Cell type-specific chromatin organization of the region that governs directionality of yeast mating type switching

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Switching of mating type in *Saccharomyces cerevisiae* is directional; *MAT α* cells recombine to transfer information from *HMR α* while *MAT α* cells switch using the silent cassette at *HML α* . Genetic analysis recently has defined a 700 bp recombination enhancer ~29 kb from the left end of chromosome III that is necessary for directionality. The chromatin structure of this region differs strikingly in *a*- and α -cells. *Mat α 2p* organizes a 3.7 kb chromatin domain that opposes interaction of *trans*-acting proteins with the enhancer. In *a*-cells lacking the α 2 repressor, two footprinted regions flank an ~100 bp section having a unique DNA structure. This structural signature probably reflects interactions of proteins that result in directional mating type switching.

Keywords: α 2/recombination enhancer/*Saccharomyces cerevisiae*/silent mating type loci

Introduction

Yeast cell type is determined by expression of master regulatory genes at the *MAT* locus, near the center of chromosome III. Silent copies of the mating type genes are present at *HML α* and *HMR α* loci, near the telomeres of the same chromosome (Herskowitz *et al.*, 1992). In homothallic strains, which express the HO endonuclease that initiates switching, switching has been known to be directional for 20 years. Over 80% of the cleavages at *MAT* by HO in strains containing both *HML α* and *HMR α* lead to a change at *MAT*, i.e. *MAT α* preferentially interacts with *HML α* and *MAT α* recombines with *HMR α* (Hicks and Herskowitz, 1976). Directionality does not depend on any sequences within or flanking by 1–2 kb the silent loci *HML* or *HMR* (Weiler and Broach, 1992). Rather, the importance of chromosomal location in directionality of mating type interconversion is emphasized by the observation that at least 40 kb of the left arm of chromosome III are activated for recombination in *a*-cells (Wu and Haber, 1995). Recently, the recombination enhancer (RE), a 700 bp segment located nearly 17 kb from *HML α* , has been identified and shown to be necessary and sufficient to confer directionality in *a*-cells (Wu and Haber, 1996).

MAT α cells adopt a different mechanism to ensure recombination with their preferred donor, *HMR α* . Nearly 150 kb of the left arm of chromosome III, including the

region activated in *a*-cells, is inactivated for recombination (Wu *et al.*, 1996). A role for the α 2 repressor in this inactivation was suggested by the observation that over-expression of this protein in *a*-cells prevented preferential switching of *MAT α* with *HML* (Wu and Haber, 1995). The dimeric homeodomain protein *Mat α 2p*, in a complex with a dimer of *Mcm1p*, represses transcription of *a*-cell specific genes (Johnson and Herskowitz, 1985). While a role for *a*-cell specific genes might be invoked, the five genes known to be regulated by α 2 have specific functions and appear unlikely to participate in global chromosomal changes. The α 2 repressor seems to have a more direct role. Interestingly, a 1.4 kb region including the RE contains two putative α 2 repressor consensus sequences (Wu and Haber, 1996). These *Mat α 2p*-binding sites are the only such sequences in the entire genome that are not within 500 bp upstream of an *a*-cell specific gene (Johnson and Herskowitz, 1985).

Within the RE, mutation of either a region bearing the α 2 operator or a region consisting nearly exclusively of TTTG sequences also abolished the preferential interaction of *MAT α* with *HML α* (J.E.Haber, personal communication), suggesting that *trans*-acting factors may activate the left arm of chromosome III for recombination through sequence-specific interactions with the RE in *a*-cells. If this is the case, previous studies of the mechanism of repression of *a*-cell specific genes by the α 2 repressor suggest a physical basis for the inactivation of the left arm of chromosome III in α -cells. We have shown for both minichromosomes and genome that the α 2 repressor organizes chromatin with positioned nucleosomes abutting the operator (Roth *et al.*, 1990; Shimizu *et al.*, 1991; Simpson *et al.*, 1993). In part, repression is achieved by precise positioning of nucleosomes precluding interactions of the transcription machinery with DNA.

In pursuit of the possibility that an analogous mechanism might underlie activation of recombination for the left arm of chromosome III in *a*-cells and its inhibition in α -cells, we have studied the chromatin organization of the RE. We present evidence for interactions of *trans*-acting factors with the RE region in *a*-cells and show that a highly organized chromatin structure is present next to the α 2 operator in α -cells, presumably capable of precluding binding of these factors. Our findings provide a structural mechanism that can explain changes in the RE that correlate directly with cell type-specific, selective directionality of mating type interconversion.

