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Mating pheromone-like diffusible factor released by *Schizosaccharomyces pombe*

Yasuhisa Fukui, Yoshito Kaziro and Masayuki Yamamoto

Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan

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We demonstrate that a diffusible factor is secreted by h^- cells of the fission yeast *Schizosaccharomyces pombe*, whose mating pheromones have not been described. This factor, tentatively named the h^--factor, affects h^+ *S. pombe* cells and induces their elongation under nitrogen-depleted conditions. Circumstantial evidence suggests its physiological significance in the mating process. Despite their sterility, h^- *ras1*$^-$ cells secrete this factor. However, h^+ *ras1*$^-$ cells have apparently lost the ability to respond to it. This may suggest that the gene product of *S. pombe ras1*, a homologue of mammalian *ras* oncogenes, is involved in the mechanism for responding to mating pheromones.

Key words: fission yeast/h^--factor/*ras1* mutants/sterility

Introduction

Many yeast species secrete mating pheromones, which mediate intercellular communication at the initial stage of mating. They have been identified as oligopeptides, either unmodified (Stötzler *et al.*, 1976; Sakurai *et al.*, 1976, 1980; Betz and Duntze, 1979) or modified (Kamiya *et al.*, 1978; Sakagami *et al.*, 1978). The mode of action of mating pheromones produced by *Saccharomyces cerevisiae*, α-factor and a-factor, has been well studied and described in detail (Thorner, 1981).

However, no description of mating pheromones of the fission yeast *S. pombe* has been made to date, although this yeast species has been used frequently in genetic studies. This is curious and one might even suspect that direct cell contact, rather than diffusible pheromones, plays an essential role in the mating process of this rather eccentric yeast.

In the analysis of *S. pombe* mutants defective in the *ras1* gene, the only homologue to mammalian *ras* oncogenes in *S. pombe* (Fukui and Kaziro, 1985), we found that they are sterile regardless of their mating type, i.e. h^+ or h^- (Fukui *et al.*, 1986). However, subsequent studies have revealed that a h^- *ras1*$^-$ strain is not completely inert in intercellular interaction. If this strain is mixed with a h^+ *ras1*$^+$ strain, the latter cells elongate markedly. This paper presents evidence to show that this elongation is mediated by a diffusible factor and that the wild-type h^- strain of *S. pombe* also releases this factor. Furthermore, the *ras1* gene product is apparently implicated in the mechanism for responding to this factor.

Results

Elongation of h^+ cells in the presence of h^- ras1$^-$ cells

When wild-type h^+ and h^- cells of *S. pombe* are mixed in the absence of a nitrogen source, they mate, form zygotes and produce ascospores (Leupold, 1969; Egel, 1971; Egel and Egel-Mitani, 1974). We have previously shown that both h^+ and h^- strains become sterile, in addition to acquiring a short and swollen cell shape, if their *ras1* gene is disrupted (Fukui *et al.*, 1986). Although no conjugation occurs at all in the mating mixture if either of the partners has a *ras1*$^-$ genotype, we found that different morphological changes take place in the mixture depending on which partner is *ras1*$^-$. As shown in Figure 1A, cells which are elongated up to 20 μm and are less refractile microscopically appear when h^- *ras1*$^-$ cells are mixed with h^+ *ras1*$^+$ cells on a sporulation agar (SPA) plate. No such elongation is seen if either of these partners is transferred alone to SPA (Figure 1B,C): they enter stationary phase and become rather shorter (5.5–7 μm) than rapidly growing cells (*ras1*$^+$: 7.5–14 μm, *ras1*$^-$: 6.5–10 μm). If h^+ *ras1*$^-$ cells are mixed with h^- *ras1*$^+$ cells, no obvious elongation of cells occurs (Figure 1D).

To investigate which cells elongate upon mixing of h^- *ras1*$^-$ and h^+ *ras1*$^+$ cells, RP771 (adenine-auxotroph) and JY294 (adenine-prototroph) were mixed on SPA. After 1 day at 30°C, elongated cells and shorter cells were separated by micromanipulation. Cells were grown up to colonies on YPD plates at 30°C and the adenine auxotrophy was judged by the red colour of colonies. As summarized in Table I, elongated cells were all white and hence h^+ *ras1*$^+$, whereas short or round cells were all h^- *ras1*$^-$.

Elongation of h^+ cells is mediated by a diffusible factor

To clarify whether direct cell-to-cell contact is necessary for inducing elongation of h^+ cells, a critical micromanipulation experiment was devised. On a thin film of SPA (1.5 mm thick), one strain is spread out so that it forms a monolayer. A small open area in the layer is searched for under the microscope, and a cell of the partner strain is placed in such an open area by micromanipulation. The thin SPA film attached to a cover glass is then kept in a humid Petri dish and incubated at 25°C for several days. At appropriate intervals, cells are examined microscopically.

