KIN10 promotes stomatal development through stabilization of the SPEECHLESS transcription factor

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Stomata are epidermal structures that modulate gas exchanges between plants and the atmosphere. The formation of stomata is regulated by multiple developmental and environmental signals, but how these signals are coordinated to control this process remains unclear. Here, we showed that the conserved energy sensor kinase SnRK1 promotes stomatal development under short-day photoperiod or in liquid culture conditions. Mutation of KIN10, the catalytic α-subunit of SnRK1, results in the decreased stomatal index; while over-expression of KIN10 significantly induces stomatal development. KIN10 displays the cell-type-specific subcellular location pattern. The nuclear-localized KIN10 proteins are highly enriched in the stomatal lineage cells to phosphorylate and stabilize SPEECHLESS, a master regulator of stomatal formation, thereby promoting stomatal development. Our work identifies a module links connecting the energy signaling and stomatal development and reveals that multiple regulatory mechanisms are in place for SnRK1 to modulate stomatal development in response to changing environments.
Stomata, the pores on the surface of leaves, regulate gas exchange between plants and the atmosphere and play critical roles for maintaining photosynthetic and water-use efficiency in plants. Stomatal development is highly plastic, and is modulated by multiple intrinsic developmental and environmental signals. The stomatal lineage in *Arabidopsis thaliana* is initiated by asymmetric divisions of undifferentiated meristemoid mother cells (MMC) to produce a small triangular cell meristemoid and a larger sister cell, stomatal-lineage ground cell (SLGC). The meristemoid either differentiates into a guard mother cell, which undergoes a single symmetric division to generate a pair of guard cells or goes through several additional rounds of asymmetric divisions to generate more SLGCs that then undergo spacing divisions to create satellite meristemoids or alternatively differentiate into pavement cells. The prime regulator linked to the initiation and proliferation of stomatal precursors is the basic helix-loop-helix transcription factor SPEECHLESS (SPCH), which controls hundreds of downstream genes to promote cell division and fate transitions. SPCH also integrates a wide range of environmental and hormonal signals to regulate stomatal development.

Maintenance of cellular and organismal energy homeostasis is a major challenge for all living organisms. Sucrose non-fermenting-1 (SNF1)-related kinase 1 (SnRK1) is a central energy sensor kinase in plants that is functionally and evolutionarily conserved with SNF1 in yeast and AMP-activated kinase (AMPK) in animals. Similar to its orthologs SNF1 and AMPK, SnRK1 activation by energy-depleting stress conditions induces catabolic reactions and represses energy-consuming anabolic processes, which redirect energy resources to support stress tolerance and survival. The eukaryotic AMPK/SNF1/SnRK1 protein kinases typically function as heterotrimeric complexes composed of one α-catalytic subunit (KIN10, KIN11, and KIN12 in Arabidopsis) and two regulatory subunits, β and γ. SnRK1 acts within complex signaling networks to maintain energy metabolism. Phosphorylation of a conserved threonine in the activation T-loop of the catalytic subunit (such as KIN10\(^{T175}\) and KIN11\(^{T176}\)) is important for SnRK1 activity. Two upstream kinases SnRK1 Activating Kinase 1 and 2 (SnAK1 and SnAK2) are Geminivirus Rep-interacting Kinase 2 and 1, respectively) were identified to interact with KIN10 and phosphorylate it at the conserved Thr\(^{175}\)-18. The phosphatases ABI1 and PP2CA, two important components of ABA signaling pathway, were found to interact with and dephosphorylate KIN10, causing its inactivation. In yeast and animal cells, SNF1 and AMPK are regulated by adenine nucleotide charge, but plant SnRK1 has been reported to be insensitive to adenosine mono- and diphosphate (AMP and ADP). Further, plant SnRK1 is known to be inhibited by diverse sugar phosphates and is highly sensitive to trehalose-6-phosphate (Tre6P). Tre6P, a signal of cellular sucrose status, binds directly to KIN10 and inhibits its interaction with the upstream kinases SnAK1/2. SnRK1 is also regulated by sumoylation- and ubiquitination-mediated degradation, as well as intracellular redox-mediated oxidation. Two recent studies reported that KIN10 was dynamically localized in endoplasmic reticulum and nucleus, and translocated to the nucleus to reprogram gene expression under metabolic stress conditions.