Results

Chromatin structure of the RE in *a*-cells

To assess the structural organization of the region involved in recombination enhancement, chromatin was analyzed by mapping micrococcal nuclease (MNase) and DNase I

Fig. 1. The recombination enhancer mapped by primer extension analysis of micrococcal nuclease-hypersensitive sites. **(A)** Chromatin structure of the Crick strand around the first $\alpha 2$ repressor-binding site ($\alpha 2\#1$) using primer a290. The 702 bp RE (29 092–29 793) is shown in its entirety. **(B)** Chromatin structure of the Crick strand around the hypersensitive region identified in **a**-cells using primer b297. **(C)** Chromatin structure of the Watson strand around the hypersensitive region identified in **a**-cells using primer b297. **a**- and α -cells are as indicated. Extensions of undigested chromatin (O) and two or three levels of MNase-digested chromatin are presented. D and F indicate protein-free DNA, either isolated from nuclei (D) or a PCR product (F), digests as control for micrococcal nuclease sequence specificity. Coordinates are positions in the published sequence of *S.cerevisiae* chromosome III (Oliver *et al.*, 1992). Rectangular boxes indicate locations of footprinted or hypersensitive regions in **a**-cells. Ellipses correspond to inferred positions of nucleosomes in α -cells. The $\alpha 2$ operator consensus sequence is shown by a filled gray box.

cutting sites in nuclei, using primer extension methodology to determine cutting sites with nucleotide level resolution. Figure 1 shows MNase digestion patterns for both strands in the region around and to the right (towards the centromere) of the MAT $\alpha 2$ p-binding consensus sequence of the

RE in **a**-cells. An ~100 bp segment to the right of the operator has a strikingly different digestion pattern from naked DNA (footprint 1 in Figure 1A). Particularly notable is a region of ~30 bp in the center of that segment containing multiple A residues that frequently

Fig. 2. Sequence of *S.cerevisiae* chromosome III (29 400–29 539) which includes the nuclease-hypersensitive region in *a*-cells (Figures 1 and 3). Positions of mapped micrococcal nuclease cuts are shown by filled arrows and DNase I cuts by open arrows.

is cut in protein-free DNA and resistant to nuclease cleavage in *a*-cell nuclei. This footprint occurs over a long stretch of repeated 5' TTTG3'/5' CAAA3' sequences, deletion of which abolishes RE function. To the right of footprint 1 is a nuclease-hypersensitive segment which appears to contain 8–9 predominant cutting sites. When examined at higher resolution (Figure 1B), many of these sites over a 110 nucleotide length segment are resolved as doublets of sites spaced by 3–5 nucleotides; these pairs are separated from one another by 10–15 nucleotides. This region contains an extensive, imperfect set of repeated YAAA sequences (Figure 2); MNase cleavage within this sequence repeat occurs primarily at TA dinucleotides. Spacing of the sites is very different from the MNase cutting in protein-free DNA, in particular in the region of the YAAA repeat. The pattern in *a*-cell chromatin is unusual and unlike any previously described MNase mapped region, suggesting a motif where chromatin DNA exists in a distinctive conformation or geometry. To the right of the hypersensitive region is another segment of ~100 bp where nuclease susceptibility is generally decreased, and the cutting sites detected in *a*-cell chromatin are very unlike those in protein-free DNA (footprint 2). This same segment, mapped in the Watson strand, also shows the two areas of protection and distinctive cutting pattern flanking a 110 nucleotide hypersensitive segment (Figure 1C). For this strand, the doublet pattern is augmented by more frequent, interspersed cutting sites. Sites in the doublet pattern of the Crick strand are also cut in the more complex pattern of the Watson strand. Precise locations of the MNase cleavage sites are summarized schematically on the sequence of this region of the RE shown in Figure 2.

The chromatin structure of the region flanking the $\alpha 2$ operator in *a*-cells mapped using DNase I digestion is shown in Figure 3. In contrast to the MNase hypersensitivity, the DNA segment of distinct geometry is only

moderately sensitive to DNase I, although it does have a specific cutting pattern with a site every four nucleotides for much of the long YAAA repeat (Figure 2). This cutting pattern is reminiscent of the two nucleotide repeat observed for poly(dAdT) (Scheffler *et al.*, 1968). Footprint 1, over the TTTG sequences adjacent to the $\alpha 2$ operator, appears as a block to cutting of several strong naked DNA sites in the *a*-cell nuclear chromatin digests; the distinctive cutting at a period of four nucleotides is not present (Figure 3). Similar results for DNase I cleavage are seen for maps of cutting sites in the Watson strand (Figure 2).

