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SEEDLING SIGNALLING

Ubiquitin ligases acting in tandem

Buried seedlings must grow both strongly, to push through soil to the surface, and fast, to reach the light as quickly as possible. A recent study finds that a pair of sequentially acting E3 ubiquitin ligases balances these conflicting imperatives.

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Sunlight may quickly dry the surface of the soil but water can remain at deeper levels, protected from evaporation. Despite being an ideal environment for seed germination, the availability of water comes at a cost; the young seedling will be in full darkness, unable to photosynthesize. Also, the aerial organs face the challenge of making their way through the considerable physical barrier posed by the soil, and emerging at the surface before the seedling's energy reserves are exhausted. Writing in *Current Biology*, Shi *et al.*[1] now show that the disparate tactics employed by the seedling to increase its chances of survival are coordinated by the tandem action of two E3 ubiquitin ligases.

During skotomorphogenesis (literally 'development in darkness') a seedling's stem (hypocotyl) grows rapidly, pushing its tip up through the soil, while the foliage (cotyledon) expands as little as possible to avoid slowing the pace of the apex to the surface. In addition, the stem curves to form an apical hook to protect the apex from scraping and damage by the soil. To conserve energy, only rudimentary synthesis of the photosynthetic apparatus takes place at this stage.

However, the physical properties of the soil through which the seedling must attempt to emerge are highly variable and changeable. For example, rain can produce hard soil crusts, while the transit of heavy machinery can compact deeper layers. When faced with a strong barrier, seedlings produce ethylene, which induces the so-called 'triple response' to increase their soil penetration ability (Fig. 1). The apical hook is reinforced (more curved), and the stem is shortened and thickened. The fast growth of skotomorphogenesis could be regarded as the seedling driving forwards in a high gear — cruising quickly but with relatively little driving force needed — and the triple response as a lower gear, slower but 'stronger'.

E3 ubiquitin protein ligases provide precise regulation of many cellular processes by ubiquitinating specific substrates to target them for rapid degradation by the 26S proteasome. The *Arabidopsis thaliana* protein CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is a RING-type E3 ubiquitin ligase whose targets are transcription factors that would otherwise terminate skotomorphogenesis[2]. The activity of COP1 is maximal in darkness and is reduced when photoreceptors in the apex of the hypocotyl perceive light as it emerges from the soil — thus allowing photomorphogenesis ('development in the light') to take over.

Physical impedance of the growing seedling triggers the synthesis of the gaseous hormone ethylene[3]. One downstream effect of ethylene is the accumulation of the transcription factor ETHYLENE INSENSITIVE 3 (EIN3)[4], which is required for the triple response[3]. In the absence of ethylene, two F-box protein components of a SCF-complex-type E3 ligase (EBF1 and EBF2) target EIN3 (and its homologue EIL1) for degradation in the 26S proteasome[5]. In the presence of ethylene, an as-yet unidentified E3 ligase (labelled X in Fig. 1) is proposed to target EBF1 and EBF2 for degradation to allow the nuclear pools of EIN3 to increase and ethylene responses to proceed.

Shi *et al.* show that mutant plants deficient in COP1 activity have a reduced ability to emerge from soils with different particle compositions[1]. The defect is apparently caused by unfolded and expanded cotyledons of *cop1* plants impeding soil penetration — much like trying to push an open umbrella through treacle. Overexpression of EIN3 in *cop1* mutants largely rescues the emergence

