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**Role of the Arabidopsis Glucose Sensor HXK1 in
Nutrient, Light, and Hormonal Signaling**

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In contrast to the role of LRP1 in the liver (23), direct cellular lipoprotein uptake is apparently not involved. Rather, this atheroprotective effect of LRP1 in the vessel wall seems to be due mainly to its role in controlling PDGFR-dependent signaling pathways and other mechanisms that increase SMC proliferation and migration (fig. S3)—events that accelerate the progression of atherosclerotic lesions in the presence of predisposing risk factors, such as elevated plasma cholesterol.

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Role of the *Arabidopsis* Glucose Sensor HXK1 in Nutrient, Light, and Hormonal Signaling

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Glucose modulates many vital processes in photosynthetic plants. Analyses of *Arabidopsis glucose insensitive2 (gin2)* mutants define the physiological functions of a specific hexokinase (HXK1) in the plant glucose-signaling network. HXK1 coordinates intrinsic signals with extrinsic light intensity. HXK1 mutants lacking catalytic activity still support various signaling functions in gene expression, cell proliferation, root and inflorescence growth, and leaf expansion and senescence, thus demonstrating the uncoupling of glucose signaling from glucose metabolism. The *gin2* mutants are also insensitive to auxin and hypersensitive to cytokinin. Plants use HXK as a glucose sensor to interrelate nutrient, light, and hormone signaling networks for controlling growth and development in response to the changing environment.

Glucose is a universal nutrient preferred by most organisms and serves fundamental roles in energy supply, carbon storage, biosynthesis, and carbon skeleton and cell wall formation. The ability to sense glucose signals is important for organisms as diverse as *Escherichia coli*, yeast, flies, mammals, and plants (1–5). In contrast to the extensive information available on glucose sensing and signaling mechanisms in

bacteria and unicellular eukaryotes (1, 3), the importance of glucose as a direct and central signaling molecule in multicellular animals and plants has only recently been recognized (2, 4, 5). In plants whose lives revolve around sugar production through photosynthesis, glucose has emerged as a key regulator of many vital processes, including germination; seedling development; root, stem, and shoot growth; photosynthesis; carbon and nitrogen metabolism; flowering; stress responses; and senescence. (4). However, it remains unclear how plants integrate environmental factors and internal sugar signals to modulate growth and development.

In *E. coli*, yeast, mammals, and plants, hexokinases (HXKs) and other sugar kinases are the most ancient and evolutionarily conserved sugar sensors (1, 3, 4). The *Arabidopsis* genome encodes two HXK and four HXK-like (HKL) genes (6–8). To determine the precise physiological functions of HXKs in plant

growth and development, we designed a two-step genetic screen to isolate loss-of-function HXK mutants in *Arabidopsis*. We first screened for *glucose insensitive (gin)* mutants that overcome development arrest on a 6% glucose Murashige and Skoog (MS) medium (6, 9). We then used a specific HXK antibody to identify two putative HXK mutants (*gin2-1* and *gin2-2*) (Fig. 1A). The mutations were mapped to chromosome IV between the cleaved amplified polymorphic sequences marker RPS2 and the simple sequence length polymorphism marker nga 1139, where *HXK1* is located (6). Both *gin2* mutants are recessive (table S1). DNA cloning and sequencing identified the molecular lesions of these two HXK1 mutants: a nonsense mutation (Q432*) in *gin2-1* and a missense mutation (G416A) in *gin2-2* (Fig. 1B). The result was consistent with reduced *HXK1* but not *HXK2* transcripts in *gin2-1* (Fig. 1C), because nonsense mutations often destabilize mRNA. The predicted truncated HXK1 protein was below detectable levels in *gin2-1*, indicating that this is a HXK1 null mutant (Fig. 1D). The antibody to HXK detected transiently expressed *Arabidopsis* HXK1 and HXK2 proteins equally well (Fig. 1D) (10), but HXK2 protein did not accumulate. Low HXK2 protein accumulation also occurred in a 35S::HXK2 transgenic line (6) (Fig. 1D). The *gin2-1* mutant was complemented by a 35S::HXK1 transgene on a 6% glucose MS medium (Fig. 1E). The glucose-insensitive phenotype displayed by the *gin2* mutants indicated that endogenous HXK2 and HKL expression cannot compensate for the loss of HXK1 function in the seedling glucose response assay. Subsequent experiments were mostly carried out with *gin2-1*.

