is that the high metabolic cost of the Na^+ and Ca^{2+} pumps in darkness results from the rod being depolarised: when stimulated by light, the rod hyperpolarises and Na^+ and Ca^{2+} channels close. With their closing, the extent of which is proportional to light level, the influx of Na^+ and Ca^{2+} ions declines, fewer ions need to be pumped out to maintain steady state, and energy expenditure plummets. Even though rods and cones are similarly expensive in the dark — and for similar reasons [11,12] — illumination of cones never closes all of the outer segment channels, even at highest light intensities [13]. This means that in bright light the Na^+ pumps of cones need to work harder to maintain steady state. In addition, recent experiments indicate that the biochemistry of transduction uses more ATP in cones than in rods. This extra energetic cost makes cones more 'expensive' than rods.

This remarkable fact — that rods are cheaper than cones — has profound implications for our understanding of the evolution of vision. As is becoming increasingly clear, the energy costs associated with maintaining neural tissues are significant [5,14,15] and have been a major selective pressure during the evolution of nervous systems, not the least the senses [3,6,15]. As Darwin certainly would have appreciated, better performance is likely in larger sensory organs with greater numbers of neurons. But in natural selection this benefit must always be weighed against the cost, since more neurons consume a greater proportion of the animal's total energy budget. Thus, the cheaper cost of running rods in bright light may explain why the vertebrate duplex retina evolved, why in most mammals (even diurnal ones) the rods greatly outnumber the cones, and why in diurnal species these relatively few cones are usually restricted to a small region of the retina (the fovea). By having two sets of photoreceptors adapted to different light levels, with one set (comprising the majority of receptors) consuming little energy for half of the day, the total cost and performance of vision over a 24-hour period can be optimised.

Energy arguments may also explain why vertebrate photoreceptors hyperpolarise in response to light. Insect photoreceptors also consume a considerable amount of ATP in darkness, and for the same reason as a rod or cone: to bias the synaptic transmitter release into a sensitive region of its range. But in contrast to rods and cones, insect photoreceptors depolarise in response to light and the energetic costs increase with light intensity [6] (Figure 1B). The benefit they gain from this investment is the ability to resolve rapid contrast changes in bright light [6]. But now, seen in the light of photoreceptor costs, it is perhaps not surprising that nocturnal arthropods, which have evolved elaborate strategies to optimise vision at night [16,17], also restrict retinal illumination during the day, by employing pupil mechanisms [18], by reducing the volume of their phototransductive membranes before dawn [19] or by simply retreating to a dark hide.

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Leaf Development: Untangling the Spirals

How do plants position their leaves and flowers around the stem in such regular patterns? Auxin is well established as an essential regulator. Now, the modification of a structural cell wall component is shown to have a dramatic impact.

Naomi Nakayama and Cris Kuhlemeier

New leaves and flowers form in ordered patterns, a process called phyllotaxis [1,2]. The most common type is spiral phyllotaxis, in which the lateral organs are initiated in an equiangular spiral with a higher order organization of overlapping spirals in opposite directions. Phyllotactic patterning takes place in the shoot apical meristem, a dome of tissue at the tip of the stem, which contains stem cells that supply cells for continuous organogenesis. New lateral organs always emerge at the flank of the meristem in the peripheral zone, where

