Volume 316, number 1, 41–47 FEBS 12010 January 1993

High levels of profilin suppress the lethality caused by overproduction of actin in yeast cells

Viktor Magdolen[a,*], David G. Drubin[b], Günter Mages[a,*] and Wolfhard Bandlow[a]

[a]*Institut für Genetik und Mikrobiologie der Universität München, Maria-Ward-Str. 1a, 8000 München 19, Germany* and [b]*Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA*

Received 30 November 1992

Overproduction of actin is lethal to yeast cells. In contrast, overexpression of the profilin gene, *PFY1*, encoding an actin-binding protein, leads to no very obvious phenotype. Interestingly, profilin overproduction can compensate for the deleterious effects of too much actin in a profilin concentration-dependent manner. Our results, thus, document that actin and profilin interact in vivo. Immunofluorescence studies suggest that suppression works by reducing actin assembly. We observed, however, that even massive overproduction of profilin fails to fully restore the wild-type phenotype (e.g. the wild-type appearance of the actin microfilament system). This may indicate that actin monomer sequestration is not the only mechanism by which the balance of actin polymerization is controlled.

Actin-binding protein; Actin microfilament system; Overexpression; *Saccharomyces cerevisiae*

1. INTRODUCTION

The architecture of the actin filament system of eukaryotic cells is controlled by accessory proteins [1–3]. One of these, profilin, has been characterized from vertebrates, unicellular organisms and plant pollen [1,4–6]. Profilin has been shown in in vitro studies to form 1:1 complexes with monomeric actin and is, thus, thought to influence actin filament formation by sequestering monomeric G-actin from the soluble actin pool and preventing it from polymerizing into F-actin [1,7–11]. In addition, reversible cap formation by profilin on the fast-growing ends of F-actin has been demonstrated [1,12,13]. Profilin also influences the rate of nucleotide exchange on actin molecules in vitro and may thereby be controlling the rate of actin polymerization [13–15]. Consequently, in the presence of high levels of ATP, profilin may even stimulate actin filament formation in vivo [12,13]. In vitro experiments with profilin from human platelets, *Acanthamoeba* and yeast [16–20] demonstrated, in addition, the reversible binding to polyphosphoinositides, thereby linking the control of actin polymerization to the growth signaling pathway and cell cycle progression [19,20]. The actin gene, *ACT1*, is essential [21]. However, we demonstrate here that overproduction of actin is deleterious to yeast cells. The structural gene for yeast profilin (*PFY1*) has been analysed recently [22]. Although viable, *PFY1*-disrupted cells display abnormal characteristics including temperature sensitivity of growth and a dramatically altered actin filament system [5]. These cells exhibit a less polarized distribution of cortical spots in both mothers and buds and lack actin cables. Instead, actin often is deposited in a bar structure, the most obvious feature observed in many of these cells after fluorescence staining with anti-actin antibodies. Similar dramatic and to a certain extent comparable effects on the cytoskeleton have been observed in null-allele mutants of other actin-binding proteins such as tropomyosin [23] and Cap2p [24] or by overproduction of the actin-binding protein Abp1p [25,26]. Overproduction of profilin, on the other hand, has no severe effect on the morphology of yeast cells or on the architecture of the actin cytoskeleton. But when profilin is overproduced together with actin, the deleterious effects of high levels of actin are suppressed. Our data confirm that actin and profilin interact directly in vivo and suggest that profilin can exert control on actin assembly.

Correspondence address: W. Bandlow, Institut für Genetik und Mikrobiologie, Maria-Ward-Strasse 1a, D-8000 Munich 19, Germany.

Present address: Max-Planck-Institut für Biochemie, 8033 Martinsried, Germany.

2. MATERIALS AND METHODS

2.1. *Yeast strains and construction of plasmids*

The *Saccharomyces cerevisiae* wild-type DL1 *MATα his3 leu2 ura3* [27] served as the recipient strain for all plasmids used in this study. To construct YEpGALPFY, a 685 bp *Eco*RI/*Bam*HI *GAL1/10* promoter fragment (derived from plasmid pBM272, obtained from M. Johnston) was cloned into YEp352 [28] to yield YEpGAL. In a second step a 977 bp *Sau*3A fragment (from pos. −11 to +966 [22]) containing the complete *PFY1* gene without its promoter was inserted into the single *Bam*HI site of YEpGAL which, in the correct orientation, yielded a *GAL1/PFY1* fusion gene. The profilin-deficient strain HPL0 *MATα ade2 his3 leu2 trp1 ura3 pfy1::LEU2* is a haploid progeny of

DWL1 *MATa/MATα PFY⁺/pfy::LEU2 ade2/+ his3/his3 leu2/leu2 trp1/+ ura3/ura3* (G. Mages, unpublished). The *PFY1* gene was disrupted by insertion of the *LEU2*-selective marker in the same manner as described for PFYD0 [22]. For growth curves, 5 ml cultures in selective minimal medium were shaken at 23°C and the absorbance at 600 nm was monitored.