To unravel the physiological functions of HXK1 in glucose sensing without the use of exogenous glucose, we grew wild-type and *gin2-1* plants under different light conditions in a growth chamber. High light intensity can boost photosynthesis, sugar production, and plausibly glucose signaling in a physiological

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Fig. 1. *Arabidopsis* HXK1 has a predominant role in glucose signaling. (A) The *Arabidopsis gin2-1* and *gin2-2* mutants. WT, wild-type. (B) *HXK1* gene structure and mutations. bp, base pairs. (C) *HXK1* and *HXK2* transcripts. The *Arabidopsis* α -tubulin gene (*TUB*) (accession no. M84697) (25) is a control. (D) *gin2-1* is a HXK1 null mutant. HXK proteins from wild-type and mutant (upper) and transgenic (35S::*HXK1* and 35S::*HXK2*, lower) plants were shown by immunoblot analysis. Higher amounts of protein were loaded to detect endogenous HXK proteins (upper); 10-fold lower amounts of protein were examined from the 35S::*HXK1* transgenic plants. Transiently expressed HXK1 and HXK2 proteins with the hemagglutinin (HA) epitope tag were detected by immunoprecipitation (middle). (E) Complementation of *gin2-1* by 35S::*HXK1* (*HXK1/gin2*). Plants were grown on a 6% glucose (Glc) or mannitol (Man) MS medium.

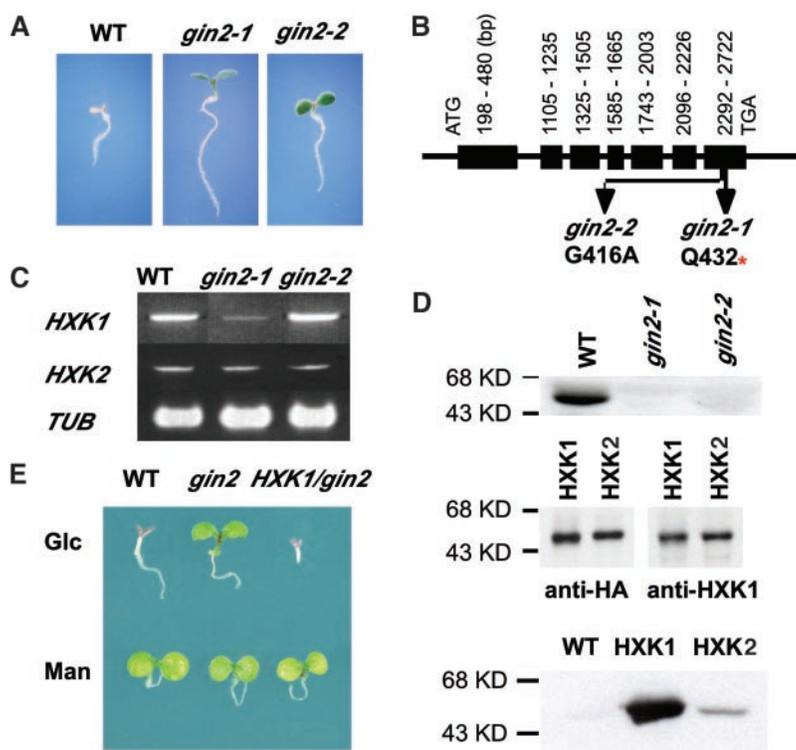
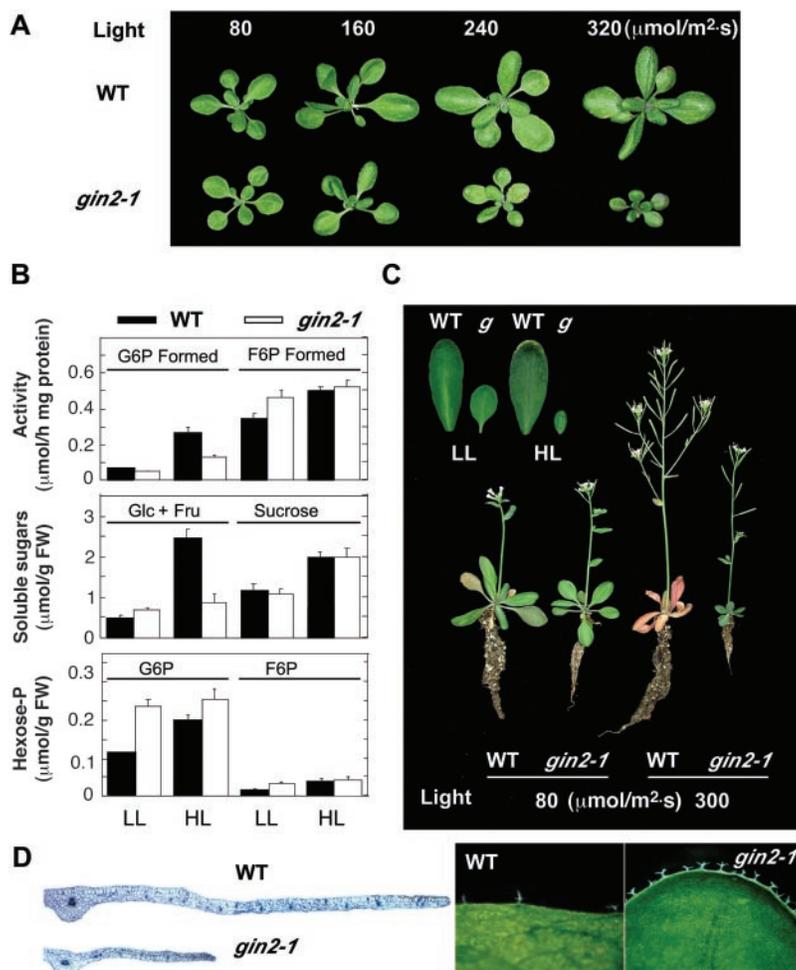