Sugar has been reported to promote stomatal formation in liquid culture condition, but the molecular mechanism remains unclear. Here, we showed that KIN10 is involved in the sugar-promoted stomatal development. When plants were grown in liquid media, sucrose treatment significantly induced the KIN10 protein accumulation by increasing its translation. Overexpression of *KIN10* resulted in the increased stomatal index, while the loss of function of *KIN10* led to the reduced stomatal index. KIN10 displayed the cell-type-specific subcellular localization pattern in the epidermal cells of leaves, mainly localized in the nucleus of the stomatal-lineage cells and guard cells, but localized in the cytoplasm of the pavement cells. In stomatal-lineage cells, the nuclear-localized KIN10 phosphorylated and stabilized SPCH to promote stomatal development. These results demonstrated that fine-tuning of KIN10 activity by environmental and developmental signals optimizes stomatal development in Arabidopsis.

### Results

**Sugar promotes stomatal development under certain conditions.** Sugar has been shown to play crucial roles in the proliferation and differentiation of plant meristems, but the molecular mechanism is still fragmentary. Stomata, the pores on plant epidermis, that facilitate gas exchange with the atmosphere, comprise an excellent system for investigating how environmental and developmental signals regulate cell fate determination in plants. To assess the effects of sugar on stomatal development, we analyzed the stomatal production under different light conditions and in the presence or absence of exogenous sucrose. Consistent with previous results, the stomatal index, which is the number of stomata relative to total epidermal cells, gradually increased with the increasing light photon irradiance (Fig. 1a). Similarly, the stomatal index of plants grown under the 16 h light/8 h dark photoperiod condition was much higher than that of plants grown under the 4 h light/20 h dark photoperiod condition (Fig. 1b). However, the decreased stomatal index of plants resulting from the low light quantity and short-time light irradiance was partially recovered by the exogenous sucrose supply (Fig. 1a, b). Prolonged darkness, which induces the carbon starvation phenotype in seedlings due to the lack of photosynthesis and the depletion of the reserved starch, inhibited stomatal development (Supplementary Fig. 1a). Under such starvation conditions, exogenous application of sucrose partly increased stomatal development, and the stomatal index increased from 11 to 15% approximately (Supplementary Fig. 1b). To evaluate the role of sugar in the development of stomata, different types of sugar were used to treat the wild-type seedlings that were grown under the 16 h light/8 h dark condition for 10 days in liquid half-strength MS medium, which might cause the mild hypoxia. Sucrose exerted the most obvious positive effects on the development of stomata (Fig. 1c). Glucose, fructose, and fructose bisphosphate (FBP) also could induce stomatal development, but glucose-6-phosphate (G6P) and mannitol (Man) had no significant effects on stomatal development (Fig. 1c). Treatment with sucrose not only promoted cell expansion but also induced cell division, and led to the increased amounts of stomata in the whole cotyledon and high ratio of clustered stomata (Supplementary Fig. 2a–h). These results indicated that sugar promotes stomatal development under certain conditions, such as low light quantity, short-day photoperiod, or in hypoxic liquid growth media.

