A NEW TYPE OF MUTATION IN SCHIZOSACCHAROMYCES POMBE: VEGETATIVE IODINE REACTION

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ABSTRACT

Colonies of Schizosaccharomyces pombe that contain ascospores (e.g., colonies of homothallic strains) turn black after treatment with iodine vapors. Heterothallic strains of S. pombe normally do not show this reaction. In experiments with the latter strains we found mutants which exhibit a positive iodine reaction though they do not contain ascospores. This phenotype is due to mutations in a new gene, vir1 (vegetative iodine reaction). The vir1 locus is not linked with the mating-type genes. Strains of mating-type h⁻⁸ are known not to give any spontaneous mating-type mutations. Mating-type mutations were also not found after treatment with nitrous acid.

LEUPOLD (1955a) has described a simple technique for the detection of ascospores in colonies of Schizosaccharomyces pombe Lindner. He reported that after a short exposure to iodine vapors, colonies of this fission yeast containing only vegetative cells appear yellow (negative iodine reaction), while colonies containing spores appear black (positive iodine reaction). This reaction is due to the presence of an amylose-like substance in the spores. This technique has proved useful for: (a) routine mating-type tests (LEUPOLD 1955a, 1970), (b) the detection of mutations from one mating type to another (GUTZ and DOE 1973), and (c) the detection and isolation of non-sporulating mutants in homothallic strains (BRESCH, MÜLLER and Egel 1968). In this paper, we describe a new gene, vir1. Mutants of this gene are exceptional as their colonies, while containing only vegetative cells, show a positive iodine reaction.

MATERIALS AND METHODS

Strains: Several S. pombe cultures were used from the strain collections of Dr. U. LEUPOLD (Berne, Switzerland), Drs. C. BRESCH and R. Egel (Freiburg, Germany), as well as of the present authors. Some specific strains will be given with the pertinent experiments. The strains were of the heterothallic mating types h⁻⁸ or h⁻⁺; for comparison, we used also a homothallic strain (mating type h₀°). The mating-type system of S. pombe has recently been summarized and discussed by GUTZ and Doe (1973) and by GUTZ et al. (1974).

Media and experimental techniques: Yeast-extract agar (YEA), malt-extract agar (MEA), and minimal agar (MMA). The compositions of these media, as well as the general techniques

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used in genetic experiments with *S. pombe*, have been described elsewhere (Leufold 1955b, 1970; Gutz 1971; Gutz *et al.* 1974).

**Mutagenesis:** The mutants were induced with nitrous acid as described by Gutz (1961). After appropriate dilution, the mutagenized cells were plated on MEA + leucine (50 mg/l) and incubated for 3 days at 30° followed by 2 days at 25°. The plates were then treated with iodine vapors and all iodine-positive sectors were picked for purification and further study.

### EXPERIMENTS AND RESULTS

**Isolation and characterization of mutants:** For the mutation experiments, an *h-s* strain (SG168) was chosen since *h-s* strains are not known to produce spontaneous mating-type mutants. If these occur, mutations to homothallism or heterothallism + would give rise to iodine-positive colonies, sectors or lines due to the presence of ascospores (Gutz and Doe 1973). Besides being *h-s*, SG168 was also leucine requiring (*leu1*).

After nitrous-acid mutagenesis, we found iodine-positive sectors in a few colonies of SG168; iodine-positive lines, which would have been indicative of mutations from *h-s* to heterothallism +, were not detected.* Twenty-six iodine-positive sectors were restreaked. The sectors yielded colonies with a homogeneously positive iodine reaction. All these colonies contained only vegetative cells; they did not self-sporulate (i.e., they were not homothallic). We call this property “vegetative iodine reaction (*vir*)”. The 26 *vir* mutants were kept for further experiments (strains JM1 to JM4 and JM6 to JM27).

The positive iodine reaction of the *vir* mutants is somewhat different from that due to the presence of spores: *vir* colonies appear dark brown rather than black. In the black and white photograph (Figure 1) the *h-s* *vir* colonies appear gray in contrast to the black homothallic colonies. The *vir* mutants grow on MMA + leucine as SG168 does; therefore, the vegetative iodine reaction is not caused by any new auxotrophic requirements.

**Tests for allelism:** Mutants JM1 and JM4 were crossed with an *h+N* strain. From the crosses, we isolated by tetrad analyses *h+N* cultures carrying the *vir* mutations of JM1 and JM4, respectively (strains JM1A and JM4A). The *h+N* strains, like *h-s* strains, also show a vegetative iodine reaction when they contain

![Figure 1](image-url)
a vir mutation. All original 26 vir mutants were crossed with JM1A and JM4A. From the latter crosses, we plated free ascospores on MEA + leucine. Per cross, approximately 1000 colonies were tested. Forty crosses yielded only vir colonies, whereas in 12 crosses few (i.e., 1–3) iodine-negative colonies were formed. Thus, all vir mutants are closely linked and seem to map at the same locus, which will be called vir1. The complementation test reported below also indicates that all vir mutants are alleles of one gene.