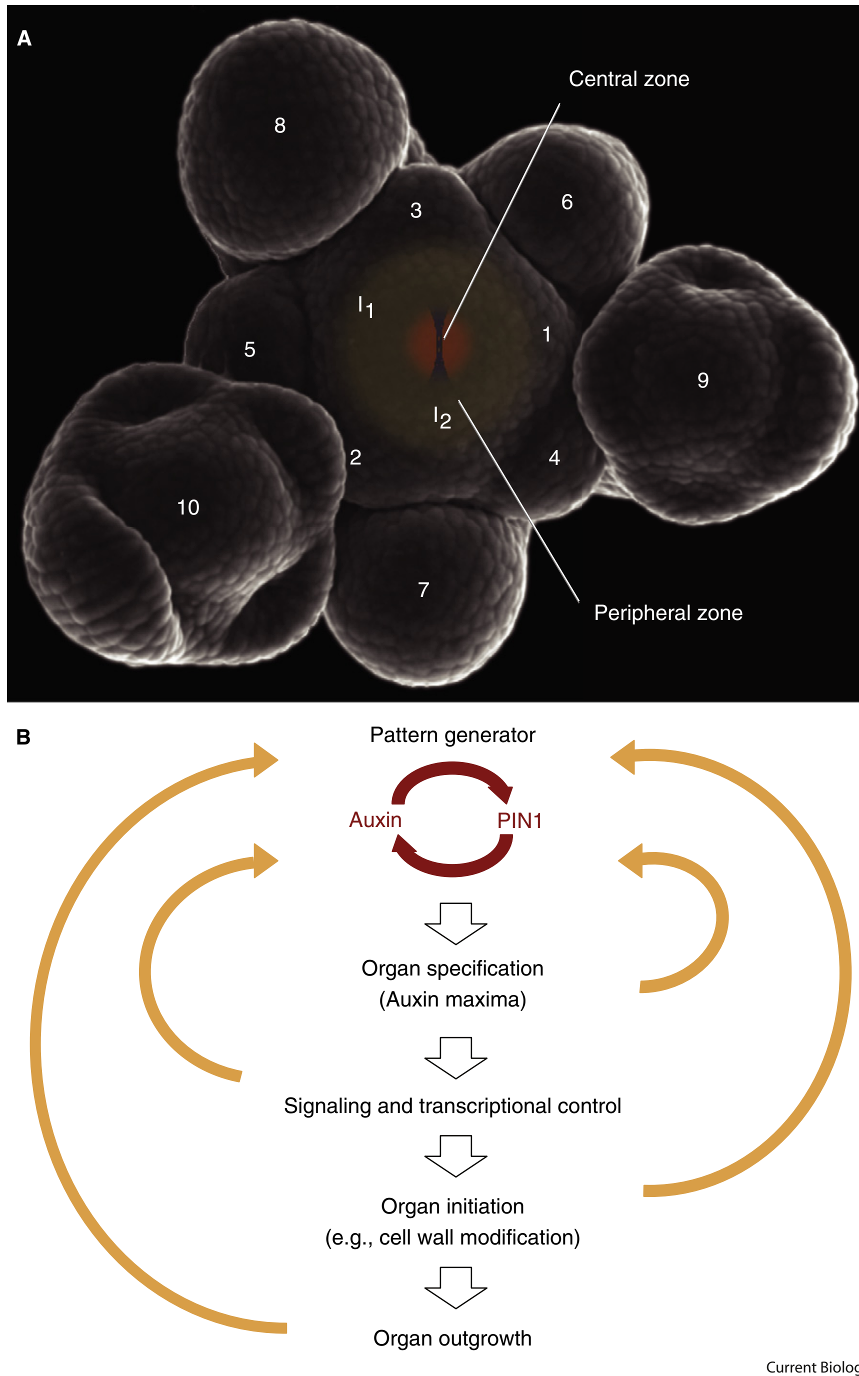

Figure 1. Pattern generation by auxin transport and feedback loops.

(A) An *Arabidopsis* inflorescence shoot apical meristem. The central zone contains the stem cells; their descendants in the peripheral zone are competent to form organs. The individual flowers (labeled 10 through 1 in order of appearance) form in a spiral phyllotaxis with divergence angles of approximately 137°. I_1 and I_2 predict the positions of incipient primordia (scanning electron micrograph by Soazig Guyomarc'h). (B) A conceptual representation of auxin-based regulation of phyllotaxis. A positive feedback loop between auxin and its transporter PIN1 creates a local auxin maximum and depletes auxin around it. The auxin maximum induces the activation of a signaling cascade, which in turn induces local wall modification. Wall modification is a prerequisite for localized outgrowth. The orange arrows indicate hypothetical feedback controls at all levels. Peaucelle *et al.* [13] demonstrate that the wall modifying enzyme PME is necessary for organ formation and that PME misexpression alters phyllotaxis.

cells are competent for differentiation and responsive to organogenic cues (Figure 1A). How plants can position their organs with such mathematical precision has tickled curious minds past and present, and much effort has been made to elucidate the mechanisms behind the pattern formation.

The heart of the phyllotactic patterning system is a pattern generator. Its primary output is a spatially and temporally restricted signal, which, through a cascade of molecular events, causes organ formation (Figure 1B). Evidence has accumulated that the phytohormone auxin is the primary output signal and that a positive feedback loop between auxin and its transporter PIN1 can pattern phyllotaxis [3–6]. Auxin accumulation precedes leaf specification and is necessary and sufficient for leaf initiation; suppression of auxin transport abolishes organogenesis and results in naked, radially symmetric meristems, while local application of auxin is sufficient to restore it [4,7,8]. Reactivation of auxin transport can re-establish normal phyllotaxis from the organless meristem, indicating that the auxin/PIN1 loop is capable of *de novo* pattern formation [8]. The auxin maxima are interpreted by transcriptional regulators and signaling molecules, which translate the primary output into activation of the organ development program.

