Protein-protein interactions of ULTRAPETALA1, a trithorax group factor in *Arabidopsis thaliana*

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ABSTRACT

ULTRAPETALA1 (ULT1) controls the size of shoot and floral meristems in *Arabidopsis thaliana* by controlling gene expression through an epigenetic mechanism, without changing the DNA sequence. ULT1 has been characterized as a Trithorax group (trxG) factor, and is therefore thought to form complexes with other proteins to carry out its function. Not all of the proteins that make up this complex have been identified. ULT1 has been observed in both the cytosol and the nucleus, but it is unknown where in the cell it functions. In this study I tested putative interaction partners to determine which proteins bind ULT1 to form complexes. I employed an intracellular imaging method of Bimolecular Fluorescence Complementation, which allowed me to examine protein interactions *in vivo* and observe where this interaction was localized in living cells. I found both the ATX2 and HAC1 proteins contained portions that bound to the ULT1 protein in localized spots throughout the cytosol and in the nuclei, suggesting that the proteins may bind in the cytosol and subsequently localize to the nucleus to carry out their regulation. Characterizing the complexes ULT1 forms in *Arabidopsis* may help us understand how epigenetic silencing and activation functions in other plants and animals, including humans. The epigenetic regulation of gene expression is more flexible than mutations in the DNA sequence, and therefore may be the mechanism through which plants respond to changes in their environment.

KEYWORDS

epigenetics, ATX2, HAC1, BiFC, shoot apical meristem

INTRODUCTION

Epigenetics is the study of mechanisms that create and maintain different patterns in gene expression without changing the nucleotide sequence (Roudier, Teixeira, & Colot, 2009). Epigenetic regulation of genes in response to different external and endogenous cues is critical for maintaining cell fate and the timely deployment of developmental programs (Schuettengruber, Chourrout, Vervoort, Leblanc, & Cavalli, 2007). For example, the gene *Flowering Locus C* (*FLC*), which promotes flowering, is epigenetically repressed in *Arabidopsis* until an extended period of cold provides the cue to end repression (Gendrel & Colot, 2005). Epigenetic changes in gene expression are heritable through rounds of cell division; and although they do not change the nucleotide sequence, they therefore act as a cellular memory mechanism (van Nocker, 2003). A large portion of epigenetic information is carried through the modification of chromatin, DNA wrapped around proteins, in response to environmental or developmental signals (Guitton & Berger, 2005). Specialized proteins may remodel the chromatin and change its composition, so that different parts of the genome are made accessible or inaccessible for transcription (Shen & Xu, 2009). Two sets of such proteins, the Polycomb group (PcG) and Trithorax group (trxG), are thought to work antagonistically to determine whether certain genes are repressed (PcG) or activated (trxG) (Breiling, Sessa, & Orlando, 2007). However, there is still much to learn about the precise proteins involved and the mechanism through which epigenetic states are changed or maintained.

The *ULTRAPETALA1 (ULT1)* gene in *Arabidopsis thaliana*, one of the first trxG genes characterized in plants (Carles & Fletcher, 2009), is required to control the size of shoot and floral meristems (Fletcher, 2001); maintaining a stable pool of stem cells is crucial for a plant's continued growth, even as it produces new organs (Fletcher, 2002). In *ult1* loss-of-function mutant plants, shoot apical meristems (SAM) are enlarged, and floral meristems are also enlarged and produce a larger number of floral organs (Fletcher, 2001). The mechanism through which *ULT1* restricts floral meristem growth is likely the transcriptional activation of the gene *AGAMOUS*, which in turn represses the downstream genes in the meristem-regulating pathway (Carles, Choffnes-Inada, Reville, Lertpiriyapong, & Fletcher, 2005). The ULT1 protein is hypothesized to activate the

expression of the *AG* gene through a chromatin-mediated trxG-like mechanism, by opposing the action of the PcG gene *CURLY LEAF* (*CLF*), a direct repressor of *AG* transcription (Carles & Fletcher, 2009). Ultimately, epigenetic changes could play a crucial role in stem cells, as they are stable yet reversible, and might account for a plant's plasticity under changing environmental conditions (Shen &Xu, 2009). The trxG and PcG groups likely play an important role.

PcG and trxG proteins are thought to not work independently, but rather to form complexes to carry out their chromatin remodeling functions. Large multi-protein complexes of PcG genes, Polycomb Repressive Complexes (PRC) and their homologues, have been well characterized in *Drosophila* and *Arabidopsis* (Guitton & Berger, 2005). At least three trxG multi-protein complexes have also been identified in *Drosophila* from embryonic extracts (Breiling et al., 2007), and the high level of conservation between PcG and trxG group structure and function in animals and plants suggests that they would also form complexes in *Arabidopsis* by analogy. The trxG protein ARABIDOPSIS TRITHORAX1 (ATX1) has been found to bind ULT1 protein in the nucleus (Carles & Fletcher, 2009), but it is likely just one part of a larger complex. There are still many proteins that display similar mutant phenotypes to *ult1* or structures similar to ATX1 that need to be tested for interactions with ULT1. ULT1 has been observed in both the cytosol and the nucleus (Carles et al., 2005), but it is unknown whether it serves a function in only one or both parts of the cell.

In this study I will test putative interaction partners to find other proteins that bind ULT1 to achieve a chromatin remodeling function. I hypothesize that, as a trxG-like protein, ULT1 will bind to other trxG proteins that it recruits to form a complex. My second objective is to identify where in the cell these interactions occur. I hypothesize that this interaction will take place in the cell nucleus, as this is where the target genes for ULT1 are located. Characterizing these protein-protein interactions is an important step in elucidating the mechanisms through which cellular memory is created in plants through epigenetic regulation.

METHODS

Study system

Protein of interest

To gain a better understanding of how trxG proteins function in plants, I studied the ULT1 protein from *Arabidopsis thaliana*. *Arabidopsis* is used extensively in plant genetics research, because it has a relatively simple genome and reproduces rapidly. It therefore has the most well characterized and understood genome of plant species.

