This is a repository copy of *Plant Development: Suppression the Key to Asymmetric Cell Fate*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/117261/

Version: Accepted Version

Article:

Casson, S.A. orcid.org/0000-0001-5702-6947 (2016) Plant Development: Suppression the Key to Asymmetric Cell Fate. Current Biology, 26 (21). R1137-R1139. ISSN 0960-9822

https://doi.org/10.1016/j.cub.2016.09.042

Article available under the terms of the CC-BY-NC-ND licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

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 *Caenorrhabditis 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 CO_2 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.

References

1. Tajbakhsh, S., Rocheteau, P., and Le Roux, I. (2009). Asymmetric cell divisions and asymmetric cell fates. Annu. Rev. Cell Dev. Biol. *25,* 671-699.

2. De Smet, I., and Beeckman, T. (2011). Asymmetric cell division in land plants and algae: the driving force for differentiation. Nat. Rev. Mol. Cell Biol. *12,* 177-188.

3. Wu, Y., Zhang, H., and Griffin, E.E. (2015). Coupling between cytoplasmic concentration gradients through local control of protein mobility in the Caenorhabditis elegans zygote. Mol. Biol. Cell *26,* 2963–2970.

4. Greenspan, L.J., de Cuevas, M., and Matunis, E. (2015). Genetics of gonadal stem cell renewal. Annu. Rev. Cell Dev. Biol. 31, 291-315.

5. Long Y, Scheres B, Blilou I. (2015) The logic of communication: roles for mobile transcription factors in plants. J. Exp. Bot. *66,* 1133-1144.

6. ten Hove, C.A., Lu, K.J., and Weijers, D. (2015) Building a plant: cell fate specification in the early Arabidopsis embryo. Development *142,* 420-430.

7. Zhang, Y., Guo, X., and Dong, J. (2016). Phosphorylation of the polarity protein BASL differentiates asymmetric cell fate through MAPKs and SPCH. Current Biol. XXX-XXX.

8. Han, S.K., and Torii, K.U. (2016). Lineage-specific stem cells, signals and asymmetries during stomatal development. Development *143,* 1259-1270.

9. MacAlister, C.A., Ohashi-Ito, K., and Bergmann, D.C. (2007). Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. Nature *445,* 537-540.

10. Bergmann, D.C., Lukowitz, W., and Somerville C.R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. Science *304,* 1494-1497.

11. Lampard, G.R., Macalister, C.A., and Bergmann, D.C. (2008). Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. Science *322,* 1113-1116.

12. Wang, H., Ngwenyama, N., Liu, Y., Walker, J.C., Zhang, S. (2007). Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell *19,* 63-73.

13. Richardson, L.G., and Torii, K.U. (2013). Take a deep breath: peptide signalling in stomatal patterning and differentiation. J. Exp. Bot. *64,* 5243-5251

14. Dong, J., MacAlister, C.A., and Bergmann, D.C. (2009). BASL controls asymmetric cell division in Arabidopsis. Cell *137,* 1320-1330.

15. Zhang Y, Wang P, Shao W, Zhu JK, Dong J. (2015). The BASL polarity protein controls a MAPK signaling feedback loop in asymmetric cell division. Dev Cell. *33,* 136-149.

16. Reese, K.J., Dunn, M.A., Waddle, J.A., and Seydoux, G. (2000). Asymmetric segregation of PIE-1 in C. elegans is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. Mol. Cell. *6,* 445-455.

17. DeRenzo, C., Reese, K.J., and Seydoux, G. (2003). Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation. Nature *424,* 685-689.

18. Casson, S.A., Hetherington, A.M. (2010). Environmental regulation of stomatal development. Curr. Opin. Plant Biol. *13,* 90-95.

19. Engineer, C.B., Ghassemian, M., Anderson, J.C., Peck, S.C., Hu, H., and Schroeder, J.I. (2014) Carbonic anhydrases, EPF2 and a novel protease mediate CO2 control of stomatal development. Nature *513,* 246-250.

20. Lau, O.S., Davies, K.A., Chang, J., Adrian, J., Rowe, M.H., Ballenger, C.E., and Bergmann, D.C. (2014). Direct roles of SPEECHLESS in the specification of stomatal self-renewing cells. Science *345*, 1605-1609.

Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, UK.

E-mail: S.Casson@Sheffield.ac.uk

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).