SHORT COMMUNICATION

Genetical Analysis of a Sterile Mutant by Protoplast Fusion in the Fission Yeast Schizosaccharomyces pombe

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The genetical analysis, by protoplast fusion, of the sterile strain ED22 of Schizosaccharomyces pombe is described. Two major mutations are harboured by this strain. One, cdc 25.22, is conditionally defective in mitosis. The other mutation, ste 1.1, causes sterility in strains of h-, h+ or mat 2.102 mating-type. Sterility is due to the failure of cell agglutination. We present evidence that ste 1.1 is defective in the production of a non-diffusible and non-mating-type specific factor. ste 1 and cdc 25 both map on chromosome I and are loosely linked.

INTRODUCTION

A number of temperature-sensitive strains of Schizosaccharomyces pombe have been isolated which are specifically defective in cell division (Nurse et al., 1976). One of these strains (ED22), conditionally defective in mitosis, is also sterile, even at 25 °C, a temperature which is permissive for nuclear and cell division. We describe here the genetical analysis of ED22, with the aid of protoplast fusion, a new and powerful method for the analysis of sterile strains of fungi. We have shown that ED22 originally carried at least three mutations, and have separated them genetically. The mutations are cdc 25.22 causing the inability to undergo mitosis (Fantes, 1979), a temperature-sensitive partial lethal mutation, and ste 1.1. The ste 1.1 mutation defines a new gene whose function is necessary for conjugation: characterization of the mutation suggests that ste 1+ may code for a factor required for cell agglutination. This factor is not mating-type specific and appears to be non-diffusible.

METHODS

Strain ED22 was obtained by nitrosoguanidine mutagenesis of the standard wild-type of S. pombe, strain 972 of h+ mating-type (Nurse et al., 1976). The mat 2.102 mating-type mutant (previously called mei 1.1B102) is described by Egel (1973). The auxotrophic mutants ura 5.294, lys 1.131 and ade 6.616 were induced by u.v. irradiation of strain 975, the h+ wild-type (U. Leupold, personal communication). Growth media and genetical procedures were as described by Gutz et al. (1974) and Kohli et al. (1977), except for protoplast fusion and regeneration, which were done according to Sipiczki & Ferenczy (1977, 1978). Minor differences were in the use of Geotrichum candidum instead of S. pombe to induce the production of digestive sap in Trichoderma viride. Because strain ED22 gave a much lower protoplast yield than wild-type S. pombe, incubation in the presence of the cell wall-degrading enzymes was continued for 8 h. KCl (0-6 m) was replaced by mannitol (0-8 m) for protoplast washing and in the regeneration medium, as the
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Protoplasts of ED22 were found to be labile in the presence of KCl. The regeneration fraction of protoplasts was measured by transferring the protoplasts of the parental strains separately to minimal medium supplemented with adenine, uracil and mannitol. Yields were 11% for sterile cdc-, and 14% for h+ ade 6.616. After protoplast fusion in polyethylene glycol, about 1% of the protoplasts present in the mixture yielded a prototrophic colony growing on minimal medium supplemented with mannitol only.

Cellular DNA content was measured as described by Bostock (1970).

RESULTS AND DISCUSSION

Genetical analysis of ED22

As a first attempt to cross the sterile strain ED22, actively growing cells of this strain and of mat 2.102 ura 5.294 were mixed in roughly equal amounts on MEA medium, which normally promotes conjugation, and incubated for 5 d at 25°C. The resulting mating mix was inoculated heavily on to minimal medium and incubated at 35°C. Neither parent strain would grow under these conditions, but heterozygous diploids were expected to do so provided all the lethal or auxotrophic mutations present were recessive. A few prototrophic colonies were observed after 5 d incubation, several of which were judged to be diploid on the basis of their large cell size. None of these strains could sporulate, consistent with their expected h+ mat 2.102 mating-type constitution (Egel, 1973). Haploid segregants were, however, recovered from one strain after haploidization with m-fluorophenylalanine (Kohli et al., 1977). The haploids were either cdc- and sterile or cdc+ and fertile, indicating that the cdc and sterility factors were on the same chromosome. Several sterile cdc segregants were ura-, showing that the sterility character was not on chromosome II which harbours ura 5 (Kohli et al., 1977). Within the group of fertile cdc+ segregants, a temperature-sensitive partial lethal mutation was observed to segregate. (Segregation of this character could not be observed for the sterile cdc- group, since its lethal effect would be masked by the cdc lesion). Either ura- or the partial lethal mutation (but never both or neither) was found in the cdc+ segregants, showing that the partial lethal character was originally present in ED22, and mapped on chromosome II.

