MATING-TYPE MUTATIONS IN *SCHIZOSACCHAROMYCES POMBE*: ISOLATION OF MUTANTS AND ANALYSIS OF STRAINS WITH AN h^- or h^+ PHENOTYPE[1,2]

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ABSTRACT

Mutants defective in various steps of the sexual cycle have been isolated from homothallic strains of *Schizosaccharomyces pombe* by BRESCH, MÜLLER and EGEL (1968). These mutants include heterothallic $h+$ and h^- strains. We have isolated additional $h+$ and h^- mutants from homothallic strains. Those mutants which are due to mutations in the mating-type region were analyzed in detail. Our results show that the mating-type gene *mat2* not only has a function in copulation and meiosis, but that it also regulates the formation of the *map1* gene product (*map1* is a mating-type auxiliary gene). Some of the h^- mutants have lost only one of the three functions while others are defective in at least two, and perhaps all three, functions. Further, we show that the *mat1*$^-$ allele of h^{90} strains can mutate to *mat1*+ but that mutations in *mat2* appear to affect the mutational behavior of *mat1*. Finally, we describe a new inactive mating-type allele, *mat2**, which is different from *mat2*^{0} in that it can mutate to *mat2*+.

IN this paper we report experiments on the mating-type genes of *Schizosaccharomyces pombe*. *S. pombe* is a haploid fission yeast; its life cycle and genetics have recently been reviewed by GUTZ *et al.* (1974).

Homothallic and heterothallic mating types in *S. pombe* (LEUPOLD 1950) are determined by a short region on chromosome II (LEUPOLD 1958; GUTZ *et al.* 1974). LEUPOLD (1958) distinguished two closely linked subunits in the mating-type region. Whether these subunits are part of one gene or whether they represent two distinct genes is not known. Further, the use of the term "gene" in connection with the subunits may be an oversimplification; both subunits may be part of one gene cluster or may represent two different gene clusters rather than single cistrons. LEUPOLD (1958) has proposed a two-gene model for this region; the postulated mating-type genes are now called *mat1* and *mat2* (GUTZ and DOE 1973). The various mating types known in *S. pombe* and their interpretation in LEUPOLD's two gene scheme are summarized in Table 1. The *mat1* gene can

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TABLE 1

Mating types of Schizosaccharomyces pombe

Phenotype	Mating-type symbol	Genotype*	Spontaneous mutation to:†
homothallic	h^{90}	*mat1*$^-$ *mat2*+	h^+, h^-
heterothallic + (h^+)	h^{+N}	*mat1*+ *mat2*+	h^{90}, h^{-U}
	h^{+R}	*mat1*+ *mat2*^{0}	h^{-S}
heterothallic — (h^-)	h^{-S}‡	*mat1*$^-$ *mat2*^{0}	none
	h^{-U}§	?	h^+, h^{90}

* The genotypes were postulated by LEUPOLD (1958). The genes *mat1* and *mat2* are 1.1 map units apart and cooperate in the determination of the mating reaction. The superscripts + and — indicate alleles of opposite heterothallic function; the superscript 0 indicates an inactive allele. Originally, LEUPOLD used the gene symbols *h1* and *h2*; GUTZ and DOE (1973) replaced these by *mat1* and *mat2*.

† Compiled from LEUPOLD (1958); BRESCH, MÜLLER and EGEL (1968), and GUTZ and DOE (1973).

‡ h^- in LEUPOLD (1958); the symbol h^{-S} was introduced by GUTZ and DOE (1973).

§ The mating type h^{-U} was described by GUTZ and DOE (1973); its genotype has still to be determined by recombination experiments.

have one of two complementary active alleles, + or —, while *mat2* has either a + allele or an inactive 0 allele. As can also be seen from Table 1, the various mating types are characterized by specific patterns of spontaneous mutation.

For two haploid cells to copulate, one cell must have at least one *mat*$^+$ allele and the other at least one *mat*$^-$ allele. Although the diploid phase of *S. pombe* is normally confined to the zygote, diploid strains can also be obtained. Experiments with diploid strains showed that cells must have at least one *mat*$^+$ and one *mat*$^-$ allele in order to initiate meiosis (LEUPOLD 1958; EGEL and EGEL-MITANI 1974). Therefore, the mating-type loci are required for copulation and meiosis.

Nonsporulating mutants were isolated from h^{90} strains by BRESCH, MÜLLER and EGEL (1968); as explained in MATERIALS AND METHODS, they are easily recognized by treating colonies with iodine vapors. These mutants can be divided into five general groups. (1) Mutants which are sterile, i.e., they can neither copulate among themselves, nor with h^{+N}, h^{-S} or h^{90} strains. (2) Phenotypic h^+ strains which copulate and sporulate with h^{-S} and h^{90} strains, but not with h^{+N}. (3) Phenotypic h^- strains which copulate and sporulate with h^{+N} and h^{90} strains, but do not sporulate with h^{-S} strains. Several mutants in this group can still self-copulate and can copulate with h^{-S} strains (the resulting zygotes are blocked in meiosis). (4) Mutants which start to self-copulate but are unable to complete cell fusion. (5) Mutants which can self-copulate and copulate with h^{+N}, h^{-S} or h^{90} strains but sporulate only in the latter three crosses.

The mutants in groups (2) to (5) were studied in more detail (BRESCH, MÜLLER and EGEL 1968; EGEL 1973a, 1973b; EGEL and EGEL-MITANI 1974). Not all are located in the mating-type region. Some 27 other loci involved in different steps of the sexual cycle were identified. Mutants of groups (2) and (3) either have mutations in the mating-type region or in a mating-type auxiliary gene (*map1*, *map2*, *mam1*, or *mam2*). Mutants in groups (4) and (5) represent 23 genes required for cell fusion (*fus1*), meiosis or sporulation.

