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DISPATCH

Plant Development: Suppression the Key to Asymmetric Cell Fate

Stuart A. Casson

A new study shows that SPEECHLESS determines cell fate in the stomatal lineage, but is inherited equally by daughter cells following an asymmetric cell division. The polarity determinant BASL acts as a MAPK scaffold, targeting SPEECHLESS for degradation in the larger daughter cell.

Asymmetric cell divisions (ACDs) are a common mechanism of generating different cell fates in daughter cells in a wide range of organisms [1,2]. There are several instances where ACDs are preceded by intrinsic cellular asymmetries — for example, after fertilisation the *Caenorhabditis elegans* zygote undergoes an ACD along the anterior–posterior axis to generate a larger anterior cell and a smaller posterior cell with different fates. This ACD requires partitioning defect (PAR) proteins and segregates the Zn-finger determinants MEX-5 and MEX-6 to the larger anterior cell, and PIE-1 to the smaller posterior cell [3]. Conversely, extrinsic signals often play a role in regulating cell fate within stem cell niches, as is seen in the *Drosophila melanogaster* female germline [4]. There are several examples of cell-fate defining ACDs in plants, and a range of mechanisms from cell-to-cell movement of transcription factors to hormone gradients define daughter cell fates [5,6]. In this issue of *Current Biology*, an elegant new study by Zhang and colleagues now provides further insight into cell fate decisions in the stomatal lineage, and sheds new light on the varied mechanisms by which cell fate decisions are determined in plants [7].

Stomata are the small pores found primarily on the surface of leaves and enable the exchange of gases – significantly CO₂ and water vapour – between the plant interior and the atmosphere. Stomatal development has emerged as a model system for examining cell fate decisions in plants with much of our current knowledge derived from studies in the model plant, *Arabidopsis thaliana*. Entry into the stomatal lineage involves the asymmetric cell division (ACD) of a meristemoid mother cell (MMC) to generate a larger stomatal lineage ground cell (SLGC) and a smaller meristemoid (M). Ms may undergo further rounds of self-renewing ACDs before ultimately differentiating into a pair of guard cells, which flank the stomatal pore [8]. Asymmetric divisions within the stomatal lineage require the activity of a nuclear bHLH transcription factor, SPEECHLESS (SPCH), which defines the fate of ACD daughter cells [9]. A mitogen-activated protein kinase (MAPK) signalling cascade that includes the MAPKKK YODA (YDA) and the MAPKs MPK3 and MPK6, phosphorylates SPCH, leading to its degradation [10,11,12]. This YDA–MAPK module operates downstream of a cell surface receptor complex, whose ligands are a series of extracellular EPIDERMAL PATTERNING FACTOR (EPF) peptides, which include both negative and positive regulators of stomatal development [13]. In this

respect, the ACDs within the stomatal lineage are observed to be extrinsically regulated, however additional mechanisms point to intrinsic regulation.

Mutations in *BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE* (*BASL*) result in a loss of asymmetry in entry divisions and causes defects in fate assignment with both daughter cells of defective ACDs capable of expressing genes specific to stomatal fate and forming guard cells [14]. *BASL* is phosphorylated by MPK3/6, and this facilitates *BASL* functioning as a scaffold for YDA at the plasma membrane (PM) [15]. Prior to the ACD of a MMC, *BASL* localises to both the nucleus and the cell periphery distal to the future division plane of the MMC, and is therefore predictive of the future ACD plane. While nuclear *BASL* is inherited by both daughter cells (SLGC and M), only the SLGC inherits *BASL* at the polar PM site, and hence in turn the YDA–MAPK module, is asymmetrically inherited. What does this asymmetry achieve in terms of both activity of the MAPK module, SPCH and cell fate decisions?

To address these questions, Zhang and colleagues used plants expressing functional GFP–*BASL* to perform fluorescence recovery after photobleaching (FRAP) to first examine the intracellular dynamics of *BASL* within SLGCs. The nuclear *BASL* pool was found to be relatively static and is not replenished following FRAP. *BASL* at the polar peripheral site did show recovery, though the dynamics were relatively slow. This slow mobility of *BASL* was specific to the polar site since *BASL* localised to non-polar sites on the PM showed greater mobility, demonstrating that distinct mechanisms may direct or retain *BASL* at polar versus non-polar sites. Targeting to the polar site requires phosphorylation of *BASL* by MPK3/6 [15]. By mutating the MAPK phosphorylation sites in *BASL* with phospho-mimicking residues, FRAP analysis demonstrated that *BASL* phosphorylation actually reduces mobility at the polar PM site, which has significant consequences. Plants expressing phospho-mimicking *BASL* variants having significantly reduced stomatal development, due to a reduction in spacing divisions of SLGCs.