When RP771 (h^- *ras1*$^-$) cells were spread and a JY336 (h^+ *ras1*$^+$) cell was placed in an open area, elongation of the latter was seen after some residual cell divisions (Figure 2). Thus some diffusible factor must be responsible for the induction of elongation of h^+ cells.

The same micromanipulation experiment was carried out using a h^- *ras1*$^+$ strain (L972) as the monolayer. Elongation of JY336 cells placed in an open area could also be detected in this case (Figure 3). By analysis of varius combinations, it was concluded that only h^+ *ras1*$^+$ cells placed among h^- cells (*ras1*$^+$ or *ras1*$^-$) display elongation. JY336 cells placed alone on SPA did

Table I. Cell type determination in the mixture of RP771 (h^- *ras1*$^-$) and JY294 (h^+ *ras1*$^+$)

Cell shape	No. of cells tested	No. of cells grown up	Ade$^-$ (RP771)	Ade$^+$ (JY294)
Elongated	32	29	0	29
Short or spherical	24	21	21	0

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Fig. 1. Morphology of cells transferred to SPA. After incubation for 3 days at 25°C, cells were suspended in distilled water and phase contrast micrographs were taken. **A:** JY505 (h^- $ras1^-$) and JY336 (h^+ $ras1^+$) mixed approximately in a 1:1 ratio. **B:** JY505 alone. **C:** JY336 alone. **D:** JY504 (h^+ $ras1^-$) and JY333 (h^- $ras1^+$) mixed approximately 1:1. The bar shown in **D** stands for 10 μm. For full description of the genotype of each strain, see Materials and methods.

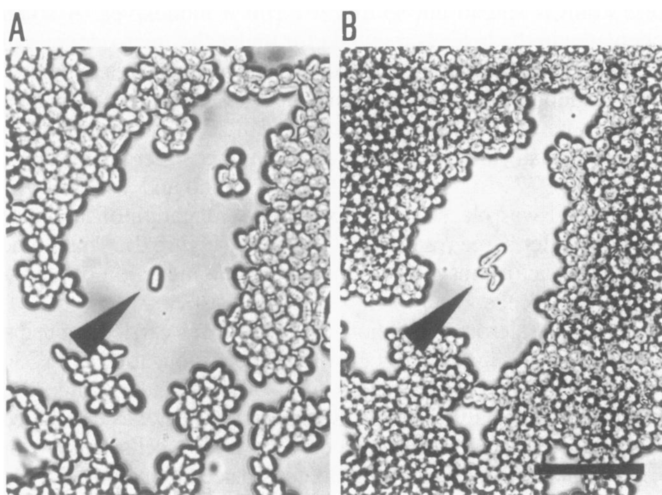

Fig. 2. Elongation of JY336 (h^+) cells surrounded by RP771 (h^- $ras1^-$) cells. **A:** The arrowhead indicates a growing JY336 cell just transferred to SPA. **B:** The same scope as **A**, but after 2 days incubation at 25°C. The arrowhead points to JY336 which has divided into three cells. Elongation of JY336 is evident, in contrast with shrinkage of surrounding JY504 cells. The bar in **B** represents 50 μm.

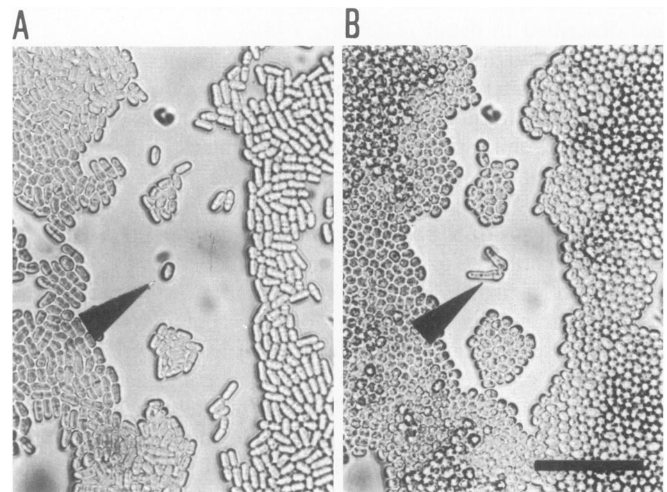

Fig. 3. Elongation of JY336 (h^+) cells surrounded by L972 (h^-) cells. Details of the experimental procedure are as in Figure 2. JY336 has divided into two cells and their elongation is more evident in this case. The bar in **B** represents 50 μm.

