enter or leave the active site as H^+, and electrons must do so in single, long-range transfers. For the enzyme, the five-coordinate, 16-valence-electron, low-spin Fe(II) would be the first guess for the primary target of H_2 binding, the paradigm being Kubas' compound (*4*), in which sideways coordination of H_2 uses the metal's d orbitals for backbonding with the σ_u antibonding orbital. Nonetheless, the inhibitor CO binds at the Ni site of the enzyme (*5*), and calculations indicate that H_2 binding is equally likely at Ni or Fe (*6*).

There is no evidence that Fe exists in any other state than diamagnetic Fe(II) during catalysis, so how could the sixth site on the Fe remain empty, and what role does the Fe perform? In the model complex, before reaction with H_2, the sixth site is occupied by a solvent MeCN molecule, yet the location of the H^- ligand in the product does not prove that Fe is also the site for transient binding of H_2. No H_2 adduct of hydrogenases has ever been detected, suggesting that H–H cleavage occurs immediately upon binding; the proton transfers to a nearby base, most likely a terminal cysteine S atom.

In [FeFe]hydrogenases, a pendant (overhanging) amine lies within 3.5 Å of the Fe atom likely to bind H_2, and the best functional analogs, which interestingly contain a single Ni atom, also depend on a pendant base (*7*). The NiFe analog requires a strong base, methoxide, to abstract H^+ and form the H^- complex (highlighted in blue in the figure). The need for such a strong base reflects both the inefficient bimolecular nature of this reaction and the importance of synchronous proton transfer.

The H^- complex, structurally characterized by neutron diffraction and nuclear magnetic resonance spectroscopy, is equivalent to the enzyme state known as Ni-R, formulated as a hydrido Ni^{II}-Fe^{II} species. Ogo *et al*. focus our attention on the Fe by unambiguously showing that Fe is the site of H^- binding after heterolytic cleavage and also establish its viability for the same function in the enzyme. The analogous product that formed with a similar complex, in which Fe is replaced by ruthenium (Ru), is a metal-metal bonded Ru(I)-Ni(I) species with a bridging proton located closer to the Ni (*8*). The $Ni(N_4S_4)$ geometry is unchanged when the Fe–H bond is formed, although the Ni–Fe distance contracts from 3.32 to 2.79 Å. The retention of ligand geometry about Ni argues against its being directly involved in the initial activation step.

In the enzyme, oxidation of Ni-R gives the intermediate Ni-C—a Ni(III) species in which H^- is coordinated to the Ni but in a bridging position with respect to Fe, as shown by EPR studies (*9*). If H^- formed first at Fe, one-electron oxidation might require that it relocate to the Ni, which can be formally oxidized to Ni(III), unlike the Fe atom, which is difficult to oxidize above Fe(II) because it bears a CO ligand. In the next stage, the H^- is oxidized to H^+. Departure as H^+ necessitates synchronous reduction to Ni(I) or to an intermediate with Ni(II) and Fe(I). Such a Ni(I) species has not been established as an intermediate but may be analogous to a state known as Ni-L produced upon low-temperature illumination (*9*). Whatever the nature of this intermediate, its one-electron oxidation completes the cycle.

Most H^- transfers in biology use NADH (dihydronicotinamide adenine dinucleotide). In contrast, transition-metal H^- ligands such as that reported by Ogo *et al*. can be generated and activated by successive electron transfers to increase hydridicity for more demanding reactions. The H^- ligand adds "hidden" two-electron capacity to a d metal, and we are likely to see many more examples.

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PLANT SCIENCE

Jack of All Trades, Master of Flowering

A sugar derivative links nutrient availability in plants to the control of flowering.

