Acta Bot. Neerl. 43(1), March 1994, p. 65–69

SHORT NOTE

Reversion of deformed *ras1*$^-$ cells of *Schizosaccharomyces pombe* to rod-like cells by nutrient stimulation

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SUMMARY

The reversion of the highly spherical *ras1*$^-$ phenotype of the sterile mutant of *Schizosaccharomyces pombe* was examined by fluorescence microscopy of Calcofluor- and rhodamine phalloidin-labelled cells and by freeze-fracturing. Rapid and dramatic changes occurred in cell shape after a nutritional stimulus, with cells regaining their rod-like cell shape during successive cell divisions. This was achieved by restoration of the cell wall architecture and of the invaginated state of the plasma membrane. This is the first demonstration of a nutrient-induced reversion of *ras1*$^-$ *S. pombe* cells from rounded to rod-like cell shape.

Key-words: cell shape deformation, cell wall, F-actin, *Schizosaccharomyces pombe*, *ras* genes.

INTRODUCTION

Schizosaccharomyces pombe contains a single *ras* protooncogene homolog, designated *ras1* (Fukui & Kaziro 1985; Nadin-Davis *et al.* 1986a,b; Lund *et al.* 1987). It exhibits significant homology to mammalian *ras* genes (Barbacid 1987), which is documented by the finding that human *H-ras* can substitute for *S. pombe ras1 in vivo* (Nadin-Davis *et al.* 1986b).

S. pombe cells lacking the *ras1* gene are viable, but are defective in conjugation and sporulation, and their cell body is greatly deformed. Loss of *ras1* function renders mutant cells unable to undergo morphological changes in response to mating pheromones (Fukui *et al.* 1986a,b; Nadin-Davis *et al.* 1986b) and inhibits pheromone-induced transcription of the mating type locus under nutrient deprivation (Nielsen *et al.* 1992). The spectacular cell shape deformation conferred by *ras1* gene disruption (Fukui *et al.* 1986a) or missense mutation in the *ras1* gene (Hughes & Yamamoto, unpublished

This paper is dedicated to Prof. M.M.A. Sassen on the occasion of his retirement.

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results) cannot be explained by a defect in pheromone signalling (Hughes *et al.* 1991). Thus, the *ras1* gene product has to be involved in the control of cell morphology.

In a previous paper we showed that the rounding-up phenomenon of *ras1*$^-$ cells of *S. pombe* was associated with a collapse of the actin-containing cytoskeleton and induced a wide-ranging effect on the cell wall (Pichová & Streiblová 1992). The aim of this study was to provide cytological evidence for a reversion of the deformed *ras1*$^-$ phenotype of *S. pombe* to the wild-type morphology as induced by nutrient stimulation.

MATERIALS AND METHODS

Strains and culture conditions

The *ras1*$^-$ strain RP 91 (h$^+$ *leu ras1::LEU2*) of *S. pombe* (Fukui & Kaziro 1985; Fukui *et al.* 1986a,b) used in this study was kindly provided by the group of Prof. Yamamoto. The strain was grown for 50 h in YPG medium to achieve depletion of nutrients, after which the cells stopped dividing and acquired a spherical cell shape (Pichová & Streiblová 1992). Five ml of this culture (10^8 cells per ml) was resuspended in 50 ml of fresh YPG medium and cultivated with gentle agitation at 30°C. Rounded *ras1* cells reverting to rod-like cells were studied under the phase contrast microscope at 30-min intervals for 6 h. Selected samples were prepared for cell wall and F-actin staining and for freeze-fracturing.

Fluorescence microscopy

Calcofluor staining was carried out as described previously (Streiblová 1984). To stain F-actin, the fixation procedure developed by Hašek *et al.* (1987) was used with slight modifications (Jochová *et al.* 1991). Specimens were examined in an epifluorescence microscope (JenaLumar microscope; Zeiss Jena), equipped with appropriate filter systems.

