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Nuclear actin-related proteins at the core of epigenetic control

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and

Kandasamy MK, McKinney EC, Deal RB, Smith AP, Meagher RB. Arabidopsis actin-related protein ARP5 in multicellular development and DNA repair. Dev Biol 2009; 335:22–32; PMID: 19679120; DOI: 10.1016/j.ydbio.2009.08.006.

and

Smith AP, Jain A, Deal RB, Nagarajan VK, Poling MD, Raghothama KG, Meagher RB. Histone H2A.Z regulates the expression of several classes of phosphate starvation response genes, but not as a transcriptional activator. Plant Physiol 2010; 152:217–25; PMID: 19897606; DOI: 10.1104/pp.109.145532.

Nuclear Actin-Related Proteins (ARPs) and actin combine as heterodimers to bind a large helicase subunit and form a core complex essential to the assembly and function of most chromatin remodeling and modifying machines. They are the most common shared subunits of these large and diverse assemblies in eukaryotes. We recently argued that most nuclear ARPs evolved directly from actin prior to the divergence of the eukaryotic kingdoms and did not evolve from pre-existing ARPs.[2] Arabidopsis plants defective in nuclear ARP4, ARP5, ARP6 or ARP7 have extreme developmental phenotypes. Our recent publication demonstrates that ARP5-defective plants are not only dwarfed and have aberrant cell sizes, but are also hypersensitive to mutagenic agents that cause double strand DNA breaks.[5] In Smith et al.[6] we show that ARP6-defective plants, in addition to their extreme developmental phenotypes like small organs and early flowering, present an apparent "Phosphate Starvation Response" with strong morphological and molecular phenotypes. Herein, we interpret our latest data in the light of a hypothesis stating that *in addition to their roles in overcoming DNA compaction that affects basal gene expression and silencing, nuclear ARP-containing chromatin complexes exert primary epigenetic control over high-level regulatory factors.*

Epigenetic control[1] is most often elaborated via alterations to chromatin structure such as changes to nucleosome position, exchange of histone isovariants within nucleosomes, histone modification and DNA base modification.[2-4] In eukaryotic microorganisms like yeast and green algae, phenotypes including mating type, carbon source utilization, chromosome segregation and DNA replication and repair are all under strong epigenetic control. In mammals and higher plants, other multicellular traits including tissue and organ development and behavior are added to the list of phenotypes now believed to be under the epigenetic control. We have just reported that Arabidopsis alleles deficient in nuclear actin-related proteins ARP5 or ARP6 show severe defects in cell, tissue and organ development, in DNA repair, and in nutrient metabolism,[5,6] phenotypes that are distinct from those reported previously for nuclear ARP-deficient plants.[2,7-12]

The nuclear actin-related proteins (ARPs) are the most common and conserved subunits of the macromolecular chromatin remodeling machines that carry out nucleosome re-positioning and histone variant exchange (e.g., SWI/SNF, SWR1, RSC, INO80, p400) or nucleosomal histone modification (e.g., NuA4 HAT).[2,13] At least two different nuclear ARP subunits or an ARP along with a conventional actin subunit are found at the core of each of these complexes. ARPs and actin bind as heterodimers to the helicase SANT-associated (HSA) domain found within a large subunit distinct to each subclass of complexes (e.g., Snf2 in SWI/SNF, Swr1/PieI in SWR1, SthI in SRC, Ino80 in INO80, Vid21/Eaf1 in NuA4).[2,14,15] This trimer of ARP, ACTIN and HSA-domain containing protein is thought to initiate joining of nine or more other subunits individually or as part of other sub-complexes to build a functional chromatin machine. The nuclear ARPs have secondary activities such as binding to

particular histone isovariants and modified histone side chains attracting this machinery to chromatin.[2] Hence, the nuclear ARPs are at the core of most of the macromolecular machinery responsible for the chromatin modification necessary for epigenetic control. With the exception of the well-known duplication of ARP4 in mammals, producing Baf53a and Baf53b isovariants, most nuclear ARPs are singlet genes. Hence, ARPs are the smallest family of subunits that are found in the largest number of chromatin altering complexes.

