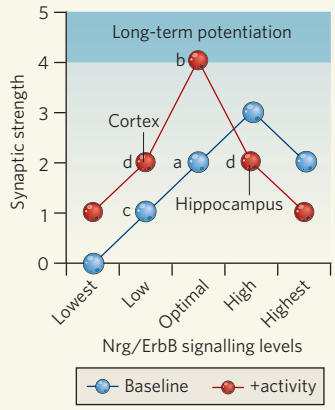

Figure 2 | An 'inverted-U model' of Nrg1 signalling. This model, based on all data to date (refs 1, 2, 5–11; L.W.R. and D.A.T., unpublished observations), attempts to resolve inconsistencies in the effects on long-term synaptic potentiation of manipulation of Nrg1 levels combined with different types of patterned stimulation. The model assumes that the initial conditions of a synaptic connection — including the local concentration of Nrg1 and ErbB signalling components, the type(s) of neuregulin expressed and the exact pattern of incoming neural activity — are all important determinants of subsequent shifts in synaptic strength from baseline activity to a long-term potentiated state. Receptor numbers either increase or decrease depending on whether the initial level of Nrg1–ErbB signalling is low, optimal or high. Lowering natural Nrg1 levels decreases potentiation, as does adding high concentrations of soluble Nrg1 to a synapse at normal signalling levels. Letters refer to panels in Fig. 1.

the recent studies[1,2] provide new perspectives and underscore how useful animal models can be, even for studying 'uniquely human' diseases of affect. They should encourage the use of further basic analyses to study the biological plausibility of genetic and environmental risk factors for susceptibility to psychiatric disorders, and thus to assess the therapeutic potential of treatment strategies. ■

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PLANT BIOLOGY

Time for growth

Ghislain Breton and Steve A. Kay

Analyses of growth kinetics in seedlings reveal exquisite connections between the signalling pathways controlled by the circadian clock and by light, and illuminate the molecular mechanisms involved.

Using infrared light imaging to observe cell elongation in the dark, Nozue and colleagues (page 358 of this issue)[1] have made a fascinating discovery — that the growth rate of plant seedlings, specifically of a structure called the hypocotyl, is differentially regulated during a day–night cycle. Intriguingly, the maximal rates occur at dawn. To determine the molecular nature of this observation, the authors designed a series of experiments using several well-characterized mutants of *Arabidopsis thaliana*, a favoured subject in experimental plant biology. Their results allow them to separate the distinct contribution of light perception and the associated responses from that of the plant's circadian clock. Furthermore, they have identified two transcription factors — mediators of the production of messenger RNA from DNA — that regulate this cyclic mode of growth.

Genetic screens of *Arabidopsis* mutants have revealed the complex nature of growth regulation in the hypocotyl; this is a small region of about 20 epidermal cells in length that lies between the root and the embryonic leaves of young seedlings, and that grows mostly through longitudinal cell expansion[2]. Many mutations in genes involved in hormonal, light-perception and circadian pathways result in short or long hypocotyls[2,3]. Measurement of hypocotyl length in constant light or constant dark is commonly used to characterize light-signalling or clock mutants. Growth in constant darkness is thought to mimic the conditions experienced by seedlings that are emerging from the soil, and reaching for light at

