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Review

Actin-Related Proteins in the Nucleus and Their Involvement in the Function of Chromatin

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Summary

Actin-related proteins (Arps), which share the basal structure with skeletal actin but possessing distinct functions, have been found in a wide variety of organisms. Individual Arps were classified based on the relatedness of their sequences and functions, and the Arps of *Saccharomyces cerevisiae* have been named Arps 1p-10p, where Arp1p is the most similar to actin, and Arp10p is the least similar. While Arps 1p-3p and their orthologs in other organisms are localized exclusively in the cytoplasm, Arp4p (also known as Act3p) of *S. cerevisiae* is present in the nucleus and is shown to be involved in transcriptional regulation through interaction with core histones. In addition, Arps 7p and 9p of *S. cerevisiae* are components of both Swi/Snf and RSC chromatin remodeling complexes. In vertebrates, it has been shown that human hArpNα and β, which show sequence similarity to Arp4p of *S. cerevisiae*, are localized in the nucleus. hArpNα and β are expressed mutually selectively in brain and other tissues, respectively, and hArpNβ is a component of Swi/Snf-like human BAF complex. Taken together, in both of vertebrates and invertebrates, some of Arps are localized in the nucleus, and they are involved in transcriptional regulation through contribution to structural modulation of chromatin.

Introduction to Actin-Relate Protein (Arp)

The actin-related proteins (Arps) constitute a recently characterized protein family that exhibits moderate sequence similarity among 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 with an ATP binding pocket, including 70-kDa heat shock cognate (Hsc70) and hexokinase (5). Since the identification of the first Arp in 1992 (6-9), various Arps, including members less similar to actin, have been found in a wide range of eukaryotic organisms with the progress of genome

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sequencing projects (3, 10). 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 have not yet been addressed.

S. cerevisiae, whose entire genome sequence has been determined, is a particularly appropriate organism to classify and analyze the functions of Arps. The 10 *ARP* genes of *S. cerevisiae* have been classified according to their similarity to actin and are designated *ARP1* to *ARP10*, where Arp1p is the most similar and Arp10p is the least similar to actin (11, 12) (Fig. 1). Arps closely related to Arps 1p-3p of *S. cerevisiae* have been characterized in various organisms including vertebrates, and these Arps are classified as Arps 1-3, respectively (Fig. 1). 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, each Arps 1-3 has 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 (13). A remarkable feature of Arp1 is that it is the only Arp known to polymerize into filaments (14). 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, 15-17). Although the information about more divergent Arps, which include Arps 4p-10p of *S. cerevisiae*, is still limited, it appears that at least some of the Arps

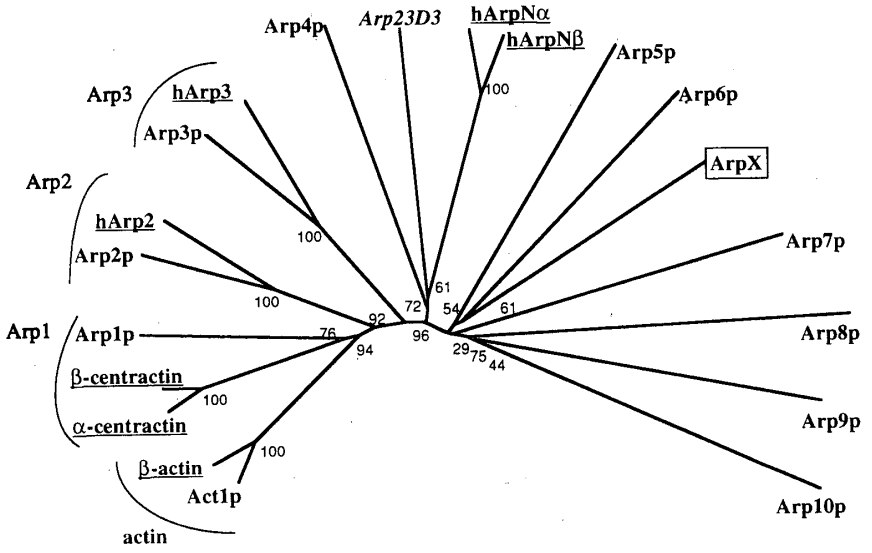

FIG. 1. Unrooted phylogenetic tree showing relatedness of actin family proteins. β-actin and human Arps (underlined), actin and all known Arps of *S. cerevisiae*, Arp 23D3 of *S. pombe* (italic), and Arp X of *Drosophila* (boxed) were shown on the tree. Numbers at branch points refer to the percentage trials in which a given pairing occurred.

have distinct subcellular localization and functions from Arps 1-3.

