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Induction of F-actin rings in regenerating protoplasts of the *cdc16* mutant of *Schizosaccharomyces pombe*

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SUMMARY

Formation of continuous F-actin rings was induced in regenerating protoplasts of the temperature-sensitive mutant of *Schizosaccharomyces pombe* defective in *cdc16* gene. The proportion of ring-containing protoplasts freshly prepared from *cdc16* mutant cells exponentially growing at permissive temperature (10%) corresponded to the proportion of mitotic cells in the starting culture. This suggests that the actin rings survived protoplast formation as was found in the wild type strain of *S. pombe* (Jochová *et al.* 1991). In 87% of the *cdc16* mutant protoplasts prepared under permissive conditions and then cultivated at 37°C in the absence of digesting enzyme, F-actin bundle assembly was initiated within 3 h. F-actin formed either abundant cables (in 63% of these spheroplasts) or distinct continuous rings (in 37% of the spheroplasts). In some spheroplasts two rings or partially split single rings were formed. These results indicate that F-actin rings not only persist during protoplast formation but also can be induced when the protoplasts are further cultivated under reversion conditions.

Key-words: F-actin ring, spheroplast, fission yeast, *Schizosaccharomyces pombe.*

INTRODUCTION

In *Schizosaccharomyces pombe* the F-actin distribution coincides with the cell-wall material deposition to specify the sites of cell surface growth, the cell polarity (Marks & Hyams 1985; Kanbe *et al.* 1989) and the sites of septum formation (Streiblová & Girbardt 1980; Marks & Hyams 1985; Kanbe *et al.* 1989). The changes in F-actin organization during *S. pombe* protoplast formation and cultivation have been studied by Kobori *et al.* (1989). The authors found that protoplast reversion in *S. pombe* correlated with the redistribution of cortical F-actin patches to the growing poles. We previously demonstrated the presence of continuous F-actin rings in freshly prepared and reverting protoplasts of the *S. pombe* and *S. japonicus* var. *versatilis* wild-type strains (Jochová *et al.* 1991). The rings originated from those in the cells used for protoplast preparation. They remained adjacent to the plasma membrane which resulted in an increase of their diameter during protoplast formation. Constriction of F-actin rings and furrowing of the spheroplasts was induced during the protoplast reversion. Here we describe the formation of

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

F-actin rings in spheroplasts of the temperature-sensitive mutant strain of *S. pombe cdc16-116*. The terminal phenotype of this mutant is characterized by formation of multiple septa and by a block in cytokinesis (Minet *et al.* 1979). This has been interpreted as a consequence of multiple signals for septation (Streiblová 1981). Marks *et al.* (1992) have reported that the signals might be generated by the regulatory complex which fails to be inactivated once the first septum is completed due to the loss of the *cdc16* function.

MATERIALS AND METHODS

The temperature-sensitive mutant strain *cdc16-116 h*$^-$ of *S. pombe* was used in all experiments. For protoplast preparation the cells were grown at the permissive temperature (25°C) in YPG medium (Jochová-Svobodová *et al.* 1989) till the early exponential phase was reached. Then, cells were transferred to Novozyme 234 digesting mixture stabilized with 1·2 M sorbitol (Jochová *et al.* 1991) to the final density 5×10^7 cells ml^{-1}. After 1·5–2 h incubation at 25°C the mixture was diluted with 0·1 M KH_2PO_4 (pH 6·5) to reach a final sorbitol concentration of 1 M. Subsequently the protoplasts were transferred into YPG/2 medium (Jochová-Svobodová *et al.* 1989) supplemented with 1·5 mg ml^{-1} Novozyme 234 (reversion-preventing conditions) or without the enzyme (reversion conditions). They were cultivated at 37°C for 3 h at the concentration 10^7 ml^{-1}. Fixation with 3·7% formaldehyde for 10 min after 5 min pre-incubation in stabilizing buffer (Jochová *et al.* 1991) was used for the visualization of F-actin, DNA and cell walls. The fixed and permeabilized protoplasts or spheroplasts were stained with Rh-phalloidin for 10 min to visualize F-actin and with 4′,6-diamidino-2-phenylindole (DAPI) to visualize DNA (Hašek *et al.* 1986). Cell walls of the whole cells were stained with Calcofluor M2R (Streiblová 1988).

RESULTS AND DISCUSSION

Cells of the *cdc16-116* mutant of *S. pombe* display multiple septa at 37°C (Minet *et al.* 1979; Streiblová 1981). Correspondingly, multiple F-actin rings (Fig. 1a) were assembled at the sites of septum formation between separated nuclei (Fig. 1b) during the 3 h cultivation at the restrictive temperature. In fresh protoplast culture prepared from the cells grown at the permissive temperature, 10% of the protoplasts contained F-actin rings. The same proportion of ring-containing cells was found in the starting culture. This supports our previous suggestion that the rings in freshly prepared protoplasts originate from the cells of the starting culture (Jochová *et al.* 1991).

To induce the formation of multiple rings we transferred the mutant protoplasts to the restrictive temperature. In the presence of Novozyme 234 (1·5 mg ml^{-1}) the number of ring-containing protoplasts did not significantly change within 3 h. On the other hand, in the Novozyme 234-lacking medium the content of continuous F-actin assemblies dramatically increased in 87% of spheroplasts during the same period. In 63% of those spheroplasts F-actin formed numerous cables extensively pervading the cytoplasm (Fig. 1c), whereas in the rest (37%) distinct F-actin rings adjacent to the plasma membrane were clearly identified (Fig. 1d). Occasionally, two (Fig. 1e) or partially split (Fig. 1f) rings or rings with extremely reduced diameter were seen (Fig. 1g). In all cases the formation of F-actin arrangements was associated with nuclear division, similarly to the wild-type protoplasts and spheroplasts (Jochová *et al.* 1991).