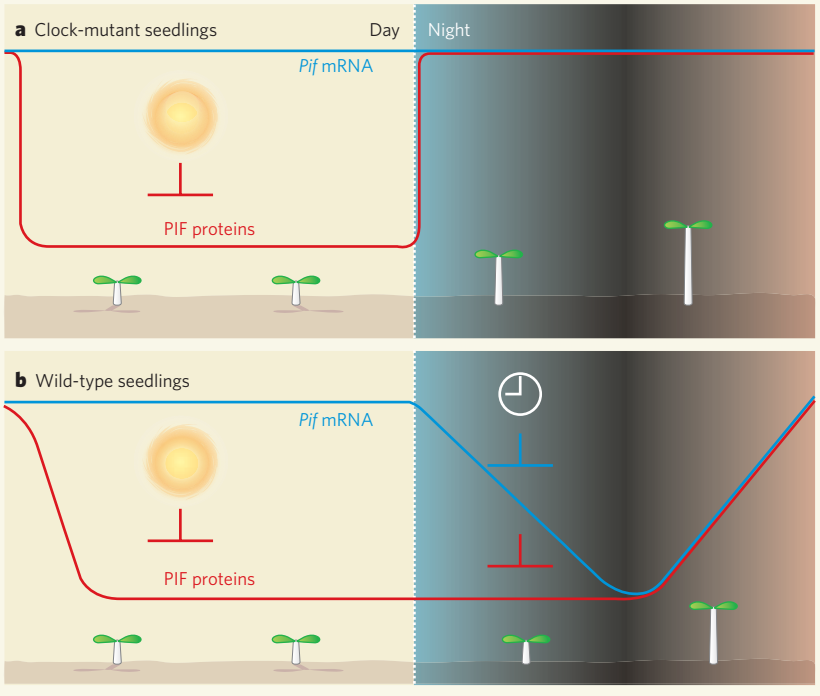

Figure 1 | From physiological observation to molecular mechanism. Nozue and colleagues' comparison[1] of hypocotyl growth rhythms in (**a**) clock mutants and (**b**) wild-type seedlings under light–dark cycles reveals the repressing effects of the circadian clock in the early night and of light during the day. **a**, Unregulated growth during the night; **b**, the normal growth pattern. Molecular studies identified two growth-promoting factors (PIF4 and PIF5), the messenger RNAs of which are normally clock-regulated (blue line) by a repressing action in the early night. In addition, the protein levels are reduced in a light-dependent manner (red line) possibly through a light-receptor-mediated mechanism. Thus, internal cues (clock) restrict transcriptional activation in the late night leading to hypocotyl elongation before dawn, whereas an external cue (sun) inhibits growth during the day by targeting the proteins for degradation. It is the coincidence of both cues that leads to the observed maximum growth at dawn.

the surface. In the dark, seedlings enter a form of development termed etiolation, in which most of the plants' resources are channelled into elongation of the hypocotyl. In contrast, seedlings grown in the light follow a different path, including the inhibition of etiolation and initiation of the greening process that enables light capture through photosynthesis[2,3].

Nozue and colleagues[1] have integrated study of these two conditions, which are normally considered separately, by measuring hypocotyl growth rate under diurnal conditions — that is, cycles of light and dark. They first noticed that, following a few days of non-consolidated growth, seedlings seem to tune their maximum growth at dawn. They showed that seedlings with specific defects in light perception had weak or no growth rhythms, suggesting that light signalling is essential for rhythmic growth under diurnal cycles.

To distinguish the role of the circadian clock on hypocotyl growth from that of light, they performed similar experiments using plants with clock defects. The output of the clock creates a temporal matrix that is used to drive overt rhythms, such as photosynthesis and protective mechanisms against cold at night, and can also serve to anticipate changes between day and night. One of the hallmarks of plant circadian clocks is their capacity to confer cycling behaviour under constant light conditions, and mutants with a disrupted clock have been used[4] to define a role for the clock in the timing of cell elongation.

Interestingly, Nozue *et al.* showed that, under conditions of diurnal cycles, maximal growth of mutants with an impaired clock occurred from dusk to dawn instead of being restricted to a few hours at dawn, suggesting that those seedlings were hyper-responsive to darkness. Additional experiments confirmed this observation, which implies that, under diurnal conditions, hypocotyl growth in wild-type (non-mutant) seedlings is partly controlled by light–dark transitions, whereas the circadian clock acts to suppress growth in the early part of the night.