Selection of interaction proteins

To determine which proteins interact with the trxG protein ULT1 to form a complex, I selected putative partners based on their similarities to other genes and on their mutant phenotypes. ARABIDOPSIS TRITHORAX-LIKE PROTEIN2 (ATX2) is similar in structure to the ATX1 protein, and displays partial redundancy in function with ATX1 (Saleh et al., 2008). However, ATX2 is expressed during different times in development and it is thought that ATX1 and ATX2 use different mechanisms when activating shared genes (Saleh et al., 2008). The protein Histone Acetyltransferase1 (*At*HAC1) in *Arabidopsis* is an ortholog of a well-characterized trxG protein in animals (Deng et al., 2007). Loss-of-function *hac1* mutants display irregularities in flowering time (Deng et al., 2007). Both of these proteins are very large. Consequently, I used genes that had been split into parts to find the specific binding sites on the proteins.

Study design

To test for protein-protein interactions *in vivo,* I employed an intracellular imaging method of Bimolecular Fluorescence Complementation (BiFC) as described by Kerppola (2006). BiFC is a method of visualizing protein interactions by transforming fusion proteins bound to Yellow Fluorescent Protein (YFP) fragments into living cells. I used BiFC instead of the yeast two-hybrid system, which is commonly used to test for protein-protein interactions. The high number of cysteine residues that compose the

ULT1 protein would have likely resulted in a high number of false positives using the yeast two-hybrid system (Fletcher, pers. comm.).

To employ this system of BiFC, I used constructs of the ULT1 protein fused with either the –SPYNE (YN) or –SPYCE (YC) fragment of the Yellow Fluorescent Protein (YFP) and transformed them into onion epidermal cells along with constructs of one of the possible interaction partners fused to the complementary YFP fragment (Fig. 1). When the two partners bind, the YFP fluoresces under UV black light.

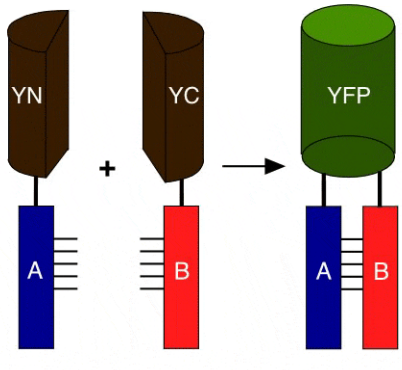

Figure 1. Diagram of the basic principle behind the BiFC assay. The two prospective interaction partners (A and B) are each fused to one half (-YN or -YC) of the yellow fluorescent protein (YFP). If A and B bind, a yellow fluorescent signal is produced. (Image reproduced from Hu, Grinberg, & Kerpolla, 2005)

DNA growth and extraction

I isolated DNA constructs of my genes of interest fused with BiFC vectors, pEZS-CL-ULT1-NY, pEZS-CL-ULT1-YC, pE-SPYCE-ULT1, pE-SPYNE-ULT1 (Carles & Fletcher, 2009), pE-SPYCE-ATX2/A, pE-SPYNE-ATX2/A, pE-SPYCE-ATX2/B, pE-SPYNE-ATX2/B, pE-SPYCE-HAC1/A, pE-SPYNE-HAC1/A, pE-SPYCE-HAC1/B, pE-SPYNE-HAC1/B, pE-SPYCE-HAC1/C, and pE-SPYNE-HAC1/C (J.H. Jun, unpublished). *E. coli* were previously transformed (J.H. Jun, unpublished) through a method of electroporation. I grew *E. coli* colonies from frozen cell stocks of transformed *E. coli* in Carb 100 solution at 37°C for 12-16 hours. I then performed the DNA extraction using a Plasmid Mini Extraction Kit (Bioneer, South Korea) and measured the concentration of DNA extracted.

Transformation

I transformed onion cells by binding the constructs to gold particles and then using a Biolistic PDS-1000/He unit (BioRad, Richmond, CA) to perform particle bombardment, as described by Sanford, Smith, and Russell (1993). I used onion epidermal cells, because they are plant cells that are large and grow in a single layer, making them easier to view and image under a light microscope (Kerppola, 2006).

Microscopy

Following bombardment, I incubated the onion for 24 to 36 hours and then examined the epidermal peels using a Zeiss Axiovert Microscope (Carl Zeiss, Inc., Germany) at 10x and 20x magnification under darkfield conditions. In cases where the ULT1 and prospective interaction partner bound, I observed an emitted fluorescent signal when observed under UV light (Fig. 1). This signal was acquired and digitally photographed under dark field conditions. I prepared at least 3 samples for each experimental combination of proteins to ensure that a lack of signal was not simply the result of an inefficient bombardment. To confirm my results I tested the interactions for both pairs of directions (bound to both the –YC and the –NY fragment of the YFP protein) (Table 1). In the case of ATX2/B, I tested the interaction using two different vectors for ULT1 to confirm that the unique signal I observed was not simply a result of the vector used.

Comparison to negative controls

To determine if there was a presence or absence of interaction, I compared the signals in my experimental samples to negative controls. The negative controls tested one of the interaction partners fused to a YFP fragment against the complementary YFP fragment alone (Table 1). Although some background fluorescence was expected in some negative controls because of the nature of the BiFC procedure, real interactions are expected to emit a clearly stronger and more localized signal than their negative controls (Kerppola, 2006). In addition, I used NY and YC vectors bearing bZIP proteins, which

are known to interact, as positive controls to ensure the efficiency of each set of transformations.

Table 1. **Different combinations of proteins tested for interactions.** Under Protein A putative binding partners are listed. Each putative binding partner was tested when bound to both the –N and –C terminal of the YFP protein. Each putative binding partner was tested for interaction with the complementary YFP fragment alone as a negative control (NC), and against ULT1 in two different vectors.

Protein A	**Expression Vector**	**Protein B**	**Expression Vector**
ATX2/A			
	pE-SPYCE-ATX2/A	NC	pE-SPYNE
		ULT1	pE-SPYNE-ULT1
	pE-SPYNE-ATX2/A	NC	pE-SPYCE
		ULT1	pE-SPYCE-ULT1
ATX2/B			
	pE-SPYCE-ATX2/B	NC	pE-SPYNE
		ULT1	pE-SPYNE-ULT1
			pE-ZS-CL-ULT1-NY
	pE-SPYNE-ATX2/B	NC	pE-SPYCE
		ULT1	pE-SPYCE-ULT1
			pE-ZS-CL-ULT1-YC
HAC1/A			
	pE-SPYCE-HAC1/A	NC	pE-SPYNE
		ULT1	pE-SPYNE-ULT1
	pE-SPYNE-HAC1/A	NC	pE-SPYCE
		ULT1	pE-SPYCE-ULT1
HAC1/B			
	pE-SPYCE-HAC1/B	NC	pE-SPYNE
		ULT1	pE-SPYNE-ULT1
	pE-SPYNE-HAC1/B	NC	pE-SPYCE
		ULT1	pE-SPYCE-ULT1
HAC1/C			
	pE-SPYCE-HAC1/C	NC	pE-SPYNE
		ULT1	pE-SPYNE-ULT1
	pE-SPYNE-HAC1/C	NC	pE-SPYCE
		ULT1	pE-SPYCE-ULT1

