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ANALYSIS OF MUTATIONS AFFECTING MATING AND SPORULATION IN YEAST

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ANALYSIS OF MUTATIONS AFFECTING MATING AND SPORULATION IN YEAST

By

Laurence Melnick

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1981

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This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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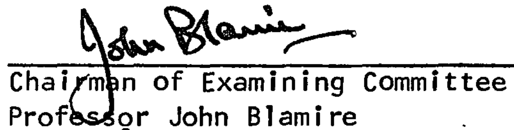

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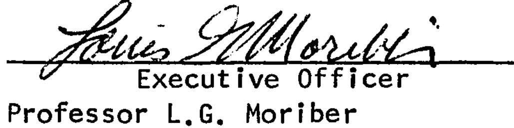

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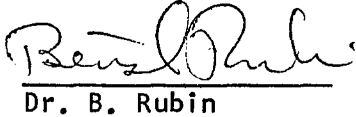

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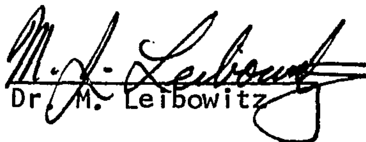

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ABSTRACT

Two mutations are described which alter certain features of mating and sporulation activity in the yeast *Saccharomyces cerevisiae*. The first mutation, the *dmt* (*d*ual *m*ating *t*ype) gene allows *Mat α dmt* cells to enter into otherwise prohibited mating with cells of the same mating type to form α mating, nonsporulating diploids.

dmt gene facilitated *α*x*α* matings result from deletion of mating locus information in *Mat α dmt* cells allowing these cells to mate as *a* mating type cells.

The second mutation, the dominant *ARM* (*a*ctive *r*egulation of *m*eiosis) gene allows the otherwise prohibited sporulation of diploid cells of the genotypes *Mat α/Mat α*, *Mat α*/ deletion of *Mat* and *Mat α/mat a** (*mat a** is a recessive mutation of the *Mat a* locus. *mat a*/Mat α* diploids are unable to sporulate). *Mat a/Mat a* diploids carrying the *ARM* gene are unable to sporulate.

Mat α ARM strains show enhanced ability to undergo switch of mating type as compared to *Mat α* wild-type strains.

The *ARM* gene was used to study the nature of the *dmt* gene by allowing sporulation of the otherwise nonsporulating diploids produced by the mating of *Mat α* cry_1^s *dmt* with *Mat α* cry_1^r *ARM*. In this cross, 246 colonies, each the product of an independent mating event were examined. Tetrad analysis of sample colonies demonstrated the presence of four major classes of diploids defined by the following

tetrad configurations:

2 Mat α $cry_1{}^r$: 2 nonviable

4 Mat α $cry_1{}^r$

2 Mat α $cry_1{}^r$: 2 Mat α $cry_1{}^S$

2 Mat a $cry_1{}^r$: 2 Mat α $cry_1{}^S$

This data also supports the model of dmt gene-facilitated rare matings where dmt induced loss of Mat α information allows a cell to mate as an a mating type cell. In addition, this data shows that dmt induced chromosome III damage can be repaired with homologous information after mating has occurred.

The ARM gene, which was used to allow sporulation of diploids formed by dmt gene-facilitated rare matings, appears to be a cryptic copy of Mat a information located on the right arm of chromosome III, distal to thr_4.

Another gene, ARM α, is described which appears to be a cryptic form of Mat α information which is expressed in a manner analogous to the Mat a information of the ARM gene.

The ability of the ARM gene to replace HMRa in allowing homothallic switch of mating type is examined.

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I am grateful for the excellent ideas, guidance, friendship and encouragement of Dr. John Blamire, who has made this thesis possible. I am also grateful for the ideas and encouragement of Dr. Jim Nishiura, Dr. Nasim Khan and Dr. Carl Beam.

My close friends and fellow graduate students at Brooklyn College, Berish Rubin, Henrietta Kazan, Howard Caplan, Lee Huang, Michael Goldenthal and Rolf Martin, helped make my stay at Brooklyn College a valued experience.

I am also grateful to my outside friends, Alison Nash, David Green and Marian Evans, who have supported me in this project.

Dedication

I dedicate this thesis to my mother and father, Mrs. Beatrice Melnick and Dr. Jacob Melnick.

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INTRODUCTION

Introduction

This thesis describes a genetic analysis of two mutations which affect mating and sporulation activity in the baker's yeast *Saccharomyces cerevisiae*. One of these mutations, the *dmt* gene, allows otherwise prohibited mating events to occur. The other mutation, the *ARM* gene, allows otherwise prohibited sporulation of certain yeast strains.

The thesis introduction includes a general background on the events related to yeast mating and sporulation, and on the genetic regulation of these events. A number of elegant genetic and more recently biochemical studies done by many investigators have demonstrated that the determination of yeast cell mating type involves a genetic system which includes transposable genetic elements. This introduction, therefore, includes a discussion of certain other genetic systems where transposable genes are involved in gene regulation.

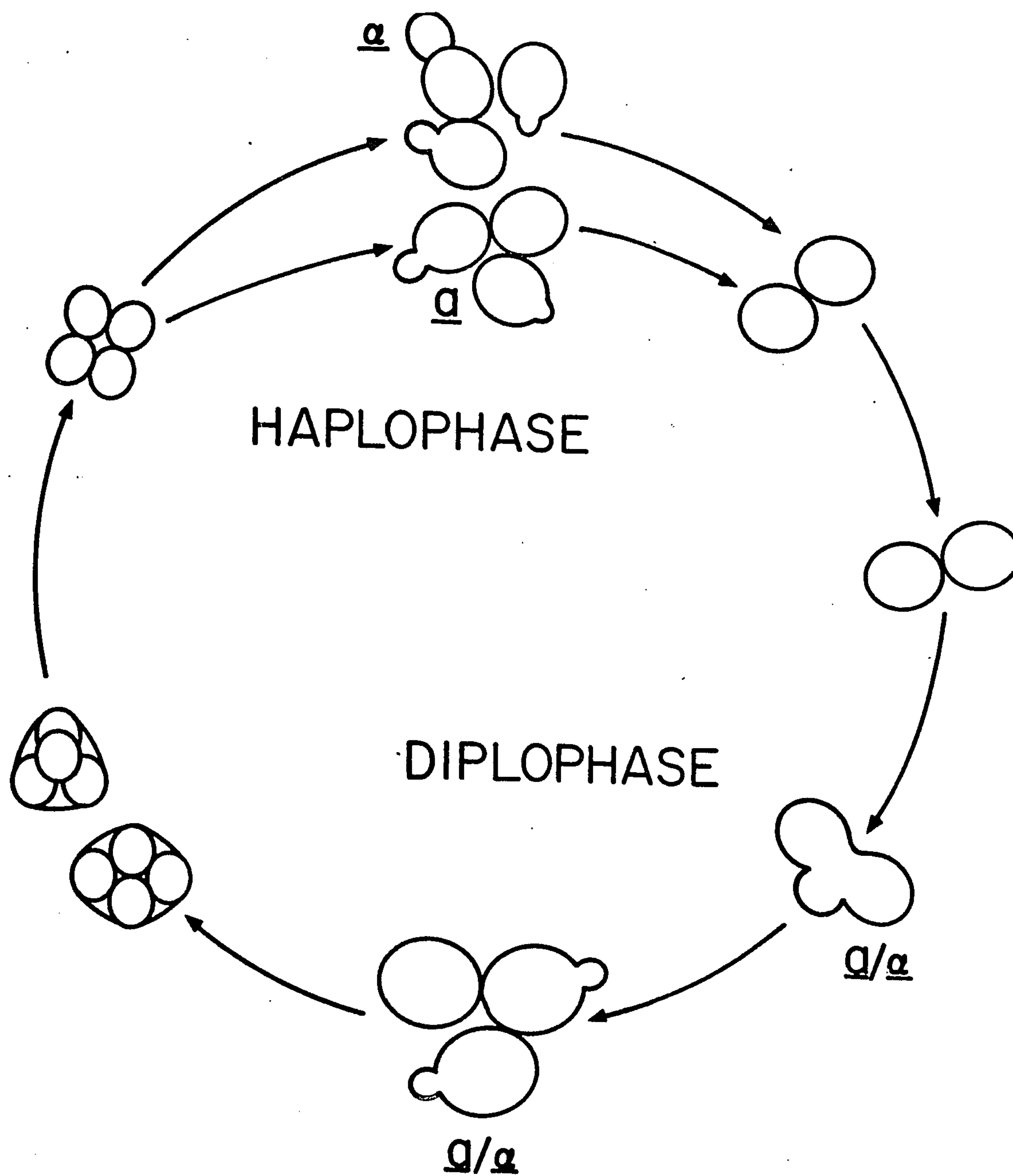
I The Mating System in Heterothallic Yeast

a) Yeast life cycle

Yeast cells can exist in haploid, diploid or higher ploidy forms, all of which are able to propagate asexually by mitosis. Haploid yeast cells may be either of two mating types, *a* or *α*, and cells of different mating types may conjugate with each other to form diploid cells of *a*/*α*

Figure I1

Yeast Life Cycle



mating type. These diploid cells, under conditions of nitrogen deprivation, are induced to undergo meiosis giving rise to asci containing two a mating type and two α mating -type haploid cells. Yeast may be either heterothallic, having stable mating types, or homothallic, having mating -types which switch from a to α or α to a in a patterned manner. The life cycle of yeast is diagrammed in Figure 1.

Yeast cells of the three different mating types, a, α and a/α differ in a variety of characteristics. However, the mating type of a cell is determined by a single pair of genetic loci, Mat a and Mat α which segregate as single allelic nuclear genes. The mating locus, Mat a in a cells, Mat α in α cells is located on the right arm of chromosome III. The genes are codominant in that diploid cells of the genotype Mat a/Mat α display neither a nor α functions and have properties found in neither of the two haploid mating-type cells.

Properties of a, α and a/α mating-type cells.

The mating-type of a yeast cell, either a or α, is determined by a single complex gene, the mating locus, which may be either of two codominant alleles, Mat a or Mat α. Haploid cells can mate with other haploid cells of opposite mating type but are prohibited from mating with cells of the same mating type. This mating results in the formation of an a/α (genotype Mat a/Mat α) diploid cell which is unable to mate and which can be induced to undergo meiosis and

sporulation under conditions of nitrogen deprivation. Diploid yeast cells or cells of higher ploidy, which are homozygous for the mating locus, cannot be induced to sporulate. These cells, however, are able to mate with cells homozygous for the opposite mating type.

α mating-type cells constitutively secrete an oligopeptide pheromone, *α*-factor, which induces a complex, reversible response in *a* mating type cells. *a* mating type cells similarly secrete an oligopeptide pheromone, *a*-factor, which has an analogous effect on *α* mating-type cells (Bücking-Throm *et al*., 1973; Hereford and Hartwell, 1973; MacKay and Manney, 1974a; Manney and Meade, 1977; Wilkinson and Pringle, 1974).

The reversible nature of the *a* cell response to *α*-factor is due at least in part to the barrier effect of *a* cells which is the ability of these cells to destroy *α*-factor activity. This activity appears to be the result of a constitutive, *α*-factor cleaving proteolytic activity specific to cells of *a* mating type (Chan, 1977; Ciejek and Thorner, 1979; Hicks and Herskowitz, 1976a).

a and *α* cells, but not *a/α* cells, produce agglutination factors, glycoproteins involved in forming bonds between cells of opposite mating type as part of the mating process. In *a* cells, agglutination factor production is inducible by encounter with *α*-factor (Sakai and Yanagishima, 1972; Sena *et al*., 1973; Yanagishima, 1973).

a/α cells differ from cells of *a* or *α* mating type in

the location of origin of bud generation. In a/α cells, buds emerge colinear to a lengthwise cellular axis, whereas in a or α cells, or diploid cells homozygous for mating type, buds emerge at an angle to a lengthwise cellular axis (Freifelder, 1960).

a/α cells also differ from cells which are homozygous for mating type in the greater ability of the former to survive irradiation by X-rays (Mortimer, 1958; Lashowski, 1960).

b) The mating reaction

Diffusable mating factors and GI arrest.

The diffusable mating factors, a-factor and α-factor, produced in a mixture of a and α cells causes cessation of budding in cells of both mating types. Cell division is arrested at the GI interval. Evidence that GI arrest is required for mating comes from observations of a variety of temperature sensitive cell division cycle (cdc) mutants (Reid and Hartwell, 1977) which at restrictive temperature arrest cell division at different points in the cell cycle. Of the various classes of cdc mutants observed, only cdc 28, which arrests at the GI interval, is competent to mate at restrictive temperature.

Although cell division is arrested at GI, certain other cellular functions are not arrested. Cell growth continues and cells become elongated. These morphologically altered cells, called "shmoos", display characteristic patterns of

binding to the lectin concanavalin A (Tkacz and MacKay, 1979). Cell wall synthesis during shmoo formation contrasts biochemically with cell wall synthesis during normal growth (Lipke *et al*., 1976; Schekman and Brawley, 1979).

Agglutination reaction

Before conjugation, cells of opposite mating type are able to adhere to each other. Upon first encounter, adhesion is weak but stronger than the adhesion of cells for other cells of the same mating type. Preconditioned cells, having been exposed to cells of opposite mating type for 20 to 60 minutes, show enhanced adherence to newly encountered cells of the opposite mating type (Yanagishima *et al*., 1974; Fehrenbacher *et al*., 1978; Betz *et al*., 1978). The agglutinability of *a* cells is inducible by exposure to *α*-factor and requires protein synthesis (Sakai and Yanagishima, 1972; Doi and Yoshimura, 1978).

Conjugation

Cells fuse at the tips of their shmoo formations. Freeze etch electron micrographs of shmooing *a* mating type cells show a thinning of the cell wall at the shmoo tip. Vesicles, possibly containing cell wall digesting enzymes, aggregate at this area (Duntze, *In* MacKay, 1978).

Cell fusion is followed by a fusion of nuclei of the two parent cells. This event has not yet been well characterized.

c) The Nature of Mating Locus Regulation of a, , and a/ Mating Types

Although a, α and a/α mating type cells differ in a variety of features, cell mating type is determined by a single pair of alleles, Mat a and Mat α.

MacKay and Manney isolated nonmating (sterile) mutants by selection using alleles for resistance and sensitivity to canavanine (MacKay and Manney, 1974; 1974a). Cells which are heterozygous for resistance-sensitivity are canavanine sensitive. Nonmating mutants were screened for among canavanine resistant cells by encountering these cells with an excess of sensitive cells of opposite mating type. After allowing time for mating, only those resistant cells which did not mate were able to survive on media containing canavanine.

Certain of the ste (sterile) mutants isolated by MacKay and Manney were at the Mat α locus. None were isolated at the Mat a locus. Other sterile mutants were not at the mating locus and of these, certain could cause sterility only in a cells, and were termed a specific. Others, α specific mutations, caused sterility only in α cells. a, α specific ste mutations caused sterility in cells of either mating type.

The sterile mutations formed the basis for a model of mating locus regulation of cell mating type in which Mat a and Mat α are regulatory alleles which control the expression of mating genes found at other loci. In a/α diploid cells, both Mat a and Mat α coordinately turn on

genes which allow sporulation while turning off genes which allow mating.

Genetic analysis of mutations at the mating locus

Two sterile mutations isolated by MacKay and Manney from *Mat α* cells, *mat α* 1⁻ and *mat α* 2⁻ map at the mating locus and are recessive to *Mat α*. These mutations complement each other in that the diploid *mat α* 1⁻/*mat α* 2⁻ behaves as an *α* cell, and therefore, these mutations are considered to define two functional units of the *Mat α* locus (Herskowitz *et al*., 1980).

The double mutant *mat α* 1⁻, *mat α* 2⁻ has been constructed and mates as an *a* cell (but not as an *α* cell). Unlike *Mat a* which is codominant to *Mat α*, the double mutation is recessive to *Mat α*.

These observations support the idea that in an *α* cell, one functional unit of *Mat α* "turns on" *α* specific mating functions, and another functional unit "turns off" *a* specific mating functions. In the double mutant, *mat α* 1⁻, *mat α* 2⁻, *α* specific functions are not "turned on" but *a* specific functions are not "turned off." This cell therefore mates as an *a* cell. This model implies that *a* mating functions are constitutive in the absence of one of the functional units of *Mat α*.

Which of the two *Mat α* functional units "turns on" *α* mating functions and which "turns off" *a* mating functions? Although both *mat α* 1⁻ and *mat α* 2⁻ confer sterile phenotype,

each has a number of distinguishing features.

The following observations on the phenotypes of the *mat* *α* 1^- and *mat* *α* 2^- mutations were made by MacKay (1974a) and Rine and Sprague (unpublished, see Herskowitz *et* *al*., 1979):

mat *α* 1^- cells are deficient in certain *α* cell mating related activities; for example, they cannot mate with *a* cells, produce *α*-factor or agglutinate specifically with *a* cells. However, the *mat* *α* 1^- mutation does display "*Mat* *α*-like" activity in that diploid cells of the genotype *mat* *α* 1^-/*Mat* *a* are able to sporulate. In contrast, *mat* *α* 2^-/*Mat* *a* diploid cells are unable to sporulate. In this comparison, *mat* *α* 1^- cells are more "*α*-like" than *mat* *α* 2^- cells. *mat* *α* 2^- cells in certain respects are "*a*-like", for example, these cells, like *a* cells, express barrier function, respond to *α*-factor and show greater ability to mate with *α* than with *a* cells. These observations can be explained by a model of *Mat* *α* regulation in which the *mat* *α* 1^- mutation defines a *Mat* *α* subunit, called α_1, which in wild-type cells positively regulates *α* specific genes. In *mat* *α* 1^- mutant strains, *α* specific genes fail to be "turned on", causing these cells to be unable to mate. The *mat* *α* 2^- mutation defines a different *Mat* *α* region, called α_2, which in wild type cells negatively regulates *a* specific genes. In the *mat* *α* 2^- mutant, *a* specific genes are not turned off, as they are in wild-type *α* cells. In this mutant, the α_1 region is intact and therefore *α*

functions are turned on. mat α 2^- sterility is postulated to be the result of simultaneous, antagonistic expression of both a and α specific genes.

In summary, the α 1, α 2 model of mating locus regulation postulates that α specific genes are positively regulated by the α 1 functional unit of the Mat α locus, and that a specific genes are negatively regulated by the α 2 functional unit of the Mat α locus. In the absence of α 2 expression, a specific genes are constitutive. Cells carrying the mat α 1^- mutation express neither α specific nor a specific genes. Cells carrying the mat α 2^- mutation express both a and α specific genes which antagonistically cause cell sterility.

Certain observations by Sprague, Rine and Herskowitz (1979) support the α 1, α 2 model explanation of mat α 2^- sterility. The model proposes that a and α genes are antagonistic to each other and therefore their simultaneous expression in mat α 2^- cells results in impaired expression of both a and α functions. If this is the case, then in mat α 2^- cells, second mutations in a or α specific genes could upset this antagonism and "release" expression of certain mating functions.

Two mutations which fit this criterion have been discovered. The ste 3-1 mutation is α specific in that it causes sterility in α but not in a cells. mat α 2^-, ste 3-1 double mutant cells, unlike mat α 2^- cells, mate as a cells! The loss of ste 3-1 function has released a

specific gene function from antagonism.

mat α 2^- cells do not produce detectable α-factor, however, mat α 2^- cells carrying the a specific mutation bar 1 produce α factor. bar 1 appears to be a deficiency in the mating-type specific ability of a cells to degrade α-factor allowing recovery from α-factor induced arrest (Chan, Sprague, personal communication).

d) Rare Mating and the dmt Gene

Whereas MacKay's sterile mutations prohibit mating, a mutation found by Dr. John Blamire and myself allows otherwise prohibited mating events to occur (Blamire and Melnick, 1975; Melnick and Blamire, 1978; and this thesis). α mating -type strains containing the single nuclear mutation, dmt, are able to mate with other α mating type strains at unusually high frequency. The dmt gene has no effect on α times a matings and did not increase the low frequency at which a cells rare mate with cells of the same mating type. Examination of the diploid progeny of Mat α dmt x Mat α + rare matings revealed that certain genetic information present in the parents was not found in the diploid rare mating progeny. In over 90% of the rare mating diploid colonies examined, the information lost had been on the chromosome III of the dmt gene containing parent.

These observations were taken to indicate that loss of chromosome III including the Mat α locus of dmt gene containing cells allows these cells to mate as a cells.

Rare matings between Mat α wild-type cells, while occurring at much lower frequency than those dmt gene induced rare matings described above, often show similar patterns of loss of chromosome III information. For this reason it was concluded that in certain of these rare matings, the event which allows diploidization is loss of Mat α information in one of the haploid parents.

These observations are consistent with the idea that a specific mating function genes are under negative regulation by information on the Mat α locus, and these a specific genes are expressed in the absence of this information. The pattern of chromosome III information loss in rare mating diploid formation is discussed in chapters I, II and IV of the Results section of this thesis.

II Homothallic Yeast

In the previous sections of the Introduction, the yeast described contained stable mating loci. These are heterothallic. Homothallic yeast, to be described in this section, change mating type in a patterned manner during mitotic growth.

a) Genetics of Homothallism

Homothallic strains differ genetically from most laboratory heterothallic strains by a single gene, Ho, for homothallism or ho for the absence of this gene, the condition of heterothallic strains (Winge and Roberts, 1949).

Certain Ho containing strains are deficient in ability to switch mating type, and the study of these strains has resulted in the identification of the HMa and HMα* loci required for homothallism (Harashima and Oshima, 1976; Harashima et al., 1974; Naumov and Tolstorukov, 1973). These genes, and their respective recessive alleles hma and hmα are located on chromosome III, HMa on the left arm and HMα on the right arm, distal from the mating locus.

The following chart demonstrates the homothallic abilities of strains of a variety of genotypes.

				type of mating type switch	
				a → α	α → a
Mat a or α	Ho	HMα	HMa	+	+
Mat a or α	Ho	hma	hmα	+	+
Mat α	Ho	HMα	hma	-	+
Mat a	Ho	HMa	hmα	+	-
Mat a	Ho	HMα	hma	-	-
Mat α	Ho	HMa	hmα	-	-

HMa and hmα, also HMα and hma appear to be functionally equivalent and further genetic studies support their equivalence. For example, Arima and Takano (1979) constructed diploid strains homozygous for mating type using the method of protoplast fusion. In this way the anthors

* HMa and HMα are now called HM_L α and HM_L a, respectively. The use of the older terms is employed here to facilitate a historical discussion.

were able to observe the action of the *Ho* gene in cells of a variety of genotypes. The results shown below are consistent with the idea of functional equivalency of *HMa* and *hmα* and also *HMα* and *hma*.

Protoplast fusions producing strains which are able to switch mating type:

Mat α Ho hma HMa x *Mat α ho hmα hma*

Mat α Ho hmα HMa x *Mat α ho HMα hma*

Mat α Ho hma HMa x *Mat α ho HMα HMa*

Mat a Ho HMα hma x *Mat a ho hmα hma*

Mat a Ho HMα hma x *Mat a ho hmα HMa*

Mat a Ho HMα hma x *Mat a ho HMα HMa*

Protoplast fusions producing strains which are not able to switch mating type:

Mat α Ho hma HMa x *Mat α ho hmα HMa*

Mat a Ho HMα hma x *Mat a ho HMα hma*

Oshima and Takano (1971) and Harishima (1974) proposed that the *HMa* and *HMα* loci were or contained controling elements similar to those proposed to regulate the expression of mutable loci in maize. The mating locus was proposed to act as an affinity site which expresses *a* or *α* mating information depending upon which of the controling elements was present at that locus.

b) Mechanism of Homothallic Switch of Mating Type

Donald Hawthorne selected rare diploid colonies formed by rare matings between complementing haploid α strains (Hawthorne, 1963). He discovered a nonmating, sporulating diploid colony which, through meiosis, produced only two viable spores per ascus, these being α cells. Non-viable spores could be rescued by cell-spore mating and these spores mated as *a* to produce diploids which were phenotypically *a*/α. By mating nonviable spores with α thr_4 MAL_2 in cells it was shown that these spores contained a deletion which included the thr_4 but not the MAL_2 locus on chromosome III. Hawthorne concluded that a deletion in chromosome III had changed an α cell to an *a* linked lethal, and proposed that the mating locus is complex, made up of *a* and α portions, as shown below.

"Mat α"

proposed complex
mating locus

"Mat a"

proposed result of deletion

The nature of the Hawthorne deletion led to speculation on the nature of mating-type switch in homothallic strains. Hawthorne proposed that the *Ho* gene acted to repress the *a* or α portion of a complex mating locus, and that after

diploidization, the a/α loci, in combination acted to repress the action of the Ho gene and thus to prevent further switch.

Two other possible explanations of homothallic switch are as follows:

Ho mediated switch of mating type could occur if a promotor were located within the mating locus, between proposed a and α subloci. The direction of transcription of an RNA polymerase which begins transcription at this promotor could be determined by the state of modification (base methylation for example) of the promotor, or by the orientation of a promotor which is able to reverse its orientation, or "flip-flop" and in this way change the direction in which it guides a polymerase (Hicks and Herskowitz, 1976a).

The control of a complex locus can be mediated by controling elements similar to those described in maize (McClintock and section II of this Introduction), which by integrating at a functional unit of the mating locus, could specifically inactivate that functional unit (Oshima and Takano, 1971; Harashima et al., 1974).

To probe the nature of a proposed complex mating locus, Hicks and Herskowitz (1977) examined the homothallic switching behavior of the mutation mat α 1-5 which is at the Mat α locus. The following diploid strain was constructed:

Mat a/mat α 1-5 Ho/ho

This diploid was able to sporulate.