Large eukaryotic genomes, 84 millimeters of DNA for Arabidopsis and 2,000 millimeters for humans, are compacted 10,000 to 100,000-fold, into a small nuclear space, typically a 5 to 10 micron diameter nucleus. Chromatin remodeling is essential for regulating access to this highly compacted DNA. Thus, remodeling involves unfolding sections of chromatin for transcription, recombination, replication and repair, and their subsequent re-compaction, when specific sequences are not in use. It is hard to reconcile the global dynamic activities of ARP-containing complexes with some of the specific multicellular, developmental and metabolic phenotypes we have reported for alleles deficient in ARP4, ARP5, ARP6 and ARP7.[5,6,8,10,11,16] Hence, we have considered the following hypothesis*: that in addition to their roles in regulating DNA compaction to allow basal gene expression and silencing, nuclear ARP-containing chromatin complexes exert primary epigenetic control over high-level regulatory factors*.[2,17] Furthermore, considering the global chromatin activities of the various ARP-containing complexes, it is surprising that severely silenced alleles *ARP4* and *ARP7* and null alleles of *ARP5* and *ARP6* are viable in Arabidopsis. Perhaps the incomplete penetrance typical of epigenetic phenotypes is partially responsible for the survival of plants with extreme phenotypes. On the other hand, no such nuclear ARP-defective alleles have been reported in the Drosophila or mouse models. The plasticity of plant development and more open embryonic development may account for the survival of ARP-defective plants relative to mammals.

The nuclear ARPs are significantly more divergent from conventional actin than cytoplasmic ARP2 or ARP3,[2] and higher plants lack the cytoplasmic ARP1 that participates in the flagella complex.[18] Based on ancient insertions and deletions in the various nuclear ARP sequences, we have recently argued that the conserved and ancient classes of nuclear ARPs (e.g., ARP4, ARP5, ARP6) are individually evolved from actin in an ancient common ancestral eukaryote and that ARP5 and ARP6 and most other nuclear ARPs are not evolved from ARP4, the sequence most closely related to actin and basal to the other nuclear ARPs.[2] The pathway for their evolution from actin is conceivable considering that conventional actin participates in many of the same nuclear complexes. By this model, duplicated actin genes were mutated and sub-functionalized into nuclear ARPs. Plants contain a full complement of nuclear ARPs relative to animals and fungi, while protists have variable nuclear ARP compositions.[2] The characterized Arabidopsis nuclear ARP proteins ARP4, ARP5, ARP6 and ARP9.[5,7,10] Are clear homologs of their counterparts in yeast and vertebrates.[2] Whereas Arabidopsis ARP7 and ARP8 may be the orthologs of vertebrate ARP4 and yeast ARP9, but their phylogenetic relationships are not definitive. Furthermore, we have shown plant ARP4, ARP5, ARP6 and ARP7 are concentrated in the nucleoplasm, and Arabidopsis ARP8 is the only ARP in any organism to be sub-localized to the nucleolus instead of the nucleoplasm.[8,19] Neither the molecular nor developmental functions of plant nuclear ARP9 have been characterized to date.[20]

ARP5 homologs from yeast and mammals are only known to participate in

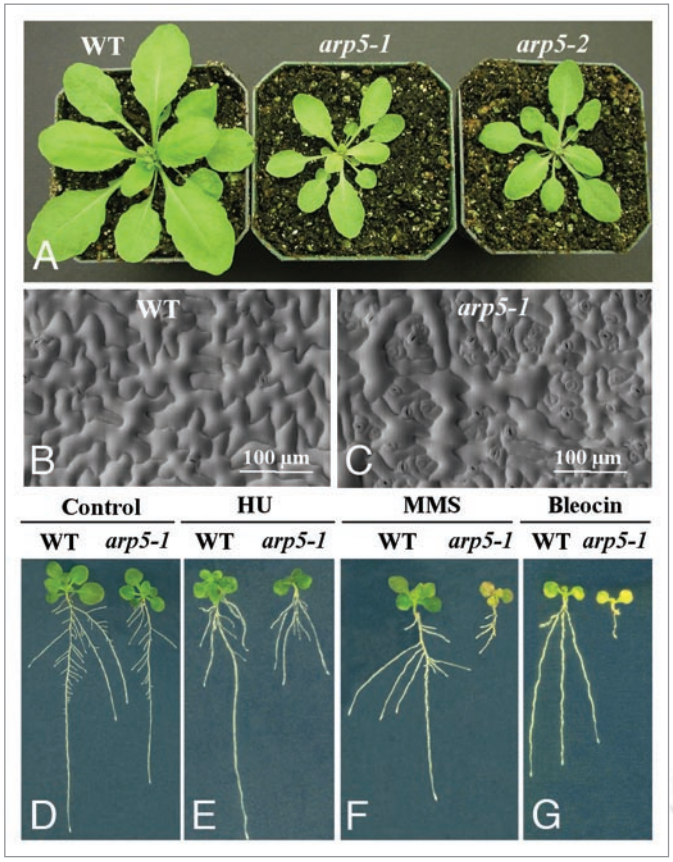

Figure 1. Phenotypes of ARP5-defective Arabidopsis plants. (A and D) ARP5 null alleles, *arp5-1* and *arp5-2*, produce dwarfed plants and seedlings slightly smaller than wild type (WT). (B and C) ARP5-defective leaves are composed of a variety of large and small sized cells with an excess of underdeveloped stomata relative to wild type. (E–G) ARP5-defective seedlings are hyper-sensitive to mutagenic agents causing double strand DNA breaks including hydroxyurea (HU), methyl methanesulfonate (MMS) or bleomycin (Bleocin).[5] (D) Control seedlings showing growth after no treatment with mutagenic agents.

