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The Actin-related Protein Act3p of *Saccharomyces cerevisiae* Is Located in the Nucleus

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Actin-related proteins, a group of protein families that exhibit about 50% sequence identity among each other and to conventional actin, have been found in a variety of eukaryotic organisms. In the budding yeast *Saccharomyces cerevisiae*, genes for one conventional actin (*ACT1*) and for three actin-related proteins (*ACT2*, *ACT3*, and *ACT5*) are known. *ACT3*, which we recently discovered, is an essential gene coding for a polypeptide of 489 amino acids (Act3p), with a calculated molecular mass of 54.8 kDa. Besides its homology to conventional actin, Act3p possesses a domain exhibiting weak similarity to the chromosomal protein HMG-14 as well as a potential nuclear localization signal. An antiserum prepared against a specific segment of the *ACT3* gene product recognizes a polypeptide band of approximately 55 kDa in yeast extract. Indirect immunofluorescence experiments with this antiserum revealed that Act3p is located in the nucleus. Nuclear staining was observed in all cells regardless of the stage of the cell cyle. Independently, immunoblotting experiments with subcellular fractions showed that Act3p is indeed highly enriched in the nuclear fraction. We suggest that Act3p is an essential constituent of yeast chromatin.

INTRODUCTION

As a major cytoskeletal component of all eukaryotic cells, actin is involved in a variety of cellular processes such as cell motility, maintainance of cell shape, organelle movement, and chromosome segregation (see e.g. Kabsch and Vandekerckhove, 1992; Bretscher *et al.*, 1994). Actins isolated from a broad range of phyla are about 90% identical to each other (Herman, 1993)–thus, actin is one of the most highly conserved eukaryotic proteins. In recent years, however, a number of proteins more distantly related in primary sequence to actin have been identified in a variety of organisms including fungi (Lees-Miller *et al.*, 1992a; Schwob and Martin, 1992; Clark and Meyer, 1994; Harata *et al.*, 1994; Muhua *et al.*, 1994; Murgia *et al.*, 1995), fruit flies (e.g. Frankel *et al.*, 1994; Fyrberg *et a1.*, 1994), chickens (Schafer *et al.*, 1994; Michaille *et al.*, 1995), and humans (Clark and Meyer, 1992; Lees-Miller *et al.*, 1992b). In contrast to conventional actins, these "actin-related proteins" (Arps) form a heterogeneous group with regard to their primary sequence, length, and the position of peptide insertions. According to the classification scheme proposed by Schroer *et al.* (1994), three main Arp subfamilies–Arp1, Arp2, and Arp3–can be distinguished based on their level of sequence similarity to conventional actin and among each other. Whereas members of each class from different species are highly conserved, the different classes of actin-related proteins are no more similar to each other than they are to actin, suggesting that each of the families fulfills a distinct cellular function. Three additional actin-related proteins, *Drosophila* Arp53D (Fyrberg *et al.*, 1994), *Drosophila* Arp13E (Frankel *et al.*, 1994), and *S. cerevisiae* Act3p (Harata *et al.*, 1994) do not fit into the classification

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scheme mentioned above; therefore we assume that they represent members of three further Arp subfamilies.

In budding yeast, one conventional actin, Act1p (Gallwitz and Sures, 1980) and three actin-related proteins–Act2p (Schwob and Martin, 1992), Act3p (Harata *et al.*, 1994; GenBank X75317), and Act5p (Muhua *et al.*, 1994) have been identified. Note that a second gene that was also termed *ACT3* (Clark and Meyer, 1994) turned out to be identical to the *ACT5* gene described earlier by Muhua *et al.* (1994).

Despite the presence of peptide insertions in the primary sequence, the three-dimensional structure of the actin molecule (Kabsch *et al.*, 1990) seems to be conserved in all three *S. cerevisiae* Arps. Two of them (Act2p and Act3p) are known to be essential for cell survival, but much remains to be learned about their functions. Act2p has been proposed to be involved in cytoskeletal reorganization during the cell cycle, whereas Act5p, the best characterized *S. cerevisiae* Arp, is a nonessential homologue of vertebrate centractins, which is involved in spindle orientation and nuclear migration.

