Eukaryotic DNA is packaged into chromatin, the basic unit of which is the nucleosome, consisting of DNA wound around histone protein complexes. There are four core histones, known as H2A, H2B, H3, and H4, and two copies of each of these proteins come together to form a histone octamer complex around which DNA is wrapped in a nucleosome. Covalent modifications of these histones play crucial roles in chromatin structure, especially related to transcriptional activation, DNA repair, and chromatin condensation during mitosis. Common modifications of histones include phosphorylation, methylation, acetylation, and ubiquitination. These diverse modifications often affect each other, and it has been proposed that different combinations of specific modifications constitute a histone code that dictates the epigenetic state of chromatin (Strahl and Allis, 2000; Turner, 2000; Jenuwein and Allis, 2001).

For example, it has been shown in yeast that monoubiquitination of a Lys residue of H2B is required for methylation of a Lys on H3, which is a key modification associated with transcriptionally active chromatin (Henry et al., 2003). In yeast, BRE1 encodes a RING finger domain ubiquitin E3 ligase that is required for H2B monoubiquitination (Hwang et al., 2003), and homologs of BRE1 have been found to perform the same function in other eukaryotes, including Drosophila (Bray et al., 2005) and human (Kim et al., 2005; Zhu et al., 2005). In this issue of The Plant Cell, independent studies by Fleury et al. (pages 417–432) and Liu et al. (pages 433–444) show that the homolog of BRE1 in Arabidopsis, named HISTONE MONOUBIQUITINATION1 (HUB1), influences the expression of downstream genes associated with cell cycle regulation affecting early leaf and root development (Fleury et al.) and also genes associated with seed dormancy (Liu et al.).

**HUB1 AND EARLY LEAF AND ROOT GROWTH**

Fleury et al. investigated leaf morphogenesis and, in particular, aspects of cell cycle regulation that influence leaf size and shape. In this work, the authors identified and characterized the gene responsible for the phenotype of the angusta4-1 mutant of Arabidopsis, which has small plant size and narrow laminae relative to the wild type (see figure). The gene was identified as \textit{HUB1}, and the mutant accordingly renamed \textit{hub1-1}.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{hub1-1.png}
\caption{Arabidopsis HUB1 Mutants. \textit{hub1-1} and \textit{hub1-2} mutants were isolated based on phenotypes in overall plant size and leaf morphology (top) and seed dormancy (bottom), respectively.}
\end{figure}
IN THIS ISSUE

The authors measured cell division and cell proliferation parameters in the mutant and found that the main cause of reduced leaf size was a decrease in cell numbers due to an increase in duration of the cell cycle in the early stages of leaf development. They also found that hub1-1 primary roots grew more slowly than those of the wild type, and this appeared to be the result of both reduced cell production in the meristem and reduced postmitotic cell expansion.

The authors looked more closely at cell cycle progression using flow cytometry to measure ploidy levels of wild-type and mutant leaves throughout development. This technique can reveal changes in the relative duration of the G1 (associated with 2C ploidy level) and G2 (4C ploidy) gap phases of the mitotic cell cycle. The results showed that the hub1-1 mutation was associated with an increased duration of G2, indicating a block at the G2-to-M transition point of the cell cycle. This suggests that HUB1 promotes the progression of cell cycle from G2 into M. The authors next conducted genome-wide gene expression analysis using the Affymetrix ATH1 array. Statistical analysis identified a large number of genes (12% of expressed genes present on the array) that were differentially expressed in the hub1-1 mutant relative to the wild type, and a substantial number of these (both upregulated and downregulated genes) were known to be associated with cell cycle regulation. These included, for example, cyclins and cyclin-dependent kinases, kinesins, and genes involved in cell plate formation and endoreduplication.

Using an in vitro ubiquitination assay with recombinant HUB1 protein, Fleury et al. showed that HUB1 mediates monoubiquitination of histone H2B and therefore is a functional ortholog of the human and yeast BRE1 proteins. Arabidopsis contains a second HUB gene, designated HUB2. Through complementation testing and creation of the hub1 hub2 double mutant, the authors determined that HUB2 acts in the same pathway as HUB1.

**HUB1 AND SEED DORMANCY**

Meanwhile, Liu et al. investigated seed dormancy in Arabidopsis with a mutant designated reduced dormancy4 (rdo4), originally isolated based on its reduced dormancy phenotype (see figure). In addition to reduced dormancy, they found a defect in seedling establishment relative to the wild type. Map-based cloning of the mutant locus revealed that it corresponded to HUB1, and this mutant was correspondingly renamed hub1-2. Unlike hub1-1, plant size is only slightly affected in the hub1-2 mutant.