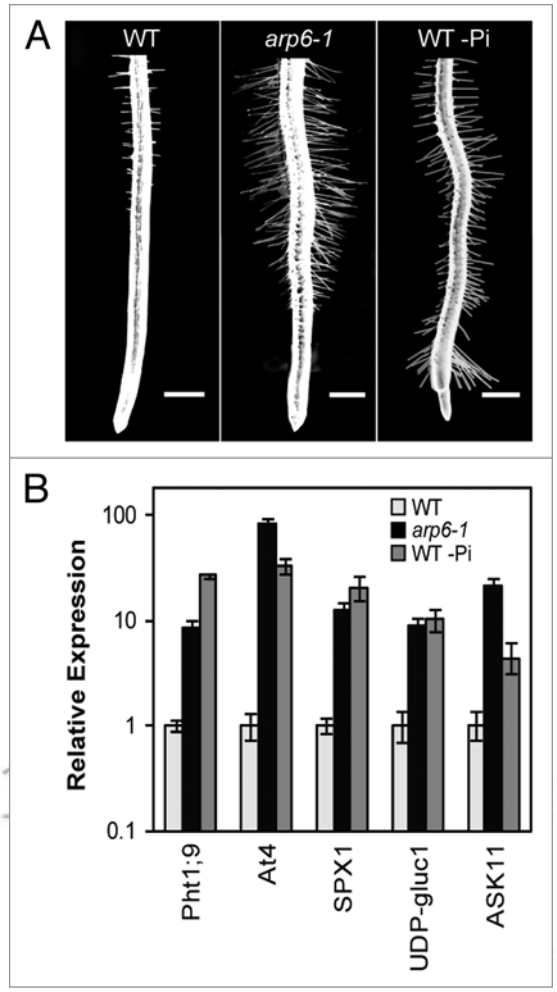

Figure 2. Phosphate Starvation Response phenotypes of ARP6-defective Arabidopsis seedlings. (A) Wild type seedlings display short, sparse root hairs, when grown on phosphate replete medium (WT left) and dense, long root hairs, when starved for phosphate (WT-Pi, right). The ARP6 null allele *arp6-1* (and *arp6-2*, not shown) exhibit this root hair phenotype on phosphate replete medium (middle). (B) Several phosphate starvation response genes including *Pht1;9, At4, SPX1, UDP-gluc1* and *ASK11* are induced in ARP6-defective seedlings grown on phosphate replete medium (compare WT to *arp6-1*), similar to their induction in Pi-starved wild type (WT-Pi).[6]

INO80 chromatin remodeling complexes. ARP4, ARP5, ARP8, actin, and the DNA dependent ATPase Ino80 combine to form the core of the 12-subunit INO80 complex.[14,21-23] The activities of INO80 have been well studied in yeast and animal cells. INO80 is shown to be associated with global gene regulation, DNA damage induced DNA replication and recovery of DNA replication forks stalled for repair, and it may also be essential for migration of normal S-phase replication forks.[24-29] In yeast, ARP5-deficiency compromises the INO80 complex for ATPase activity, DNA binding and nucleosome movement.[30] In mammalian cells, ARP5-depletion slows DNA repair.[31] Thus, we might expect the phenotypes of ARP5-deficiencies in a multicellular organism to be expansive.