Tetrads produced from this diploid are expected to contain two a mating type spores and two mat α 1-5 spores. Those a mating type spores in which Ho segregates are expected to produce a mixture of α and a mating-type cells which will mate with each other to form nonmating, sporulating a/α diploid cells. In this sense, Mat a Ho spores are self-diploidizing. In contrast, mat α 1-5 spores carrying Ho are not expected to be self-diploidizing. These spores might be expected to produce a mating-type cells, but these cells should not be able to mate with mat α 1-5 cells, whose phenotype is sterile. Therefore, most tetrads derived from Mat a/mat α 1-5 Ho/ho should contain less than two self-diploidizing segregants. In contrast to this expectation, all tetrads showed 2:2 segregation of self-diploidization. mat α 1-5 Ho cells were able to diploidize as if the mat α 1-5 mutation had been healed!

This healing process can be observed more closely by examination of haploid cells which arise by mitotic division of the strain Ho mat α 1-5. Mating type of these cells can be determined by observing response to α-factor. In such an analysis, an Ho mat α 1-5 cell, which does not respond to α-factor, gives rise to two cells, both of which are nonresponders and cannot mate (both are mat α 1-5). One of these cells gives rise to two nonmating cells, but the other gives rise to two cells which respond to α-factor

(Mat a cells). One of these gives rise to two nonresponding cells; however, unlike the mat α 1-5 nonresponders, these cells are able to mate as wild type Mat α cells.

These α mating cells can be crossed to Mat a ho cells. α mating, ho segregants derived from this cross are indistinguishable from wild-type Mat α cells.

In addition to being able to heal mat α 1-5 mating locus mutations, the following mating locus mutations have also been healed by homothallic strains:

mat α 1-2	(Hicks and Herskowitz, 1977)
mat α 10-73 mat α 1-5 mat α 10-73 double mutant	(Strathern, personal communication)
mat α-ochre	D. Hawthorne
mat a*	(Strathern, 1977)

The ability of homothallic cells to heal mating locus mutations led to the proposal that extra copies of Mat a and Mat α are present in the genome, and that these extra copies can replace the expressed copy at the mating locus under the influence of the Ho gene. Since the HMa and HMα loci were known to be necessary for switch to occur, it was proposed that these genes are the silent, extra copies of mating locus information.

This explanation of the mechanism of homothallic mating type switch is called the cassette hypothesis (Hicks and Herskowitz, 1976b). HMa and HMα are considered to be silent

"library" cassettes of mating locus information. These copies become active upon insertion at the mating locus.

Support for the cassette model

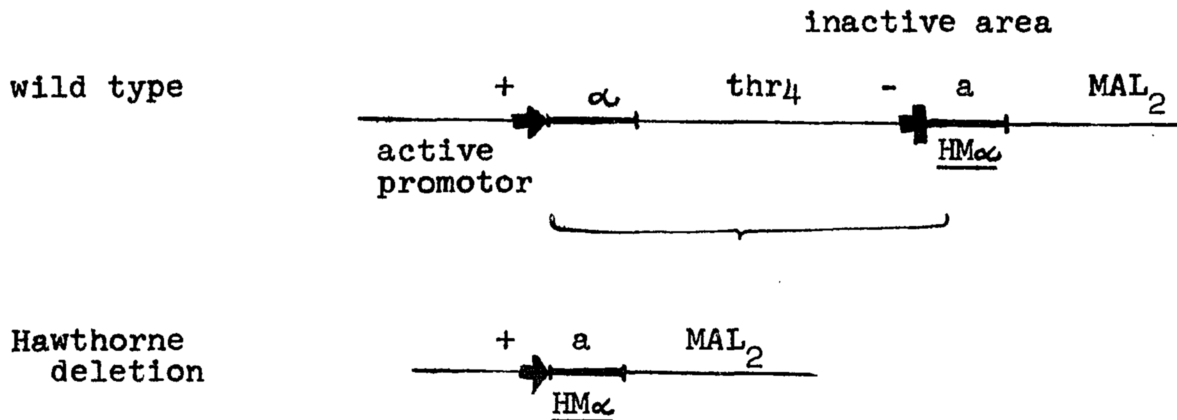
In this section a number of observations are reported which provide strong evidence in support of the cassette theory of mating type interconversion.

(a) Reevaluation of a linked lethal mutations derived from rare matings between α strains.

The Hawthorne deletion mutation described previously was originally interpreted to support the notion of a compound mating locus containing both a and α structural genes at the mating locus, each of which was proposed to be expressed only under certain conditions mediated by Ho. This idea does not explain the homothallic healing phenomenon. The Hawthorne deletion, surmised to include the mating locus and extend to within six recombinational units of MAL_2 (Hawthorne, 1963), reaches an area close to the map location of HMα, the proposed silent copy of Mat a information.

An alternative interpretation of Hawthorne's deletion is that it includes both Mat α and all of chromosome III between a Mat α active promotor region and HMα. HMα is fused to the active mating promotor and consequently is itself the active determinant of cell mating type. This is diagrammed below (Hicks and Herskowitz, 1976b).

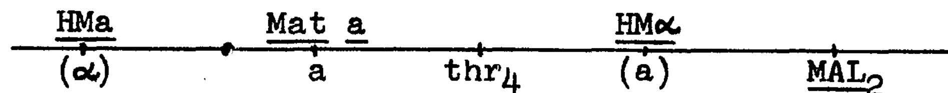
Alternate explanation of Hawthorne's deletion:

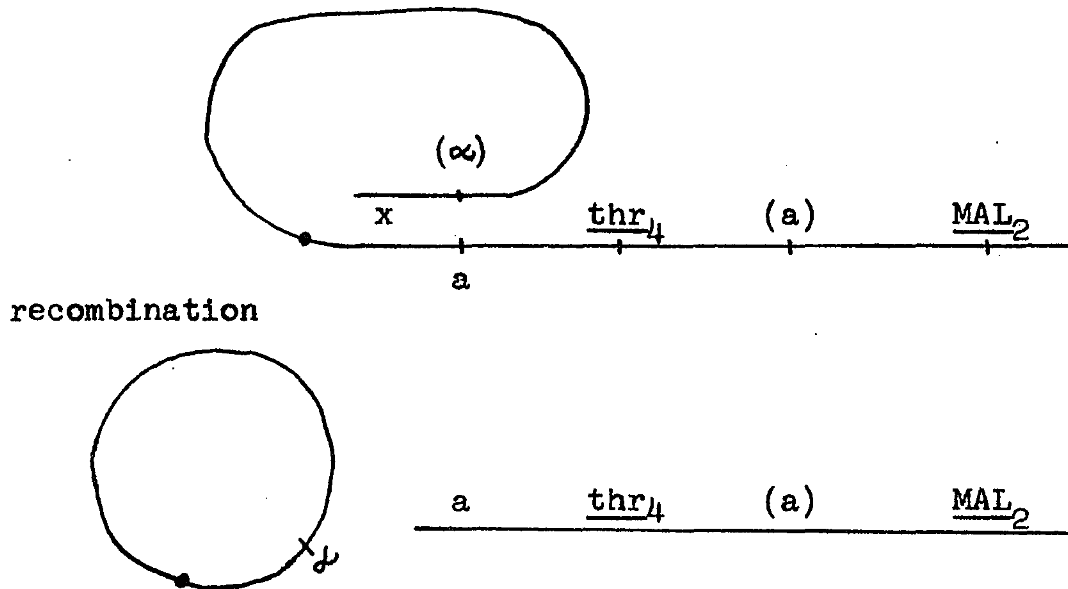


Strathern (1977) crossed a lethal cells derived from Mat α HMα cells, with Mat α hmα cells and looked for Mat α HMα recombinants. That these were not found supports the cassette theory interpretation of the Hawthorne deletion.

(b) α-linked lethal mutations (Strathern et al., 1979).

Rare matings between a cells with complementing genetic markers produce certain diploids which are phenotypically a/α and which sporulate to produce asci containing 2 a spores and 2 α-lethal spores. These α-lethal spores may be rescued by mating with a cells. Several lines of evidence support the notion that the a to α-lethal switch of mating type is the result of the fusion of HMa (silent α) to the active area on chromosome III at which Mat a had been located. The result is the formation of a circular chromosome as shown below.





The following observations support the cassette theory explanation of the a → α-lethal switch of mating type.

(a) Physical characterization of the DNA of α-lethal carrying diploid strains reveals a supercoiled, covalently closed circular portion of DNA which is not found in wild type yeast strains. This circular DNA hybridizes specifically with plasmids containing segments of chromosome III including the his_4 and leu_2 genes. DNA fragments not from chromosome III do not hybridize to the circular DNA.

(b) Formation of the α-lethal mutation results in loss of the thr_4 and MAL_2 genes on the right arm of chromosome III. Although the left arm markers his_4 and leu_2 are not lost, map distance between these markers is altered, as would be expected of a circularized chromosome segment.

(c) HMa cannot be recovered from α-lethal mutations. α-lethals derived from Mat α HMα strains were rescued by mating with Mat a Ho HM_ hma strains. Of 141 segregants

analyzed, none had the ability to switch mating type. This was interpreted to show that HMa is inseparable from the α-lethal mutation.

(3) HMa and HMα dependent mutations that cause a sterile phenotype.

The sir 2, 3 and 4 mutations (Rine and Herskowitz, 1979), the mar 1-1 mutation (Klar et al., 1979) and the cmt mutation (Haber and George, 1979) cause sterility in strains of ho HMa HMα genotype. These mutations do not cause sterility in strains of the genotypes Mat α ho HMa hmα or Mat a hma HMα. In order for sterility to be expressed, a strain must contain both Mat a and Mat α information (at the mating locus and at one of the "silent cassette" sites). The sterile phenotype of strains carrying the sir, mar or cmt mutations can be interpreted to be the result of expression of mating type information from the HMa and HMα loci. Simultaneous expression of Mat a and Mat α information from these sites results in the sterile phenotype of an a/α diploid. This interpretation is supported by the following observations:

(a) sir 2, in HMa containing strains, suppresses the mat α 1-5 sterile mutation at the Mat α locus (Rine and Herkowitz, 1979). In HMα containing strains, the cmt gene suppresses the sporulation deficiency of the mat a* mutation at Mat a (Haber and George, 1979). These suppression events can be explained as the result of expression of wild type mating locus information from the

HMa or HMα loci in sir 2 or cmt strains.

(b) Strains of the genotype Mat α/Mat α sir 1/sir 1 HMα/HMα are able to sporulate. This appears to be the result of Mat a expression (necessary for sporulation) from the HMα loci.

The healing of mating locus mutations by homothallic switch, and the discovery of HMa and HMα dependent nonmating mutations provide strong support for the cassette model but do not prove that HMa and HMα are silent mating locus cassettes. An alternate possibility is that HMa and HMα are necessary for some aspect of cassette activation but are not themselves the cassettes. This possibility has been eliminated by experiments using strains which were selected to contain amber and ocher mutations at the HMa and HMα cassettes. When these strains undergo homothallic switch of mating type, the amber or ocher mutation becomes localized at the mating locus (Klar, Hicks, Strathern, Broach, 1979).

e) Physical Properties of the Mating Locus

Recently, the Mat α locus has been integrated into a recombinant plasmid and physical characterization has been accomplished (Nasmyth and Tatchell, 1980; Strathern et al., 1980). Mat α DNA has the following properties.

1) Plasmids containing Mat α hybridize with Mat a, HMRa*, HMLα as well as Mat α containing yeast DNA restriction fragments.

*HMRa = HMα, HMLα = HMa. R and L stand for right and left cassettes respectively.

2) Mat α and HM_L differ from Mat a and HMRa in that the former contain a 900 bp α specific region and the latter a 700 bp a specific region. In all of the above fragments, the mating type specific regions are flanked by a 230 bp region to the left and a 700 bp region to the right. These flanking regions are the same in the four forms of mating locus information mentioned.

3) An additional 700 bp region is found to the right of the already described DNA in Mat a, Mat α and either HM_L or HM_L . This region is absent in either the HMRa or HM_R forms of mating locus information.

Homothallic yeast employ gene transposition in the regulation of gene expression. Other examples of genetic regulation by transposable elements include controlling elements in maize and a variety of transposable elements in the genomes of bacteria and viruses. These transposable elements are discussed in the following section.

III Transposable Genetic Elements

Barbara McClintock demonstrated the presence of transposable genetic elements which are involved in the regulation of gene expression in maize. These discoveries occurred long before a molecular level view of gene activity was possible. Certain recently discovered genetic sequence transposition events in prokaryotic cells are in many ways analogous to maize gene transposition events, and these prokaryote

events, which are accessible to analysis at a molecular level suggest models to account for the eukaryote genetic rearrangements involved in maize gene regulation.

a) Controlling Elements in Maize

The work of Barbara McClintock and others on the genetics of common maize (Zea mays) has resulted in the description of two types of genetic elements which roughly correspond to what are now called structural and regulatory genes. The regulatory genes were called controlling elements.

Through elegant genetic analysis, McClintock was able to demonstrate many aspects of gene regulation by controlling elements. Alterations in gene expression by controlling elements (in some cases tightly linked to regulated genes; in some cases acting from a distance) explained the changes in expression of certain genes called mutable or unstable loci, that is genes which are subject to unusual mutation-like changes in expression. An interesting observation about certain controlling elements is that under certain circumstances, these elements may be observed to change their genetic map positions. Controlling elements, however, in many fundamental ways, behave exactly like more traditional genes, that is, these elements, under definable conditions, show map stabilityand normal Mendelian patterns of inheritance.

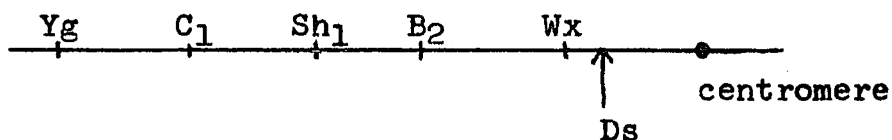
It is suspected (McClintock, 1956) that the integrated

activities of controlling elements upon "genes" and upon other controlling elements results in the coordination of complex genetically mediated developmental processes in *Zea mays*.

In maize, the expression of mutable genes resulting in variegated kernel pigmentation, is influenced by controlling elements which are able to modify the expression of these mutable loci. Two examples of patterns of mutable locus activity are presented here: the activator-dissociator and the suppressor-mutator systems.

1) Activator-dissociator

Barbara McClintock (1965, 1967), using genetic and cytological techniques, discovered that in early stages of kernel development, certain cells showed a pattern of frequent chromosome breaks at a specific locus proximal of *Wx* on the short arm of chromosome 9.



chromosome 9

The locus of frequent breaks was called *Ds* (dissociator) It was discovered that an additional genetic element, unlinked to *Ds* was required for *Ds* expression. This element was called *Ac* (activator). *Ds* cannot be detected, i.e. has no phenotype in the absence of *Ac*.

A pollen grain derived from a *Wx* linked *Ds* parent was shown to carry a new modification of the C_1 (anthocyanin in

aleurone) locus which had become modified to c_1^{m-1} (no anthocyanin in aleurone). Ac containing cells derived from this pollen grain by fertilization, showed no chromosome breaks near the Wx locus, but instead showed frequent breaks specifically at the c_1^{m-1} locus. This was interpreted to indicate a transposition of the Ds element to a new position on chromosome 9.

The transposition of Ds to the C_1 locus results in "mutation" of that locus to c_1^{m-1} only in cells which contain Ac. In this respect, the Ds element can be viewed as analogous to a prokaryote operator which, when acted upon by Ac, causes repression of C_1 gene expression. It is not known why the presence of Ds is associated with chromosome breaks.

The Ac-Ds system can cause variations in phenotype by the partial or complete inactivation of genes in which Ds has become integrated. Another system of control of gene expression in maize is Suppressor-Mutator (Spm).

2) Suppressor-Mutator

The following example demonstrates properties of the a_1^{m-1} variant of the A_1 gene for anthocyanin pigment biosynthesis. Expression of this gene is influenced by the suppressor-mutator (Spm) system (McClintock, 1967, 1965).

Corn kernels homozygous for the a_1^{m-1} gene are light red in color, a function of the activity of the a_1^{m-1} variant of the A_1 locus. Homozygous A_1 genotype kernels are a darker red color. In contrast, kernels of the same

genotype but which also carry an unlinked Spm segregating element are white with spots of dark red pigmentation. The Spm element displays two types of modification of the a_1^{m-1} locus. In the white portions of kernels, pigment synthesis is suppressed. In the dark red spots, pigment production is produced at higher degree than in a_1^{m-1} kernels which do not contain Spm. This latter activity is called mutator function. A model explaining the influence of the Spm locus on the a_1^{m-1} locus is as follows:

a_1^{m-1} represents a mutational condition of the A_1 locus which, in this condition, contains an integrated genetic element. This integrated element has two properties. First, it causes lower levels of gene expression in that a_1^{m-1}, which contains this element, produces less pigment (kernels are lighter in color) than A_1 which is the same gene without the integrated element. Kernels not containing Spm, but homozygous for a_1^{m-1} are uniformly light red, whereas kernels not containing Spm but homozygous for A_1 are uniformly dark red.

The second property of the integrated element which alters A_1 to form a_1^{m-1} is the responsiveness of this element to an Spm genetic element. Spm containing kernels homozygous for a_1^{m-1} show two distinct areas of pigmentation. Most of the kernel is white while small areas are dark red. In the white areas, a_1^{m-1} expression is "turned off" by the suppressor component of the suppressor mutator element. In the dark areas, a_1^{m-1} has undergone Spm directed mutation

which allows the a_1^{m-1} to be expressed at the level of the A_1 gene.

In summary, two genetic elements are involved in the Spm system described here. One element is located at the modifiable gene. This is a receptor element which, in the absence of Spm, decreases activity at the A_1 locus to the a_1^{m-1} lower level. The second element, Spm, may act at a distance from the receptor element by suppressing a_1^{m-1} gene activity or by mutating a_1^{m-1} to higher levels of expression. In the example described, Spm acts on a receptor element at a distance, however, in other cases, the Spm element is located at the locus of the gene whose activity it modifies.

Spm also modifies the expression of certain genes which can undergo changes of state, i.e. types of changes in frequency of schedule of or intensity of expression. For example, kernels of corn homozygous for a_1^{m-1} in a certain state, and in the presence of Spm, are white with dark red spots as described previously. Kernels homozygous for another state of the same a_1^{m-1} locus and also carrying Spm contain large diffuse regions of lighter red over a smaller white area. Kernels heterozygous for both of these states show superimposed pigmentation patterns.

The state of a gene is stable in the absence of Spm. In the presence of Spm genes may change state at low frequency.

The Spm locus itself may undergo changes in its own

activity. For example, mutational activity or suppressor-mutator activity can be lost. Suppressor is never lost without loss of mutator function. These losses of activity may last for varying periods of time.

A model of Spm activity based upon known DNA rearrangements in prokaryotes is presented later in this section.

b) Transposable Elements in Prokaryotes

Genetic and physical studies of prokaryotic DNA have demonstrated the presence of genetic elements which, like the postulated control elements in maize, can transpose within the genome and modify gene expression by positioning at specific loci. These prokaryote transposable elements include insertion sequences, drug resistance transpositions and the temperate bacteriophage Mu (Starlinger, 1977).

Prokaryote transposable elements were first discovered as mutations which, by systematic genetic and physical analysis, proved to result from DNA transpositions. For example, Starlinger (1977) observed mutations in the E. coli gal operon which displayed a strong polar influence. These mutations reverted at rates of 10^{-5} to 10^{-8} and therefore could not be deletions. Reversion rate was not enhanced by applications of mutagens, an observation distinguishing the gal polar mutations from point mutations. It therefore seemed likely that these mutations must either be inversions, duplications or translocations.

In order to determine if the gal polar mutations were missing DNA (were deletions) or contained extra DNA (duplications, translocations), λ gal transducing phages were constructed which carried the gal operon mutations (Shapiro, 1969, Jordan et al., 1968). DNA from λ gal phages carrying gal mutations showed increased buoyant density as compared to λ gal phage DNA carrying the wild type gal operon. The gal mutants contained added DNA. Michaelis et al. (1969) hybridized λ gal mutant RNA to wild type λ gal DNA and was able to show that the gal mutants were not duplications.

The conclusion of the above analysis is that the polar gal mutations result from DNA translocations.

Additional mutations having similar qualities to the ones described above have been identified in E. coli and bacteriophage λ. Comparison of these mutations by heteroduplex analysis has shown homology between certain of the translocated or inserted sequences. Most of these inserted sequences were found to belong to two classes: IS 1 and IS 2. Two other classes, IS3 and IS 4 were found less frequently (Fiandt et al., 1972; Hirsh et al., 1972a, b; Malamy et al., 1967, 1970, 1972; Brachet et al., 1970).

Insertion sequences (IS) are small, between from 800 to 1,500 base pairs, and express no phenotype of their own. Transposons are similar to IS elements but carry drug resistance markers which allow them to be easily detected by genetic methods. Phage Mu is a temperate bacteriophage

of E. coli which can become integrated at a wide range of loci. All of these transposable elements can insert in bacterial chromosomal or plasmid DNA or temperate phage DNA.

Polar gene inactivation

Insertion of a transposable element in an operon results in polar inactivation of genes downstream from the locus of insertion. This polar effect appears to result from rho-dependent transcriptional termination in that IS 1 causes in vivo transcriptional termination when integrated in the gal operon of wild type E. coli cells but not when integrated in the gal operon of rho-deficient mutant E. coli cells (Malamy et al., 1972; Das et al., 1976; Besemer and Herpers, 1977). IS 1 has been sequenced and shows no known rho factor associated termination sequences, however it does contain nonsense codons at all three reading frames in either orientation of insertion (Ohtsubo and Ohtsubo, 1978). Adhya et al. (1974) postulate that the rho-dependent termination requires that the termination signal be preceded by a stretch of untranslated mRNA. IS 1 nonsense sequences could provide this untranslated RNA which would precede a termination signal within the gal operon.

In contrast to IS 1, IS 2 does contain rho-dependent termination signals at either orientation. IS 2, however, appears to cause polar gene inactivation only if integrated in a specific orientation (orientation I) (Saedler et al., 1974).

Mechanism of transposition

The mechanism by which prokaryotic transposable elements assume new genetic loci is not yet known, however certain observations have been made.

IS 1, IS 2, IS 3, IS 4 and all Tn elements are flanked by sequences which deviate from inverted repeats only by small insertions and substitutions. Phage Mu, however, does not have flanking inverted repeats. These areas may be recognition sites for symmetrically operating enzymes (Shapiro, 1979).

It is not yet clear whether transposition includes excision and insertion, or alternately if replication of the entire element is a necessary part of the transposition process. Although precise excision of IS, Tn and Mu sequences is observed in the reversion of certain transposable element caused mutations, certain other evidence supports the idea that replication is necessary in order for integration to occur. For example, phage Mu has been observed to replicate and transpose without leaving its original site of origin (Ljunquist and Bukhari, 1977).

In the *E. coli* genome, only a single copy of IS 4 is present, but certain Gal T inactivated derivative strains carry two copies of IS 4 (Chadwell *et al.*, 1978).

A model of the mechanism of transposition proposed by Shapiro (1979) has a requirement that transposition be accompanied by replication.

Specificity of integration

Different transposable elements appear to have different specificities of site of integration. For example, 20% of all insertions of IS 1 and IS 2 into the Gal operon are in the leader sequence which comprises only about 1% of this operon (Pfeifer *et al.*, 1977).

IS 4 is only known to integrate into a single locus of the Gal T region (Shapiro and Adhya, 1969).

Studies of Tn 5, Tn 10, and Mu integration into the *E. coli* genome show that of those cells which integrate an element, only 1-3% become auxotrophic. This is a lower number than expected for a randomly integrating element (Taylor, 1963; Berg *et al.*, 1975; Botstein and Kleckner, 1977).

Is IS 2 a flip-flop promotor?

IS 2 is of particular interest in that it appears to display promotor activity when integrated in one orientation (orientation II) and causes polar repression of downstream genes when integrated in inverted orientation (orientation I) (Saedler *et al.*, 1974). This was demonstrated by the observation that in certain cells which constitutively express *E. coli Gal* operon functions, the *Gal* structural genes have become fused to an IS 2 element in orientation II. Certain *gal*$^-$ cells derived from the constitutive producers have lost IS 2. *Gal*$^+$ revertants of these *gal*$^-$ cells were found to carry IS 2 in orientation II fused to the structural genes of the *Gal* operon. These fused

structural genes express higher levels of enzyme activity than wild type *Gal* operon structural genes. When IS 2 is integrated in orientation I, in the leader sequence of the *Gal* operon, structural gene activity is impaired.

IS 2 promotor activity is also observed in certain phage λ mutants which have become constitutive for the *int* gene and which carry IS 2 upstream of this gene (Pilacinski *et al*., 1977). In yeast, cloned *trp* genes carrying promotor up mutations have been shown to carry IS 2 (Walz *et al*., 1978).

Whereas these observations suggest IS 2 promotor activity, Boyen *et al*. (1978) have observed reduction in expression of the *Arg H* structural gene of *E. coli* associated with IS 2 integration at either orientation. A possible explanation is that internal rearrangements within IS 2 could alter promotor activity.

IS 2 is particularly interesting in that it provides an example of a possible way that IS-like elements can regulate gene expression. Nevers and Saedler (1977) propose a model to account for the activities of controlling elements in maize. This model employs an IS 2-like controlling element which can, by integrating at forward or inverted orientation, differentially influence the expression of genes.