Electron microscopy

Samples for freeze-fracturing were prepared as described by Moor *et al.* (1961). The freeze-fracture nomenclature of Branton *et al.* (1975) was used. The specimens were studied under a Philips EM 400 electron microscope.

RESULTS AND DISCUSSION

In stationary *ras1*$^-$ *S. pombe* cells, nutrient starvation induces complete loss of cell polarity, manifested by a spectacular morphological transformation (Fukui *et al.* 1986b; Pichová & Streiblová 1992). Within 50 h, the *ras1*$^-$ cells with a rod-like cell shape developed into stationary-phase cells with a largely homogeneous size and spherical shape. Between 50 and 70 h, the *ras1*$^-$ phenotype was characterized by a redistribution of the actin-based cell cortex, structural changes of the plasma membrane and a total dislocation of cell wall deposition (Pichová & Streiblová 1992). This behaviour has not been observed so far in *S. pombe* cells. The *ras1*$^-$ cells consistently retained their viability and specific morphology for at least 100 h. Prolonged nutrient depletion resulted in cell death.

To define these phenomena more clearly, in parallel experiments 50-h *ras1*$^-$ cells were transferred into fresh YPG medium and cultivated as described above. Calcofluor

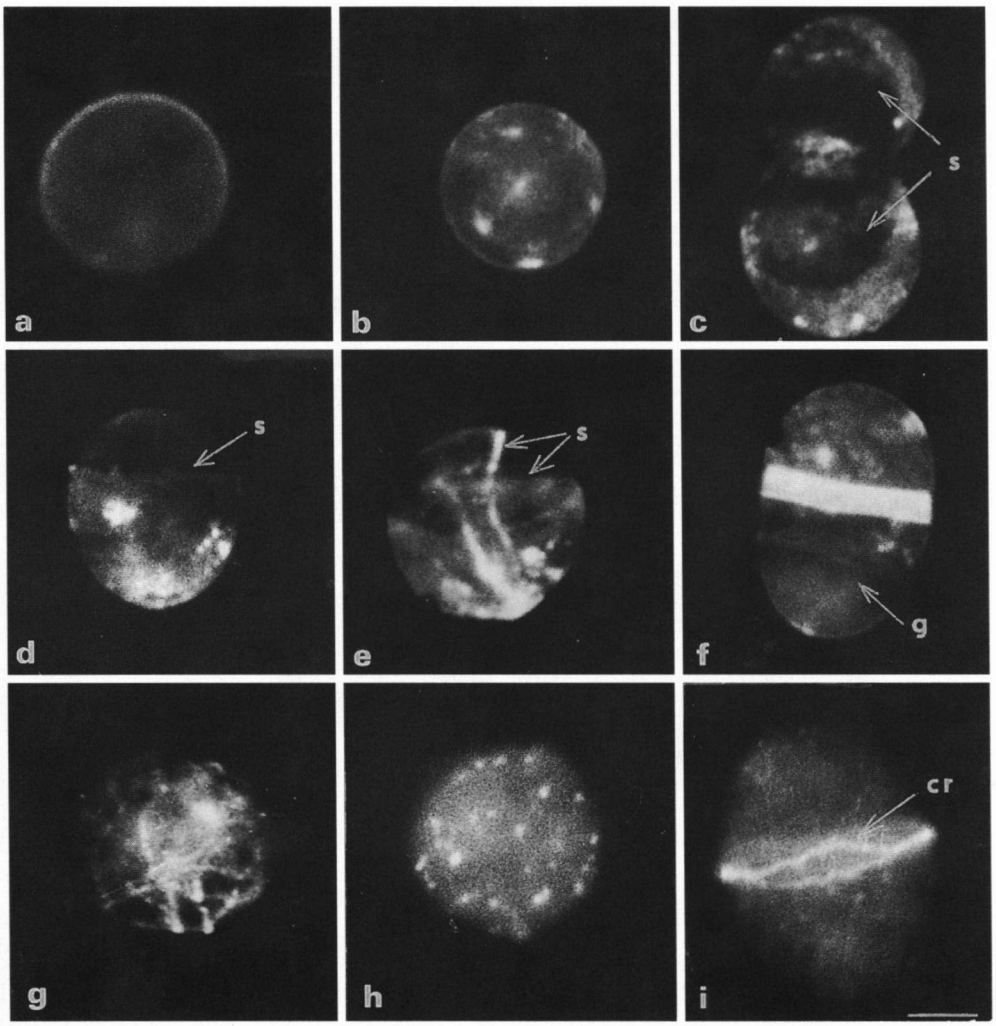