Localization within the cell

I employed a method that allowed me to examine protein interactions *in vivo*, so I could determine not only whether or not an interaction existed, but also where this interaction was localized in living cells. I performed intracellular imaging as a qualitative analysis of what the pattern of interaction of the proteins looked like (Solid= So, Spotted= Sp) and where it was occurring in the cell, in the cytosol (C), the nucleus (N) or both (C+N). I was able to distinguish a true interaction from the background glow of the negative controls based on the strength of the signal.

RESULTS

ATX2/A

The assay between the first segment of the ATX2 protein, ATX2/A, and ULT1 showed no signs of a distinct signal relative to the controls (Table 2). When I combined pE-SPYCE-ATX2/A with pE-SPYNE (the N-terminal half of the YFP alone) as a negative control, I observed a very weak signal emitted from the cytosol and nucleus (Fig. 2a). I observed the same signal in one of the two negative control samples where ATX2/A was fused with the other half of the YFP protein, pE-SPYNE-ATX2/A: pE-SPYCE. In the second sample a weak signal came only from the nucleus (Fig. 2b). In the assays between ATX2/A and ULT1, I observed no noticeable differences. In both the combinations, pE-SPYNE -ATX2/A: pE-SPYCE-ULT1 and pE-SPYCE -ATX2/A: pE-SPYNE -ULT1, I observed a weak signal in both the cytosol and the nucleus. All but one sample expressed this signal.

Table 2. ATX2/A Vector combinations and expression signals. Control (shaded) and experimental assays for interaction with the first half of the ATX2 protein. The location of the signal emitted (C=cytosol, N=nucleus) and the pattern of the signal (So=solid, Sp=spotted) are listed for each of the vector combinations. The number of samples describes in how many replicates at least one cell displayed a certain signal. More than one type of signal was sometimes displayed in different cells of the same sample.

Vector Combination		Location	Pattern	# of Samples
pE-SPYCE-ATX2/A:	pE-SPYNE	C+N	So	2/2
pE-SPYCE-ATX2/A:	pE-SPYNE-ULT1	C+N	So	2/3
pE-SPYNE-ATX2/A:	pE-SPYCE	C+N	So	1/2
		N	So	1/2
pE-SPYNE-ATX2/A:	pE-SPYCE-ULT1	C+N	So	3/3

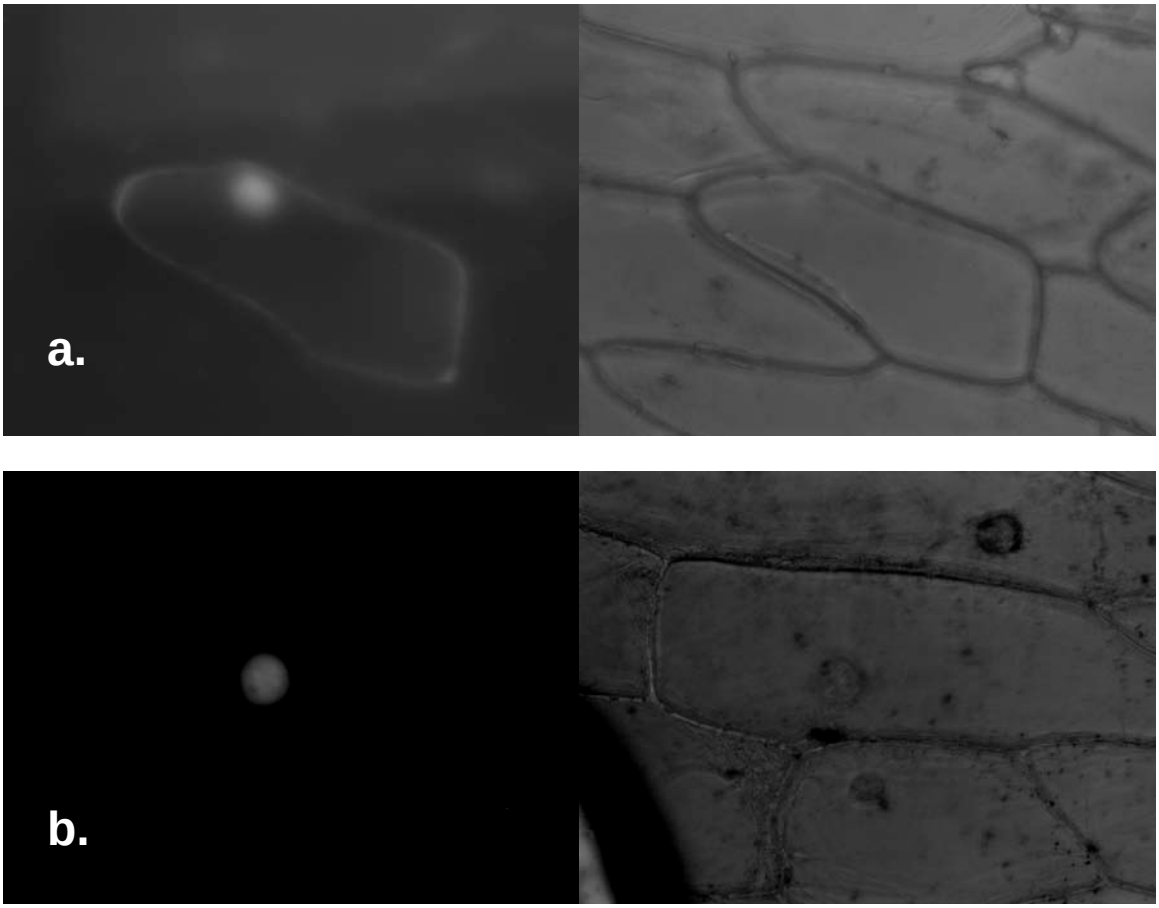

Figure 2. ATX2/A interactions. Fluorescence under darkfield conditions to the left and the same cell under halogen light to the right. Both the negative control and experimental assays showed (a) a solid signal from both the cytosol and nucleus. In some cases (b) the signal in the negative control came only from the nucleus.

