yet been investigated. Interestingly, although Pin1 binding is dependent on phosphorylation of PML within its C-terminal region, PML SUMOylation blocks this interaction[10]. Given the link between PML SUMOylation and its degradation, this seems paradoxical but the study by Lallemand-Breitenbach *et al.* may provide an explanation[5]. They show that wild-type PML is SUMO-1 modified on Lys 490 and mutation of this residue enhances degradation of PML in response to ATO. This suggests a stabilizing effect of PML Lys 490 SUMOylation, possibly due in part to inhibition of Pin1-mediated degradation. Furthermore, mutation of the 'pro-degradation' PML Lys 65 residue promotes Lys 490 SUMOylation, suggesting that SUMO modification of PML is subject to strict control by a variety of factors, whose activities can have antagonistic effects on its stability.

Questions arising from the results presented in the two papers are whether RNF4 recognizes other polySUMOylated proteins and also, whether there are other proteins that contain multiple SUMO-interaction motifs. RNF4 has been reported to associate with a number of transcription factors and steroid hormone receptors[11–15] but it is not known whether these interactions are mediated through the binding of polySUMO chains and/or related to the function of RNF4 as a ubiquitin E3 ligase. Although *Schizosaccharomyces pombe* contains two RNF4 homologues (Rfp1 and Rfp2), so far only RNF4 has been identified as possessing multiple SIMs in humans. However, a search of the Entrez database at the NCBI revealed the presence of a highly homologous 4 × SIM-containing region in a predicted but as yet unverified isoform of a testis-specific RING protein encoded by the human *RNF36* gene (accession number EAW77280). Interestingly, both RNF36 (TRIM69) and PML (TRIM19) are members of the tripartite motif (TRIM) family of RING domain-containing proteins and, given that mouse RNF36 localizes to PML nuclear bodies, the function of its human counterpart perhaps merits a more detailed investigation[16].

In conclusion, results presented in the two manuscripts identify RNF4 as a polySUMO chain-binding protein and also PML as a *bona fide* RNF4 target; this is the first example of a protein degraded by this SUMO-dependent ubiquitination pathway. Moreover, the findings by Lallemand-Breitenbach *et al*[5] reinforce the scientific rationale for the use of low-dose ATO in differentiation therapy for PML–RARα-associated APL. However, given the role of PML as a growth/tumour suppressor[17], the finding that ATO also targets PML presents a 'catch 22' situation with regard to its use as a treatment for cancers in general. Nevertheless, the success of ATO in APL therapy has provoked renewed interest in its use and a large number of clinical trials investigating its potential in haematological malignances and solid tumours are currently underway or recently have been completed.[1] This, along with the development of other organic arsenic compounds could well see the emergence of new applications for this semimetal in the wider range of cancer therapies.

1. Dilda, P. J. & Hogg, P. J. *Cancer Treat Rev.* **33,** 542–564 (2007).
2. Zhu, J. *et al. Proc. Natl Acad. Sci. USA* **94,** 3978–3983 (1997).
3. Kamitani, T. *et al. J. Biol. Chem.* **273,** 26675–26682 (1998).
4. Lallemand-Breitenbach, V. *et al. J. Exp. Med.* **193,** 1361–1371 (2001).
5. Lallemand-Breitenbach, V. *et al. Nature Cell Biol.* **10,** 547–555 (2008).
6. Tatham, M. H. *et al. Nature Cell Biol.* **10,** 538–546 (2008).
7. Matic, I. *et al. Mol. Cell Proteomics* **7,** 132–144 (2008).
8. Mukhopadhyay, D. *et al. J. Cell Biol.* **174,** 939–949 (2006).
9. Hayakawa, F. & Privalsky, M. L. *Cancer Cell* **5,** 389–401 (2004).
10. Reineke, E. L. *et al. Mol. Cell Biol.* **28,** 997–1006 (2008).
11. Poukka, H., Aarnisalo, P., Santti, H., Janne, O. A. & Palvimo, J. J. *J. Biol. Chem.* **275,** 571–579 (2000).
12. Fedele, M. *et al. J. Biol. Chem.* **275,** 7894–7901 (2000).
13. Lyngso, C. *et al. J. Biol. Chem.* **275,** 26144–26149 (2000).
14. Kaiser, F. J., Moroy, T., Chang, G. T., Horsthemke, B. & Ludecke, H. J. *J. Biol. Chem.* **278,** 38780–38785 (2003).
15. Wu, S. M. *et al. Mol. Pharmacol.* **66,** 1317–1324 (2004).
16. Shyu, H. W., Hsu, S. H., Hsieh-Li, H. M. & Li, H. *Exp. Cell Res.* **287,** 301–313 (2003).
17. Wang, Z. G. *et al. Science* **279,** 1547–1551 (1998).
18. Hecker, C. M., Rabiller, M., Haglund, K., Bayer, P. & Dikic, I. *J. Biol. Chem.* **281,** 16117–16127 (2006).

