

## A novel ATP-dependent protein unfolding factor unfoldin involved in yeast cell morphology

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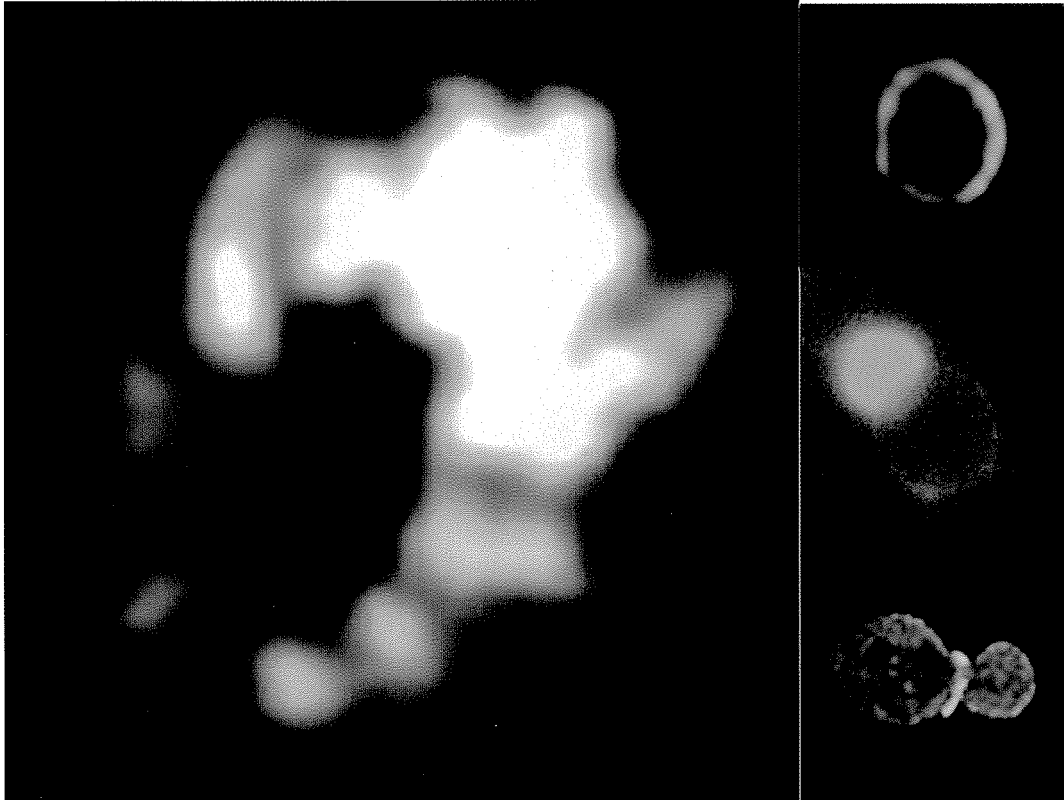
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Proteins inside a cell cannot function correctly unless they assume the proper three-dimensional conformation. On the other hand, the folded structure of proteins can also be a hindrance during a translocation across the organelle membranes. Is there any factor that has the activity of "protein unfolding"? To attempt address this question, we developed *in vitro* protein-unfolding assay based on trypsin sensitivity. As substrate of this assay, *E. coli* expressed yeast alpha factor precursor was used. The addition of the low concentrate trypsin did not attack alpha factor precursor, however, when a yeast microsomes salt extracted fraction was added to the assay mixture and incubated at 30 °C for 15 min, it was efficiently degraded by the protease. This efficiency correlate to the amounts of the extract and suggests that this fraction contains an activity which increases protease sensitivity of the substrate protein, as a result of exposure of trypsin sensitive sites due to conformational changes, i.e. "unfolding" of it. We purified the activity using this assay system to a homogeneous state (600-fold) and named "unfoldin" because of its activity.

From the *in vitro* and *in vivo* experimental data, I will describe today that

- (1) unfoldin recognized substrate proteins in the presence of ATP.
- (2) unfoldin has broad substrate specificity.
- (3) unfoldin requires ATP-binding for its activity.
- (4) unfoldin forms a large oligomeric complex consisting of 10~12 individual subunits arranged to ring-like structure with a ~2 nm central cavity
- (5) unfoldin overexpression and deletion both caused cell deforming.
- (6) unfoldin localized and surrounds yeast bud neck at the log phase.

Taken together we concluded that ATP-dependent novel protein unfolding factor unfoldin involved in yeast cell morphology, especially cell polarity.



**The rotary shadowing image of unfoldin  
and its localization in yeast cells**

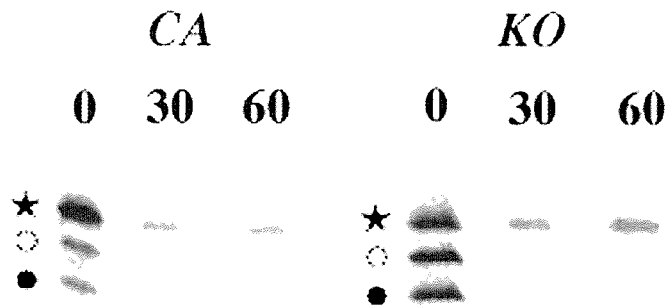
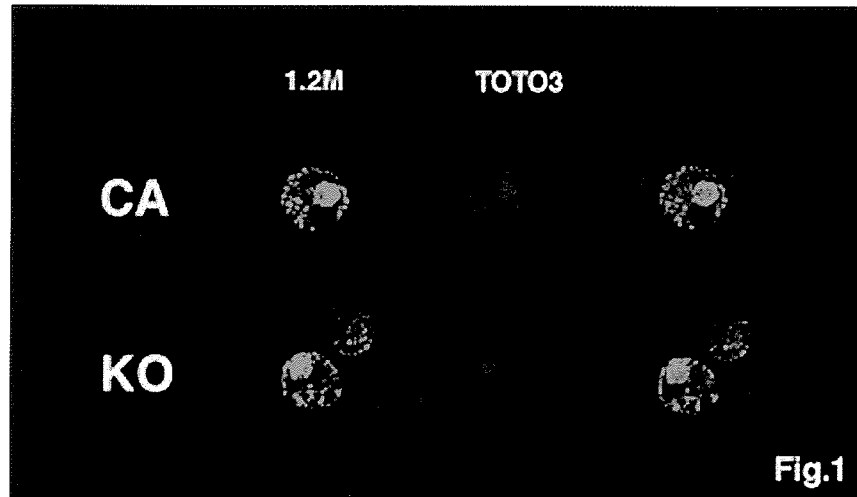


Fig.2

Open circle shows pre-sequenced form.

Closed circle shows mature form.

Star shows glycosylated form.

Chase times are indicated at the top in minutes.