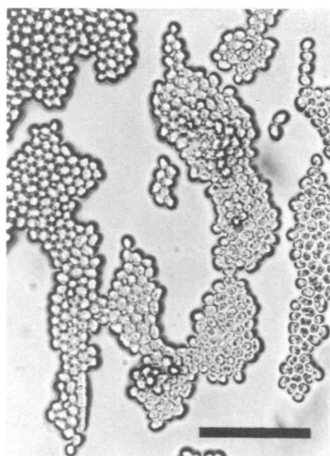

Fig. 4. JY336 cells spread alone on SPA, as a control for Figures 2 and 3. The micrograph was taken after incubation for 2 days at 25°C. Note that no elongation, but only shrinkage is seen here, even in those cells isolated from the mass.

Table II. *Schizosaccharomyces pombe* strains used in this study

L972	h^- wild-type
JY294	h^+ *ade6-704 sup3-5* (Ade$^+$)
JY333	h^- *ade6-M216 leu1*
JY336	h^+ *ade6-M210 leu1*
JY504	h^+ *ade6-M216 leu1 ras1::LEU2*$_{IK9}$
JY505	h^- *ade6-M216 leu1 ras1::LEU2*$_{IK9}$
RP771	h^- *ade6-M216 leu1 ras1::LEU2*$_{IK7}$

not elongate but shrank (Figure 4). No elongation of a h^- cell could be seen whether it was placed in the midst of h^+ *ras1*$^-$ cells or h^+ *ras1*$^+$ cells, confirming the observation described above (data not shown).

Discussion

This study demonstrates that an h^+ cell of *S. pombe* can respond to spatially separated h^- cells. As no pili-like structure has been observed in this microbe either microscopically or electron microscopically, the effect is most likely to be mediated by a diffusible factor. We tentatively call this factor the h^--factor. Although more evidence is required before we can conclude that the h^--factor is indeed a mating pheromone, this seems a strong possibility for the following reasons. (i) The wild-type strain produces this factor. (ii) The elongation of h^+ cell bodies induced by this factor is not uniform but apparently shows directionality. In most cases one end of the cell body projects out and becomes narrower than the other. Thus, it is distinct from cell growth. (iii) Elongated cells as mentioned above are often seen in cell aggregates formed under inefficient mating conditions (e.g. liquid sporulation medium). (iv) Some zygotes produced in a normal mating process possess cell shapes which strongly suggest the elongation of one partner.

In *S. pombe*, the mating reaction is induced by nutritional deprivation, especially of nitrogen. It is presently unknown whether the h^- factor is secreted constitutively or is induced by nitrogen starvation. Similarly, it is unclear whether h^+ cells secrete any diffusible factor, either in response to the h^--factor or quite independently.

The observations shown here are also of importance in that they shed some light on the function of the *ras1* gene product in *S. pombe*. As h^- *ras1*$^-$ cells apparently secrete the h^--factor as efficiently as the wild-type cells, the *ras1* protein is not required for the production of this factor. On the other hand, h^+ *ras1*$^-$ cells appear to have lost the ability to respond to the h^--factor, as shown in Figure 1D. This suggests a possible involvement of the *ras1* gene product in the mating pheromone recognition cascade, although there remains the possibility that the *ras1* protein is required to establish the state of readiness for mating, for instance, in the assessment of nutritional conditions. Further analysis, including purification of the h^--factor, will be necessary to distinguish between these possibilties.

Materials and methods

Strains

Schizosaccharomyces pombe strains used in this study are summarized in Table II, some of which were kindly supplied by J.Kohli. They are derivatives of those originally described by Leupold (1950). The construction of *ras1*$^-$ strains and the symbolization of their genotype have been previously described (Fukui *et al.*, 1986).

Media

Complete medium YPD contains per l 10 g yeast extract (Nakarai), 20 g polypeptone (Daigo) and 20 g glucose. Fifteen grams of agar is added to solidify it. Nitrogen-free SPA contains per l 10 g glucose, 1 g KH_2PO_4, 30 g agar and four vitamins (Gutz *et al.*, 1974).

Genetic methods

Standard genetic techniques described for *S. pombe* by Gutz *et al.* (1974) were followed. Micromanipulation of *S. pombe* cells was performed using Narishige MN-100 micromanipulator in a similar manner to standard tetrad analysis of yeast.

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Note added in proof

After submission of the manuscript, we were informed that K.L.Friedmann and R.Egel had suggested the existence of diffusible compounds released by *S. pombe* h^+ and h^- cells, measuring the effect of medium exchange on the pattern of protein synthesis of these strains (*Z. Naturforsch.*, **33c**, 84–91, 1978).