Liu et al. also identified the HUB2 gene and show that mutations in hub2 and hub1 result in reduced seed dormancy. These authors tested functionality of HUB1 and HUB2 by transforming plants with a construct containing FLAG-tagged H2B driven by a constitutive 35S promoter. After isolation of histone-enriched protein from wild-type seedlings transformed to express this construct, separation by electrophoresis, and immunoblotting with FLAG antibodies, monoubiquitinated H2B could be detected as a slower migrating form of H2B. The slower-moving monoubiquitinated H2B could not be detected in hub1 or hub2 mutants transformed with the FLAG-tagged construct, offering further confirmation for the role of both proteins as functional orthologs of human and yeast BRE1. Based on their interest in the seed dormancy phenotype, Liu et al. examined the expression of a number of dormancy-related genes and found that the expression of several of these was significantly reduced in the hub1-2 mutant relative to the wild type.

The authors speculate that, as in other eukaryotes, HUB1-mediated monoubiquitination of H2B plays a role in transcriptional activation of target genes through its effect on chromatin remodeling. They note that the dormancy genes downregulated in the hub1-2 mutant could be direct targets of HUB1 or could act downstream of primary targets. They also note that the function of HUB1 is not restricted to seed dormancy, as indicated by pleiotropic defects observed in plants, including seedling establishment and alterations in leaf color and plant architecture.

**HUB1: A FUNDAMENTAL REGULATOR OF DEVELOPMENT?**

It is tempting to speculate that the direct targets of HUB1 could be a relatively small number of key developmental regulatory genes. In humans, it has been found that some of the key target genes transcriptionally activated by H2B monoubiquitination are HOX genes, which encode transcription factors that are key developmental regulators (Zhu et al., 2005). The HOX family consists of highly conserved homeobox transcription factors that specify the identity of the body segments along the anteroposterior axis of the embryo (Kosaki et al., 2002). Bray et al. (2005) found that H2B monoubiquitination by the BRE1 homolog in Drosophila is critical for the transcription of Notch target genes. The Notch signaling pathway is highly conserved in animals and controls numerous cell fate decisions during development (Artavanis-Tsakonas et al., 1999).

The work on these proteins in yeast and other organisms has shown that H2B ubiquitination is involved in chromatin remodeling that results in transcriptional activation. Fleury et al. found that 12% (1758) of the expressed genes represented on the ATH1 array were differentially expressed between hub1-1 mutant and wild-type Arabidopsis shoot apices. Slightly less than half of these (46.8%) were downregulated, and the rest (53.1%) were upregulated in the mutant. This is consistent with the hypothesis that some of the direct targets of H2B monoubiquitination are transcription factors, some of which likely function as repressors of other genes, while others function as activators, and many of these differentially expressed genes likely represent secondary targets. Future work might seek to measure histone H3 methylation in hub mutants at specific sites in the genome to help uncover specific target loci.

Zhu et al. (2005) found that human homologs of yeast BRE1, called RNF20 and RNF40, function together in a tetrameric complex as the E3 ligase responsible for monoubiquitination of H2B. H2B monoubiquitination is part of a transcriptional cascade whereby H2B monoubiquitination leads to methylation of a Lys on H3, which in turn leads to transcriptional activation of target genes. Accordingly, Zhu et al. found that overexpression of RNF20 led to elevated H2B monoubiquitination and subsequently higher levels of H3 methylation.
and stimulation of HOX gene expression. Disruptions in the RNF20/40 complex using RNA interference produced the opposite effects. Zhu et al. (2005) speculated that the two nonidentical E3 ligases in the complex could be regulated differently and trigger different outcomes with respect to downstream methylation. Interestingly, Liu et al. found that HUB1 and HUB2 are both required for H2B monoubiquitination in Arabidopsis, leading them to postulate that these proteins might also function as a tetramer.

**HUB1 LESSONS**

These studies provide new information on the role of chromatin remodeling in plant development. They suggest that H2B monoubiquitination is an important component of the histone code in plants and animals and influences transcription of downstream target genes involved in regulating key transition points in early leaf and root development and during seed dormancy. The work of Fleury et al. suggests that at least some of these results may be related to effects of H2B monoubiquitination on regulation of the mitotic cell cycle.

These two articles also serve as an important reminder of the primary difficulties faced when investigating gene function. Namely, that loss- and gain-of-function mutant phenotypes can be easy to miss, and observing a phenotype in one tissue or developmental program (such as cell cycle regulation) does not preclude the possibility of an important function somewhere else (such as seed dormancy). As objective as we might attempt to be, and as carefully as we might conduct experiments, we are naturally predisposed to making observations and drawing conclusions most closely related to our own areas of expertise. These two tales of HUB1 remind us always to remain open to possibilities outside the circles we draw around our own favorite pathways.

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