2.2. *Western blotting and immunofluorescence*

Protein extracts were prepared by breaking the cells with glass beads after boiling for 2 min in the presence of SDS-PAGE sample buffer [25]. Immunoblots of total proteins were performed as described previously [5] using the immunogold detection system (Amersham). Polyclonal rabbit antisera against actin [25] and profilin [5] were used.

For immunofluorescence studies, cells were grown on selective medium containing 2% glucose at 23°C. Immunofluorescence microscopy was performed as described previously [29].

3. RESULTS

We have overproduced profilin in haploid wild-type yeast transformed with multicopy plasmids containing the profilin gene (*PFY1*) [22]. In a Western blot of cell homogenates, a polyclonal anti-profilin antiserum detects a single protein of apparent molecular mass 13,000 Da (Fig. 1). A four- to tenfold overproduction of the protein (based on densitometric analysis, not shown; see e.g. Fig. 1) is achieved using either its own or, after induction by galactose, the *GAL1* promoter. The strain harbouring the *GAL1/PFY1* fusion on the multicopy vector YEp352 grows very slowly on galactose. Since in a control experiment, using a transformant containing the vector with the *GAL1/GAL10* promoter but lacking profilin sequences (YEpGAL), the same effect is observed, we attribute this slow-growth phenotype to competition for the binding of the transactivator Gal4p [30]. It is very likely that the vast excess of binding sites for the transactivator Gal4p present on the multiple plasmid copies leads to only partial induction of chromosomal-encoded genes for galactose catabolizing enzymes and, in consequence, to an increased generation time on galactose. Transformant cells overexpressing profilin under the control of its own promoter are viable and grow at the same rate or even slightly faster than the same strain transformed with a control plasmid lacking *PFY1*. In a strict contrast to profilin-deficient cells, overproduction of profilin does not result in a temperature sensitive phenotype (tested at 23, 30 and 37°C). These cells exhibit normal size and ellipsoidal morphology and do not display multiple buds. In indirect immunofluorescence experiments, cells overexpressing *PFY1* show only minor differences in the organization of the actin cytoskeleton in comparison to the wild-type. Budding wild-type cells contain an asymmetric distribution of actin [31,32]: actin filaments or cables are aligned parallel to the mother-daughter cell axis, whereas cortical patches occur predominantly in the bud at sites of active growth of the cell surface (Fig. 2A). Cells that overexpress profilin, on the average, show slightly shorter and thinner cables, whereas the number of patches appears less affected, the organization and asymmetric distributions being maintained (Fig. 2B). The background fluorescence is significantly higher as compared to wild-type control cells, probably caused by a higher concentration of unpolymerized profilactin complexes in these cells. In general the features of profilin overexpressing cells indicate that, although the actin organization is slightly affected in vivo, the changes have no major consequences for cell growth in contrast to profilin-deficient cells. In *PFY1*-disrupted strains, on the other hand, completely abnormal actin filament organization is observed [5]. Immunofluores-

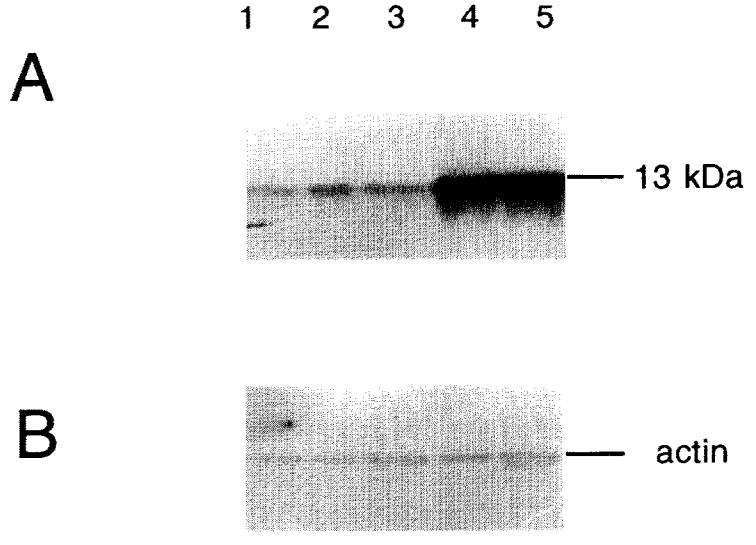

Fig. 1. Overexpression of yeast profilin. Protein extracts, prepared from yeast transformant cells grown on selective synthetic medium containing 2% glucose (lanes 1,3,5) or 3% galactose (lanes 2,4), were separated by SDS-PAGE and decorated with (A) a profilin-specific polyclonal antibody and (B) with an anti-actin antibody. Strain DL1 *MATa his3 leu2 ura3* was either transformed with control shuttle vector YEp352 (lanes 1 and 2) or plasmid YEpPFY, containing the profilin gene under its own promoter [22] (lane 5), or plasmid YEpGALPFY, which allows regulated overexpression of profilin (lanes 3 and 4).