Coming closer to a stoma ion channel

Laura Serna

Plant stomata, which consist of paired guard cells placed on the surface of leaves, control gas exchange with the atmosphere. Anion transport by unidentified guard-cell channels closes the stomatal pore and the first component for this channel function has now been found.

Stomata are like windows on the surface of leaves: they close to prevent water vapour loss during transpiration, but open to allow CO_2 uptake for photosynthesis. The stomatal pore aperture depends on the transport of ions and organic metabolites across guard-cell membranes[1–3]. Malate and Cl^- efflux from the guard cells causes membrane depolarization, which drives K^+ efflux through K^+ channels during stomatal closure (Fig. 1a). Despite their proposed role, until now no component of the plant membrane anion channel has been identified. Two studies published in *Nature*, from Negi *et al.*[4] and Vahisalu *et al.*[5], make a convincing case that *SLOW ANION CHANNEL-ASSOCIATED1* (*SLAC1*) is a component of the slow (S-type) anion efflux channel function that controls stomatal pore closure in *Arabidopsis thaliana* in response to multiple signals.

Laura Serna is in the Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, E-45071 Toledo, Spain. e-mail: laura.serna@uclm.es

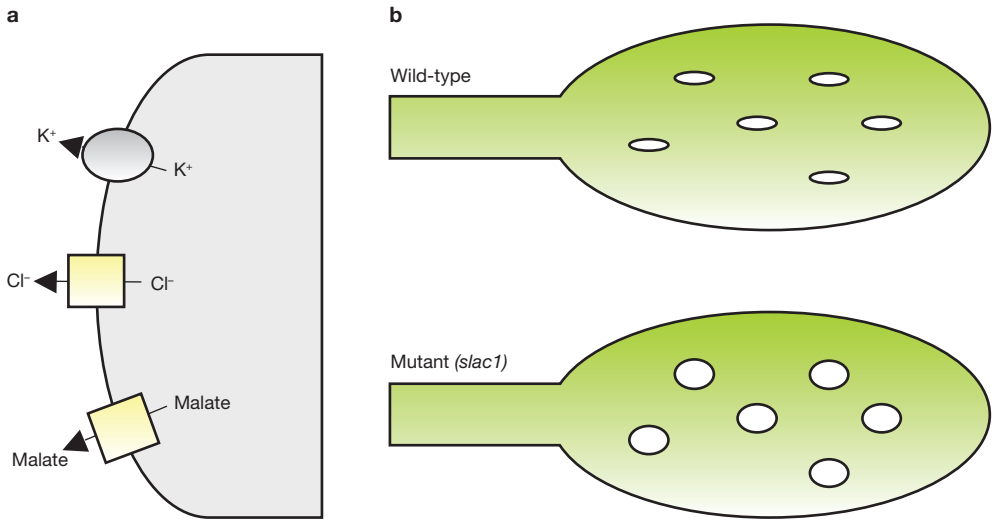

Figure 1 *SLOW ANION CHANNEL-ASSOCIATED1* (*SLAC1*) controls stomata S-type anion channel function in *Arabidopsis*. Gas exchange between the plant and the atmosphere takes place through the stomata, which consist in two bean-shaped guard cells surrounding a pore. (**a**) Malate and Cl^- efflux from the guard cells induces membrane depolarization, which drives K^+ efflux through K^+ channels, inducing stomatal closure[1–3]. Both S- and R-type anion efflux channels account for Cl^- and malate efflux from guard cells[1,3,13]. *SLAC1* regulates S-type anion channel function during stomatal closure[4,5]. (**b**) Multiple stresses trigger stomatal pore closure in normal plants, but not in plants with a mutated *SLAC1* gene (*slac1*; refs 4, 5).

