Nuclear Actin-Related Proteins as Epigenetic Regulators of Development[1]

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Complex regulatory networks control cell fate and the development of organs and tissues in multicellular organisms. But what mechanisms initiate the necessary global changes in patterns of gene expression? What regulates the regulators of organismal development? The nuclear actin-related proteins (ARPs) participate in macromolecular chromatin-remodeling machines that regulate the transcription of developmentally important genes. In Arabidopsis (*Arabidopsis thaliana*), ARP4, ARP6, and ARP7, which are predominantly localized in the nucleus, participate in the regulation of several pathways affecting cell proliferation and organ development. The diverse plant phenotypes resulting from deficiencies in these nuclear ARPs include reduced cell size or numbers, photoperiod-dependent and -independent early flowering, delayed floral senescence, altered leaf, stem, and flower organ morphology, embryo lethality, and an assortment of male and female reproductive defects. A working hypothesis emerging from these and other plant and animal data is that diverse isoforms of nuclear ARP-containing chromatin-modifying complexes exert epigenetic control over global regulators of multicellular development. In support of this hypothesis, we herein examine nuclear ARP phylogeny, the chromatin-remodeling activities of ARP-containing complexes that lead to epigenetic control of gene expression, the expanding developmental roles assigned to several putative plant ARP-containing complexes, as well as the evidence that a large number of ARP complex isoforms may have evolved in concert with the significant demands of multicellular development.

The ARPs share limited sequence identity (15%–60%) with conventional actins, but they appear to maintain the actin fold, a nucleotide-binding pocket and hinge region that enables a conformational change in actin. Eight to 10 ancient classes of ARPs are found in most eukaryotes that have been examined, and all appear to participate in protein complexes (McKinney et al., 2002; Blessing et al., 2004; Kandasamy et al., 2004). Several of the more divergent ARPs are homologs of yeast (*Saccharomyces cerevisiae*) Arp4, 5, 6, and 8, which are found primarily in the nucleus. This location distinguishes the nuclear ARPs from actin or Arp2 and Arp3, which have been found in the nucleus, but are primarily concentrated in the cytoplasm. None of the nuclear ARPs are believed to form polymers such as actin microfilaments or the short filaments formed from ARP1 in the centractin complex, which contains both ARP1 and ARP10.

Nuclear ARPs act as essential subunits of macromolecular machines that remodel chromatin structure, and their only demonstrated functions are within such complexes. Specifically, ARP-containing complexes are involved in nucleosome phasing and movement, histone acetylation, and exchange of histone subunit isovariants within nucleosomes. The chromatin-modifying activities of these complexes can serve the basal regulatory function of reinforcing or alleviating the nucleosomal suppression of transcription that affects most genes (Yuan et al., 2005). However, they may also exert more precise epigenetic control over development via the particular activities of ARP-containing complexes on the transcription of a small subset of regulatory genes. The activities of nuclear ARP complexes on genes that direct global changes in cell proliferation and ontogeny of organs and tissues are the subjects of our working hypothesis and the major focus of this *Update* article.

NUCLEAR ARP PHYLOGENY

The majority of genes encoding the nuclear ARPs evolved from a common ancestral actin gene prior to the divergence of the four eukaryotic kingdoms, yet the nuclear ARP proteins share 15% to 40% amino acid sequence identity with conventional actin (Blessing et al., 2004). Because the initial characterization of the entire family of ARPs was performed in yeast, most nuclear ARPs in other organisms are named relative to the yeast nuclear ARPs (Arp4, 5, 6, 7, 8, and 9; Poch and Winsor, 1997). The increasing numbers in this ARP nomenclature represent increasing divergence from conventional actin, with ARP4 being most closely related (approximately 40% amino acid identity to actin) and ARP9 the most divergent (approximately 15% identity; McKinney et al., 2002; Kandasamy et al.,

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2004). While the nuclear ARPs share the backbone of actin sequence and are predicted to retain the actin fold, they contain several, sometimes large, insertions and small deletions that distinguish each ARP clade. The phylogenetic relationships of the yeast nuclear ARPs to those in humans, Arabidopsis, and rice (*Oryza sativa*) are illustrated in Figure 1. Humans contain clear homologs of yeast ARP4 (HsaBAF53), Arp5 (HsARP5), Arp6 (HsARP6x), and Arp8 (HsARP8). Arabidopsis and rice ARP4, ARP5, ARP6, and ARP9 are clear plant homologs of ARP4 (Baf53), Arp5, Arp6, and Arp8, respectively, in animals and yeast. Thus, these four clades of ARPs (labeled in Fig. 1) certainly predate the divergence of the three kingdoms represented from a common ancestor. We have shown that Arabidopsis ARP4, ARP6, and ARP7 are localized to the interphase nucleus (Kandasamy et al., 2003; Deal et al., 2005), and by their phylogenetic position in the sequence tree the plant homologs of yeast ARP5, ARP8, and ARP9 are predicted to encode nuclear ARPs (Kandasamy et al., 2004). The six phylogenetically paired sets of plant ARP proteins from Arabidopsis and rice are 52% to 87% identical at the amino acid sequence level. Because these two angiosperms have not shared a common ancestor for 200 million years, it is likely that homologs to these six ARPs are reasonably well conserved and may be universally present in all higher plants (McKinney et al., 2002; Kandasamy et al., 2004). The phylogenetic relationships of Arabidopsis ARP7 and ARP8 to ARPs in other eukaryotic kingdoms are not clear, and hence, have an orphaned status (Blessing et al., 2004).