So, what is the functional significance of *BASL* localisation on both MAPK activity and SPCH in the ACD daughter cells? Here again, Zhang and colleagues' elegant use of FRAP and cell biology addressed these questions. MPK6-mRFP was expressed in stomatal lineage cells and FRAP was used to examine MPK6 mobility in the nuclei of ACD daughter cells. MPK6 mobility was significantly higher in SLGC nuclei compared with M cell nuclei, and this was dependent on polar *BASL*. Experiments utilising MAPK activators and inhibitors support a model whereby increased mobility represents active MPK6 shuttling to the nucleus.

What, then, was the impact on SPCH protein in these cells? Quantification found that more nuclear SPCH was found in Ms compared with SLGC, which was dependent on polarised *BASL* in the SLGC. Significantly, *basl* mutants or plants expressing a degradation resistant SPCH variant, have equal amounts of SPCH in SLGC and Ms. Therefore, the key fate determinant SPCH is not differentially segregated during the ACD. Instead, asymmetric segregation of polar *BASL* in the SLGC leads to an active YDA MAPK signalling cascade that differentially degrades SPCH in the nucleus of SLGC post ACD (Figure 1A). In this respect, there are similarities to the PAR system in *C. elegans*, which sees the fate determinant PIE-1 distributed in posterior cells

following zygote division, which involves both diffusion and regulated degradation of PIE-1 [16,17]. The key difference however, is that SPCH asymmetry is determined after ACD.

This represents a novel mechanism of generating different fates in plants, so why might this be the case? There are a number of examples, such as in plant roots or the female germ line in *Drosophila*, where stem cells occupy a defined niche (SCN). The leaf epidermis however, does not have a defined SCN, and in *Arabidopsis* at least, stomata are relatively randomly distributed. SPCH confers limited stem cell fate, but the young leaf epidermis shows environmentally regulated flexibility in its development and is also constrained by the fact that stomata should not form adjacent to each other [8,18]. EPFs and their receptor complexes enable communication between neighbouring cells, and BASL polarity orientation is disrupted in some receptor and EPF mutants showing the role of extrinsic regulation of cell fate decisions [14]. By asymmetrically degrading SPCH post ACDs, it is likely that SPCH levels in the SLGC can be fine-tuned by extrinsic signals, such as the EPFs in response to environmental conditions [19]. Having the same environmentally modulated MPKs differentially regulate both BASL persistence at the polar site and SPCH stability in SLGC is an efficient means of manipulating the fate of the SLGC (Figure 1B).

Additionally, the slow dynamics of polar BASL may buffer against brief environmental episodes, enabling a more integrated response to noisy environments. However, an additional level of complexity exists in that BASL is also a direct target of SPCH [20]. It will therefore be interesting to determine whether BASL dynamics are modulated by environmental signals, whilst at the mechanistic level, how is BASL targeted and delivered to the polar site? Does it associate with any components of the receptor complex that perceives EPFs and does this explain the differences in mobility of polar versus non-polar BASL? There is also the question of what is the source of polar BASL during self-renewal divisions of Ms. If a M undergoes an ACD, is the nuclear BASL mobilised to the polar site, or does SPCH direct expression of BASL *de novo*? It will certainly be interesting to determine whether similar mechanisms can be found in systems that share the plastic fate of the leaf epidermis.

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Figure 1. BASL asymmetry defines daughter cell fate by directing post-division degradation of SPEECHLESS.

(A) The upper panel shows BASL, YDA and MPK3/6 localisation before and after ACD of the MMC. The lower panel shows localisation of the fate determinant SPCH in the same cells, including immediately post-ACD. (B) The polar BASL-mediated post-ACD degradation of SPCH in SLGC may enable extrinsic signals to modulate the fate of SLGC. If inhibitory, SPCH levels remain low in the SLGC, which prevents ACD. If signals promoting stomatal development are perceived, MPK3/6 phosphorylation of BASL is reduced and results in loss of polar BASL and active nuclear MPK6 leading to increased SPCH and ACD of the SLGC. A similar mechanism may operate in *Ms*, however they already have increased SPCH and hence are more likely to undergo further rounds of ACD (not shown).