One of the h^- mutants of group (2), B102, is of particular interest. B102 has a mutation in the mating-type region, is still able to self-copulate like the h^{90} strain from which it was isolated, but has lost the capability to self-sporulate. Also, B102 copulates and sporulates with h^{90} and h^{+N} strains; it can copulate with h^{-S} strains, but the resulting zygotes do not undergo meiosis (EGEL 1973a; EGEL and EGEL-MITANI 1974). In previous publications the mutant B102 was called *mei*-1-102 (BRESCH, MÜLLER and EGEL 1968), *mei*-1 (B102) (EGEL 1973a), and *mei*1 (B102) (EGEL and EGEL-MITANI 1974); the corresponding gene was called *mei*-1 or *mei*1. We will refer to this strain by its original isolation number, B102; since the mutation present in B102 appears to be in *mat2*, we designate it *mat2*+-B102.

In this paper, we report additional h^+ and h^- mutants from h^{90} strains, and an analysis of those in the mating-type region. We show that *mat2* not only has a function in copulation and meiosis, but is also required for the formation of the *map1* gene product. Mutations in *mat2* either inactivate all three functions simultaneously or each function independently. We also find that *mat2* appears to affect the mutational behavior of *mat1*.

MATERIALS AND METHODS

Strains: The strains and their genotypes are listed in Table 2; several of these cultures were obtained from Dr. U. LEUPOLD (Berne, Switzerland), DR. C. BRESCH and DR. R. EGEL (Freiburg im Breisgau, Germany). All strains were derived from those originally introduced by LEUPOLD (1950, 1958) into genetic research. The h^{+N} strains (*mat1*+ *mat2*+) contain the mating type of the L975 culture and the h^{-S} strains (*mat1*$^-$ *mat2*^{0}) containing the mating type of the L972 culture. All h^{90} strains are independently isolated spontaneous mutants from h^{+N} strains. From our h^+ mutants, we isolated spontaneous h^- mutants; the latter are designated by a Roman numeral following the original strain number (e.g., JM31-I). Strains isolated from tetrads are designated by a T before the isolation number.

SG14 (h^{90}) and its derivatives contain an additional mutation, which decreases growth on MMA plates and, in particular, on MMA plates supplemented with histidine. To eliminate this mutation from four strains, asci were dissected from crosses of JM35 × SG173, JM 48 × SG173, SG16 × JM35-I, and SG16 × JM48-I, and strains containing the same h^+ or h^- mutations (and *leu1* gene) as the above mutants, but no longer containing this mutation, were isolated. These new strains are designated JM35A, JM48A, JM35-IA and JM48-IA, respectively.

Media: Yeast-extract agar (YEA), malt-extract agar (MEA), minimal agar (MMA) and synthetic sporulation agar (SPA) are described elsewhere (GUTZ *et al.* 1974). The media were supplemented with adenine (75 mg/l), leucine (50 mg/l) and histidine (100 mg/l) as necessary.

Mating-type tests and mating-type terminology: Ascospores of *S. pombe* contain a starch-like compound not found in vegetative cells. A simple method for the detection of spores is to treat colonies, or replicas of colonies on lawns of L975 (h^{+N}) or L972 (h^{-S}), with iodine vapors: material containing spores turn black (positive iodine reaction) while material containing only vegetative cells turns yellow (negative iodine reaction). This technique is useful for the detection of mating-type mutants as well as for routine mating-type test (LEUPOLD 1970; GUTZ and DOE 1973; GUTZ *et al.* 1974). Vegetative cells of *vir1* mutants (*vir*: vegetative iodine reaction) also show a positive iodine reaction (MEADE and GUTZ 1975); these mutants can be easily distinguished from spore-forming material by microscopic examination.

When strains are known to have a h^{+N} or h^{-S} mating type, these symbols will be used. For newly isolated heterothallic + or — mutants, we will use the less specific symbols h^+ and h^-.

TABLE 2

Genotypes of Schizosaccharomyces pombe *strains*

Strain	Genotype*
Wild type:	
L975 (ATCC 24970)	*mat1*+ *mat2*+
L972 (ATCC 24969)	*mat1*$^-$ *mat2*^{0}
Parents of mutants:†	
L975 (h^{90})	*mat1*$^-$ *mat2*+
SG14 (h^{90})	*leu1 mat1*$^-$ *mat2*+
M216 (h^{90})	*mat1*$^-$ *mat2*+ *ade6*–M216
M26 (h^{90})	*mat1*$^-$ *mat2*+ *ade6*–M26
L84 (h^{90})	*mat1*$^-$ *mat2*+ *ade7*–L84
h$^-$ mutants from C. BRESCH and R. EGEL‡	
A10	*mat1*$^-$ *mat2*+–A10
A11	*mat1*$^-$ *mat2*+–A11
A91	*mat1*$^-$ *mat2*+–A91 *ade6*–M210
A149	*mat1*$^-$ *mat2*+–A149
A204	*mat1*$^-$ *mat2*+–A204 *ade6*–M210
A207	*mat1*$^-$ *mat2*+–A207 *ade6*–M210
B102	*mat1*$^-$ *mat2*+–B102 *ade6*–M210
B266	*mat1*$^-$ *mat2*+–B266 *ade6*–M210
Other haploid strains:	
SG16	*mat1*+ *mat2*+ *his2*
SG378	*leu1 mat1*+ *mat2*+ *his2*
SG393	*mat1*$^-$ *mat2*^{0} *his2 ade6*–M210
JM111	*mat1*+ *mat2*+ *fus1*–B20 *vir1 ade6*–M210
A80	*mat1 mat2*+ *map1*–A80 *ade6*–M210
B20	*mat1*$^-$ *mat2*+ *fus1*–B20 *ade6*–M210
Diploid strains:	
JM218‡§	*mat1*$^-$ *mat2*+–JM161 *fus1*–B20 *ade6*–M210 / *mat1*$^-$ *mat2*+–JM161 *fus1*–B20 *ade6*–M216
JM247	*mat1*$^-$ *mat2*+ *map1*–A80 *ade6*–M210 / *mat1*$^-$ *mat2*+ *map1*–A80 *ade6*–M216