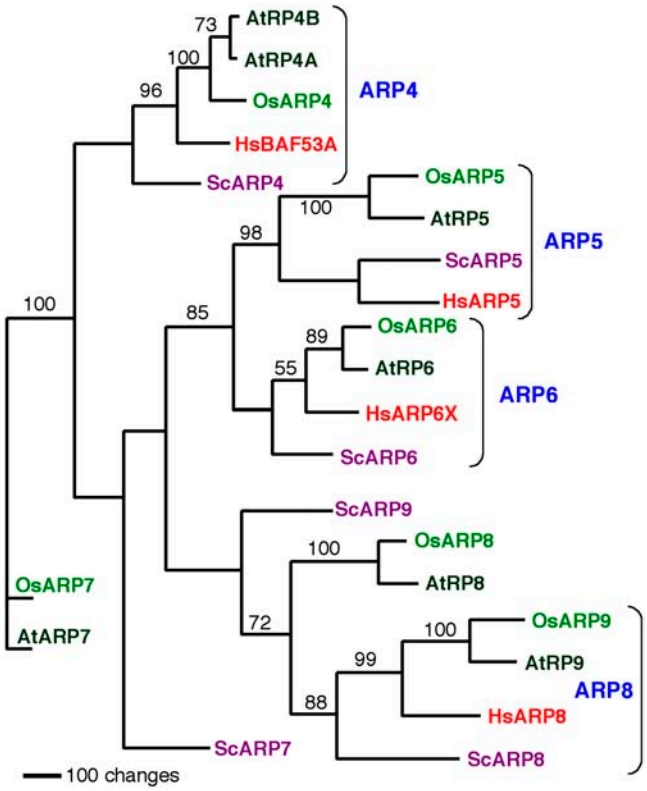

Figure 1. Phylogenetic relationships among the nuclear ARPs. The phylogenetic relationships among the nuclear ARPs from yeast (purple), Arabidopsis (dark green), rice (light green), and human (red) are shown. The four clades of nuclear ARPs that are clearly conserved in all eukaryotes and named after the closest yeast ARP homolog are indicated (uppercase blue font). ClustalW was used to align the sequences. The phylogram presented used Parsimony to create the tree's topography based on sequence similarity and a heuristic search with 100 replicates to generate bootstrap values within PAUP (Swofford, 1998). Bootstrap values below 50% are not indicated. A tree with very similar branching was obtained using the neighbor-joining tree-building method.

THE ACTIVITIES OF NUCLEAR ARPS AND ARP-CONTAINING CHROMATIN-MODIFYING COMPLEXES

All of the nuclear ARPs that have been examined to date are constituents of either ATP-dependent nucleosome-remodeling (NR) complexes, histone acetyltransferase (HAT) complexes, or histone variant exchange (HVE) complexes, all of which are involved in the modification of chromatin structure (Olave et al., 2002; Mizuguchi et al., 2004). The ATP-dependent NR and HVE complexes all contain a DNA-dependent ATPase and use the energy of ATP hydrolysis to drive the repositioning of nucleosomes on DNA or the exchange of one histone type for another within nucleosomes. The NR and HVE complexes share other similar subunits, and there are mechanistic arguments that all NR complexes can carry out HVE activities (Flaus and Owen-Hughes, 2004). In contrast, the HAT complexes transfer an acetyl group onto histone N-terminal tails within nucleosomes, thereby altering chromatin structure indirectly, but they do not contain a DNA-dependent ATPase. The modifications that HAT and related complexes perform on the termini of histone polypeptides generate a histone code that can be decoded to define active and inactive regions of chromatin (Margueron et al., 2005). Surprisingly, the majority of the well-characterized chromatin-modifying complexes include monomeric actin as a subunit in addition to one or more ARPs (Olave et al., 2002).

A number of important themes have emerged regarding the biochemical functions of the ARPs and actins in NR, HVE, and HAT complexes, but no single function stands out as universally conserved in all complexes (Blessing et al., 2004). Recalling that ARPs belong to the actin superfamily of proteins that includes cytoskeletal actins, heat shock proteins, ATPases, and sugar kinases, most models for ARP and actin function center on the actin fold and hinge region that impart the ability to shift between distinct conformational states upon nucleotide binding, ATP hydrolysis, and ATP/ADP exchange (Sunada et al., 2005). Through this change in conformation, the ARP and actin subunits may act as molecular switches controlling the assembly, stability, and/or the activity of these machines. However, to date, only ARP2 and ARP4 are

known to bind and hydrolyze nucleotides (Sunada et al., 2005). Thus, ARPs may possess some of their own ATPase activity, but they are more likely to act indirectly by activating the DNA-dependent ATPase subunit found in NR and HVE complexes, given that mutations in the predicted ATPase domains of yeast ARP7 and ARP9 did not alter the ATPase activity of the RSC (remodel the structure of chromatin) or SWI/SNF (mating-type switch/Suc nonfermenting)-related complexes (Cairns et al., 1998; Szerlong et al., 2003). In addition, yeast ARP4 and ARP8 can bind histones directly, and thus, may recruit chromatin complexes directly to nucleosomes (Shen et al., 2003). Finally, it has been proposed that ARPs and actins may connect one chromatin complex to another to create higher order chromatin complexes, in this way acting similarly to conventional actin, but in the polymerization of higher order structures instead of filaments (Blessing et al., 2004).