Fig. 2. The *gin2-1* null mutant defines physiological functions of HXK1. (A) The *gin2-1* phenotype is enhanced by increased light intensity. (B) The *gin2* phenotype is not attributable to leaf hexose phosphorylation activity or hexose-phosphate content. Fru, fructose; FW, fresh weight; HL, high light conditions; LL, low light conditions. (C) Diverse growth defects in the flowering *gin2-1* mutant. The inset shows the same-age sixth leaves of wild-type and *gin2* (*g*) plants grown under low or high light conditions before senescence. (D) Profiles of trichome densities and leaf cell size.



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context in plants. The results showed that increased light intensity resulted in marked phenotypes in *gin2-1* mutant plants (Fig. 2A). For example, the leaves remained small and dark green with little cell expansion under high light conditions, although the same number of leaves appeared at the same time as in the wild type, indicating that the developmental program of leaves was not delayed in *gin2-1* plants. Previous studies showed that HXK1 overexpression inhibits growth (6, 7). The observed growth defects of *gin2-1* mutants support a role for HXK1 in growth promotion as well (Fig. 2A).

Leaves of *gin2-1* plants had about one-half the glucose phosphorylation (GP) capacity of wild-type leaves when grown under low or high light conditions (Fig. 2B). Thus, other endogenous enzymes may have important roles in glu-

cose metabolism and may account for the HXK1 mutation being nonlethal. Fructokinase (FK) is abundant (Fig. 2B) and can direct carbon flow into the glycolytic pathway and tricarboxylic acid cycle (11). The growth defect in *gin2-1* plants under high light is unlikely to be due to a general deficiency in carbon metabolism or insufficient amounts of glucose 6-phosphate (G6P) (Fig. 2B). The levels of GP activity and hexoses increased substantially in a HXK1-dependent manner when light intensity was increased, suggesting a possible coordination between endogenous glucose signals and signaling (Fig. 2B).

