Profilin is required for the normal timing of actin polymerization in response to thermal stress

Juddi Yeh, Brian K. Haarer*

Department of Zoology and Institute for Cellular and Molecular Biology, 219 Patterson, The University of Texas, Austin, TX 78712-1064, USA

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Abstract **We have used a fluorometric assay to determine the relative amounts of polymerized actin (F-actin) in wild-type and profilin mutant yeast cells. Our results indicate that profilin plays a role in maintaining normal F-actin levels in response to shifts to high temperature. Cells lacking profilin display a greater drop in F-actin levels upon such temperature shifts, and are slower to recover to initial F-actin levels than are wild-type cells. Interestingly, shifts to cold temperatures result in rapid increases of F-actin levels in wild-type and profilin null cells. We have further determined that shifting to high-osmolarity growth conditions causes a relatively slow decrease in F-actin levels in wild-type cells, and a small but rapid increase in the F-actin levels in profilin null cells. Profilin null cells contain normal concentrations of F-actin while growing exponentially at room temperature, indicating that profilin is not essential for maintaining F-actin concentrations during steady-state growth. Our data suggest that actin is inherently unstable in vivo at high temperatures, and that profilin helps to maintain actin in its filamentous state at these temperatures, perhaps by stimulating actin polymerization in a proper temporal and spatial fashion.**

Key words: Actin; Profilin; Cytoskeleton; *Saccharomyces cerevisiae*

1. Introduction

Profilin is an ~13 kDa protein that supports a surprisingly large number of interactions with other molecules. Originally identified as an actin-monomer sequestering protein, profilin was subsequently shown to enhance the rate of nucleotide exchange on actin and to retard the rate of hydrolysis of bound ATP [1,2]. Profilin also interacts with the acidic phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) in vitro, which has the combined effects of blocking profilin-actin interactions and preventing PIP_2 cleavage by unphosphorylated (unactivated) phospholipase Cγ1 [3–6]. This has led to the hypothesis that profilin may play a key role in linking signal transduction pathways to control of the actin cytoskeleton. Another property of profilin is that it binds to stretches of proline residues. Recent evidence suggests that this property is important for profilin's interactions with the *Cappuccino* gene product in *Drosophila* [7], and for its involvement in the motility of various pathogenic bacteria and viruses [8–11]. Less well characterized profilin interactions include the copurification of profilin with a seven-protein complex from *Acanthamoeba* [12], and our recent two-hybrid results that indicate a direct interaction between yeast profilin and adenylate cyclase (S. Palmieri and B. Haarer, unpublished data).

Despite these many interactions attributed to profilins from various species, it is still unclear which roles are central to profilin function, and how such properties relate to the in vivo control of the actin cytoskeleton. Convincing arguments can be made for an in vivo role as an inhibitor [13–15], or stimulator [8,16] of actin polymerization. Indeed, it is entirely likely that profilin could be playing both roles, depending on species, cell type, or temporal regulation within an individual cell. To further address profilin's potential in vivo functions, we have utilized a fluorometric assay to measure the relative filamentous actin content in cells before and after changing growth conditions. Our findings are consistent with profilin's playing a significant role in the stimulation of actin polymerization under conditions that are perturbing to the yeast actin cytoskeleton.

2. Materials and methods

2.1. Yeast strains and culture conditions

Saccharomyces cerevisiae strains used in this study are listed in Table 1. Cells were grown in YEPD medium (1% yeast extract, 2% bactopeptone, 2% glucose) or YEPD+1.8 M sorbitol. Growth of yeast strains was at room temperature (~22°C), 37°C, or 15°C, as indicated.