ATX2/B

The second half of the ATX2 protein, ATX2/B, exhibited a variety of different signals when bound to ULT1 (Table 3). In the SPYNE negative control samples I observed a signal from both the cytosol and nucleus in 3 out of 7 replicates, and no signal in the other 4 samples. When I performed the assay between pE-SPYCE -ATX2/B and pE-SPYNE-ULT1, I observed a solid signal from the cytosol and nucleus in one sample or weakly only in the cytosol in another. In two samples the signal came from localized spots in the cytosol. In one sample, cells contained many small speckles that emitted signals. In another sample, I observed a high number of cells that emitted a signal from a single larger spot that was separate from the nucleus. In the confirmation assay between pE-SPYCE-ATX2/B and pEZS-CL-ULT1-NY, I again saw a solid signal from both the cytosol and nucleus or only the cytosol. I also observed a strong speckled signal in the cytosol of a majority of replicates (Fig. 3). In one replicate I saw a larger spot in addition to many small speckles. Overall the signals I observed using the two different vectors were qualitatively similar.

In the negative control assay between pE-SPYNE-ATX2/B and SPYCE, I did not observe a signal emitted in any of the replicates. In the assay between pE-SPYNE-ATX2/B and pE-SPYCE-ULT1, I observed a very strong and distinct spotted signal in both cytosol and nucleus. I also observed a very weak signal coming from both the cytosol and nucleus from a small number of cells in only one of the samples. In a confirmation assay using the pEZS-CL-ULT1-YC vector for ULT1, in all but one sample (which lacked any signals), I observed either spots in both the cytosol and nucleus, or a solid background in the cytosol with brighter localized spots in the cytosol. In many cases this speckled signal was very strong and distinct.

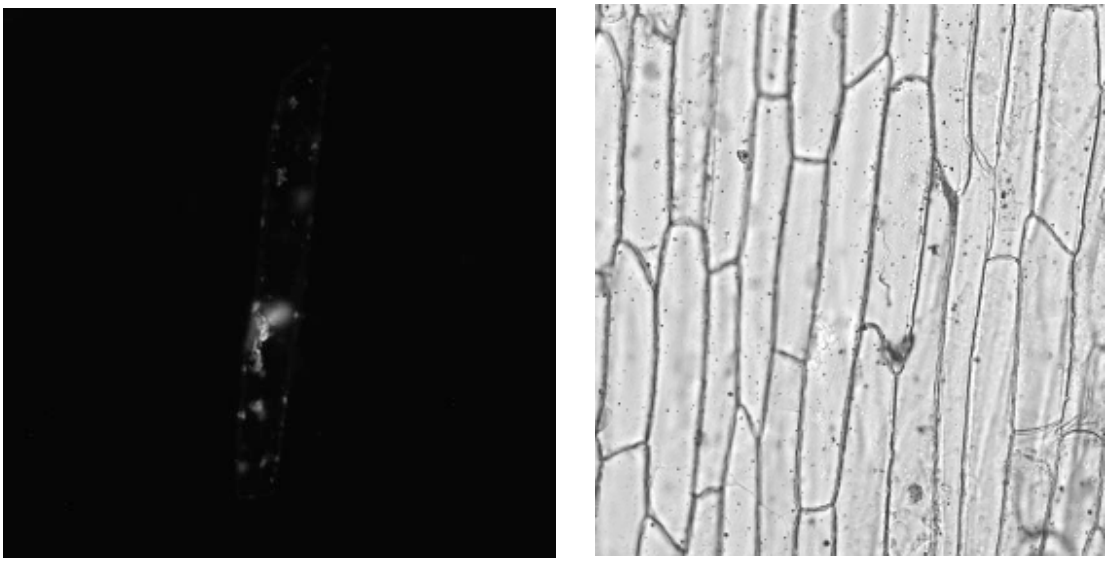

Figure 3. ATX2/B interactions. ATX2/B bound to ULT1 in localized speckles throughout the cytosol, and in some samples the nucleus.

Table 3. ATX2/B Vector combinations and expression signals. Control (shaded) and experimental assays for interaction with the second half of the ATX2 protein. The location of the signal emitted (C=cytosol, N=nucleus) and the pattern of the signal (So=solid, Sp=Speckled) are listed for each of the vector combinations. Two different vectors for ULT1 were used to confirm results. The number of samples describes in how many replicates at least one cell displayed a certain signal. More than one type of signal was sometimes displayed in different cells of the same sample.

Vector Combination		**Location**	**Pattern**	**# of Samples**
pE-SPYCE-ATX2/B:	pE-SPYNE	C+N	So	3/7
		none		4/7
pE-SPYCE-ATX2/B:	pE-SPYNE-ULT1	C	Sp	2/5
		C+N	So	1/5
		C	So	1/5
	pEZS-CL-ULT1-NY	C+N	So	3/6
		C	So	2/6
		C	Sp	5/6
		none		1/6
pE-SPYNE-ATX2/B:	pE-SPYCE	none		6/6
pE-SPYNE-ATX2/B:	pE-SPYCE-ULT1	C+N	Sp	3/3
		C	So	1/3
	pEZS-CL-ULT1-YC	C+N	So	3/6
		C+N	Sp	3/6
		C	Sp	3/6
		none		1/6

HAC1/A

The first segment of the HAC1 protein, HAC1/A, also showed a speckled pattern in the experimental assays (Table 4). The negative controls of both orientations emitted a signal from both the cytosol and the nucleus 5 out of 8 samples (Fig. 4a). In some cases the signal was very clear and strong. In other samples the signal was only a faint background glow. I didn't observe strong localized spots in any of the controls. Some of the experimental assays between HAC1/A and ULT1 showed similar patterns as the controls, where signals were apparent in both the cytosol and nucleus, but ten out of twelve samples had cells with brighter localized spots (Fig. 4b).

Table 4. HAC1/A Vector combinations and expression signals. Control (shaded) and experimental assays for interaction with the first segment of the HAC1 protein. The location of the signal emitted (C=cytosol, N=nucleus) and the pattern of the signal (So=solid, Sp=Speckled) are listed for each of the vector combinations. The number of samples describes in how many replicates at least one cell displayed a certain signal. More than one type of signal was sometimes displayed in different cells of the same sample.