The simple concept of a pattern generator with downstream signaling and effector genes is complicated by the feedbacks that operate in the system. A critical feedback is the negative influence of pre-existing primordia: they inhibit new organogenesis in their vicinity. Developing organs are thought to be sinks for auxin; they help prevent auxin accumulation around them and thereby enable pattern formation [4]. Other feedbacks are necessary both to stabilize the system and to enable it to respond to external stimuli. For instance, auxin-dependent transcription factors not only induce downstream genes but also feedback on the pattern generator by regulating the expression of PIN1 [9]. In principle, any factor involved in organ initiation or formation could alter phyllotaxis.

Further downstream effectors can also feedback on the central pattern

generator. Changes in the structure or composition of the cell wall, which are conventionally regarded as housekeeping functions, have been shown to be able to affect organ position. Plant cells are under osmotic pressures of five atmospheres or more. In order for the cell not to explode, a rigid cell wall must be in place to withstand this pressure. At the same time, plant cells must be able to grow, which is accomplished by ordered loosening of the structural polysaccharide network of the wall. Expansins and other wall-associated proteins are implicated in altering the cohesion between cellulose microfibrils and hemicellulose [10]. Expansin is expressed before leaf initiation and localized activation of expansin can induce ectopic leaf formation. The resulting leaf influences the position of subsequent primordia and can even reverse the direction of the phyllotactic spiral [11,12].

In a recent issue of Current Biology, Peaucelle *et al.* [13] introduced a new player — pectin. Pectins form a hydrated gel phase in the cell wall and are implicated in adhesion between cells and control of wall porosity [14]. They are deposited in the extra-cellular space in the methylesterified form. Subsequent removal of the methylester group by the enzyme pectin methylesterase (PME) results in structural stabilization and wall stiffening in the presence of bivalent cations, or degradation and wall loosening in acidic conditions [15,16]. Now, Peaucelle *et al.* [13] have shown that this pectin modification has instructive roles in flower formation in *Arabidopsis*. First of all, de-methylesterification of pectins precedes primordia initiation. Furthermore, overexpression of a PME inhibitor completely yet reversibly blocks organogenesis and leads to the formation of naked meristems. And local application of PME induces ectopic organ formation. It should be noted, however, that the commercial enzyme preparation may contain contaminating wall activity (e.g., expansin). This caveat does not apply to the final observation: overexpression of a PME gene results in irregular phyllotaxis. Thus, pectin modification clearly affects organ initiation and positioning.

This work leads to a number of exciting questions. First, how does the pectin modification enhance organ initiation? Does it alter cell wall rigidity and, if so, does it soften or stiffen it? To answer this question, more information about cell walls in the meristems, such as pH and Ca^{2+} distributions, is required. Does pectin de-methylesterification interact with expansins? By loosening the mesh of carbohydrate polymers, they could expose other wall components to the action of modifying enzymes, as might happen during fruit ripening [17]. In addition, the PME-mediated reaction itself acidifies the local apoplastic environment [18]. Interestingly, auxin accumulation also triggers rapid acidification of the extra-cellular space but through the action of an outwardly directed proton pump. Thus, in both cases, local cell wall acidification might be the key activator of organogenesis.

A further thought-provoking issue concerns the relationship between wall-modifying factors and auxin-based phyllotactic patterning. Could the roles of auxin and PME be reversed? Could PME- and expansin-induced local tissue softening be at the heart of a mechanics-based pattern generator with auxin maxima forming in its wake? Such biophysical patterning mechanisms have been postulated ever since the dawn of developmental biology. The meristem could be patterned through mechanical constraints operating at the tissue level. The mechanical stress that growing primordia exert on the meristem would inhibit organ formation around them and generate a regular pattern of organogenesis. In some scenarios, bulges arise analogous to the way a pattern of wrinkles arises in our skin after an extended soak in the bathtub [19].

Indeed, pectin modification has the potential not just to induce organ initiation but also to direct organ positioning. But does this mean that the pattern generator is based on mechanics rather than auxin? The data presented by Peaucelle *et al.* [13] (Figure 1) suggest that localized PME-induced de-methylesterification occurs after the auxin maxima are established [4,20]. Thus, a more conservative interpretation is that auxin maxima set up wall modification, which in turn feeds back to the auxin-based pattern generator. The important question is where in the auxin patterning loop does it connect to? Does the modification of the cell wall influence auxin prior to organ specification or during organ development? In the midst of all these feedback loops, the exact role of each player in phyllotactic patterning is not easy to untangle, but curious minds will surely continue to take on the challenge of solving this great puzzle.

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Meiosis: DDK Is Not Just for Replication

How is the chromosome segregation machinery modified to segregate homologs during meiosis I? The Dbf4-dependent Cdc7 kinase (DDK) has now been identified as a key regulator in this process.

