig. 7). The yeast competitor in these experiments appears to be distinct from MCM-1 but, like MCM-1, binds to α2. Taken together, these results suggest that a component of the mammalian silencer factor is analogous to yeast α2.

The mammalian silencer factor consists of at least two distinct components (28), one of which is similar to yeast α2. The nature of the other component is unknown; however, it does not appear to bind yeast α2, since addition of purified yeast α2 to the PD1 silencer complex had no effect (Fig. 6C). This second component is unlikely to be SRF, which is a mammalian homolog of MCM-1 (10, 21, 22), since its DNA-binding site is distinctly different from that of the PD1 silencer element, making it unlikely that SRF is a component of the PD1 silencer factor. In addition, the separated components of the PD1 silencer do not independently bind DNA, whereas SRF does so. It is possible that the second mammalian component is similar to the yeast competitor which binds the mammalian α2 analog.

In conclusion, we have found a striking parallel between yeast and mammalian cells in one molecular mechanism of negative regulation. The overall similarity with respect to DNA sequence, protein interactions, and function is particularly striking if considered in light of the function of the genes being regulated. The gene regulated by the PD1 silencer encodes a major histocompatibility antigen, which is involved in self-nonself recognition in the immune system. The genes regulated by α2 are those which determine mating type and mating-type recognition in yeast cells, which is also

a self-nonself recognition system. It is intriguing to speculate that the two regulatory systems have derived from a common evolutionary origin and that although the products which the MHC genes encode have completely diverged from those of the yeast mating-type genes, the molecular mechanisms which regulate their expression have been conserved.

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