Other examples of control of gene expression by inversion of DNA sequences

There are several examples of genetic systems in which a segment of DNA may be found in either a certain

orientation, or in the inverse of that orientation. Two examples of this phenomenon are the phage Mu G segment and Salmonella flagella antigen determination.

In phage Mu, the G segment of the phage genome, 3,000 base pairs long is able to invert. The orientation of this segment determines the infectiousness of a lysogen (Bukhari, 1976).

In Salmonella, flagella antigen type is determined by the orientation of a DNA sequence 800 base pairs in length which allows expression of certain genes in one orientation but prevents expression in inverted orientation (Zeig *et al*., 1978).

IV The dmt Gene and the ARM Gene

In this thesis experiments are described which characterize two new mutations which influence mating locus regulated functions in *Saccharomyces cerevisiae*. One new mutation, the *dmt* gene, allows *Mat α dmt* cells to mate with *Mat α* wild-type cells. This is accomplished by *dmt* gene induced loss of the mating locus of *Mat α dmt* cells allowing these cells to mate as *a* mating type cells.

Another new mutation, the *ARM* gene, allows the otherwise prohibited sporulation of cells of the genotype *Mat α/Mat α, ARM/ARM*. This anomalous sporulation is mating-type specific in that cells of genotype *Mat a/Mat a ARM/ARM* are unable to sporulate. Arguments will be presented to

support the idea that the ARM gene expresses Mat a information from a region distal to thr_4 on the right arm of chromosome III.

MATERIALS AND METHODS

Section 1

Table 1

Strains of *Saccharomyces cerevisiae*

STRAIN NUMBER	GENOTYPE	REMARKS
123.1c	Mat α ura_3 his_7	Lab stock
102.10A	Mat a lys_2	Lab stock
103.1A	Mat α his_1	Lab stock tester
103.1B	Mat a his_1	Lab stock tester
104.2A	Mat a ura_3 lys_1	Lab stock
A10700B	Mat α thr_4	G.R. Fink collection
A10701C	Mat a thr_4	G.R. Fink collection
H641	Mat α dmt ura_3 his_7	Original dmt mutant
3H.211.5D	Mat α dmt ura_3 his_7 lys_1	
3H211.4B	Mat a dmt ura_3 lys_1	
3H.215	Mat a/Mat α dmt/dmt ura_3/ura_3 his_7/+ lys_1/+	omt
3H2171	Mat a/Mat α dmt/dmt ura_3/ura_3 his_7/+ lys_1/lys_1	omt
C76.1D	Mat α $cry_1{}^s$ dmt his_7 ura_1 lys_1	
C82.2B	Mat α $cry_1{}^r$ dmt his_4 leu_2 thr_4 ura_3 lys_2	

STRAIN NUMBER	GENOTYPE	REMARKS
C82.17A	Mat α $cry_1{}^r$ dmt his_4 leu_2 thr_4 ura_3 lys_2	
C122.29A	Mat α $cry_1{}^s$ dmt ura_3 lys_1 ade_2 his_7	
SH732Ar	Mat α/Mat α dmt/+ $cry_1{}^r$/$cry_1{}^r$ his_4/+ leu_2/+ thr_4/+ ura_1/+ ura_3/+ lys_1/+ lys_2/+	Rare mating progeny of C76.1DX C63.8D
Ar137B	Mat α $cry_1{}^r$ ARM leu_2 his_4	
Ar104D	Mat α $cry_1{}^r$ ARM his_4 his_7 ura_3	
Ar105D	Mat α $cry_1{}^r$ ARM his_4 leu_2 ura_3	
Ar108A	Mat α $cry_1{}^r$ ARM his_4 leu_2 ura_3	
Ar129C	Mat α $cry_1{}^r$ ARM his_4 his_7 leu_2 thr_4 ura_3	Meiotic progeny of SH732Ar
SH732ArE7D	Mat α $cry_1{}^r$ ARM ura_1 lys_2	
SH732ArE1B	Mat α $cry_1{}^r$ ARM ura_1 ura_3 lys_1	
Ar23A	Mat α $cry_1{}^r$ ARM his_4 leu_2 ura_3 lys_2	
L88.25B	Mat α $cry_1{}^s$ ARM lys_1 lys_2 his_4	
I14.13D	Mat α $cry_1{}^r$ ARM his_4 leu_2 lys_1 ura_1	
17-15	Mat a* ade_2 ura_3 can_{1-11} leu_1 cyh_{2-21} csp	Gift from Dr. Yona Kassir
C63.8D	Mat α $cry_1{}^r$ his_4 leu_2 thr_4 ura_3 lys_2	
C76.12B	Mat α $cry_1{}^s$ ura_1 lys_1	
C70.10D	Mat α $cry_1{}^s$ ura_3 lys_2	
C60.8A	Mat α $cry_1{}^r$ his_4 leu_2 thr_4 ura_1 lys_1	
C60.15A	Mat α $cry_1{}^r$ his_4 leu_2 thr_4 ura_1 lys_2	
L63.1C	Mat α $cry_1{}^r$ his_4 ura_3 ura_1	

STRAIN NUMBER	GENOTYPE	REMARKS
L63.14B	Mat α cry_1^r his_4 ura_3 leu_2 thr_4	
L100.2C	Mat α cry_1^s lys_1 lys_2 his_4	
Ber 1	Mat α cry_1^s ura_3 his_7 lys_1	Gift from Dr. Berish Rubin
XJ9	Mat a/Mat a cry_1^r/cry_1^r his_4/his_4 leu_2/leu_2 ade_6/ade_6 lys_2/lys_2	Kindly donated by Dr. Jeff Strathern
I11.102D	Mat a cry_1^r ARM ura_1 lys_1 lys_2	
Ar31C	Mat α cry_1^r ARM ura_1 lys_1	
C128.33.2D	Mat α cry_1^r ARM ura_3 ade_2	
L29.34D	Mat α cry_1^s ARM his_4 leu_2 thr_4 lys_2 ura_3	
L72.3C	Mat a* ARM his_7 ura_3	
C27.13B	Mat a cyr_1^r his_4 leu_2 thr_4 lys_2	
C63.16A	Mat a cry_1^s his_4 ura_3 lys_2	
C29.11B	Mat α cry_1^s leu_2 thr_4 lys_2	
C128.110.3	Mat α/Mat α ARM/ARM cry_1^r/cry_1^r ura_3/ura_3 $his_4/+$ $his_7/+$ $lys_1/+$ $lys_2/+$	
L103 G1	Mat a/Mat a ARM/ARM cry_1^r/cry_1^r his_4/his_4 $leu_2/+$ $lys_1/+$ $lys_2/+$ $ura_3/+$	
6D131.10B	Mat α Ho HML(α) HMR(α) leu_1 ura_2	Kindly donated by Dr. Harry Gruenspan

Section 2: Media

NAD: Difco Nutrient Agar, 23.2 g/l; Difco yeast extract, 10g/l; dextrose, 20 g/l.

Synthetic Minimal (SM): Yeast nitrogen base without amino acids, 6.7 g/l; dextrose, 20 g/l; agar 17 g/l.

SM + X: SM media supplemented with designated supplements in the following concentrations: L histidine, 20 mg/l, L leucine 40 mg/l, D+L threonine, 300 mg/l.

KAc: Potassium acetate 9.65 g/l; dextrose 1 g/l; Difco yeast extract 2.5 g/l; agar 17 g/l (taken from MacKay and Manney, 1974).

Liquid YEPD: Dextrose 20 g/l; Bacto peptone 20g/l; yeast extract 10 g/l.

YEPD-Difco Yeast Extract, 10g/l; Difco Bacto-Peptone, 20 g/l; dextrose 20 g/l; agar 17 g/l (omitted in liquid media).

CS: Sm media supplemented with the following: adenine, 30 mg/l; arginine, 30 mg/l; histidine, 20 mg/l; leucine, 40 mg/l; lysine, 40 mg/l; methionine, 20 mg/l; threonine, 150 mg/l; tryptophan, 30 mg/l; uracil, 20 mg/l.

Dropout media: In order to determine specific nutritional requirements of strains, dropout media was employed. Dropout media is CS media missing a single nutritional supplement. For example, uracil dropout media is the equivalent of CS media with uracil omitted.

Preparation of slants for storage of strains: Media is prepared as follows: 1% Bacto-yeast extract, 2% Bacto-

peptone, 2% Dextrose, 0.003% Adenine sulfate, 2% Bacto agar, distilled water. Before autoclaving, 1.5 ml aliquots of media were placed in 1 dram vials and then autoclaved (15 pounds per square inch). Vials were then tilted while cooling to allow formation of the proper angle of slant for optimal surface area for strain growth.

Cryptopleurine Media: 750 ml of NAD media was autoclaved (250°, 15 pounds per square inch) and cooled in a temperature regulated water bath to 60°C. 7.5 ml of 300 uM cryptopleurine in 95% ethanol was then added to the NAD forming a 3.0 uM solution that was dispersed into sterile petri dishes and allowed to solidify.

Section 3: Tetrad Analysis

Preparation of Asci

Diploid cells competent to undergo sporulation were incubated on sporulation media at 30°C for 5 days and were then examined microscopically for the presence of asci. Cell populations containing asci were suspended in a preparation of snail digestive enzyme (glusulase, obtained from Endo Laboratories, Garden City, N.Y., diluted 1 part in 20 parts sterile distilled water) for from 2-15 min (different strains required different digestion times). A drop of this preparation was transferred, using a sterile inoculation loop, onto a prepared slab of dissection agar.

Dissection Agar

1% yeast extract, 2% peptone, 2% dextrose, 2.5% Bacto

agar.

Preparation of Dissection Agar

Dissection agar was prepared and stored in 11 ml aliquots in test tubes. Before use, agar was melted in a water bath at 100°C for 5 min and poured while still warm into plastic petri dishes and allowed to harden and dry at room temperature for approximately 24 h. A slab of this agar, 18 mm x 45 mm, was removed using a sterile knife and transferred to a glass slide, 35 mm x 50 mm, which had been previously sterilized by dipping in 95% ethanol and flaming. Onto this slab of agar, using a sterile inoculation loop, the asci-glusulase preparation was transferred. The slide containing the inoculated agar slab was placed face down on a dissection chamber.

Preparation of Needle

A glass microneedle was prepared by stretching a 2 mm glass rod over a small flame, forming a right angle and drawing to a thickness of from 10 u to 100 u. The needle was cut with a razor blade. This technique is described in <u>Methods in Cell Biology</u>, Volume XI, page 196.

Micromanipulation Tetrad Analysis

A micromanipulator produced by Lawrence Precision Machines was employed for microneedle movement. The dissection chamber was placed on a two axis stage produced by the same manufacturer.

Section 4: Interpretation of Cryptopleurine Plates

Master plates were replica-plated onto cryptopleurine containing plates, which were then incubated at 30°C for 24 h before a first scoring was made. After this initial scoring the cryptopleurine plates were again incubated for another 48 h and rescored.

On the first observation, confluent growth of a patch of cells was scored as resistance (R) and no growth was scored as sensitivity (S) to the drug cryptopleurine. Certain patches on these plates,however, showed localized clones which grew into colonies despite the absence of growth throughout the greater area of the patch. These patches were scored as papillated (P). After 48 h a second observation was carried out. Plates were scored as they had been in the first observation. The following cryptopleurine resistance phenotypes were observed in homozygous diploids heterozygous for cryptopleurine resistance:

First observation	Second observation
R	R
P	P
S	P
S	S

Procedural note on interpretation of cryptopleurine phenotypes

Interpretation of the events taking place at the mating locus is based to some extent on the observed behavior of the cryptopleurine resistance locus. To under-

stand the somewhat ambiguous nature of the expression of this gene we constructed several normal a/α diploids heterozygous for the cryptopleurine locus (cry^s/cry^r). Fifty single colonies of each of these diploids were transferred to NAD master plates and, after 24 h, replica-plated onto cryptopleurine containing plates. In all cases after 24-48 h the papillated phenotype for cryptopleurine resistance was observed.

We interpreted this phenotype to be the result of the presence of homozygous resistant mitotic recombinant subclones within the majority heterozygous cell population of the clone examined.

In our phenotypic analysis of homozygous α diploids many of the prototophic diploids examined displayed a similar papillated phenotype which we attributed to the heterozygous condition of these strains with respect to cryptopleurine resistance.

Certain of the auxotrophic diploids isolated in this study have been shown to display low viability on enriched growth media. No explanation of this phenomenon has yet been obtained; however, as a result of this low viability, growth on cryptopleurine plates is also affected. Once again, a similar papillated phenotype for cryptopleurine resistance is obtained after 24-48 h. However, in contrast to that explained by mitotic recombination, this phenotype is considered to be true resistance in these diploids. The non-confluency of growth (papillated growth) of these cells

on cryptopleurine media is accounted for by the poor viability that these strains suffer in association with their auxotrophy. This hypothesis was tested by isolating several such strains and allowing them to grow over a greater period of time than allotted for the general phenotypic assays used here. These strains, thus treated, displayed the true cryptopleurine resistant phenotype once the problem of initial low viability was overcome.

Determination of Cryptopleurine Genotype of Diploid Strains

Diploid strains to be tested were placed on master plates of enriched media in approximatly ¼ inch by ¼ inch square patches using sterile toothpicks. After 24 h growth these strains were replica-plated onto cryptopleurine containing media and incubated for 48 h at 22°C. Haploid strains showed either confluent growth or no growth indicating the presence of either the $\underline{cry_1}^{r}$ or the $\underline{cry_1}^{s}$ allele respectively. Homozygous diploid strains similarly showed either confluent growth or no growth.

In contrast, diploid strains heterozygous for the $\underline{cry_1}$ alleles ($\underline{cry_1}^{s}/\underline{cry_1}^{r}$) formed drug resistant sub-populations which, on cryptopleurine containing media, formed papillations of growing cells within a sensitive, non-growing background. The major portion of heterozygous diploid cells displayed the sensitive phenotype of the dominant $\underline{cry_1}^{s}$ allele, however, mitotic recombinant and gene convertant homozygous resistant subpopulations were able to grow on cryptopleurine containing media.

Section 5: Mating Assay

Vertical streaks of the strains under investigation were made using toothpicks on YEPD plates and on separate plates tester strains, Mat a and Mat α, were streaked horizonatally. After one day's growth the tester strains and the experimental strains were replica-plated together on the surface of a fresh YEPD plate and incubated for 24 h at 30°C. After mating this plate was then re-replica-plated onto SM or SM + X so that only diploids would be able to grow. Confluent growth at any intersection was taken as being a strong positive result and the experimental strain assigned the appropriate mating type. Strains showing no growth were designated as sterile. Confluent growth at both intersections was designated as omni-mating type and confluent growth with the Mat a tester, and reduced growth (1-10%) with the Mat α tester was designated Mat a dmt (nominal Mat α, dual mating type).

Section 6: Sex Factor Assay

The production of α factor was detected by streaking the experimental strain on CS media and allowing it to grow for 24 h; after this time a dilute suspension of tester Mat a cells was laid down adjacent to the experimental strain and the morphology of the Mat a cells followed microscopically. The test was considered positive for α factor when a significant number of the Mat a cells took on the characteristic "schmoo" shape as described by MacKay

and Manney (1974a).

Section 7: Assay for Sporulation

Strains to be tested were placed on KAc sporulation media (see above) and incubated at 30°C for 5 days before being checked microscopically for the presence of asci. Ability to sporulate was scored by degrees as follows: preparations in which greater than 90% of the cells observed had formed asci were scored as ++++; 80-90% asci, +++; 40-80% asci, ++; 3-40% +. Less than 3% ascus formation was scored as sporulation negative.

Section 8: Mutagenesis

Mutagenesis using ethylmethane sulphonate is described in Chapter I, section 1 of the Results.

Section 9: Relative Ultraviolet Induced Reversion Rates of Threonine Auxotrophic Strains

10^8 cells of each threonine auxotrophic strain to be tested were plated onto petri dishes containing solid drop-out media lacking threonine. These preparations were exposed to ultraviolet radiation from a General Electric G15T8 15 watt germicidal lamp at a distance of about 18" from lamp to platform for varying lengths of time ranging from 10 sec to 35 sec. Control plates received no radiation. Following this treatment, plates were incubated at 30°C for 48 h, after which time prototrophic colonies were observed to grow.

Plates containing different strains but which had been irradiated for equivalent time intervals were compared in order to achieve a rough comparison of relative reversion rates between strains.

Section 10: Rare Mating Procedure

Each haploid Mat $\underline{\alpha}$ strain to be mated was inoculated into 5 ml of sterile liquid YEPD media and allowed to grow at 30°C for 24 h. Serial dilutions of each culture were then made in test tubes of sterile distilled water. Those pairs of haploid strains which were to be mated were plated together in 0.1 ml aliquots on solid NAD mating plates at dilutions of 10^{-1}, 10^{-2} and 10^{-3} respectively. The mating plates were incubated at 30°C for 30 h and then replica-plated onto selective media, SM + histidine + leucine + threonine (SM was supplemented with histidine, leucine and threonine to assure that loss of chromosome III information concomitant with rare mating did not result in diploid auxotrophy (Melnick and Blamire, 1978)). These plates were incubated at 30°C for 3 to 5 days during which time prototrophic diploid colonies appeared. Since mating had taken place on solid media, each colony resulted from an independent rare mating event.

All colonies growing on selective media were transferred, using sterile toothpicks, to fresh selective media master plates and allowed to grow for a minimum of 24 h at 30°C.

Section 11: Determination of Relative Frequences of Rare Matings between Mat α Cells

Each parental strain to be tested was inoculated into 5 ml of liquid YEPD media in a test tube and incubated overnight at 30°C. Cell density was determined using a Klett meter. Each parental strain was added to a test tube such that the concentration of each strain was 1 x 10^6 cells per ml in 5 ml of sterile liquid YEPD. Mating mixtures were precipitated by centrifugation and incubated at 30°C for 6 h to allow mating without allowing cell division of newly formed diploid cells. After incubation, mating mixtures were resuspended by stirring and were plated in 0.1 ml aliquots onto petri dishes containing solid selective media on which only complementing diploid cells were able to grow. These preparations were incubated for 48 h at 30°C after which time diploid cells appeared and were counted in order to determine relative mating frequencies.

Section 12: Construction of Homozygous Mat a ARM and Mat α ARM Diploid Colonies (see Figure 1)

a) Rare Matings

Since it is possible to determine the ARM phenotype only in Mat α cells, it was necessary to begin all constructions with Mat α ARM parents. Rare matings were performed as described in this section between parents which were Mat α $cry_1{}^r$ ARM a B c and Mat α $cry_1{}^s$ ARM a b C where a is an auxotrophic genetic marker common to both parents and B, b

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Figure 1. Construction of strains homozygous for Mat and ARM.

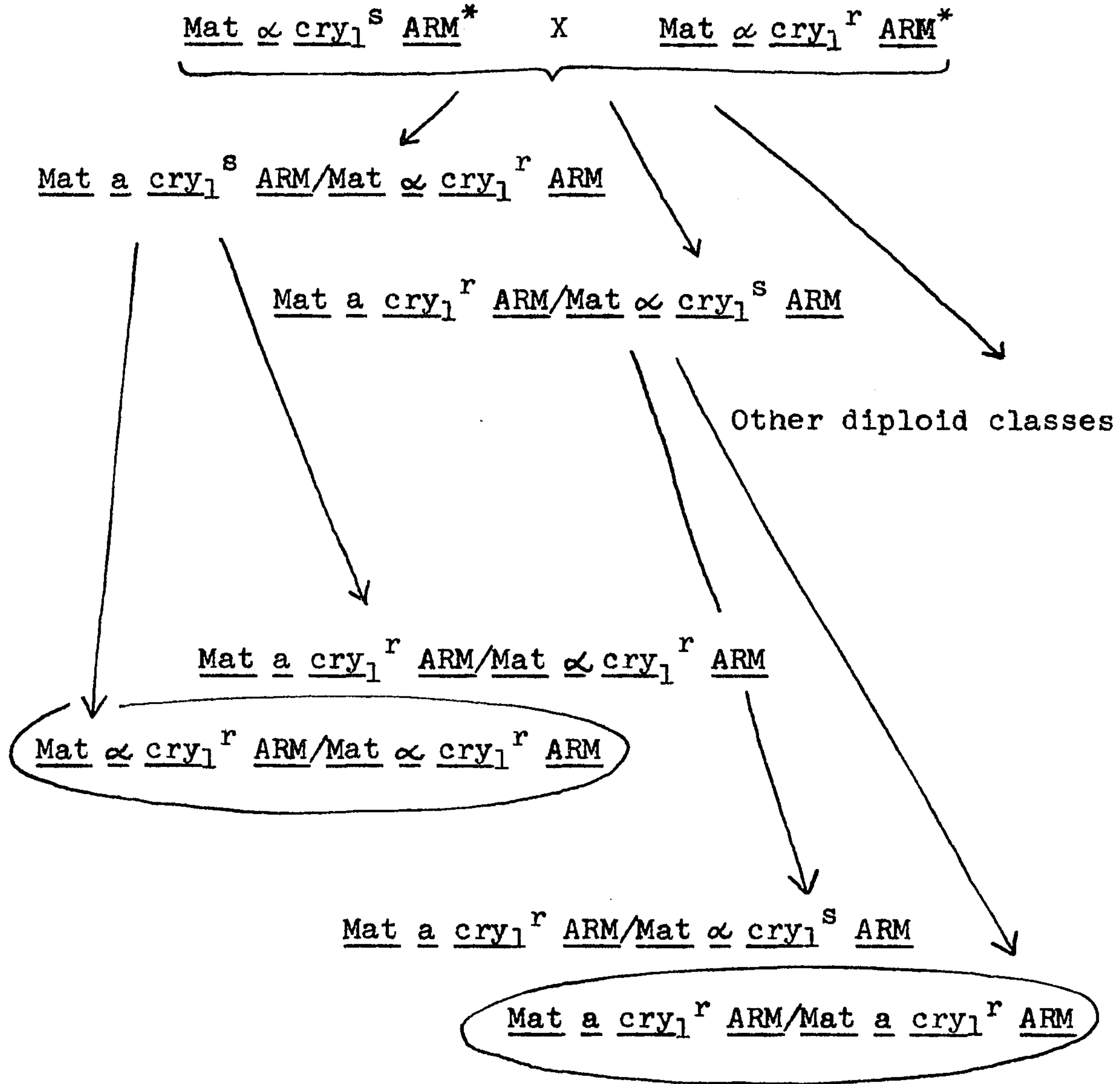
* Mat α ARM strains were used because their ARM genotype was easily confirmed.

** Selection for cry_1^r/cry_1^r mitotic recombinants and gene convertants was by growth and selection on cryptopleurine containing media.

The circled strains were homozygous for Mat and ARM. Mat α ARM/Mat α ARM was able to sporulate, demonstrating that the ARM gene was not lost in the construction process. Mat a ARM/Mat a ARM did not sporulate. To confirm the genotype of the Mat a ARM homozygotes, tetraploids were formed by mating with Mat α ARM diploid homozygotes. Tetraploid analysis showed the expected segregation of the ARM gene.

Figure MM1

Construction of strains homozygous for Mat and ARM



Circled strains are homozygous for Mat and for the ARM gene.

and C, c represent auxotrophic markers which will allow for the selection of diploids by complementation.

Mating mixtures were incubated on solid enriched media for 24 h and then replica-plated onto solid SM + a' media where a' supplied the nutritional need created by the a genetic marker. After 48 h diploid colonies appeared and were transferred onto fresh SM + a' media using sterile toothpicks.

b) Diploid analysis

Mating type, sporulation ability and cryptopleurine phenotype were determined as described in Materials and Methods, sections 4 and 5. Those colonies which displayed the a/α diploid phenotype, i.e., were non-mating, able to sporulate, cry_1 heterozygotes, were selected for further analysis.

c) Selection of cry_1^r Mat homozygotes

Diploid strains heterozygous for cry_1 (i.e. cry_1^s/cry_1^r) produced mitotic recombinant and gene convertant substances which were cry_1^r homozygotes and could be selected for on cryptopleurine containing media. The mitotic recombinant cryptopleurine resistant cells became homozygous for the mating locus which was tightly linked to this cry_1^r allele. Conversely, cryptopleurine resistant subclones which arose by gene conversion were cry_1^r homozygotes, but mating type heterozygotes (i.e., non-mating cry_1^r/cry_1^r a/α diploids).

Colonies able to mate were considered to be homozygous

for the mating locus. Selected sporulating mating colonies were examined by tetrad analysis to confirm this genotype. Selected non-sporulating colonies were mated to diploid strains homozygous for the opposite mating type and tetraploid analysis was performed to confirm their genotype.

Construction of homozygous Mat a ARM and Mat α ARM diploid colonies is diagrammed in Figure 1.

Section 13: Determination of the identity of the Mat α parental cell which underwent heterothallic mating type switch during the formation of rare mating a/α diploid colonies from Mat α x Mat α- matings

This process takes advantage of the tight linkage between the mating locus and the cry_1 alleles.

a) Rare matings

Rare matings were performed as described in section 12a between parents which were Mat α cry_1^s a B c and Mat α cry_1^r a b C where a represented an auxotrophic genetic marker common to both parents and B, b and C, c represented auxotrophic genetic markers which would allow for selection of diploids by complementation. Mating mixtures were incubated on solid enriched media for 24 h and then replica-plated onto selective media, SM + a' where a' supplied the nutritional need created by the auxotrophy of the a gene. After 48 h colonies appeared on selective media and these were transferred using sterile toothpicks to fresh solid SM + a' media in patches $\frac{1}{4}$ inch by $\frac{1}{2}$ inch.

b) Diploid analysis

a/α phenotype diploid colonies were selected for further analysis as described in Materials and Methods, section 12b).

c) Selection of cry_1^r Mat homozygotes

See Materials and Methods, section 12c.