* Gene symbols: *mat* (mating-type gene), *leu* (leucine auxotroph), *ade* (adenine auxotroph), *his* (histidine auxotroph), *fus1* (prevents fusion between cells of compatible mating types), *vir1* (produces a positive iodine reaction in vegetative cells), and *map1* (mating-type auxiliary gene required for plus activity; necessary both for copulation and meiosis). Where multiple alleles are known for a particular gene, the heteroalleles are given after the gene symbol (e.g. *ade6*–M216, *ade6*–M210 and *ade6*–M26).

† From these h^{90} strains, we isolated non-sporulating mutants. For simplicity, the h^{90} strains have the same collection number as the h^{+N} strains from which they were isolated, but the h^{90} mating type is given in brackets behind the numbers for distinction.

‡ The h^- mutants donated by C. BRESCH and R. EGEL and the diploid strain JM218 are listed as having mutant *mat2* alleles. This point will be proven in this paper.

§ This diploid strain was prepared from a mutant, JM161, isolated in our experiments.

Nitrous-acid mutagenesis: Nitrous acid was used for the induction of nonsporulating mutants from h^{90} strains. The strains were grown for 48 hours on YEA slants, suspended in 0.2 M acetate buffer (pH 4.0), washed twice with this buffer, and resuspended to a final titer of approximately 2×10^7 cells/ml. Following the incubation of these suspensions for 10 minutes at 30°, 0.3 M sodium nitrate was added to a final concentration of 0.03 M. At various times (3 to 7 minutes) samples were removed and appropriately diluted with 0.2 M phosphate buffer (pH 7.2). The mutagen treated cells were plated on MEA and incubated for three days at 30° and two days at

25°. The above nitrite treatment resulted in an inactivation of 45 to 97%. The MEA plates were treated with iodine vapors. Many of the colonies had iodine-negative sectors and a few homogeneous iodine-negative colonies were also found. Material from sectored colonies were restreaked on MEA; from each of these purification streaks, only one homogeneous iodine-negative colony was saved. In each experiment, in order to avoid selection of duplicate mutants of the same origin, no more than one homogeneous iodine-negative colony was picked from the original MEA plates.

Reversion experiments with ultraviolet light: We irradiated several of our h^- mutants with UV in order to test whether they could revert to homothallism or mutate to a h^+ mating type. Cells grown for 48 hours on YEA slants were suspended in 0.05 M K_2PO_4, washed, and diluted to a final titer of approximately 1×10^5 cells/ml. The cells were irradiated at 2000 ergs/mm^2 and all further manipulations were done under yellow lights. After irradiation, the cells were diluted, plated on MEA and incubated for three days at 30° and two days at 25°. Approximately 15% of the cells survived. The plates were treated with iodine vapors and checked for the presence of iodine-positive sectors or colonies.

Other experimental techniques: In *S. pombe*, crosses can be analyzed by tetrad analysis with the use of micromanipulators or by the plating of large numbers of free spores. These procedures, as well as other experimental techniques used with this yeast, have been reviewed in detail by LEUPOLD (1970) and GUTZ *et al.* (1974). In most crosses two flanking markers for the mating-type region, *leu1* (11.3 map units) and *his2* (1.1 map units), were included.

EXPERIMENTS AND RESULTS

Isolation of mutants: We were interested in isolating, from h^{90} strains, mutants that were h^+ and h^- due to mutations in the mating-type region. Following nitrous-acid mutagenesis of three h^{90} strains, 450 iodine-negative sectors were picked and restreaked on MEA in order to obtain pure isolates. A total of twelve spontaneous mutants were also isolated. All mutants were tested for their ability to form spores in crosses with L975 (h^{+N}) or L972 (h^{-S}). Fifty-three h^+ mutants (able to form spores with L972) and forty-one h^- mutants (able to form spores with L975) were identified. The remaining mutants were either sterile or contained mutations in fusion, meiosis or spore formation genes and are not discussed further.

From previous studies (EGEL 1973b) it was known that h^+ and h^- mutants isolated from h^{90} strains could be due to mutations in the mating-type region or in one of four mating-type auxiliary genes which are not closely linked to the mating-type region. These possibilities can easily be distinguished by examining the progeny from crosses between a mating-type mutant and either L975 or L972. Strains which contain mutations in the mating-type region are expected to yield approximately 1% or less h^{90} spores while the auxiliary gene mutants are expected to yield approximately 25% h^{90} spores. The 53 h^+ and 41 h^- mutants were crossed with L972 and L975, respectively. From these crosses, free ascospores were streaked in a manner that, after incubation, approximately 200–500 single colonies were present on each plate. The plates were treated with iodine vapors. Thirty-two of the 53 h^+ strains and 12 of the 41 h^- strains yielded only a few h^{90} colonies and thus seemed to have mutations in or close to the mating-type region. The remaining mutants yielded many h^{90} colonies and thus contain mutations in auxiliary genes. We will describe the properties of the h^- and h^+ mutants separately.

I. *The h⁻ mutants*

Characterization of the h⁻ *mutants*: In this section we describe the properties of those 12 h^- mutants which seem to have mutations in the mating-type region. We include 8 h^- mutants (also having mutations in the mating-type region) isolated by Drs. C. BRESCH and R. EGEL. The various h^- mutants are listed in Tables 2 and 3.