Vector Combination		Location	Pattern	# of Samples
pE-SPYCE-HAC1/A:	pE-SPYNE	C+N	So	3/4
pE-SPYCE-HAC1/A:	pE-SPYNE-ULT1	C+N	Sp	5/6
		C+N	So	3/6
		N	So	1/6
pE-SPYNE-HAC1/A:	pE-SPYCE	C+N	So	2/4
pE-SPYNE-HAC1/A:	pE-SPYCE-ULT1	C+N	Sp	5/6
		C+N	So	5/6

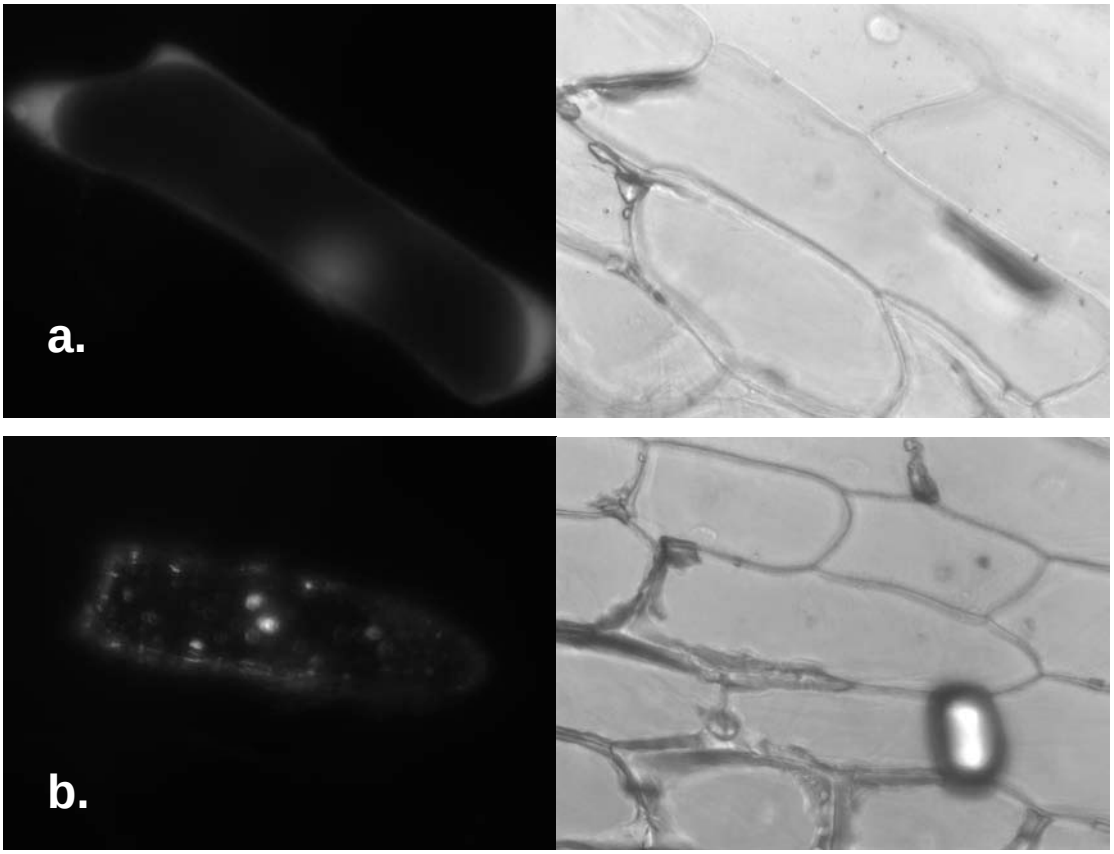

Figure 4. HAC1/A interactions. Fluorescence under darkfield conditions to the left and the same cell under halogen light to the right. The negative control (a) a solid signal from both the cytosol and nucleus. The experimental assays also exhibited a clear speckled pattern (b) from the cytosol and nucleus.

HAC1/B

The assays between HAC1/B, the second segment of the HAC1 protein, and ULT1 proteins showed no distinct signal from their negative controls (Table 5). In both the control and experimental samples I observed weak signals from either the cytosol and nucleus or only the nucleus.

Table 5. HAC1/B Vector combinations and expression signals. Control (shaded) and experimental assays for interaction with the second segment of the HAC1 protein. The location of the signal emitted (C=cytosol, N=nucleus) and the pattern of the signal (So=solid, Sp=Speckled) are listed for each of the vector combinations. The number of samples describes in how many replicates at least one cell displayed a certain signal. More than one type of signal was sometimes displayed in different cells of the same sample.

Vector Combination		Location	Pattern	# of Samples
pE-SPYCE-HAC1/B:	pE-SPYNE	C+N	So	1/2
		N	So	1/2
pE-SPYCE-HAC1/B:	pE-SPYNE-ULT1	C+N	So	2/3
pE-SPYNE-HAC1/B:	pE-SPYCE	N	So	2/2
pE-SPYNE-HAC1/B:	pE-SPYCE-ULT1	C+N	So	3/3

HAC1/C

The third segment of the HAC1 protein showed varying results in the experimental assays depending on the orientation of the protein (Table 6). When I tested pE-SPYCE-HAC1/C: pE-SPYNE as a negative control, I observed a very strong and clear signal from the cytosol and nucleus in every sample (Fig. 5a). When I combined pE-SPYCE-HAC1/C with pE-SPYNE-ULT1 I observed a signal from the cytosol and nucleus that was weaker than that of the controls.

In the pE-SPYNE-HAC1/C: pE-SPYCE control I saw a weak but clear signal from the cytosol and nucleus. In the assay between pE-SPYNE-HAC1/C and pE-SPYCE-ULT1 all replicates included some cells with signals from the nucleus and cytosol that looked similar to the negative controls or moderately stronger. All replicates also included some cells with brighter localized spots in the cytosol and nucleus (Fig.5b,c). The spotted signal for this interaction was very strong and clear.