2.2. Fluorometry assay

Relative levels of filamentous actin were measured using a slight modification of the method of Lillie and Brown [17], which is based on the method of Howard and Oresajo [18]. Yeast cells were grown in YEPD medium at room temperature to ~10^7 cells/ml. 2 ml of culture was added to 8 ml YEPD equilibrated at the appropriate temperature (37°C or 15°C) in a shaking water bath. Cells were fixed at various times from 30 s to 120 min by adding 1 ml of 37% formaldehyde that had been equilibrated to the experimental temperature; for 0 s time points, 2 ml of culture was added directly to 8 ml YEPD+1 ml formaldehyde. Cultures were removed from shaking 30 min after the addition of formaldehyde and were placed at room temperature overnight. Cells were harvested by centrifugation and resuspended in 100 μl of 0.33 μM rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR, USA), 0.2% Triton X-100 in phosphate-buffered saline (PBS). Cells were incubated for 30 min in the dark on a rotator, then washed three times with PBS to remove unbound rhodamine-phalloidin. Bound phalloidin was eluted by incubating the cells in 1.5 ml methanol for 1.5 h in the dark on a rotator. The methanol was removed after centrifugation, and fluorescence of the extracted rhodamine-conjugated phalloidin was measured with a Turner model 450 fluorometer using a standard rhodamine filter set. Corrected fluorescence values (see below) are presented in arbitrary units.

2.3. Cell number and volume corrections

Cell densities were estimated by counting cells on a hemacytometer. Data sets for each strain were normalized by dividing fluorescence readings by average cell counts over the first five time points (through 5 min).

Wild-type (22ABΔ1-6A) and profilin null (22ABΔ1-6D) yeast cells were photographed on a hemacytometer slide and the resulting nega-

*Corresponding author. Fax: (1) (512) 471-9651.
E-mail: haarer@mail.utexas.edu

Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; F-actin, filamentous actin; G-actin, globular actin

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tives were converted to digital images using a Nikon slide scanner. Areas of unbudded cells, mother cells, and buds were measured using the program NIH Image (v. 1.59) and volumes were estimated from these areas using the equation for spherical volume ($4/3\ \pi r^3$). The hemacytometer 50 μm division provided scale for conversion of volumes to cubic microns. Combined data for all cells indicated that the average volume of profilin null cells is 4.54 times that of comparable wild-type cells; fluorescence measurements of profilin null cells were divided by 4.54 to correct for cell volume differences.

Data were further normalized (Figs. 1–4B) by calculating fluorescence changes relative to the zero time point (given the arbitrary value of 1) for each trial. Such treatment of the data was meant to correct for aging of the rhodamine-phalloidin and day to day differences in fine adjustments of the fluorometer; this further allowed for standard deviation calculations on combined trials (Figs. 2–4B).

3. Results

The polymerization state of actin filaments can be examined in vivo using the F (filamentous)-actin-specific drug phalloidin conjugated to a fluorochrome such as rhodamine [17]. An indication of the relative amounts of F-actin in a cell can be determined by exposing cells to varying conditions (such as heat shock), followed by rapid fixation at specific time points. Cells are then exposed to rhodamine-conjugated phalloidin, followed by washing and elution of the F-actin-bound phalloidin, and the associated fluorescence is measured with a fluorometer. Thus, the fluorescence measurement is an indirect indication of the relative amount of F-actin in the cell at the time of fixation. Using this method, Lillie and Brown [17] have shown that a shift to high temperature causes a rapid but transient depolymerization of actin, which is accompanied by a transient redistribution of actin-containing structures [17,19,20]. Similar effects on actin organization have been noted upon increasing the osmotic strength of the medium [21].

Initially, we sought to determine if various point mutations in the profilin gene affected the ability of yeast strains to properly regulate their actin networks in response to similar cellular stresses. To address this question, we used this assay to assess the relative amounts of F-actin in wild-type and profilin point-mutant cells before and after temperature shifts from room temperature to 37°C (Fig. 1). Although there was some fluctuation between strains, there does not appear to be a significant defect in the ability of these mutant strains to respond to a high temperature shift. Wild-type and mutant strains showed similar initial drops in F-actin, followed by a recovery to starting levels within ~10–15 min (Fig. 1). We are currently examining these mutants further to determine the potential significance of minor fluctuations seen with these strains.