Adele L. Marston

Meiosis is a specialized cell cycle that generates haploid gametes from a diploid cell. This is achieved through two consecutive chromosome segregation events, meiosis I and meiosis II, which follow a single round of DNA replication. Meiosis I is a unique kind of segregation event because homologs are separated, in contrast to mitosis or meiosis II during which sister chromatids are separated (Figure 1). Recent reports by the Hollingsworth, Ohta and Zachariae labs [1–4] have established the Dbf4-dependent Cdc7 kinase (DDK), hitherto famous for its role in the initiation of DNA replication, as being important for setting up the so-called reductional pattern of chromosome segregation during meiosis I in budding yeast.

The segregation of homologs during meiosis I requires three modifications to the chromosome segregation machinery (Figure 1) [5]. First, meiotic recombination generates chiasmata, which hold homologs together owing to the sister-chromatid cohesion on chromosome arms. Second, sister kinetochores attach to microtubules from the same spindle pole body (monoorientation) during meiosis I, rather than opposite spindle pole bodies (biorientation) as they do in mitosis and meiosis II. Third, the cohesion that holds sister chromatids together is lost only from chromosome arms during meiosis I and is protected around centromeres to allow the accurate segregation of sister chromatids during meiosis II. In recent years, a basic molecular explanation for how these modifications are set up in budding yeast has emerged, but how they are coordinated with each other has remained less clear. DDK is now shown to control three events that promote meiosis I segregation: (1) the initiation of DNA replication, (2) the initiation of meiotic recombination, and (3) the recruitment of monopolin to kinetochores, which is required for monoorientation. The finding that DDK controls multiple processes necessary to prepare the chromosomes for reductional segregation during meiosis I [1–4] implicates DDK as a global coordinator of the meiosis I program.

DDK is a Ser/Thr kinase whose activity depends on the association of the constitutive Cdc7 catalytic subunit with a regulatory protein, Dbf4 [6], the levels of which are highest during metaphase I [4]. In vegetative cells, DDK phosphorylates components of the replicative complex, thereby triggering DNA replication. DDK also controls DNA replication during meiosis. Using an ‘analog sensitive’ version of the Cdc7 kinase (*cdc7-as*), which has an enlarged ATP-binding pocket and can be specifically inactivated by the addition of purine analogs to the medium, replication was shown to be greatly delayed, although it eventually occurred [7]. In a different approach, depletion of Dbf4 almost completely prevented DNA replication [8]. Therefore, DDK plays an important role in meiotic DNA replication, and an essential role cannot be ruled out as DDK may not be completely inactivated in these experiments.

After undergoing DNA replication, *cdc7-4* (a temperature-sensitive allele) and *cdc7-as* mutants arrest in prophase I [7,9]. To analyze the requirement for DDK in later meiotic events, the Ohta [2], Hollingsworth [3] and Zachariae [4] groups made use of the *bob1* allele. The *bob1* allele encodes a point mutation in a component of the Mcm complex (thought to constitute the replicative helicase), and completely bypasses the requirement for DDK in DNA replication [10]. Use of the *bob1* mutation relieved the delay in DNA replication caused by DDK inactivation [2–4]. However, in the Ohta [2] and Hollingsworth [3] studies, the *bob1* mutation did not bypass the prophase I arrest of *cdc7Δ* (a mutant lacking the *cdc7* gene) or *cdc7-as* mutants. The prophase I arrest appears to be due to a failure in inducing transcription of *NDT80* [2,3], a global meiotic transcriptional regulator that is required for exit from prophase I and progression into meiosis I [11]. Indeed, ectopic expression of *NDT80* in *bob1 cdc7-as* cells allowed progression beyond prophase I [3]. In contrast, the Zachariae group [4] observed no defect in either meiotic gene expression in *bob1 cdc7Δ* cells or the ability of *bob1 cdc7Δ* cells to exit prophase I. It was suggested [4] that replication defects activate checkpoints that cause the prophase I arrest and block *NDT80* transcription [12]. However, inactivation of various checkpoint genes did not relieve this arrest [3], so it is unclear whether DDK has a role in prophase I exit and, if so, whether this is direct or indirect.

DDK is required for the initiation of meiotic recombination [1,2,4,7]. Specifically, DDK inactivation abolishes the formation of double strand breaks (DSBs) by the Spo11 endonuclease [13]. DDK promotes meiotic recombination, in part, by phosphorylation of one of the Spo11 accessory factors, Mer2, on Ser29 [1,2]. The S-phase, cyclin-dependent kinase, Cdc28–Clb5,6 (S-CDK), which is required for DNA replication, also phosphorylates Mer2, but on Ser30 [14]. Furthermore, DDK-dependent phosphorylation of Mer2 on Ser29 is enhanced by prior S-CDK-dependent phosphorylation on Ser30 [1]. These phosphorylation events are essential