J. Biochem. 128, 665–671 (2000)

Multiple Actin-Related Proteins of *Saccharomyces cerevisiae* Are Present in the Nucleus[1]

Masahiko Harata,*[,2] Yukako Oma,* Teruko Tabuchi,* Yan Zhang,†[,3] David J. Stillman,† and Shigeki Mizuno*

**Laboratory of Molecular Biology, Department of Molecular and Cell Biology, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Tsutsumidori-Amamiyamachi 1-1, Aoba-ku, Sendai 981-8555; and †Division of Molecular Biology and Genetics, Department of Oncological Science, Huntsman Cancer Institute, University of Utah Health Science Center, Salt Lake City, Utah 84132, USA*

Received July 17, 2000; accepted August 7, 2000

An increasing number of actin-related proteins (Arps), which share the basal structure with skeletal actin but possess distinct functions, have been found in a wide variety of organisms. Individual Arps of *Saccharomyces cerevisiae* were classified into Arps 1–10 based on the relatedness of their sequences and functions, where Arp1 is the most similar to actin, and Arp10 is the least similar. While Arps 1–3 and their orthologs in other organisms are localized exclusively in the cytoplasm, Arp4 (also known as Act3) is localized in the nucleus and is involved in transcriptional regulation. Here we examined the more divergent Arps for possible nuclear functions. We show that Arps 5–9 are localized in the nucleus, but Arp10 is not. The nuclear export signals identified in actin are well conserved in the cytoplasmic Arps, Arps 1–3, but less conserved in the nuclear Arps. Gel filtration chromatography experiments show that the nuclear Arps are larger than monomer in size and thus are present in multi-protein complexes. Since nuclear protein complexes containing Arps are found to be responsible for histone acetylation and chromatin remodeling, it is suggested that most of the divergent Arps are involved in the transcriptional regulation through chromatin modulation.

Key words: actin-related protein, chromatin, nuclear localization, protein complex, transcriptional regulation.

The actin-related proteins (Arps) constitute a recently characterized protein family whose members exhibit moderate sequence similarity to each other and to conventional actin (for reviews, *1–4*). Arps and conventional actin appear to share a common ancestor and compose the branch of the actin family within a superfamily of proteins that have an ATP-binding pocket, including 70-kDa heat shock cognate (Hsc70) and hexokinase (*5*). Various Arps, including members less similar to actin, have been found in a wide range of eukaryotic organisms with the progress of genome sequencing projects (*3*, *6*), and these comparative studies show that this protein family is much more divergent than previously thought. While previously analyzed Arps possess important and distinct functions from actin, the function of many of the Arps has not yet been addressed. The budding yeast *Saccharomyces cerevisiae*, whose entire genome sequence has been determined, is a particularly appropriate organism to classify and analyze the functions of Arps.

The 10 Arps (Arps 1–10) of *S. cerevisiae* have been classified according to their similarity to actin, where Arp1 is the most similar and Arp10 is the least similar (*7*). Arps closely related to Arps 1–3 of *S. cerevisiae* have been characterized in various organisms including vertebrates, and these Arps are classified as Arps 1–3, respectively. It has been shown that Arps 1–3 are predominantly localized in the cytoplasm as a component of multi-protein complexes that are the basal units of their function. Moreover, Arps 1–3 each have a similar function across eukaryotic phyla. Arp1 is the major subunit of dynactin, a 1–2 MDa macromolecular complex initially identified as a factor that promotes dynein-mediated movement along microtubles, with nine other polypeptides (*8*). A remarkable feature of Arp1 is that it is the only Arp known to polymerize into filaments (*9*). Arps 2 and 3 are present in a macromolecular complex with six other polypeptides, and the complex is an essential component of actin-filament network in the cytoplasm (for reviews, *10–12*).

Although information about the more divergent Arps, including Arps 4–10 of *S. cerevisiae*, is still limited, it appears that some of them have distinct subcellular localization and/or functions from Arps 1–3. We have shown that Arp4 (also known as Act3) is an essential protein for growth of *S. cerevisiae* (*13*) and is the first example of an Arp localized in the nucleus (*14*). Act3/Arp4 interacts with core histones, and is involved in transcriptional regulation through the establishment, remodeling, or maintenance of chromatin structure (*15*, *16*). Recently it was shown that

[1] This research was supported by a Grant-in-Aid for science research, 11760051, from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Phone: +81-22-717-8771, Fax: +81-22-717-8883, E-mail: mharata@biochem.tohoku.ac.jp

[3] Present address: Department of Microbiology and Molecular Genetics, UMDNJ–New Jersey Med. School, Newark, NJ 07103, USA

Abbreviation: GFP, green fluorescent protein.

NuA4 histone acetyltransferase complex (*17*) contained Act3/Arp4 together with Act1, the yeast actin (*18*). Arps 7 and 9, whose subcellular localization has not been examined, are included in both Swi/Snf and RSC chromatin remodeling complexes (*19*, *20*). The subcellular localization of Arps 7 and 9 can not be concluded only from this observation, because actin, which exists primarily in the cytoplasm, is a component of a histone acetyltransferase complex (*18*) and of a Swi/Snf-like chromatin remodeling complex (*4*, *21*). Therefore, the subcellular localizations of Arps 1–4 only were reported so far.

Here we examined subcellular localization of the more divergent Arps of *S. cerevisiae* using fusion to green fluorescent protein (GFP). Surprisingly, most of the divergent Arps were found to be localized in the nucleus. Although these proteins are functionally distinct from the cytoplasmic Arps 1–3, these nuclear Arps are like cytoplasmic Arps in that they are present in high molecular mass complexes.