Determination of the mating type of mitotic recombinant cryptopleurine resistant, mating subclones indicated the mating locus allele which was tightly linked to the cry_1^r allele. This identified which parent had switched mating type prior to diploid formation.

RESULTS

CHAPTER I

Discovery and phenotypic characterization of the dmt gene

Introduction

In wild type yeast, mating takes place only between cells of opposite mating types. Matings between pairs of Mat α cells or pairs of Mat a cells are prohibited.

In this chapter, a mutant gene is described, whose phenotype is to allow otherwise prohibited matings between Mat α mating type cells. This gene, termed dmt for dual mating type is not linked to the mating locus and has no observed effect upon the mating of Mat a or Mat α cells with cells of the opposite mating type. Mat α strains carrying the dmt gene can mate illegitimately with strains of the same mating type, however, Mat a cells containing the dmt gene show no unusual phenotypic qualities.

Section 1: Isolation and phenotypic description of the dmt gene

A stationary culture of a parental strain, X123.1C (Mat α ura_3 his_7) was mutagenized with ethylmethane sulphonate (EMS) in 0.1 M phosphate buffer pH 7.0 at a concentration of 40 ug/ml. Potential mutants were selected after 80 min incubation at 30°C by plating on rich media (YEPD). Each potential mutant colony was streaked in a vertical line and crossed to Mat a his_1 and Mat α his_1

testing strains as described in Materials and Methods (Section 5). In such experiments, the haploid strains tested normally exhibited strong diploidization only with tester strains of opposite mating types. Control streaks of Mat a/Mat α diploid strains and known sterile strains did not mate with either tester strain.

Mutant H641 on day one, following transfer of the mating crosses to SM, showed the normal confluent growth of prototrophic diploids at the intersection of the Mat a tester, and on day two showed a reduced but still significant mating with the Mat α tester. This phenotype, strong mating with Mat a testers (for convenience in later discussion this is abbreviated to: hmwa: high frequency mating with Mat a) and weaker or lower frequency mating with Mat α testers (lmwα) has been termed dual mating type (dmt) and therefore strains carrying this gene are written: H641 Mat a dmt ura_3 his_7 (and phenotypically: lmwα hmwa).

The original mutant colony was taken and single cells isolated. Each cell was in turn allowed to grow into a distinct colony and then tested for the presence or absence of the dmt gene. All such experiments gave positive results, indicating that the mutant strain was homogeneous and not a mixture of Mat a and Mat α haploids.

Various types of control experiments have been carried out and the results of these indicate that the phenotypic expression of the dmt gene in the mating reaction is independent of the other auxotrophic markers in either the

mutant or Mat a and Mat α tester strains; is independent of temperature (i.e. 24, 30 or 36°C), and is independent as to whether the strain carrying the dmt gene is grande or petite.

Section 2: Analysis of Diploids

Diploids produced by crosses of H641 Mat α dmt to both Mat a and Mat α testers have been examined.

a) Mat α dmt x Mat α + Diploids. Diploids produced from H641 x Mat α have been isolated and single diploids purified repeatedly on selective media. Isolates were then tested in several ways. Each colony was transferred to KAc media for up to three weeks and monitored for ascus formation. Each colony was tested for α factor production and crossed with either Mat a or Mat α haploid tester strains. The results showed that all the diploids would produce α factor in large amounts (as compared with the parental and other Mat α haploids), would not sporulate and would mate further with Mat a haploids but not with Mat α haploids. It appeared, therefore, that these strains were Mat α/Mat α diploids and not Mat a/Mat a diploids. Repeated testing of these and other diploids eventually located one such diploid which would sporulate. This strain was taken and tetrad analysis carried out. Each ascus contained four spores, however only two spores per ascus were viable and were capable of growing into colonies. These were analyzed and found to be all of the Mat α mating type with a random assortment of input auxotrophic markers including the dmt

gene. This sporulating diploid has the same phenotype as diploids formed by an event called the Hawthorne deletion (Hawthorne, 1963; Strathern _et al_., 1979). Further descriptions of this type of diploid are provided in the Introduction and Chapter IV of this thesis.

b) _Mat a_/_Mat_ _α_ Diploids. Diploids produced by crossing H641 with _Mat a_ testers appeared perfectly normal. They neither produced nor responded to α factor and would not mate further with either _Mat a_ or _Mat α_ haploid testers. On KAc media they sporulated at high frequency and tetrad analysis has now been carried out on a wide variety of such crosses. The results can be summarized as follows.

X3H.201 (H641 X _Mat a_ _lys_$_2$). Sporulation of diploids produced in this cross gave asci in which all four spores were viable. In each case two _Mat a_ and two _Mat α_ spores were produced; however the phenotype produced by the _dmt_ gene segregated with 2 _Mat a_ 2 _Mat α_ _dmt_; 2 _Mat a_ 1 _Mat α_ 1 _Mat α_ _dmt_ or 2 _Mat a_ 2 _Mat α_. These results suggested that the _dmt_ gene was segregating independently of the mating type locus, in a 2 : 2 manner, but could only be phenotypically expressed in a _Mat α_ cell. This was confirmed when back crosses of _Mat a_ spores carrying a "hidden" _dmt_ gene were carried out with normal _Mat α_ tester strains. Sporulation of these back crosses once again produced _Mat α_ _dmt_ haploids. These _Mat a_ strains with an unexpressed _dmt_ gene are now referred to as _Mat a_(_dmt_). In all the analyses

carried out so far no unequivocal Mat a haploid spores have been produced in which a phenotype equivalent to Mat α dmt but with a Mat a mating locus have been observed (i.e. hmw α lmw a).

Section 3: Relative mating frequencies of diploid formation between wild type and dmt gene containing strains of α mating type

Relative mating frequencies were determined as described in Materials and Methods.

The mating combinations tested were the following (these same crosses are further analyzed in Chapter II, Tables RII and RIII).

Cross A738

Strain C70.10D Mat α + cry_1^s + + + ura_3 lys_2

Strain C60.8A Mat α + cry_1^r his_4 leu_2 thr_4 ura_1 lys_1

Cross RH735

Strain C82.2B Mat α dmt cry_1^r his_4 leu_2 thr_4 ura_3 lys_2

Strain C76.12B Mat α + cry_1^s + + + ura_1 lys_1

Cross SH732

Strain C76.1D Mat α dmt cry_1^s + + + ura_1 lys_1 his_7

Strain C63.8D Mat α + cry_1^r his_4 leu_2 thr_4 ura_3 lys_2

Cross 0731

Strain C76.1D Mat α dmt cry_1^s + + + ura_1 lys_1 his_7

Strain C82.17A Mat α dmt cry_1^r his_4 leu_2 thr_4 ura_3 lys_2

Relative mating frequencies are shown in Table RI.

These results confirm the effect of the dmt gene of greatly enhancing the frequency at which Mat α strains mate with other strains of the same mating type.

Conclusions

1) Strains of the genotype Mat α dmt are able to mate illegitimately with other strains of α mating type. The frequency at which dmt gene containing α mating type strains mate with other α mating type strains is considerably higher than the frequency of Mat α X Mat α mating without the dmt gene.

2) Strains of genotype Mat a dmt show no indication of unusual mating ability. The dmt gene has no observed phenotype in a mating type cells.

3) Strains of either a or α mating type, containing the dmt gene, are able to mate normally with strains of opposite mating type to produce sporulating diploids. Mat α dmt strains produce α factor and Mat a dmt strains are able to respond to α factor.

4) The dmt gene segregates as a Mendelian gene unlinked to the mating locus.

Table R1

Relative Mating Frequencies of Wild-type and dmt Strains

Cross	Parental dmt and Mat genotype	Observed mating frequency	Relative mating frequency
A738	Mat α + Mat α +	5.0×10^{-7}	1
RH735	Mat α dmt Mat α +	7.0×10^{-3}	1.4×10^{4}
5H732	Mat α dmt Mat α +	1.0×10^{-2}	2.0×10^{4}
0731	Mat α dmt Mat α dmt	1.6×10^{-2}	3.2×10^{4}

CHAPTER II

The Effects of the dmt Gene on Chromosome III

Introduction

The dmt gene greatly increases the frequency at which haploid Mat α cells mate with cells of the same mating type. Examination of the diploid progeny of these matings shows a pattern of genetic information loss from chromosome III, the chromosome which contains the mating locus. This pattern of information loss as well as other properties of diploids formed by dmt gene facilitated Mat α x Mat α matings, suggests a mechanism by which the dmt gene allows these illegitimate mating events to occur.

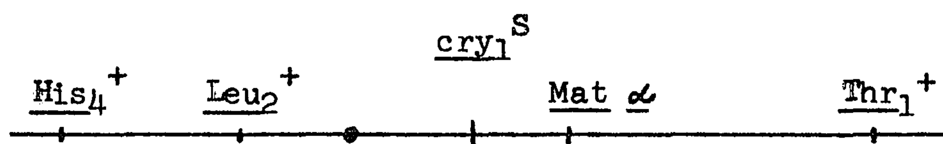
In this chapter, strains are described which were constructed to contain several chromosome III genetic markers. Using these strains it was possible to observe patterns of chromosome III information loss associated with dmt gene influenced rare matings. Section 1 of this chapter examines this pattern of information loss. Section 2 examines the mating and sporulation properties of diploids produced by dmt gene influenced rare matings.

Section 1: The dmt gene and chromosome III genetic marker loss

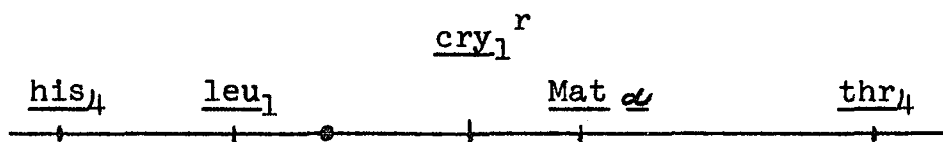
The majority of diploid progeny of dmt rare matings showed loss of expression of certain mating locus linked, chromosome III genetic markers. This marker loss is

specific to the chromosome III of the parent which carried the dmt gene.

Table RII shows the results of five different rare mating crosses. Each cross involved the same chromosome III parental combinations with respect to genetic markers, but different placements of the dmt gene (not on chromosome III) among the parents. In each cross, one of the parents contained a chromosome III marked as follows:



This chromosome III was termed nutritionally positive (nut +). The other parent of each cross contained a nutritionally negative (nut -) chromosome III.



In different crosses the dmt gene was introduced with the nut^+ or nut^- chromosome III as shown in Table RII.

Diploids formed by mating parents containing nut^+ and nut^- chromosome III's should be heterozygous for chromosome III genetic markers. The heterozygous condition for chromosome III, nut^+/nut^- should express His^+, Leu^+, and Thr^+ prototrophy. The heterozygous cry_1^s/cry_1^r phenotype can be distinguished from the homozygous sensitive phenotype on a drug media assay by the presence in the heterozygote of mitotic recombinant cryptopleurine resistant subpopulations of a sensitive population (see Materials and Methods,

Table RII

Chromosome III marker loss in diploids formed by α rare matings

Cross	Parental Combinations (w.t. = wild type, i.e. not dmt)	I no chromosome III marker loss	II nut⁻ chr III marker loss (cry_1^s)	III nut⁺ chr III marker loss (see columns IV, V & VI)	IV** cry_1^r	V** thr_4^-	VI** his_4^- leu_2^- cry_1^r thr_4^-
A738	α w.t. nut^+ α w.t. nut^-	55*	57*	38*	1*	18*	19*
SH732	α dmt nut^+ α w.t. nut^-	4	0	146	2	76	68
SH734	α dmt nut^+ α w.t. nut^-	5	0	90	0	48	42
RH735	α w.t. nut^+ α dmt nut^-	2	81	1	1	0	0
0731	α dmt nut^+ α dmt nut^-	0	17	74	1	21	52

* There is no dmt gene in this cross. The progeny are produced at much lower frequency than those progeny of rare matings whichinvolve the dmt gene.

** Degree of nut^+ marker loss among diploids is shown in column III. Columns IV, V and VI are subsets of Column III.

Table RII continued

CROSS A738

nut$^+$ strain C70.10D Mat α + $cry_1{}^s$ + + + ura_3 lys_2

nut$^-$ strain C60.8A Mat α + $cry_1{}^r$ his_4 leu_2 thr_4
ura_1 lys_1

CROSS SH732

nut$^+$ strain C76.10 Mat α dmt $cry_1{}^s$ + + + ura_1 lys_1 his_7

nut$^-$ strain C63.8D Mat α + $cry_1{}^r$ his_4 leu_2 thr_4
ura_3 lys_2

CROSS SH734

nut$^+$ strain C76.1D Mat α dmt $cry_1{}^s$ + + + ura_1 lys_1 his_7

nut$^-$ strain C63.18A Mat α + $cry_1{}^r$ his_4 leu_2 thr_4
ura_3 lys_2

CROSS RH735

nut$^+$ strain C76.12B Mat α $cry_1{}^s$ + + + ura_1 lys_1

nut$^-$ strain C82.2B Mat α dmt $cry_1{}^r$ his_4 leu_2 thr_4
ura_3 lys_2

CROSS 0731

nut$^+$ strain C76.1D Mat α dmt $cry_1{}^s$ + + + ura_1 lys_1 his_7

nut$^-$ strain C82.17A Mat α dmt $cry_1{}^r$ his_4 leu_2 thr_4
ura_3 lys_2

Section 4).

In the diploid, expression of any of the nut⁻ chromosome genes $\underline{his_4}^-$ $\underline{leu_2}^-$ or $\underline{thr_4}^-$ indicates loss of expression of their nut$^+$ chromosome homologous alleles. Cryptopleurine complete sensitivity indicates loss of the resistant allele and complete resistance indicates loss of the sensitive allele (see Materials and Methods, Section 4). Each diploid represented in Table RII represents an independent rare mating event (see Materials and Methods, Section 10).

Diploids produced by the crosses SH732, SH734 and RH735 were derived from Mat α dmt x Mat α + parents. Of 329 diploid cells assayed from these crosses, 318 indicated loss of some chromosome III information. The loss of information corresponds to the chromosome III which was derived from the dmt containing parent. In crosses SH732 and SH734, the dmt parent contained the nut$^+$ chromosome III. The diploid progeny of this cross showed loss of nut$^+$ chromosome III information. Alternatively, in cross RH735, the dmt gene containing parent carried the nut$^-$ chromosome III. The progeny of this cross showed loss of nut$^-$ chromosome III information.

In cross 0731 where both parents carried the dmt gene dmt diploids showed indication of marker loss from both the nut$^+$ and nut$^-$ chromosome III's. It should be noted that no single diploid colony showed loss of information from both chromosome III's, but rather certain colonies showed nut$^-$

loss and others nut$^+$ loss.

In cross A738, neither parent contained the dmt gene. Mating frequency was considerably lower than in the dmt crosses. Certain of the diploids produced by this cross showed information loss similar to that in dmt crosses. This suggested that the dmt gene increased the frequency of a process which could also occur, but at much lower frequency, in wild type cells. For this reason, it was decided to term the event whereby Mat α dmt cells mated with Mat α cells a dmt facilitated rare mating event.

Section 2: Mating type and sporulation characteristics of diploid colonies derived from dmt gene induced rare mating events

Table RIII shows the sporulation ability of diploid colonies produced by the dmt gene facilitated rare matings SH732 and RH735. Only a small percentage of these diploids show sporulation ability. In the crosses examined in Tables RII and RIII it was not possible to determine mating ability of colonies derived from rare mating events. Cross C117 was arranged to allow determination of diploid mating types.

Table RIV indicates the mating type, chromosome III marker loss and sporulation ability of diploid colonies derived from the dmt rare mating C117, Mat α dmt nut$^+$ x Mat α + nut$^-$.

Of 312 diploid colonies examined, 302 mated as "α" and of these, 268 showed indication of nut$^+$ chromosome III

Table RIII

Sporulation of SH732 and RH735 diploids

CROSS SH 732: 120 colonies assayed, 5 showed sporulation ability.

Of these 5, 3 showed no marker loss and 2 showed loss of only the $\underline{thr_4}^+$ genetic marker of the nut$^+$ chromosome III (see Table RII).

CROSS RH735: 96 colonies were assayed, none were able to sporulate.

marker loss. Only four of 302 *α* mating diploids displayed the ability to sporulate.

Of the ten nonmating diploid colonies produced by cross C117, nine were able to sporulate. Eight of the ten nonmating colonies showed chromosome III marker loss of the thr_4 locus. These are thought to be Hawthorne mutations (Hawthorne, 1963; Strathern *et al.*, 1979), the product of a mating event allowed by a deletion associated switch of mating type by one of the parent strains. The two nonmating, sporulating diploid colonies, which showed no loss of chromosome III information are thought to have resulted from spontaneous switch of mating type by one of the haploid parents. Both Hawthorne deletions and nondeletion associated mating type switches have been observed by a number of investigators (Hicks and Herskowitz, 1977; Rabin, 1970; Strathern, *et al.*, 1979) and are not considered to be associated with the activity of the *dmt* gene.

The majority of those colonies of cross SH732 which showed the unusual ability to sporulate, showed no loss of genetic information. Therefore, the class of SH732 diploids which showed no information loss was examined more closely. This was done by selecting diploids on SM media in place of SM + histidine + leucine + threonine media (see Materials and Methods). In this way, diploid colonies were specifically selected which showed no loss of genetic information on chromosome III. Of 72 colonies which had no loss of genetic information (i.e. heterozygous for all chromosome

Table RIV

Mating and sporulation characteristics of diploid strains derived from the dmt gene facilitated rare mating, C117

CROSS C117

nut^+ strain C76.1D Mat α cry_1^s dmt his_7 ura_1 lys_1

nut^- strain C63.16A Mat α cry_1^r his_4 leu_2 thr_4 ura_1 lys_2

Diploid colonies were selected on SM + leu + thr + ura solid media.

		Total	Sporulating	Nonsporulating
nonmating colonies	no chrIII marker loss	2	2	0
	nut^+ chrIII* marker loss	8	7	1
α mating colonies	no chrIII marker loss	34	2	32
	nut^+ chrIII** marker loss	268	2	266
		312		

* These colonies had lost the thr_4^+ allele and are of the phenotype of Hawthorne deletions (see text).

** These colonies had lost thr_4^+ information. Cryptopleurine phenotype was not assayed in C117 diploids.

III genetic markers), 16 were able to sporulate.

In a similar manner, C117 colonies were selected on SM + uracil media instead of SM + leucine + threonine + uracil (Table RIV) in order to eliminate from selection those diploid colonies which had undergone loss of chromosome III information. The results of this analysis are shown in Table V.

These results are consistent with the idea that most of the sporulating colonies produced by dmt facilitated rare matings are a/α diploids. The rare α mating, sporulating colonies have not yet been explained. One such colony, SH732Ar is examined in detail in Chapter III.

Discussion

Proposed mechanism of action of the dmt gene in facilitating rare matings between Mat α cells

The observations of dmt diploids presented in this chapter suggest the following model to explain dmt induced rare matings.

Step 1: The dmt gene causes loss of chromosome III information, including the mating locus, in certain cells in a Mat α dmt haploid population. This event is likely to be lethal.

Step 2: Loss of Mat α information is proposed to allow a cell to mate as an a mating type cell. This lethal cell can be "rescued" by mating with a neighboring cell of α mating type.

Table RV

C117 diploid colonies selected on minimal media plus uracil

	Total	Sporulating	Nonsporulating
$\underline{\alpha}$ mating colonies	80	1	79
nonmating colonies	15	12	3
Total	95	13	82

It is proposed that loss of Mat α information allows a cell to mate as an a mating type cell; however, this cell should not contribute Mat a functions to the diploid it forms. Diploids formed from dmt rare matings are observed not to express Mat a diploid functions. These cells mate as α, produce α factor (Chapter I) and do not sporulate.

Diploids produced by dmt gene rare matings are expected to contain only one mating locus allele, Mat α. Since these diploids do not sporulate, this expectation had been difficult to confirm.

In studying the rare class of diploids produced by dmt gene facilitated matings which are able to mate as "α" and also sporulate, a new gene was discovered which could be used to allow sporulation of dmt diploids. This gene, termed ARM (active regulator of meiosis) is described in the following chapter. The sporulation of dmt diploids using the ARM gene is described in Chapter IV.

Conclusions

Diploid colonies formed by mating Mat α dmt strains with Mat α wild type strains have the following properties:

1) Diploid colonies belong to two general categories: (i) nonmating and sporulating colonies and (ii) α mating, nonsporulating colonies.

2) Nonmating diploid colonies are thought to result from heterothallic switch of mating type and are not related to the activity of the dmt gene. Certain of these colonies

may display loss of information of the chromosome III of the parent that has switched mating type (Hawthorne deletions). Other nonmating diploid colonies display no information loss.

3) The *α* mating, nonsporulating colonies vary depending upon which parent contained the *dmt* gene. The majority of these colonies are missing genetic information present on the chromosome III of the *dmt* gene containing parent.

4) A model is presented to explain *dmt* gene facilitated rare matings. Predictions of this model are tested in Chapter IV.

CHAPTER III

The ARM gene - a mating type specific sporulation gene

In wild type yeast, the ability to undergo meiosis and sporulation is regulated by information at the mating locus alleles, Mat a and Mat α, both of which are required by a cell in order to allow sporulation (Lindegren and Lindegren, 1943; Roman, 1953). Wild type diploid cells homozygous for either of the mating locus alleles are prohibited from sporulating but can be removed from this prohibition by the recessive genes csp (control of sporulation) (Hopper and Hall, 1975; Hopper, Kirsch and Hall, 1975), or rme (regulator of meiosis) (Kassir and Simchen, 1976). These genes appear to bypass mating locus regulation of sporulation in that their expression is not influenced by cell mating type. In this Chapter, a gene termed ARM (active regulator of meiosis) is described which allows otherwise prohibited sporulation to occur in cells of specific mating type. The dominant ARM gene allows sporulation in homozygous Mat α diploids but not in homozygous Mat a diploids.

Expression of the ARM phenotype was first observed in an α/α diploid colony, SH732Ar which was formed by a rare mating event between Mat α dmt (Blamire and Melnick, 1975; Chapter I of this thesis), and a Mat α wild type cell. Rare matings of this kind often result in the formation of α/α diploid cells (Melnick and Blamire, 1978; Chapter II of thesis); however, only in rare cases are these cells able

to sporulate. The unusual sporulation ability of SH732Ar caused it to be the focus of further attention resulting in the discovery of the ARM gene segregating among the meiotic progeny of this strain.

Another sporulation regulation mutant, termed mat a* (Kassir and Simchen, 1976) has been of value in assaying for the expression of the ARM gene. mat a* is a sporulation deficient mutation of the Mat a locus. mat a* cells mate as a cells to produce mat a*/Mat α diploids which cannot sporulate. In contrast, mat a*/Mat α diploids containing the ARM gene are able to undergo sporulation.

In this Chapter certain properties of the ARM gene and the diploid colony in which it was first observed are described.

Section 1: Detection of the ARM gene in a diploid colony formed by a dmt gene facilitated rare mating event between Mat α strains

Matings between Mat α strains occur at a low frequency. This frequency can be considerably enhanced when one or both of the mating partner strains carries the dmt gene (Blamire and Melnick, 1975; Melnick and Blamire, 1978; Chapters I and II). Matings between Mat α dmt and Mat α wild-type parents produce a variety of different diploid cell types, each the product of an independent rare mating event.

A rare mating cross was carried out between the following parents:

Cross SH732

C76.1D Mat α dmt cry_1^S his_7 ura_1 lys_1

C63.8D Mat α + cry_1^r his_4 leu_2 thr_4 ura_3 lys_2

Several hundred diploid colonies produced by this cross, each the product of an independent rare mating event, were examined. The colony SH732Ar was selected for further analysis because it expressed a cry_1^r/cry_1^r homozygous phenotype instead of the expected heterozygous cry_1^r/cry_1^S phenotype, and because it was able to undergo sporulation. Tetrad analysis was therefore performed on SH732Ar and the results are shown in Table RVI.

The auxotrophic genetic markers, ura_1, ura_3, lys_1, lys_2, and leu_2 show the expected 2 : 2 segregation. Three genetic markers, cry_1^r, Mat α and thr_4, all on chromosome III, show unusual segregation. Both cry_1^r and Mat α display 4 : 0 segregation. As far as could be determined, none of these Mat α cells displayed any unusual mating or sporulation characteristics when mated with a strains and all Mat α cells examined segregated normally in a variety of crosses. The thr_4 genetic marker, however, showed 3+ : 1- segregation in about half of the tetrads examined. The remaining tetrads showed 2+ : 2- segregation of thr_4 alleles.

The diploid SH732Ar, therefore, has three unusual features. (1) It has become homozygous for cry_1^r although its parents contain both the cry_1^r and cry_1^S alleles. (2) It displays unusual segregation of the thr_4 locus. (3) It

Table RVI

Segregation of genetic markers among the meiotic progeny of SH732Ar

Parents of SH732Ar (doubly underlined markers are on chromosome III)

C76.1D Mat α dmt cry_1^s his_7 ura_1 + lys_1 + + + +

C63.8D Mat α + cry_1^r + + ura_3 + lys_2 his_4 leu_2 thr_4

Genetic Marker	Tetrads Analyzed	Ratios observed 2+:2-	3+:1-	1+:3-	Other
Mat α	113				113 (4Mat α:0Mat a)
cry_1^r	113	0	0	0	113 ($4cry_1^r$:$0cry_1^s$)
thr_4	113	54	55	1	3 (4+:0-)
leu_2	113	108	3	2	0
lys_1	34	29	2	2	1 (0+:4-)
lys_2	34	32	1	1	0
ura_1	34	32	1	1	0
ura_3	34	32	1	0	1 (0+:4-)

is able to sporulate although it does not have the characteristic *a*/*α* configuration of the mating locus which allows sporulation in wild type cells.