In this first examination of ARP5 in an intact multicellular eukaryote, Kandasamy et al.[5] show that Arabidopsis ARP5 is constitutively expressed in the nucleoplasm of essentially all cells and organs. ARP5-null and knockdown alleles develop small organs with altered ratios of cell types.[5] Intriguingly, the dwarf leaves are composed of a heterogeneous mixture of cells ranging from normal size and shape to extremely small sizes (**Fig. 1A–C**). The leaf surfaces contain several times higher ratios of stomatal complexes relative to normal cells than wild type, although most of the stomata were incompletely developed (**Fig. 1B and C**). These defects in cellular development of leaves are consistent with ARP5 playing a role in normal S-phase replication, as in yeast.[28] Surprisingly, the defects reported in cell and organ development for *ARP5* mutants were far more severe than those reported for Arabidopsis *Ino80* null alleles.[29] Moreover, the ARP5-defective plants were extremely sensitive to DNA damaging agents with the potential to cause double strand DNA breaks including hydroxyurea, MMS and bleomycin. Growth of the ARP5-defective plant mutants appears more sensitive to chemical DNA damage than growth of either yeast or Arabidopsis *Ino80* alleles.[25,29] These and other data suggested that plant ARP5 might also participate in chromatin remodeling machines other than the classical INO80 complexes.[5] It seems reasonable to propose that Arabidopsis ARP5 might participate in novel chromatin complexes, combining for example, with one or more of the 43 DNA dependent ATPases other than Ino80, resulting in independent chromatin activities.[17] Hence, the pleiotropic phenotypes observed for ARP5-defective plants may result, not only from the loss of normal INO80 activities on high-level regulatory machinery and basal gene expression, but also by its participation in other unknown chromatin altering complexes.

ARP6 homologs are only known to participate in the chromatin remodeling and assembly of the histone variant exchange complex SWR1. SWR1 moves nucleosomes and exchanges the histone variant H2AZ for the more common H2A subunit within nucleosomes, an activity thought to poise genes in a transcriptionally active state.[11,32,33] In contrast, in fission yeast, ARP6 plays a role in telomere silencing, and in animal cells, ARP6 also appears to participate in silencing heterochromatin.[34-36] ARP4, ARP6, actin and the DNA dependent ATPase Swr1 assemble the core of SWR1 and are joined by ten or more other subunits to form the final complex. ARP6 and ARP4 together are necessary for assembly of several of these other subunits into SWR1 and binding of the completed complex to nucleosomes.[14,37,38]

We recently reported that Arabidopsis ARP6-null alleles exhibit an apparent

phosphate starvation response (PSR) phenotype, when grown in phosphate (Pi) replete medium. The PSR in ARP6-deficient plants includes the development of three times more root hairs of twice the length found in wild type plants grown in parallel as shown in **Figure 2A**. Shoots accumulate excessive starch and express higher than normal levels of phosphatases consistent with their scavenging for Pi. Of the ten PSR genes examined that are induced in wild type during Pi starvation, all are expressed at 2- to 80-fold higher levels in ARP6-defective plants, when grown in Pi replete media as shown for a few examples in **Figure 2B**. In addition, we observe a significant drop in H2AZ abundance at all ten PSR genes assayed. Further, transcripts encoding a known activator of PSR, SPX1 (**Fig. 2B**), are upregulated in ARP6 mutants, consistent with the PSR phenotype we observed. However, the expression of some of the best-characterized Pi signaling factors *PHR1, miR399* and *PHO2* are not altered in ARP6 mutants. Clearly, ARP6 deficiency produces a palpable global PSR, and ARP6-dependent activities normally repress many PSR genes. These data suggest that in the regulation of the PSR, both halves of our hypothesis may be true with nuclear ARP6 "*regulating DNA compaction to allow basal gene expression and silencing*" and the "*nuclear ARP-containing chromatin complexes exerting primary epigenetic control over high-level regulatory factors*."

In contrast to these results, ARP6-defective alleles flower early in long and short day growth conditions, because of the significant downregulation of three related MADS box repressors of flowering, FLC, MAF4 and MAF5.[10,11] FLC is considered the master repressor of flowering being central to the highly networked regulation of flowering time in Arabidopsis. ChIP assays showed that all three MADS box genes had a distinct and uncommon bimodal distribution of H2AZ deposition in wild type. There are peaks of H2AZ containing nucleosomes at the proximal and distal ends of each gene, which are lost in the ARP6-defective alleles. These data support the latter part of our hypothesis, suggesting that ARP6 function is essential to potentiate the expression of high-level regulators of flowering.

In conclusion, the nuclear ARPs are central to epigenetic control due to their role in assembling the core of most chromatin remodeling and modifying machines. In recent publications, we have discussed the novel evolutionary origin of the nuclear ARPs and the dramatic metabolic, DNA repair, cellular, tissue and organ level phenotypes of plants defective in normal ARP expression. Nuclear ARP activities control both gene activation and silencing that affect basal gene expression and expression of high-level regulatory factors. Our data support the growing body of evidence that epigenetic control may be as essential as genetic control to the morphology and development and to the evolution of multicellular eukaryotes. The extreme sensitivity of ARP5-defective plants to DNA damaging agents and PSR response in ARP6-defective plants suggest an interesting interplay between epigenetic control and the environment that is of significant interest to both agriculture and medicine.

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