The activities of several nuclear ARP-containing chromatin-remodeling complexes are known. In yeast, for example, *ARP4* is an essential gene and ARP4 protein is a component of the NUA4 (nucleosomal acetyltransferase of H4, HAT), the INO80 (inositol requiring) NR, and the SWR1 HVE complexes (Galarneau et al., 2000; Krogan et al., 2004; Mizuguchi et al., 2004). The 1.3-megadalton NUA4 complex contains 11 subunits, including ARP4 and conventional actin, and it is known to primarily acetylate histone H4. In temperature-sensitive *arp4* mutants the NUA4 complex is absent (Galarneau et al., 2000), indicating that ARP4 is required to assemble or stabilize the complex. These same mutants display defects in the transcription of certain target genes at the restrictive temperature, coincident with changes in the chromatin structure around those genes (Jiang and Stillman, 1996; Harata et al., 2002). In addition, ARP4 has been shown to bind all four core histones in vitro (Harata et al., 1999). Thus, ARP4 may serve to recruit components and/or stabilize complexes and could also provide a targeting function by interacting with core histones. In addition to yeast, ARP4 orthologs are also found in at least three different mammalian ATP-dependent NR complexes, in at least one remodeling complex in Drosophila, and in one HAT complex in mammals (Olave et al., 2002).

In addition to ARP4, the yeast INO80 NR complex also contains ARP5, ARP8, monomeric actin, and seven other subunits as shown in Table I. The loss-of-function mutations for the Ino80 subunit, a DNA-dependent ATPase, are defective in transcribing the genes involved in inositol biosynthesis and are hypersensitive to DNA-damaging agents (Ebbert et al., 1999; Shen et al., 2003), suggesting a role for the INO80 complex not only in transcriptional control, but also in DNA damage repair. Deletion mutants lacking the *ARP5* and *ARP8* genes also display the *ino80*$^-$ inositol requiring phenotype, indicating that these ARP proteins are critical to the function of the complex. INO80 complexes purified from *arp5Δ* or *arp8Δ* yeast strains still retain most other subunits, but are deficient in Ino80 ATPase activity, DNA binding, and NR functions. In addition, the complex purified from *arp8Δ* cells also lacks ARP4 and actin, indicating that ARP8 is needed to recruit these components to the complex (Shen et al., 2003). The finding that ARP8 also binds histones H3 and H4 in vitro (Shen et al., 2003) suggests that, in addition to stimulating the ATPase activity of the Ino80 subunit, the ARPs may also serve as points of contact between the complex and chromatin.

For some time, our knowledge of ARP6 function was limited to the observation that this protein was localized to the nucleus in both yeast and Drosophila. However, two groups have recently isolated the yeast SWR1 complex and found that it contains not only ARP6, but also ARP4, actin, and 10 other subunits as listed in Table I (Krogan et al., 2004; Mizuguchi et al., 2004). This complex carries out a new class of chromatin-remodeling activity: the exchange of one histone variant for another within the nucleosome. The SWR1 HVE complex replaces histone H2A with the variant H2A.Z at specific chromosomal locations (Krogan et al., 2004; Mizuguchi et al., 2004). The H2A.Z histone variant is conserved among eukaryotes, and thus the HVE activity of ARP6-containing complexes is probably ancient and present in most eukaryotes. Histone H2A.Z-containing nucleosomes act partly to antagonize the spread of silent heterochromatin into euchromatic regions, but they also have important heterochromatic functions (Meneghini et al., 2003; Dryhurst et al., 2004).

Yeast SWI/SNF and RSC are two related ATP-dependent NR complexes containing 11 and 15 subunits, respectively, including ARP7 and ARP9. Table I shows the composition of the yeast SWI/SNF complex. Unlike the complexes described above, neither SWI/SNF nor RSC contain monomeric actin. The SWI/SNF complex was identified independently in genetic screens for genes involved in mating-type switching and Suc fermentation, and the RSC complex was later isolated based on homology to SWI/SNF complex components. Yeast strains lacking ARP7 or ARP9 show typical *swi/snf*$^-$ phenotypes, indicating that these proteins play an essential role in the function of SWI/SNF. In addition to the *swi/snf*$^-$ phenotype, mutations in *ARP7* or *ARP9* also lead to other transcriptional defects not related to SWI/SNF function, indicating that RSC plays a role in transcriptional regulation as well (Cairns et al., 1998). Surprisingly, RSC complexes isolated from *arp7Δ/arp9Δ* cells are fully intact and retain the ability to remodel nucleosomes in vitro. However, a screen for suppressors of *arp7* and *arp9* mutations identified the transcription factor Nhp6, which interacts physically with RSC and enhances the activity of the complex in vitro (Szerlong et al., 2003). This finding suggests that ARP7 and ARP9 serve to connect the RSC complex to interacting proteins or other complexes, allowing functionality in vivo. The animal SWI/SNF NR complexes that have been characterized contain homologs of yeast ARP4 and actin instead of homologs of ARP7 or ARP9 found

Table I. *Predicted Arabidopsis subunit isovariant diversity for three chromatin-remodeling complexes*