Act3p/Arp4p of S. cerevisiae, *the First Identified Nuclear Arp*

In 1994, we reported the discovery of a third member of Arp in *S. cerevisiae*, which we named Act3p (18). According to the classification of nowadays as described above, Act3p is called as Arp4p. Arp4p/Act3p consists of 489 amino acids (Mr=54,831) (Fig. 2), and is essential for viability. While Arp4p possesses two unique hydrophilic insertion segments, named insertion I and II, the basal core structure of actin may be well conserved in Arp4p. If we pay no regards to the insertion peptides, Arp4p is 55% similar to actin (18).

At the time, Arps were expected to be predominantly localized in the cytoplasm. However, Arp4p possesses insertion II exhibiting weak similarity to the chromosomal protein HMG-14 as well as insertion I containing a putative nuclear localization signal. We raised a polyclonal antibody against insertion II of Arp4p, and used the antibody to check the subcellular localization of Arp4p. Indirect immunofluorescence with the antibody revealed that Act3p was localized in the nucleus (19). Nuclear staining of the antibody was observed in all cells regardless of the stage of the cell cycle.

The nuclear-localization of Arp4p was confirmed by the expression of Arp4p

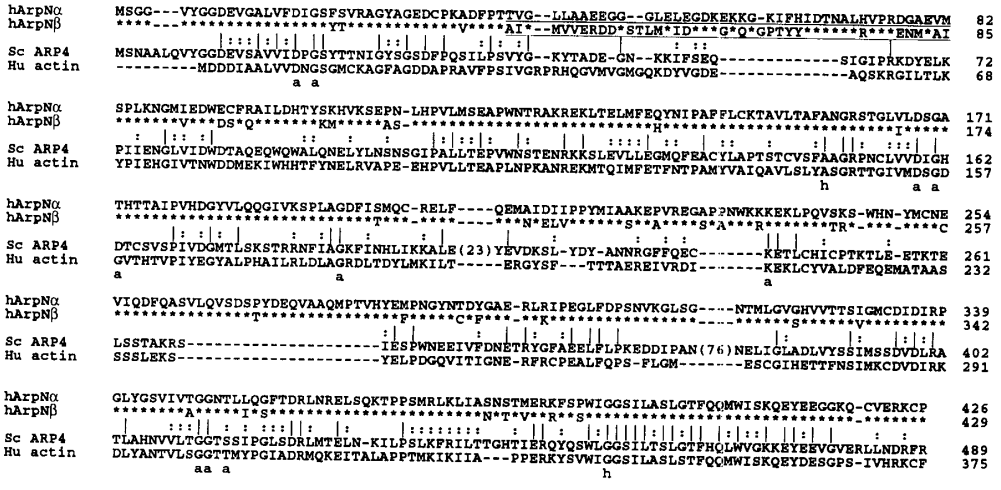

FIG. 2. Alignment of the deduced amino acids sequences of hArpNα and β with the sequences of *S. cerevisiae* Arp4p (Sc ARP4) and human β-Actin (Hu actin). Identical amino acids between hArpNα and β are represented by asterisks (*) and stretches of amino acids existing in Arp4p only are indicated by the respective numbers of amino acids in parentheses. The centerline designates identical (|) and conserved (:) residues among all four actin family proteins. In the sequences of hArpNα and β, the less conserved regions are underlined. The letters of "a" under the sequence of β-actin mark amino acids conserved within the ADP environment between rabbit skeletal actin and bovine Hsc70 and "h" shows the predicted hinge residues for a structural transition accompanying the hydrolysis of ATP.

conjugated with green fluorescent protein (GFP) (Fig. 3). 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 (Fig. 3, upper panels). 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, Arp4p-GFP was observed to be concentrated uniformly in the nucleus (Fig. 3, lower panels). As Arp4p-GFP is not able to diffuse through the nuclear pore because of its larger molecular mass, its nuclear localization represents that Arp4p-GFP is actively transported into the nucleus probably using its nuclear targeting signal (19).