Jonas Å. H. Danielson and Wolf B. Frommer

Artists and scientists have long pondered the beauty and mystery of flowers and their origins. To Maurice Maeterlinck, the Nobel laureate in literature, the most striking feature of plants was the diversity of flowers, organs that evolved to enhance sexual reproduction (*1*). Charles Darwin was fascinated with the rapid expansion and dominance of flowering plants (angiosperms) in the late Cretaceous, a phenomenon he called "the abominable mystery" (*2*). Along with the evolution of flowers came the need for plants to trigger flowering at the right time. On page 704 of this issue, Wahl *et al*. report that an internal cue—the sugar metabolite trehalose-6-phosphate (T6P)—helps to ensure that flowering occurs at the time optimal for successful reproduction (*3*).

The evolution of flowering plants accelerated greatly ~100 million years ago with the development of flowers and their role in recruiting animals to help distribute pollen and seeds. The daily and seasonal timing of flowering evolved to ensure sufficient energy and nutrients to produce vigorous and healthy seeds (*4*, *5*). In 1936, the Russian scientist Mikhail Chailakhyan proposed that a mystery substance acts as a mobile signal that underlies flowering. Analogous to a morphogen—a mobile substance that helps to create asymmetry during development and patterning of organs in animals—he named this substance "florigen." Since then, the nature of florigen remained an enigma. In the past decade, research on the model flowering plant *Arabidopsis thaliana* has led to the widely accepted hypothesis that a protein called Flowering Locus T (FT) acts as a florigen (*4*, *5*). FT production is initiated in leaves in response to changes in the photoperiod (the relative lengths of the light and dark periods), determined by light receptors and the plant's internal circadian clock. FT then translocates, through vascular cells comprising the phloem, to the shoot apical meristem, where it triggers flowering. Although FT is the best-characterized trig-

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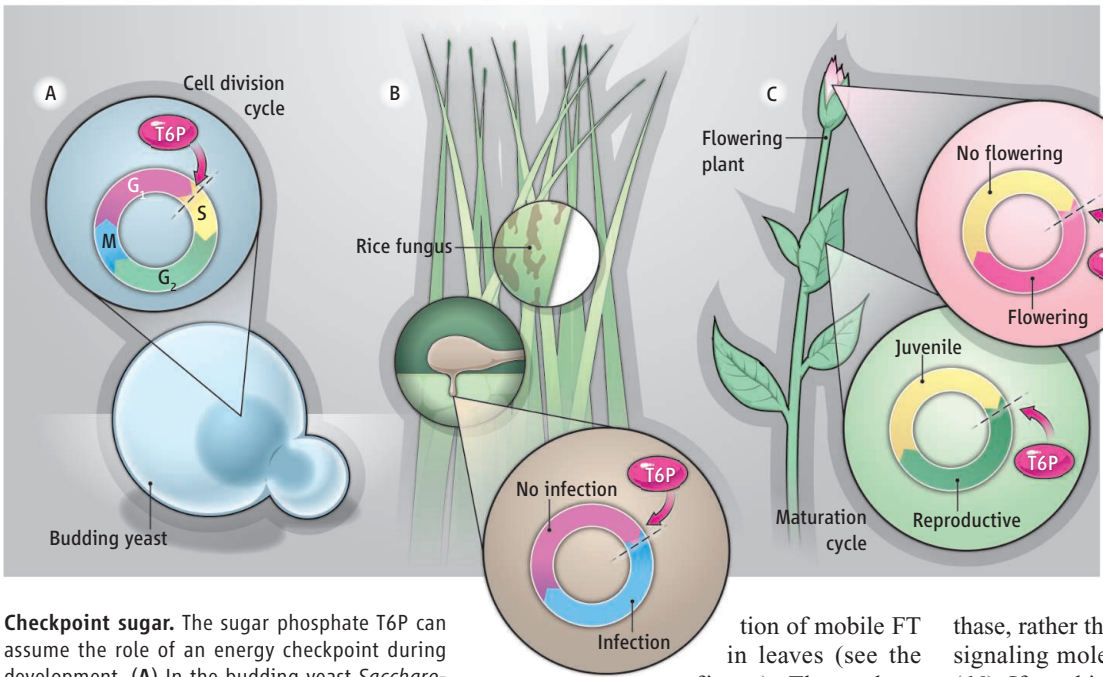