At the flowering stage, the *gin2-1* mutant had a smaller root system, tiny leaves with delayed senescence, shorter petioles and inflorescence, and a reduced number of flowers and

silques (Fig. 2C and table S2). Despite marked differences in plant architecture, *gin2-1* plants had normal trichomes and flower size and shape and produced fertile seeds, albeit at reduced numbers (Fig. 2, C and D, and table S2). The distance between trichomes was shorter in *gin2* leaves, indicating a lack of cell expansion (Fig. 2D). Under high light growth conditions, wild-type plants underwent accelerated growth with earlier leaf senescence. These results reveal functions of HXK1 in enhancing light utilization to promote cell expansion in roots, leaves, and inflorescence.

Whether the signaling functions of HXK1 rely on its GP activity remains unproven. We first designed experiments to perturb GP activities and glucose responses, reasoning that their possible correlation would suggest that the glucose responses may result from HXK1-dependent metabolism. Because glucose responses are antagonized by nitrate (12), quantitative analyses of photosynthesis gene repression, chlorophyll (Chl) accumulation, and GP activity were measured in the absence and presence of nitrate in wild-type and *gin2* seedlings on a 2% glucose medium in the absence of MS salts. Although nitrate completely abolished the glucose repression of seedling development, photosynthesis gene expression, and Chl accumulation, GP activity was actually increased in wild-type plants (Fig. 3A). Thus, there was no correlation between GP activity and quantitative glucose responses (Fig. 3A). We extended these analyses to seedlings grown under five different combinations of sugar and nitrogen concentrations. In both wild-type and *gin2* seedlings, GP activities positively correlated with G6P levels but not with Chl levels, a quantitative indicator of glucose repression (Fig. 3B). The previous lack of correlation in mature leaves between enzyme activity and G6P levels (Fig. 2B) may be due to storage and multiple metabolic pools of G6P that are known to occur in the well-differentiated leaf organ. Nonetheless, the seedling response does indicate that G6P metabolism is uncoupled from HXK1-dependent glucose signaling.

Next, we used targeted mutagenesis to make HXK1 mutants that might retain signaling functions but not catalytic activities. The GP activities and signaling functions of mutants were analyzed with a protoplast transient assay (10). We identified two catalytically inactive HXK1 mutants, one that presumably eliminated adenosine triphosphate binding [Gly¹⁰⁴ → Asp¹⁰⁴ (G104D)] and another that likely prevented phosphoryl transfer [Ser¹⁷⁷ → Ala¹⁷⁷ (S177A)] but both maintained the glucose-binding site (13, 14). The HXK1 protein expression of wild-type and G104D and S177A mutants was similar, as detected with ³⁵S-methionine labeling and immunoprecipitation (Fig. 3C). However, the GP activity was abolished in both the G104D and S177A mutants (Fig. 3D). Using a well-established glucose repression assay in