The 20 h^- mutants were mapped in crosses with h^{+N} strains. From each cross ascospores were plated on MEA and, following incubation, the plates were treated with iodine vapors and the total number of colonies and the number of h^{90} colonies determined. The low frequency of h^{90} progeny (.0032–.0098) justifies the conclusion that our strains contain mutations in the mating-type region.

During several years the 20 h^- mutants were streaked repeatedly on MEA and no iodine-positive material due to reversion to h^{90} or mutation to h^+ was found. Thus, the h^- mutants appear to be stable. Furthermore, we irradiated the mutants JM54, JM55, JM56, and B102 with UV (approximately 15% survival). Approximately 30,000 colonies from surviving cells were examined from each mutant. No h^{90} or h^+ mutants were found.

Strains B102 and B266 are able to self-copulate but are blocked at an early stage of meiosis (BRESCH, MÜLLER and EGEL 1968; EGEL 1973a). We tested our h^- mutants for self-copulation. All h^- strains were spotted on MEA plates, incubated at 25° and 2–5 days later samples were picked and examined microscopically. All strains, except B102 and B266, contained only vegetative cells. B102 and B266 contained many zygotes but no spores or asci (Table 4).

Complementation studies with B102: Each h^- strain, including B266, was mixed with B102 to test complementation for meiosis and spore formation. Within 5 days 9 of the 19 mixtures contained zygotic asci in addition to zygotes and vegetative cells. Data are summarized in Table 4.

Production of map1 *substance by the* h⁻ *mutants*: EGEL (1973b) reported that the *map1* gene is necessary for copulation and meiosis. EGEL noted that h^{90}/h^{90} *map1/map1* diploids (e.g. strain JM247), while unable to self-sporulate, can be induced to sporulate (azygotic asci) if h^{+N} *map1*$^+$ or h^{90} *map1*$^+$ cells are present in the same culture. The exact function of *map1* is not yet known, and

TABLE 3

h⁻ *mutants*

Experiment	Parent strain	Nitrous acid treatment	Mutants isolated
1	SG14(h^{90})	3 minutes (~55% survival	JM54
		4.5 minutes (~12% survival)	JM55
		7 minutes (~3% survival)	JM56
2	L975(h^{90})	none (spontaneous mutant)	JM103
3	M216(h^{90})	none (spontaneous mutant)	JM108
4	M25(h^{90})	5 minutes (~8% survival)	JM150, JM161, JM164
5	L84(h^{90})	5 minutes (~8% survival)	JM167, JM169, JM171, JM178

TABLE 4

Properties of h^- mutants

Mutant class		Ability to self-copulate	Ability to complement B102	Ability to induce JM247 to sporulate*	Sporulation of diploid strains†	
					Self-Sporulation	induction by h^{+N} and h^{90}
Class I:	B102, B266	+	—	+	—	‡
Class II:	JM54, JM103, JM108, A10, A11, A91, A159, A204, A207	—	—	—	—	‡
Class III:	JM161, JM167, JM171, JM178	—	+	—	—	+§
Class IV:	JM55, JM56, JM150, JM164, JM169	—	+	+	+	‖

* JM247 is the diploid strain $\frac{mat1^- mat2+ \; map1\text{–A80} \; ade6\text{–M210}}{mat1^- mat2+ \; map1\text{–A80} \; ade6\text{–M216}}$.

† These diploid strains are homozygous for the shown mating-type mutants.

‡ Not tested; these mutants would not be expected to be induced to sporulate by h^{+N} or h^{90} strains since they have *mat2+* alleles nonfunctional for meiosis. The products of the mating-type alleles are not diffusible (EGEL 1973b).

§ Only JM161 was tested.

‖ Not applicable since class IV mutants can self-sporulate.

attempts to isolate its product have so far been unsuccessful (EGEL 1973b, personal communication). We found that h^{-S} *map1+* strains cannot induce JM247 to sporulate. We will call the inducing principle the *map1* substance.

It was of interest to determine if any of the h^- mutants could induce JM247 to sporulate. We mixed JM247 with each of the h^- mutants (including B102 and B266) and checked after 2–4 days for the presence of azygotic asci. Of the 20 mixtures, seven contained azygotic asci (see Table 4). From the mixture of JM247 with JM56, 21 asci were dissected. From 19 of the asci all four spores germinated; each tetrad showed a segregation 2 h^{90} *map1 ade6*-M210 : 2 h^{90} *map1 ade6*-M216.

Occasionally, we observed iodine-positive material in colonies of JM55, JM56, JM150, JM164 and JM169. This reaction is due to the presence of azygotic asci. Fourteen asci were dissected from JM56, JM150 and JM164. From 38 of the 42 asci, all 4 spores germinated. In all cases the tetrads had 4 h^- spores and carried the markers of the strain from which they were isolated. The asci, therefore, were not due to contamination by another strain or to spontaneous mutation of the h^- mutants to h^+ or h^{90}. These asci apparently originated from diploid cells in the cultures. *S. pombe* strains usually contain a few diploid cells which probably originate by endomitosis (LEUPOLD 1955).

Iodine-positive material was never observed in colonies of JM161, JM167, JM171 and JM178, although diploid cells should also be formed spontaneously in these cultures. A diploid strain (JM218)

$$\frac{mat1^- mat2+\text{-JM161}}{mat1^- mat2+\text{-JM161}} \quad \frac{fus1\text{-B20}}{fus1\text{-B20}} \quad \frac{ade6\text{-M210}}{ade6\text{-M216}}$$

was constructed. JM218 did not sporulate by itself or when grown in culture with L972 (h^{-S}) or A80 (h^{90} *map1*). Azygotic asci were formed, however, when

JM218 was mixed with B20 (*h*^{90} *fus1*) or JM111 (*h*$^{+N}$ *fus1*). Twenty-seven asci were dissected from the mixture with B20. From 17 asci all four spores germinated; each tetrad contained 2 *h*$^{-}$ *fus1 ade6*-M210 and 2 *h*$^{-}$ *fus1 ade6*-M216 spores. JM218, though not able to sporulate directly, can, like JM247 (*h*^{90}/*h*^{90} *map1/map1*), be induced to sporulate if mixed with *h*$^{+N}$ or *h*^{90} strains (Table 4).