To further verify the effects of sucrose on the stomatal-lineage cell fate transition, we analyzed the sucrose-treated stomatal development phenotype in different stomatal cell-type-specific marker lines, including: SPCH::mucGFP that was mostly expressed in the cells of the stomatal lineage, SPCH::SPCH-GFP that was only expressed in the MMCs and meristemoids, BSL::GFP-BASL that was expressed in the asymmetrically dividing MMCs, meristemoids and the larger SLGCs, and MUTE::MUTE-GFP that was expressed in the guard mother cells. Sucrose treatment dramatically increased the number of cells marked by these markers: SPCH::mucGFP, SPCH::SPCH-GFP, MUTE::MUTE-GFP and BSL::GFP-BASL relative to total cells.
Fig. 1 Sucrose promotes stomatal development and alters cell fate in Arabidopsis epidermis. a The stomatal index changes in response to light quantity. Seedlings of wild-type plants were grown on ½ MS solid medium with or without 1% sucrose under 16 h light/8 h dark photoperiod for 10 days. b Quantification of the effects of photoperiod and exogenous sucrose on stomatal development. Seedlings of wild-type plants were grown on ½ MS solid medium with or without 1% sucrose under different photoperiod with 100 μMol m⁻² s⁻¹ for 10 days. Numbers between bars indicated the relative fold changes of average means in the indicated conditions. c Quantification of the effects of different sugars on stomatal index. Seedlings of wild type Col-0 were grown in ½ MS liquid medium containing 30 mM mannitol (Man, 0.55%), sucrose (Suc, 1%), glucose (Glu, 0.54%), fructose (Fru, 0.54%), glucose-6-phosphate (G6P, 0.78%), and fructose-1,6-bisphosphate (FBP, 1%) under 16 h light/8 h dark photoperiod with 100 μMol m⁻² s⁻¹ continuously for 10 days. Error bars indicate standard deviation (S.D.) (n = 15–20). Different letters above the bars indicated statistically significant differences between the samples (ANOVAs analysis followed by Uncorrected Fisher’s LSD multiple comparisons test, p < 0.05). d–o Sucrose alters cell fate in Arabidopsis epidermis. Seedlings of pSPCH::nucGFP, pSPCH::SPCH-GFP, pBASL::GFP-BASL and pMUTE::MUTE-GFP were grown in ½ MS liquid medium with or without 1% sucrose for 3 days under long-day condition. Quantification of the percentage of GFP-expressing cells over total epidermal cells of different transgenic plants. The fluorescent signals of GFP or GFP fused proteins are in green, PI-marked cell outlines are in purple. Scale bars in confocal images represent 20 μm. Error bars indicate S.D. (n = 15–20). Asterisk between bars indicated statistically significant differences between the samples (Student t test, ****p < 0.0001).

(Fig. 1d–o). These results indicated that sucrose alters the epidermal cells fate in the Arabidopsis leaves.

**KIN10 positively regulates stomatal development.** Previous study showed that the prolonged darkness and hypoxia could activate the energy sensor SnRK1 to regulate the plant growth and stress response. Our study showed that sucrose promoted stomatal development only when plants were grown under the short-day photoperiod or in hypoxic liquid culture conditions, indicating that SnRK1 might be involved in this process. To test this hypothesis, we analyzed the stomatal phenotype of SnRK1-related materials under different growth conditions. First, when plants were grown in the liquid half-strength MS medium with 1% sucrose, we observed that the transgenic plants overexpressing **KIN10**, a catalytic subunit of SnRK1, displayed the increased stomatal index phenotype comparing to wild-type plants (Fig. 2a).
Because the KIN10-Ox genetic material that we obtained from Arabidopsis Biological Resource Center is in the Landsberg (Ler) background, in which ERECTA (ER), a key component of stomatal development, is mutated, it showed the increased number of stomata\(^2\). To rule out the effects of ER mutation, we generated KIN10-overexpressing transgenic plants in the Columbia-0 (Col-0) background. The results showed that, in the presence of sucrose, overexpression of KIN10 in the Col-0 background also led to the increased stomatal index, elevated stomata number in whole cotyledon, and higher ratio of clustered stomata compared to Col-0 plants. (Fig. 2b and Supplementary Fig. 3a–i). In addition, both kin10 and kin11 single mutants exhibited the lower stomatal index in liquid medium containing 1% sucrose, indicating that SnRK1 kinase is required for sucrose-induced stomatal development (Fig. 2c and Supplementary Fig. 4a–c). Second, we analyzed the stomatal phenotypes of wild type Col-0, KIN10-Ox, and kin10 seedlings that were grown on half-strength MS solid medium with or without 1% sucrose under different photoperiod conditions. The results showed that overexpression of KIN10 significantly induced stomatal development in the presence of sucrose, particularly under the 4 h light/20 h dark photoperiod condition (Supplementary Fig. 5a–c). Third, to determine whether the positive effect of KIN10 on stomatal development is similar in the cotyledons and true leaves, the fifth rosette leaves of wild type, KIN10-Ox and kin10 plants grown in soil under the 4 h light/20 h dark photoperiod for 5 weeks were used for stomatal index analysis. The stomatal index of rosette leaves of KIN10-Ox was higher, and that of kin10 mutant was lower than the stomatal index of wild type rosette leaves (Supplementary Fig. 6a–d). These results indicated that KIN10 positively regulates stomatal development under certain conditions.