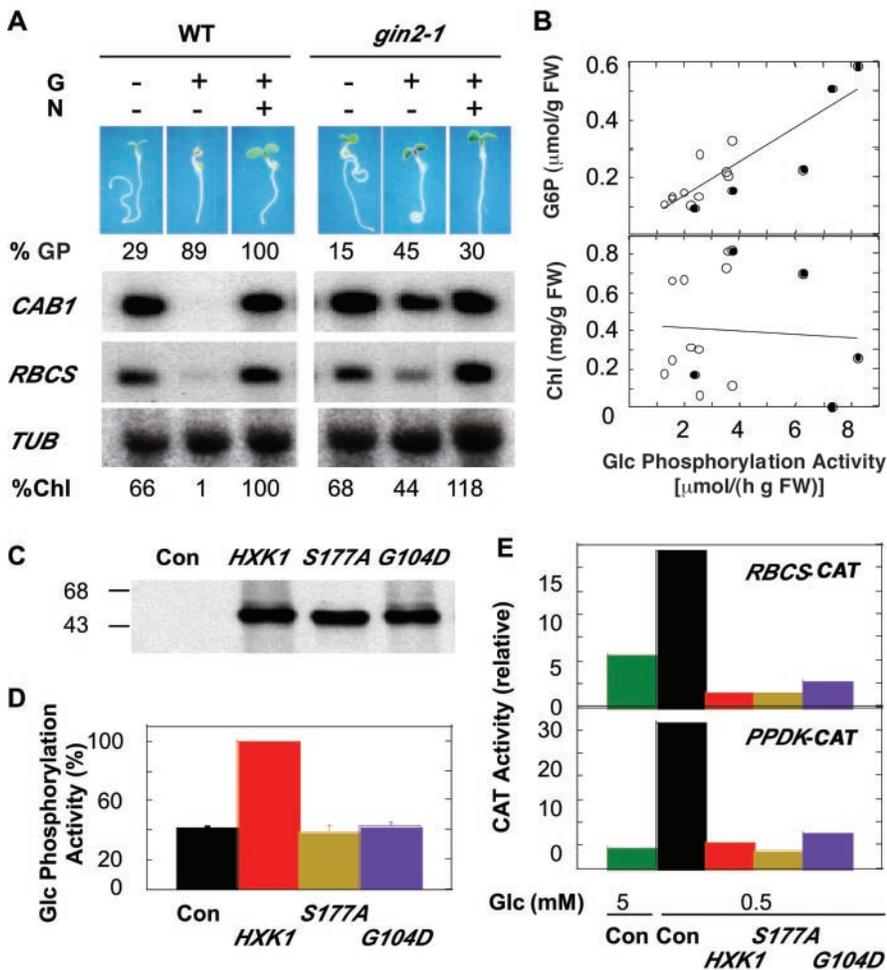


Fig. 3. Uncoupling glucose signaling and metabolism. (A) Glucose responses are affected by nitrate but are not correlated with GP activity. G, glucose; N, KNO₃. RNA blot analysis was done with Chl *a/b* binding protein (*CAB1*), ribulose-1,5-bisphosphate carboxylase small subunit (*RBCS*), and *TUB* probes (9, 25). Chl content was measured as in (26). (B) G6P levels are correlated with GP activity but not with glucose repression of Chl accumulation in wild-type (solid circles) and *gin2* (open circles) seedlings. (C) Protein expression of wild-type or mutated HXK1 in transfected maize mesophyll protoplasts. Con, control. (D) GP activities (± 1 SD, $n = 4$ transfections) of wild-type and mutated (S177A and G104D) HXK1 in transfected maize mesophyll protoplasts. The control shows endogenous enzyme activity. (E) Catalytically inactive HXK1 mediates transcription repression. Control protoplasts were transfected with vector DNA. CAT, chloramphenicol acetyltransferase; PPDK, pyruvate orthophosphate dikinase.

protoplasts (10), we found that the catalytically inactive G104D and S177A mutants exhibited a similar glucose signaling function as the wild-type HXK1 in repressing two promoters of photosynthesis genes (Fig. 3E). These results were unexpected, because the corresponding yeast Hxk2 G89D mutant was assumed to have lost both catalytic and signaling functions (13). The corresponding yeast Hxk2 S158A mutant abolished glucose signaling responses and 90% of the GP activity but retained fructose repression and 50% of the fructose phosphorylation activity (14).

To investigate glucose signaling and metabolism in plants, we generated transgenic plants that expressed the two catalytically inactive HXK1 mutant alleles in the *gin2-1* null mutant background. The two HXK1 mutant alleles mediated glucose-dependent developmental arrest and glucose repression of Chl accumulation (Fig. 4A and fig. S1). When grown under high light intensity in soil, both HXK1 mutants also supported substantial leaf expansion (Fig. 4B) without affecting GP and FK activities or G6P and F6P levels (Fig. 4C and Fig. S3). To explore other physiological functions of HXK1, we tested the growth of wild-type, *gin2*, and transgenic plants in the absence of high exogenous glucose and high light. The *gin2* mutant showed defects in the elongation of hypocotyls, roots, and cotyledons under dim light (15 $\mu\text{mol}/\text{m}^2/\text{s}$) (Fig. 4D and table S2). Both the G104D and S177A mutants supported HXK1-mediated cell elongation in different organs (Fig. 4D and table S2). Under the same growth condition, the addition of 2% glucose repressed three classes of photosynthesis genes in wild-type but not *gin2-1* seedlings (Fig. 4E and fig. S2). The G104D and S177A mutants could restore HXK1-mediated gene repression to the levels detected in wild-type seedlings (Fig. 5E). Because the wild-type HXK1 protein accumulated to higher levels in transgenic *gin2* plants (Fig. 4B), a stronger level of glucose repression was observed, as seen also in other glucose signaling assays (Fig. 4, A and B). Thus, catalytically inactive HXK1 mutants support both growth-inhibiting and growth-promoting roles of HXK1 under different growth conditions.