One sterile segregant of constitution cdc- ura- was crossed by protoplast fusion with h+ ade 6.616, giving numerous prototrophic colonies at 25°C (Fig. 1). These colonies sporulated well on MEA, and tetrad analysis of the asci gave a 2:2 segregation for ura and ade, as well as for the cdc and sterility (ste) characters. Thus the colonies obtained by regenerating fused protoplasts are genuinely diploid. Furthermore, the ste and cdc characters are due to single gene mutations.

Tetrad analysis showed that the cdc and ste genes are on distinct but linked genes (21 parental ditypes, 9 tetratypes and 1 non-parental ditype, giving a map distance of 15 centimorgans). The cdc mutation is not closely linked to any of the 24 cdc genes so far identified in S. pombe and has been designated cdc 25.22. Mitotic haploidization and tetrad analysis have permitted its localization on chromosome I, not far from ura 1 (36 parental ditypes, 39 tetratypes and 1 recombinant ditype, giving a map distance of 29 centimorgans). Recent results (Fantes, 1979) suggest that cdc 25 participates in a previously described control determining the time of mitosis during the vegetative cell cycle (Nurse, 1975; Thuriaux et al., 1978). The sterile mutation also shows a 2:2 segregation and therefore defines a single gene. The mutation, designated ste 1.1, is unlinked to the mating-type locus and is expressed in a h-, h+ or mat 2.102 mating-type background.

Physiological basis of ste 1.1

Sexual mating in yeasts is preceded by two major steps (see Crandall et al., 1977, for review). First, the vegetative cells become arrested in G1. In S. pombe, this is brought about by transferring the cells to nitrogen-limiting medium (Egel, 1971). It is not known whether a diffusible sex hormone of the type found in Saccharomyces cerevisiae (Duntze et al., 1970)
Fig. 1. Regeneration of fused and unfused protoplasts on minimal medium. Protoplasts were prepared as described in Methods and plated on to minimal medium supplemented with mannitol. The plates were incubated at 25 °C. Left, parent strain h+ ade 6.616; middle, mixture of parent strains; right, parent strain ste 1.1 ura 5.294 cdc 25.22.

also participates in the G1 arrest. To test whether ste 1.1 cells were able to arrest in G1, cultures were starved of nitrogen at 25 °C as described by Nurse & Thuriaux (1977), and the DNA content per cell was determined. This was 19.2 fg for ste 1.1 and 17.9 fg for the wild-type (972) control. Both these values are consistent with the 1c content of 16.9 fg reported previously (Nurse & Thuriaux, 1977), showing that ste 1.1 cells arrest in G1 under these conditions. The DNA content of exponentially growing ste 1.1 cells is 30.5 fg, very similar to the wild-type value of 29.9 fg.

The second step preceding conjugation is agglutination of cells destined to conjugate, which occurs rapidly after G1 arrest when cells of h- and h+ mating-types are mixed together (Crandall et al., 1977). This agglutination does not occur when ste 1.1 is one of the mating partners, whether the other partner is h-, h+ or mat 2.102.

While G1 arrest (at least in Saccharomyces cerevisiae) involves mating-type specific diffusible factors (Duntze et al., 1970), cell agglutination appears to require the interaction between mating-type specific glycoproteins of the cell wall (Crandall & Brock, 1968). Such mating-type factors are not defective in ste 1.1, whose sterility is expressed in an h-, h+ or mat 2.102 mating-type strain. We can also rule out the possibility that ste 1.1 is defective in the production of a diffusible factor required for cell agglutination. This is because in a mating mixture of three strains h- lys 1.131, h+ lys 1.131 and mat 2.102 ste 1.1 ade 6.616, the only meiotic products recovered were ade+ lys-. This shows that the ste 1.1 cells could not participate in the cross, even though any diffusible factor required for agglutination would be provided by one of the other strains present.

In conclusion, ste 1.1 is defective in a non-diffusible and non-mating-type specific factor which is required for cell agglutination, and consequently for sexual conjugation.
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Since agglutination is believed to require an intercellular interaction between glycoprotein components of the cell walls (Crandall & Brock, 1968), it may be rewarding to investigate the glycoprotein composition of the wall in this mutant.

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REFERENCES