Homozygosis of the $\underline{cry_1}^r$ locus is not unusual in rare matings between *α* strains. The implication of this event in rare mating mechanisms is discussed in Chapter IV.

The unusual $\underline{thr_4}$ segregation in SH732Ar and in several other crosses which involve the progeny of SH732Ar (Chapter V) is not yet understood. Two possibilities will be considered in Chapter V and in the Discussion section of this thesis. These are (i) that unusual $\underline{thr_4}$ segregation is the result of a phenotypic property of the same gene that allows sporulation in SH732Ar, and (ii) unusual $\underline{thr_4}$ segregation is the result of a genetic or chromosomal rearrangement artifactual to the rare mating event which resulted in the origin of the diploid strain SH732Ar.

The unusual ability of SH732Ar to undergo sporulation has been determined to be one of the phenotypic properties of a gene we have termed *ARM* (active regulation of meiosis) and which is the focus of the remainder of this chapter.

Section 2: Phenotypic properties of the *ARM* gene

a) Sporulation of the *α*/*α* diploid SH732Ar

The *ARM* gene allows the diploid SH732Ar to sporulate despite the absence of *Mat a* information in this diploid. In so doing, the *ARM* gene replaces a missing *Mat a*

contribution in the regulation of sporulation. In order to determine whether the ARM gene could replace Mat a deficiency in other types of diploids, meiotic segregants from SH732Ar were mated to strains carrying the mat a* mutation of the Mat a locus. mat a* is recessive to either wild type mating locus allele and, important to this experiment, does not support sporulation in mat a*/Mat α diploid cells (Kassir and Simchen, 1976).

b) Sporulation of mat a*/Mat α ARM/+ diploid cells

Meiotic segregants of SH732Ar were mated with the strain 17-15 (Kassir and Simchen, 1976) which carries the mat a* mutation. The diploids formed were incubated on sporulation media and assayed for presence of asci. Certain of the meiotic segregants of SH732Ar supported sporulation in diploids formed with 17-15, indicating that the ARM gene can replace the Mat a deficiency of these diploid strains. The fact that the sporulating Mat α/mat a* ARM/+ diploids are heterozygous for the ARM gene indicates the dominant nature of this gene.

mat a* strains can be used to ascertain the ability of Mat α ARM strains to overcome the Mat a deficiency of mat a*/Mat α diploids. This provides an assay for determining the presence of the ARM gene in Mat α cells. Using this assay, the pattern of segregation of the ARM gene among the meiotic products of SH732Ar was examined.

Table RVII

Pattern of ARM gene segregation among the meiotic products of SH732Ar

Ability to sporulate in diploids formed by crossing with mat a*	Number of tetrads
4+ : 0-	9
3+ : 1-	41
2+ : 2-	42
1+ : 3-	3
0+ : 4-	0
Total	100

c) Pattern of segregation of the ARM gene among SH732Ar meiotic progeny

The pattern of ARM gene segregation among SH732Ar meiotic progeny (Table RVII) is indicative of neither the homozygous nor the heterozygous condition of this gene in the parent SH732Ar.

There is a number of possible explanations for the pattern of ARM gene segregation shown in Table RVII. For example, an additional gene or genes may be interfering with the expression of the ARM phenotype. Alternatively, more than one sporulation gene may be segregating among the meiotic products of SH732Ar. Certain observations, however, support the explanation that the ARM gene is unstable. For example, certain SH732Ar meiotic segregants have been observed to lose their ability to express the ARM phenotype after the passage of time. Also, in crosses between parents, both of which carry the ARM gene, certain meiotic segregants do not express the ARM phenotype.

The homozygous condition of an unstable ARM gene in the diploid SH732Ar would account for these observations and for the segregation of ARM phenotype shown in Table RVII.

d) Varying degrees of expression of the ARM gene and interference with ARM gene expression

The ARM gene shows nonuniform patterns of segregation among tetrads of SH732Ar. In addition, the ARM gene is non-uniform in the degree of expression among the meiotic progeny of SH732Ar. This difference has been scored as percentage of diploid cells which have formed asci after

incubation on KAc media for five days (see Materials and Methods) and is shown in Table RVIII . It was noticed that the expression of the ARM gene could also be influenced by certain properties of strains containing this gene. For example, strains containing the thr_4 auxotrophic genetic marker on chromosome III expressed the ARM gene less strongly than did strains that were wild type for thr_4 (Table RVIII).

This interference with the expression or stability of the ARM gene appears to be linked to or at the thr_4 genetic marker in that not a single thr_4^- segregant of SH732Ar expresses the highest degree (90-100%) of sporulation displayed by 50 of 242 assayed thr^+ segregants.

e) Expression of the ARM gene in diploid strains homozygous for mating type

Table RIX shows the sporulation of Mat α ARM and Mat a ARM homozygous diploid strains. a/a and α/α diploid strains were constructed as described in Materials and Methods. In the rare mating crosses L101, L102, L103, L105 and L106, the parental combinations were Mat α cry_1^r ARM x Mat α cry_1^s ARM. Each of these combinations produced colonies which were a/α diploids and cry_1^s/cry_1^r heterozygotes. From these colonies cry_1^r/cry_1^r homozygous mitotic recombinant colonies were selected on cryptopleurine containing media. Many of these resistant colonies had also become homozygous for the mating locus which is tightly linked to the cry_1 allele. By this procedure each of the

Figure R1

Sporulation of THR^{+} ARM vs thr^{-} ARM segregants of the parent SH732 Ar

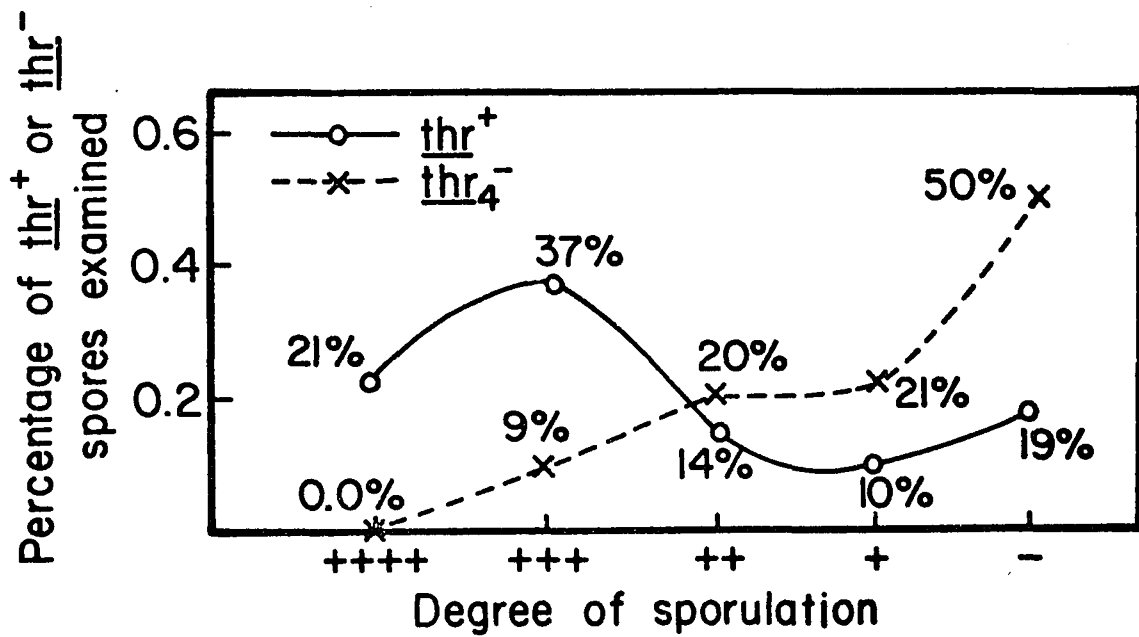


Table RVIII

Variation in expression of the ARM phenotype and influence of the thr4 genetic marker on the expression of the ARM gene

	Total spores	Percentage of diploid a*/α ARM/+ cells which sporulate				
		90-100	80-90	40-80	5-40	5
		++++	+++	++	+	-
	388	50	103	62	54	119
thr^{+} cells	242	50	90	33	23	46
thr_4^{-} cells	146	0	13	29	31	73

crosses L101, L102, L103, L105 and L106 gave rise to diploid cells homozygous for either Mat a or Mat α. To confirm that the phenotypes of the constructed strains were homozygous for the mating locus, sample colonies of those diploids which could sporulate were examined by tetrad analysis for 4 : 0 mating locus segregation. Samples of nonsporulating diploids were mated to diploid strains homozygous for the opposite mating type to form tetraploids. These tetraploids were examined by tetraploid analysis (Table RX).

All mating locus homozygous diploid strains were transferred to sporulation media, incubated, and assayed for ability to form asci. Of 104 α mating diploid colonies examined (Table RIX), 91 showed sporulation ability. This confirms the result seen in SH732Ar that the ARM gene allows sporulation of α/α diploids, i.e. replaces a deficiency of Mat a sporulation information in these diploids. However, of the 82 a/a diploid colonies examined, none were able to undergo sporulation despite the fact that they should have been homozygous for the ARM gene. This implies that the ARM gene cannot replace missing Mat α information needed to complement Mat a information in the regulation of sporulation.

The segregation of mating ability among the meiotic products of the tetraploids shown in Table RX is the expected segregation of the meiotic products of tetraploids of the genotype a/a/α/α.

Table RIX

Sporulation of Mat α ARM and Mat a ARM homozygous diploid strains

	Cross	Total colonies observed	α/α diploid colonies sporulating	α/α diploid colonies nonsporulating	a/a diploid colonies sporulating	a/a diploid colonies nonsporulating
L101	L88.25B Ar137B	43	12	0	0	31
L102	L88.25B Ar104D	50	43	0	0	7
L103	L88.25B Ar105D	46	34	3	0	9
L105	L88.25B Ar108A	40	7	0	0	33
L106	L88.25B Ar129C	20	8	10	0	2

Table RX

Analysis of tetraploids

a mating diploid strains (described in Section 2e) were mated to the α/α diploid strain C128.110.3. The meiotic products of these tetraploids were examined with respect to mating ability as is shown below.

strain C128.110.3   Mat α/Mat α

strain L103.G1   a mating diploid produced from cross L103 (section 2e)

L106.I1   a mating diploid produced from cross L106 (section 2e)

L101.I1   a mating diploid produced from cross L101 (section 2e)

L105.B1   a mating diploid produced from cross L105 (section 2e)

	4 nm	1a',1α',2nm	2a' : 2α'	other
L128.110.3 x L103.G1	5	3	3	
L128.110.3 x L106.I1		2	1	
C128.110.3 x L101.I1	3	2	1	
C128.110.3 x L105.B1	2	3	2	

Table RXI

Mating and sporulation characteristics of diploids formed from rare matings between Mat α ARM and Mat α + strains

Cross L109

strain L85.25B Mat α cry_1^s ARM lys_1 lys_2 his_4

strain L63.14B Mat α cry_1^r his_4 leu_2 thr_4 ura_3

Diploid phenotype	Total	Sporulating	Nonsporulating
nonmating no chromosome III marker loss	61	61	0
nonmating loss of thr_4^+ allele	16	16	0
α mating no information loss	16	12	4
α mating loss of chromosome III information			
nut^+ loss	3	3	0
nut^- loss	23	2	21

Cross L107

strain L85.25B Mat α cry_1^s ARM lys_1 lys_2 his_4

strain L63.1C Mat α cry_1^r his_4 ura_3 ura_1

Diploid phenotype		Sporulating	Nonsporulating
nonmating	70	70	0
α mating	25	9	16

f) Influence of the ARM gene on rare matings between Mat α cells.

(i) The ARM gene increases the rate at which heterothallic Mat α cells switch mating type from α to a

The rare mating cross Mat α ARM x Mat α + produces diploid colonies, a high proportion of which display the nonmating, sporulating phenotype of a/α diploids (Table RXI). This suggests that the ARM gene increases the frequency of switch of mating type in Mat α ARM cells allowing these switched cells to mate with neighbors in forming a/α diploids. To test this supposition, matings were performed between several Mat α ARM strains and several Mat α wild type strains. Observations of the mating locus linked cry_1^r, cry_1^s alleles allowed determination of the mating partner, which had switched mating type allowing diploid formation (see Materials and Methods, Section 13). The results of this determination, shown in Table RXII, indicate that, with rare exception, in a/α diploids derived from Mat α ARM x Mat α + parents, the Mat a locus is linked to the cry_1 allele of the ARM gene containing parent. This indicates that the ARM gene increases the rate of heterothallic switch in Mat α ARM cells.

(ii) Sporulation of α mating diploids produced by Mat α ARM x Mat α +

Table RXI shows that a high proportion of the α mating diploids produced by crosses L109 and L107 are able to sporulate. This is in contrast to those α mating diploid cells produced by dmt rare matings, very few of

Table RXII

Identity of parent which switches mating type in α x α rare matings

Rare mating Cross	ARM strain		Wild type strain		a/α diploid colonies	
					α ARM parent has switched mating type	wild type parent has switched mating type
C135	Ar23A	α cry^r ARM	Ber 1	α cry^s +	83	1
C140.13D	I14.13D	α cry^r ARM	Ber 1	α cry^s +	46	0
L107	L85.25B	α cry^s ARM	L63.1C	α cry^r +	38	0
L109	L85.25B	α cry^s ARM	L63.14B	α cry^r +	54	0
IAE	SH732E7d	α cry^r ARM	C70.10D	α cry^s +	114	1
IIAE	SH732E1B	α cry^r ARM	L100.2C	α cry^s +	30	0
C128*	Ar23A	α cry^r ARM	C122.29A	α cry^s + dmt	18	0

* Cross C128 involved the dmt gene contained in parent C122.29A. The diploid colonies produced by this cross are analyzed in detail in Chapter IV.

which can sporulate (Tables RIII, RIV, RV).

The ARM gene is located distal of thr_4 on the right arm of chromosome III (Chapter V). The nonsporulating α mating progeny of L109 and L107 are thought to have lost the ARM gene in the rare mating process in a manner analogous to the loss of thr_4 information, also on the right arm of chromosome III, which is lost in many of the rare mating diploids shown in cross A738 in Table RII.

Discussion

It appears that the ARM gene replaces Mat a information in the regulation of sporulation. It can be considered either to be a gene involved directly in the Mat a mediated control of sporulation, or alternatively, to be a form of incompletely expressed Mat a information residing at a place other than the mating locus.

As a specific regulation of sporulation gene, ARM could be a constitutive mutant allele of a gene (arm) which is normally under positive regulation by the Mat a locus. The ARM gene, or the induced arm gene, in this model, would produce a product which in conjunction with a Mat α produced or induced product would positively regulate other genes whose products conduct the processes of meiosis and sporulation.

As a form of Mat a information, the ARM gene has phenotypic similarity with certain other known genes that can substitute for missing mating locus information in the

regulation of sporulation. In certain ways the action of the ARM gene is similar to the action of certain genes which appear to allow expression of information at the usually silent mating locus cassette loci HMa (Mat α) and HMα (Mat a) (Hicks *et al.*, 1977). For example, diploid cells that are homozygous for Mat a or Mat α and also homozygous for the sir_1 (silent information regulator, Rine *et al.*, 1979) allele, can sporulate. Similarly, mat a*/Mat α, sir_1 homozygous diploid cells also sporulate. This sir_1 expression is dependent upon the presence of the HMa and HMα mating locus cassettes which appear to be derepressed in the absence of the Sir^+ product. These cells express both Mat a and Mat α information and are therefore able to sporulate. Other genes similar to sir_1, for example cmt (Haber and George, 1979; Hopper and Hall, 1975b), sir_2, sir_4 and sir_5 (Rine *et al.*, 1979) allow more complete expression of cassette mating type information. Haploid cells carrying these genes display the nonmating phenotype of a/α diploids. In contrast, in sir_1 haploid cells, mating activity is determined by the allele at the mating locus.

The ARM gene, like the sir_1 gene, allows sporulation of α/α and a*/α diploids and does not interfere with mating activity. In contrast to sir_1, however, the ARM gene is dominant and does not allow sporulation in a/a diploid cells. The sir_1 gene acts at a distance to allow derepression of both HMa and HMα alleles. It is tempting to speculate that the ARM gene is a form of HMα which is derepressed in a way

similar to $\underline{sir}_1$ influenced cassettes. The effect of the ARM gene of increasing the rate at which heterothallic Mat α ARM cells can switch mating type could be an additional outcome of a regulatory mutation of HMα which could confer ARM phenotype.

Conclusion

A dominant gene termed ARM (active regulator of meiosis) is described which allows sporulation of α/α but not a/a diploid cells. This gene was first observed in a sporulating α $\underline{cry}_1{}^r$/α $\underline{cry}_1{}^r$ diploid strain (SH732Ar) which was derived from a rare mating event between strain C76.10, Mat α $\underline{cry}_1{}^S$ dmt and strain C63.8D, Mat α $\underline{cry}_1{}^r$ +. The diploid SH732Ar displayed the following properties:

1) It exhibited homozygosity of the $\underline{cry}_1{}^r$ allele.
2) It could be induced to undergo sporulation.
3) Tetrads produced by this diploid contain four Mat α spores.
4) Meiotic products of this diploid showed unusual segregation of the ARM gene.
5) Meiotic products of this diploid showed unusual segregation of the $\underline{thr}_4$ gene on the right arm of chromosome III.

The ARM gene allows sporulation of mat a*/Mat α, ARM/+ diploids and therefore can be assayed for in α cells by examining the sporulation ability of diploids formed by mating with strains carrying the mat a* sporulation

deficient mutation (Kassir and Simchen, 1976).

Heterothallic strains carrying the ARM gene mate normally but display increased ability to spontaneously switch mating types from α to **a**.

CHAPTER IV

ARM Gene influenced Sporulation of dmt Diploids

Heterothallic yeast cells of the same, Mat α, mating type are normally prohibited from mating with each other, however, by setting up appropriate selective conditions it is possible to isolate the products of occasional rare mating events. These diploid colonies, with rare exception, are composed of either nonmating but sporulating cells, or of α mating but nonsporulating cells. The first group consists of a/α diploids. Their formation results from a switch of mating type from α to a by one of the haploid parents, allowing this parent to mate with a neighboring α cell (Hicks and Herskowitz, 1977; Rabin, 1970; Melnick and Blamire, 1978). In certain of these a/α diploids, switch of mating type is associated with a deletion (Hawthorne, 1963, Strathern et al., 1979) on the right arm of chromosome III.

Certain of the α mating diploid cells were proposed to have been formed as the result of loss of chromosome III information, including the mating locus, in one of the haploid parents. This loss of Mat α information is thought to allow this cell to mate as an a mating type cell, enabling diploid formation with a neighboring α cell (Melnick and Blamire, 1978; Chapter II). Since these α mating diploids do not sporulate, it was difficult to con-

firm this model of rare mating diploid formation.

In the work described in this chapter, two genes, ARM and dmt are employed. Both of these genes influence the outcome of rare matings between α cells. The ARM gene (Chapter III), which allows otherwise prohibited sporulation of α/α diploids, also increases the frequency of heterothallic switch of mating type from α to a. This gene increases the size of the a/α diploid class produced from rare matings between α cells (Chapter III, section 2f). The dmt gene greatly increases the size of the α mating, nonsporulating class of diploids produced by α cell rare matings (Chapter II, section 2). By crossing Mat α ARM + x Mat α + dmt it was possible to produce α mating diploid cells which were able to sporulate. The meiotic products of samples of these cells were analyzed and the implications of this analysis towards an understanding of the mechanisms of rare matings are discussed.

Section 1: The Mat α dmt, Mat α ARM Rare Mating Cross, C128

a) Diploid categories

The rare mating cross C128 was carried out between the strain C122.29A, Mat α dmt and strain Ar23A, Mat α ARM. Table RXIII shows the distribution of phenotypes of 246 diploid colonies, each the result of an independent mating event between a cell from strain C122.29A and one from Ar23A (see Materials and Methods, section 10).

To simplify discussion, diploid colonies are assigned

Table RXIII

Phenotypes of diploid strains produced by the rare mating cross C128

Parents were mated at 1:1 ratio of cell numbers, and all colonies on a single plate were assayed (see Materials and Methods).

Cross C128

Strain C122.29A Mat α cry_1^S dmt ura_3 lys_1 ade_2 his_7

Strain Ar23A Mat α cry_1^r ARM his_4 leu_2 ura_3 lys_2

Mating phenotype	Chromosome III phenotype	Number of colonies isolated	Designated class
nonmater	Leu^+ cry^{p*}	152	A
α mater	Leu^+ cry^{p*}	38	B_2
α mater	Leu^+ cry^r	30	B_1
α mater	leu^- cry^{p*}	19	D
α mater	leu^- cry^r	1	C
α mater	Leu^+ cry^S	6	G

* cry^p is the phenotype assigned to diploids of cry_1^s/cry_1^r phenotype, or to diploids which contain only a single copy of the cry_1^r allele and no copies of the cry_1^s allele (for example, diploids monosomic for chromosome III). "p" stands for the papillations of cryptopleurine resistant cells which are able to grow on cryptopleurine solid media plates despite the sensitivity of the major proportion of cells in cry^p populations. In contrast, in cry^s populations all cells are sensitive.

to classes. Class A diploids are nonmating and are considered to be similar to those nonmating diploids produced in Mat α ARM x Mat α crosses L107 and L109 (Table RXI), and also by those crosses shown in Table RXII. Of 18 class A colonies analyzed all produced cryptopleurine resistant mitotic recombinant subclones of a mating type (Table RXII), an indication that these colonies arose as a result of switch of mating type by the ARM gene carrying parent, Ar23A.

The α mating diploid colonies of classes B_1, B_2, C and D are similar to diploids derived from the α dmt x α + mating C117 (Table RIV) in their mating characteristics and, in classes B_1, D and C, in their loss of the cry_1^s allele of the dmt gene carrying parent, C122.29A. Unlike α mating diploids from cross C117, classes B_1, B_2, C and D cells from cross C128 are able to undergo sporulation (Table RXIV). Class G α mating diploid colonies, which do not sporulate (Table RXIV) show loss of the cry_1^r allele of the parent which did not contain the dmt gene. This class is thought to result from background rare mating events which do not involve the dmt gene, similar to those events observed in cross A738 (Mat α + x Mat α +) shown in Chapter II, Table RII).

b) Meiotic products of C128 diploid colonies.

The results of analysis of the meiotic products of sample colonies from each diploid class described in Section 1 are shown in Table RXV.

Table RXIV

Sporulation of C128 diploids*

Class	Number Examined	Sporulating	Nonsporulating
A	85	85	0
B_1	24	23	1
B_2	32	30	2
C	3	2	1
D	30	28	2
G	10	1	9

* The diploid cells displayed in this chart are not the same diploids shown in Table RXIII. Table RXIII diploids were isolated using a procedure which was designed to insure that the parental strains were introduced in equal quantities (see Materials and Methods). In Table RXIV, C128 diploids of the same phenotypic classes as shown in Table RXIII are examined but these diploids were selected in a procedure that was not designed to insure a 1:1 numerical ratio of parental strains.

Table RXV

Results of tetrad analysis of sample diploids from the cross C128

Diploid class see Table RXIII	Rare mating colonies analyzed	Tetrad properties	Comments
Class A	C128.116.4 C128.45.1 C128.7a C128.107.2 C128.108.1 C128.115.2 C128.117.1 C128.109.1 C128.108.3 C128.108.2 C128.107.4 C128.117.2	2Mat a cry^r: 2Mat α cry^s 2Mat _ cry^S	The Mat a spores carry the $cry_1{}^r$ allele of the ARM gene carrying parent expected phenotype of Hawthorne deletion
Classes B_1 and C	C128.12 C128.23 C128.D_L1.4 C128.$D_1$3.2 C128.110.3	4Mat α cry^r, 2leu⁻ : 2+ Class C 4Mat α cry^r leu⁻	The $cry_1{}^r$ allele has become homozygous leu_2 in addition to $cry_1{}^r$, has become homozygous
Class B_2	C128.133 C128.109.2 C128.111.3 C128.112.2 C128.33	2Mat α $cry_1{}^r$: 2Mat α $cry_1{}^s$ 2leu⁻ : 2+	
Class D	C128.41 C128.108.4 C128.109.3 C128.110.4 C128.111.1	2Mat α cry^r:0	The $cry_1{}^s$ contribution to the dmt gene containing parent is lost. Results of tetraploid analysis (Table IX) is consistent with haploid state of the mating locus in class C diploids.