Due to the unique sequence of this region, replete with TTTR motifs, UV photofootprinting (Axelrod and Majors, 1989) is particularly suitable for assessing protein–DNA interactions. Thymidine dimer formation in the region which is hypersensitive to MNase in *a*-cells occurs as readily as dimer formation in naked DNA (data not shown), consistent with the absence of protein binding to the region. Permanganate treatment of isolated nuclei (1–35 mM KMnO_4 , 1 min, 22°C) did not lead to thymidine oxidation in the MNase-hypersensitive region (data not shown); thus, we could not detect totally unwound, single-stranded DNA in this region.

Chromatin structure of the RE in α -cells

The chromatin structure of the RE in α -cells was mapped by nuclear digestion using MNase (Figure 1) and DNase I (Figure 3). Flanking the $\alpha 2$ operator in α -cells are regions ~140 nucleotides in length that are largely protected from MNase digestion (Figure 1A) and are separated by shorter segments with strong susceptibility to MNase. We interpret this characteristic pattern as indicating the presence of positioned nucleosomes. Similar indications of positioned nucleosomes in α -cells are seen in maps of both strands over the TTTR-rich region which is MNase hypersensitive in *a*-cells (Figure 1B and C) and extending into the low resolution part of the electrophoretic gel for maps in

Fig. 3. Recombination enhancer chromatin structure mapped by primer extension analysis of DNase I cutting sites. (A) The region around the MNase-hypersensitive region in α -cells was mapped after DNase I digestion using primer b297. For each cell type (as indicated), undigested (0) and two levels of DNase I digestion are presented. (D) and (C) indicate respectively the protein-free control digest and the dideoxycytosine-terminated sequencing reaction showing positions of G residues in the mapped strand of the PCR DNA. Rectangular boxes indicate locations of the unusual TTTT repeat sequences. The location of the α 2#1 operator consensus sequence is shown by a filled box. Ellipses indicate inferred positions of nucleosomes in α -cells. (B) A scan of the cutting pattern (lower DNase I concentration lane) of the central nucleosome in α -cells (29 560–29 403) demonstrates its tight rotational positioning.

either direction. This chromatin organization results in the incorporation of the sequence corresponding to the hypersensitive TTTR motif and the two footprinted regions in α -cells in positioned nucleosomes in α -cells. The extent of the alternating pattern of cleavage and protection suggests that a large domain of chromatin may be organized around the α 2 repressor-binding site, similar to that seen for repressed α -cell-specific genes (Y. Tsukagoshi and R. T. Simpson, unpublished observations).

In the region of chromatin flanking the operator, DNase

I digestion produces a series of bands spaced at ~ 10 nucleotide intervals (Figure 3), reinforcing the assignment of positioned nucleosomes in α -cells. The map and the accompanying scan in Figure 3 demonstrate in particular the tight rotational positioning of the nucleosome at location 29 560–29 403 in α -cells. The ~ 10 nucleotide spaced bands decrease in intensity from the periphery towards the dyad of the nucleosome. Intensities of the bands are roughly symmetrical around the central cutting site. Precise positioning of nucleosomes which lack anisotropy in-phase with the ~ 10 bp helical repeat of DNA is surprising and reinforces the conclusion that non-histone proteins generate the highly organized chromatin structure next to the α 2-binding site. Assignment of the nucleosome location based on DNase I digestion allows us to interpret the presence of some bands near the end of an inferred nucleosome in MNase digests (e.g. those from ~ 29 360 to 29 380 in Figure 1A) as being due to either exonuclease activity or nicking of DNA on the surface of a nucleosome, rather than a long linker. Binding of α 2 repressor to its operator is suggested by UV photofootprinting of this site in α -cells (data not shown), and is likely to be responsible for the organization of chromatin as positioned nucleosomes spanning a considerable region adjacent to the operator.