**Complementation tests:** Complementation experiments were performed to determine whether the vir1 mutations are dominant or recessive, and whether or not they represent a single cistron. Since *S. pombe* is a haploid organism, some experimental tricks were necessary for the selection of diploid cultures. We made use of strains carrying the mutation “me11–102” of Bresch, Müller and Egel (1968) and an auxotrophic marker (ade6) complementary to leu1. me11–102 cultures copulate with h– strains but the zygotes do not undergo meiosis. By plating material from crosses of the type

\[
\text{me11–102 leu1+ ade6 vir1-X} \times h– \text{leu1 ade6+ vir1-Y}
\]

on MMA, it was easy to select hybrid diploid strains. In the above genotypes, the symbols vir1-X and vir1-Y stand either for the wild-type and a mutant allele, or for two vir1 mutations of different origin.

The following diploid strains were prepared: vir1+/vir1–1, vir1+/vir1–2, vir1+/vir1–3, vir1+/vir1–4 and vir1+/vir1–6. All strains produced only iodine-negative colonies. Furthermore, we prepared 26 diploid strains each carrying a different vir1 allele from the mutants JM1 to JM4 and JM6 to JM27 in combination with vir1–3. All 26 diploids gave rise to iodine-positive colonies when plated on MEA. Thus, vir1 is recessive, and all 26 mutants appear to belong to the same cistron.

**Tetrad analyses:** Five of the vir1 mutants (*h– leu1 ade6+ vir1*) were crossed with a strain being *h+N leu1+ ade6 vir1+*. A total of 167 tetrads were analyzed from these crosses (approximately 30 tetrads from each cross). One hundred and sixty-four tetrads showed a 2 vir1+: 2 vir1 segregation. Three gene conversion tetrads were found: two were 3 vir1+: 1 vir1, and one was 1 vir1+: 3 vir1 (the other markers segregated 2:2). In the 164 tetrads with a normal vir1 segregation, vir1 showed free recombination with the mating-type genes (PD = 28, NPD = 24, T = 112) and leu1 (PD = 25, NPD = 32, T = 107) as well as with ade6 (PD = 33, NPD = 26, T = 105).

**DISCUSSION**

The “vegetative iodine reaction” mutants show a new property, a positive iodine reaction of heterothallic (i.e., non-sporulating) colonies. The reaction is not due to an auxotrophic requirement. All 26 mutants of independent origin map at a single locus, vir1.

The most interesting question which arises in connection with the discovery of vir1 is: does this gene have a function in the sexual cycle of *S. pombe*? During sporulation of *S. pombe*, an amylase-like substance is formed which is incorpor-

† The mating types of *S. pombe* are determined by two closely linked genes (see Gutz and Dog 1973). These and leu are located on chromosome II, whereas ade6 is located on chromosome III (Gutz et al. 1974). Jürg Kohli (personal communication) has recently determined by haploidization experiments that vir1 is on chromosome I.
ated into the ascospores. Whether the iodine-positive substance of the \textit{vir1} mutants is biochemically related to the "spore substance" is not known, and the above question cannot be answered at this time. Since \textit{vir1} does not map in the mating-type region, we can only infer that, if \textit{vir1} has a function in the sexual cycle, it should be one which is different from the function of the mating-type genes.

For a discussion of the \textit{vir1} gene, it also has to be taken into consideration that the mutated alleles are recessive. However, the mutants and not the wild type contain an iodine-positive substance. This fact is incompatible with the idea that \textit{vir1}+ may code for an enzyme which takes part in the synthesis of an iodine-positive substance. The \textit{vir1} mutations could be recessive for either one of the following reasons. (1) The wild-type gene may have a regulatory function as to the synthesis of an iodine-positive compound. Normally, the synthesis is repressed in vegetative cells. (2) The \textit{vir1} mutants may have a biochemical block in a non-essential pathway which did not become apparent in our experiments (i.e., the mutants can grow on MMA). Due to this block, an iodine-positive substance (or an intermediary product which later on is transformed to such a substance) is accumulated.

Aside from the \textit{vir1} mutants, our mutation experiments are also of interest with regard to the mating types of \textit{S. pombe}. The different mating types of this yeast are characterized by specific patterns of spontaneous mutations; the mutants are detectable by iodine-vapor treatment (Gutz and Doe 1973; Gutz et al. 1974). Strains being \textit{h}s are not known to give spontaneous mating-type mutants. In our present experiments, a mutagen was applied, and the experiments included a search for iodine-positive colonies, sectors or lines. It is noteworthy that also in these experiments no mating-type mutants were found in the \textit{h}s strain SG168 while 26 independent mutants were found at the \textit{vir1} locus.

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LITERATURE CITED