MATERIALS AND METHODS

Yeast Strains, Media, and General Methods—Strain MZ3 (*MAT***a** *pep4-3 trp1 leu2-Δ1 ura3-Δ1*) was derived from 20B12 (*22*). Yeast strains DY2864 (*MAT***a** *his4-912δ-ADE2 his4-912δ lys2-128δ can1 trp1 ura3 ACT3*) and DY4519 (*MAT***a** *his4-912δ-ADE2 lys2-128δ can1 leu2 trp1 ura3 act3-ts12*) were used for complementation analysis. To isolate a new temperature-sensitive allele of *ACT3/ARP4*, a plasmid shuffle was performed as described (*23*). A 3.1 kb *Xba*I–*Xho*I fragment with *ACT3/ARP4* was cloned into *Xba*I- and *Xho*I-digested pRS315, creating plasmid M2997. This plasmid was mutagenized with hydroxylamine and transformed into strain DY3677 (*act3:TRP1 ade2 can1 his3 leu2 trp1 ura3*) with plasmid M2706, a *URA3* plasmid with the *ACT3/ARP4* gene. Transformants were grown on 5-fluoroorotic acid to select for colonies that have lost the *URA3* M2706 plasmid but retain the mutagenized *LEU2* plasmid. These strains, which grew well at 30°C, were screened for complete lack of growth at 37°C. Plasmids with the *act3-ts12* allele were recovered and retransformed to verify that the phenotype is plasmid-dependent. DNA sequencing shows that the *act3-ts12* allele has a single amino acid substitution of G455S. The native *ACT3/ARP4* allele was replaced with the *act3-ts12* allele using strain DY3460 as described (*15*), and crosses were performed to yield strain DY4519.

Plasmid Constructs—Primers used for PCR amplification of genes are listed in Table I. The genes coding for a yeast Arp were amplified with the primer sets for each *ARP* with yeast genomic DNA as a template. All reactions were done in a total volume of 50 μl with final concentration of 1 × *Pfu* DNA polymerase buffer (Stratagene, La Jolla, CA), 0.4 μM dNTPs, and 0.25 μM each primer with 2.5 units of cloned *Pfu* DNA polymerase (Stratagene). Plasmid vector pGAL-GFP, which can express a GFP gene fusion under the control of *GAL1* promoter, was constructed: the first PCR amplified the entire coding sequence for GFP with primers that introduce *Sal*I and *Xho*I sites at each end, then this *Sal*I–*Xho*I fragment was cloned into plasmid pYEUra3. Using the primer-derived *Bam*HI and *Sal*I sites of the amplified PCR products for the various Arps, the entire coding sequences for Arps were subcloned into the corresponding cloning sites upstream of *GFP* in pGAL-GFP, and plasmids coding for an Arp fused in frame at its carboxyl-terminus to GFP were constructed.

TABLE I. **Primers used in this study.**

GFP	forward	CGCGTCGACTATGGGTAAAGGAGAAGAAC
	reverse	CCGCTCGAGCTTGTATAGTTCATCCAT
ARP4	forward	AAGGATCCATGTCCAATGCTGCTTTG
	reverse	CGCGTCGACCTAAACCTATCGTTAAG
ARP5	forward	GTGGATCCATGTCTAGCAGAGACGC
	reverse	CGCGTCGACAGGTCTTCAAAATACTTCG
ARP6	forward	TCGGATCCATGGAAACACCACCCATTG
	reverse	CCGGTCGACGATATCCAATTCTGGTAAC
ARP7	forward	GCGGATCCATGACATTGAATAGGAAG
	reverse	CCAGTCGACAGGTTTGTTGCGTTCGTAGC
ARP8	forward	CGGGATCCATGTCGCAAGAAGAAGCAG
	reverse	CCTGTCGACGAGTACGTGAAAATACATTT
ARP9	forward	GAGGATCCATGGTCCCATTTAGGCAG
	reverse	CGCGTCGACGAAAATTGCACGTCCCAAAG
ARP10	forward	GCGGATCCATGTCGAATACTATTGTG
	reverse	CCTGTCGACGATTGTAAGCGCTTCACTCC

Cell Culture—The yeast transformants were grown in 20 ml of synthetic complete media minus uracil in the presence of 2% glucose as sole sources of carbon and energy at 24°C overnight to early log phase. After being harvested and washed twice with water, cells were inoculated in 100 ml of 2% polypeptone/1% yeast extract/2% galactose and cultured at 24°C for 10 h. For preparation of yeast extract, cells were harvested by centrifugation, washed once with water, and kept at −70°C for at least 1 h. Cells were resuspended in two volumes of gel filtration buffer (25 mM HEPES, pH 7.4, 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 1 mM DTT, protease inhibitors). After addition of DNaseI (TaKaRa Shuzo, Shiga) at 350 U/ml, cells were disrupted by vortexing with an equal volume of glass beads.

Western Blot Analysis—Endogenous Act3/Arp4 and GFP-fusion proteins on the blots were detected with a polyclonal rabbit antibody against Act3/Arp4 (*14*) and with a polyclonal rabbit antibody against GFP (Santa Cruz Biotechnology, Santa Cruz, CA). The anti-GFP antibody was used for Western blot analysis after incubation with yeast extract prepared from MZ3 (100 μl/ml of working solution) at room temperature for 30 min to absorb antibodies that recognize yeast proteins non-specifically.

Gel-Filtration Column Chromatography—Yeast extracts prepared from 300-ml culture of cells were applied to a TSK-gel HW65S (Toyo Soda Manufacturing, Tokyo) column (2.2 × 60 cm) which had been equilibrated with the gel filtration buffer, and proteins were eluted with the same buffer at a flow rate of 2 ml/min. Two-milliliter fractions were collected from 55 min after the start of gel filtration, and 1 ml of each fraction was subjected to Western blot analysis after TCA-precipitation of proteins. Alternatively, eluted proteins were pooled into two fractions corresponding to 55 to 81 min and 82 to 90 min in elution time as >150 kDa and <150 kDa fractions, respectively, and one twentieth of each fraction was subjected to Western blot analysis after TCA-precipitation.