To further verify the effects of KIN10 on stomatal development, we analyzed the stomatal phenotype of wild-type plants grown in liquid medium with Tre6P, which is a known inhibitor of KIN10 through weakening the interaction between KIN10 and its upstream kinases SnAK1 and SnAK2\(^3\). The results showed that cotreatment with Tre6P strongly counteracted the sucrose effects and reduced the stomatal index (Supplementary Fig. 7a). Trehalose-6-Phosphate Synthase1 (TPS1) is the key enzyme for the biosynthesis of Tre6P in plants\(^3\). Mutation of TPS1 resulted in a significantly increased stomatal index comparing to that of wild-type plants in the presence of sucrose (Supplementary Fig. 7b). Furthermore, we found that the transgenic plants of pKIN10::KIN10\(^{175E}–\)myc, pKIN10::KIN10\(^{175E}–\)myc and p3SS::SnAK2-GFP showed the increased stomatal index comparing to wild-type plants (Supplementary Figs. 8a–c and 9a, b). These results further confirmed that KIN10 is a positive regulator for stomatal development.

Next, we analyzed whether KIN10 regulates the cell fate transition of stomatal-lineage cells. The expression levels of stomatal cell-type-specific markers were monitored in KIN10-Ox and wild-type plants. The results showed that a higher fraction of cells labeled by pSPCH::SPCH-GFP in KIN10-Ox than in wild-type plants (Fig. 2d–f). The difference between KIN10-Ox and wild-type plants for SPCH-GFP accumulation was much higher than the difference for stomatal index. This might be due to that the accumulated SPCH protein induces cell division to form many small cells, but only a few small cells will eventually develop into stomata\(^4\). These results indicated that KIN10 promotes the differentiation of epidermal cells into stomatal-lineage cells, leading to enhanced stomatal formation.
Sucrose induces KIN10 protein accumulation. To investigate how sugar optimizes stomatal development through KIN10, we first analyzed the effects of sucrose on the protein levels of KIN10 in the pKIN10::KIN10-myc transgenic plants that were grown in liquid half-strength MS medium without any sugar for 3 days and then treated with 1% sucrose or 1% mannitol for different time spans. Sucrose treatment increased the protein levels of KIN10-myc, which became more obvious after 6 h of treatment, while mannitol showed no significant effects on the protein levels of KIN10-myc (Fig. 3a). To examine whether the accumulation of KIN10 protein was due to the increased transcriptional levels of KIN10 protein, we performed quantitative reverse transcription-PCR (qRT-PCR) analysis of the expression of KIN10 in response to sucrose. Wild-type seedlings were grown in sugar free 1/2 MS liquid medium for 3 days and then treated with 1% sucrose for different times. PP2A gene was analyzed as an internal control. Error bars represent standard deviation of four independent experiments. Different letters above the bars indicated statistically significant differences between the samples (ANOVA analysis followed by Uncorrected Fisher’s LSD multiple comparisons test, *p < 0.05). Sucrose induces KIN10 protein accumulation in p35S::KIN10-myc transgenic plants. Seedlings of p35S::KIN10-myc transgenic plants were grown in sugar free 1/2 MS liquid medium for 3 days and then treated with 1% sucrose or 1% mannitol for different time periods. Immunoblot analysis of the effects of cycloheximide (CHX) on the Sucrose-induced the KIN10 protein accumulation. Seedlings of pKIN10::KIN10-myc transgenic plants were grown in sugar free 1/2 MS liquid medium for 3 days and then treated with sucrose or mannitol for different time periods. **p < 0.01; ****p < 0.0001).