Given the lack of significant effect of point-mutated profilins in responding to a high temperature shift, we next examined cells that completely lack profilin (Fig. 2). In this case, it is clear that profilin null cells show a much more pronounced initial drop in their F-actin content (32% versus 15% for wild

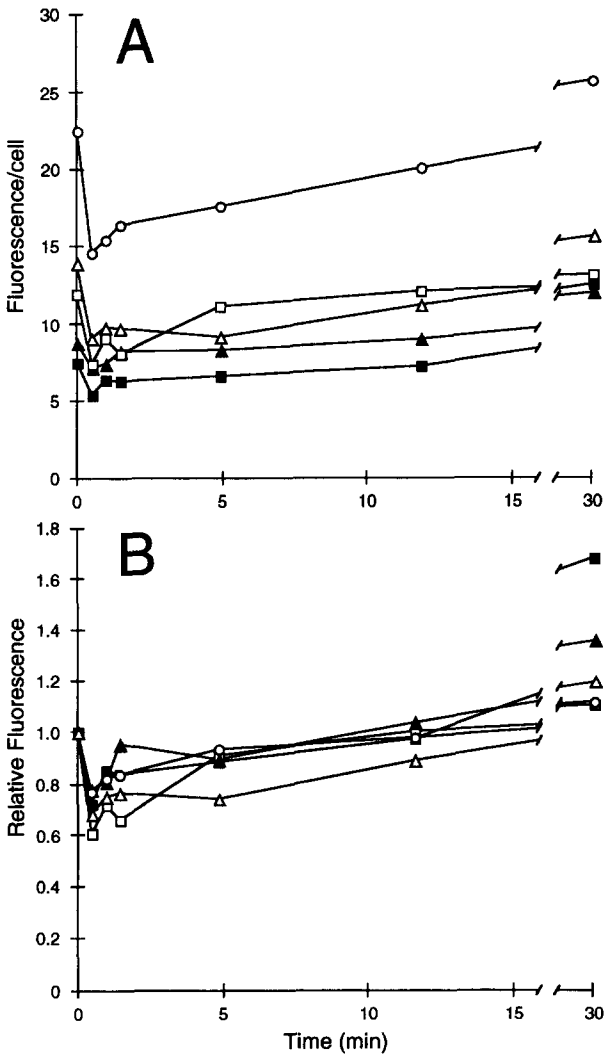

Fig. 1. Changes in F-actin levels of wild-type and profilin point-mutant yeast strains upon shifting to 37°C. A: Data are from the combination of two independent trials, each of which was corrected for cell number before combining. BHY14 (*pfy1-111*) cells tend to be larger than wild-type cells, generating higher fluorescence/cell measurements for this strain. B: Same data as in A, but fluorescence measurements were normalized to the 0 min time point for each strain prior to combining trials. Open squares, (DC5×Y388)2D; closed squares, (DC5×Y388)13A; circles, BHY14 (*pfy1-111*), open triangles, BHY31 (*pfy1-112*); closed triangles, BHY32 (*pfy1-116*).

Table 1
Saccharomyces cerevisiae strains used in this study

Strain	Relevant genotype	Profilin mutation[a]	Source
22ABΔ1-6A	*MATα PFY1*	none	[36]
22ABΔ1-6D	*MATa pfy1Δ*	complete deletion	[36]
(DC5×Y388)2D	*MATα PFY1*	none	[37]
(DC5×Y388)13A	*MATa PFY1*	none	[37]
BHY14	*MATα pfy1-111*	Arg^{72} to Glu	[37]
BHY31	*MATa pfy1-112*	Arg^{76} to Gly	lab strain
BHY32	*MATa pfy1-116*	Arg^{76} to Glu	lab strain

[a]See Haarer et al. [36] for a description of the in vitro properties of the profilins expressed by strains BHY14, BHY31, and BHY32.

type), and that the recovery time to initial F-actin levels is also significantly longer (~50 min versus ~12 min for wild type; Table 2). It is interesting to note that, despite being significantly larger than wild-type cells, profilin null cells contain concentrations of F-actin similar to those of wild-type cells (Figs. 2–4A).