Tetrad analyses from crosses of four h^{-} *mutants with SG16*: A few *h*^{90} spores were found among the progeny from crosses of the *h*$^{-}$ mutants with *h*$^{+N}$ strains. To obtain more detailed information on the origin of these *h*^{90} spores, tetrad analyses were made from crosses of JM54, JM56, JM161, and B102 with SG16 (*h*$^{+N}$). All tetrads were checked for the presence of *h*^{90} spores. Tetrads with *h*^{90} spores were picked up for further tests. For the determination of linkage relationships between the mating-type region and *leu1* and *his2*, some of the tetrads without an *h*^{90} spore were also analyzed. The results are similar to those found for *h*$^{+N}$ × *h*$^{-S}$ crosses as far as the frequency of *h*^{90} spores (~0.6%) and the map distances (*leu1* – MT: ~14.5 map units; MT – *his2*: ~1.2 map units) are concerned. The tetrads with one *h*^{90} spore are consistent with the assumption that the *h*$^{-}$ mutants have mutations in *mat2*. In addition to the above tetrads, two were found that had more than one *h*^{90} spore. One contained 2 *h*^{90} *his2* and 2 *h*$^{+}$ *his2* spores and the other 4 *h*^{90} *his2* spores. These tetrads are obviously due to the presence of a few spontaneous *h*^{90} mutants in the strain SG16; no further work was done with these tetrads.

In all tetrads with an *h*^{90} spore, one of the *h*$^{+}$ spores should contain a *mat1*$^{+}$ allele from SG16 and a *mat2* allele from the *h*$^{-}$ mutant used in the cross. These *h*$^{+}$ strains will be referred to as "*h*$^{+}$ recombinants"; they were identified by the combination of their *leu1* and *his2* markers (e.g., *leu1*$^{+}$ *h*$^{+}$ *his2*$^{+}$).

Spontaneous mutation pattern of the h^{+} *recombinants*: According to the LEUPOLD scheme (1958), a *mat1*$^{+}$ allele can mutate to *mat1*$^{-}$. If our assumption regarding the *h*$^{+}$ recombinants is correct, the *h*$^{+}$ recombinants should mutate spontaneously to *h*$^{-}$ but not to *h*^{90}. The new *h*$^{-}$ mutants should have properties like the *h*$^{-}$ mutants from which the *h*$^{+}$ recombinants were derived. To test this prediction, the *h*$^{+}$ recombinants were streaked on MEA and the resulting colonies were treated with iodine vapors in order to detect spontaneous mating-type mutants.

All eight *h*$^{+}$ recombinants isolated from the cross SG16 × JM54 (T1b to T8d) mutate spontaneously to *h*$^{-}$, and also to *h*^{90}. The latter observation was unexpected and cannot be explained at this time. Twenty-eight *h*$^{-}$ mutants were isolated from the *h*$^{+}$ recombinants T1b to T8d. Twenty-seven of these *h*$^{-}$ mutants appear to be stable (no iodine-positive material has been found). One unstable *h*$^{-}$ mutant was isolated from T5c; this *h*$^{-}$ strain mutates spontaneously to *h*^{90} and *h*$^{+}$.

The seven *h*$^{+}$ recombinants isolated from the crosses of JM56 (T9b and T10b), JM161 (T11b–T13b) and B102 (T14b and T15b) with SG16 mutate spontaneously only to *h*$^{-}$; no *h*^{90} mutants were found. Altogether 27 *h*$^{-}$ mutants were isolated from these *h*$^{+}$ recombinants. All of the *h*$^{-}$ mutants appear to be stable; no mutations to *h*$^{+}$ or *h*^{90} were found.

As has been described above, JM56 can complement B102 for meiosis and it can produce the *map1* substance. We tested whether the h^- mutants isolated from T9b and T10b show the same characteristics as JM56. The nine h^- mutants isolated from T9b and T10b were mixed with B102 and the mixtures were spotted on MEA. Microscopic examinations of the spots were made after three days. All nine mixtures contained zygotic asci in addition to zygotes and vegetative cells; therefore, all h^- mutants which have been isolated from T9b and T10b can complement B102 for meiosis. To determine if these h^- mutants can produce the *map1* substance, we mixed the nine mutants with the diploid strains JM218

$$\left(\frac{mat1^-\,mat2^+\text{-JM161}}{mat1^-\,mat2^+\text{-JM161}}\ \frac{fus1\text{-B20}}{fus1\text{-B20}}\ \frac{ade6\text{-M210}}{ade6\text{-M216}}\right)$$

and JM247 $\left(\frac{mat1^-\,mat2^+}{mat1^-\,mat2^+}\ \frac{map1\text{-A80}}{map1\text{-A80}}\ \frac{ade6\text{-M210}}{ade6\text{-M216}}\right)$.

The mixtures were spotted on MEA. As has been mentioned above, neither JM218 nor JM247 can self-sporulate, but both strains produce azygotic asci if mixed with cells that produce the *map1* substance. After three days all mixtures contained azygotic asci. Since these h^- mutants can induce both diploids to sporulate, all of them must produce the *map1* substance. It appears, therefore, that the nine h^- mutants isolated from T9b and T10b are very similar, if not identical, to JM56.