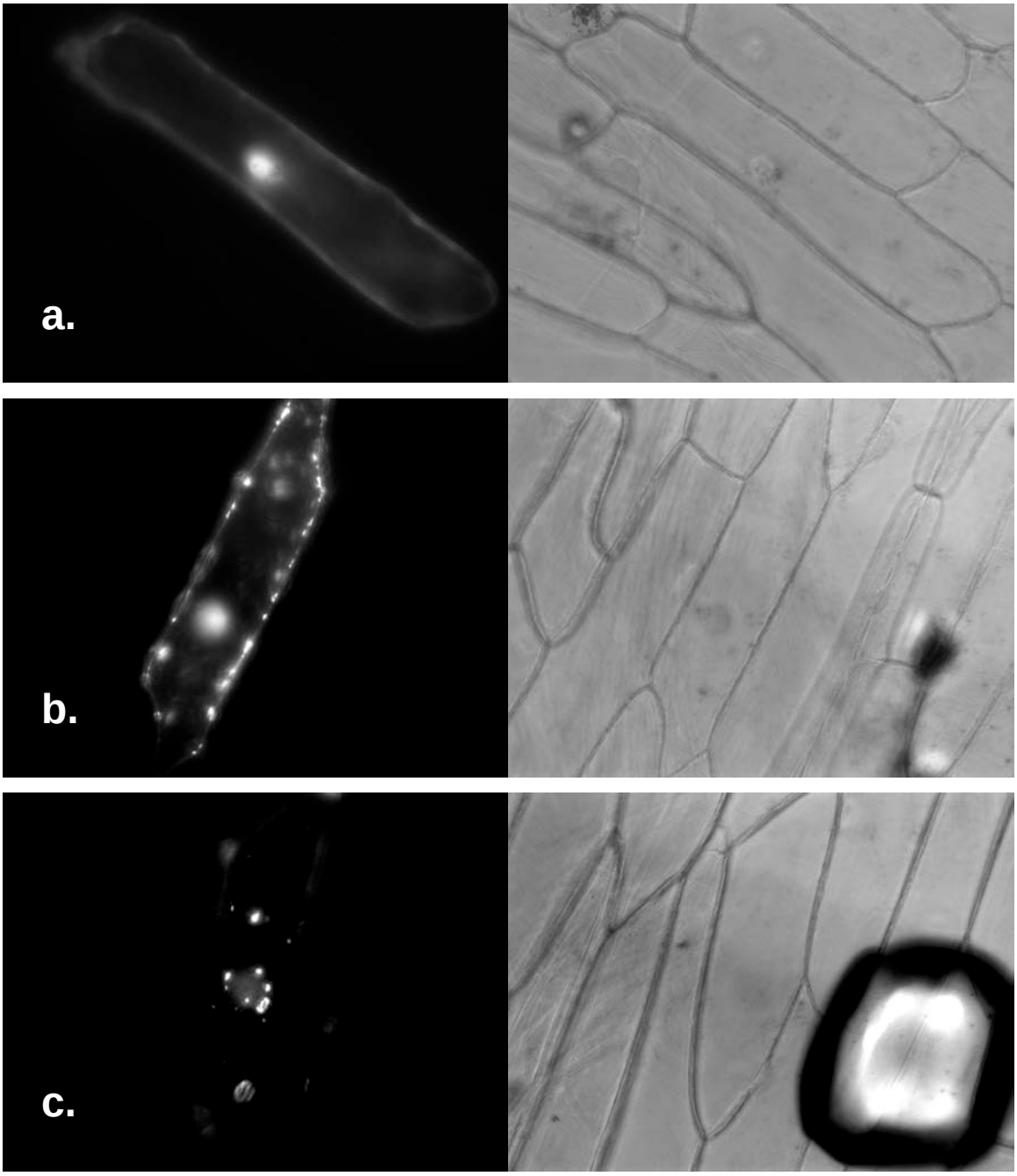

Figure 5. HAC1/C interactions. Fluorescence under darkfield conditions to the left and the same cell under halogen light to the right. Both the negative control (a) emitted a solid signal from both the cytosol and nucleus. The experimental assays (b,c) emitted signals in localized speckles in both the cytosol and nucleus.

Table 6. HAC1/C Vector combinations and expression signals. Control (shaded) and experimental assays for interaction with the third segment of the HAC1 protein. The location of the signal emitted (C=cytosol, N=nucleus) and the pattern of the signal (So=solid, Sp=Speckled) are listed for each of the vector combinations. The number of samples describes in how many replicates at least one cell displayed a certain signal. More than one type of signal was sometimes displayed in different cells of the same sample.

Vector Combination		Location	Pattern	# of Samples
pE-SPYCE-HAC1/C:	pE-SPYNE	C+N	So	2/2
pE-SPYCE-HAC1/C:	pE-SPYNE-ULT1	C+N	So	3/3
pE-SPYNE-HAC1/C:	pE-SPYCE	C+N	So	2/2
pE-SPYNE-HAC1/C:	pE-SPYCE-ULT1	C+N	So	3/3
		C+N	Sp	3/3

DISCUSSION

In this study, I found two proteins that likely bind with the trithorax group protein ULTRAPETALA1 in a complex that epigenetically silences flowering genes. Both the ATX2 and HAC1 proteins contained portions that bound to the ULT1 protein *in vivo.*
It was expected that only part of each protein would be involved in the protein-protein interactions, because it would bind at a specific binding domain. These findings are important, because ULT1 is a trithorax group (trxG) protein, and therefore works in these complexes to activate genes that control flowering in *Arabidopsis thaliana*.

ATX2 binds ULT1

The interaction of the ATX2/B protein with ULT1 in localized spots throughout the cytosol and in the nuclei suggests that ATX2 and ULT1 may dimerize in the cytosol and then localize to the nucleus. It was found, by binding ULT1 to green fluorescent proteins and GUS (beta-glucuronidase), a second type of reporter that can be seen upon staining, that ULT1 is present in both the cytosol and nucleus (Carles, Choffnes-Inada, Reville, Lertpiriyapong, & Fletcher, 2005). The large size of these three components fused together stopped the passive transport of the small ULT1 protein through nuclear pores, allowing Carles *et al*. (2005) to conclude that ULT1 must either include a nuclear localization signal (NLS), or bind to another protein with a NLS. They also observed that

when they excluded ULT1 from the cytoplasm, it was still able to perform its function when expressed in the nucleus alone. These results suggest that, although translated in the cytosol, ULT1 likely localizes to the nucleus through an undetermined nuclear localization mechanism to perform its function (Carles et al., 2005).