Yeast Complex-Protein Subunits[a]	Genes in Arabidopsis Encoding Isovariants[b,c]	Accession Nos. of Arabidopsis Genes
SWI/SNF		
Arp7 (YPR034W)	1 (ARP7[d])	At3g60830[b]
Arp9 (YMR033W)	N.D.[e]	
Snf2 (YOR290C)	4	At5g19310, At3g06010, At2g28290, At3g06400, At5g18620[b]
Snf5 (YBR289W)	1	At3g17590[b]
Snf12 (YNR023W)	2	At5g14170, At3g01890[b]
Swi1 (YPL016W)	2	At1g79000, At1g16710[b]
Swi3 (YJL176C)	4	At2g33610, At1g21700, At4g34430, At2g47620[b]
Taf14 (YPL129W)	2	At2g18000, At5g45600[c]
Rtt102 (YGR275W), Snf11 (YDR073W), Snf6 (YHL025W),	N.D.[e]	
SWR1/SWR-C		
Arp4 (YJL081C)	1 (ARP4)	At1g18450
Arp6 (YLR085C)	1 (ARP6)	At3g33520
Actin ACT1 (YFL039C)	8[f]	
Swr1 (YDR334W) Snf2 homolog	4	At3g12810, At3g57300, At3g06400, At5g19310[b]
Swc3 (YAL011W)	5	At4g23800, At1g15340, At3g16000, At4g11080, At1g65470[b]
Swc5 (YBR231C)	3	At3g28730, At1g08600, At1g58025[b]
Vps72 (YDR485C)	5	At4g23800, At5g22650, At2g06210, At4g11080, At5g18620[b]
Rvb1 (YDR190C)	1	At5g22330[c]
Rvb2 (YPL235W)	2	At5g67630, At3g49830[c]
Swc4 (YGR002C)	1	At2g47210[c]
Vps71 (YML041C)	1	At5g37055[c]
Yaf9 (YNL107W)	2	At5g45600, At2g18000[c]
Swc7 (YLR385C)	N.D.[e]	
INO80		
Arp4 (YJL081C)	1 (ARP4)	At1g18450[b]
Arp5 (YNL059C)	1 (ARP5)	At3g12380[b]
Arp8 (YOR141C)	1 (ARP9)	At5g43500[b]
Actin ACT1 (YFL039C)	8[f]	
Ino80 (YGL150C) snf2 homolog	3	At3g57300, At3g12810, At5g66750[b]
Nhp10 (YDL002C)	7	At4g23800, At4g11080, At1g20693, At3g51880, At1g20696, At3g28730, At5g23420[b]
Rvb1 (YDR190C)	1	At5g22330[c]
Rvb2 (YPL235W)	2	At5g67630, At3g49830[c]
Taf14 (YPL129W)	2	At2g18000, At5g45600[c]
Ies1 (YFL013C), Ies3 (YLR052W)	N.D.[e]	

[a]Systematic yeast gene name (dp.yeastgenome.org). [b]The Plant Chromatin Database (www.chromdb.org) and [c]The Arabidopsis Information Resource were searched for various protein homologs of the yeast proteins. The phylogeny of related gene sequences was examined to make an estimate of the number of hits in the databases with scores equal to or less than (more significant than) $E = 0.001$ (the expectation of finding two sequences with a given amount of similarity by chance). An E value of 0.001 is considered statistically significant in large databases (Gerstein, 1998; Pearson, 2000). [d]Arabidopsis has no unambiguous homolog of yeast ARP7, but the orphaned plant ARP7 could be its immediate ortholog (Kandasamy et al., 2005b). In animals, ARP4 homologs have been found in most SWI/SNF complexes and the same could be true in plants. [e]None detected based on a homology score of less than 0.001. [f]The Arabidopsis genome encodes eight isovariants of conventional actin that were not included in calculations of isoform diversity.

in the yeast complex (Olave et al., 2002). This suggests at least a functional relationship if not an undetected phylogenetic relationship exists between these more divergent ARPs and the reasonably well conserved ARP4.

In summary, nuclear ARP functions are believed to be central to the activity of the majority of the known chromatin-remodeling complexes and to NR, HAT, and HVE complexes in particular. The six yeast nuclear ARPs (ARP4–9) are all found in these three classes of complexes. Thus, the subunit compositions of the yeast NR, HAT, and HVE complexes, such as those examples presented in Table I, are likely to be predictive of the basic subunit compositions of ARP-containing complexes in other kingdoms.

THE ROLES OF NUCLEAR ARPS IN PLANT DEVELOPMENT

During the last few years, chromatin remodeling has been directly linked to numerous pathways of plant development (Wagner, 2003; Gendrel and Colot, 2005). Although it is known that ARPs are essential components of all SWI/SNF and most other chromatin-modifying complexes in yeast, there were until recently no data directly linking ARP functions to plant development. Therefore, it was exciting to find that knocking down or knocking out the Arabidopsis nuclear ARPs ARP4, ARP6, and ARP7 was associated with defects in several developmental pathways. A sampling of the developmental abnormalities associated with deficiencies in these three ARPs is illustrated in Figure 2. Reduction in ARP4 expression produces phenotypes affecting numerous aspects of plant growth and development (Kandasamy et al., 2005a). For example, the leaky *arp4-1*-deficient mutant allele is partially male sterile. Anthers retain a small, immature heart shape and make a small number of mature pollen grains (Fig. 2I), reducing the efficiency of self-pollination. Targeting the distinct 3′ untranslated region of Arabidopsis *ARP4* transcripts with RNA interference generates an epiallelic series with strong, moderate, or weak ARP4 deficiency phenotypes (Fig. 2). Strong lines with barely detectable ARP4 protein levels have smaller than normal cotyledons, leaves, inflorescence stems, flowers, and fruits (Fig. 2, C, D, and J). Under long-day but not short-day growth conditions, the strong RNAi lines flower a week early with only six rosette leaves compared to 12 leaves for wild type (Fig. 2G), suggesting a defect in the photoperiod-dependent flowering pathway. The plants with strong epialleles also show an extreme delay in floral organ senescence and abscission, with 20 or more flowers retaining their petals and sepals (Fig. 2H), whereas a wild-type inflorescence seldom retains these floral organs on more than six flowers.