The h^- mutants isolated from T11b, T12b, and T13b were tested for similarity with JM161. As described below, JM161 can complement B102 for meiosis, but cannot produce the *map1* substance. The six h^- mutants from T11b, T12b, and T13b were tested in the same way as the nine h^- mutants from T9b and T10b. All mixtures of the six h^- mutants with B102 contained zygotic asci in addition to zygotes and vegetative cells; therefore, the h^- mutants isolated from T11b, T12b, and T12b can complement B102 for meiosis. None of the mixtures of the six h^- mutants with JM218 and JM247 contained azygotic asci; these h^- mutants apparently do not produce the *map1* substance. Therefore, the six h^- mutants isolated from T11b, T12b, and T13b are very similar, if not identical, to JM161.

Among the h^- mutants from T14b and T15b, two distinct types of colonies were observed: one type was hyaline after iodine treatment, had a rough colony morphology and formed faint iodine positive lines with adjacent h^+ colonies while the other type gave yellow, smooth colonies with dark iodine positive lines between it and adjacent h^+ colonies. We tested whether the h^- mutants are similar to B102. As mentioned previously, B102 can self-copulate, but it cannot initiate meiosis. The 12 h^- mutants isolated from T14b and T15b were spotted on MEA; B102 was also spotted as a control. After three days, each culture was examined and the frequency of zygotes F was determined:

$$F = \frac{2(\text{number of zygotes})}{(\text{number of vegetative cells}) + 2(\text{number of zygotes})}.$$

B102 and eight of the twelve h^- mutants had high F values ranging from 0.65

to 0.87 while the remaining four h^- mutants had low F values from 0.00 to 0.02. All of the strains with high F values were of the rough hyaline type while all of the low F values were of the smooth yellow type. Thus, from T14b and T15b, two types of h^- mutants were found: one type which appears to be identical with B102 and another which produces few, if any, zygotes.

To see if the difference between the two types of h^- mutants from T14b and T15b is due to a mutation in auxiliary genes required for copulation or to differences in the mating-type region, two h^- mutants with a high F value (T14b-III, T15b-III) and two with a low F value (T14b-I, T15b-I) were crossed with SG378 (h^{+N}). We dissected approximately 30 asci from each cross. In 109 of 111 tetrads the mating-type segregated 2 h^+ : 2 h^-. In the cross T15b × SG378, two tetrads contained 1 h^{90}, 1 h^- and 2 h^+ spores. In each of these two tetrads, the h^{90} spore and one of the h^+ spores were recombinants for the *leu1* and *his2* markers. All of the h^- spores from the crosses with T14b-I and T15b-I produced cultures with only a few zygotes. Therefore, it appears that the mutants with low F values do not contain an additional mutation in an auxiliary gene required for copulation. No further experiments were performed to elucidate their nature.

II. *The* h$^+$ *mutants*

Characterization of the h$^+$ *mutants*: Preliminary experiments had shown that the 13 h^+ mutants are due to mutations in or close to the mating-type region. To obtain quantitative mapping data, we crossed the 13 h^+ mutants, and the derivatives JM35A and JM48A (for their origin, see MATERIALS AND METHODS) with SG173 (h^{-S}). Ascospores were plated on MEA and following incubation, the plates were treated with iodine vapors and the total number of colonies and the number of h^{90} colonies were determined. The low frequency of h^{90} progeny (.0019–.0139) justifies the conclusion that the h^+ mutants are due to mutations in the mating-type region.

To determine whether the h^+ mutants mutate spontaneously to other mating types, we streaked all 13 mutants on MEA. The resulting colonies were treated with iodine vapors. All 13 h^+ mutants mutate to h^{90} as well as h^-. We isolated one to three h^- mutants from each h^+ strain. The h^- mutants were also examined for their pattern of spontaneous mutation. All h^- mutants mutate to h^{90} and h^+.

We crossed four h^- strains (JM35-I, JM48-I, JM35-IA, and JM48-IA) with SG16 (h^{+N}). From these crosses, spores were plated on MEA. We determined the frequencies of h^{90}'s among the resulting colonies. The low frequency of h^{90} progeny (.0012–.0045) shows that the h^- mutants are due to mutations in the mating-type region.

Tetrad analyses with h$^+$ *mutants*: To determine the genotype of the h^+ mutants tetrads from crosses of JM35, JM35A, JM48 and JM48A with SG173 (h^{-S}) were analyzed. All tetrads were tested for the segregation of the mating types and those which did not show a 2 h^+ : 2 h^- segregation (recombinant tetrads) were saved for further tests. To estimate linkage between the mating-type region and *leu1* and *his2*, tetrads which segregated 2 h^+ : 2 h^- were also analyzed. The results are similar to those found for $h^{+N} \times h^{-S}$ crosses as far as the

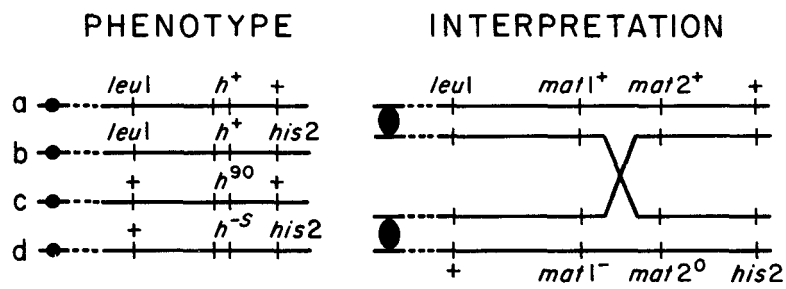

FIGURE 1.—Typical recombinant tetrad found in crosses of $h+$ mutants isolated from h^{90} strains with SG173.

frequency of h^{90} spores ($\sim 0.45\%$) and the map distances (*leu1*-MT: $\sim$ 13 map units; MT-*his2*: $\sim$ 1 map unit) are concerned.