The observed *gin2* defects in cell expansion in various organs suggest a potential link between glucose signaling and plant growth hormones that are important for cell elongation, such as auxin, gibberellins, and brassinosteroids (15–17). The delayed leaf senescence phenotype in the *gin2* mutant also suggests a link with the plant growth hormone cytokinin (18). Because the *Arabidopsis det2* mutant with brassinosteroid deficiency (16) did not display the *gin* phenotypes and because the *gal1* mutant with gibberellin deficiency showed a germination defect not found in *gin* mutants (15), we focused on finding a connection between glucose, auxin, and cytokinin signaling. We first carried out tissue culture response experiments

using explanted hypocotyls at a low concentration of exogenous glucose (2%) in an MS medium. A defect in auxin-induced cell proliferation and root formation was observed in the *gin2* mutants (Fig. 5A). Because no difference in endogenous auxin levels was detected between wild-type and *gin2* hypocotyls (19) and because indole-3-acetic acid (IAA) was applied exogenously, the lack of auxin responses indicated a deficiency in auxin signaling and/or uptake. The defect was not due to a general lack of glucose metabolism and energy supply, because the addition of cytokinin promoted extensive shoot induction in *gin2* calli but not in wild-type calli (Fig. 5A). Cytokinin treatment also eliminated seedling developmental arrest induced by 6% glucose in an MS medium (Fig. 5B). However, because cytokinin promotes ethylene biosynthesis and ethylene antagonizes glucose signaling (9), the effect of cytokinin could be indirect. We therefore tested an ethylene receptor mutant (*etr1*) and a strong ethylene-insensitive mutant (*ein2*) (20) for seedling growth repression. We found that cytokinin and

ethylene acted independently in glucose responses (Fig. 5B). To further demonstrate the antagonistic interaction between cytokinin and glucose signaling, we tested for possible seedling resistance to 6% glucose in transgenic *Arabidopsis* lines with constitutive cytokinin signaling (18). Both cytokinin histidine kinase (*CKI1*) and response regulator (*ARR2*) transgenic plants could overcome the glucose repression response (Fig. 5C). To further evaluate the interaction between glucose and auxin, we examined the auxin resistance mutants *axr1*, *axr2*, and *tir1* (17) in the seedling glucose response assay (9). These auxin signaling-deficient mutants were resistant to growth inhibition by 6% glucose (Fig. 5D). The catalytically inactive HXK1 mutants could also restore auxin-mediated cell proliferation and root formation (Fig. 5E). These results support roles of auxin in both growth promotion and growth inhibition, depending on the tissue and/or glucose concentration. These phenomena illustrate plant growth plasticity and complex nutrient and hormone interactions.