KIN10 interacts with SPCH in vivo and in vitro. SPCH is a master regulator of stomatal development downstream of a wide range of environmental and hormonal signals6–9. We found that sucrose treatment and overexpression of KIN10 both resulted in the increased levels of SPCH (Figs. 1m and 2f), indicating that sucrose and KIN10 might regulate stomatal development through SPCH. To test this hypothesis, we first analyzed the interaction between KIN10 and SPCH. Yeast two-hybrid analysis showed that KIN10 interacts with SPCH, but not MUTE and FAMA (Fig. 4a and Supplementary Fig. 10). In vitro protein–protein pull-down assays showed that glutathione S-transferase (GST)-SPCH interacts with maltose-binding protein (MBP)-KIN10, but not MBP alone (Fig. 4b). The ratiometric bimolecular fluorescence complementation (rBiFC) assays showed that the strong YFP fluorescence signal was observed in the nucleus when KIN10-YFP and SPCH-myc from 35S promoters confirmed their interaction in plants (Fig. 4d and Supplementary Fig. 11). We further analyzed the tissue expression patterns of
KIN10 using the pKIN10::KIN10-YFP transgenic plants. KIN10 displayed the ubiquitous expression pattern in all epidermal cells, and it was mainly localized in the nucleus of guard cells and the smaller cells that were smaller than 200 μm², while mainly distributed in the cytoplasm of larger cells (Fig. 4e–g). There is significantly increased nuclear localization of KIN10 in smaller cells in liquid growth media compared to that in solid growth media (Supplementary Fig. 12a–e). Whereas, the Thr175 phosphorylation of KIN10 had no significant effect on its subcellular localization in small scale dividing cells and pavement cells (Supplementary Fig. 13a–f). Co-localization analysis with pKIN10::KIN10-YFP/pSPCH::SPCH-RFP showed that KIN10 and SPCH were simultaneously distributed in the nucleus of the stomatal-lineage cells (Fig. 4h). These results indicated that KIN10 physically interacted with SPCH in stomatal-lineage cells.