Chromatin structure of the RE in *tup1* mutants

Tup1p is necessary for repression of genes regulated by a number of yeast repressors, including Mat α 2p (Williams and Trumbly, 1990; Keleher *et al.*, 1992), and disruption of the *TUP1* gene leads to a disorganized chromatin structure covering the α -cell-specific genes *STE6* and *BAR1* (Cooper *et al.*, 1994). In addition, Szeto and Broach (1997) have shown that interaction of Tup1p with MAT α 2p is required for MAT α donor preference. A *URA3* disruption plasmid was used to replace *TUP1* in α - and α -cells. The chromatin structure of the RE in the resultant *tup1* mutant strains was mapped using MNase. Both footprints and the MNase-hypersensitive region over the imperfect TTTR domain are present in α -cell chromatin maps as well as in α -cell maps; the two cell types appear to have identical chromatin structure in a *tup1* context (Figure 4). In the absence of Tup1p, the organized structure of the RE with an extensive array of positioned nucleosomes in α -cells disappears. The chromatin structure of the RE region was also mapped in α - and α -cells in which the *SSN6* gene had been disrupted. These mutant strains display identical structures in the region of the RE to wild-type α - and α -cells, respectively (data not shown). In the absence of Ssn6p, the chromatin structure of the RE in α -cells is still highly organized.

Highly regular, organized chromatin spans 3.7 kb in α -cells

The maps in Figure 1 suggest that highly organized α -cell chromatin containing precisely positioned nucleosomes extended over some distance. To determine the boundaries of this particular chromatin domain in α -cells, MNase-cut sites of nuclear digests were mapped over a 5 kb region flanking the central RE (Figures 5 and 6). The mapped region is shown schematically in Figure 7. The region contains two potential α 2 operators, one (α 2#1) in the RE and a second (α 2#2) ~ 1350 bp to the right of the first.

Fig. 4. Recombination enhancer chromatin structure in *tup1* mutants. The chromatin structure of the Crick strand was mapped in *tup1* mutants by primer extension analysis of MNase cleavage sites using primer a292. Cell types are indicated above the sets of four lanes each. Undigested (–) and three levels of MNase-digested chromatin samples are shown. (D) indicates PCR DNA that controls for sequence specificity of MNase. The gray rectangle indicates the hypersensitive region identified in wild type *a*-cells (Figure 1B).

Both are well removed from the promoter of the nearest potential transcription unit. An open reading frame (ORF), *YCL055*, is to the left of $\alpha 2\#1$ and further to the left is a gene encoding a protein of unknown function, *YCL056* (Defoor *et al.*, 1992). To the right of $\alpha 2\#2$ is an essential gene, *YCL054*. It has been identified as somehow being implicated in silencing (Loo *et al.*, 1995).

Unlike previously studied domains (Shimizu *et al.*, 1991), nucleosomes are precisely positioned flanking the two $\alpha 2$ operator consensus sequences in both directions. In α -cells, 10 nucleosomes are precisely positioned to the left of $\alpha 2\#1$ (Figure 5). Only the first of these is unique to α -cells (Figure 5A), the remaining nine which cover the *YCL055* ORF are also present in *a*-cells (Figure 5A and B). In α -cells, another nine positioned nucleosomes are present between the two operators (Figures 5 and 6). This region in *a*-cells contains the two footprints and the MNase-hypersensitive region of the RE. Beyond the RE, *a*-cell chromatin has multiple MNase cutting sites and presumably contains randomly located nucleosomes. Four nucleosomes exist to the right of the second $\alpha 2$ operator consensus sequence, $\alpha 2\#2$ (Figure 6). To the left and right sides of this array of positioned nucleosomes, chromatin structure is apparently random, identical in both cell types (Figure 5 and data not shown).

Fig. 5. Chromatin structure around and to the left of $\alpha 2\#1$. The chromatin structure of the Watson strand was mapped by primer extension analysis of MNase cleavage sites using primers b295 (A) and b275 (B) and b275 (C). In α -cells, nucleosomes are positioned precisely adjacent to the operator (A) and extend over 1.5 kb until the promoter region of the first gene, *YCL056*, a gene for a protein of as yet unknown function (B), where the chromatin becomes more randomly organized (C).

Thus, in α -cells, an organized chromatin domain spanning 3.7 kb exists over and surrounding the RE region; a summary of all the data in α -cells is shown in Figure 7. On both sides, the boundary of that domain is represented by the promoter region of genes *YCL056* (Defoor *et al.*, 1992), to the left of $\alpha 2\#1$, and *YCL054*, an essential gene (Loo *et al.*, 1995), to the right of $\alpha 2\#2$. Chromatin in *tup1* mutant α -cells is disrupted over at least the right portion of the domain (data not shown). This difference in the chromatin structure of the RE region is not a general phenomenon for the left arm of chromosome III in *a*- and α -cells, since the unique nucleosomal organization of and adjacent to *HML* α persists in both mating cell types (K.Weiss and R.T.Simpson, unpublished data). The 3.7 kb domain can be divided into two regions. The first region, to the left of base pair 29 000, is organized identically in *a*- and α -cells. The second region includes both operators and displays striking differences in structure in the two cell types. In this region, *a*-cell chromatin is globally more susceptible to MNase cleavage, does not contain positioned nucleosomes and has distinctive footprints and nuclease hypersensitivity over the RE. In α -cells, on the other hand, 14 precisely positioned nucleosomes form a distinctive chromatin structure which is likely to preclude interactions of *trans*-acting factors that bind this region in *a*-cells.