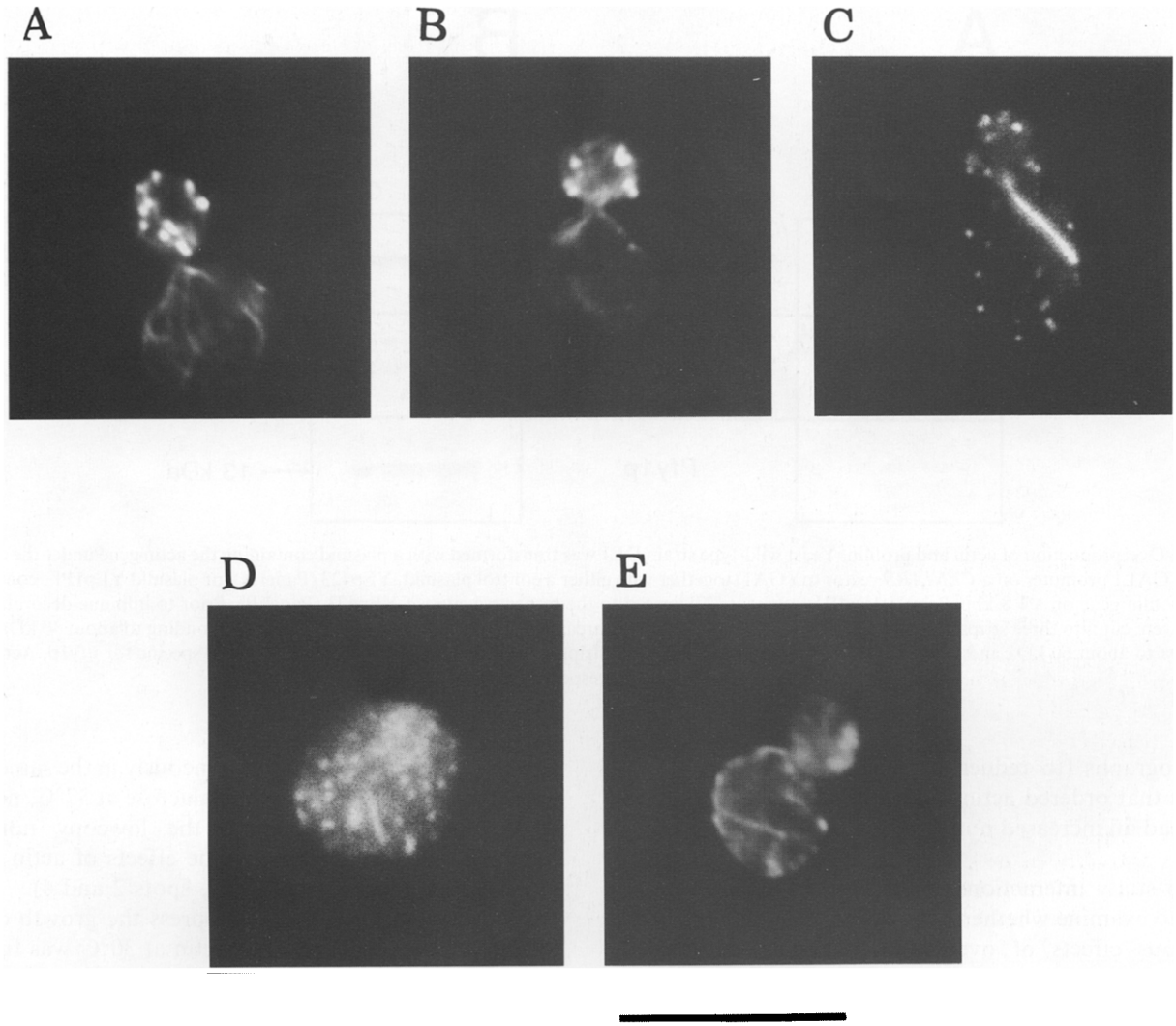

Fig. 2. Actin distribution in wild-type and cells lacking or overexpressing profilin. Fixed yeast cells were stained with affinity-purified anti-actin antibodies and analysed by indirect immunofluorescence microscopy. A, wild-type strain DL1 transformed with control plasmid YEp423 [35]; B, DL1, transformed with YEp1PF; C, profilin-deficient strain HPL0; D, actin-overexproducing strain DL1, transformed with plasmid pYGA1; E, co-overproduction of actin and profilin. In printing these panels, D was exposed 6- and E 3-fold longer than A in order to reduce strong background fluorescence. Scale bar, 10 μm.