KIN10 stabilizes SPCH to promote stomatal development. Next, we investigated whether KIN10 could control SPCH activity by phosphorylation. The in vitro kinase assays showed that MBP-KIN10 had no ability to phosphorylate MBP-SPCH by itself (Fig. 5a). However, in the presence of its upstream kinase GST-SnAK2, MBP-KIN10 was activated to phosphorylate MBP-SPCH (Fig. 5a). Mass spectrometry analysis identified in vitro phosphorylation residues in SPCH by KIN10, including Thr49, Thr50, Ser51, and Ser52 (Supplementary Fig. 14a, b). To verify that these residues of SPCH are the target sites of KIN10, we replaced them with alanine or aspartic acid to generate S/T49-52A or S/T49-52D, which we named SPCH-4A or SPCH-4D, respectively. Transformation of the pSPCH::SPCH-RFP and pSPCH::SPCH-4D-RFP constructs into spch-4 mutants both rescued stomatal development and defective growth phenotypes of spch-4 mutants, while SPCH-4A only partially rescued the growth defect phenotypes of spch-4 mutants, suggesting that these four KIN10-dependent phosphorylated residues are essential for SPCH to control stomatal development (Fig. 5b and Supplementary Figs. 15a–g and 16a, b). Quantification of the fluorescent intensity of pSPCH::mucGFP and pSPCH::SPCH-GFP revealed that the short-time sucrose treatment had marginal effects on the transcription of SPCH, but significantly induced the protein accumulation of SPCH protein (Fig. 5c). Furthermore, we showed that sugar starvation caused by washing away sucrose in liquid medium had no effects on the expression of SPCH but significantly reduced the protein levels of SPCH (Fig. 5d). Immunoblot analysis showed that sucrose treatment significantly induced SPCH protein accumulation in p35S::SPCH-myc transgenic plants (Fig. 5e). More importantly, SPCH-4A performed weaker protein stability compared to wild type SPCH and SPCH-4D in developing cotyledon epidermis (Fig. 5f, g and Supplementary Fig. 17a, b). Combined with overexpression of KIN10 leading to the accumulation of SPCH (Fig. 2d–f), these results suggested that KIN10 phosphorylates SPCH, thereby increasing SPCH protein stability and subsequently promoting stomatal development.
Discussion
SnRK1 is a central metabolic regulator of energy homeostasis in plants that is functionally and evolutionarily related to SNF1 in yeast and AMPK in mammals. Here, our genetic and biochemical analyses revealed an important role of SnRK1 in plant stomatal development under conditions that are likely associated with mild energy starvation of plants, such as short-day photoperiod or liquid cultures.Sucrose supply induces the accumulation of KIN10 by increasing its translation in the liquid culture condition. KIN10 is expressed in all epidermal cells, but displays the cell-type-specific subcellular location. The nuclear-localized KIN10 is highly enriched in the stomatal-lineage cells. Under certain stress conditions, activated KIN10 phosphorylates SPCH to increase its stability, thereby promoting stomatal development. Thus, our research demonstrates the highly conserved SnRK1 kinase as a positive regulator of SPCH and stomatal development that influences many plant responses to changing environmental conditions (Fig. 6).

SnRK1 has been reported to play central roles in the regulation of plant growth and development in response to energy signals. Mutation in the α-catalytic subunit of SnRK1 resulted in defective embryo development as well as in delayed germination and seedling development. In the present study, we demonstrated, through several lines of evidence, that SnRK1 is a positive regulator for stomatal development under certain conditions. First, overexpression of KIN10 displayed the increased stomatal index, partially under the short-day photoperiod or in the hypoxic liquid growth condition. The loss of function of KIN10 or KIN11 resulted in defective embryo development as well as in delayed germination and seedling development. In the present study, we demonstrated, through several lines of evidence, that SnRK1 is a positive regulator for stomatal development under certain conditions. First, overexpression of KIN10 displayed the increased stomatal index, partially under the short-day photoperiod or in the hypoxic liquid growth condition. The loss of function of KIN10 or KIN11 resulted in defective embryo development as well as in delayed germination and seedling development. In the present study, we demonstrated, through several lines of evidence, that SnRK1 is a positive regulator for stomatal development under certain conditions. First, overexpression of KIN10 displayed the increased stomatal index, partially under the short-day photoperiod or in the hypoxic liquid growth condition. The loss of function of KIN10 or KIN11 resulted in defective embryo development as well as in delayed germination and seedling development. In the present study, we demonstrated, through several lines of evidence, that SnRK1 is a positive regulator for stomatal development under certain conditions. First, overexpression of KIN10 displayed the increased stomatal index, partially under the short-day photoperiod or in the hypoxic liquid growth condition. The loss of function of KIN10 or KIN11 resulted in defective embryo development as well as in delayed germination and seedling development.
KIN10 promotes stomatal development through stabilization of SPCH. When plants were grown under conditions that are likely associated with mild energy starvation of plants, such as short photoperiod or liquid cultures, sucrose supply induced the KIN10 protein accumulation by increasing its translation. KIN10 displayed the cell-type-specific subcellular location pattern in the epidermal cells of leaves. The nuclear-localized KIN10 in stomatal-lineage cells phosphorylated and stabilized SPCH to promote stomatal development. Thus, the combination of multiple regulatory mechanisms controls the activity of KIN10 and thereby optimizes stomatal development.