Fig. 1. (a) Calcofluor fluoresence of the cell wall of a 50-h *ras1*⁻ cells of *S. pombe* prior to nutrient stimulation. (b) Cell wall fluoresence of a *ras1*⁻ cell 30 min after nutrient stimulation. (c) Calcofluor fluorescence of the cell wall pattern in a reverting *ras1*⁻ cell; note the presence of faintly stained division scars (s). (d) Calcofluor fluorescence showing a conspicuous division scar (s); note the difference in fluorescence of the scar plug and the cell wall. (e) Calcofluor fluorescence showing overlapping division scars (s). (f) Calcofluor staining of a *ras1*⁻ cell resuming the rod-like cell shape; note a thickened septum and a regular cell wall at the pole (g). (g) Actin pattern revealed by Rh-phalloidin in a *ras1*⁻ cell prior to nutrient stimulation. (h) Dotted pattern of actin stained by Rh-phalloidin, 30 min after nutrient stimulation. (i) Contractile actin ring (cr) revealed by Rh-phalloidin staining in the plane of the future septum formation. Abbreviations: cr, contractile actin ring; g, polarized cell wall growth; s, division scar; us, uncomplete septum. Bar: 5 μm.

staining of 50-h starved cells revealed thin-walled spheres showing a uniform dim fluorescence over the entire cell surface (Fig. 1a). It is noteworthy that these cells were osmotically stable with no sign of cell wall lysis. Patches of new cell wall material were deposited on the surface of reverting cells (Fig. 1b) within 30 min of reversion of nutritional conditions. After this, the cells started to divide in the equatorial region by weakly stained septa (not shown) pointing to deposition of small amounts of septum material in the plane of future cell separation. Correspondingly, septum cleavage