Discussion

Footprints and a distinct DNA geometry characterize the RE in α -cells

The structural data, footprints 1 and 2, indicate binding of proteins to two regions of the RE to the right of the $\alpha 2\#1$ operator. The intervening segment of DNA appears to be uniquely structured, presumably as a consequence of this protein binding. The TTTR-rich DNA is distinctively MNase hypersensitive. Thymidine dimer formation, however, is not impeded in this region. While nucleosomes only modulate UV photofootprinting due to DNA bending (Pehrson, 1995), many non-histone proteins block thymidine dimer formation (Axelrod and Majors, 1989; Murphy *et al.*, 1993). Thus, the hypersensitive region does not

appear itself bound by proteins. MNase has a strong sequence preference for AT-rich regions flanked by a 5' dC or dG (Drew, 1984; Flick *et al.*, 1986), but the hypersensitive digestion pattern does not correlate with the appearance of such a sequence in the TTTR region. MNase also preferentially degrades single-stranded over double-stranded DNA (Cuatrecasas *et al.*, 1967; Drew, 1984), but the opposite is true for DNase I (Drew, 1984). Hypersensitivity to MNase taken together with unimpeded thymidine dimer formation suggests that the distinctive region might be partially unwound to have some single-stranded character. However, lack of thymidine oxidation with permanganate treatment shows that it is not totally unwound into a single-stranded conformation.

Investigation of different subfragments of the RE that could or could not restore donor preference in α -cells led Wu and Haber to suggest the existence of at least three important regions, with the combined presence of two being a minimal requirement for enhancer activity (Wu and Haber, 1996). Our structural data correlate with these conclusions from genetic findings. What proteins might bind to the RE? Neither MAT α 1p nor MAT α 1p are involved in switching directionality (Rine *et al.*, 1980; Jensen and Herskowitz, 1984). Interestingly *CHL1*, a gene necessary for mitotic stability of certain chromosomes (Gerring *et al.*, 1990), only affects MAT α donor preference (Weiler *et al.*, 1995). In *chl1* MAT α cells, donor preference appears unchanged. It is worth noting that *CHL1* encodes a protein predicted to have a DEAH helicase motif (Gerring *et al.*, 1990). It seems likely that the interaction of presumed proteins and the alterations of DNA geometry contribute to conferring directionality in mating type interconversion. The complex present at the RE must enable a molecular communication with *HML*, 17 kb away, to promote its recombination with the MAT α locus and subsequent mating type switching.

Precisely positioned nucleosomes characterize the RE in α -cells

If the unusual DNA structure and footprinted regions in α -cells are involved in directionality, a different chromatin structure would be expected in α -cells. Dramatic differences for the two cell types are indeed observed. Flanking the operator in α -cells, positioned nucleosomes are present over the regions footprinted in α -cells, as well as the TTTR-rich segment displaying the unique structure. Several of the features distinctive in α -cell chromatin organization of the switch control region are located completely or partially in positioned nucleosomes in α -cells: note particu-

Fig. 6. Chromatin structure to the right of $\alpha 2\#1$ and around $\alpha 2\#2$. The chromatin structure of the Crick strand was mapped by primer extension analysis of micrococcal nuclease cleavage sites using primers a297 (A), a302 (B) and a304 (C). In α -cells, nucleosomes are precisely positioned to the left of (A), adjacent to (B) and to the right of the operator (C). This organized structure extends over 2 kb from $\alpha 2\#1$ to the promoter region of the first transcribed gene, *YCL054*, an essential gene (C), where the chromatin becomes more randomly organized. Adjacent ellipses in (A) indicate an area of protection of 300 bp which possibly accommodates two tightly packed nucleosomes.