Table RXV continued/

Diploid class	Diploid colony designation	Description
A	C128.116.4	5 tetrads, all 2Mat a cry_1^r : 2Mat α cry_1^s
A	C128.115.1	5 tetrads 4 2Mat a cry_1^r: 2Mat α cry_1^s 1 2Mat a cry_1^s: 2Mat α cry_1^r
A	C128.7.2	6 tetrads 5 2Mat a cry_1^r: 2Mat α cry_1^s 1 2Mat a cry_1^s: 2Mat α cry_1^r
A	C128.107.2	2 tetrads, both 2Mat a cry_1^r: 2Mat α cry_1^s
A	C128.108.1	7 tetrads 6 2Mat a cry_1^r: 2Mat α cry_1^s 1 2Mat a cry_1^s: 2Mat α cry_1^r
A	C128.115.2	5 tetrads, all 2Mat a cry_1^r: 2Mat α cry_1^s
A	C128.117.1	6 tetrads, all 2Mat a cry_1^r: 2Mat α cry_1^s
A	C128.109.1	3 tetrads, all 2Mat a cry_1^r: 2Mat α cry_1^s
A	C128.108.2	6 tetrads, all 2Mat a cry_1^r: 2Mat α cry_1^s
A (Hawthorne deletion)	C128.117.2	4 two-spore tetrads 3 2Mat α cry_1^s: 0 Mat a 1 1Mat α cry_1^r: 1Mat α cry_1^s
B_1	C128.1.2	9 tetrads all 4Mat α cry_1^r 2Leu_2^+:2leu_2^-
B_1	C128.2.3	5 tetrads all 4Mat α cry_1^r 2Leu_2^+:2leu_2^-
B_1	C128.D_L1.4	2 complete tetrads both 4Mat α cry_1^r 2Leu_2^+: 2leu_2^- 4 partial tetrads (3 spore and 2 spore) all cells Mat α cry_1^r

Table RXV continued/

Diploid class	Diploid colony designation	Description
B_1	C128.D_L3.2	3 complete tetrads 4Mat α $cry_1{}^r$ 2$leu_2{}^+$: 2$leu_2{}^-$ 4 two-spore tetrads all cells Mat α $cry_1{}^r$
C	C128.110.3	4 tetrads all Mat α $cry_1{}^r$ $leu_2{}^-$
B_2	C128.133	7 tetrads all 2Mat α $cry_1{}^r$:2Mat α $cry_1{}^s$
B_2	C128.109.2	5 tetrads 5 2Mat α $cry_1{}^r$: 2Mat α $cry_1{}^s$
B_2	C128.111.3	6 tetrads 6 2Mat α $cry_1{}^r$: 2Mat α $cry_1{}^s$
B_2	C128.112.2	6 tetrads 6 2Mat α $cry_1{}^r$: 2Mat α $cry_1{}^s$
B_2	C128.33	3 tetrads 3 2Mat α $cry_1{}^r$: 2Mat α $cry_1{}^s$
D	C128.11	3 two spore tetrads all 2Mat α $cry_1{}^r$
D	C128.108.4	5 two spore tetrads all 2Mat α $cry_1{}^r$
D	C128.109.3	2 two spore tetrads all 2 Mat α $cry_1{}^r$
D	C128.110.4	6 two spore tetrads all 2Mat α $cry_1{}^r$
D	C128.111.1	4 two spore tetrads all 2Mat α $cry_1{}^r$

Class A

Ten class A diploid colonies, each the product of an independent rare mating event, were examined by tetrad analysis. The results are shown in Table RXV. Eleven of these diploid colonies show 2Mat a cry_1^r:2Mat α cry_1^s segregation. One tetrad shows 2Mat α cry_1^s: 2 nonviable spore segregation, the expected meiotic products of a diploid carrying a Hawthorne deletion (Hawthorne, 1963; also Strathern et al., 1979). In all cases, cry_1^r, the cryptopleurine allele of the ARM gene containing parent, segregates with the a mating type spores. This indicates that in each of these independently derived diploids, the Mat α ARM parent underwent switch of mating type. The ARM gene segregates among the meiotic progeny of class A diploids indicating that it is not lost during the process of switch of mating type.

Class D

Five class D diploid colonies, each the product of an independent mating event, were examined by tetrad analysis (Table RXV). No more than two viable spores per ascus were found and all viable spores were of the genotype Mat α cry_1^r.

Class D tetrads are the expected progeny of a rare mating event brought about by dmt gene induced deletion of the mating locus of haploid Mat α dmt cells. The cry_1^s locus, the expected contribution of the dmt containing

parent strain, is not observed among the viable meiotic products of these diploids. This implies that class D diploids are haploid for the mating locus. The class D diploids C128.110.4 and C128.109.3 were mated with the Mat a homozygous diploid strain XJ9. The results of the analysis of the tetraploids produced by these crosses is shown in Table RXVI. These results are consistent with the idea that C128.110.4 and C128.109.3 are haploid for the mating locus (see Figure 2a).

Classes B_1 and C

Four class B_1 diploid colonies, each the product of an independent rare mating event, were examined by tetrad analysis (Table RXV). All of these tetrads showed 4Mat α cry^r, 2Leu^+: 2leu^- segregation. The class C diploid showed 4Mat α cry^r leu^- segregation in every tetrad.

These diploid colonies are thought to have arisen as a result of the same mechanism described to have given rise to class D diploids. No tetrads contain the cry^S information expected to have been contributed by the dmt parent C122.29A. Unlike class D diploids, class B_1 and C diploids have become homozygous for chromosome III information from the non dmt parent. The dmt induced damage which allowed mating to occur appears to have been replaced in the diploid with homologous information from the non dmt parental chromosome III (see Figure 2a).

Segregation of the ARM gene among the spores of class

Table RXVI

Results of tetraploid analysis of sample class D diploids crossed with the a/a diploid strain XJ9

a' = a mating; α' = α mating; n.m. = nonmating

CROSS L137

strain C128.110.4

strain XJ9

Segregation of mating activity	Number of tetrads
2a':2α'	5
2a':2n.m.	3
2a':1α':1n.m.	2
3a':1n.m.	1
1a':1α':2n.m.	1
2α:2n.m.	1

CROSS L138

strain C128.109.3

strain XJ9

Segregation of mating activity	Number of tetrads
2a':2α'	4
2a':2n.m.	3

B_1 and C tetrads is shown in Table RXVII and is most easily interpreted as the segregation of an unstable gene among the meiotic products of homozygous parents (see Chapter III, segregation of the ARM gene among the meiotic products of SH732Ar).

Class B_2

Five class B_2 diploid colonies, each the product of an independent rare mating event, were examined by tetrad analysis as shown in Table RXV. All tetrads examined contained 2Mat α cry_1^r: 2Mat α cry_1^s, 2Leu^+: 2leu^- spores.

Class B_2 diploids show no indication of loss of chromosome III information and could possibly have arisen by a "fusion" of two Mat α cells without the involvement of information loss. Alternatively, the information loss in these diploids may have been localized to portions of chromosome III which do not contain genetic markers to allow detection of information loss.

In cross C128, loss of chromosome III information distal of cry_1^S is not detectable as it is in dmt crosses SH732, SH734 (Table RII) and C117 (Table RIV) by virtue of the presence of the thr_4 allele in these latter crosses. The diploid dmt rare mating progeny of these latter crosses contain a high proportion of cells which display loss of Thr_4^+ information but no other marker loss. This class of diploids could be present in C128, class B_2, and would show no loss of information. A suggestion that B_2 diploids have

lost information distal to $\underline{cry_1}^s$ comes from observation of the segregation of the ARM gene among the meiotic progeny of diploids of this class (Table RXVII). The ARM gene is distal of $\underline{thr_4}$ on the right arm of chromosome III (Chapter V).

Many of the tetrads observed from class B_2 asci show other than 2:2 segregation of the ARM gene. Ten of 16 tetrads examined display the ARM gene phenotype in three or four spores per tetrad (Table RXVII). A possible explanation of this segregation can be derived from comparison with the pattern of segregation of the ARM gene in the diploid strain SH732Ar as described in Chapter III. A high proportion of the tetrads produced by meiosis of this strain displayed 3+:1- segregation of the ARM gene (Chapter III, Table RVII). This was explained as being the result of homozygous segregation of an unstable gene. Those B_2 diploids which produce 3 ARM: 1 wild type and 4 ARM : 0 wild type tetrads could have become homozygous for the ARM gene in a manner similar to the homozygosity of the $\underline{cry_1}^r$ allele in diploids of classes B_1,and C. This would indicate that in the dmt containing parental strain of cross C128, information distal to $\underline{cry_1}^s$ is lost in the formation of class B_2 diploids. This lost information is replaced in the diploid by homologous information, including the ARM gene from the non dmt gene parent's chromosome III (see Figure 2b).

Table RXVII

Segregation of the ARM gene among the meiotic products of C128 diploids

The ARM phenotype is determined by ability to sporulate in diploids of the genotype Mat α/mat a*.

Diploid class	Number of tetrads belonging to each tetrad type (+ = ARM phenotype)			
	2+:2-	3+:1-	4+:0-	other
B_1	8	6	4	0
B_2	5	9	1	1

Diploid class	ARM^+	not ARM^+
A*[1]	15	29
D*[2]	18	10

*[1] Only Mat α spores were assayed

*[2] Only two viable spores (both Mat α) per tetrad

Figure R2

Proposed pathway of dmt gene induced rare matings between Mat α cells in cross C128

Figure 2a

Step 1

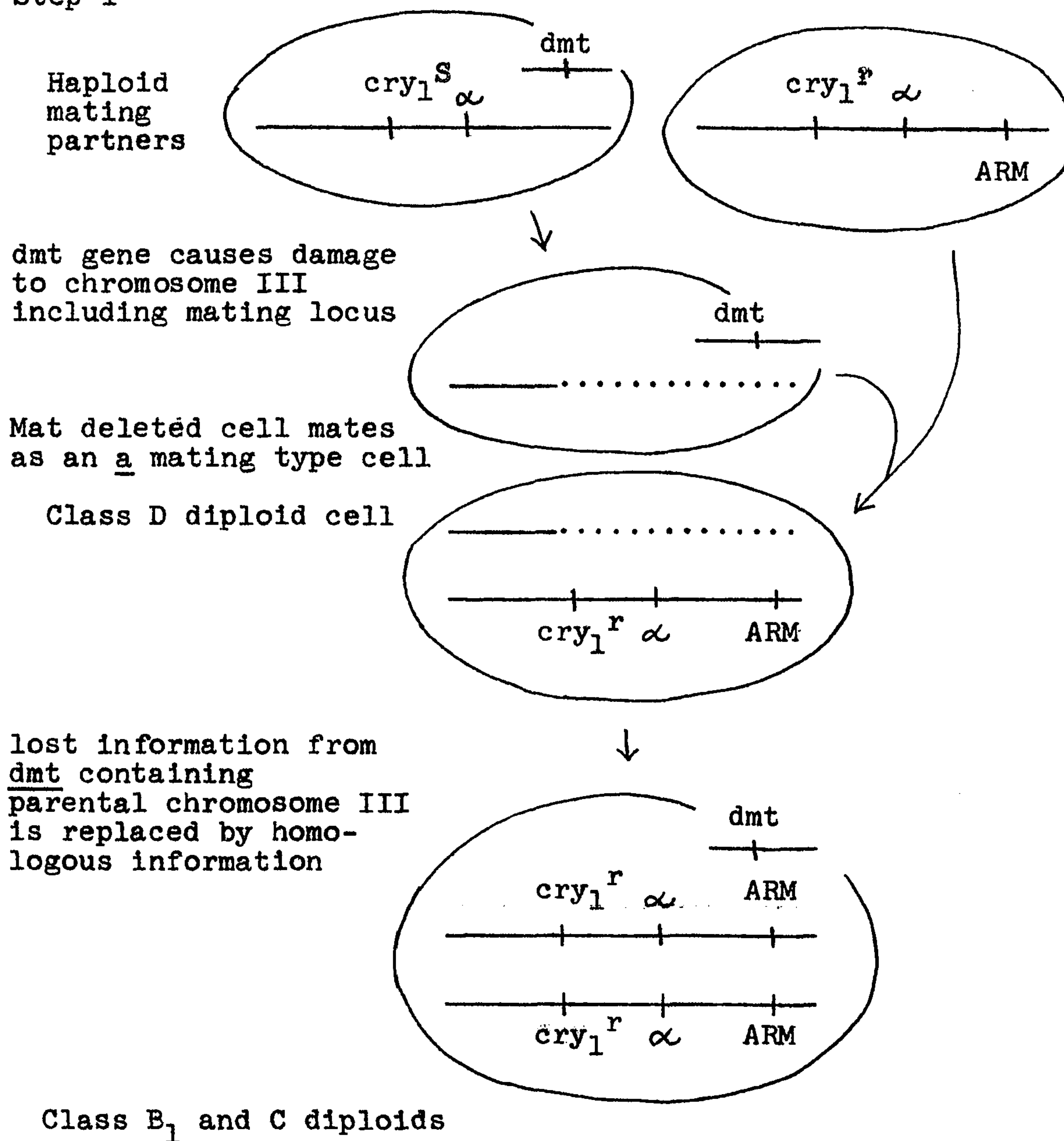
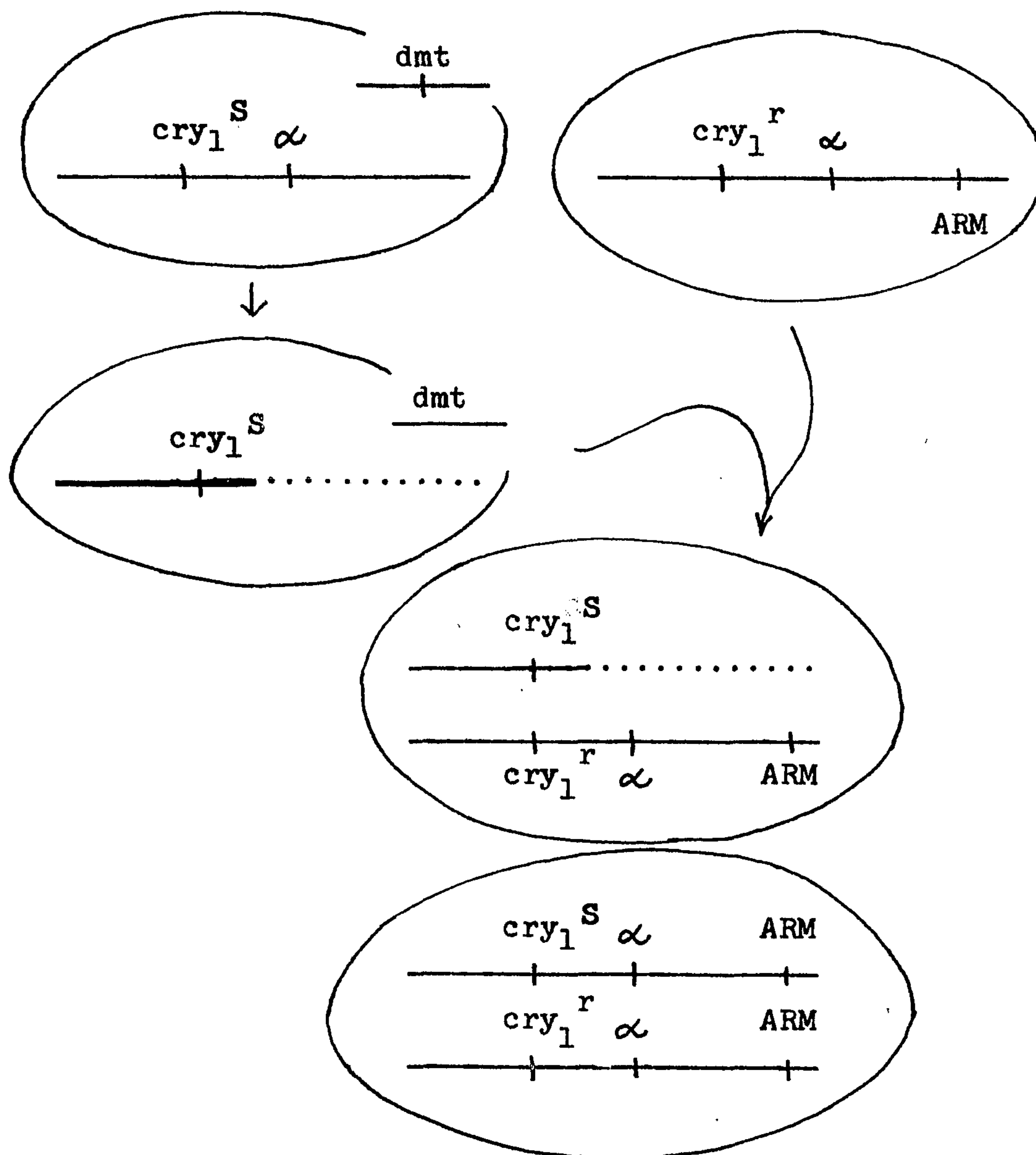


Figure 2b

Proposed pathway of dmt gene induced class B_2 diploids



Class G

These diploid colonies constitute a rare class comprising only six of 242 diploid colonies examined (Table RXIII). Class G diploids are completely sensitive to cryptopleurine and do not produce drug resistant mitotic recombinant subclones on cryptopleurine containing media (see Materials and Methods, section 4). These diploids are missing the $\underline{cry}^r$ contribution of their non dmt containing parent and are thought to have arisen from a chromosome III damage event which was not induced by the dmt gene. A similar pattern of chromosome III modification is observed in the rare mating diploids which are cryptopleurine sensitive derived from cross A738 (Chapter II, Table II). Nine of ten class G diploids isolated do not sporulate. This is expected if these diploids have lost information from the right arm of the chromosome III of the non dmt gene containing parent strain.

Discussion

In Chapter II it was proposed that loss of the mating locus of a haploid Mat α cell allows that cell to mate as an a mating type cell. We presented evidence to support the idea that the high frequency of α x α rare matings associated with the presence of the dmt gene is a result of dmt gene induced damage to the chromosome III of α dmt cells allowing them to mate as a mating type cells. Because diploids

formed by dmt gene induced rare matings do not sporulate, analysis of the meiotic products of these diploids was difficult. In this chapter, utilizing the ARM gene to allow sporulation, these meiotic products are examined.

The results of this analysis support the model of dmt rare mating proposed earlier.

Surprisingly, however, not all of the diploids which had lost chromosome III information were haploid for the mating locus or for information homologous to this lost information. In classes B_1 and C, lost cry_1^s information has been replaced with the homologous cry_1^r allele. Possibly, in class B_2, lost wild type information has been replaced with homologous ARM information (Figure 2b). This repair ability would appear to warrant further study as described in the discussion section of this thesis.

Rare matings between Mat α wild type cells not containing the dmt gene produce diploids at much lower frequency than when one or both of the parents contain the dmt gene. A high proportion of the progeny of these wild type rare matings (cross A738, Table II) (also Rabin, 1970) show loss of chromosome III genetic information present in the haploid parent strains. This suggests that the dmt gene induced rare mating pathways described in this chapter may occur also, at lower frequency, in the absence of the dmt gene.

Conclusions

The dominant ARM gene, which allows sporulation in α mating diploid cells, is employed to allow sporulation of otherwise nonsporulating diploid cells which are derived from rare matings between Mat α dmt and Mat α wild type strains.

Rare matings between Mat α dmt and Mat α wild-type strains produce α mating diploids most of which display loss of mating locus linked chromosome III genetic markers (Chapter II). The marker modification in these strains is specific to that chromosome III which was contributed by the dmt gene containing parent. These diploids do not sporulate which makes their further analysis difficult (Chapter II).

Rare matings between Mat α ARM and Mat α wild-type strains produce diploid progeny most of which display the phenotype of a/α diploids. This indicates that mating is the result of switch of mating type by one of the parent strains. By constructing these a/α diploids to be heterozygous for the mating locus linked cry_1^r, cry_1^s alleles, it is possible to determine which of the parents of these rare mating diploids has undergone switch of mating type. This parent is, with rare exception, the ARM gene carrying mating partner (Chapter III).

Diploid colonies derived from the cross Mat α ARM x Mat α dmt are either α maters or nonmaters. The presence of the ARM gene allows the α mating colonies to sporulate and

allows examination of the meiotic products of these colonies. Information gained by this examination is incorporated into models to explain dmt gene facilitated and ARM gene facilitated rare matings.

The following conclusions can be made.

The products of rare matings between Mat α dmt and Mat α ARM strains give rise to the following types of diploid colonies:

Class A: a/α diploids, the Mat a locus carries the tightly linked cry_1 allele of the ARM carrying parent.

Class D: α mating diploids haploid for the mating locus and other chromosome III genetic markers. These are missing genetic information from the dmt gene containing parent.

Classes B_1 and C: α/α diploids which are missing chromosome III information from the dmt gene containing parent and are homozygous for genes homologous to the missing genes.

Class B_2: α/α diploids which show no missing genetic information but may be homozygous for the ARM gene, which is not contained in the dmt gene containing parent.

The above diploid colonies are able to sporulate and the ARM gene segregates among their meiotic progeny.

The results of this analysis support the model proposed to explain dmt gene facilitated rare matings (Chapter II and Figure 2) and ARM gene facilitated rare matings (Chapter III, section 2f).

CHAPTER V

Segregation of the ARM Gene and thr_4 Nutritional Marker in ARM Gene-containing Crosses

Introduction

In this chapter the segregation of the ARM gene is examined in several crosses. The ARM gene maps distal to thr_4 on the right arm of chromosome III. In many crosses which involve the ARM gene, the thr_4 genetic marker shows unusual segregation.

Section 1: Segregation of the ARM Gene

Certain properties of the ARM gene make segregational analysis difficult. As described in Chapter III, the ARM gene is unstable and also displays variation in expression among cells in which it segregates. In addition to these properties, several crosses which involve the ARM gene display unusual segregation of the thr_4 locus. It is not yet clear whether or not this unusual segregation is the result of a phenotypic property of the ARM gene. Experiments are suggested in the discussion section of this thesis to investigate this possibility.

Table RXVIII lists the crosses discussed in this chapter and indicates the types of unusual segregation of the thr_4 locus encountered in each cross.

Table RXVIII

Unusual Thr_4 segregation in crosses containing the ARM gene

Cross		Unusual thr_4 segregation
L100	I11.102D x L88.25B	New thr^- alleles
	Mat a ARM Thr^+ x Mat α ARM Thr^+	12 of 76 spores examined are threonine auxotrophs
L88	C63.16A x Ar31C	New thr^- alleles
	Mat a Thr^+ x Mat α ARM Thr^+	16 of 208 spores examined are threonine auxotrophs
L61	I11.102D x C63.8D	thr_4 gene conversion
	Mat a ARM Thr^+ x Mat α thr_4^-	3+:1- 3; 3-:1+ 3; 2+:2- 22 — 28 tetrads
L29	C27.13B x C128.33.2D	thr_4 gene conversion
	Mat a thr_4^- x Mat α ARM Thr^+	3-:1+ 21; 2+:2- 61 — 82 tetrads
L45	C63.16A x L29.34D	No unusual thr_4 segregation
	Mat a Thr^+ x Mat α ARM thr_4^-	
L42	I11.102D x C29.11B	thr_4 gene conversion
	Mat a ARM Thr^+ x Mat α thr_4^-	3+:1- 20; 3-:1+ 10; 2+:2- 41 — 71 tetrads
		Introduction of osg sporulation gene with C29.11B

Table RXVIII continued

	Cross	Unusual thr$_4$ segregation
Cross L74	L72.3c x C60.8A mat a* ARM Thr$^+$ x Mat α thr$_4^-$	thr$_4$ gene conversion 3+:1- 18) 26 tetrads 2+:2- 8)
SH732Ar	(constructed by a dmt facilitated rare mating) C76.1D x C63.8D Mat α dmt Thr$^+$ x Mat α thr$_4^-$	thr$_4$ gene conversion 3+:1- 55) 3-:1+ 1) 110 tetrads 2+:2+ 54)
L125	C76.1D x A10701C Mat α dmt Thr$_4^+$ x Mat a thr$_4^-$	No unusual thr$_4$ segregation (16 tetrads)

Cross L45 Mat a x Mat α ARM thr_4

In cross L45, no unusual segregation of the thr_4 genetic marker is observed. Therefore it was believed that interpretation of the segregation of the ARM gene with respect to the thr_4 gene would not be subject to interference related to unusual thr_4 gene segregation. The segregation of the ARM gene in cross L45 is shown in Table RXIX.

Abbreviations for segregation tables:

NCO	No crossover class
SCO I	Single crossover class, crossover between Mat and thr_4
SCO II	Single crossover class, crossover between thr_4 and ARM
DCO	Double crossover class

Of the various crosses involving the ARM gene described in this section, only L45 showed no apparent unusual thr_4 segregation. The segregation of chromosome III markers in this cross establishes the gene order Mat, thr_4, ARM on the right arm of chromosome III.

Cross L29 Mat a thr_4^- x Mat α ARM

In cross L29, approximately one quarter of the tetrads observed showed 3-:1+ segregation of the thr_4 locus. Table RXX shows the segregation of the ARM gene in the tetrads which display 2+:2- thr_4 segregation, and also, separately

Table RXIX

Cross L45

L29.34D Mat α cry_1^s ARM his_4 leu_2 thr_4 lys_2 ura_3

C63.16A Mat a cry_1^s his_4 ura_3 lys_2

28 tetrads were examined. No gene conversion of thr_4 was observed in L45 tetrads.

α — thr_4 — ARM (intervals I, II) X a — + — arm*2 (intervals I, II)

Mat	thr_4	ARM*1		
α	-	+	NCO	31
α	+	-	SCO I	10
α	-	-	SCO II	13
α	+	+	DCO	2

*1 ARM phenotype can only be assayed in Mat α cells.

*2 arm is used here to represent the alternate allele of ARM. arm does not express the ARM gene phenotype and represents the wild type gene.

