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Transcriptome and Genome-Wide Analysis of the *Arabidopsis* Stem Cell Regulator WUS

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The genomic and transcriptional analysis of the transcription factor WUSCHEL (WUS), explored in this issue of *Developmental Cell*, represents the next generation of stem cell analysis in *Arabidopsis*. The resources generated provide insights into WUS function and a wealth of new information for the entire field.

The genomic and transcriptional analysis of the stem cell factor WUSCHEL (WUS) by Busch et al. (2010), detailed in this issue of *Developmental Cell*, represents the next generation in analysis of stem cell function in *Arabidopsis*. WUS is central to stem cell control because it acts to specify the stem cell niche and, in a poorly understood manner, to drive stem cell establishment and maintenance in the overlying cells (Mayer et al., 1998). While details of the signaling pathway that limits WUS expression are emerging (Song et al., 2006), the manner by which WUS drives stem cell specification is not known. A few known targets for WUS have been uncovered (Leibfried et al., 2005; Lohmann et al., 2001), but the bulk of WUS function has been left unexplained.

Busch and colleagues have now addressed this gap using genomic and transcriptomic approaches. Their analysis of transcriptional targets of WUS included a multifactoral analysis using multiple combinations of *WUS* gain- and loss-of-function plants combined with a similar slate of samples for the *WUS* repressor CLV3. All of the samples, with the exception of the *clv3* mutant, showed remarkably strong correlations for gene expression, providing a robust approach to screen for likely targets whose transcription is controlled, directly or indirectly, by WUS. By combining data from all of the samples, the authors developed a WUS Regulation Score, or WRS, and used a threshold WRS score to identify over 600 transcriptional targets.

Most of these targets are repressed by WUS activity, which is consistent with a role for WUS in maintaining undifferentiated cells. More intriguing is that the bulk of targets repressed by WUS are expressed within the *WUS* domain, based on cross-referencing WUS targets with recent transcriptional profiling of regions of the shoot meristem by Yadav and coworkers (Yadav et al., 2009). This suggests that much of WUS activity involves generating signals to repress gene expression in neighboring cells, or involves the movement of WUS protein to neighboring cells to effect this regulation directly.

Busch and coworkers also carried out ChIP-chip with WUS to identify target sites for direct WUS regulation. They compared samples from inducible WUS overexpression with those from the *wus* mutant control. Using 13 replicates, multiple algorithms, and transcriptional targets as a guide, the authors identified 136 chromatin regions bound by WUS. These target sites yielded several insights into WUS function. Specifically, a new in vivo binding site for WUS was identified. Notably, the consensus site based on ChIP-chip differed significantly from a previously identified WUS binding site that now appears to be a low-affinity site (Lohmann et al., 2001).

A second finding is that the direct binding sites for WUS in the genome show very little overlap with the WUS transcription targets, despite using the transcription targets as a guide. Ninety-three percent of genes bound by WUS show no evidence of transcriptional regulation by WUS, while nearly ninety-nine percent of WUS transcriptional targets are not bound by WUS. While the latter category is easily explained by hypothesizing that most transcriptional targets are indirectly regulated by WUS, the former category is harder to resolve. Similar results have been observed in other systems, where binding sites and transcriptional targets

show limited overlap. What is remarkable here is that the limited overlap remains in spite of the lengths to which the authors went experimentally and in developing new algorithms to narrow the list of candidates in both categories.

The limited overlap between WUS binding and transcriptional regulation raises several questions. Are most WUS binding sites nonfunctional? Are the experimental approaches identifying sites WUS does not normally bind? Are these sites transcriptionally regulated by WUS in other developmental contexts outside of the meristem (Gross-Hardt et al., 2002)? Are the genes regulated by WUS in subtle ways that are not detected by microarrays?

The most exciting WUS binding targets are the genes that are both bound directly by WUS and transcriptionally regulated by WUS. Surprisingly, the receptor-kinase CLV1, which acts to repress *WUS* transcription, is one such target. *CLV1* expression largely overlaps with *WUS* expression (Clark et al., 1997), yet WUS binds to the *CLV1 cis* elements and represses *CLV1* expression across the battery of samples tested by the authors. One possibility is that the WUS repression of *CLV1* acts to fine-tune *CLV1* expression, perhaps as a form of positive feedback. Another possibility is that WUS binds to and directly regulates *CLV1*, but that the repressive function of WUS is a largely an indirect effect. Finally, we should consider that WUS protein may move to adjacent cells to carry out this repressive function. Whatever mechanism is ultimately uncovered for WUS-CLV1 interactions, it is clear that the targets of WUS binding and transcriptional control will provide critical material for unraveling the control of stem cell specification in *Arabidopsis*. Indeed, the resources provided by Busch and coworkers, combined with recent expression profiling of domains within the shoot meristem (Yadav et al., 2009), open up many new avenues for exploration of stem cell function.

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