Fig. 7. Schematic representation of the mapped chromatin structure of 4 kb including the recombination enhancer in α -cells. Map units correspond to base pair positions of the sequence of chromosome III. Mat α 2p/Mcm1p heterodimers, shown bound to the operators for clarity, are depicted by a sphere connected to two ovals. Ellipses indicate precisely positioned nucleosomes. Filled ellipses indicate nucleosomes that are precisely positioned only in α -cells; open ellipses signify nucleosomes that are positioned in both cell types. An ORF is identified by a bracket and transcribed genes by arrows. Possible promoter-associated factors are depicted by black boxes.

larly the presumed protein footprint 1 near the $\alpha 2$ -binding site (Figure 1A) and footprint 2 distal to the hypersensitive region (Figure 1B). The hypersensitive region which has a distinctive nuclease susceptibility in **a**-cells is entirely included in a positioned nucleosome in α -cells (Figure 1B). While some *trans*-acting factors can interact with DNA in peripheral regions of positioned nucleosomes (M.Xu, R.T.Simpson and M.P.Kladde, unpublished) (Simpson, 1990; Redd *et al.*, 1996), the central ~80–100 bp of nucleosomes preclude interactions of most regulatory proteins *in vivo*. The organized chromatin extends over the entirety of the RE and 1–2 kb adjacent to it in a region which lacks transcribed ORFs (Figure 7). In α -cells, much of the left arm of chromosome III is inactivated for recombination (Wu *et al.*, 1996), although at this point in our analysis of chromosome III chromatin structure, we know of only ~2.5 kb around the RE that are packaged into a highly organized chromatin structure of positioned nucleosomes in α -cells, distinctive from the protein–DNA interactions that occur in **a**-cells. In particular, an identical chromatin structure of and adjacent to *HML α* can be observed in both **a**- and α -cells (K.Weiss and R.T.Simpson, unpublished data). Gene-sized organized chromatin domains exist for *STE6* and *BAR1* **a**-cell-specific genes (Y.Tsukagoshi and R.T.Simpson, unpublished observations). Here, a chromatin domain extending over >2 kb exists in the absence of any transcriptional context.

Which proteins are involved in silencing recombination and creating the organized chromatin structure? Mat $\alpha 2$ p is a reasonable candidate for both functional and structural differences. Inappropriate production of this protein makes **a**-cells lose their preference for interaction with *HML α* (Wu and Haber, 1995). The chromatin structure of the RE in a *MATa* strain expressing endogenous levels of $\alpha 2$ protein is identical to that observed in α -cells (data not shown). We have shown previously that Mat $\alpha 2$ p/Mcm1p binding to the $\alpha 2$ operator organizes chromatin with positioned nucleosomes spreading from the operator over promoter and coding sequences in both minichromosomes and the genome (Roth *et al.*, 1990; Shimizu *et al.*, 1991; Simpson *et al.*, 1993). Indeed, both the $\alpha 2\#1$ of the RE and the $\alpha 2\#2$ 1350 bp away seem to be functional, since UV photofootprints show a pattern indistinguishable from that for the operator upstream of *STE6* in α -cells (Murphy *et al.*, 1993). Thus, the presence of the distinctive chromatin structure around the RE is most likely due to Mat $\alpha 2$ p. Mapping of chromatin in *tup1* and *ssn6* strains further supports this interpretation. Tup1p has been shown to be necessary for establishing Mat $\alpha 2$ p organized chromatin structures (Cooper *et al.*, 1994). In the absence of Tup1p, the chromatin structure of the RE in α -cells is not only random, but also displays unique features identical to **a**-cells. *SSN6* disruption has no apparent impact on the precise positioning of nucleosomes in α -cells in the RE or flanking regions; previous studies of **a**-cell-specific genes showed much more minor effects on chromatin structure of the *ssn6* mutation than those associated with a *tup1* disruption (Cooper *et al.*, 1994). A recent study by Szeto and Broach (1997) has demonstrated that Tup1p, but not Ssn6p, is essential for preferential recombination of *MAT α* with *HMRa*. Disruption of organized chromatin structure in *tup1* α -cells is totally consistent with these

functional observations; the absence of positioned nucleosomes covering the critical RE elements allows access of the putative *trans*-acting factors and activation of the left arm of chromosome III for recombination.