Stomatal closure occurs in response to a variety of stresses, including high CO_2 levels, darkness, low air humidity, drought and ozone[6,7]. Both groups find that plants with a mutated *SLAC1* gene (*slac1*) do not close their stomata in response to elevated CO_2 levels and ozone stress, and show a modest response to darkness, air humidity and drought[4,5]. The plant hormone abscisic acid (ABA) also restrains water loss by closing the stomatal pore, and this response is also disrupted in the *slac1* mutant[4,5]. It is known that ABA, whose levels increase under drought conditions, stimulates stomatal closure through second messengers, such as ROS, cytosolic Ca^{2+} and nitric oxide[8–10]. Vahisalu *et al.* showed that mutations in the *SLAC1* gene impair stomatal closure in response to hydrogen peroxide, Ca^{2+} and nitric oxide. *SLAC1* therefore stimulates stomatal pore closure by functioning downstream of a wide variety of signals (Fig. 1b), which suggests that it controls a central step during stomatal closure.

Vahisalu *et al.* and Negi *et al.* investigated the molecular nature of the *SLAC1* gene, and found that it encodes a putative membrane protein of about 500 amino acids. The SLAC1 protein contains ten predicted transmembrane domains and hydrophilic amino- and carboxy-terminal tails; sequence analysis reveals that SLAC1 possesses features of the C4-dicarboxilate transporter family[4,5]. *SLAC1* is expressed in guard cells[4,5], and occasionally in the vascular strands[5]. When SLAC1 protein was transiently expressed in onion epidermal cells and tobacco protoplasts, it attached to the plasma membrane of these cells[5]. Negi *et al.* went on to examine the subcellular localization of SLAC1 in *Arabidopsis*. They produced transgenic plants carrying the SLAC1 gene product fused to green fluorescent protein (GFP), and found that this lit up the plasma membrane of guard cells, consistent with the presence of predicted transmembrane domains.

But what does SLAC protein do in the guard-cell plasma membrane? Given that efflux of Cl^- and malate from the guard cells triggers stomatal closure, an exciting possibility is that SLAC1 may close the stomata by providing (or regulating) a gate for anion transport across the guard-cell plasma membrane. Negi *et al.* tested this hypothesis by measuring the levels of organic and inorganic ions in guard-cell protoplasts of both *slac1* and wild-type control plants. Interestingly, *slac1* protoplasts showed higher contents of both malate and fumarate, compared with control protoplasts, whereas succinate levels were not affected[4]. The authors also found that the level of Na^+ in *slac1* protoplasts was similar to that of wild-type control protoplasts, and that *slac1* protoplasts exhibit a higher content of both K^+ and Cl^-. These results support the idea that SLAC1 provides or regulates a gate for anion transport, and also suggest that fumarate may be involved in the stomatal movements. But, why do *slac1* protoplasts accumulate K^+? Negi *et al.* proposed that the K^+ increase in *slac1* protoplasts may occur to balance the charge caused by the accumulation of malate and Cl^- anions. Future research is required to understand the role of SLAC1 in the control of K^+ efflux.

One member of the C4-dicarboxilate transporter family, Mae1 of *Schizosaccharomyces pombe*, controls malate uptake[11]. Negi *et al.* reasoned that, if SLAC1 controls malate uptake from the guard cells, *SLAC1* expression in the *mae1* mutant of *S. pombe* should rescue its defect in malate uptake. However, the *mae1* mutant was not affected by expression of *SLAC1* (ref. 4). The authors argued that, as described for the *Arabidopsis* potassium transporter AKT1 (ref. 12), perhaps *SLAC1* should be expressed together with other genes to promote malate uptake. The same argument may be used to explain the absence of a SLAC1-dependent ion current in oocytes of *Xenopus laevis* that express *SLAC1* (ref. 4). These results also suggest that these related proteins are functionally divergent in distant organisms. Certainly, Mae shows a low homology with SLAC1, and even lacks the hydrophilic tail that characterizes the SLAC1 N-terminus[4,5].