cence microscopy shows the absence of actin cables and the abolition of the polar distribution of actin spots. In addition, in many of these cells (about 50%) actin is aggregated into a bar structure which is absent from wild-type cells (Fig. 2C). On the average, these mutant cells are larger, heterogenous in size and frequently have lost their ellipsoidal cell morphology and assumed a more roundish shape. They select bud sites randomly (cf. [5]). Mother cells are often multinucleate as revealed by DAPI staining (about 5 to 10%, not shown), whereas in many cases buds emerge which have retained actin patches but lack nuclei.

Constitutive overproduction of actin from a multicopy plasmid is not tolerated in haploid yeast cells (cited in [33]). Therefore, a centromere plasmid containing a *GAL1* promoter/*ACT1* gene fusion was used to allow inducible overexpression of *ACT1*. Expression and induction by galactose was studied in a Western blot experiment using actin-specific antibodies. Sera directed against profilin and Sac6p, which is yeast fimbrin, another cytoskeletal protein [34], served as constitutive controls. Fig. 3A shows that, after induction by galactose, an increase of at least twofold in the steady state level of actin was achieved. Actin overproducing cells cease to grow shortly after a shift from glucose to galactose medium (Fig. 4). Microscopic analysis of cells induced for overproduction of actin for 16 h shows that both budded and unbudded cells are present, indicating that the cells are blocked at all stages of the cell cycle (not shown). The use of an anti-actin antibody in indirect immunofluorescence yields a very bright background in these cells, which may be caused by high concentrations of soluble actin. Although analysis of such cells is thereby impeded, longer exposure of such

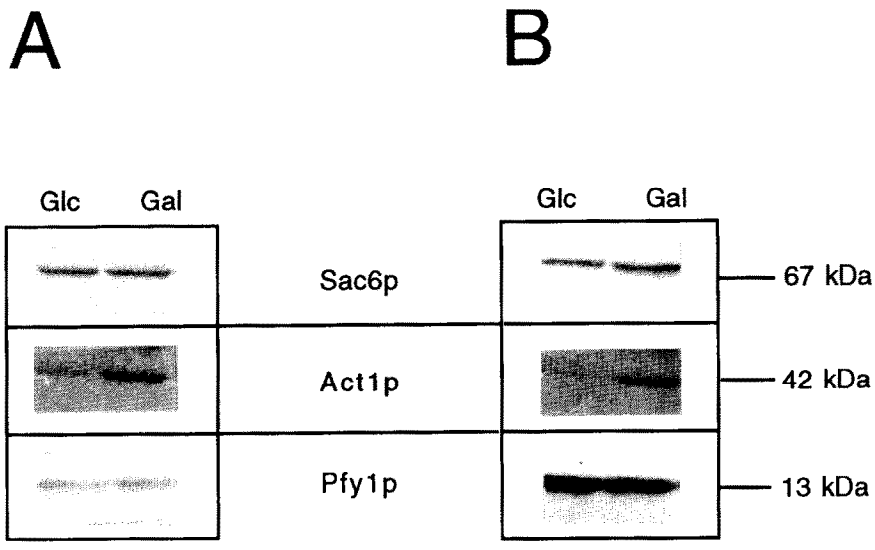

Fig. 3. Overproduction of actin and profilin. Yeast wild-type strain DL1 was transformed with a plasmid containing the actin gene under the control of the GAL1 promoter on a *CEN/ARS* vector (pYGA1) together with either a control plasmid, YEp423 (Panel A), or plasmid YEp1PF, containing the profilin gene on a 1.8 kbp *Bam*HI/*Hin*dIII fragment [22] ligated to the respective sites of YEp423 (Panel B). Prior to immune-decoration the blots were cut into three strips perpendicular to the lanes (covering the areas from the dye front to a position corresponding to about 30 kDa, from 30 kDa to about 60 kDa and from 60 kDa to the top of the gel). The strips were incubated separately with antisera specific for Pfy1p, Act1p and Sac6p, respectively.

photographs (to reduce background fluorescence) reveals that ordered actin fibers or even bars are absent. Instead an increased number of actin spots occurs (Fig. 2D).