According to Wu and Haber (1996), in the absence of a 2.5 kb segment including the 702 bp minimal recombination enhancer sequence, the entire left arm of chromosome III is inactivated for recombination in **a**- and α -cells. Insertion of the 702 bp RE restores donor preference in both cell types. The chromatin structure of the 702 bp insertion is indistinguishable from the wild-type data shown in Figure 1 (manuscript in preparation). This locally present organized chromatin structure is therefore necessary to oppose protein binding to the RE in order to preserve the inactive state of the left arm of chromosome III. The role of the second $\alpha 2$ -binding site is unclear. At least it does not appear to be necessary for nucleosome positioning over the minimal RE in α -cells.

Mechanistic considerations regarding directionality of mating type switching

Structural and functional properties of the RE must be integrated with directionality of mating type interconversion for a full understanding of this basic process. The unique geometry of the RE in **a**-cells suggests an entry point for the recombinational machinery and the flanking footprints suggest that specific *trans*-acting factors are involved in creating this DNA structure. As sequences required for RE activity overlap with the Mcm1p-binding sites of $\alpha 2\#1$ (J.E.Haber, personal communication), a role for Mcm1p in recombinational activation in **a**-cells, in addition to its requirement for Mat $\alpha 2$ p/Mcm1p complex formation in α -cells, is possible.

Identification of factors that interact with the RE in **a**-cells is a topic of great interest; the definition of likely targets in the current study should facilitate such investigations. We suggest a similarity between repression of **a**-cell-specific gene transcription and inactivation of recombination by Mat $\alpha 2$ p. In the former case, transcriptional activators or the basal transcription machinery and, in the latter case, the putative *trans*-acting factors necessary for activation of recombination in **a**-cells, may be unable to gain access to their binding sites in promoters or the RE, respectively, because they are tightly packaged into organized chromatin in α -cells. Even once these factors are identified, the location of the RE at long distances from both the donor and acceptor sequences will raise considerable problems in defining the mechanistic explanation for communication between *MAT* and *HML*.

Features of global chromosomal architecture and interactions are likely to play a role. After cleavage by HO endonuclease, sequences at *MATa* have to contact the *HML* locus for strand invasion and gene copying. The left arm of chromosome III must form a loop. The RE, located a large distance from both loci, may promote this looping event in **a**-cells. Similarly, loci at positions other than *HML* on chromosome III might benefit from a particular physical configuration or localization that the chromosome adopts in **a**-cells. However, the enhanced intra- and interchromosomal recombination frequencies reported by Haber and colleagues (Wu and Haber, 1996) suggest a different, possibly overlapping mechanism. The possible involvement of a particular DNA geometry in this com-

munication is intriguing. In contrast, the highly organized chromatin structure could inhibit initiation of such a physical transition in α -cells, by precluding access of factors necessary for creating the unusual DNA structure. Further elucidation of how a small element can influence or control recombination over large chromosomal regions will be of great interest.

The beginnings of a mechanistic understanding of the directionality of mating type switching in *Saccharomyces cerevisiae* emerge from this structural study. Presumably non-cell type-specific protein(s) interact with two regions of the RE in α -cells, creating a unique DNA structure spanning 110 bp near the $\alpha 2$ operator. By mechanisms which remain to be elucidated, the protein binding and DNA alterations communicate with *HML α* , 17 kb away, to allow recombination with the *MAT α* locus and mating type switching. In α -cells, where the $\alpha 2$ repressor is present, *Mat $\alpha 2$ p* organizes a distinctive chromatin structure wherein positioned nucleosomes preclude access of the *trans*-acting factors to the switch control elements.

Materials and methods

Saccharomyces cerevisiae strains, generously provided by J.Haber, are JKM115 (*hml::ADE1 MAT α hmr Δ ::ADE1 lys5 leu2 ura3 trp1*) and JKM111 (*hml::ADE1 MAT α hmr Δ ::ADE1 lys5 leu2 ura3 trp1*). They were propagated in YEPD complete medium.

The *TUP1* gene was replaced with the *URA3* gene by transformation with the linearized disruption plasmid pRS406-TUP1 (Cooper *et al.*, 1994). Transformation was assessed by uracil prototrophy. The disruption of *TUP1* was verified by PCR analysis. A parallel strategy was used to disrupt *SSN6*. The *tup1* and *ssn6* mutants displayed a clumpy phenotype.