Confocal Laser Scanning Microscopy—Cells were fixed in 4% formaldehyde for 30 min, washed twice in PBS, and resuspended in 20 μl of PBS containing 1.7 μg/ml propidium iodide and 1 mg/ml ribonuclease A (Sigma, St. Louis, MO). Samples were placed on a glass slide and squashed gently under a cover slip. Propidium iodide staining and ribonuclease treatment were performed at 37°C for 8–12 h. The following conditions were used for confocal microscopy (NRC-1024, Bio-Rad, Hemel Hempstead, United Kingdom): excitation at 488 nm with a krypton-argon laser and detection using 522DF32 emission filter allowed visualization of GFP-mediated fluorescence with no significant autofluorescence background. Excitation at 568 nm and an emission filter 585EFLP were used to visualize the fluorescence of nuclei stained with propidium iodide.

RESULTS AND DISCUSSION

Construction and Expression of Arp-GFP Fusions—Green fluorescent protein (GFP) is often used as a tag to characterize proteins in a wide variety of organisms including *S. cerevisiae*. In the case of actin-related proteins (Arps) in *S. cerevisiae*, it was previously shown that Arp2 and Arp3 that were fused with GFP at their carboxyl termini were both localized in the cytoplasm, and that Arp2-GFP and Arp3-GFP fusions both rescued the appropriate temperature-sensitive mutations (*24*). To examine the more divergent Arps of *S. cerevisiae* (Arps 4–10), we constructed and expressed the Arps conjugated with GFP at their carboxyl termini (Fig. 1A). Expression of the various Arp-GFP fusions was induced at 24°C for 10 h to avoid producing excessive Arp fusions (see below). Expression of the Arp-GFP fusions was detected by Western blot analysis with an anti-GFP antibody. In all of the transformants GFP-fusion proteins of the appropriate molecular mass were detected (Fig. 1B).

Comparison of Endogenous Arp and Its Fusion with GFP—We examined the effect of GFP on the divergent Arp fusions. Analysis of the individual Arps has just begun, and genetic and functional information is not available for checking all of the divergent Arp fusions. On the other hand, although sequence alignments show that Arps 4–10 all have insertions when compared to actin (Fig. 1A), it is believed that they will all show a similar dimensional structure to actin (*2*, *3*, *7*). Additionally, the amino acid sequences at their carboxyl termini, where the GFP fusion occurs, are relatively well conserved (Fig. 1A) (*7*). Therefore, Act3/Arp4, the most characterized divergent Arp, was used to represent them.

A genetic complementation experiment was performed to determine whether the Act3/Arp4-GFP fusion protein was functional. The Act3/Arp4-GFP plasmid was transformed into a strain with a temperature-sensitive *arp4* allele that is unable to grow at 37°C. Act3/Arp4-GFP expressed from the *GAL1* promoter on medium with galactose was able to rescue the growth defect of the temperature-sensitive mutant at the non-permissive temperature (Fig. 2).

Recent work has shown that 1.3 MDa NuA4 histone acetyltransferase complex (*17*) contains Act3/Arp4 (*18*). Thus we compared the native molecular mass of Act3/Arp4 and its GFP-fusion by gel filtration chromatography. An extract was prepared from yeast cells expressing both endogenous Act3/Arp4 and Act3/Arp4-GFP under the conditions for the observation of the GFP signal in cells, and the exfract was fractionated by gel filtration (Fig. 3, upper panel). Using an anti-Act3/Arp4 antibody, a part of the endogenous Act3/Arp4 was detected in a wide range of fractions in higher molecular mass (fractions 8–22) with a peak at around 1–2 MDa (fraction 16). The amount of Act3/Arp4-GFP is slightly less than that of endogenous Act3/Arp4 in these cells, but is not excessively expressed. The elution profile of Arp4-GFP from the gel filtration column was nearly the same as that of endogenous Act3/Arp4 (Fig. 3, upper panel), and GFP itself was mostly separated into the fractions covering the molecular mass of its monomer (Fig. 3, lower panel, fractions 28–32). These results, taken together with the results