The activation of slow (S-type) or rapid (R-type) anion efflux channels transmits Cl^- and malate efflux from guard cells[1,3,13], triggering stomatal closure[1–3]. Vahisalu *et al.* investigated the function of S-type and R-type anion channels in both wild-type plants and the *slac1* mutant. They found that mutations in *SLAC1* impair S-type anion channel currents that are activated by cytosolic Ca^{2+} and

ABA, but do not affect R-type anion channel currents or Ca^{2+} channel function. They concluded that *SLAC1* is required for S-type anion channel function specifically, which is consistent with its role in anion transport across the guard-cell plasma membrane. Given that *slac1* mutants are impaired in their stomatal response to a wide variety of signals, S-type anion channels must have a central role during stomatal closure.

But that's not all. Negi *et al.* went a step further by delving into the function of three *Arabidopsis* SLAC homologues, SLAHs, and showed that two of them can rescue effects exhibited by the *slac1* mutant when expressed in guard cells. As with SLAC1, these three proteins locate to the cell membrane, but are not expressed in guard cells[4]. *SLAH1* is expressed in the vascular root system, whereas *SLAH2* is present in lateral root primordia and taproot tips[4]. *SLAH3* is expressed in the whole plant, but is absent from guard cells[4]. Using a regulatory region (the promoter) from *SLAC1*, they drove expression of *SLAHs* in the guard cells and found that *SLAH1* and *SLAH3* were able to complement the CO_2-insensitive and organic/inorganic ion accumulation phenotypes of *slac1* mutants. In contrast, *SLAH2* was not able to rescue the *slac1* phenotype[4]. The high hydrophobicity of the fifth transmembrane region of SLAH2 relative to other SLAC-family proteins may explain this functional divergence between SLAH2 and other SLAC1-family proteins[4]. SLAH1 lacks the hydrophilic tail that characterizes the N-terminus of SLAC1, and which is also present in SLAH3 and SLAH2. The ability of *SLAH1* to complement the *slac1* phenotype suggests that the N-terminus of SLAC1 is not required for this function.

Together, these studies identify the first component for the function of S-type anion channels during stomatal closure in *Arabidopsis*. Much of what we know about stomatal movements comes from *Arabidopsis*, so studies on *SLAHs* in other plant species are now essential to assess whether *SLAC* function in stomatal movements is universal. The ability of *SLAHs* to complement the *slac1* phenotype illuminates the involvement of anion channels in the plasma membrane of other cells, as well as guard cells. *SLAC1* therefore provides a unique opportunity to understand not only stomatal movements, but also plant anion transport. Because stomatal closure prevents water loss, these studies should also provide a useful tool for producing drought-resistant crops by genetic engineering.

1. Schroeder, J. I. & Hagiwara, S. *Nature* **338,** 427–430 (1989).
2. Pandey, S., Zhang, W. & Assmann, S. M. *FEBS Lett.* **581,** 2325–2336 (2007).
3. Keller, B. U., Hedrich, R. & Raschke, K. *Nature* **341,** 450–453 (1989).
4. Negi *et al. Nature* **452,** 483–486 (2008).
5. Vahisalu *et al. Nature* **452,** 487–491 (2008).
6. Fan, L.-M., Zhao Z. & Assmann S. M. *Curr. Opin. Plant Biol.* **7,** 537–546 (2004).
7. Kollist, T. *et al. Physiol. Plant.* **129,** 796–803 (2007).
8. McAinsh, M. R., Brownlee, C. & Hetherington, A. M. *Nature* **343,** 186–188 (1990).
9. Pei, Z. M. *et al. Nature* **406,** 731–734 (2000).
10. Desikan, R., Griffiths, R., Hancock, J. & Neill, S. *Proc. Natl Acad. Sci. USA* **99,** 16314–16318 (2002).
11. Camarasa, C. *et al. Appl. Environ. Microbiol.* **67,** 4144–4151 (2001).
12. Xu, J. *et al. Cell* **125,** 1347–1360 (2006).
13. Schmidt, C. & Schroeder, J. I. *Plant Physiol.* **106,** 383–391 (1994).