Yeast were grown in YEPD at 30°C to mid-log phase (OD₆₀₀ ~1). Nuclei were isolated as described previously (Roth and Simpson, 1992). Zymolyase YT100 (Seikagaku), 0.5 mg/ml for 25 min, was used instead of lyticase for spheroplast formation. MNase and DNase I digestions were performed as described (Shimizu *et al.*, 1991). The nuclear pellet from 1 l of culture was suspended in 4 ml of digestion buffer (10 mM HEPES, pH 7.5, 0.5 mM MgCl₂, 0.05 mM CaCl₂). The suspension was divided into 800 μ l portions and each of them was digested at 37°C for 10 min using increasing concentrations of MNase (Worthington) (0–40 U/ml) or DNase I (Worthington) (0–4 U/ml). The reaction was terminated by adding EDTA to a final concentration of 25 mM. DNA was purified as described (Szent-Gyorgyi and Isenberg, 1983). Purified DNA was resuspended in 0.1 $\times$ TE (1 mM Tris-HCl, pH 8, 0.1 mM EDTA). Naked DNA controls were obtained by either digesting purified, previously undigested DNA with a 50-fold lower concentration of enzyme or digesting a PCR product. A total of 1.6 kb of the sequences including the RE were amplified using oligonucleotides a283 and b299 (see below) as primers. Approximately 100 ng of the PCR product was digested with 0.5 U/ml MNase or 0.05 U/ml DNase I at 37°C for 3 min in the presence of 36 μ g of carrier DNA (calf thymus). After ethanol precipitation, DNA was resuspended in 50 μ l of 0.1 $\times$ TE.

MNase and DNase I cleavage sites were located by primer extension assay using *Taq* polymerase as described (Shimizu *et al.*, 1991) with minor modifications. Oligonucleotides used as primers include [coordinates are base pair positions in the published sequence of *S.cerevisiae* chromosome III (Oliver *et al.*, 1992)]: a283, 28 362–28 383 (5'-AGGTTTCTGTT-CATCTGGGCC-3'); a290, 20 031–29 053 (5'-GCGCAAGGATTGATT-CAGTACAA-3'); a292, 29 242–29 264 (5'-GCTGGAAGTGCAG-AACAAAGAGG-3'); a297, 29 687–29 710 (5'-GGGACTACA-GTATTAGGGAAACAGG-3'); a302, 30 182–30 207 (5'-CACTA-GTCTTAAGTTCTGTTGACGC-3'); a304, 30 475–30 500 (5'-CTCAAAGTCAAGAAACACAGAACAC-3'); b299, 29 963–29 936 (5'-GCTTCTCTTGAATTGGGTATCATGGG-3'); b297, 29 710–29 687 (5'-CCTGTTTCCCTAATACTGTAGTCCC-3'); b295, 29 544–29 521 (5'-TACAAAGTTTCAATGCTTGCAAATATTG-3'); b285, 28 534–28 512 (5'-CCATCAACGTTTCATCGTCCTG-3').

Oligonucleotides were gel purified and separated over a Sep-Pak reverse phase chromatography column (Waters). Primers used in individual experiments are indicated in the corresponding figure legends.

For chromatin mapping, multiple cycle primer extension was performed as follows: 5 μ l of DNA was combined with 2 μ l of 10 $\times$ *Taq* buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 30 mM MgCl₂, 0.5% NP-40, 0.5% Tween-20) (Shimizu *et al.*, 1991), 2.5 μ l of a deoxynucleotide triphosphate mixture (2.5 mM dNTP; 2.5 mM each dATP, TTP, dCTP, dGTP in H₂O) and H₂O to a final volume of 17 μ l. Then 0.4 pmol of ³²P-end-labeled primer in a 3 μ l volume was added, followed by 5 μ l of 1 U of *Taq* polymerase suspended in 1 $\times$ *Taq* buffer. The cycling pattern was 94°C, 1 min, 55°C, 1 min, 72°C, 1 min 30 s. The cycle was repeated 20 times and then followed by a 5 min chase at 72°C and cooling to 4°C. DNA was precipitated in 0.3 M sodium acetate with 3 vols of 100% ethanol. DNA was dissolved in sample buffer, heated to 95°C for 5 min and electrophoresed on a 6% polyacrylamide–8 M urea, sequencing gel at 58 W for 300 min. A gradient buffer system was used. The upper chamber contained 0.5 $\times$ TBE buffer, the lower one contained 0.66 $\times$ TBE, 1 M sodium acetate buffer. The gel was dried at 80°C for 60 min and exposed on X-ray film or a phosphorimager screen.

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Note added in proof

YCL055 is the *KAR4* gene (Kurihara,L.J., Stewart,B.G., Gammie,A.E. and Rose,M.D. (1996) Kar4p, a karyogamy-specific component of the yeast pheromone response pathway. *Mol. Cell. Biol.*, **16**, 3990–4002).