The interaction between ATX2 and ULT1 in both the cytosol and nucleus suggest that ATX2 must bind to ULT1 in the cytosol and then travel together with ULT1 into the nucleus. ATX1 and ATX2 are paralogs that resulted from a segmental chromosomal duplication event (Baumbusch et al., 2001) and therefore have highly conserved gene sequences. A strong physical interaction in the nucleus has been observed in a BiFC assay between ATX1 and ULT1; the subnuclear localization pattern was also speckled, but was not seen in the cytosol (Carles & Fletcher, 2009). The close evolutionary relationship between ATX1 and ATX2, along with my findings that ATX2 similarly binds to ULT1, suggests that ATX1 and ATX2 perform similar or redundant functions in *A. thaliana*. My findings contradict previous findings that the two homologs have divergent functions, with ATX2 playing a more limited role, by comparing mutant phenotypes (Saleh et al., 2008). However, ATX1 and ATX2 do activate some shared genes using distinct mechanisms (Saleh et al., 2008), and it is possible that both these genes interact with ULT1 through different mechanisms as well, or that they both bind to ULT1 for different purposes. In addition, ATX1 and ATX2 are present during different times in development (Saleh et al., 2008), a factor that would not be accounted for in the BiFC assay. The interaction I observed between ATX2 and ULT1 suggest that ATX2 may be part of a complex that silences genes in the flowering pathway.

HAC1 binds ULT1

HAC1 also interacted with ULT1 in localized spots in the cytosol and nucleus. This localization pattern suggests that HAC1 also binds with ULT1 in the cytosol, before the complex is transported to the nucleus. Similar results have been found for the CLOCK-BMAL1 complex that regulates the circadian clock in mammals (Lee et al., 2010). A BiFC assay of the CLOCK-BMAL1 proteins showed an interaction that was speckled in the cytosol and nucleus and was likely a result of dimerization in the cytosol

and subsequent nuclear localization; tracked movement of specific speckles also confirmed this observation (Lee et al., 2010). In addition, the protein CREB-binding protein (CBP) has been found to be involved in activating the CLOCK-BMAL complex by binding to it. CBP is a HAC1 homologue in animals (Deng et al., 2007), suggesting that the similarity in BiFC results is unlikely a coincidence, but rather results of the highly conserved functions of these trxG complexes across plant and animal kingdoms.

Orientation of the proteins bound to YFP was an additional factor affecting the interaction of HAC1 with ULT1. To determine which site on the large HAC1 protein was interacting, I split it into three parts. For HAC1/C, I saw an interaction that was weaker than the negative controls in one direction. The weaker signal likely results from the orientation of the proteins blocking the YFP fragments from binding. This possibility stresses the importance of testing each interaction twice, with each protein bound to both the Y- and N- terminal halves of the YFP (Kerpolla, 2006). Given that it has been established that HAC1 affects flowering time by epigenetically modifying gene expression in the *FLC* pathway (Deng et al., 2007), my results support the hypothesis that HAC1 is working in conjunction with ULT1 to control the timing and size of floral meristem development.

Limitations

Using a method of BiFC shows interactions *in vivo* in an onion cell gives a view of interactions within a living plant cell instead of an assay that simply tests whether the proteins would bind in a test tube. However, even though these interactions occur in a living cell of a different plant species, we cannot assume that these interactions would actually occur in *A. thaliana* or that both proteins would be expressed at the same time in the cell developmentally. Potentially, ULT1 and ATX2 would interact as seen in this study if expressed at the same time in the *Arabidopsis* cell, but in nature they may only be present at different times in the plant's life cycle. Differences in expression timing is less likely than the possibility that these proteins do in fact form complexes, and could easily be tested for in a future study.

The BiFC assay requires that each interaction be tested one at a time, rather than a large variety of proteins at once. I was only able to test a limited number of proteins, which I hypothesized to interact with ULT1 based on previous knowledge about the function of those proteins. It is very likely that there are other proteins that remain untested.

Future Directions

My next step will be to test whether these protein-protein interactions translate to an observable phenotypic effect in an *Arabidopsis* plant. To test whether these proteins interact in the plant, I will grow double mutants that have lost the function of both the *ult1* and *atx2* or *hac1* genes and observe if these mutant plants vary phenotypically from the single mutants. Double mutants conferring a characterizable phenotypic change, or perhaps a more dramatic *ult1* mutant phenotype, will further confirm that the proteins work together in complexes to control flowering.

To further characterize and find additional members of the trithorax complex, I will need to test more proteins using the same BiFC assay. Other candidates include other *HAC* genes that are closely related to *HAC1* and the *PIE* genes, which are also chromatin remodeling proteins in *Arabidopsis*.

Broader Implications

Characterizing the complexes ULT1 forms in *Arabidopsis* will help us understand how epigenetic silencing and activation functions in all plants. Additionally, finding homologous structures to epigenetic proteins in animals helps us see how far we can carry our analogies of these systems in plants and animals. ULT1 was the first gene in plants that was characterized to contain a SAND domain (Carles et al., 2005), which makes it of particular interest, because homologous SAND domains are found in genes of animals and humans, including the Autoimmune Regulator (AIRE1) gene in humans (Pitkänen et al., 2000). Mutations in the AIRE1 gene cause the disorder Autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy (Heino et al., 2001) and homologous genes in *Arabidopsis* may provide a powerful tool for studying the genetic

basis for these autoimmune diseases. The epigenetic function of PcG and TrxG proteins is particularly interesting, because the chromatin modifications are passed on through rounds of cell division, although the nucleotide sequence remains unaltered, creating a mechanism for cellular memory (Ringrose & Paro, 2007). There is still much to learn about how these epigenetic changes are maintained, and an important step in doing so is learning the different proteins involved.

The regulation of gene expression through chromatin remodeling is more flexible than permanent mutations in the DNA sequence, and therefore may be the mechanism through which plants show plasticity in their development (Pfluger & Wagner, 2007). PcG-mediated repression of gene expression is often reversible in plants in response to certain environmental cues (Pien et al., 2008), and this plasticity in cell fate is possible, because trxG proteins counteract the PcG complexes and promote the transcription of specific genes in a spatially and temporally restricted manner (Carles & Fletcher, 2009). The ability for plant gene expression to change in response to environmental changes is especially crucial for the stem cells in the shoot apical meristem (SAM). The timely termination of stem cell activity in the SAM during flower development through a number of complicated genetic pathways is critical for the proper development of the plant (Fletcher, 2002). Numerous studies have shown that global changes in temperature have already begun to alter the flowering time of many plants, including agricultural crops (Grab & Craparo, 2011; Marta et al., 2010; McEwan, Brecha, Geiger, & John, 2011; among others). The ability to understand and manipulate the epigenetic genes involved in the timely switch from SAM to floral meristem may become increasingly important in a changing climate.