Here, we report that *S. cerevisiae* Act3p, the most divergent actin-related protein known so far, is located in the nucleus, and, in view of the data that will be presented here, we propose that it might be a constituent of chromatin.

MATERIALS AND METHODS

Yeast Strains and Media

Strain AK300 (*MAT a/α; ho/ho ade2–1/ade2–1 trp1–1/trp1–1 can1–100/can1–100 leu2–3, 112/leu2–3,112 his3–11,15/his3–11,15 ura3/ura3 ssd1/ssd1*) was constructed by crossing strains K699 and K700, which were obtained from K. Nasmyth (Vienna, Austria). Strain MZ3 (*MAT α; pep4–3 trp1 leu-Δ1 ura3-Δ1*) was derived from 20B12 (Wintersberger *et al.*, 1995). Strain HK1 was obtained by transformation of K699 with the plasmid pYEUra3/ACT3 (see below). Yeast extract-peptone-dextrose (YPD) growth medium was used as standard medium for the culture of yeast cells. Synthetic complete-galactose (ScGal) medium was prepared as described by Sherman (1991). LB-broth medium was obtained from Life Technologies (Gaithersburg, MD).

Plasmid Constructions

To construct a plasmid for the $(his)_6$-Act3-fusion protein, a DNA-fragment coding for amino acids 269S to 399D of Act3p (nucleotides 803 to 1198 in Harata *et al.*, 1994; see also Figure 1) was isolated by *Sau3A*I digestion and inserted into the *Bam*HI site of the pQE30 vector (Qiagen, Hilden, Germany). For Act3p overexpression the plasmids pFL1/6–1 and pYEUra3/ACT3 were used. To obtain the high copy number 2μ-plasmid pFL1/6–1, a 8.8-kb *Bam*HI fragment containing the *ACT3* open reading frame under its own promoter was inserted into the pFL1-*Bam*HI cloning site. The low copy number plasmid pYEUra3/ACT3 was obtained by integration of a 1.8-kb *Dra*I segment (nucleotides −134 to 1682; Harata *et al.*, 1994) into the *Sal*I site of pYEUra3 (Clontech, Cambridge, UK) adjacent to the strong galactose-inducible GAL1 promoter. The precise integration of the fragments into the vectors was confirmed by digestion of the plasmids with restriction enzymes, by determination of the amino acid sequence of the fusion protein and by the observation of Act3p overexpression, respectively.

Preparation and Purification of Antibodies

Escherichia coli (M15[pREP4]) was transformed according to Inoue *et al.* (1990) with the pQE30 vector containing the *ACT3* fragment. Transformants isolated from LB-broth agar containing 100 μg/ml ampicillin, 25 μg/ml kanamycin, and 2% glucose were grown to an OD_{600} of 0.8 in LB-broth medium with the same additives as above. Expression of the $(his)_6$-Act3-fusion protein was induced by addition of 2 mM isopropyl-β-D-thiogalactopyranoside. After further incubation at 30°C for 5 h, cells were harvested by centrifugation and the cell pellet was resuspended in 50 mM sodium phosphate (pH 8.0) containing 300 mM NaCl. The suspension was frozen at −20°C and cells were lysed by sonication on ice. The lysate was centrifuged and the fusion protein was purified from the supernatant using a Ni^{++}-NTA column (Qiagen) according to the instructions of the manufacturer. Purity of the fusion protein was checked by SDS-PAGE. Two rabbits were immunized with about 400 μg of purified fusion protein each, which was applied in four portions at intervals of 2 wk. Antisera were taken at 28 and 48 days after the first injection.

Act3p-specific antibodies were affinity-purified from the crude antiserum using nitrocellulose strips containing the $(his)_6$-Act3-fusion protein as affinity matrix (Lillie and Brown, 1987). Anti-Act3p antibodies were eluted with 0.2 M glycine-HCl (pH 2.8), 1 mM EGTA, and the eluate was immediately neutralized with 1 M Tris-HCl (pH 8.0) as described by Lillie and Brown (1987). To assess antibody specificity and the success of the affinity-purification, blots of total yeast proteins were probed with the affinity-purified antiserum.