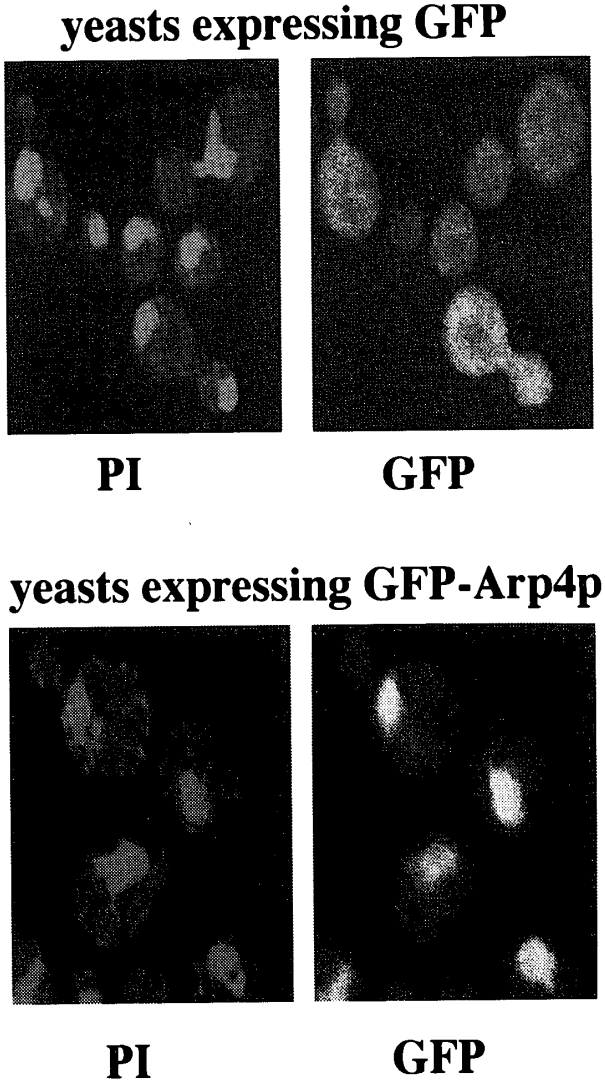

FIG. 3. Subcellular localization of Arp4p. Cells expressing GFP (upper panels) and Arp4p-GFP fusion protein (lower panels) were observed under a confocal laser-scanning microscope. DNA-staining with propidium iodide (PI) and the distribution of the fluorescence of GFP (GFP) were observed.

In addition to the cytological analysis, western blot analysis with subcellular fractions showed that Arp4p is indeed highly enriched in the nuclear fraction, and that Arp4p is a component of chromatin (19). The report is the first example showing the presence of Arp in the nucleus, and the possible involvement of Arp in the function of chromatin.

Genetic Analysis of Arp4p

Independently genetic studies indicated that Arp4p is involved in transcriptional regulation (20). *S. cerevisiae* contains a retrovirus-like transposable element, Ty1, which possesses two domains with strong promoters, the δ element. Insertion of Ty1 or a δ element into the 5′ region of genes often causes inactivation of the adjacent gene because of interference or competition between transcriptional signals in the δ element and the native gene promoter (21). Selections for extragenic suppressors of Ty1- or δ element-inactivated-genes have identified numerous *SPT* (Suppressor of Ty), and many of them were shown to be involved in transcriptional regulations via effects on chromatin structure (21). As certain non-lethal mutations in the *ARP4* gene suppress the transcriptional defect caused by the insertion of a δ element into the yeast *HIS4* promoter (*his4-912δ*), *ARP4* belongs to the group of *SPT* genes.