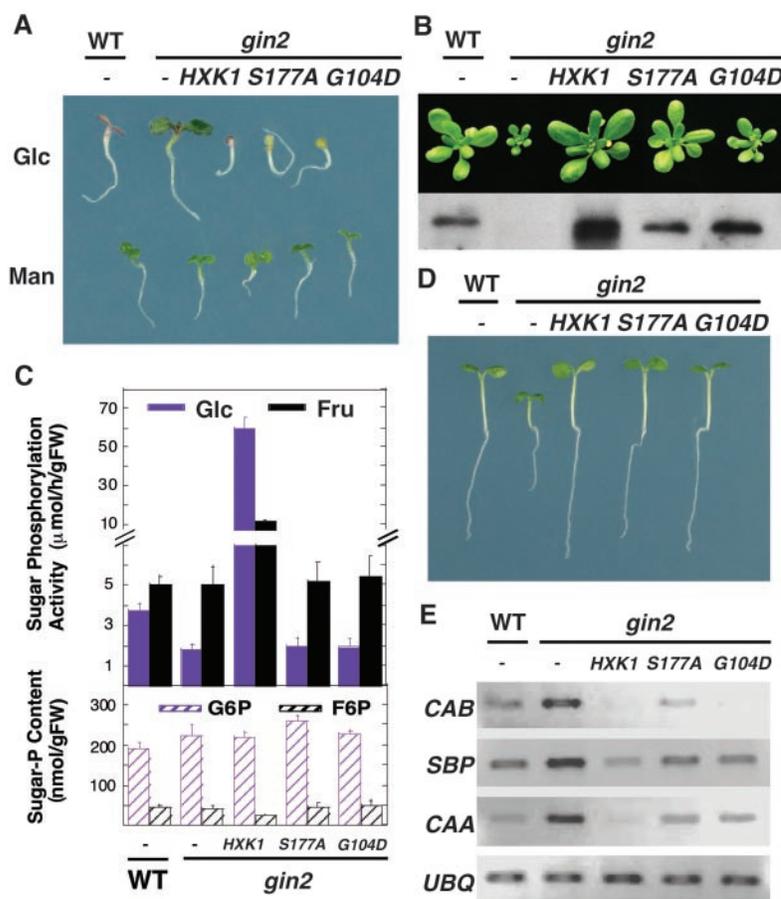


Fig. 4. Catalytically inactive HXK1 mutants support glucose signaling in transgenic *gin2* plants. (A) Glucose-mediated developmental arrest in seedlings. Plants were grown on a 6% Glc or Man MS medium. (B) Top: Soil-grown plants under high light. Bottom: Leaf HXK1 protein expression is shown by immunoblot. (C) Leaf hexose phosphorylation activities (with Glc and Fru) and hexose-phosphate content (G6P and F6P). P, phosphate. (D) Complementation of the root, cotyledon, and hypocotyl growth defects. (E) HXK1-mediated glucose-dependent gene repression. CAA, carbonic anhydrase (accession no. AL391149); SBP, sedoheptulose-bisphosphatase (accession no. AL391149); UBQ, ubiquitin 4 (accession no. AF296832).

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The ability to sense and transduce glucose signals to regulate metabolism, growth, and stress responses is critical for survival in *E. coli*, yeasts, flies, mammals, and plants (1–5). Plants use sunlight as their sole source of natural energy supply, and it has been well documented that sugar signals control numerous activities in sugar-producing (source) and sugar-consuming (sink) tissues (4, 21). We show here that *Arabidopsis* HXK1 has a unique function in a broad spectrum of glucose responses, including gene expression, cell proliferation, root and inflorescence growth, leaf expansion and senescence, and reproduction. A prevailing view has been that glucose promotion of plant growth is due to its metabolic effects. However, many new HXK1 functions identified here can be mediated at least partially by catalytically inactive mutants, demonstrating the dual functions of HXK1 in glucose signaling and metabolism. The roles of HXK1 in growth promotion or growth inhibition depend on glucose concentration, cell type, developmental state, and environmental condition. The HXK1 glucose-signaling pathway interacts intimately with the signaling pathways

regulated by auxin and cytokinin. Thus, the hormone-like effects of glucose on plant growth and development reflect the outputs of a complex signaling network governed by nutritional, hormonal, and environmental inputs (4, 21) that are integrated in part through HXK1 regulatory functions.