Biochemical Procedures

Protein Determination, Gel Electrophoresis, and Protein Blotting. Protein concentration was determined by the method of Bradford (1976). Discontinuous SDS-PAGE was performed in a 'Mighty small SE 250' cell (Hoefer, San Francisco, CA) with gels containing 12 or 16% acrylamide, respectively. Protein bands were visualized with Coomassie blue G250.

Proteins were blotted to nitrocellulose or polyvinylidene difluoride-sheets using a semi-dry blotter (NovaBlot, Pharmacia, Piscataway, NJ) and stained with Ponceau S to assess the amounts of proteins transferred. Blots were developed with the ProtoBlot alkaline phosphatase detection system from Promega (Madison, WI).

Preparation of Yeast Extract. Log phase cells were harvested by centrifugation, washed once with 20 mM Tris-HCl, 1 mM EDTA, 10% glycerol (pH 7.9), frozen in liquid nitrogen, and kept at −70°C for at least 1 h. An equal volume of the washing buffer containing 2 M NaCl, 1 μl/ml 2-mercaptoethanol, and protease inhibitors (1 mM each of phenylmethylsulfonylfluoride and sodium sulfite, pH 8.0; 0.1 mM sodium tetrathionate; 1 μM each of *Nα*-p-tosyl-L-lysine-chloromethylketone, *N*-tosyl-L-phenylalanine-chloromethylketone, pepstatin A, and antipain) as well as chilled glass beads were added to the frozen cell pellet. The mixture was vortexed vigorously 8–10 times for 30 s and the extract was kept on ice between the vortexing steps. Glass beads, undisrupted cells, and large cell fragments were removed by centrifugation for 30 min at 17.500 rpm in a Sorvall SS34 rotor. Aliquots of the supernatant were either diluted with SDS-sample buffer and subjected to SDS-PAGE or frozen in liquid nitrogen and stored at −70°C.

Preparation of Yeast Nuclei. Yeast nuclei were prepared using a procedure adapted from Mann and Mecke (1980). All steps were carried out at 4°C and protease inhibitors (see above) were included in all buffers. Briefly, the haploid wild-type strain MZ3 was grown to mid log phase in 2.5 l of YPD at 30°C and spheroplasted by

addition of 10 mg Zymolyase 100T (WAK Chemie, Bad Homburg, Germany)/g pelleted cells. Spheroplasts were suspended in ice-cold 20 mM phosphate buffer (pH 6.5) containing 0.5 mM $MgCl_2$ and 18% Ficoll, and the suspension was homogenized with 20 strokes in a tight-fitting Dounce homogenizer. The homogenate was cleared of unbroken cells and cell walls by a spin of 15 min at 5000 × *g* and the resulting pellet was homogenized and centrifuged as above. Both supernatants were pooled and subjected to centrifugation at 30,000 × *g* for 30 min, yielding a crude nuclear pellet and the postnuclear supernatant. For further purification, the nuclear pellet was suspended in 20 mM phosphate buffer (pH 6.5), 1 mM $MgCl_2$, and 20% Ficoll, layered onto a cushion of 50% Ficoll in the same buffer, and centrifuged at 100,000 × *g* for 90 min.

Extraction of Yeast Nuclei. Nuclei isolated as described above were resuspended in 20 mM potassium phosphate (pH 6.5) containing 10% Ficoll, 0.5 mM $MgCl_2$, and protease inhibitors (see above). Twenty-microliter aliquots of this suspension (corresponding to 50 μg of protein) were treated with 3 volumes of sucrose extraction buffer (SE-buffer: 0.5 M sucrose, 20 mM potassium phosphate, pH 6.5, 0.5 mM $MgCl_2$) plus salt or DNase I (Mirzayan *et al.*, 1992). The following extraction conditions were used: 1) SE + 0.2 M NaCl, 2) SE + 0.5 M NaCl, and 3) SE + 0.25 mg/ml DNase I. As a control, nuclei were treated with SE buffer alone. Incubation was performed on ice for 20 min, except for the nuclease digestion, which was performed for 1 h. Samples were separated into pellet and supernatant by centrifugation in an SS 34 rotor at 20,000 rpm for 15 min. Supernatants were carefully removed and both supernatants and pellets were precipitated with 10% trichloroacetic acid (final concentration) for 1 h on ice. The resulting protein pellets were washed with acetone, suspended in SDS-sample buffer, and subjected to immunoblot analysis.