To determine if the sudden drop in F-actin content upon temperature shift is a general response to cellular stress or changing environmental conditions, we examined the response of wild-type and profilin null cells upon shifting to 15°C, or to high-osmolarity medium. Surprisingly, when shifted to 15°C from room temperature, these strains showed rapid increases in F-actin concentrations of 32% and 18% for wild-type and profilin null cells, respectively (Fig. 3, Table 2). Shifting cells to high-osmolarity medium also failed to elicit a rapid drop in the F-actin concentration (Fig. 4), though minor differences were observed between wild-type and profilin null cells. Most

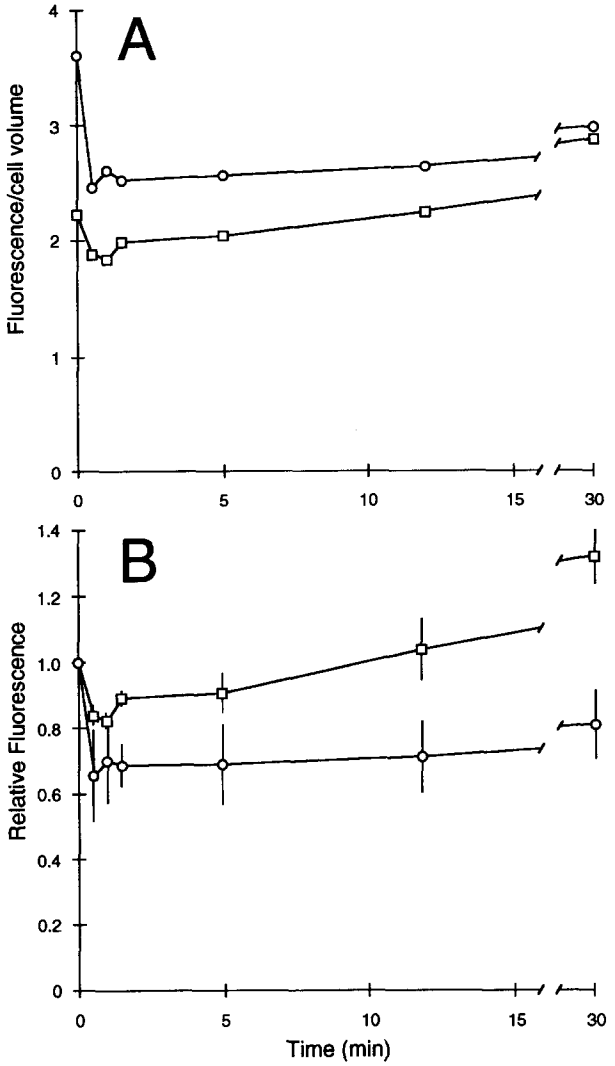

Fig. 2. Changes in F-actin levels of wild-type and profilin null strains upon shifting to 37°C. A: Data are from the combination of two independent trials, each of which was corrected for cell number before combining; measurements of profilin null cells were also corrected for volume differences (see Section 2). B: Same data as in A, but fluorescence measurements were normalized to the 0 min time point for each strain prior to combining trials. Bars in B are standard deviations. Squares, 22ABΔ1-6A (wild type); circles, 22ABΔ1-6D (profilin null).