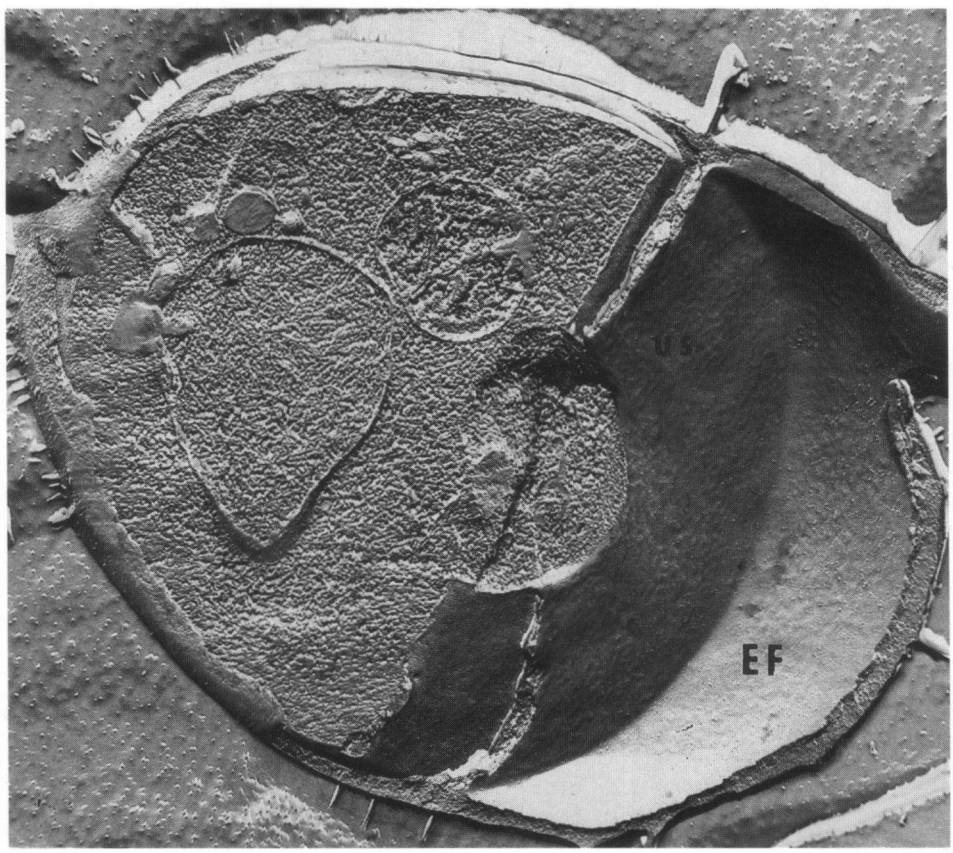

Fig. 2. Freeze-fracture electron micrograph of a *ras1*$^-$ cell exposing the exoplasmic fracture face (EF) of the plasma membrane and an uncomplete septum (us) × 50 000. See Fig. 1 for explanation of abbreviations.

resulted in the formation of slightly distinct, faintly stained division scars (Fig. 1c). Throughout subsequent divisions, wall fluorescence, and thus the amount of material deposited, appeared to increase. Division scars became more distinct (Fig. 1d) after separation by aberrant septa. It should be noticed that reverting cells showed overlapping division scars (Fig. 1e), which strongly suggests a role for *ras1* in determining the plane of cell division. By 6 h, the cells had resumed a cylindrical shape, with polar wall growth (not shown). Cell division occurred by slightly aberrant septa (Fig. 1f). The conspicuous walls of reverted *ras1*$^-$ cells consist of wall rings that impose mechanical restraints and limit cell expansion to the poles as described elsewhere (Streiblová 1981).

F-actin in starved 50-h cells occurred in the form of transcellular filaments seen throughout the cytoplasm of deformed cells (Fig. 1g). Within 30 min of nutrient stimulation, actin cables gradually rearranged into randomly distributed cortical spots (Fig. 1h). Conspicuous actin rings were regularly found during cytokinesis in all dividing cells (Fig. 1i). Their location was coincident with that of aberrant septa, confirming that the F-actin rings are required for localization of the septa (Streiblová 1981). They were comparable to the contractile rings described in *S. pombe* protoplasts (Jochová *et al.* 1991, 1993). Finally, in reverted cells, actin showed the usual polarization of cortical dots with few axial actin filaments as shown by Marks *et al.* (1985).

At 50 h of nutrient depletion the plasma membrane of freeze-fractured *ras1*$^-$ cells showed large uninvaginated areas, both at the cell periphery and in the septum region (not shown). After 4 h of reversion, the cells predominantly divided by incompletely closed septa (Fig. 2). By 6 h, the plasma membrane of reverted cells resumed its invaginated state (not shown).

Although it is unclear at present how the *ras1* protein restores the polarity of cell growth and the plane of division in *S. pombe* cells, the present observations clearly demonstrate a close interaction between the *ras1* and nutrition-sensing pathways in the fission yeast. The fact that human *H-ras* (Nadin-Davis *et al.* 1986b) and mammalian *RAP* genes (Xu *et al.* 1990) can suppress the morphological defects of *ras1*$^-$ *S. pombe* cells indicates that further studies in the given direction may be particularly rewarding for the understanding of malignant *ras* phenotypes in higher systems.

REFERENCES

Barbacid, M. (1987): *ras* genes. *Annu. Rev. Biochem.* **56:** 779–827.