Immunofluorescence

Indirect immunofluorescence of a diploid wild-type strain and a strain overproducing Act3p was performed according to the method of Pringle *et al.* (1991). Cells were grown to mid log phase in the appropriate liquid medium at 30°C and fixed by adding 37% formaldehyde directly to the culture to give a final concentration of 3.7%. After 15 min, cells were transferred to 100 mM potassium phosphate (pH 6.5), containing 0.5 mM $MgCl_2$ and 3.7% formaldehyde and were incubated at room temperature for 3 h with gentle shaking. After washing in buffer A (1.2 M sorbitol/phosphate, pH 7.5), cell walls were removed from ~10^9 cells in 2 ml buffer A containing 2 μl 2-mercaptoethanol and 40 μl Zymolyase 100T stock solution (1 mg/ml in buffer A). After incubation for 30 min at 30°C with gentle agitation, spheroplasts were harvested by centrifugation, washed, and attached to slides coated with poly-D-lysine (Boehringer Mannheim, Indianapolis) at a density of ~1 × 10^7 cells/cm^2. Dried slides were successively treated with methanol (6 min at −20°C) and acetone (30 s at −20°C). After blocking with phosphate-buffered saline/bovine serum albumin (1 mg/ml) for 15 min, the slides were incubated overnight (~16 h) at 4°C with affinity-purified primary antibody (undiluted or diluted 1:10). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Pierce, Rockford, IL) that had been preadsorbed against yeast spheroplasts for 2 h at 4°C was applied as secondary antibody for 2 h at room temperature. DNA was stained by adding 2.25 μg/100 ml 4′,6-diamidino-2-phenylindole (DAPI) to the mounting medium (90% (v/v) glycerol, 0.1% (w/v) p-phenylenediamine in phosphate-buffered saline).

For standard immunofluorescence, cells were viewed with a Leitz Orthoplan microscope equipped for epifluorescence and photographed on Fujichrome 400 film. Confocal microscopy was performed with a LEICA scanning microscope equipped with a krypton/argon laser.

RESULTS

The ACT3 Gene Encodes a Polypeptide of 55 kDa

Although the three-dimensional structure of conventional actin seems to be well conserved in the Act3p molecule, its primary sequence is characterized by the presence of three unique hydrophilic peptide insertions termed I, IIa, and IIb, which consist of 24, 11, and 68 amino acid residues, respectively (compare Harata *et al.*, 1994, and Figure 1). To reduce the possibility of cross-reactivity with conventional actin and with other actin-related proteins, an antiserum was raised not against the entire *ACT3* gene product, but against a 131-amino acid segment encompassing peptide insertions IIa and IIb (Figure 1). The corresponding 0.4-kb DNA-segment was cloned into the pQE 30 vector and a fusion protein consisting of the Act3 protein fragment tagged by six histidine residues at the amino-terminus was expressed in *E. coli*. The histidine tag allowed the purification of the fusion protein over Ni^{++}-NTA resin (see MATERIALS AND METHODS). The antiserum obtained by immunization of a rabbit with the purified fusion protein was affinity-purified and tested for specificity on blots of yeast extract. The affinity-purified antibody recognized one major polypeptide at 55 kDa, which is in good agreement with the calculated molecular mass of 54.8 kDa for