Nineteen recombinant tetrads were found among the 1400 tetrads dissected. One type of recombinant tetrad and its interpretation is shown in Figure 1. Ten tetrads were of this type. Four tetrads contained an additional crossing over between *leu1* and *mat1*, one appeared to be due to a four-strand double crossover between *mat1* and *mat2* while the remaining four tetrads appeared to result from gene conversion. The data are consistent with the h^+ mutants having the genotype *mat1*+ *mat2*+.

In the crossover tetrads, one of the h^+ spores should contain a *mat1*+ allele from a h^+ mutant and a *mat2*^{0} allele from SG173. These h^+ strains will be referred to as "h^+ recombinants"; they were identified by the combination of their *leu1* and *his2* markers (e.g., *leu1* h^+ his*2*). To examine their pattern of spontaneous mutation, we streaked the h^+ recombinants on MEA. The resulting colonies were treated with iodine vapors. All h^+ recombinants mutated only to h^-. We isolated two to three h^- mutants from each h^+ recombinant and tested them for spontaneous mating-type mutations; no mutations to other mating types were found.

Tetrad analyses with h$^-$ *mutants*: As mentioned above, the h^+ mutants isolated from h^{90} strains can mutate spontaneously to h^{90} and h^-. We made tetrad analyses from crosses of JM35-I, JM35-IA, JM48-I and JM48-IA with SG16 (h^{+N}) in order to determine the genotype of these h^- mutants. All tetrads were tested for mating-type segregation and those which did not show a 2 h^+ : 2 h^- segregation (recombinant tetrads) were saved for further tests. Other tetrads were also analyzed for linkage estimates. The results are similar to those found for $h^{+N} \times h^{-S}$ crosses. The frequency of h^{90} spores ($\sim 0.17\%$) is slightly less than expected ($\sim 0.5 - 1.0\%$); the map distances for *leu1*-MT ($\sim$ 13 map units) and MT-*his2* ($\sim$ 1 map unit) are as expected.

Eight recombinant tetrads were found among the 1200 tetrads dissected. Five recombinant tetrads were of the type shown in Figure 2. One tetrad contained an additional crossing over between *leu1* and *mat1* while the other two tetrads appeared to result from gene conversion. The data are consistent with a genotype of *mat1*$^-$ *mat2** for the h^- mutants. The gene symbol *mat2** stands for an inac-

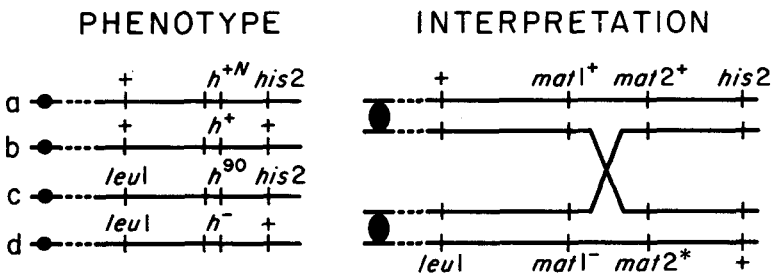

FIGURE 2.—Typical recombinant tetrad found in crosses of h^{-U} strains with SG16.

tive *mat2* allele; the reason for the use of the superscript * rather than a zero will be given in the discussion.

In the crossover tetrads, one of the h^+ spores should be a recombinant containing a *mat1*+ gene from SG16 and a *mat2** gene from the h^- mutant. The h^+ recombinants were identified by the combination of their *leu1* and *his2* markers. To examine their pattern of spontaneous mutation, we streaked the h^+ recombinants on MEA. The resulting colonies were treated with iodine vapors. All six recombinants mutated to h^{90} as well as h^-. The frequencies of h^{90} mutants, although not measured, were distinctly lower than those observed in streaks of h^{+N} strains. We isolated three h^- mutants from each of the six h^+ recombinants and tested them for spontaneous mating-type mutations: all mutated to h^{90} and h^+.

DISCUSSION

In this paper we describe the properties of h^- and h^+ mutants isolated from h^{90} strains of *S. pombe*; the mutants are due to mutations in the mating-type region. Mutants mapping in the mating-type region of *S. pombe* were previously isolated by BRESCH, MÜLLER and EGEL (1968); the h^- mutant B102 maps in *mat2*+ (EGEL 1969).[4] The phenotypes of all 20 h^- mutants described in section I can be explained by assuming that they have a normal *mat1*$^-$ locus and that the mutations are in *mat2*. The tetrad data are consistent with this hypothesis since the mutations map between *mat1* and *his2*, and, therefore, in or near the *mat2* locus.

Two of the h^- mutants isolated by BRESCH, MÜLLER and EGEL (1968), B102 and B266, are still able to self-copulate, but cannot initiate meiosis. Among our h^- mutants, none were found which still can self-copulate. When we mated our h^- mutants, and those of BRESCH and EGEL, with B102, an important fact became apparent: in half of the mating mixtures ascospores were formed. Thus, some of the h^- mutants can complement B102 for meiosis.

These results could mean that the mutant B102 has a mutation which inactivates only the meiosis function and that the mutants which complement B102 have mutations which inactivate only the copulation function of the *mat2* locus and that mutations can affect the copulation and meiosis functions of *mat2* independently. Further experiments indicated that the situation is more complex:

[4] EGEL (1969) uses "*h2*+", the previous designation of *mat2*+ (see GUTZ and DOE 1973).

the h^- mutants also differ with respect to production of the *map1* substance. Since this is needed for copulation as well as meiosis, inability to produce it will make the mutant unable to self-copulate and, if diploid, to self-sporulate. Therefore, it appears that the *mat2* gene normally contributes three functions to the sexual cycle: one required only for copulation, a second required only for meiosis, and a third required by both, namely the production of the *map1* substance. As can be seen from Tables 4 and 5, the mutants can be grouped in four different classes. Mutants of class I and class IV have defects in only one of the three functions. Since the mutants of classes II and III cannot produce the *map1* substance, no conclusion can be made regarding the function that is required only for copulation. From their ability to complement B102, however, it is obvious that the class III mutants have no defect in the function required only for meiosis while those in class II have lost their function.