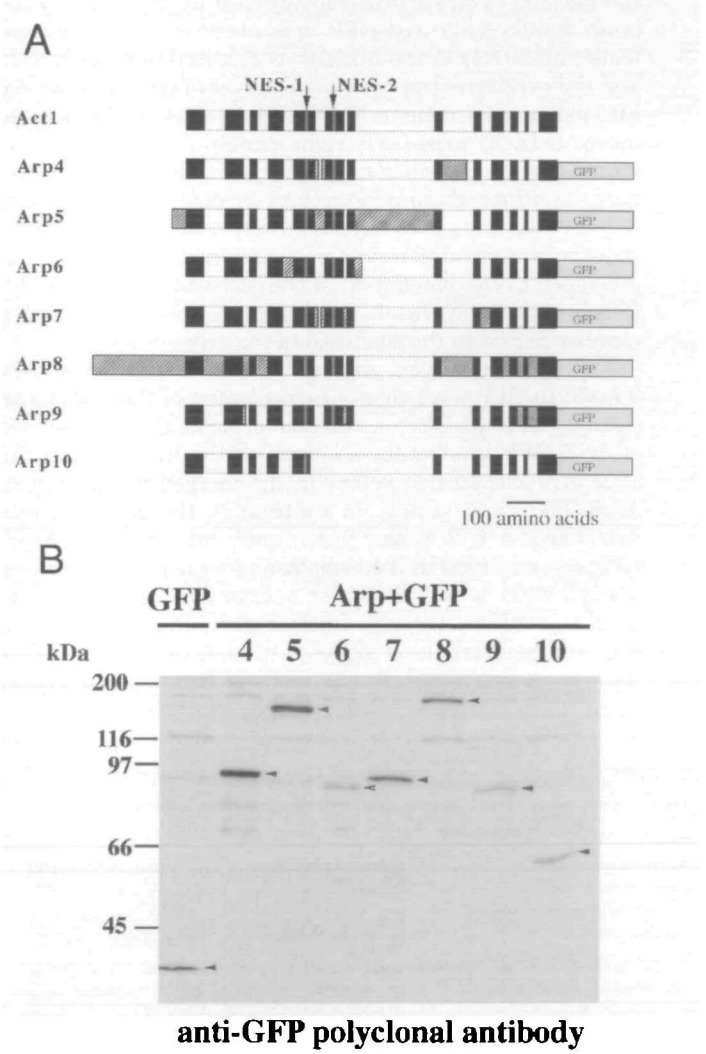

Fig. 1. Construction and expression of Arp-GFP fusion proteins for Arps 4–10. (A) Schematic representation of Arps of *S. cerevisiae*. The blocks of conservation and sequence insertions are indicated by solid boxes and hatched boxes, respectively, while the dotted lines represent gaps introduced for alignment. Boxes distinguished by arrows indicate the positions of motifs corresponding to NES-1 and NES-2 of actin. Boxes marked by GFP represent the position of GFP conjugated to the Arps. (B) Detection of expressed fusion proteins by Western blot analysis. Numbers over the panel represent the Arp gene number. Extracts from cells expressing each of the fusion proteins were subjected to Western blot analysis according to the "MATERIALS AND METHODS." Arrowheads show fusion proteins detected with appropriate molecular mass.

of complementation experiments with Act3/Arp4-GFP and the findings on Arp2-GFP and Arp3-GFP described above, suggest that the fusion of GFP to the carboxyl termini of Arps is unlikely to have a significant effect on protein function.

Subcellular Localization of More Distantly Related Arps than Arps 1–3—Yeast transformants expressing the Arps-GFP fusions were fixed, stained with propidium iodide, and observed with a confocal laser microscope (Fig. 4). In cells expressing GFP that was not fused to another protein, the fluorescence of GFP was dispersed throughout the cell and was not concentrated in the nucleus. As the molecular mass of GFP itself is small enough to go through the nuclear pore, the results show that GFP, which is not localized in the nucleus by itself, passively diffused in the cells. On the other hand, Act3/Arp4-GFP was observed to be concentrated uniformly in the nucleus as reported previously (*14*), and the overlap of the two fluorescences of GFP and propidium iodide turned the color of nuclei yellow in the merged image (Fig. 4, Arp4-GFP, right panel). Along with many studies using GFP-fusion proteins (*25*), these results support the utility of the GFP-system for determining the subcellular localization of Arps of *S. cerevisiae*. We noted that there were some differences in the intensity of the GFP-fluorescence in the population of transformed cells. These differences probably result from variation in plasmid copy number and/or in the condition of the cells.

Among the divergent Arps of *S. cerevisiae*, Arps 5 and 7–9 fused to GFP were clearly concentrated in the nucleus as strongly as Arp4-GFP. In the case of Arp6, the fluorescence of Arp6-GFP was rather observed in the nucleus, and the color of nuclei turned yellow in the merged image (Fig. 4, Arp6-GFP, right panel). In contrast to the other nuclear Arps (Arps 4, 5, 7, 8, and 9), a significant amount of Arp6-GFP was observed in the cytoplasm. As the molecular mass of Arp6-GFP, as well the other nuclear Arps, is too large to passively diffuse through nuclear pore, this difference in subcellular distribution suggests that Arp6 has a weaker nuclear targeting signal or a more modest nuclear export signal than the other nuclear Arps. Arp10-GFP showed a quite different pattern from Arps 4–9. Arp10-GFP was not observed predominantly in the nucleus, but was dispersed throughout the cells. The nuclei appear red in the merged image of GFP and propidium iodide (Fig. 4, Arp10-GFP, right panel). Arp10 is the smallest Arp ever reported, and Arp10-GFP is small enough to diffuse through the nuclear pore. Thus, this result suggests that Arp10 is not localized predominantly in the nucleus, but Arp10 translated in the cytoplasm passively enters the nucleus as well as GFP.

Comparison of the Motifs Corresponding to Nuclear Export Signals of Actin—In spite of its small molecular mass, monomeric actin exists primarily in the cytoplasm. The subcellular distribution of actin is controlled by nuclear export signals (NESs) present in its amino acid sequence (*4*, *26*). NESs were first identified as short sequence motifs that are necessary and sufficient to mediate the nuclear export of large carrier proteins (*27*, *28*). Important for their function is a characteristic spacing of hydrophobic residues. Actin contains two NES sequences (NES-1 and NES-2, Figs. 1A and 5A), and both are conserved in all isoforms of actin from higher eukaryotes to yeast. Each NES contains four hydrophobic residues with a specific spacing (Fig. 5A, boxed residues) (*26*).