Interestingly, *arp4* mutations cause variegated suppression of the δ element-inactivated *HIS4* gene (the *his4-912δ* allele) in cells of identical genetic background (20), suggesting an epigenetic effect of the mutated Arp4p proteins on transcription. On and off states of transcription of the *his4-912δ* allele in the *arp4* mutants were found heritable with a low degree of reversibility. This situation seems to be comparable with the position effect variegation (PEV) originally observed for genes adjacent to heterochromatin in *Drosophila* (22). Since PEV in *Drosophila* is associated with an altered chromatin structure (23), it is likely that the *arp4* mutations cause a defect in chromatin structure that affects gene regulation.

Interaction of Arp4p with Core Histones

As described above, Arp4p contains two inserted peptides, named insertion I and II, the latter of which is predicted to form a loop-like structure protruded from beyond the surface of the molecule. Since Arp4p is a constituent of chromatin but itself does not bind to DNA, we hypothesized that insertion II might be responsible for an Arp4p-specific function through it interaction with some other chromatin protein. Far western blot analysis of a chromatin fraction from yeast with a labeled Arp4p-insertion II fusion polypeptide pointed to a possible interaction with core histones. This finding was substantiated by the two hybrid experiments (Fig. 4). Fusion constructs were prepared by cloning the *ARP4* gene and the yeast core histone genes into plasmids encoding the DNA-

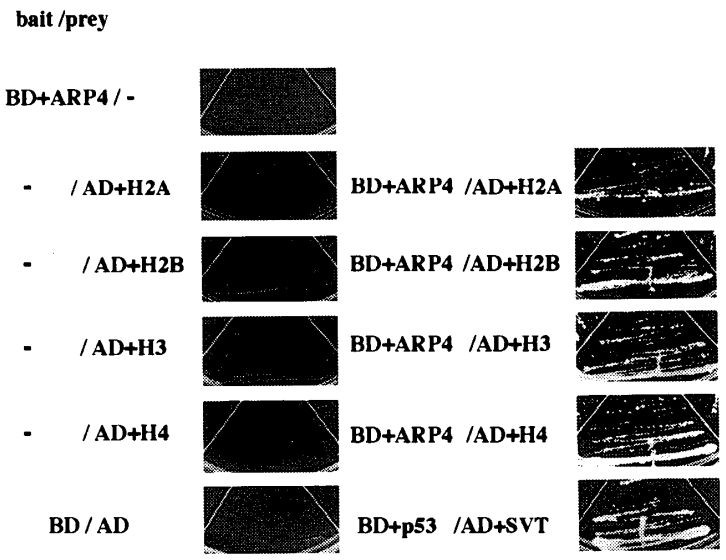

FIG. 4. Two hybrid analysis for the detection of the interaction between Arp4p and yeast core histones. Panels show the growth of the transformants on the dropout media containing 25 mM 3-amino-1, 2, 4-triazole and no histidine after 4 days of incubation. Plasmids encoding the listed sequences were introduced into yeast strain Y190. BD (control bait vector), plasmid pAS2-1 containing the Gal4p DNA binding domain alone; BD+ARP4, pAS2-1 containing an additional peptide 269S-413G of Arp4p, including insertion II; AD (control prey vector), plasmid pACT2 containing the Gal4p AD alone; H2A, H2B, H3, and H4: plasmids containing each of the years histone genes fused with AD. Positive controls were plasmids containing the fused gene of the following proteins: BD+p53, the murine p53 gene fused with Gal4p-DBD; AD+SVT, the SV40 large T-antigen fused with Gal4p-AD.

binding domain (DBD) or the activation domain (AD) of the Gal4p protein, respectively. Interactions between insertion II of Arp4p and core histones were expected to result in the activation of *HIS3* transcription within the transformants. Yeast cells coexpressing Gal4p-DBD+Arp4p (insertion II) together with each of the Gal4p-AD histone fusions grew on plates lacking histidine, showing the interaction between insertion II and each of core histones (Fig. 4). Taken together with the genetic analysis with *ARP4*, these results suggest that Arp4p is involved in the establishment or the maintenance of chromatin structure through its interaction with core histones (20, 24).