ARP7 is an essential protein whose knockdown results in aberrant cell expansion and retarded plant development (Kandasamy et al., 2005b). Knocking out the expression of Arabidopsis ARP7 protein in the homozygous *arp7-1* T-DNA mutant produces morphologically abnormal embryos that are arrested early in development (torpedo stage, Fig. 2B). Moreover, knocking down the expression of ARP7 protein levels with RNA interference produces an epiallelic series of plant lines with dosage-dependant, heritable defects in multiple developmental pathways. ARP7-defective plants are severely to moderately dwarfed, with highly retarded roots having reduced elongation zones at their tips (Fig. 2F). Moreover, the dwarf plants contain fewer rosette leaves that were small and severely curled as compared to wild type. These smaller leaves have a similar number of cells to wild type, but the cells are about one-half the size of wild-type cells (Fig. 2E; Kandasamy et al., 2005b). ARP7-deficient plants show a significant decrease in fertility due to defects in pistil and anther development, and poor pollen production. Silenced lines also show delayed abscission of the petal and sepals (Fig. 2H).

Null alleles lacking ARP6 protein (*arp6-1*, *arp6-2*) display defects during several stages of Arabidopsis development (Choi et al., 2005; Deal et al., 2005). Rosette and cauline leaves, inflorescence stems, flowers, and fruits are dwarfed due to poor cell proliferation, with fewer but normal-sized cells constituting each organ. Under long-day growth conditions the leaves are smaller and narrower than wild type, while under short-day growth the leaves have normal length but are narrower than wild type (Fig. 2G, top), and the mutant leaves are deeply serrated along the margins (Fig. 2D). The *arp6*$^{-}$ mutants also show defects in inflorescence and flower organ morphologies. Some organs appear underdeveloped relative to mature wild-type organs (Fig. 2K), and under some growth conditions the flowers contain extra petals. There is also a loss of apical dominance, a trait often associated with the loss of normal hormone-stimulated stem cell development. Mutant plants flower earlier than wild type, and early flowering occurs in both long- and short-day photoperiods, implying defects in the photoperiod-independent flowering pathway (Fig. 2G). The mutants are relatively infertile due to a defect in female fertility but not pollen fertility.

In plants, the mechanisms by which ARP-containing chromatin-remodeling complexes are targeted to specific genes are poorly understood, but there are several examples of the subunits of these complexes acting as high-level regulators of cell proliferation and development. The control of flowering time in Arabidopsis is the most thoroughly studied plant developmental pathway regulated by changes in chromatin structure, as summarized in Figure 3 (He and Amasino, 2005). *ARP4*- and *ARP6*-deficient Arabidopsis lines revealed distinct photoperiod-dependent and photoperiod-independent early-flowering phenotypes, respectively. Defective *ARP4* and *ARP6* alleles flowered in approximately half the time required for wild-type plants, when grown under long-day growth conditions. The complex regulation of a master repressor of flowering