The peptide blocks of actin where the NESs are localized (Fig. 1A, boxes indicated by NES-1 and NES-2) are relatively well conserved in Arps in *S. cerevisiae*, except Arp10 (Fig. 1A). NES-1 and -2 of actin were aligned with the corresponding sequences in Arps 1–9 of *S. cerevisiae*, and the four hydrophobic residues in the NESs were boxed (Fig. 5A). In Arps 1–3, the four residues in each motif are mostly conserved or substituted by other hydrophobic residues

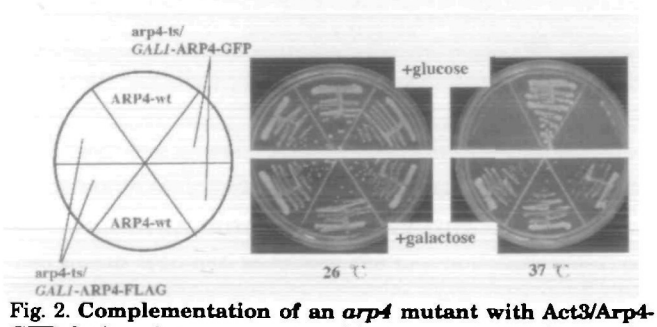

Fig. 2. **Complementation of an *arp4* mutant with Act3/Arp4-GFP fusion.** A temperature-sensitive mutant possessing a point mutant in *ACT3/ARP4* was transformed with a plasmid coding for an Act3/Arp4-GFP under *GAL1* promoter (arp4-ts/*GAL1*-ARP4-GFP), or with a plasmid coding for an Act3/Arp4 tagged with a FLAG epitope under *GAL1* promoter (arp4-ts/*GAL1*-ARP4-FLAG). The transformants and a strain possessing a wild-type Act3/Arp4 (ARP4-wt) were grown in the presence of glucose (upper panels), or galactose (lower panels) as the only carbon source at 26°C (middle panel) or at 37°C (right panel) for 3 days. The left panel shows the positions of yeast strains on the plates.

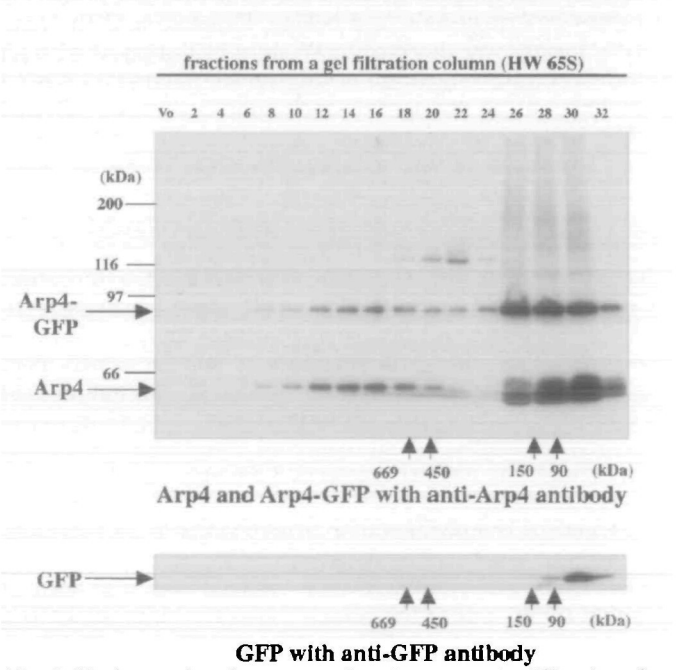

Fig. 3. **Native molecular mass of endogenous Act3/Arp4 and its conjugation with GFP.** Extracts from cells expressing endogenous Act3/Arp4 and Act3/Arp4-GFP (upper panel) or GFP (lower panel) were fractionated on a HW65S gel filtration column, and the proteins in the fractions were detected on a Western blot with an anti-Act3/Arp4 antibody (upper panel) or an anti-GFP antibody (lower panel). The positions of proteins detected on the blot are shown by arrows on the left of each panel. The numbers of fractions and the positions of molecular mass standard proteins are shown over and under each panel, respectively.