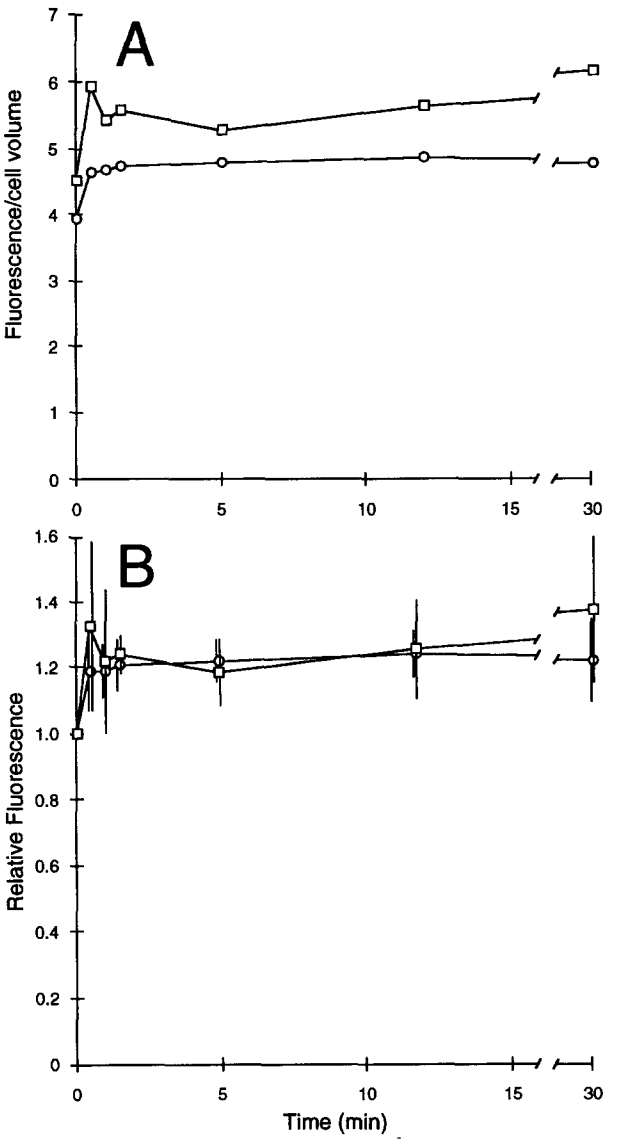

Fig. 3. Changes in F-actin levels of wild-type and profilin null strains upon shifting to 15°C. See Fig. 2 legend for details. Data are from the combination of three independent trials.

Table 2
Changes in F-actin levels and recovery times upon treatment

Strain[a]	Treatment[b]	Initial change[c]	Recovery time[d]
WT	Heat	−15%	12 min
Δ	Heat	−32%	50 min
WT	Cold	+32%	NA
Δ	Cold	+18%	NA
WT	Sorbitol	−2.5% (−14%)[c]	65 min
Δ	Sorbitol	+8%	NA

[a]WT, strain 22ABΔ1-6A; Δ, profilin null strain 22ABΔ1-6D.
[b]Heat, shift from room temperature to 37°C; Cold, shift from room temperature to 15°C; Sorbitol, shift from YEPD to YEPD+1.8 M sorbitol at room temperature.
[c]Percent change in fluorescence from 0 min to 0.5 min post-shift; negative and positive values indicate reduction and increase in fluorescence, respectively. Value in parentheses represents percent change at 12 min.
[d]Approximate time at which fluorescence reaches the level measured at 0 min.

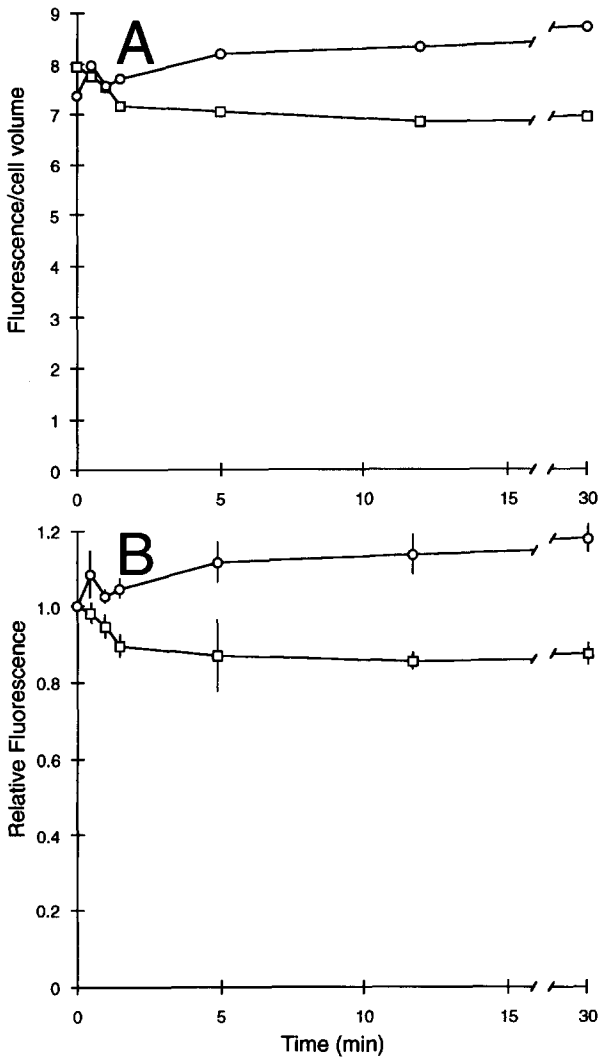

Fig. 4. Changes in F-actin levels of wild-type and profilin null strains upon shifting to high-osmolarity medium. See Fig. 2 legend for details. Data are from the combination of three independent trials.

notably, wild-type cells displayed a slow but reproducible drop in F-actin levels through 12 min post-shift, while profilin null cells displayed a small but rapid increase in F-actin levels (Fig. 4, Table 2).