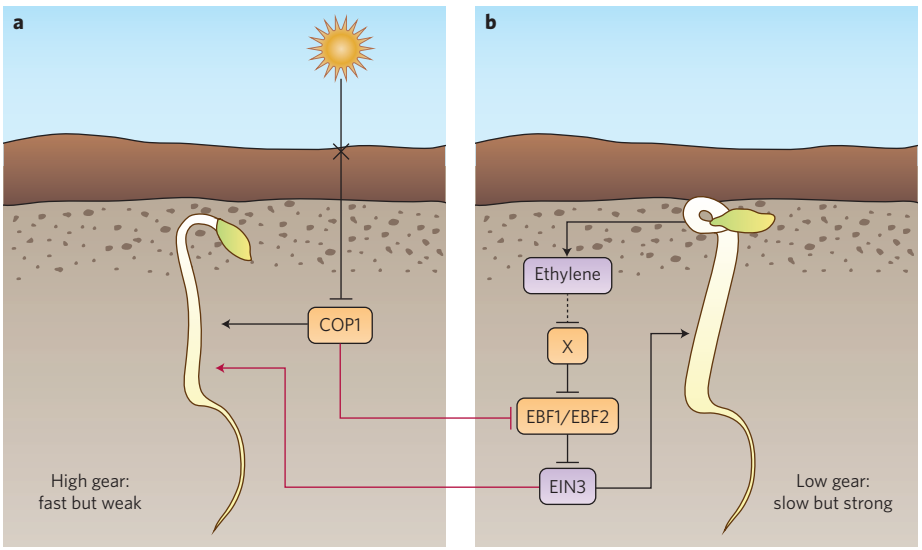

Figure 1 | A tandem of E3 ubiquitin ligases connect skotomorphogenesis and the ethylene triple response. **a**, Light is perceived by photoreceptors and inhibits COP1. Light cannot penetrate deep soil where COP1 is allowed to promote the skotomorphogenesis program, including fast hypocotyl growth. **b**, In the presence of a physical barrier (such as compacted soil), seedlings produce ethylene. This triggers the 'triple response', including slower but stronger hypocotyl growth. Both pathways are connected by COP1, which ubiquitinates the ubiquitin ligases EBF1 and EBF2. This relieves their suppression of EIN3 abundance, which in turn promotes skotomorphogenesis. Red connections indicate links identified by Shi *et al.*[1]; orange boxes are ubiquitin ligases (X, not yet identified).

and cotyledon phenotypes of *cop1*. In the mutants, genes whose expression is promoted or inhibited by EIN3 showed reduced or enhanced expression levels, respectively. COP1 stabilizes EIN3 by ubiquitinating EBF1 and EBF2 and causing their 26S-proteasome-mediated degradation (Fig. 1). In other words, thanks to COP1, in darkness there is enough residual EIN3 activity even in the absence of a strong ethylene signal.

Although the signalling pathways that maximize either speed or strength are represented as separate and sequential in Fig. 1, it is important to remember that in the plant they coexist simultaneously. The softest soil will trigger some ethylene production and triple response[3], and a seedling facing even the most compacted soil follows some aspects of skotomorphogenic growth — there would have to be a link that coordinates these pathways. Shi *et al.* show that this point of convergence occurs when COP1 interacts with EBF1/EBF2 to control EIN3. It is very likely that there are other, as-yet unknown, regulatory links, as suggested by a number of unexplained observations. For example, the enhancement of EIN3 abundance by ethylene is actually stronger in the *cop1* mutant than in the wild type, despite the fact that COP1 itself is shown to increase EIN3 abundance. Also, de-repression of cotyledon expansion and unfolding does not seem to be a phenotype of the *ein3* mutant in the presence of COP1[4], again suggesting interactions between EIN3 and other signalling components downstream of COP1.

The identification of a tandem arrangement of E3 ubiquitin ligases in this signalling system — that is, one binding ubiquitin to another and marking it for subsequent degradation — may have wider implications. The existence of an 'E3 ligase cascade' analogous to the well-characterized and widespread kinase cascade has already been suggested to control timely cell cycle transitions in humans[6]. In *Arabidopsis*, the stability of the transcription factor PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) is known to be enhanced by COP1[7]. It is intriguing to speculate that this could occur through COP1 targeting for destruction some other E3 ligase that negatively regulates the abundance of PIF3. Tandem E3 ubiquitin ligases may turn out to be a more common signalling motif than expected. ❐

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