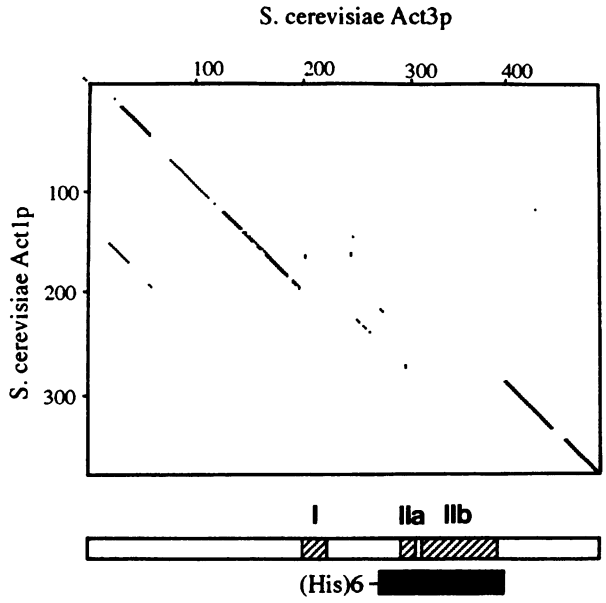

Figure 1. Homology analysis between Act3p and yeast conventional actin (Act1p), and schematic representation of the fusion protein for the preparation of an antiserum against Act3p. Harr plot analysis was done using the GENETYX program (unit size 25; score 1.1). The upper bar under the plot represents the Act3 polypeptide with hatched boxes corresponding to Act3p-specific peptide insertions (I, IIa, and IIb as described in Harata *et al.*, 1994), and the bar below indicates the histidine-tagged fusion protein that was used for the generation of the anti-Act3p antiserum.

Act3p (Figure 2). Minor bands that were occasionally observed in addition to the 55-kDa band varied from sample to sample and were most probably due to proteolysis. The signal obtained with extracts from a strain containing the *ACT3* gene on a multicopy-plasmid under its own promoter (MZ3 pFL1/6–1) was stronger than that obtained with extracts from wild-type cells; this can be taken as additional evidence that the affinity-purified antibody specifically recognized Act3p.

Immunolocalization of Act3p

As actin-related proteins are generally expressed at low levels (e.g., attempts to detect wild-type levels of *S. cerevisiae* Act5p by immunofluorescence microscopy have as yet been unsuccessful; Muhua *et al.*, 1994), we performed the initial immunofluorescence-experiments with the strain HK1 in which *ACT3* is placed under control of the strong galactose-inducible GAL1 promoter. For this experiment, the primary antibody was affinity-purified as described in MATERIALS AND METHODS, and FITC-conjugated secondary antibody was preadsorbed to yeast spheroplasts to reduce background staining. Anti-Act3p staining was visible throughout the nucleus and appeared to be uniform (Figure 3, A and B). Nuclear staining was observed in cells having no, small, or large buds as well as in cells with dividing nuclei, suggesting that the distribution of Act3p within the cell does not change during the vegetative cell cycle. When we repeated the experiment with wild-type cells (Figure 3, C and D), nuclear staining was considerably weaker, but clearly detectable, especially with prolonged incubation with primary antibody (overnight at 4°C instead of 2 h at room temperature). No staining above background was observed when preimmune serum (Figure 3, E and F) or FITC-conjugated secondary antibody alone was used. Confocal scanning microscopy confirmed the results obtained by conventional fluorescence microscopy; FITC staining was evenly distributed over the nucleus both in wild-type cells and in cells overproducing Act3p (Figure 3, G–J).

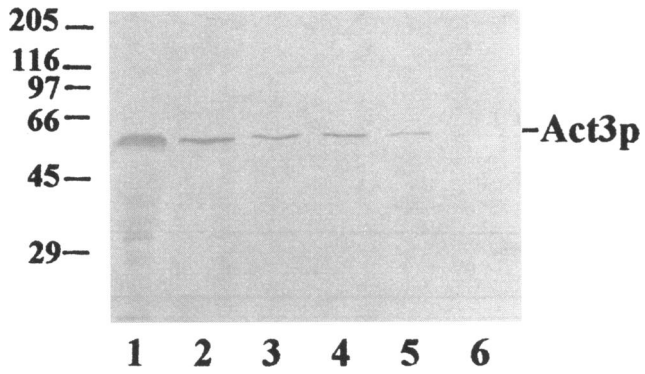

Figure 2. Demonstration of antibody specificity by immunoblotting. Blots of yeast extract from strain MZ3 prepared as described in MATERIALS AND METHODS were probed with antiserum affinity-purified against the $(his)_6$-Act3-fusion protein. Lanes 1–3: 12, 6, and 2 μg of protein extracted from a strain overproducing Act3p (MZ3 pFL1/6–1) were loaded; lanes 4–6 contain the same amounts of protein from the wild-type strain MZ3. Numbers on the left indicate the positions of the molecular weight markers.