The finding that JM56 (and 8 other h^- mutants) can "complement" B102 for meiosis needs some further discussion. It could be that both mutants have a defect in the meiosis function of *mat2*$^+$, and that the observed sporulation is due to some type of interallelic complementation (see FINCHAM 1966). Such an interpretation cannot be correct since spontaneous diploids of JM56 do form azygotic asci. This result shows that JM56 must have a *mat2*$^+$ locus which is fully active as to meiosis. Whether the *mat2* locus comprises one or more cistrons cannot be determined from our data.

The h^+ recombinants isolated from crosses of an h^{+N} strain (SG16) with the h^- mutants appear to have a wild-type *mat1*$^+$ allele and a defective *mat2*$^+$ allele corresponding to the mutant used in the cross. This conclusion is supported by the fact that, in most cases, when *mat1*$^+$ mutates to *mat1*$^-$ in these recombinants, the resulting h^- strains have properties resembling the original h^- mutants. The behavior of the h^+ recombinants from SG16 × B102 is more complex: they give rise to two different types of h^- mutants. Thus, in some cases, the mutation from an h^+ to an h^- phenotype (*mat1*$^+ \rightarrow$ *mat1*$^-$) appears to also affect the expression

TABLE 5

Interpretation of the defects in the four classes of mat2 *mutants*

Mutant class*	*mat2* functions		
	Copulation only	Meiosis only	Copulation and meiosis†
I B102 (2 mutants)	+	—	+
II JM54 (9 mutants)	?	—	—
III JM161 (4 mutants)	?	+	—
IV JM56 (5 mutants)	—	+	+

* One typical mutant from each class and the number of mutants in each class are given.
† Mutants which are shown to be — in this column do not produce the *map1* substance even if they have a *map1*+ gene.

of the *mat2* allele. This pattern of mutation appears to be the same as that observed for h^{+N} strains (*mat1*+ *mat2*+). These can either mutate to h^{90} (*mat1*- *mat2*+) or to h^{-U} (*mat1*- *mat2**) (for a discussion of this see below). The ability of the h^+ recombinants isolated from the crosses of SG16 × JM54 to yield *both* h^{90} and h^- mutants was unexpected and cannot be explained at this time.

As regards the h^+ mutants isolated from h^{90} strains, LEUPOLD (1958) stated that, on the basis of their mutational behavior, they are similar to h^{+N} strains. He did not, however, describe the mutation pattern in detail, nor did he determine the genotype of the h^+ mutants. EGEL (1973b) found that h^+ mutants isolated from h^{90} strains could be due either to mutations in the mating-type region or in one of the mating-type auxiliary genes; he did not determine the genotype of the h^+ mutants that were due to mutations in the mating-type region.

Our results are in agreement with the statement of LEUPOLD: h^+ mutants (isolated from h^{90}) have a mutation pattern similar to h^{+N} strains. They both mutate to h^{90} and to unstable h^- mating types. Further, our recombination experiments with two h^+ mutants (one of spontaneous origin, the other isolated after nitrous-acid treatment) indicate that they have the same genotype as h^{+N} (*mat1*+ *mat2*+).

The h^- mutants isolated from the h^+ mutants can mutate spontaneously to h^{90} and to h^+. Such unstable h^- mutants (h^{-U}) have also been isolated from h^{+N} strains (GUTZ and DOE 1973) (as described above, our h^+ mutants isolated from h^{90} strains appear to be identical with h^{+N}). The results of experiments with h^{-U} mutants derived from LEUPOLD's original h^{+N} strain (F. J. DOE, personal communication), as well as with our h^{-U} mutants, indicate that they have a *mat1*- allele. Their *mat2* allele appears to be nonfunctional, but unlike the *mat2*^{0} allele which is stable, this *mat2* allele can mutate back to *mat2*+. We designate the new allele as *mat2** to distinguish it from *mat2*^{0}. Whether the mutation from h^{+N} (*mat1*+ *mat2*+) to h^{-U} (*mat1*- *mat2**) occurs through one or two mutational steps cannot be determined from our data.

One final point remains to be discussed. We found that h^{90} strains (*mat1*- *mat2*+) can mutate to h^+ (*mat1*+ *mat2*+), but that *mat2* mutants such as B102 (*mat1*- *mat2*+ — B102) or JM56 (*mat1*- *mat2*+ — JM56) are unable to mutate to h^+. It is also known that h^{-S} strains (*mat1*- *mat2*^{0}) cannot mutate to h^+. At this time, no explanation can be given as to why no h^+ mutants can be detected in these h^- strains.

In summary, our studies on the mating-type region indicate: (1) Mutations from h^{90} to h^- are due to mutations in *mat2*, whereas (2) mutations from h^{90} to h^+ are due to mutations in *mat1*. An important difference exists between these two types of mutation: in going from h^{90} to h^-, *mat2*+ mutates to partial or total inactivity, while in going from h^{90} to h^+, *mat1* mutates from one activity to a complementary activity (*mat1*- to *mat1*+). (3) The *mat2* locus has at least three different functions (for copulation, meiosis, and production of the *map1* substance) which can mutate independently, and (4) a wild-type *mat2*+ appears to be required for the mutation of h^{90} to h^+.

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