In other words, within 3 h the proportion of ring-containing spheroplasts increased about threefold compared to the fresh protoplasts under the reversion conditions. Under

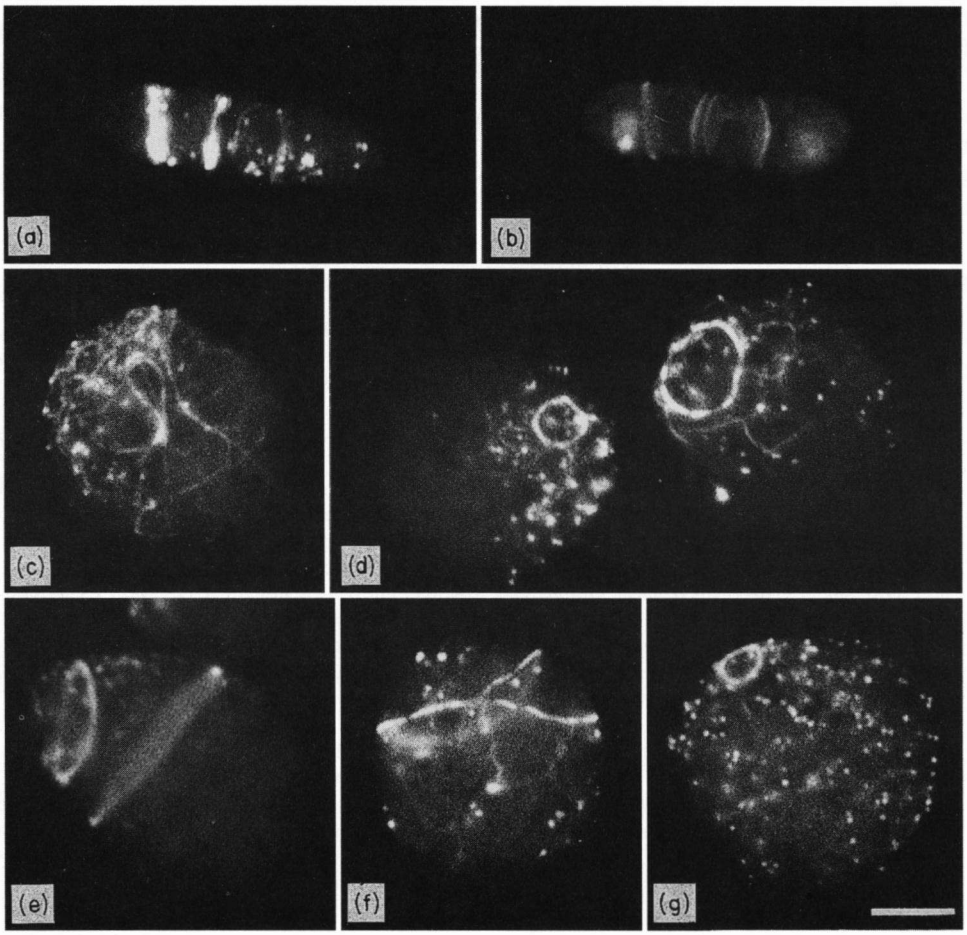

Fig. 1. *Schizosaccharomyces pombe cdc16-116h*$^-$ mutants cultivated at 37°C for 3 h. (a,b) Triple-stained cell; (a) F-actin stained by Rh-phalloidin; (b) the cell wall stained by Calcofluor M2R and DNA by DAPI. (c–g) Rh-phalloidin staining of F-actin in the spheroplasts; (c) F-actin cables pervading the cytoplasm; (d) F-actin rings adjacent to the plasma membrane; (e) two F-actin rings in a spheroplast; (f) partially split F-actin ring; (g) extremely small F-actin ring. Bar: 5 μm.

reversion-preventing conditions no increase occurred. This suggests that in the spheroplasts of the *cdc16-116* mutant of *S. pombe* the presence of newly synthesized cell-wall material may enhance the induction of the F-actin ring assembly. More probably, the formation of F-actin cables is inhibited by non-specific activities of the Novozyme 234 enzymatic mixture. The incomplete cell envelope interferes considerably with the formation of regular rings and often bunches of irregular cables appear instead. Since it is generally accepted that the dividing nucleus is a signal source for positioning of the contractile ring (Satterwhite & Pollard 1992), the occurrence of irregular actin cables might be a consequence of random movements of mitotic nuclei in the spheroplasts (Streiblová 1981). The formation of the extremely abundant F-actin cables has not been observed in wild-type protoplasts and spheroplasts (Jochová *et al.* 1991). Therefore, it is also possible that the mechanism of ring induction requires some functional linkages between actin

filaments and the cell surface which may be affected by the loss of the *cdc16* gene product function.

Reverting protoplasts of the wild-type strain are somewhat furrowed at the cell division plane (Jochová *et al.* 1991). On the contrary, no such furrowing was observed after 3 h reversion of the ring-containing *cdc16-116* mutant spheroplasts. This indicates that the furrowing of wild-type spheroplasts corresponds to the cell separation process which is inhibited in the *cdc16* mutant cells (Minet *et al.* 1979).

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