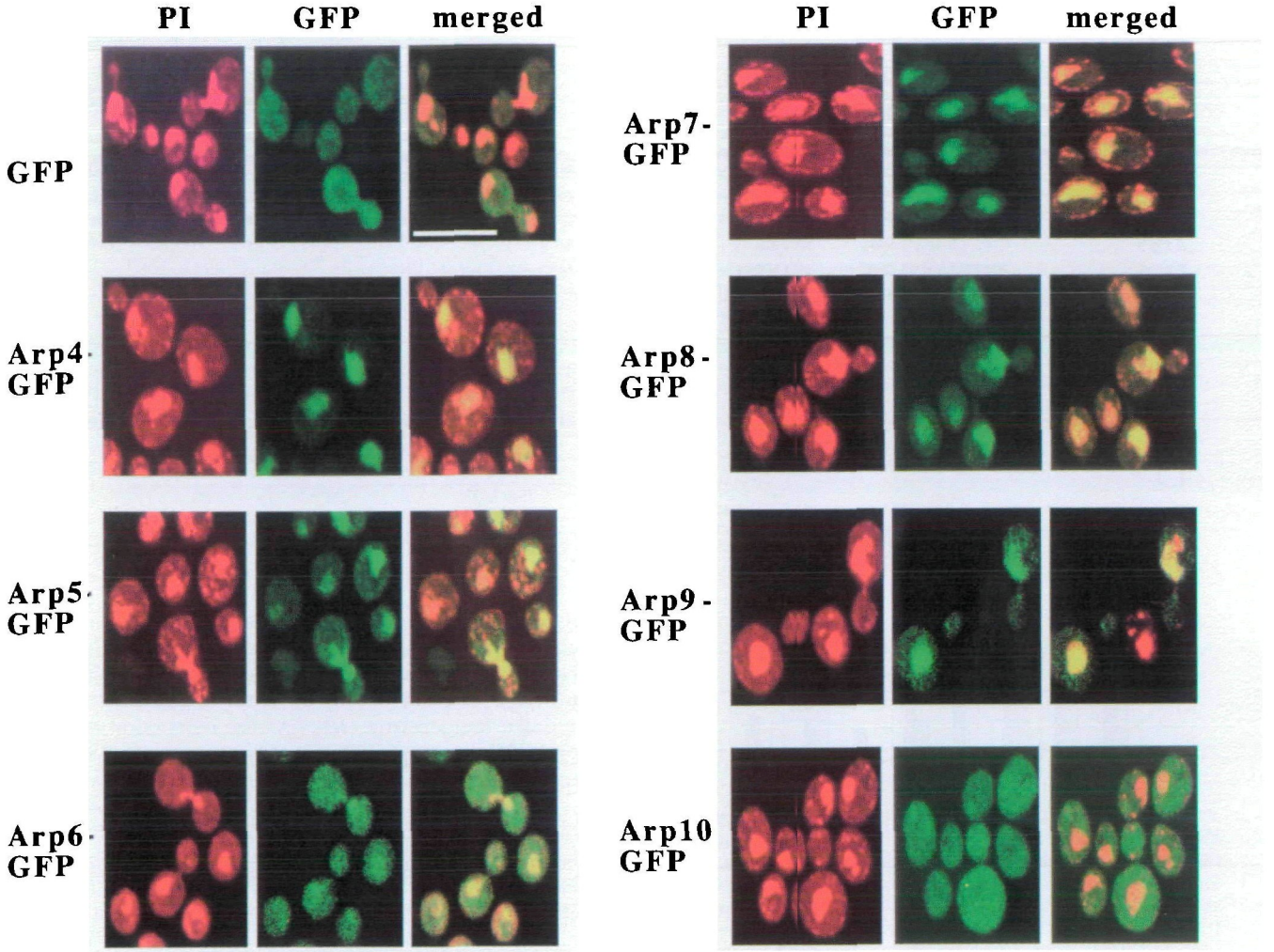

Fig. 4. **Subcellular localization of more distantly related Arps than Arps 1–3.** Cells expressing an Arp-GFP fusion protein were observed under a confocal laser scanning microscope. DNA-staining with propidium iodide (red color in left panels of each set) and the distribution of the fluorescence of GFP (green color in middle panels of each set) were observed. These two images were merged in the right panels of each set, and the overlap of the fluorescences of GFP and propidium iodide showed yellow. (Bar = 10 μm.)

(Fig. 5A), and the sums of the hydrophobic value of the four residues in the motifs are not much lower than those of actin (Fig. 5B). As Arps 1–3 are localized exclusively in the cytoplasm, we expect that the NES motifs in Arps 1–3 function as well as those of actin. On the other hand, in nuclear Arps 4–9, the sums of hydrophobicity value of the residues are smaller than those of the cytoplasmic actin-family proteins, actin and Arps 1–3 (Fig. 5B), showing that the conservation of the motifs is correlated with subcellular localization of the Arps. We note that among the nuclear Arps of *S. cerevisiae*, Arp6 has the highest value in the hydrophobicity of the residues in the motifs, and this protein had significant cytoplasmic accumulation. Taken together, these findings suggest that, while the NES motifs do not seem to be functional in most of the nuclear Arps (Arps 4, 5, 7, 8, and 9), the motifs in Arp6 might possess some ability for nuclear export.

The Presence of Nuclear Arps in High Molecular Mass Complexes—Arps 1–3 of *S. cerevisiae* and their orthologs in other organisms including vertebrates have been characterized relatively well, and it has been shown that they form multi-protein complexes that are the basal units of their function (*8*, *29*). In addition, Arp10 was recently shown to interact with Arp1 by two-hybrid analysis (*30*), suggesting that the cytoplasmic Arps are contained in multi-protein complexes. To investigate whether Arps in the nucleus are also present in large complexes, we determined the native molecular mass of the nuclear Arps. Extracts from cells expressing each of the Arps conjugated with GFP were separated by gel-filtration into two fractions corresponding to >150 kDa (Fig. 6, lanes H) and <150 kDa (Fig. 6, lanes L) in molecular mass, and the presence of the fusion proteins in the fractions was detected with an anti-GFP antibody. The amounts of the fusion proteins present in the two fractions are summarized in Table II. For Arps 5–9, a significant fraction of the GFP fusion was present in the >150 kDa fraction. This suggests that these nuclear Arps, like Act3/Arp4, are able to form high molecular mass complexes with other proteins. For Arp7 and Arp9, these results are consistent with previous reports showing that these proteins are present in multi-protein complexes, the Swi/Snf and RSC chromatin remodeling complexes (*19*, *20*). As