To study interactions of actin with profilin in vivo and to examine whether profilin can counteract the deleterious effects of overproduction of actin on cell growth, we transformed yeast wild-type cells simultaneously with *GAL1/ACT1*- and profilin-encoding plasmids. Expression of the profilin gene was driven by its own (moderately strong) constitutive promoter. As in cells overexpressing only *PFY1* (Fig. 1), a comparison of Panels A and B of Fig. 3 reveals that also in the double transformant profilin is overexpressed about four- to tenfold from the *PFY1* multicopy plasmid. The constitutive expression of fimbrin (Sac6p) serves as an internal control.

The transformant strains were examined for effects of the simultaneous and separate overexpression of *PFY1* and *ACT1* on cell growth. As controls, transformants containing either the actin construct together with the 2μ- or *CEN/ARS*-based control plasmids (lacking a profilin gene, spots 1 and 3 in Fig. 4A, Glc and Gal), or the profilin gene together with the *CEN/ARS* vector (lacking an actin gene, spot 5), were assayed for growth on glucose and galactose media. Fig. 4A shows that glucose allows growth of all strains at 30 and 37°C whereas induction of actin expression by galactose at 30°C is lethal to cells overproducing actin alone. Overproduction of profilin alone leads to no obvious phenotype (as observed above). Profilin overproduction suppresses the lethal effect of high actin levels when both genes are overexpressed simultaneously in the same cells (spots 2 and 4 in Fig. 4A). On galactose at 37°C, neither expression of profilin from the lowcopy nor the highcopy plasmid suppresses the effects of actin overproduction (Fig. 4A, Gal 37°C, spots 2 and 4).

The ability of profilin to suppress the growth defect caused by overproduction of actin at 30°C was further tested by measuring growth rates of strains containing the *ACT1* gene on a *CEN/ARS* vector together with either a control vector or a plasmid containing the *PFY1* gene under its own promoter on a single- or a multicopy plasmid. The data in Fig. 4B demonstrate that, at 23°C on glucose minimal medium, the velocity of mass doubling is relatively slow in all cases, presumably due to the presence of two different plasmids in the same cell. However, it is very similar for all of the transformants on glucose. When the cells are transferred to galactose-containing minimal medium, the strain induced for actin overproduction alone ceases to grow, whereas the double transformants carrying both the *ACT1* and *PFY1* plasmids grow at a rate proportional to the copy number of the *PFY1*-encoding plasmids. This observation suggests that the compensation of the deleterious effect of the overproduction of actin is dependent on the level of profilin in the cell. However, a full suppression of the phenotype of actin-overproducing cells cannot be reached (compare e.g. growth rates of transformants YEp1PF *plus* pYGA1 and YEp1PF *plus* YCp50 in Fig. 4B).

Addition of uracil to the selective medium, leads to the rapid loss of the centromere plasmid containing the *GAL1/ACT1* fusion gene (which is normally retained