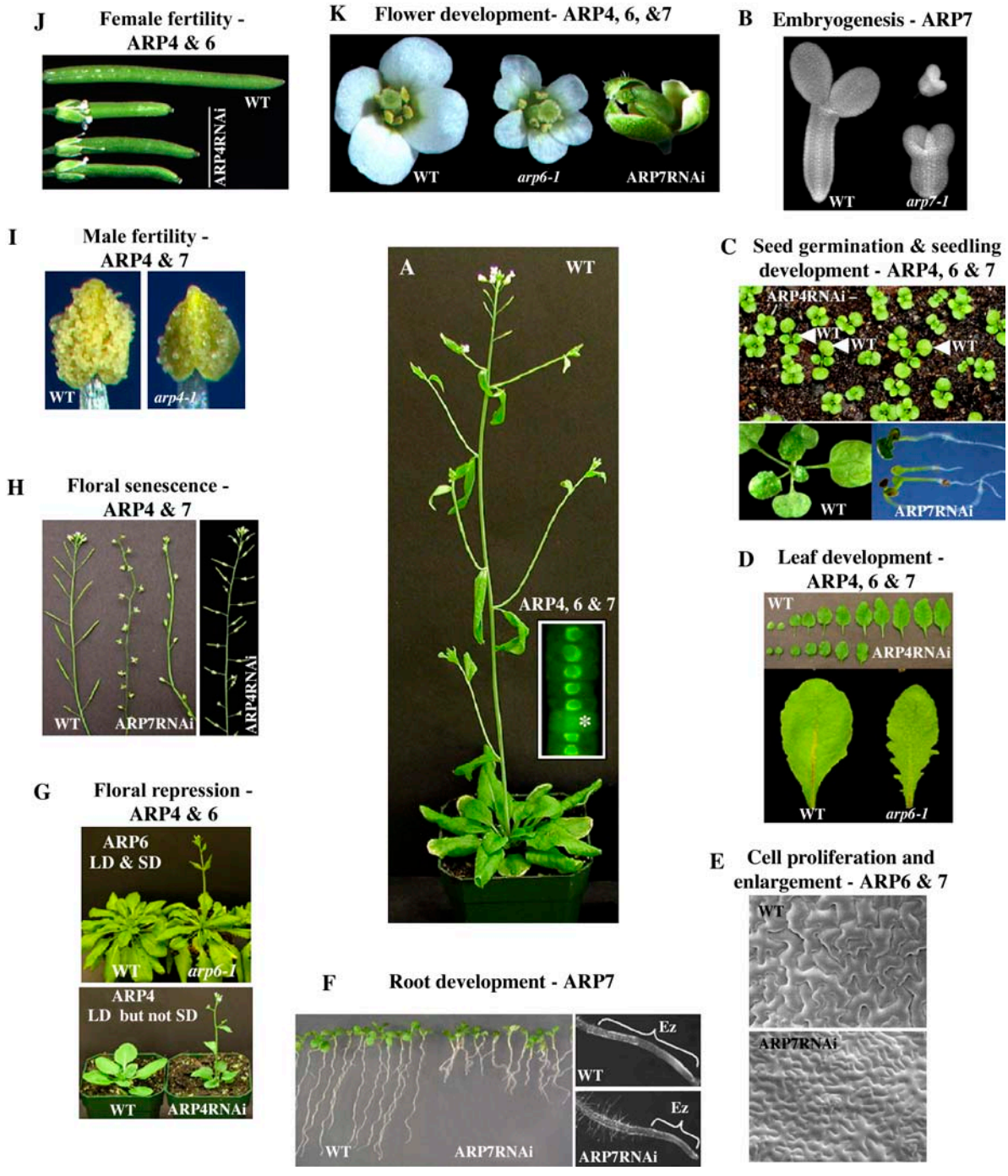

Figure 2. Deficiencies in Arabidopsis ARPs ARP4, ARP6, and ARP7 alter cell proliferation and plant development. A, Wild-type Arabidopsis plant. ARP4, 6, and 7 are localized to the interphase nucleus. In mitotic cells lacking intact nuclear membrane (*) they are dispersed throughout the cytoplasm. For example, the insert depicts the subcellular distribution of ARP4 in wild-type root apical cells. B, Knockout *arp7-1* mutation causes homozygous embryo lethality. Mutant embryos (right) are arrested at the heart (top) or torpedo (bottom) stage of development. A wild-type embryo at the cotyledon stage from the same silique (fruit) is shown at the left. C, Knocking out or knocking down ARP4, 6, and 7 causes poor seed germination or early seedling development, depending upon the level of reduction in the expression of these proteins. Dwarf ARP4RNAi seedlings (10-d-old) with stunted cotyledons are shown with three wild-type seedlings (arrow heads) in the top section. Two-week-old normal wild-type (WT) and arrested ARP7RNAi seedlings from a strong line showing more than 85% reduction in ARP7 expression are shown in the bottom section. D, Deficiencies in the expression of ARP4, 6, and 7 affect leaf size, number, and/or morphology. The *arp6-1* knockout mutants produce narrow and highly serrated leaves under short-day photoperiod (bottom section). All the leaves from an adult ARP4RNAi plant are compared with those from wild type (top section). E, Scanning electron microscopy of the smaller leaves of ARP7RNAi plants reveals similar number, but smaller sized leaf epidermal cells. For comparison, knocking out ARP6 in

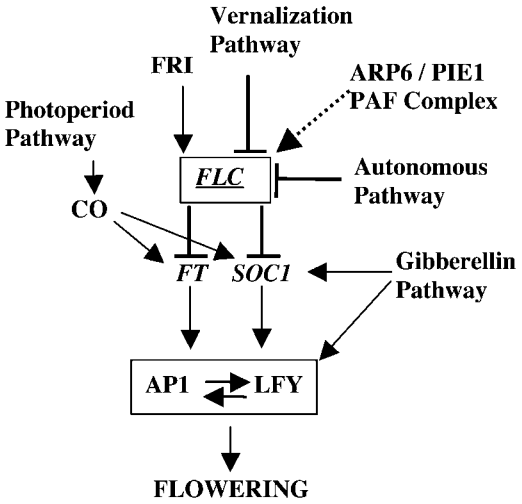

Figure 3. Regulatory networks controlling flowering time in Arabidopsis. Flowering time is controlled by the activities of the global regulator, FLC. A large number of environmental factors and several plant signaling molecules stimulate or repress the transcription of the *FLC* gene (underlined) via chromatin remodeling.

time, *FLOWERING LOCUS C* (*FLC*; Fig. 3) and other related transcription factors in this pathway, are controlled by modulations of chromatin structure (He et al., 2004; Oh et al., 2004). Nearly two-dozen genes in several pathways are known to exert competing signals to stimulate or repress chromatin-mediated *FLC* expression and therefore repress or activate flowering, respectively (He et al., 2003).