Many genes involved in glycolysis, respiration, gluconeogenesis, carbon storage, fatty acid biosynthesis, amino acid metabolism, cell cycle, and stress are similarly regulated by glucose in yeast, animals, and plants (4, 5, 21). Thus, glucose is an evolutionarily conserved metabolic signal used by all eukaryotic cells to control the energy budget and resource utilization. Under high light conditions with ample sugar supplies, plants grow fast, reproduce abundantly, and senesce early. HXK1 may coordinate high light intensity and high endogenous glucose signals, presumably for both metabolism and signaling (Fig. 2B). The *gin2* mutant plants cannot use increased light energy input and are miniature with delayed senescence and reduced reproduction (Fig. 2C). This phenomenon is reminiscent of caloric restriction by which life-span is extended in yeast and animals (22, 23). The yeast glucose sensor

Hxk2 plays a critical role in caloric restriction. Unique to plants, however, increased sensitivity to cytokinin and reduced glucose repression of genes important for photosynthesis could also delay senescence (24). Future analyses of other HXK1 mutants with altered subcellular localization, signaling, or metabolic functions and of protein-protein interactions in *Arabidopsis* are likely to reveal molecular mechanisms of glucose signaling mediated by this ancient enzyme and sensor. Studies of glucose responses in flowering plants improve our general understanding of glucose sensing and signaling mechanisms conserved in other eukaryotes.

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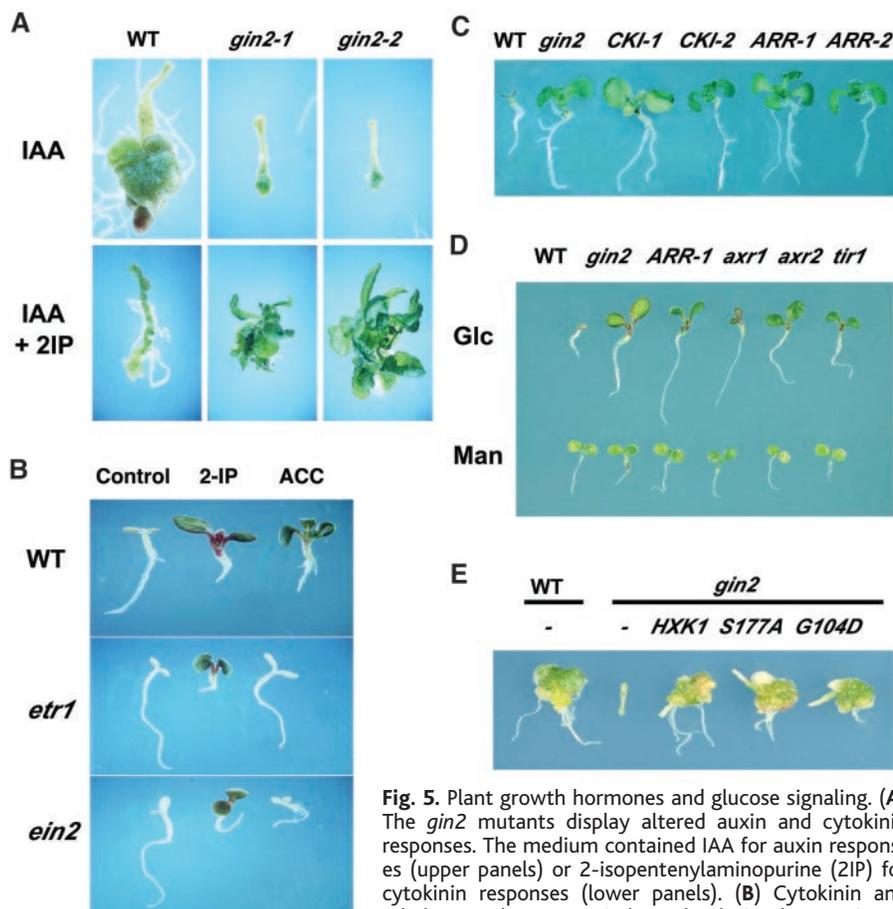


Fig. 5. Plant growth hormones and glucose signaling. (A) The *gin2* mutants display altered auxin and cytokinin responses (upper panels) or 2-isopentenylaminopurine (2IP) for cytokinin responses (lower panels). (B) Cytokinin and ethylene pathways act independently in glucose signaling. Ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) was used. (C) Transgenic *CKI1* and *ARR2* plants are glucose insensitive. Two independent lines (*CKI-1* and *CKI-2*; *ARR-1* and *ARR-2*) (18) were tested. (D) Auxin signaling mutants are glucose insensitive. (E) Catalytically inactive HXK1 restores auxin sensitivity for callus and root induction.