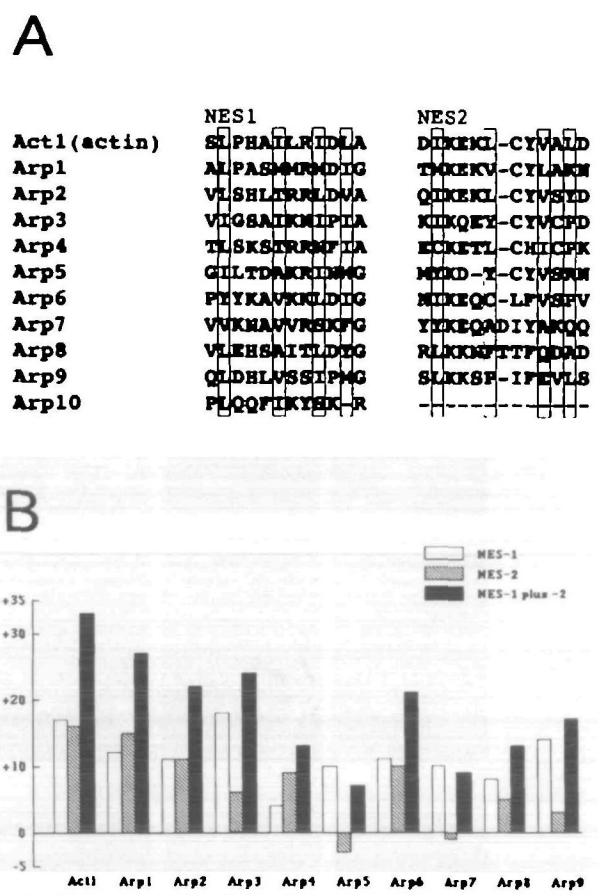

Fig. 5. **Conservation of nuclear export sequences (NESs) among Arps of *S. cerevisiae*.** (A) The amino acids motifs of NES-1 and -2 in actin of *S. cerevisiae* were compared with the corresponding sequences of Arps 1–10 of *S. cerevisiae* according to the alignment by Poch and Winsor (*7*). Four hydrophobic residues reported to be required for NES function in actin are boxed. (B) The sums of the hydrophobic value of the four residues boxed in A. The values of the motifs corresponding to NES-1 (open bar), NES-2 (hatched bar), and the total of both NES-1 and -2 (closed bar) are shown for Arps 1–9.

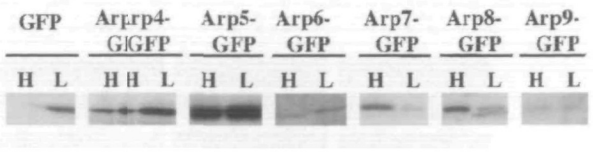

Fig. 6. **Extracts from cells expressing the GFP fusion proteins containing each nuclear Arp were applied to the gel filtration column and separated into two fractions containing proteins of >150 kDa (lanes H) and <150 kDa (lanes L) in native molecular mass.** The fusion proteins in the fractions were detected with the anti-GFP antibody.

TABLE II. **Relative distribution (%) of the fusion proteins in the two fractions from a gel filtration column HW65S corresponding to <150 kDa and >150 kDa of molecular mass.**

Molecular mass	GFP	Arp4-GFP	Arp5-GFP	Arp6-GFP	Arp7-GFP	Arp8-GFP	Arp9-GFP
>150 kDa	<1	34	48	31	90	69	44
<150 kDa	>99	66	52	69	10	31	56

these complexes do not contain any other Arps, the nuclear Arps would be contained in several distinct complexes.

Possible Function of the Nuclear Arps—The subcellular distribution of all Arps in *S. cerevisiae* is now disclosed, and surprisingly, it was found that not only Act3/Arp4 but also most of the divergent Arps of *S. cerevisiae* are localized in the nucleus. As none of the nuclear Arps except Act3/Arp4 is able to rescue temperature-sensitive *act3/arp4* mutants (Harata, M. and Tabuchi, T., unpublished observation), Arps 5–9 must have distinct functions from Act3/Arp4. In spite of such divergent functions of Arps both in the cytoplasm and the nucleus, here we showed that the nuclear Arps were contained in high molecular mass complexes like cytoplasmic Arps, suggesting that all Arps function as subunits of the complexes. Since, a cytoplasmic Arp, Arp1, and a nuclear Arp, Act3/Arp4, have the ability to bind ATP as well as actin (*2*, *16*), Arps may regulate the function and/or formation of the complexes by using this ability.

In contrast to Arps 1–3, information about the functions of the nuclear Arps is limited. However, protein complexes containing nuclear Arps are shown to be involved in the function of chromatin. As mentioned above, NuA4 histone acetyltransferase complex (*18*) and both Swi/Snf and RSC chromatin remodeling complexes (*19*, *20*) contain the nuclear Arps of *S. cerevisiae*. Temperature-sensitive *act3/arp4* mutants lose the activity of NuA4 complex at the non-permissive temperature (*18*), and the deletion of either *ARP7* or *ARP9* gene causes the *swi/snf* phenotype (*19*, *20*), showing the importance of the Arps in the complexes.

Nuclear Arps have been reported also in metazoan. In human, two Arps, hArpNα and hArpNβ/BAF53, both of which show similarity to Act3/Arp4 of *S. cerevisiae*, are localized in the nucleus (*21*, *31*). Interestingly, hArpNβ/BAF53, together with actin, was shown to be a component of Swi/Snf-like BAF chromatin remodeling. Actin and hArpNβ/BAF53 are required for optimal ATPase activity of the complex (*21*). In addition, *Drosophila* Arp6/ArpX (also known as Arp4, but note this is not the ortholog of *S. cerevisiae* Act3/Arp4) is co-localized in the nucleus with HP1 (heterochromatin protein 1), and is suggested to be involved in the formation of heterochromatin by forming a complex with HP1 (*32*).

Observations on these nuclear Arps both in yeast and metazoans show that they are required for integrity and/or regulation of the activity of the complexes involved in the modulation of chromatin structure. Functional analyses of nuclear Arps have just begun; however, it seems likely that most of the divergent Arps in the nucleus are involved in the regulation of gene expression through the chromatin modulation.