4. Discussion

Using a phalloidin-based fluorometric assay, we have determined that profilin is necessary to properly reverse the transient depolymerization of actin that occurs upon shifting yeast cells to elevated temperature [17]. A possible explanation for this observation is that profilin normally serves to stimulate actin polymerization, perhaps by enhancing the rate of nucleotide exchange on actin monomers [22,23], or by shuttling actin monomers to the barbed/plus ends of growing filaments [16,24]. Alternatively, profilin may enhance the stability of existing actin filaments, countering their apparent thermal instability.

Potential roles for profilin's involvement in stabilizing actin filaments, or in directing filament assembly, have been noted in other experimental systems [25–27]. Perhaps most convincing are studies (reviewed by Cossart [8]) that have implicated profilin in the stimulation of actin polymerization to promote the actin-based locomotion of various pathogenic bacteria. Thus, in wild-type yeast cells, profilin may help to reduce the initial drop in F-actin levels by immediately stimulating actin polymerization in response to the signal generated by an increase in temperature.

Interestingly, upon shift to low temperature, we detected a rapid increase in F-actin levels for both wild-type and profilin null cells. Our initial expectation was that any cellular stress might cause a transient decrease in F-actin levels as the cytoskeleton readjusted to accommodate a new set of growth conditions. Contrary to this expectation, these data suggest that the F-actin decrease that accompanies a shift to high temperature may reflect of an inherent thermal instability of F-actin, which would also explain the increase in F-actin levels seen with shifts to low temperature.

Upon shifting to a high osmolarity medium, wild-type strains show a small, relatively slow decrease in their F-actin content to ~12 min post-shift. Cells expressing mutant actins, or reduced levels of wild-type actin, exhibit sensitivity to high osmolarity media [21,28], suggesting that actin may be somewhat osmosensitive. However, this fails to explain why elimination of profilin should stabilize F-actin under high osmolarity conditions, unless profilin also plays a role in the initial reorganization of the actin cytoskeleton that is observed upon increasing osmotic pressure [21]. An alternative explanation is that the cytoplasm of profilin null cells, which are also osmosensitive [29], may undergo significantly different changes upon exposure to high osmolarity medium. For example, profilin null cells may shrink proportionately more than wild-type cells, causing an increase in the concentration of cytoplasmic actin, and thus driving additional actin polymerization.

Our results have also demonstrated that, although profilin null cells tend to be significantly larger than corresponding wild-type cells, the initial concentrations of F-actin in these cell types are similar (see Figs. 2–4A). As there is only a single profilin gene in *S. cerevisiae*, this would suggest that profilin is not required for maintaining steady-state F-actin levels in yeast. Our previous results [29, and unpublished data] indicate that the ratio of actin to total protein in profilin null cells is roughly equivalent to that of wild-type cells, suggesting that G-actin concentrations are also unaffected by loss of profilin. The observation that overproduction of profilin in yeast can suppress the lethality associated with actin overproduction [14] indicates that, under such conditions, profilin may be sequestering actin monomers, and that elimination of profilin might be expected to cause a significant increase in the cellular F-actin concentration. A possible explanation for these apparent discrepancies is that profilin's primary role may be to stimulate actin polymerization, but that it is also capable of sequestering actin monomers, particularly when it and/or actin are overproduced. Perhaps in normal situations, the bulk of profilin is bound by PIP_2 or a poly-proline-containing substrate, thereby preventing it from performing a major actin-sequestering role. In support of this notion, Ostrander et al. [30] have found that up to 80% of yeast profilin is associated with the plasma membrane. If most of the profilin in yeast cells is not available to bind actin monomers, the role of sequestering G-actin may normally fall to other yeast G-actin-binding proteins such as cofilin [31,32] or Cap/Srv2p [33–35].

In summary, our data support a major role for profilin in the stimulation of actin polymerization in yeast. Further, they suggest that F-actin in yeast is thermolabile, and that profilin's activity contributes to the apparent stability of F-actin at elevated temperatures, presumably via this enhancement of polymerization.

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