Checkpoint sugar. The sugar phosphate T6P can assume the role of an energy checkpoint during development. (**A**) In the budding yeast *Saccharomyces cerevisiae*, the decision to proceed through cell division is controlled by T6P (*11*). (**B**) The rice blast fungus *Magnaporthe oryzae* uses T6P to sense the environment and initiate infectious growth as a response to the increased sugar concentrations in the plant cell. (**C**) T6P regulates flowering both in the leaf and in the shoot apical meristem.

ger for flower development, day length is but one of several cues affecting flowering regulation. Other pathways, such as vernalization (prolonged cold treatment) and age, as well as substances, particularly sugars and the plant hormone gibberellin, also have been implicated in floral induction. Thus, the transition to flowering is complex and involves the convergence of multiple signals onto the flowering gene circuitry (*6*). In the face of this complexity—which is probably key to evolutionary bet hedging on the part of the plant (*7*)—we still lack an understanding of fundamental components of the process of floral regulation, specifically how the nutritional status, which is key to the production of viable seed, affects this transition.

Wahl *et al*. show how the plant's metabolic status may feed into the developmental decision to flower through the sugar phosphate T6P. Trehalose, while present at low amounts in most plants, is a jack of all trades: Insects use it as an energy source; worms and some plants use it as an osmolyte; and it protects enzymes from dehydration (trehalose stabilizes enzymes, for example, when water is scarce) (*8*). Wahl *et al*. reveal that T6P is sufficient to trigger flowering in *Arabidopsis* when it is produced in the shoot apical meristem and that T6P contributes to the production of mobile FT in leaves (see the figure). The authors show that in leaves, the T6P biosynthetic pathway has a gating role for the photoperiod pathway—that is, T6P allows the plant to mature from a juvenile stage to a reproductive, flower-producing stage. Because sucrose can trigger flowering, and because T6P concentrations correlate strongly with sucrose availability (*9*), T6P creates a link between the carbohydrate status of the leaf and the FT-flowering pathway. This link provides a possible means to ascertain that flowering is initiated when sufficient resources are expected to be available (energy reserves and nutrients) to complete the plant's reproductive life cycle. Independent of this function, T6P also affects the transition to flowering directly at the shoot apical meristem. Wahl *et al*. observed that in the meristem, the action of T6P partially overlaps with the age-related signaling pathway that involves miR156, a microRNA that represses flowering.

The role of a unique and low-abundance sugar phosphate in decision-making in plant development is evolutionarily interesting. That T6P is a key regulator of developmental decisions in plants and fungi (*10*) points to the possibility of an evolutionarily conserved and ancient function in developmental transitions. Information on energy and nutritional status—whether conditions are such that enough reserves will likely be available for completion of flowering and seed production—is likely key for the decision to flower by the plant. It may be that plants use checkpoints in this process, analogous to the role of checkpoints, in the decision for cells to divide. Perhaps T6P, acting as a proxy for energy status, has to increase to a concentration that meets the checkpoint requirement (*11*). When multiple criteria are met (plant size, energy status, and favorable conditions), florigen is produced and exported from the leaf. Although T6P may act as an allosteric effector—that is, by binding to a regulatory protein and thereby changing its activity—analysis of T6P synthesis in the rice blast fungus *Magnaporthe oryzae* indicates that the biosynthetic enzyme T6P synthase, rather than its product T6P, is the key signaling molecule in fungal development (*10*). If so, this would be similar to the idea that hexokinase has a dual function as an enzyme and glucose sensor (*12*).

It remains unclear exactly how plant sucrose affects the concentration of T6P, or how T6P or T6P synthase act mechanistically as a signal. The findings of Wahl *et al*. will enable the unraveling of T6P signaling networks and further exploration of how various signals are integrated to control flowering. In many organisms, T6P serves as a stress signal in cold temperatures and during drought. It is possible that stress-induced T6P production in plants could override repressive signals and trigger premature flowering. T6P may thus not only act in gating checkpoints to optimize plant reproduction, but under less favorable conditions, it may trigger premature flowering in a last-ditch effort to produce descendants.

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