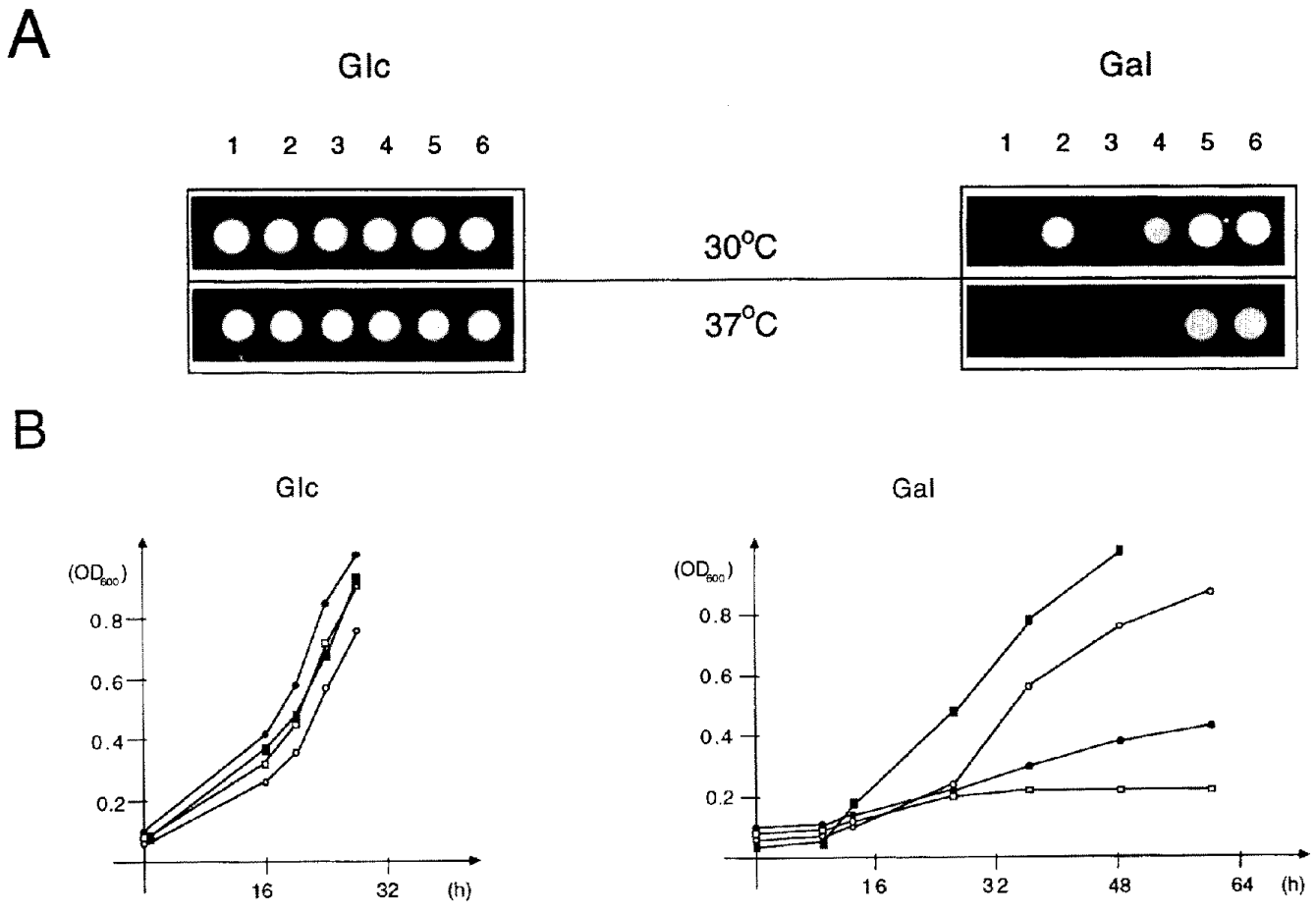

Fig. 4. Effects on growth of overexpression of actin and profilin. Double transformant DL1 strains were grown on either 2% glucose (Glc) or 3% galactose(Gal) synthetic medium. A, liquid pre-cultures in glucose medium were spotted onto minimal medium plates supplemented with 0.01% histidine and incubated at the specified temperatures for 3–4 days (glucose) and 8–10 days (galactose) at 30 and 37°C, respectively. Dot 1, DL1 transformed with the multicopy control vector YEp423 (control vector without *PFY1*) *plus* pYGA1; 2, YEp1PF *plus* pYGA1; 3, single copy vector YCp402 (control vector without *PFY1*) *plus* pYGA1; 4, YCpPFY (contains the 1.8 kbp PFY1-fragment [22] inserted into YCp402) *plus* pYGA1; 5, YEp1PF *plus* the single copy vector YCp50 (control vector without *ACT1*); 6, DL1, transformed with YCp402 *plus* YCp50 (two control vectors). B, Growth rates of wild-type yeast containing *ACT1* and *PFY1*-encoding plasmids. Pre-cultures in glucose medium were washed twice with water and diluted with either 2% glucose or 3% galactose selective medium. DL1 was transformed with the centromere vector pYGA1 (containing *ACT1* under *GAL1* control). Additionally, the transformants contained either the 2μ-based control plasmid YEp423 (□; corresponding to dot 1 in Fig. 4A) or *PFY1* under its own promoter on a multicopy vector (○; dot 2 in Fig. 4A) or *PFY1* on a singlecopy plasmid (●; dot 4 in Fig. 4A). As another control strain, lacking pYGA1, DL1 was transformed with the *CEN* plasmid YCp50 together with YEp1PF (■; dot 5 in Fig. 4A). All growth curves were averaged from two experiments.

because of selection for the *URA3*-selective marker of the plasmid), whereas addition of leucine does not lead to a loss of the 2μ-based plasmid (with the marker gene *LEU2*) containing the *PFY1* gene when grown on galactose. In contrast, the same experiment performed with glucose as the carbon source leads to a much faster loss of the 2μ-based plasmid as compared to the *CEN/ARS* plasmid, which is expected if there is no selection for either loss or retention of the plasmids (data not shown). The observation that the cells loose the centromere plasmid containing the overexpressed actin gene rapidly after addition of uracil in spite of the overproduction of profilin supports the conclusion that high levels of profilin only partially suppress the lethal effects caused by actin overproduction.

The incomplete suppression is also reflected by the inability of the strains co-overexpressing actin and profilin to grow into colonies at elevated temperatures (37°C; Fig. 4A). In addition, observation of these cells by light microscopy (not shown) reveals that they exhibit abnormal, elongated buds or are multi-budded and sometimes multinucleate (as evident from DAPI staining), even at the lower temperature.