What might be the molecular mechanism associated with growth control? To address this question, Nozue *et al.* carried out transcription profiling using whole-genome arrays. Taking advantage of the fact that clock mutants are hyper-responsive to darkness, they designed a strategy to find genes whose expression is associated with maximal growth rates. They identified two transcription factors — PIF4 and PIF5 (also known as PIL6), which belong to a family known as phytochrome-interacting factors (PIFs)[5] — to be good candidates as light-regulated components of the mechanism. Overexpression of each factor alone in *Arabidopsis* led to seedlings that were hyper-responsive to the dark. Furthermore, double mutants had a very short hypocotyl that didn't display any growth rhythmicity. These striking results confirm the growth-promoting nature of the PIF4 and PIF5 proteins.

Finally, two different experiments helped to determine how both genes might be regulated by light and the circadian clock. Expression-profiling experiments revealed that both are under circadian regulation and are expressed at high levels in seedlings without a functional clock (Fig. 1a). Because in wild-type seedlings the expression maxima are at dawn, and the minima are at the beginning of the night, the authors propose that, during the early night, growth suppression by the circadian clock must in part occur through transcriptional repression of the *PIF4* and *PIF5* genes (Fig. 1b). Further experiments with transgenic plants overexpressing PIF4 and PIF5 showed that the abundance of both proteins decreased in the light and increased in the dark. These results complete an elegant model of events, in which one of the essential processes involved in the reduction of growth rate after dawn is the light-dependent degradation of PIF4 and PIF5.

Questions remain, of course. Which factor negatively regulates *PIF4* and *PIF5* mRNA in the early night? Which molecular complex controls their degradation? And which genes are targeted by PIF4 and PIF5? More generally, there is the issue of whether Nozue *et al.*[1] have uncovered design principles that apply to growth regulation in other tissues, given that growth of the stem and leaf seem to be under similar control[6,7]. For the moment, however, publication of their discovery provides a considerable step forward in understanding the factors that shape the young seedling's quest for photons. ■

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CELL BIOLOGY

Caught in the traffic

Aparna Lakkaraju and Enrique Rodriguez-Boulan

In mice, deletion of the Rab8 protein disrupts organized molecular distribution to membranes of intestinal epithelial cells. Death by starvation follows, exactly as it does in humans with microvillus inclusion disease.

The gut, like other tubular structures in the body, is lined with a monolayer of epithelial cells, which forms the main interface between the external and internal environments of an organism. A defining characteristic of these cells is their polarity — that is, their apical and basolateral surfaces have different molecular compositions and different functions, allowing them to manage the different environments they face. This spatial asymmetry is achieved through sophisticated intracellular sorting mechanisms. Newly synthesized membrane proteins are packaged into specific carrier vesicles at the Golgi complex — the cell's 'post office' — and transported to their apical or basolateral destinations on the cell surface[1].

Crucial controllers of polarized membrane trafficking are a family of enzymes known as Rab GTPases, which are involved in vesicle targeting, docking and fusion. On page 366 of this issue, Sato *et al.*[2] report an unexpected function for one particular Rab GTPase, Rab8 — involvement in vesicle transport to the apical membrane of gut epithelial cells. These findings contribute to a better understanding of both the formation of the apical surface in epithelial cells and the pathogenesis of microvillus inclusion disease — a rare congenital human disorder for which no causative gene is known.

The most abundant type of gut epithelial cell, the enterocyte, has many finger-like projections at its apical surface called microvilli, which are rich in digestive enzymes known as hydrolases. These enzymes break down food into individual molecules (such as amino acids and sugars), which the enterocyte then transports into the blood.

Sato *et al.*[2] generated mice that lack the *Rab8* gene and found that these animals develop a lethal condition in which a type of vacuolar compartment rich in microvilli forms and apical hydrolases are mislocalized to these vesicles. The absence of hydrolases from their normal location at the apical surface resulted in death by starvation a few weeks after birth. This outcome, as well as pathological features of the condition, resembles that of human microvillus inclusion disease[3].

The work of Sato *et al.* is pioneering because it is the first study to examine Rab8 function in epithelial trafficking *in vivo*, and the team's observations raise some truly intriguing questions. First, why are only apical proteins affected by Rab8 deficiency, with the distribution of basolateral proteins being unchanged?