Branton, D., Bullivant, S., Gilula, N.B., Karnovsky, J.M., Moor, H., Muhlethaler, K., Northcote, D.H., Packer, L., Satir, B., Satir, P., Speth, V., Staehelin, L.A., Steere, R.L. & Weinstein, R.S. (1975): Freeze-etching nomenclature. *Science* **190:** 54–56.

Fukui, Y. & Kaziro, Y. (1985): Molecular cloning and sequence analysis of a *ras* gene from *Schizosaccharomyces pombe*. *EMBO J.* **4:** 687–691.

Fukui, Y., Kozasa, T., Kaziro, Y., Takeda, T. & Yamamoto, M. (1986a): Role of a *ras* homolog in the life cycle of *Schizosaccharomyces pombe*. *Cell* **44:** 329–336.

Fukui, Y., Kaziro, Y. & Yamamoto, M. (1986b): Mating pheromone-like diffusible factor released by *Schizosaccharomyces pombe*. *EMBO J.* **5:** 1991–1993.

Hašek, J., Rupeš, I., Svobodová, J. & Streiblová, E. (1987): Tubulin and actin topology during zygote formation of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **133:** 3355–3363.

Hughes, D., A., Imai, Y. & Yamamoto, M. (1991): Regulation of the *ras* pathway in the fission yeast *Schizosaccharomyces pombe*. In: D.A. Spandidos (ed.): *The Superfamily of ras-Related Genes*, pp. 41–47. Plenum Press, New York.

Jochová, J., Rupeš, I. & Streiblová, E. (1991): F-actin contractile rings in protoplasts of the yeast *Schizosaccharomyces*. *Cell Biol. Internat. Rep.* **15:** 607–610.

Jochová, J., Rupeš, I. & Streiblová, E. (1993): Induction of F-actin rings in regenerating protoplasts of the *cdc16* mutant of *Schizosaccharomyces pombe*. *Acta Bot. Neerl.* **42:** 233–236.

Lund, P.M., Hasegawa, Y., Kitamura, K., Shimoda, C., Fukui, Y. & Yamamoto, M. (1987): Mapping of the *ras1* gene of *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* **209:** 627–629.

Marks, J., Hagan, I.M. & Hyams, J.S. (1985): Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. *J. Cell Sci.*, Suppl. 5: 229–241.

Moor, H., Muhlethaler, K., Waldner, H. & Frey-Wyssling, A. (1961): A new freezing ultramicrotome. *J. biophys. biochem. Cytol.* **10:** 1–13.

Nadin-Davis, S.A., Yang, R.C.A., Narang, S.A. & Nasim, A. (1986a): The cloning and characterization of a *ras* gene from *Schizosaccharomyces pombe*. *J. Mol. Evol.* **23:** 41–51.

Nadin-Davis, S.A., Nasim, A. & Beach, D. (1986b): Involvement of *ras* in sexual differentiation but not in growth control in fission yeast. *EMBO J.* **5:** 2963–2971.

Nielsen, O., Davey, J. & Egel, R. (1992): The *ras1* function of *Schizosaccharomyces pombe* mediates pheromone-induced transcription. *EMBO J.* **11:** 1391–1395.

Pichová, A. & Streiblová, E. (1992): Features of the periphery in the deformed *ras1*$^-$ cells of *Schizosaccharomyces pombe*. *Exp. Mycol.* **16:** 178–187.

Streiblová, E. (1981): Fission. In: J.W. Arnold (ed.): *Yeast Cell Envelopes: Biochemistry, Biophysics and Ultrastructure*, pp. 80–92. CRC Press, Boca Raton, USA.

Streiblová, E. (1984): The yeast cell wall—a marker system for cell cycle controls. In: P. Nurse and E. Streiblová (eds): *The Microbial Cell Cycle*, pp. 117–142. CRC Press, Boca Raton, FL.

Xu, H.P., Wang, Y., Riggs, M., Rodgers, L. & Wigler, M. (1990): Biological activity of the mammalian *RAP* genes in yeast. *Cell Regulation* **1:** 763–769.