Indirect immunofluorescence experiments performed with actin-specific antibodies reveal that simultaneous overproduction of profilin and actin seems to revert to a certain degree the deleterious phenotype observed when actin is overexpressed alone (compare Fig. 2E with 2D). The identification of fluorescent structures in these micrographs is again complicated by the strong

background fluorescence. However, it appears that additional profilin partly restores the asymmetric distribution of actin. It can also be seen and is remarkable that in many dividing cells, the buds appear to be packed with actin, while the mother cells have apparently normal actin contents. Thus, even the four- to tenfold overexpression of profilin is insufficient to compensate completely for about only two-fold higher levels of actin.

4. DISCUSSION

In yeast, the formation of actin filaments is delicately balanced by a complex system of accessory actin-binding proteins. Mutations which impair this balance generally lead to deleterious consequences for cell morphology and/or proliferation. Deficiency in one of the components which are involved in the structure or regulation of the actin cytoskeleton frequently leads to a phenotype which is similar to that produced by overproduction of the respective protein [36,37]. This generalization is corroborated by the analysis of actin overproduction in this paper. Insofar, we were surprised to find that overproduction of profilin led to no obvious phenotype. This finding was the less expected as it is known that profilin apparently fulfills multiple functions in the cell. This protein is thought to exert control on the extent and kinetics of microfilament formation, possibly at different steps [1,12]. It also has been implicated in the regulation of phosphoinositide metabolism [17] and in Ras1,2p- and adenylate cyclase-dependent signaling [19,20]. We could show that profilin, in fact, interacts with F-actin in vivo, confirming earlier results obtained in vitro. Similar conclusions have been derived from experiments using microinjection of profilin into rat kidney cells [38] and from the analysis of the cytological consequences of a deletion of the chickadee gene, encoding profilin, in *Drosophila* which leads to the displacement of nurse cell nuclei through the involvement of a misshaped cytoplasmic actin filament system early in egg development [39]. By using simultaneous overproduction of actin and profilin we could demonstrate that profilin, by some unproven mechanism, can suppress actin toxicity due to high level expression. Those cells simultaneously overproducing actin and profilin grow and show a more normal-appearing actin cytoskeletal architecture as when actin is overproduced alone. However, we have failed to restore completely the cytological wild-type appearance of the actin cytoskeleton even when vastly overproducing profilin: a more than fourfold overproduction of profilin fails to compensate fully for about only twofold higher levels of actin expressed from an additional *ACT1* copy on a lowcopy plasmid under the guidance of the *GAL1* promoter. Assuming a cellular concentration of actin of about 60 μM (corresponding to about 0.3% of the total cellular protein) and of profilin of about 20 μM (corresponding to about 1/3 of actin in analogy to the conditions *Acanthamoeba* [13]) in the yeast wild-type, this means that in the transformant overproducing both proteins about 100–200 μM profilin are insufficient to balance excessive F-actin formation in the presence of 120 to 150 μM actin. This conclusion is particularly underlined by the failure of profilin overproduction to balance an about twofold increase in cellular actin concentration at the elevated temperature of 37°C. How this suppression operates and why it is incomplete remains unknown at present. One possible explanation of the observed effect could be that additional accessory proteins are playing a role in the maintenance of the balance of actin polymerization. Such proteins may be titrated away by the excess of F-actin under these conditions.

Acknowledgements: We would like to thank M. Johnston, Washington University School of Medicine, St. Louis, and J.D. Beggs, Edinburgh, for the gifts of plasmids and A.E.M. Adams, The University of Arizona, Tucson, for anti-profilin antibodies. V.M. wishes to thank the members of D.G.D.'s laboratory for their various support during his stays. The work was financially aided by grants from the Deutsche Forschungsgemeinschaft to W.B., by the National Institute of General Medical Sciences (GM-42759) and the Searle Scholars Program/The Chicago Community Trust to D.G.D. and by a travel support from the Deutsche Forschungsgemeinschaft to V.M.