In this study, we found that KIN10-YFP fluorescent signals were present in all epidermal leaf cells, but the nuclear localization signals of KIN10-YFP were significantly enhanced in the small cells that might belong to the stomatal-lineage cells, leading to phosphorylation of SPCH by KIN10 in the nucleus to increase SPCH protein stability and promote stomatal development. The nuclear/cytoplasmic ratios of KIN10-YFP in stomatal-lineage cells of plants grown in liquid growth media were significantly higher than that grown on solid media in the presence of 1% sucrose, indicating KIN10 activity in stomatal-lineage cells is more sensitive for use of liquid growth condition. Consistent with this, plants grown in liquid media with 1% sucrose showed the increased stomatal index comparing to the plants grown on solid medium in the presence of 1% sucrose, indicating KIN10 activity in stomatal-lineage cells is higher than that grown on solid media in the presence of 1% sucrose, which may be due to the increased KIN10 activity by the mild hypoxia of the liquid culture.

Phosphorylation of a highly conserved threonine residue in the T-loop is essential for the catalytic activity of AMPK, SNF1, and SnRK1 in mammalian, yeast and plant cells, respectively. The phosphorylation of KIN10 has been reported to be activated by submergence. However, submergence treatment had no significant effect on the transcriptional level and total protein level of KIN10, although it significantly increased the phosphorylation of KIN10 at the T-loop conserved threonine residue. In this study, a liquid culture system was used to assess stomatal index and KIN10 activity. The significant promotion of stomatal development in plants grown in liquid medium with sucrose might be due to the activation of KIN10 by mild hypoxia of the liquid culture, similar to submergence treatment. Accordingly, exogenous sucrose supply had no obvious promoting effects on stomatal index of plants grown on solid medium under the 16 h light/8 h dark photoperiod condition, but it significantly increased the stomatal index of plants grown on solid medium under the 4 h light/20 h dark photoperiod condition. This may be due to the activation of KIN10 under prolonged darkness. These results indicated that sugar induces the accumulation of KIN10 by enhancing its translation, while liquid culture or prolonged darkness might activate KIN10 through inducing its phosphorylation. Subsequently, the activated and nuclear-localized KIN10 in stomatal-lineage cells phosphorylated and stabilized SPCH to promote stomatal development. Overall, the combination of multiple regulatory mechanisms controls the activity of KIN10 and thereby optimizes stomatal development.

The promotion of KIN10 on stomatal development may occur when plants encounter sunny days after consistent cloudy weather for many days, or when plants recover from the flooding stress. The cloudy weather or flooding stresses resulted in the energy starvation of plants. When plants encounter the sunny days again, plants restart photosynthesis, and produce sugar to induce the accumulation of KIN10. The activated KIN10 phosphorylates and stabilizes SPCH to promote stomatal formation and then increase the ability of plant photosynthesis and carbon assimilation, thus forming a positive feedback loop to help plants recover from stress. Taken together, our research not only establishes the highly conserved SnRK1 kinase as a positive regulator for stomatal development, but also provides a tractable system for investigating how environmental stresses integrate with metabolic signals to modulate stomatal development.