Localization of Act3p by Cell Fractionation

To independently demonstrate the nuclear localization of Act3p, homogenates of yeast spheroplasts, nuclei, and postnuclear supernatant (see MATERIALS AND METHODS) were tested for the presence of Act3p. Equal amounts of proteins were separated by SDS-PAGE, blotted, and probed with affinity-purified anti-Act3p antibodies. As shown in Figure 4A (upper panel), Act3p, which is only weakly visible in the homogenate, is highly enriched in the nuclear fraction but practically absent in the postnuclear supernatant. Histones were used as marker proteins to assess the enrichment of nuclei (Figure 4A, lower panel).

To get information about the nature of the association of Act3p with nuclei, nuclear pellets were treated with salt or DNase I and the supernatant and pellet fractions were assayed by immunoblotting (see MATERIALS AND METHODS). About 50% of Act3p was released from the nuclear pellet after incubation with 0.2 M NaCl, and incubation with 0.5 M NaCl resulted in almost complete removal of Act3p from the nuclear pellet (Figure 4B, upper panel). Moreover, Act3p was progressively removed from nuclei upon digestion with DNase I (Figure 4B, lower panel).

The Polypeptide Sequence of Act3p Contains a Potential Nuclear Localization Signal and a Domain with Similarity to Vertebrate High Mobility Group Proteins (HMG-14)

Upon searching for potential nuclear localization signals in the Act3 sequence, we found that the sequence ^{195}**KK**ALEPKEIIPLFAI**KO**R**K**^{213}, which is situated on insertion peptide I of the Act3 polypeptide (Figure 1 in

Figure 3 (facing page). Immunolocalization of Act3p. Cells were labeled with affinity-purified anti-Act3p-antibody and FITC-conjugated anti-rabbit IgG as described in MATERIALS AND METHODS. DAPI-staining (blue; panels A, C, E, and J) defines the nucleus. Pictures A–F were obtained with a conventional fluorescence microscope, whereas pictures G–J where obtained by confocal scanning microscopy. (A and B) Cells from the haploid strain HK1 overproducing Act3p. (C and D) Diploid wild-type cells (strain AK300). (E and F) HK1 incubated with preimmune serum. In G, H, and I, three sections through the same field of cells from the strain HK1 grown on ScGal, i.e., overproducing Act3p, are shown. A resting cell, a budded cell that has not yet undergone nuclear division, and a cell with a dividing nucleus are depicted.