FLC and a few other related transcription factors appear to be the primary global regulators of flowering time that integrate signals from the various information pathways. Thus, the flowering-time signaling pathway in Arabidopsis is relevant to dissecting subsets of *ARP4* and *ARP6* functions. The photoperiod-independent early flowering of *ARP6* knockout mutants is associated with reduced expression of *FLC* as well as *MADS AFFECTING FLOWERING 4* and *MADS AFFECTING FLOWERING 5* (Deal et al., 2005). Consistent with the reduced expression of *FLC*, the downstream targets of FLC, *FT*, and *SOC* are increased in expression in *arp6* mutants. In addition, *arp6* mutations suppress the *FLC*-mediated late flowering of a dominant *FRIGIDA* allele, indicating that ARP6 is required for the activation of *FLC* expression to levels that inhibit flowering (Choi et al., 2005; Deal et al., 2005). Finally, strong overexpression of ARP6 resulted in no abnormal plant phenotypes, consistent with ARP6 activity being limited by other components of a chromatin-modifying complex. Thus, *FLC* expression appears to be directly controlled by chromatin remodeling that is dependent upon the normal expression of ARP6.

PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 and *ARP6* encode the closest Arabidopsis homologs of two subunits in the yeast SWR1 complex. The *photoperiod-independent early flowering1* and *arp6* mutants have many of the same diverse developmental phenotypes, suggesting they participate in the same chromatin-remodeling complex(es) (Noh and Amasino, 2003). In addition, Arabidopsis encodes putative homologs of nearly all of the subunits of the yeast SWR1 complex, consistent with the existence of such complexes in plants (Table I).

In summary, there are abundant examples of chromatin remodeling being required for normal plant development. Deficiencies in Arabidopsis ARP4, ARP6, and ARP7 each result in multiple distinct developmental abnormalities including defects in cell expansion and proliferation, dwarfing, and/or alterations in the shape of every vegetative and reproductive organ, and alterations in the timing of developmental pathways such as flowering and floral senescence. The defects observed are consistent with their roles in chromatin-remodeling complexes effecting epigenetic changes in the expression of global regulators like FLC. The possible roles of putative plant nuclear ARPs, ARP5, ARP8, and ARP9 in plant development are as yet unknown.

A COMBINATORIAL ARGUMENT FOR THE EXISTENCE OF MULTIPLE ISOFORMS OF ARP-CONTAINING CHROMATIN-MODIFYING COMPLEXES

There is substantial evidence that diverse isoforms of ARP-containing chromatin-modifying complexes exist with the potential to exert epigenetic control over development. To define what is meant by isoforms of chromatin complexes, consider the ARP-containing

Figure 2. (*Continued.*)
the *arp6-1* mutant results in dwarfed leaves with a smaller number of normally sized cells (not shown). F, A knockdown of ARP7 in RNAi plants severely affects root growth. The retarded roots of RNAi plants have highly reduced cell elongation zone compared to wild type (WT). G, Deficiencies in ARP4 and ARP6 expression affect flowering time. ARP4RNAi plants flower early only under long-day (LD) conditions (bottom section), whereas the *arp6-1* mutants flower early both under long- and short-day (SD) conditions (top section). Thus, ARP4 and ARP6 are involved in photoperiod-dependent and photoperiod-independent flowering pathways, respectively. H, A knockdown in ARP4 and ARP7 in the RNAi plants causes delayed senescence and abscission of floral organs. Wild-type inflorescences each contain five or six flowers with intact sepals and petals, whereas the RNAi plants have 15 to 20 flowers in each inflorescence with intact sepals and petals. I, A strong reduction in ARP4 and ARP7 expression affects stamen development and male fertility. The aberrant heart-shaped anthers in the *arp4-1* mutant contain less pollen than wild type. J, Deficiencies in ARP4 and ARP6 expression affect female fertility. Defects in pistil development and pollination results in stunted fruits with reduced seed set compared to wild type. K, Knocking out or knocking down ARP4, 6, and 7 expression affect flower morphology and/or organ number.

SWI/SNF, SWR1, and INO80 complexes. All three complexes contain 12 to 13 different protein subunits, summarized in Table I. Protein isovariants are classically defined as closely related polypeptides with altered sequences encoded by different gene family members. In plants and animals, divergent gene families encode multiple isovariants of several subunits in each complex. Substituting a single subunit with a different isovariant would generate a new complex isoform with the potential to recognize a new target gene or carry out a slightly different chromatin-modifying reaction (i.e. different phasing of nucleosomes or different histone modifications). Sequentially substituting three different isovariants of, for example, the Snf2 DNA-dependent ATPase subunit of a SWI/SNF complex would generate three isoforms of the complex. By extension, substituting three different isovariants of two different subunits has the potential to generate nine isoforms, and so on. Besides being derived from gene families, protein isovariants could be generated from single genes by alternate splicing or polyadenylation site selection, alternate initiation and termination of translation, and posttranslational protein modifications.

Direct and indirect support for the existence of multiple isoforms of chromatin-remodeling complexes can be found in the plant and animal literature and from further examination of their complex genomes. Several human and mouse chromatin-remodeling complexes were first isolated as mixtures of isoforms. For example, the purified mammalian BAF or SWI/SNF complex was shown to have a basic composition of about nine to 12 proteins, but it existed in several isoforms. Isoforms purified from various organs varied in their Baf60 subunit and Swi2/Snf2 subunit compositions (Wang et al., 1996; Debril et al., 2004). BAF60a is constitutively expressed in all organs, whereas BAF60b and BAF60c are expressed in neural and muscle tissues and the pancreas. These Baf60 protein isovariants appear to contribute to the target gene specificity of the complex. Phosphorylation of BAF60 subunits creates an additional isovariant that appears to target a subset of BAF complex isoforms to particular target genes required for muscle development (Simone et al., 2004).