REFERENCES

1. Frankel, S. and Mooseker, M.S. (1996) The actin-related proteins. *Curr. Opin. Cell Biol.* **8**, 30–37
2. Mullins, R.D., Kelleher, J.F., and Pollard, T.D. (1996) Actin' like actin? *Trends Cell Biol.* **6**, 208–212
3. Schafer, D.A. and Schroer, T.A. (1999) Actin-related proteins. *Annu. Rev. Cell Dev. Biol.* **15**, 341–363
4. Rando, O.J., Zhao, K., and Crabtree, G.R. (2000) Searching for a function for nuclear actin. *Trends Cell Biol.* **10**, 92–97
5. Holmes, K.C., Sander, C., and Valencia, A. (1993) A new ATP-binding fold in actin, hexokinase and Hsc70. *Trends Cell Biol.*

3, 53–59
6. Eckly, D.M., Gill, S.R., Melkonian, K.A., Bingham, J.B., Goodson, H.V., Heuser, J.E., and Schroer, T.A. (1999) Analysis of dynactin subcomplexes reveals a novel actin-related protein associated with the Arp1 minifilament pointed end. *J. Cell Biol.* **147**, 307–319
7. Poch, O. and Winsor, B. (1997) Who's who among the *Saccharomyces cerevisiae* actin-related proteins? A classification and nomenclature proposal for a large family. *Yeast* **13**, 1053–1058
8. Schroer, T.A., Bingham, J.B., and Gill, S.R. (1996) Actin-related protein 1 and cytoplasmic dynein-based motility—What's the connection? *Trends Cell Biol.* **6**, 212–215
9. Bingham, J.B. and Schroer, T.A. (1999) Self-regulated polymerization of the actin-related protein, Arp1. *Curr. Biol.* **9**, 223–226
10. Mullins, R.D. and Pollard, T.D. (1999) Structure and functions of the Arp2/3 complex. *Curr. Opin. Struct. Biol.* **9**, 244–249
11. Machesky, L.M. and Gould, K.L. (1999) The Arp2/3 complex: a multifunctional actin organizer. *Curr. Opin. Cell. Biol.* **11**, 117–121
12. Welch, M.D. (1999) The world according to Arp: regulation of actin nucleation by the Arp2/3 complex. *Trends Cell Biol.* **9**, 423–427
13. Harata, M., Karwan, A., and Wintersberger, U. (1994) An essential gene of *Saccharomyces cerevisiae* coding for an actin-related protein. *Proc. Natl. Acad. Sci. USA* **91**, 8258–8262
14. Weber, V., Harata, M., Hauser, H., and Wintersberger, U. (1995) The actin-related protein Act3p of *Saccharomyces cerevisiae* is located in the nucleus. *Mol. Biol. Cell* **6**, 1263–1270
15. Jiang, Y.W. and Stillman, D.J. (1996) Epigenetic effects on yeast transcription caused by mutations in an actin-related protein present in the nucleus. *Genes Dev.* **10**, 604–619
16. Harata, M., Oma, Y., Mizuno, S., Jiang, Y.W., Stillman, D.J., and Wintersberger, U. (1999) The nuclear actin-related protein of *Saccharomyces cerevisiae*, Act3p/Arp4, interacts with core histones. *Mol. Biol. Cell* **10**, 2595–2605
17. Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L., Workman, J.L., and Côté, J. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *EMBO J.* **18**, 5108–5119
18. Galarneau, L., Nourani, A., Boudreault, A.A., Zhang, Y., Heliot, L., Allard, S., Savard, J., Lane, W.S., Stillman, D.J., and Côté, J. (2000) Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol. Cell* **5**, 927–937
19. Cairns, B.R., Erdjument-Bromage, H., Tempst, P., Winston, F., and Kornberg, R.D. (1998) Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol. Cell* **2**, 639–651
20. Peterson, C.L., Zhao, Y., and Chait, B.T. (1998) Subunits of the yeast SWI/SNF complex are members of the actin-related protein (ARP) family. *J. Biol. Chem.* **273**, 23641–23644
21. Zhao, K., Wang, W., Rando, O.J., Xue, Y., Swiderek, K., Kuo, A., and Crabtree, G.R. (1998) Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**, 625–636
22. Wintersberger, U., Kuehne, C., and Karwan, A. (1995) Scp160p, a new yeast protein associated with the nuclear membrane and the endoplasmic reticulum, is necessary for maintenance of exact ploidy. *Yeast* **11**, 929–944
23. Sikorski, R.S. and Boeke, J.D. (1991) *In vitro* mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. *Methods Enzymol.* **194**, 302–318
24. Madania, A., Dumoulin, P., Grava, S., Kitamoto, H., Scharer-Brodbeck, C., Soulard, A., Moreau, V., and Winsor, B. (1999) The *Saccharomyces cerevisiae* homologue of human Wiskott-Aldrich syndrome protein Las17p interacts with the Arp2/3 complex. *Mol. Biol. Cell* **10**, 3521–3538
25. Roberts, P.M. and Goldfarb, D.S. (1998) In vivo nuclear transport kinetics in *Saccharomyces cerevisiae*. *Methods Cell Biol.* **53**, 545–557
26. Wada, A., Fukuda, M., Mishima, M., and Nishida, E. (1998) Nuclear export of actin: a novel mechanism regulating the subcellular localization of a major cytoskeletal protein. *EMBO J.* **17**, 1635–1641
27. Gorlich, D. and Mattaj, I.W. (1996) Nucleocytoplasmic transport. *Science* **271**, 1513–1518
28. Nigg, E.A. (1997) Nucleocytoplasmic transport: Signals, mechanisms and regulation. *Nature* **386**, 779–787
29. Welch, M.D., DePace, A.H., Verma, S., Iwamatsu, A., and Mitchson, T.J. (1997) The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. *J. Cell Biol.* **138**, 375–385
30. Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J.M. (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* **403**, 623–627
31. Harata, M., Mochizuki, R., and Mizuno, S. (1999) Two isoforms of a human actin-related protein show nuclear localization and mutually selective expression between brain and other tissues. *Biosci. Biotechnol. Biochem.* **63**, 917–923
32. Frankel, S., Sigel, E.A., Craig, C., Elgin, S.C., Mooseker, M.S., and Artavanis-Tsakonas, S. (1997) An actin-related protein in *Drosophila* colocalizes with heterochromatin protein 1 in pericentric heterochromatin. *J. Cell Sci.* **110**, 1999–2012