REFERENCES

[1] Pollard, T.D. and Cooper, T.A. (1986) Annu. Rev. Biochem. 55, 987–1035.
[2] Way, M. and Weeds, A. (1990) Nature 344, 292–294.
[3] Drubin, D.G. (1990) Cell Motil. Cytoskel. 15, 7–11.
[4] Hartwig, J.H., Chambers, K.A., Hopica, K.L. and Kwiatkowski, D.J. (1989) J. Cell. Biol. 109, 1571–1579.
[5] Haarer, B.K., Lillie, S.H., Adams, A.E.M., Magdolen, V., Bandlow, W. and Brown, S.S. (1990) J. Cell. Biol. 110, 105–114.
[6] Valenta, R., Duchêne, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D. and Scheiner, O. (1991) Science 253, 557–560.
[7] DiNubile, M.J. and Southwick, F.S. (1985) J. Biol. Chem. 260, 7402–7409.
[8] Cooper, J.A. (1991) Annu. Rev. Physiol. 53, 5585–5605.
[9] Hartwig, J.H. and Kwiatkowski, D.J. (1991) Curr. Op. Cell Biol. 3, 87–97.
[10] Vandekerckhove, J.S., Kaiser, D.A. and Pollard, T.D. (1989) J. Cell Biol. 109, 619–626.
[11] Lal, A.A. and Korn, E.D. (1985) J. Biol. Chem. 260, 10132–10138.
[12] Goldschmidt-Clermont, P.J. and Janmey, P.A. (1991) Cell 66, 419–421.
[13] Goldschmidt-Clermont, P.J., Machesky, L.M., Doberstein, S.K. and Pollard, T.D. (1991) J. Cell Biol. 113, 1081–1089.
[14] Mockrin, S.C. and Korn, E.D. (1980) Biochemistry 19, 5359–5364.
[15] Nishida, E. (1985) Biochemistry 24, 1160–1164.
[16] Lassing, I. and Lindberg, U. (1988) J. Cell. Biochem. 37, 255–267.
[17] Machesky, L., Goldschmidt-Clermont, P.J. and Pollard, T.D. (1990) Cell Reg. 1, 937–950.
[18] Goldschmidt-Clermont, P.J., Machesky, L.M., Baldassare, J.J. and Pollard, T.D. (1990) Science 247, 1575–1578.
[19] Goldschmidt-Clermont, P.J., Kim, J.W., Machesky, L.M., Rhee, S.G. and Pollard, T.D. (1990) Science 251, 1231–1233.
[20] Vojtek, A., Haarer, B., Field, J., Gerst, J., Pollard, T.D., Brown, S. and Wigler, M. (1991) Cell 66, 497–505.

[21] Shortle, D., Haber, J.E. and Botstein, D. (1982) Science 217, 371–373.
[22] Magdolen, V., Oechsner, U., Müller, G. and Bandlow, W. (1988) Mol. Cell. Biol. 8, 5108–5115.
[23] Liu, H. and Bretscher, A. (1989) Cell 57, 233–242.
[24] Amatruda, J.F., Cannon, J.F., Tatchell, K., Hug, C. and Cooper, J.A. (1990) Nature 344, 352–354.
[25] Drubin, D.G., Miller, K.G. and Botstein, D. (1988) J. Cell Biol. 107, 2551–2561.
[26] Drubin, D.G., Mulholland, J., Zhu, Z. and Botstein, D. (1990) Nature 343, 288–290.
[27] Van Loon, A.G.P.M., Van Eijk, E. and Grivell, L.A. (1983) EMBO J. 2, 1765–1770.
[28] Hill, J.E., Myers, A.M., Koerner, T.J. and Tzagoloff, A. (1986) Yeast 2, 163–167.
[29] Pringle, J.R., Preston, R.A., Adams, A.E.M., Stearns, T., Drubin, D.G., Haarer, B.K. and Jones, E.W. (1989) Methods Cell. Biol. 31, 357–435.
[30] Baker, S.M., Okkema, P.G. and Jaehning, J.A. (1984) Mol. Cell. Biol. 4, 2062–2071.
[31] Kilmartin, J.V. and Adams, A.E.M. (1984) J. Cell Biol. 98, 922–933.
[32] Adams, A.E.M. and Pringle, J.R. (1984) J. Cell Biol. 98, 934–945.
[33] Huffaker, T.C., Hoyt, M.A. and Botstein, D. (1987) Annu. Rev. Genet. 21, 259–284.
[34] Adams, A.E.M., Botstein, D. and Drubin, D.G. (1991) Nature 354, 404–408.
[35] Ma, H., Kunes, S., Schatz, P.J. and Botstein, D. (1987) Gene 58, 201–216.
[36] Burke, D., Gasdaska, P. and Hartwell, L. (1989) Mol. Cell. Biol. 9, 1049–1059.
[37] Shortle, D., Novick, P. and Botstein, D. (1984) Proc. Natl. Acad. Sci. USA 81, 4889–4893.
[38] Cao, L.-G., Babcock, G.G., Rubenstein, P.A. and Wang, Y.-L. (1992) J. Cell Biol. 118, 1023–1030.
[39] Cooley, L., Verheyen, E. and Ayers, K. (1992) Cell 69, 173–184.