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REFERENCES

Baumbusch, L. O., Thorstensen, T., Krauss, V., Fischer, A., Naumann, K., Assalkhou, R., Schultz, I., Reuter, G., & Aalen, R. B. (2001). The Arabidopsis thaliana genome contains at least 29 active genes encoding SET-domain proteins that can be assigned to four evolutionary conserved classes. *Nucleic Acid Research, 29*, 4319-4333.

Breiling, A., Sessa L., & Orlando, V. (2007). Biology of polycomb and trithorax group proteins. *International Review of Cytology, 258,* 83-136.

Carles, C. C., Choffnes-Inada D., Reville K., Lertpiriyapong K., & Fletcher, J. C. (2005). *ULTRAPETALA1* encodes a SAND domain putative transcriptional regulator that controls shoot and floral meristem activity in *Arabidopsis. Development, 132,* 897-911.

Carles, C. C., & Fletcher, J. C. (2009). The SAND domain protein ULTRAPETALA1 Acts as a trithorax group factor to regulate cell fate in plants. *Genes & Development, 23,* 2723-2728.

Deng, W. W., Liu C. Y., Pei Y. X., Deng X., Niu L. F., & Cao, X. F. (2007). Involvement of the Histone Acetyltransferase AtHAC1 in the regulation of flowering time via repression of *FLOWERING LOCUS C* IN Arabidopsis. *Plant Physiology, 143,* 1660-1668.

Fletcher, J. C. (2001). The *ULTRAPETALA* gene controls shoot and floral meristem size in *Arabidopsis*. *Development, 128,* 1323-1333.

Fletcher, J. C. (2002). Shoot and floral meristem maintenance in Arabidopsis. *Annual Reviews in Plant Biology, 53,* 45-66.

Gendrel, A. V., & Colot, V. (2005). *Arabidopsis* epigenetics: when RNA meets chromatin. *Current Opinion in Plant Biology, 8,* 142-147.

Grab, S. & Craparo, A. (2011). Advance of apple and pear tree full bloom dates in response to climate change in the southwestern Cape, South Africa: 1973-2009. *Agricultural and Forest Meteorology, 151,* 406-413.

Guitton, A. E. & Berger, F. (2005). Control of reproduction by Polycomb Group

complexes in animals and plants. *International Journal of Developmental Biology, 49,* 707-716.

Heino, M., Peterson, P., Kudoh, J., Shimizu, N., Antonarakis, S. E., Scott, H.S, & Krohn, K. (2001). APECED mutations in the autoimmune regulator (AIRE) gene. *Human Mutation, 18,* 205-211.

Hu, C. D., Grinberg A. V., & Kerppola, T. K. (2005). Visualization of protein interactions in living cells using bimolecular fluorescence complementation (BiFC) analysis. *Current protocols in protein science, 19.10.1–19.10.21*

Kerppola, T. K. (2006). Design and implementation of bimolecular fluorescence Complementation (BiFC) assays for the visualization of protein interactions in living cells. *Nature Protocols, 1*(3), 1278-1286.

Lee, Y., Lee, J., Kwon, I., Nakajima, Y., Ohmiya, Y., Son, G. H., Lee, K. H., & Kim, K. (2010). Coactivation of the CLOCK-BMAL1 complex by CBP mediates resetting of the circadian clock. *Journal of Cell Science, 123,* 3547-3557.

Marta, A. D., Grifoni, D., Mancini, M., Storchi, P., Zipoli, G., & Orlandini, S. (2010). Analysis of the relationships betweens climate variability and grapevine phenology in the Nobile di Montepulciano wine production area. *Journal of Agricultural Science, 148,* 657-666.

McEwan, R. W., Brecha, R. J., Geiger, D. R., & John, G. P. (2011). Flowering phenology change and climate warming in southwestern Ohio. *Plant Ecology, 212,* 55-61.

Pfluger, J., & Wagner, D. (2007). Histone modifications and dynamic regulation of genome accessibility in plants. *Current Opinions in Plant Biology, 10,* 645-652.

Pien, S., Fleury, D., Mylne, J. S., Crevillen, P., Inze, D., Avramova, Z., Dean, C., & Grossniklaus, U. (2008). *ARABIDOPSIS* TRITHORAX1 dynamically regulates *FLOWERING LOCUS C* activation via histone 3 lysine 4 trimethylation. *Plant Cell, 20,* 580-588.

Pitänken, J., Doucas, V., Sternsdorf, T., Nakajima, T., Aratani, S., Jensen, K., Will, H., Vähämurto, P., Ollila, J., Vihinen, M., Scott, H. S., Antonarakis, S. E., Kudoh, J., Shimizu, N., Krohn, K., & Peterson, P. (2000). The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common co-activator CREB-binding protein. *Journal of Biological Chemistry, 275,* 16802-16809.

Ringrose, L., & Paro, R. (2007). Polycomb/Trithorax response elements and epigenetic Memory of cell identity. *Development, 134,* 223-232.

Roudier, F. Teixeira F. K., & Colot, V. (2009). Chromatin indexing in *Arabidopsis*: an

epigenomic tale of tails and more. *Trends in Genetics, 25,* 511-517.

Saleh, A., Alvarez-Venegas, R., Yilmaz, M., Le, O., Hou, G., Sadder, M., Al-Abdallat, A., Xia, Y., Lu, G., Ladunga, I., & Avramova, Z. (2008). The highly similar *Arabidopsis* homologs of trithorax ATX1 and ATX2 encode proteins with divergent biochemical functions. *The Plant Cell, 20,* 568-579.

Sanford, J., Smith, F.D., & Russell, J.A. (1993). Optimizing the biolistic process for different biological applications, *Methods in Enzymology, 217,* 483–509.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B., & Cavalli, G. (2007). Genome regulation by polycomb and trithorax proteins. *Cell, 128,* 735-745.

Shen, W. H. & Xu, L. (2009). Chromatin remodeling in stem cell maintenance in *Arabidopsis thaliana. Molecular Plant, 2*(4), 600-609.

Van Nocker, S. (2003). CAF-1 and MSI1-related proteins: linking nucleosome assembly with epigenetics. *TRENDS in Plant Science, 8*(10), 471-473.