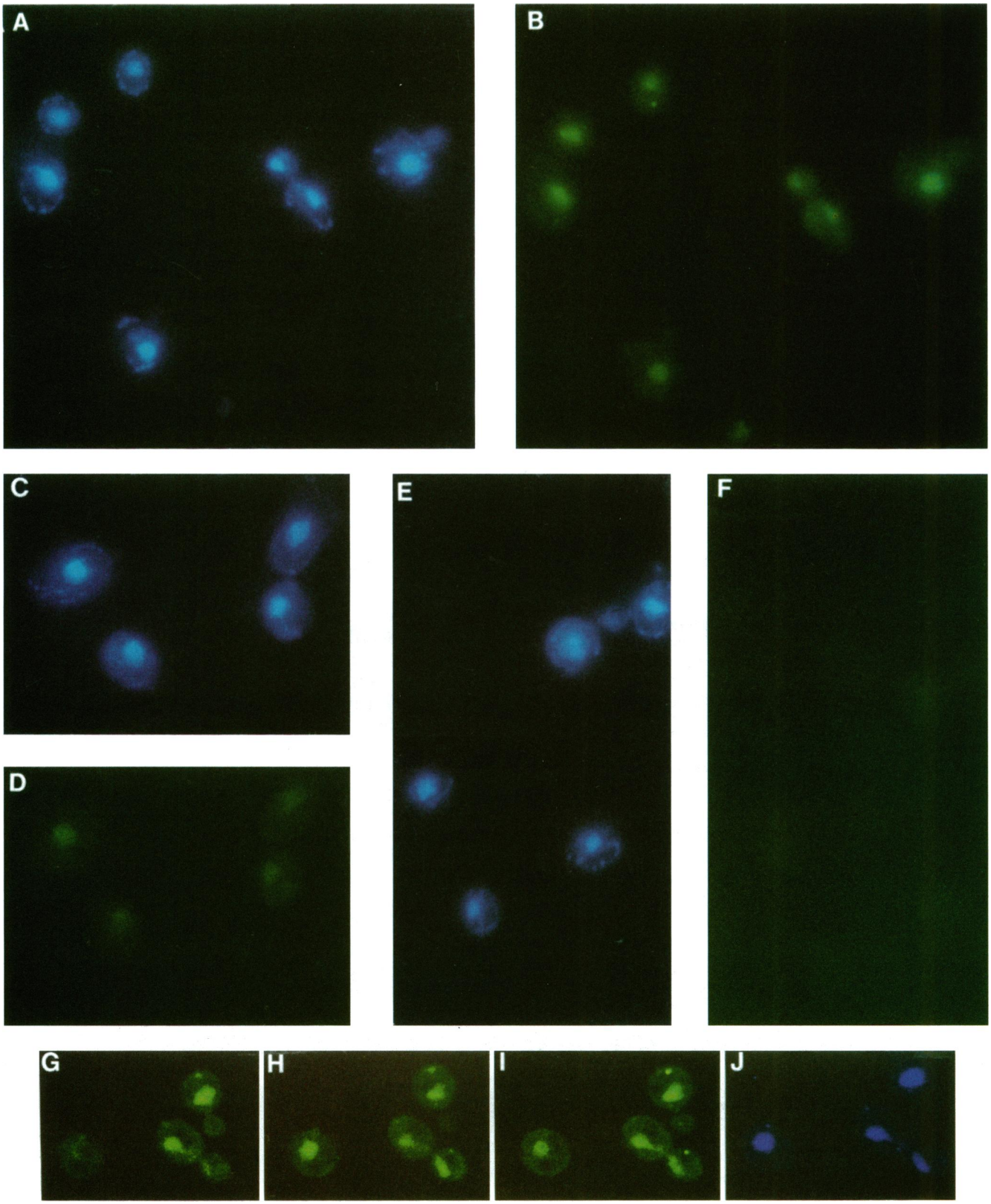

Figure 3.

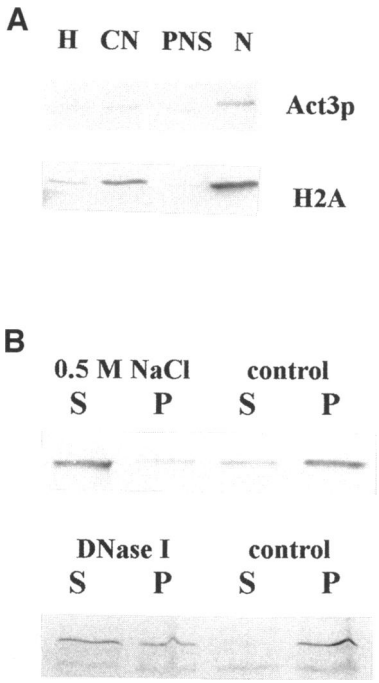

Figure 4. Localization of Act3p by cell fractionation. (A) Act3p cofractionates with nuclei. Nuclei were prepared from the protease-deficient strain MZ3 as described in MATERIALS AND METHODS. Equal amounts of protein (20 μg per lane) from homogenized spheroplasts (H), crude nuclei (CN), post-nuclear supernatant (PNS), and nuclear pellet (N) were separated by SDS-PAGE and subjected to immunoblotting using primary antibodies directed against Act3p (upper panel) and against histone H2A (lower panel). (B) Immunoblots of nuclear fractions after extraction with salt or nuclease treatment. Nuclei were treated with buffer containing 0.5 M NaCl or with buffer alone (upper panel) and with DNase I or buffer alone (lower panel). Extracted proteins recovered from the supernatant (S) and pellet (P) fractions were separated by SDS-PAGE, blotted, and probed with anti-Act3p antibodies.

this paper and Figure 3 in Harata *et al.*, 1994), shows all characteristics of a bipartite nuclear targeting signal, which typically comprises two basic amino acids, followed by a spacer of about 10 residues (13 in the case of Act3p), and a basic cluster in which three of the next five amino acids must be basic (Dingwall and Laskey, 1991; Garcia-Bustos *et al.*, 1991). Although there is not yet proof that this motif of Act3p actually functions as a nuclear targeting signal in vivo, its presence supports the idea of nuclear localization of Act3p.