Table RXX

Cross L29

C27.13B Mat a cry_1^r his_4 leu_2 thr_4 lys_2

C128.33.2D Mat α cry_1^r ARM ura_3 ade_2

82 tetrads were examined:

61 tetrads showed 2+:2- segregation of thr_4

21 tetrads showed 1+:3- segregation of thr_4

All threonine auxotrophic alleles in the gene conversion class belong to the same complementation group as thr_4 in parental stocks. All thr_4 alleles in the gene conversion group also revert at approximately the same frequency (see section 2, ii, a, of this chapter).

a) Segregation of spores from tetrads where thr_4 shows 2+:2- segregation

L29 α + ARM (intervals I, II) X a thr_4 arm (intervals I, II)

Mat	thr	ARM*		
α	+	+	NCO	70
α	-	-	SCO I	12
α	+	-	SCO II	30
α	-	+	SCO	10
				122

* ARM phenotype can only be assayed in α spores.

b) Segregation in spores from tetrads where $\underline{thr_4}$ shows 1+:3- segregation

Mat	thr	ARM*	
$\underline{\alpha}$	+	+	12
$\underline{\alpha}$	-	-	7
$\underline{\alpha}$	+	-	8
$\underline{\alpha}$	-	+	16

Mating type and $\underline{thr_4}$ genotype of segregants from tetrads in which $\underline{thr_4}$ segregates 1+:3-

$\underline{Mat}$ $\underline{a}$ $\underline{thr_4}^-$	41
$\underline{Mat}$ $\underline{a}$ $\underline{Thr}^+$	1
$\underline{Mat}$ $\underline{\alpha}$ $\underline{thr}^-$	22
$\underline{Mat}$ $\underline{\alpha}$ $\underline{Thr}^+$	20

shown, in the tetrads which display 3-:1+ thr_4 segregation.

If only those tetrads, which display 2+:2- thr_4 segregation are considered, gene order is the same as in crosses L45, L61 and L74. In contrast, however, is the distance between thr_4 and ARM which is inflated in cross L29. Also inflated is the double crossover frequency which is expected to be 0.025 (i.e. SCO I x SCO II = (.10)(.25). However, the observed DCO class is .83. This unexpected segregation could be the result of the same phenomenon as that which results in gene conversion of thr_4^- in other tetrads of cross L29.

Segregation of the ARM gene among tetrads which display 1+:30 thr_4 segregation is shown in Table RXXb. Table RXXb shows that in those tetrads where threonine segregates 1+:3-, the thr^- phenotype is closely associated with the a mating phenotype. As of yet we have no explanation to account for this observation.

Cross L61 Mat a ARM x Mat α thr_4

Segregation of the ARM gene in cross L61 is shown in Table RXXI.

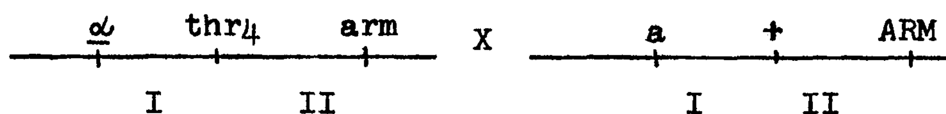
In this cross the SCO II class is unusually small as compared to that class in crosses L45 and L29. This unexpected result could be due to the same cause which results in the unusual segregation of thr_4 observed in this and other crosses involving the strain I11.102D (see section 2, Unusual thr_4 segregation, below).

Table RXXI

Cross L61

I11.102D	Mat a $cry_1{}^r$ ARM ura_1 lys_1 lys_2
C63.8D	Mat α $cry_1{}^r$ his_4 leu_2 ura_3 lys_2

23 tetrads were examined, of which three showed 3+:1- segregation of thr_4, and three showed 1+:3- segregation of thr_4.



Mat	thr	ARM		
α	-	-	NCO	26
α	+	+	SCO I	17
α	-	+	SCO II	2
α	+	-	DCO	1

A similar tight linkage of Thr_4^+ and ARM is observed in cross L42 which has the same ARM contributing parent as cross L61; that parent being the strain Ill.102D.

Cross L42 Mat a ARM x Mat α thr_4

A high proportion of the tetrads from cross L42 show 3+:1- and 3-:1+ segregation of the thr_4 alleles (Table RXXII). Based on observations of other crosses (Table RXVIII) the unusual thr_4 segregation appears to result from the input of the parental strain Ill.102D.(Mat a ARM). From observations presented below, it was concluded that the cross L42 parental strain, L29.11B, contributes an as yet uncharacterized gene which allows sporulation in diploids formed by mating Mat α cells with mat a* cells. This gene, termed, for the purpose of this discussion, OSG (other sporulation gene) is discussed below.

In those tetrads where thr_4 segregates at a 2+:2- ratio, the ratio of Mat α segregants which allow sporulating in mat a*/Mat α diploids to those which do not is 75:29. This ratio is close to 3:1, the expected ratio of segregants whose diploid parent is heterozygous for two unlinked sporulation genes.

The segregation of sporulation ability among the Mat α segregants of cross L42 demonstrates the presence of a new gene which allows sporulation in mat a*/Mat α diploids and which appears not to be linked to the markers observed on the right arm of chromosome III. This new gene which was

Table RXXII

Cross L42

I11.102D Mat a cry_1^r ARM ura_1 lys_1 lys_2

L27.11B Mat α cry_1^s leu_2 thr_4 lys_2

82 tetrads were analyzed.

20 showed 3+:1- segregation for the thr_4^- genetic marker.

10 showed 1+:3- segregation of this genetic marker.

52 showed 2:2 segregation of the thr_4 alleles.

Analysis of tetrads which displayed 2+:2- segregation for the thr_4 alleles

Mat	thr_4	Ability to sporulate when mated to mat a*	
α	+	+	50
α	+	-	2
α	÷	+	25
α	-	-	27

Segregation of sporulation genes among L42 tetrads which show 3+:1- thr_4 gene conversion

Mat	thr_4	Sporulation of diploids formed with mat a*	
α	+	+	25
α	+	-	0
α	-	+	11
α	-	-	4

Table RXXII continued

Segregation of the sporulation genes among L42 tetrads which show 3-:1+ segregation of thr_4

Mat	thr_4	Sporulation of diploids formed by mating with mat a*	
α	+	+	5
α	+	-	1
α	-	+	7
α	-	-	7

The segregation Mat and thr_4 in tetrads which show unusual $\frac{thr_4}{thr^+}$ segregation

Cross L42

Tetrads where thr_4 segregates 3+:1- (20 tetrads)

Mat	thr	
α	+	25
a	+	35
α	-	15
a	-	5

Tetrads where thr_4 segregates 1+:3- (10 tetrads)

Mat	thr	
α	+	6
a	+	4
α	-	14
a	-	16

introduced in strain L29.11B (see cross L47, Table RXXIII) is, for the purpose of discussion termed OSG for other sporulation gene.

The data presented in Table RXXII is consistent with the idea that the ARM gene, introduced with parent I11.102D, is tightly linked to THR_4^+. The OSG gene, introduced with parent L29.11B, does not appear to be linked to either Mat or thr_4. The linkage of ARM and THR^+ in this cross is indicated by the very low number of Mat α THR^+ segregants that do not sporulate when crossed with mat a*. This observation is consistent with segregation data from cross L61 which also shows THR_4^+ and ARM to be tightly linked in the parent I11.102D.

The unusual thr_4 segregation in cross L42 is discussed in section 2.

Cross L47 Mat α OSG x Mat a +

Cross L47 was carried out in order to observe the segregation of the OSG in the absence of the ARM gene. The results are shown in Table RXXIII.

Cross L47 demonstrates the segregation of a gene which allows sporulation in mat a*/Mat α diploids but does not appear to be linked to thr_4. This gene is contained in the parental strain L29.11B, a fact that is confirmed by observations of crosses involving the other L47 parent, C63.16A. In these crosses, no unusual sporulation activity is observed.

Table RXXIII

Cross L47

L29.11B Mat α cry_1^s leu_2 thr_4 lys_2 (OSG)

C63.16A Mat a cry_1^s ura_3 lys_2

18 tetrads were analyzed. None showed any departure from 2+:2- segregation of the thr_4 alleles.

Mat	thr	Sporulation of diploids formed with mat a*	
α	+	+	6
α	+	-	6
α	-	+	13
α	-	-	11

Cross L74 mat a* ARM x Mat α thr_4

In this cross, the ARM gene was introduced in a mat a* strain. The ARM phenotype can be detected in mat a* strains by observing the sporulation ability of diploids formed by mating with wild type Mat α strains. It was therefore possible to observe the segregation of the ARM gene among all of the meiotic progeny of cross L74.

Of the 26 tetrads examined from cross L74, 18 showed 3+:1- segregation of the thr_4 genetic marker. This unusual segregation is discussed in section 2 of this chapter.

In the class of tetrads where thr_4 segregates at a 2+:2- ratio, the segregation of chromosome III genetic markers is consistent with the gene order Mat, thr_4, ARM.

In the class of tetrads where the thr_4 locus segregates 3+:1- (Table RXXIVb), the wild type allele segregates with the mat a* locus in all but one segregant. This linkage is discussed in section 2.

Section 2: Unusual Segregation of the thr_4 Gene

In the majority of crosses involving the ARM gene described in this chapter, the thr_4 locus segregates in an unusual manner. Three unusual patterns of segregation have been observed: (i) high levels of 3+:1- gene conversion, (ii) high levels of 1-:3+ gene conversion, and (iii) the emergence of thr_4^- segregants among the meiotic progeny of homozygous wild type parents (Table RXVIII). Although these

Table RXXIV

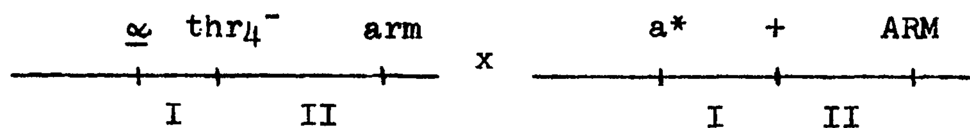
Cross L74

L72.3C *mat* *a** *ARM* his_7 ura_3

C60.8A *Mat* *α* $cry_1{}^r$ his_4 leu_2 thr_4 ura_1 lys_1

26 tetrads were examined. 8 showed 2+:2- segregation of the thr_4 genetic marker, 18 showed 3+:1- segregation of the thr_4 genetic marker.

a) Segregation of chromosome III genetic markers in tetrads where thr_4 segregates 2+:2-



	Mat	thr_4	ARM		
thr_4 2:2 class only	α	-	-	NCO	8
	a*	+	+		10
	α	+	+	SCO I	2
	a*	-	-		2
	α	-	+	SCO II	6
	a*	+	-		4
	α	+	-	DCO	0
	a*	-	+		0

b) thr_4 gene conversion class

Mat	thr_4	
α	+	19
α	-	17

Mat	thr_4	
a*	+	35
a*	-	1

patterns of segregation are observed in crosses which also include the ARM gene, it is not yet certain that the unusual thr_4 segregation is the outcome of a phenotypic property of the ARM gene. Experiments to determine this possibility are described in the discussion section of this thesis.

Crosses which display unusual thr_4 segregation have the following properties.

(i) 3+:1- gene conversion

This phenomenon is observed in cross L74 (18 of 26 tetrads are 3+:1- thr_4), which displays no 3-:1+ gene conversion class. L42 (20 of 71 tetrads are 3+:1- thr_4) also produces 3-:1+ thr_4 tetrads. L61, to a lesser extent (3 of 23 tetrads are 3+:1-, 3 are 3-:1+), displays gene conversion in both directions. Finally, SH732Ar, the parental diploid of all subsequently crossed ARM gene carrying strains, contains 55 of 113 tetrads which display 3+:1- thr_4 gene conversion and only a single tetrad displaying 3-:1+ thr_4.

3+:1- segregation could be the result of segregation of an extra copy of the Thr_4^+ allele in one of the parental strains. All ARM gene containing strains were derived from the diploid strain SH732Ar, in which the ARM gene was first detected. About 50% of the tetrads derived from this diploid strain show 3+:1- segregation of the thr_4 alleles and therefore it is proposed that thr_4 segregation observed in other crosses is a result of the same event which causes this segregation in SH732Ar. A model to account for the 3+:1- segregation of thr_4 in ARM gene crosses is shown in

Figure 3.

This model also accounts for the emergence of new $\underline{thr_4}^-$ segregants from parental diploids which are homozygous for $\underline{Thr_4}^+$ (Table RXVIII, crosses L100 and L88).

According to this model, the extra $\underline{Thr_4}^+$ allele is located on the chromosome III of the $\underline{Thr_4}^+$ parent strain (C76.10) of the diploid SH732Ar. This strain, C76.1D, does not appear to contain an extra $\underline{thr_4}^+$ allele in that when crossed with the strain 10701C, a $\underline{thr_4}$ (cross L100), normal, 2+:2- segregation of the $\underline{thr_4}$ alleles is observed among 16 tetrads. It is therefore proposed that the event which allowed 3+:1- $\underline{thr_4}$ segregation originated in the strain SH732Ar.

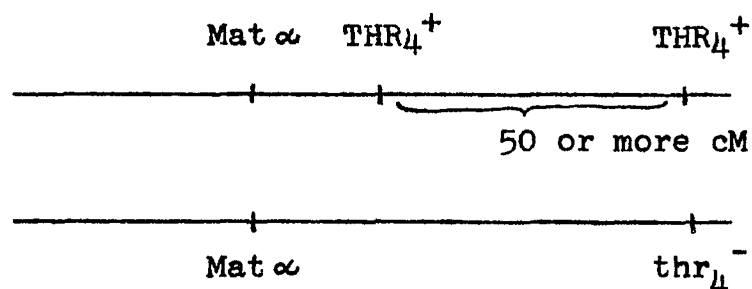
In cross L74, Table RXXIV, as described earlier, the pattern of unusual $\underline{thr_4}$ segregation is consistent with the idea that the mat a* locus is closely linked to a copy of $\underline{Thr_4}^+$ in addition to a $\underline{Thr_4}^+$ allele at its usual locus. The mat a* carrying this unusual proposed $\underline{Thr_4}^+$ configuration also carries the ARM gene and was derived from strain SH762Ar. The segregation of L74 meiotic products, therefore, is consistent with the model proposed in Figure 3.

The model described in this section can account for 3+:1- gene conversion of the $\underline{thr_4}$ locus and also for the emergence of "new" $\underline{thr_4}$ alleles in the meiotic segregants of homozygous parents, however, this model does not explain tetrads where $\underline{thr_4}$ segregates 1+:3-.

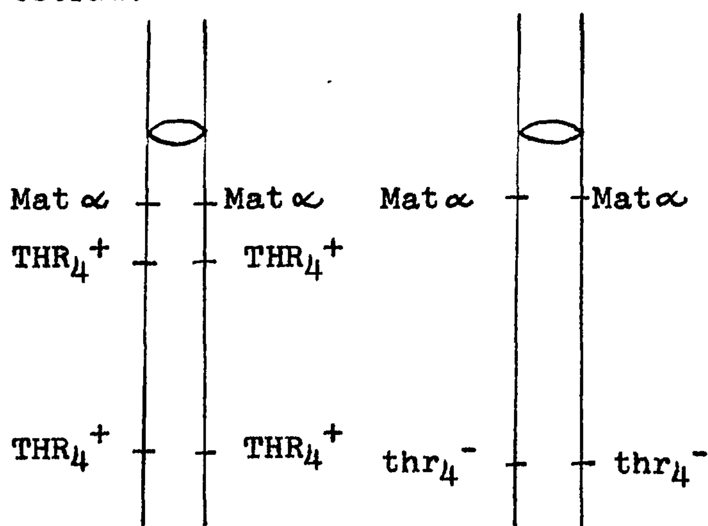
Figure R3

Hypothesis to explain 3+:1- segregation of the $\underline{thr}_4$ locus in certain crosses involving the $\underline{ARM}$ gene

The diploid strain SH732Ar was formed by a $\underline{dmt}$ gene facilitated rare mating event (Chapter III). It is proposed that the chromosome III's of this diploid strain are of the following configuration:



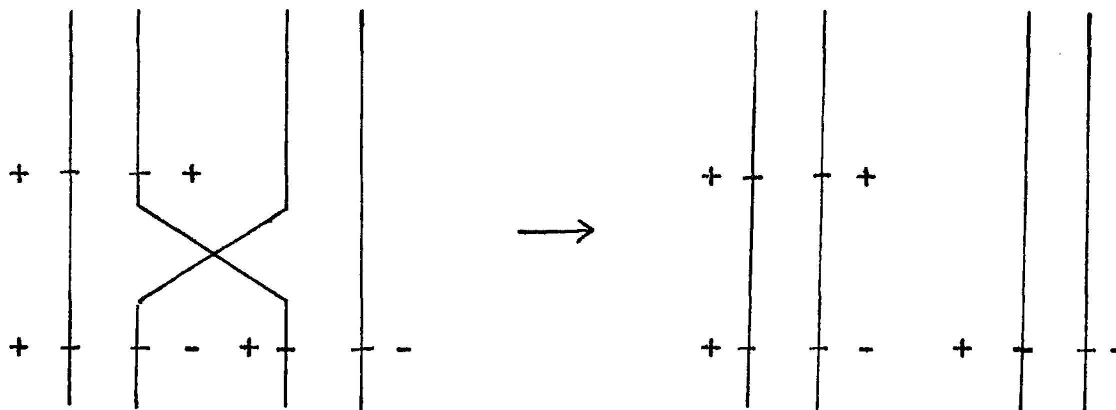
This diploid will produce the following meiotic prophase tetrad:



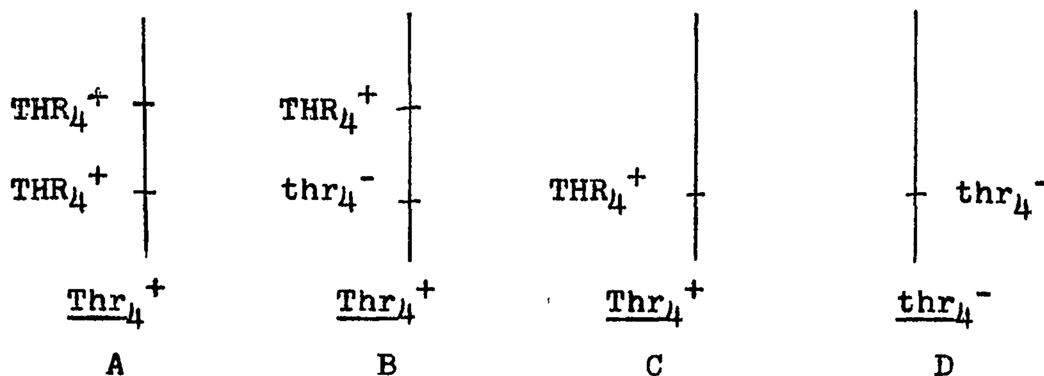
If no recombination occurs, asci produced from this tetrad will display 2+:2- segregation as do appxoximately half of the asci produced by SH732Ar (Chapter III, Table RVI).

Figure 3 continued/

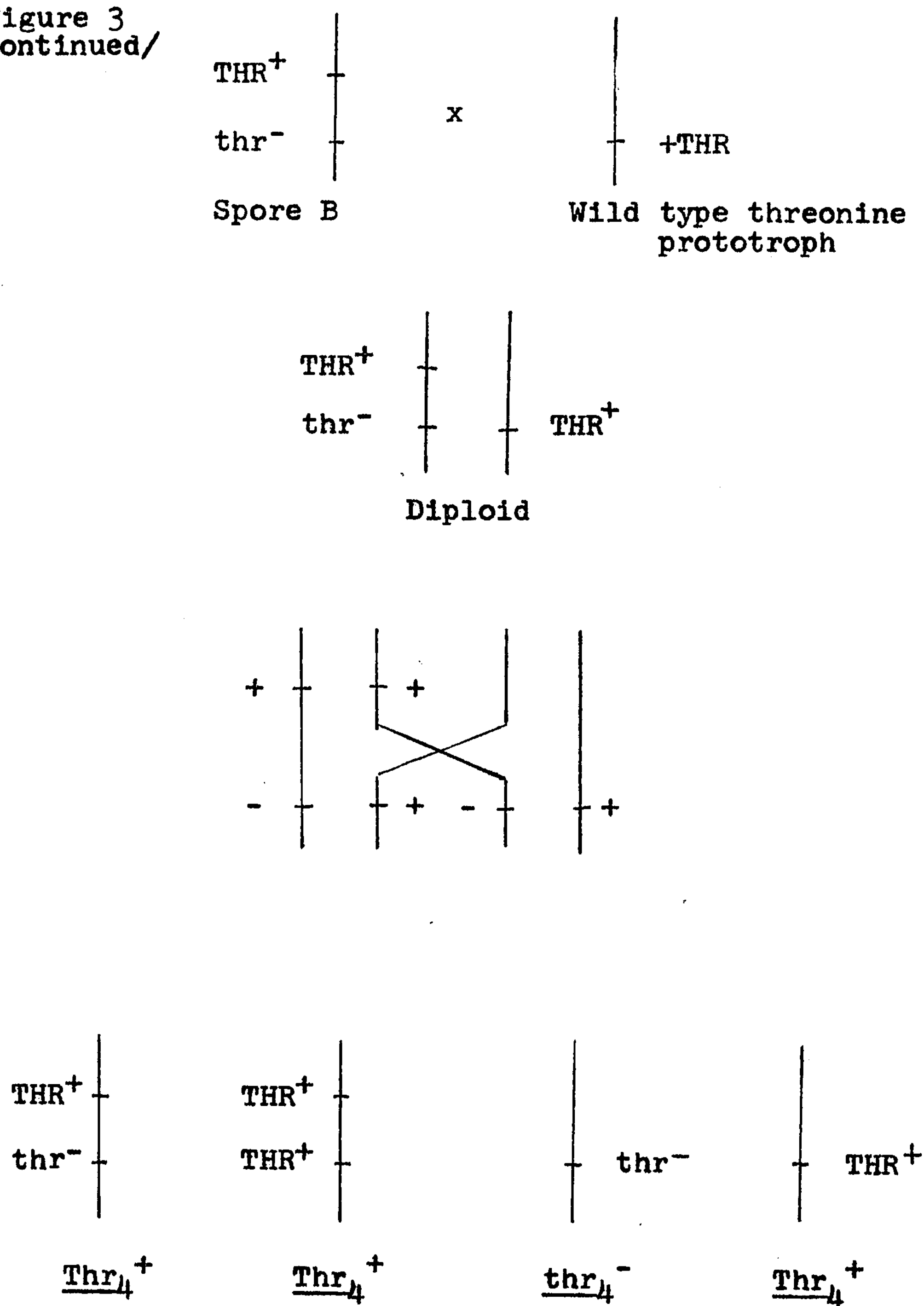
The result of a single crossover between $\underline{THR_4}^+$ loci is the following:



Resulting in the production of an ascus containing the following spores:



Spores derived from this tetrad will express 3+:1- segregation for the $\underline{thr_4}$ genetic marker. Spore B will be peculiar in that, when crossed to spores prototrophic for threonine, it will allow the segregation of $\underline{thr_4}^-$ segregants, as follows:



This phenomenon would explain the origin of $\underline{thr_4}^-$ segregants in the meiotic progeny of crosses L100 and L88 (Table RXVIII).

(ii) 1+:3- gene conversion of the <u>thr_4</u> alleles.

Cross 29 displays a high proportion of tetrads in which the <u>thr_4</u> gene segregates 1+:3- (21 of 82 tetrads). This cross gives rise to no tetrads in which <u>thr_4</u> segregates in a 3+:1- ratio. Cross L42 produces meiotic progeny which display high levels of both 1-:3+ and 3+:1- gene conversion of <u>thr_4</u>.

Sample tetrads which display 1+:3- <u>thr_4</u> segregation, derived from cross L29, have the following properties:

a) None of the threonine auxotrophic spores complement each other, nor do they complement strains carrying the stock <u>thr_4</u> markers derived from the strains A10700B and A10701C (Materials and Methods). In crosses from which new threonine auxotrophic segregants are derived from homozygous wild type parents (L100, L88, see Table RXVIII), these new auxotrophic cells do not complement L29 <u>thr^-</u> segregants or laboratory stock <u>thr_4^-</u> strains.

b) All threonine auxotrophic segregants have approximately equivalent UV induced reversion rates (Materials and Methods).

c) In cross L29, the threonine auxotrophic phenotype is tightly linked to the <u>a</u> mating type in those tetrads where <u>thr_4</u> shows 1+:3- segregation.

At this time we have no explanation for this unusual auxotrophic segregation.

d) Threonine auxotrophic segregants have arisen from crosses where both parents are threonine prototrophs.

Crosses L100 and L88 (Table RXVIII) were produced by mating haploid parents, all of which were wild type for threonine biosynthesis. These crosses produce segregants which are threonine auxotrophs and which do not complement stock thr_4 strains and which show similar rates of UV induced reversion to thr_4 stock strains. An explanation of the origin of these alleles is presented in Figure 3.

Conclusions

Analysis of the segregation of the ARM gene leads to the following conclusions:

1) The ARM gene is on chromosome III distal to the thr_4 locus.

2) In many crosses which involve the ARM gene, unusual segregation of the thr_4 marker is observed. In certain cases, the presence of the ARM gene is associated with the presence of an "extra" copy of the Thr_4^+ allele on chromosome III.

CHAPTER VI

ARM α: A Gene Which Allows Sporulation of Diploids Which Lack Mat α Information

Introduction

In this section a gene is described which allows sporulation of certain Mat a/Mat a and Mat a/mat a* diploid cells. This gene was first observed in the meiotic products of tetraploid, Mat a/Mat a/Mat α/Mat α which was presumably homozygous for the ARM gene. Mat a cells carrying this new gene are able to mate at low frequency with cells of the same mating type. mat a* cells carrying this new gene mate confluently with both Mat a and Mat α tester strains.