Evidence for large numbers of isoforms of ARP-containing complexes in plants comes indirectly from the existence of small gene families encoding subunit isovariants, which we will discuss briefly in the context of the Arabidopsis genome. The three yeast complexes SWI/SNF, SWR1, and INO80 collectively contain all the yeast nuclear ARPs: ARP4, ARP5, ARP6, ARP7, ARP8, and ARP9 (www.yeastgenome.org). As a case study, the plant chromatin (www.chromdb.org) and The Arabidopsis Information Resource (www.arabidopsis.org) databases were searched for homologs of the subunits of these three yeast complexes. Table I summarizes this preliminary examination of Arabidopsis subunit isovariants. The yeast SWI/SNF, SWR1, and INO80 complexes each contain a single, high-M_r snf2-related ATPase subunit, Snf2, Swr1, and Ino80, respectively. The yeast genome encodes a total of 10 distinct Snf2-related proteins (www.yeastgenome.org), and all 10 ATPases are known to participate in a different chromatin-remodeling complex. By contrast, Arabidopsis encodes 42 genes that can be classified as homologs of these ATPases, with one or more being closely allied with each of the 10 yeast sequences in a protein sequence tree (www.chromdb.org). The Arabidopsis clade encoding the immediate homologs of yeast Snf2 contains four genes (Table I). Because the Snf2-related ATPases are not known to participate in any processes other than chromatin modification and have been found primarily in ARP-containing chromatin-remodeling complexes, it is likely that these four closest relatives of the Snf2-ATPase will participate in different isoforms of the Arabidopsis SWI/SNF complex. A similar analysis shows that in addition to the four Snf2 isovariants, Arabidopsis encodes two, two, four, and two isovariants of yeast Snf12, Swi1, Swi3, and Taf14 subunits, respectively (Table I). If these 14 subunit isovariants from the five Arabidopsis gene families are freely interchangeable in SWI/SNF complexes, 128 isoforms of the Arabidopsis complex (i.e. $4 \times 2 \times 2 \times 4 \times 2 = 128$) would be generated. Each would have the potential to target different genes or carry out variations in NR complex function.

A recent analysis of mutants in the four Arabidopsis Swi3 (Table I) isovariants provides direct support for the functional significance of four SWI/SNF complex isoforms each with a different Swi3 isovariant (Zhou et al., 2003; Sarnowski et al., 2005). Mutants in each isovariant subunit displayed a distinct set of phenotypes, including embryo lethality and vegetative and reproductive defects. The sum of the phenotypes described for these four mutants include most of the phenotypes we described for plants deficient in ARP7, which is likely to be a universal member of this complex (Kandasamy et al., 2005b). For example, the *swi3a* and *swi3b* mutants display an embryo-lethal phenotype similar to the homozygous *arp7-1* mutant (Fig. 2B). In addition, the *swi3d* mutations cause severe dwarfism and male and female infertility like the *ARP7* knockdown alleles (Figs. 2K and 3H). Plants carrying *atswi3c* mutations shared the retarded root and plant growth, curly leaf, and reduced fertility phenotypes of the ARP7-silenced lines.

Moreover, homologs of several subunit proteins within the yeast SWR1 and INO80 complexes are also encoded by small gene families in Arabidopsis, as shown in Table I. There are 21 subunit isovariants in six families with the potential to produce 1,200 SWR1 isoforms. Similarly, there are 14 isovariants in four families with the potential to produce 84 INO80 isoforms. Thus, Arabidopsis ARP4, ARP5, ARP6, and perhaps ARP7 and ARP8 are each predicted to participate in numerous distinct isoforms of complexes distinguished by their isovariant subunit compositions. Each individual ARP complex with a different isovariant composition might control only one or a few target genes participating in the global regulation of development.

It seems reasonable to ask how diverse isoforms of ARP-containing chromatin-remodeling complexes became functionally linked to the macroevolution of multicellular development. One evolutionary view would be that as plants and animals diversified from single-celled protist ancestors, there was a combinatorial expansion in the number of nuclear ARP complex isoforms following an expansion and diversification of the gene families encoding their various subunit isovariants. An increase in chromatin-modifying complex isoforms allowed the natural selection of more specialized control over chromatin dynamics and target gene transcription, which generated more specialized epigenetic control over multicellular development. It is logical to assume that greater target gene specificity and more finely tuned epigenetic control were selective advantages to multicellular organisms.

SUMMARY

Eukaryotic genomes encode several ancient classes of nuclear ARPs that participate in macromolecular machines affecting chromatin dynamics. Nuclear ARPs are variously required for assembly, chromatin binding, and/or the enzymatic activities of different complexes. ARP-containing chromatin-modifying complexes carry out NR, chemical modifications of histones, or HVE reactions. These activities exert epigenetic control over cell proliferation and multicellular development along with controlling basal levels of transcription. In Arabidopsis, developmental pathways affecting every plant organ require normal levels of ARP4, ARP6, and/or ARP7 expression, providing strong evidence for this role. Diverse isoforms of ARP complexes may provide the greater target gene specificity and wider variety of chromatin-modifying activities needed for multicellular development, relative to development of single-celled organisms like yeast.

Sequence data from this article can be found in the GenBank/EMBL data libraries under locus numbers given in Table I.

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