As we assumed that the three hydrophilic insertions I, IIa, and IIb that are peculiar to the Act3 polypeptide sequence (Figure 1) might play an important role for the specific function of Act3p, we searched for homologues of these segments. No protein with a strikingly high similarity to any of the three segments was found; yet the largest one (IIb) exhibits low, but significant similarity to HMG-14 proteins (Walker *et al.*, 1979), which are components of vertebrate chromatin (Figure 5).

DISCUSSION

Although actin-related proteins have become the subject of intensive research during the last few years, and although two of the three *S. cerevisiae* genes coding for actin-related proteins (*ACT2* and *ACT3*) were found to be essential, comparatively little is known about their cellular roles.

One reason for the lack of knowledge concerning the function(s) of *S. cerevisiae* actin-related proteins may be the fact that compared with conventional actins, ARPs are expressed at relatively low levels. For instance, the yeast *ACT3* gene is transcribed at a level which is less than one-tenth that of *ACT1* (Harata *et al.*, 1994). Nevertheless, by creating a polyclonal antiserum against a specific segment of the Act3p-molecule and thus avoiding cross-reaction with actin or with other actin-related proteins, we were able to determine the cellular localization of the 55-kDa Act3-polypeptide. The two methods employed, namely indirect immunofluorescence microscopy and immunoblotting of proteins from different cellular fractions, independently demostrated that Act3 protein, which contains a potential nuclear localization signal, is located in the nucleus. The distribution of Act3p within the cell does not vary markedly during the cell cycle, because nuclear staining appears at comparable intensity in unbudded cells as well as in cells with small or large buds and in cells with dividing nuclei.

Concerning the nature of association of Act3p with the nuclear fraction, its extraction behavior observed

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Act3p: 312:                                   V K - - T W R N D Y
Hmg14p:  1: P K R K V S S A E G A A K E E P K R R S A R L S A

Act3p: 319: V P - - - L K R T K P - - - S G V N K - S D K K V
Hmg14p: 26: K P A P A K V E T K P K K A A G K D K S S D K K V

Act3p: 337: - - - - - T P T E E K E Q E A V - - - S K S T S P
Hmg14p: 51: Q T K G K R G A K G K Q A E V A N Q E T K E D L P

Act3p: 354: A A N S A D T P N E T G K R P L E - E E K P P K E N
Hmg14p: 76: A E N - G E T K N E E S P A S D E A E E K E A K S D
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Figure 5. Alignment of Act3p insertion peptide IIb with bovine Hmg14p. The sequence comparison was carried out with the aid of GENE WORKS and the maximum matching program of GENETYX. Identical residues are boxed.

upon treatment of yeast nuclei with sodium chloride indicates that Act3p most probably is bound to a nuclear constituent by ionic interactions; it is not associated with the nucleus as tightly as proteins that have been identified as structural components of the yeast nucleus, such as Nuf1p and Nuf2p (Mirzayan *et al.*, 1992; Osborne *et al.*, 1994). Moreover, the progressive release of Act3p from nuclei upon treatment with DNase I, which again is in clear contrast to the behavior of the nucleoskeletal proteins mentioned above, suggests that the integrity of the DNA structure is critical for maintainance of the nuclear location of Act3p. In this context, the resemblance of Act3p insertion peptide IIb to HMG-14 proteins, polypeptides known to affect histone-DNA interactions, gains significance. HMG-14 polypeptides are nonhistone chromosomal proteins that are preferentially associated with transcriptionally active chromatin. Recently, they have been shown to bind to the inner side of nucleosomal DNA, thus influencing the interaction between DNA and histones and thereby modulating chromatin structure (Alfonso *et al.*, 1994; Grosschedl *et al.*, 1994). In vitro, HMG-14 proteins enhance transcription on chromatin-, but not on naked DNA-templates (Ding *et al.*, 1994). Taken together, our data suggest that Act3p may be an essential constituent of yeast chromatin, which probably influences the degree of its compactness.

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