This new gene was proposed to be a form of cryptic Mat α information functioning in analogous fashion to the proposed cryptic Mat a function of the ARM gene. This new gene is termed ARM α. To avoid confusion, in this chapter the ARM gene is referred to as ARM a.

Section 1: The Detection of the ARM α Gene

The ARM α gene was first detected in one of the diploid meiotic products of a tetraploid (A11) constructed by mating a Mat a ARM a homozygous diploid strain with a Mat α ARM a homozygous diploid strain. This diploid product, termed A11.B7B showed a mating ability but was unusual in its ability to undergo sporulation.

Cross A11

Strain C128.110.3	Mat α/Mat α, ARM a/ARM a, cry_1^r/cry_1^r, ura_3/ura_3, his_4/+, leu_2/+, his_7/+, lys_1/+, lys_2/+,
Strain L103G1	Mat a/Mat a, ARM a/ARM a, cry_1^r/cry_1^r, his_4/his_4, leu_2/+, lys_1/+, lys_2/+, ura_3/+.

Results of tetraploid analysis of strain A11

15 tetrads examined.

n.m. = nonmating

a' = a like mating

α' = α like mating

Tetrad type	Number of tetrads
4 n.m.*	5
1 a', 1α', 2 n.m.	3
2 a', 2 α'	3
Other*	4

* These tetrads contain prototrophic segregants, the mating types of which could not be determined.

In all tetrads examined, with the exception of tetrad .B7, all α mating meiotic products (α/α, ARM a/ARM a) were able to sporulate and no "a" mating segregants (a/a, ARM a/ ARM a) showed sporulation ability.

The meictic products of tetraploid A11, with the exception of tetrad .B7, are the expected segregants of a

parent of the genotype:

Mat a/Mat a/Mat α/Mat α   ARM a/ARM a/ARM a/ARM a

Tetrad A11.B7 showed the following segregation of mating and sporulation ability.

	Mating activity	Sporulation	
7A	n.m.	+++	
B	a'	+++	(!)
C	α'	-	(!)
D	n.m.	+++	

Because of its unusual sporulation ability, segregant A11.B7B was set aside for further analysis.

Section 2: Properties of A11.B7B

1) A11.B7B is a sporulating Mat a/Mat a diploid.

The diploid A11.B7B mates as an a mating type strain. This diploid also shows proficient sporulation ability in that, when incubated on KAc media for five days, over 80% of the cells observed have formed asci. This is in contrast to much lower levels of sporulation observed in homozygous Mat a diploid cells which are allowed to sporulate by csp genes (Hopper and Hall, 1975) or rme genes (Kassir and Simchen, 1976).

2) The meiotic products of A11.B7B are four Mat a cells per tetrad. These Mat a cells display the unusual ability to mate at low frequency with cells of the same mating type.

Twelve tetrads of A11.B7B were examined. All spores mated confluently with Mat α tester strains. Also, all spores displayed papillated, low frequency mating with a mating type tester strains. This papillated mating is unusual for Mat a strains and is considered to be a phenotypic property of the same gene that allowed sporulation of the a/a diploid A11.B7B. This gene is termed ARM α (Active Regulator of Meiosis, α).

A11.B7B is considered to be homozygous for the ARM α gene and its meiotic products are of the genotype Mat a ARM α.

3) Products of papillated matings between A11.B7B meiotic products and mat a* tester strains are able to sporulate.

The Mat a ARM α strains A11.B7B.1A, A11.B7B.2A, A11.B7B.4D, A11.B7B.5C and A11.B7B.6C mated with the mat a* strain 17-15 by the rare mating procedures described in the Materials and Methods section. Diploid colonies were formed at considerably higher frequencies than are formed by rare matings between Mat a wild type cells. The sporulation of these diploid colonies, after incubation on sporulating media, is shown in Table RXXV. Each colony is the result of an independent (ARM α facilitated) rare mating event.

4) Certain of the diploids produced by mating Mat a ARM α strains with mat a* strains (section 3) mate as a mating type strains.

Table RXXVI shows the mating phenotype and

Table RXXV

Sporulation of colonies formed by mating Mat a ARM with mat a*

L140 A11.B7B.1A x 17-15 (mat a*)

4 colonies observed, all showed 60-90% ascus formation after incubation on KAc media.

L141 A11.B7B.2A x 17-15 (mat a*)

3 colonies observed, all showed 80-90% ascus formation.

L142 A11.B7B.4D x 17-15 (mat a*)

4 colonies observed, all showed 60-90% ascus formation.

L144 A11.B7B.5C x 17-15 (mat a*)

4 colonies observed, all showed 80-90% ascus formation.

L145 A11.B7B.6C x 17-15 (mat a*)

2 colonies observed, both showed 60-90% ascus formation.

Table RXXVI

Mating ability of colonies formed by mating Mat a ARM α with mat a*

	Colony	Mating	cry_1 phenotype
Cross L143	1	nonmater	sensitive
	2	a mater	heterozygous
	3	a mater	heterozygous
	4	a mater	heterozygous
Cross L144	1	a mater	heterozygous
	2	nonmater	resistant
	3	nonmater	resistant
	4	nonmater	sensitive

cryptopleurine phenotype of the crosses L143 and L144 (see Table 1). Note strains A11.B7B.4D and A11.B7B.5C contain the cry_1^r allele. Strain 17-15 contains the cry_1^s allele. These alleles are tightly linked to the mating locus.

The a like mating ability of diploids formed by mating Mat a ARM α strains with mat a* strains demonstrates that these strains did not form as a result of switch of mating type by one of the parental cells. Since these diploids lack a Mat α locus, sporulation is unusual and is considered to be the result of the influence of the ARM α gene. The ARM α gene is considered to replace the lacking Mat α locus in allowing sporulation of strains of the genotype Mat a/mat a*, ARM α/+.

While the ARM α gene parallels the sporulation activity of the ARM a gene, it remains to be demonstrated that the ARM α gene is mating type specific, and does not also allow sporulation in Mat α/mat a* strains. However, certain other observations of the ARM α gene in strains containing mat a* suggest that ARM α expresses α functions in addition to the already mentioned sporulation functions.

5) mat a* ARM α cells mate confluently with both Mat a and Mat α tester strains.

Strain L141.iso 2 was constructed by mating A11.B7B (Mat a ARM α) with 17-15 (mat a* +) (see section 4). This diploid was able to sporulate giving rise to the meiotic products shown in Table RXXVII.

Table RXXVII

L141 iso 2 Meiotic Products

Tetrad		Mating activity	Sporulation* of diploids formed by mating with Mat α	Sporulation of diploids formed by mating with Mat a
1	A	a'	+	
	B	a'	+	
	C	a' and weak α'	+	-
	D	a', α', both confluent	-	-
2	A	a', α', both confluent	-	-
	B	a'	+	
	C	a'	+	
	D	a' and weak α'	+	-
3	A	a', α', both confluent	-	-
	B	a'	+	
	C	a'	+	
	D	a', α', both confluent	-	-
4	A	a'	+	
	B	a'	+	
	C	a' and weak α'	+	-
	D	a' and weak α'	+	-

Table RXXVII continued

Tetrad		Mating activity	Sporulation* of diploids formed by mating with Mat α	Sporulation of diploids formed by mating with Mat a
5	A	a', α', both confluent	-	-
	B	a'	+	
	C	a', α', both confluent	-	-
	D	a'	+	

* In all cases, score of "+" represents >60% ascus formation.

In each of these tetrads, all four spores mate confluently as a cells. This is the expected mating phenotype of both Mat a and mat a* cells. Two spores in each tetrad show unusual ability to mate as α cells, in addition to mating as a cells. Certain of these α maters show confluent α mating, others show low frequency or papillated α mating. This segregation pattern is explained as follows:

a) L141 iso 2 has the genotype Mat a/mat a* ARM α/+

b) Strains of genotype Mat a ARM α (L141 iso 2 1D, 2A, 3A, 3D, 5A, 5C) mate confluently with α tester strains and at low frequency with a tester strains. This was shown in section 4.

c) mat a* ARM α strain (L141 iso 2 1C, 2D, 4C, 4D) mate confluently with cells of both mating types. This could be explained by instability of the ARM α gene which causes an original mat a* ARM α population to revert to a mixed population of a maters (mat a* +) and α maters (mat a* ARM α).

Sporulation characteristics of L141 iso 2 meiotic progeny

L141 iso 2. 1C, 2D, 4C and 4D, putative mat a* ARM α cells, do not support sporulation in diploids formed by mating with Mat α cells (Table RXXVII). This observation, that mat a*/Mat α ARM α/+ diploid cells do not sporulate, is consistent with the idea that ARM α is mating type specific in function and requires Mat a information in order to allow sporulation.

With the exception of the segregants just mentioned,

all other L141 iso 2 segregants support sporulation in diploids formed by mating with Mat α strains. This result was unexpected. Whereas Mat a ARM α and Mat a + segregants are expected to support sporulation in these diploids, mat a* + segregants (of the same mating phenotype as Mat a +) should not support sporulation. A possible explanation of this unexpected sporulation is that the ARM a gene segregates among the meiotic progeny of L141 iso 2. This is not unlikely considering that L141 iso 2 was derived from parents which carried the ARM a gene (see section 1). Examination of larger numbers of L141 iso 2 tetrads will determine the segregation of ARM a and ARM α in this cross.

Another unexpected sporulation characteristic of L141 iso 2 is that none of the meiotic products of this cross support sporulation in diploids formed by mating with Mat a strains (Table RXXVII). Mat a/mat a* ARM _/+ diploids shown in Table RXXV and also the Mat a/Mat a ARM α/ARM α diploid, A11.B7B, are able to sporulate. Therefore, it was expected that diploids formed by mating Mat a cells with L141 iso 2 segregants containing ARM α would also sporulate.

A possible explanation for this sporulation inability is that the ARM α is not stable in the nonsporulating diploids. This idea is supported by observations of decrease with the passage of time, of the ability of Mat a ARM α strains to mate with Mat a cells.

Additional crosses involving the ARM gene

Cross 114 Mat a ARM α x Mat a +

Strain A11.B7B.9B Mat a ARM α

Strain 103.1B Mat a his_1

Cross 114, a rare mating, produced diploid colonies at low frequency but considerably higher frequency than rare matings between Mat a wild type cells.

Nine tetrads from Cross L114 were examined. All showed 2 Mat a cry_1^r : 2 Mat α cry_1^s segregation of mating type indicating that a switch of mating type of the ARM α containing parent had occurred in the formation of this strain.

Eight of 18 Mat α spores examined allowed sporulation in diploids formed by mating with mat a* strains. This indicates that ARM a segregates among the α spores of L114.

In this isolated case, an ARM α gene facilitated rare mating between Mat a cells, mating resulted from switch of mating type by the ARM α containing parent strain. Experiments are planned to determine what proportion of the diploid colonies arising from ARM α facilitated Mat a rare matings are a/α diploids.

Cross L113

Strain A11.B7B.1B Mat a ARM α

Strain C60.8A Mat α

Twenty-two tetrads were examined. All show 2 Mat a : 2 Mat α segregation. The ARM a gene segregates among the

meiotic progeny of L113.

Discussion

In certain ways, the ARM α gene is similar to the ARM a gene. Both allow otherwise prohibited sporulation of diploids which are homozygous for the mating locus. Both genes also enhance rare mating frequencies. In contrast, ARM a appears to supply Mat a information, or replaces Mat a function, in allowing anomalous sporulation and in enhancing rare mating frequencies. ARM α appears to replace Mat α information in performing these functions.

One explanation of the apparently analogous activities of ARM a and ARM α is that both are forms of cryptic mating locus cassette information. ARM α is proposed to be a Mat α (HMa) cassette which has become inserted at the ARM a locus. Under certain conditions mating type cassettes can insert at other cassette locations, i.e. HMa can insert at and replace HMα, instead of inserting at the mating locus as it normally does during homothallic switch of mating type (Haber, 1980). For example, a strain carrying the Mat α^{stk} mutation which prevents homothallic switch of mating type, can, at low frequency, change from HMa, HMα to HMa, HMa. The possibility that ARM α originated as the result of insertion of HMa information at the ARM a locus is examined in the Discussion section of this thesis.

Conclusions

The ARM α gene was first discovered in a Mat a/Mat a diploid strain which was isolated as a meiotic product of an a/a/α/α tetraploid homozygous for the ARM gene.

The ARM α gene has the following properties:

1) Mat a/Mat a ARM α/ARM α is able to sporulate.

2) Mat a ARM α shows enhanced rare mating frequency in crosses with Mat a.

3) Mat a/mat a* ARM α/+ sporulates.

4) mat a* ARM α mates confluently with strains of either mating type.

CHAPTER VII

A Cross Between a Cassette Deficient Homothallic Strain and an ARM Gene Containing Strain

Introduction

The ARM gene increases the rate of mating type switch from α to a in heterothallic strains (Chapter III, Table RXII). In this section, the effect of the ARM gene on homothallic switch is examined. The ARM gene may be expected to influence homothallic switch in at least two ways. First, if the ARM gene contains a copy of Mat a (see Chapter III and Discussion), then this gene may be able to support homothallic switch in HMR(a) (silent cassette a) deficient strains. Another influence that the ARM gene may have is in homothallic regulation. In a/α diploids the Ho gene is inactive. The ARM gene which can replace the Mat a influence in sporulation, might be expected to exert similar influence in the regulation of the Ho gene in Mat α Ho ARM cells.

In order to examine the effect of the ARM gene on homothallic switch, cross L110 was performed.

Cross L110

Strain I11.102.1D Mat a ho HML(α) HMR(a) ARM ura_1 lys_1 lys_2

Strain 6D131.10B Mat α Ho HML(α) HMR(α) leu_1 ura_2

Expected segregation of mating ability:

Strain 6D131.10B, although it contains the Ho gene for

homothallism, cannot switch mating type because it lacks the HM allele necessary for switch from α to a mating type (see Introduction, section). According to the cassette theory of mating locus interconversion (Introduction, section), this strain can be visualized as follows:

Mat α

Ho

HML(α) HMR(α)

The other parental strain, I11.102D, is heterothallic and contains a copy of HMα.

A cross between Mat α Ho HML(α) HMR(α) and Mat a ho HML(α) HMR(a) is expected to give rise to meiotic products of which 25% are expected to be of the genotype Ho HML(α) HMR(a) and therefore able to undergo homothallic switch of mating type. In the Cross 110, deviations from this expectation can be the result of the influence of the ARM gene.

Results

L110 meiotic segregants displaying the following mating activities were observed:

- α' — confluent mating with a tester strains
- a' — confluent mating with α tester strains
- a' > α' — high frequency mating as an a strains (with α tester), lower frequency mating as an α strain
- α' > a' — high frequency mating as α, lower frequency as a

a',α' confluent mating with both a and α tester strains

n.m. mates at very low frequency or does not mate with either tester strain

The meiotic products of Cross L110, shown in Table RXXVIII contain a higher proportion of homothallic segregants than is expected if the ARM gene were not influencing this segregation. These homothallic segregants are unusual in their high proportion of a' > α' mating strains. These results are consistent with an explanation that the ARM gene is capable of substituting for $HMR_{(a)}$ in allowing homothallic mating type switch. However, in the absence of suitable controls this conclusion is preliminary. Controls which are needed include further characterization of both parental strains to eliminate the possibility that properties of these strains, other than the ARM gene influence, are contributing to the observed unusual segregation of mating activity.

Conclusions

A cross between an $HMR_{(a)}$ cassette deficient homothallic strain (6D131.10B, Mat _ Ho, $HML_{( )}$ $HMR_{( )}$) and an ARM gene containing strain (I11.102D, Mat a ARM) produces a higher proportion of homothallic meiotic segregants than is produced if the ARM gene does not influence homothallism.

Table RXXVIII

Comparison of the expected and the observed segregation of mating activity among meiotic segregants of L110

Mating activity	Observed segregants	Observed percents		Expected percents	Expected genotypes
a'	57	22.4%		25%	Mat a ho HML(α) HMR(a)
					Mat a ho HML(α) HMR(α)
α'	63	24.8%		50%	Mat α ho HML(α) HMR(a)
					Mat α ho HML(α) HMR(α)
					Mat α Ho HML(α) HMR(α)
					Mat a Ho HML(α) HMR(α)
a' > α'	70	27.6%			
α' > a'	14	5.5%	52.8%	25%	Mat a Ho HML(α) HMR(a)
a', α'	38	15.0%			Mat α Ho HML(α) HMR(a)
n.m.	12	4.7%			
Total	254				

DISCUSSION

The Results section of this thesis describes two genes, the dmt gene and the ARM gene. Mat α dmt cells are able to mate with Mat α wild-type cells at a considerably greater frequency than Mat α wild type cells can mate with each other. The diploid cells produced by these dmt gene-influenced matings do not contain active Mat a information and consequently are unable to sporulate. The ARM gene was discovered to be able to allow sporulation in diploid cells containing Mat α but lacking Mat a information. Cells of the genotype Mat α dmt were mated to Mat α ARM cells. The diploids produced by this dmt gene-influenced mating were able to sporulate allowing the genotypes of these diploids to be determined. The information obtained from this analysis supports a model of dmt gene induced rare matings whereby Mat α dmt cells, as a result of dmt gene-induced loss of Mat α information, are able to mate as a mating type cells.

We also discovered that the ARM gene, independent of the action of the dmt gene, increases the frequency of mating between Mat α cells. This is a result of the increased frequency of spontaneous switch of mating type observed in Mat α ARM strains as compared to wild-type Mat α strains.

In Chapters V, VI, VII certain additional properties of the ARM gene are described. It is our view that the ARM gene is a form of expressed Mat a information which is

distal to the thr_4 locus on chromosome III.

In this Discussion, certain as yet unanswered questions about the dmt gene and the ARM gene are considered.

I The dmt Gene

The focus of the work presented in this thesis, as it concerns the dmt gene, is on the influence of this gene on rare matings between Mat α cells. Certain additional ideas concerning the dmt gene are presented here.

1) The Influence of the dmt Gene on Genetic Loci which are not on Chromosome III

Certain cells carrying the dmt gene were shown to have lost chromosome III information (Chapters II and IV). Is the action of the dmt gene specific to chromosome III, or does the dmt gene cause information loss in other chromosomes as well?

Solomon Bryski (1978) observed the effect of the dmt gene on a variety of genetic markers both on chromosome III and elsewhere. He constructed homozygous dmt strains which were heterozygous for a number of recessive auxotrophic genetic markers. Thousands of subclones of each of these constructed strains were screened in search of subclones which express recessive genetic markers. This expression of recessive genes in subclones of diploids heterozygous for these genes was taken to indicate loss of dominant genetic

information in these subclones. Similar experiments were performed using control diploid strains containing the same auxotrophic genetic markers but not containing the dmt gene. The results of these studies are as follows:

a) In homozygous dmt diploids, approximately 1.0% of subclones screened expressed recessive genetic markers which had been heterozygous in the parental colony. These recessive genetic loci were on chromosome I, II, III, V, XI, XII and XV. In control strains, not containing the dmt gene, only between 0.01 and 0.1 percent of diploid subclones screened expressed recessive genetic markers.

b) Recessive markers linked to Mat a were shown to be expressed as often as recessive markers linked to Mat α in homozygous dmt strains.

c) Linked recessive genetic markers, for example lys_2 and tyr_1 on chromosome II, were shown to be expressed in the same diploid subclones.

d) Whereas the dmt gene appeared to increase the frequency at which heterozygous auxotrophic strains lost dominant genes, not all dominant genes which were lost were lost at the same frequency. For example, the frequency of dmt gene associated loss of the ADE_1^+ allele on chromosome I was much greater than the frequency of loss of the URA_3^+ allele on chromosome V or the URA_1^+ allele on chromosome XI.

In conclusion, the dmt gene appears to cause loss of information which is not on chromosome III. Different loci

under the influence of the dmt gene, are lost at different frequencies, suggesting that the loci of information loss (dmt gene action) are not randomly distributed.

2) Strains of the Genotype Mat α $HM_L\alpha$ $HM_R\alpha$ Show Mating Patterns Similar to α dmt Strains

Observations by Jim Hicks (personal communication), Harry Greunspan (1979) and ourselves show that α strains containing Ho but lacking a silent Mat a cassette (HM_{Ra}) show enhanced ability to undergo rare mating with α strains. One explanation is that these strains, while in a transition state of a homothallic switch which they are incompetent to complete, are unable to express Mat α information. These cells would therefore mate as a cells during the absence of negative regulation of a specific genes by Mat α.

It would be of interest to screen the products of these rare matings for chromosome III marker loss to determine if, in this way, these diploids are similar to those formed by rare matings which involve the dmt gene, and if a similarity in rare mating mechanism is suggested.

3) Repair of dmt Gene Induced Damage with Homologous Information

As the result of rare matings between Mat α + and Mat α dmt cells, certain diploid cells are formed which are homozygous for certain chromosome III genes despite the fact that each parent contained a different allele of these genes (Melnick and Blamire, 1978; Chapter IV of this thesis).

For example, the cross Mat α cry_1^s dmt x Mat α cry_1^r + produced diploids of the genotype Mat α/Mat α cry_1^r/cry_1^r dmt/+. The missing cry_1^s allele is proposed to have been lost in one of the haploid parent cells by action of the dmt gene in this cell. It is further proposed that after mating, the lost information becomes replaced with information from the homologous, nondamaged chromosome III of the non-dmt parent.

The dmt gene rare mating system described in Chapters II and IV supplies a means of studying this repair event. It would be of interest to characterize this repair with respect to, for example, the extent of information that can be repaired in this way and the genetic requirements which allow competence in this repair mechanism.

II The ARM Gene

In Chapter III the nature of the ARM gene was discussed. Two alternative ideas were proposed. Either the ARM gene is a mutation in a gene which is directly involved in the events which allow sporulation, or alternatively, the ARM gene is a form of Mat a information which, in cells otherwise lacking Mat a, complements Mat α to allow sporulation. In this discussion, a number of observations are presented which support the idea that the ARM gene is a form of partially expressed Mat a information, and further experiments are suggested to confirm this possibility.

1) Sporulation Influence of the ARM Gene

The ARM gene is dominant and allows sporulation in α/α but not a/a diploids. This is precisely the expectation of a gene which expresses Mat a information. The ARM gene differs from Mat a in that, whereas a/α cells are unable to mate, Mat α ARM cells mate as Mat α. This situation may be similar to the expression of sporulation functions in α/α diploid cells homozygous for the sir_1 gene (Rine and Herskowitz, 1979). These cells are proposed to be allowed to sporulate as a result of expression of Mat a information at the HM_Ra cassette, however, these cells are able to mate. Herskowitz et al. (1980) propose that the sir_1 gene allows only low level expression of cassette information, sufficient to allow sporulation but insufficient to confer the nonmating phenotype of a/α cells.

2) Influence of the ARM Gene on Heterothallic Mating Type Switch

Heterothallic Mat α ARM cells switch mating type at increased frequency as compared to wild-type heterothallic Mat α cells (Chapter III, section 2b). The same regulatory mutation that causes expression of Mat a information from the cryptic ARM gene could also be responsible for the anomalous switch function of the ARM gene.

3) ARM Gene Instability

A regulatory mutation which allows anomalous patterns

of switch of a mating locus cassette might also interfere with the stability of this gene. For example, incomplete "switches" could conceivably result in removal of cassette information without integration of this information.

4) The ARMα Mutation Appears in a Segregant Derived from Homozygous ARM_a Parents (Chapter VI)

The ARMα gene appears to express Mat α information but is not at the mating locus. ARMα appears to parallel ARMa in function while expressing Mat α instead of Mat a information. The ARMα gene could possibly have originated as a result of insertion of Mat α or $HM_{L\alpha}$ information at a proposed ARMa locus which had contained Mat a information. As suggested previously, if ARMa contains a regulatory mutation allowing partial or low level expression of a mating type cassette at this locus, then a new cassette inserted at this locus might also be affected by the same mutation and therefore exhibit partial or low level expression. Under certain conditions, mating type cassettes have been observed to "switch" the type of mating locus information within those cassettes (Haber, 1980).

A straightforward method of determining if the ARM gene contains Mat a DNA is to determine if any homology can be detected between the ARM gene and between a radioactively labeled DNA probe containing the mating locus gene. This method was used by Klar et al. (personal communication) in determining that the SAD gene (Hopper and MacKay, 1980;

Kassir and Herskowitz, 1980) which has a number of similar properties to the ARM gene, contains Mat a DNA.

5) Unusual Segregation of Genetic Markers in Crosses Involving the ARM Gene

In certain crosses where one or both parent strains carry the ARM gene, and where one parent carries the thr_4^- allele and the other the THR_4^+ allele, unusual segregation of thr_4 alleles is observed (see Table RXVIII, Chapter V). Certain of these crosses produce an unusually high proportion of tetrads in which three out of four of the spores express THR^+. In Chapter V it is suggested that this unusual segregation is the result of an extra THR^+ allele contributed to the cross by one of the parents. In certain other crosses, a high proportion of the tetrads display 3- : 1+ segregation of thr_4 alleles. This segregation is difficult to explain because an extra segregating thr_4^- allele would not be expressed in a strain which also carries a dominant THR_4^+ allele. It is important to establish whether or not unusual thr_4 segregation is caused by the ARM gene. One approach towards determining this possibility is to work with ARM gene containing parent strains which are shown to display unusual thr_4 segregation in crosses with wild-type strains. Since the ARM gene is unstable (Chapter III), it should be possible to select subclones of ARM gene parents which have lost the ARM phenotype. If reversion of the ARM phenotype results in loss of unusual thr_4

segregation in these strains, then this would suggest a causal relationship between the ARM gene and the observed unusual thr_4 segregation.

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