**Methods**

**Plant materials and growth conditions.** Arabidopsis ecotype Columbia (Col-0) was used as the wild-type except where indicated. Plants were grown in a greenhouse with white light at 100 μmol m⁻² s⁻¹ and relative humidity of 50% under a 16 h light/8 h dark cycle at 22 °C for general growth and seed harvesting. Mutants and transgenic plants used in this study were spch-4, p35S::KIN10-HA, pSPCH::nucGFP, pSPCH::SPCH-GFP, pMUTE::MUTE-GFP, pBASL::BASL-GFP, p35S::KIN10-Myc, pKIN10::KIN10-YFP, pKIN10::KIN10T175D, YFP, p35S::SnAK2-GFP, pSPCH::SPCH-RFP, pSPCH::SPCH-4A-RFP, and pSPCH::SPCH-4D-RFP. The T-DNA insertion mutants of GABI_579_E09 (kin10/snark1) and WiscDsLox384F5 (kin11/snark2) were in Col-0 background and ordered from The European Arabidopsis Stock Centre. The GABI_579_E09 mutant has been used the knock out mutant of KIN10 to determine its functions in hypocotyl elongation, root growth and stress response in plants. The mutant WiscDsLox384F5 has not been used in previous study. Our RT-PCR analysis showed there are some amount of KIN10 transcript in kin10 mutant, but no KIN11 transcript in kin11 mutant. Immunoblot further confirmed that no significant amount of KIN10 protein and KIN11 protein could be detected in kin10 and kin11 mutants, respectively.
Coimmunoprecipitation assays. In 1/2 MS liquid medium containing transgenic Arabidopsis seedlings were grown for 10 days under 16 h photoperiod. For protein extraction, seedlings of the indicated genotypes were frozen in liquid nitrogen, ground in 50 μl of 5 × LDS buffer, and then treated with or without sucrose, CHX for different times. Pull-down assays. KIN10-p35S::RFP-p35S::cYFP-x and RFP were determined by ImageJ software. Immunoblot analyses. Antibodies were visualized by using the following settings: excitation 488 nm and emission 530 nm for GFP and RFP, respectively. The rBiFC assays were performed in Arabidopsis leaves and imaged by using the following settings: excitation 488 nm and emission 670 nm. Laser intensity was adjusted to optimize signal-to-noise ratios. Immunofluorescence complementation assays. GFP and RFP were determined by ImageJ software.

In vitro kinase assay and phosphopeptide analysis. MBP-KIN10, MBP-SPCH and GST-SnAk2 proteins were expressed and purified from Escherichia coli. MBP-Sp-chip was incubated with MBP-KIN10 and/or GST-SnAk2 as indicated in the kinase buffer (25 mM HEPES, pH 7.4, 10 mM MgCl₂, 50 mM KCl, 1 mM DTT, and 30 μM cold ATP) containing [γ-³²P] ATP (10 μCi). The reaction was stopped by addition of 10 μl of 5 × LDS buffer. Proteins were resolved by 10% SDS-PAGE. After nonradioactive in vitro kinase assays, proteins were digested with trypsin and endopeptidase Asp-N. The digested peptide mixtures were injected into Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer (ThermoFisher Scientific) for Mass spectrometer analysis. The phosphorylated residues in MBP-SPCH were identified by Maxquant software.

Data availability
All data in this study are available in the main text or the supplementary materials. Source data are provided with this paper.

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Author contributions
C.H. and M.Y.B. together designed the experiments. C.H. performed statistical analysis of stomatal index of wild type and various mutants in our experimental conditions, microscopy analysis, western blot, subcellular location analysis, pull down, RNAi, CoIP. W.S., Y.L., Y.T., M.F., L.W., and Y.Q. generated KIN10 related mutants and transgenic plants, pKIN10::KIN10-YFP, p35S::KIN10-YFP/p35S::SPCH-myc and pSPCH::SPCH-RRP. C.H. and Z.D. performed performed the kinase assays and Mass spec analysis. O.I. and G.J. provided the critical discussion on the work. C.H. performed all other experiments. C.H. and M. B. wrote the paper.

Competing interests
The authors declare no competing interests.

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