Other Possible Nuclear Arps in S. cerevisiae

Arps 7p and 9p, whose subcellular localization has not been examined, are components of both Swi/Snf (mating type SWItching/Sucrose Non Fermenting) and RSC (Remodel of Structure of Chromatin) complexes (25, 26). These chromatin remodeling complexes are important for overcoming nucleosome-mediated repression of transcription. The fact that Arps are components of the

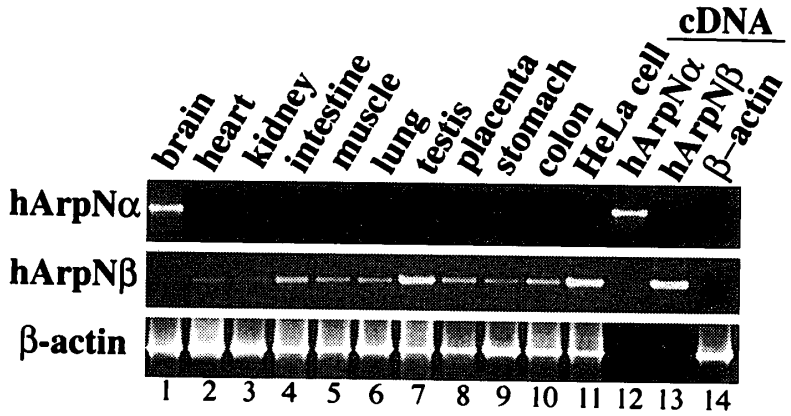

FIG. 5. Agarose gel electrophoresis of RT-PCR products generated by using primer sets for hArpNα (top), hArpNβ (middle), and β-actin (bottom). To prepare a template for the PCR, RNAs from various human tissues (lanes 1-10) and from HeLa cells (lane 11) were reacted for synthesis of the first strand of cDNA with reverse transcriptase. To show expected products generated by the primer sets, cloned full length cDNAs for hArpNα, β, and human β-actin were used as templates (lanes 12-14).

complex shows the involvement of the nuclear Arps in transcriptional regulation.

Putative Human Orthologs of Arp4p

To search nuclear Arps in vertebrates we tried to find human orthologs of Arp4p of *S. cerevisiae*, and we identified two human isoforms of an Arp, designated hArpNα and hArpNβ (27). hArpNα and β are highly similar each other (83% identical and 94% similar), and show apparent similarity to actin and Arps (Fig. 2).

In a phylogenetic tree composed of actins, all comparable human Arps 1-3, all *S. cerevisiae* Arps, a selected *Schizosaccharomyces pombe* Arp, and a selected *Drosophila* Arp, hArpNα and β were the most distantly related Arps to actin among reported human Arps, and apparently belonged to a more distantly related class than Arp3 (Fig. 1). The branch of hArpNα and β were most close to that of *S. pombe* Arp23D3, which is a putative ortholog of Arp4p recently found by the *S. pombe* genome project, and then to that of *S. cerevisiae* Arp4p (18). To check the subcellular distribution of hArpNα and β, both proteins conjugated with GFP were expressed in HeLa cells. In the cells, the fluorescence of both GFP-fusions was observed in the nucleus, showing that hArpNα and β are localized in the nucleus (27).

Interestingly, in different human tissues, hArpNα and β were found expressed mutually exclusively. The mutually selective expression was shown by RT-PCR with RNAs from human tissues and HeLa cells (Fig. 5). The expression of hArpNβ was detected in all tested tissues, but it was very faint in brain. On the other hand, the expression of hArpNα was only detected in brain. Taken

together with the high sensitivity of RT-PCR for the detection of purposive RNA, the expression of hArpNα was shown to be strictly restricted to brain.

After completing the analyses of hArpNα and β, Zhao *et al.* reported a human actin-related protein BAF53 as a subunit of human Swi/Snf-like BAF chromatin remodeling complex, and BAF53 was identical to hArpNβ (28). BAF53/hArpNβ is required for association of human BAF complex, which can disrupt nucleosome *in vitro* (29).

General Property of Nuclear Arps

In addition to hArpNα and β of human, *Drosophila* Arp4/ArpX (note that this is not the ortholog of *S. cerevisiae* Arp4p) was co-localized in the nucleus with HP1 (heterochromatin protein 1), which is involved in the formation of heterochromatin (30). Functional analyses of the unclear Arps have been just begun. However, based on these observations described above, nuclear Arps are most likely to be involved in the regulation of gene expression through the organization and/or modulation of chromatin structure.

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