PLANT SCIENCE

NIN-like protein 7 transcription factor is a plant nitrate sensor

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Nitrate is an essential nutrient and signaling molecule for plant growth. Plants sense intracellular nitrate to adjust their metabolic and growth responses. Here we identify the primary nitrate sensor in plants. We found that mutation of all seven *Arabidopsis* NIN-like protein (NLP) transcription factors abolished plants' primary nitrate responses and developmental programs. Analyses of NIN-NLP7 chimeras and nitrate binding revealed that NLP7 is derepressed upon nitrate perception via its amino terminus. A genetically encoded fluorescent split biosensor, mCitrine-NLP7, enabled visualization of single-cell nitrate dynamics in planta. The nitrate sensor domain of NLP7 resembles the bacterial nitrate sensor NreA. Substitutions of conserved residues in the ligand-binding pocket impaired the ability of nitrate-triggered NLP7 to control transcription, transport, metabolism, development, and biomass. We propose that NLP7 represents a nitrate sensor in land plants.

Nitrogen, the main limiting factor for plant growth, is fundamental to agricultural productivity, animal and human nutrition, and sustainable ecosystems (*1*, *2*). Photosynthetic plants drive the terrestrial nitrogen cycle by assimilating inorganic nitrogen into biomolecules (DNA, RNA, proteins, chlorophyll, and vitamins) that sustain the plants and the food webs that depend on them (*1*–*4*). To compete with microbes that prefer organic nitrogen or ammonium in the soil, most plants have evolved regulatory pathways that respond to fluctuating nitrate availability (*3*–*5*). Plants that sense available nitrate will, within minutes, orchestrate transcriptome, metabolism, hormone, system-wide shoot and root growth, and reproduction responses (*5*–*8*). Here we identify the transcription factor NIN-like protein 7 (NLP7) as the primary nitrate sensor. We show that NLP7 acts as an intracellular nitrate sensor distinct from the plasma membrane extracellular nitrate transporter-sensor (transceptor) NRT1.1 (also known as CHL1 or NPF6.3) (*9*–*13*).

NIN-like proteins 6 and 7 (NLP6/7), which were identified by homology to the regulator NODULE INCEPTION (NIN) that controls root nodule initiation and nitrogen fixation (*14*), are master transcription factors in nitrate signaling and widely conserved in land plants and major crops (*9*, *15*–*19*). There are nine NLPs in *Arabidopsis thaliana* featuring an N-terminal nitrate response region, a conserved RWP-RK DNA binding domain for the nitrate response cis-element (*NRE*), and a C-terminal PB1 protein-protein interaction domain (*13*, *15*, *17*, *20*). The N-terminal nitrate-response region of NLP1 to NLP9 (NLP1-9) encompasses an evolutionarily conserved regulatory phosphorylation site critical for nitrate-induced nuclear retention and transcription activation (*18*, *20*). Functions of putative GAF (cGMP-specific phosphodiesterase, adenylyl cyclases, and FhlA)–like domains and other conserved motifs remain unclear (*17*). Nitrate activates group III calcium-sensor protein kinases (CPKs), which phosphorylate NLP7 at S205 to retain NLP7 in the nucleus, where it activates primary nitrate responses that shape organ biomass and architecture (*18*). Nitrate-dependent transactivation activity of NLP2 requires the conserved S202 residue (*20*). Nitrate is required to activate the primary reporter gene despite coexpression of NLP7 and constitutively active CPK10ac in nitrate-free leaf cells (*18*), suggesting a missing nitrate sensor action. Here, we demonstrate that NLP7 is an intracellular nitrate sensor. The nitrate-binding domain seems evolutionarily ancient and is conserved in plant NLPs and the bacteria nitrate sensors NreA (*21*). Nitrate directly binds to NLP7 through residues evolutionarily conserved in other NLPs and NLP7 orthologs, triggers a conformational change, and derepresses NLP7 as a transcription activator.

Combinatorial NLPs control primary nitrate responses

All nine *NLP* genes are expressed in *Arabidopsis* shoots. Analyses of individual *nlp1-9* single mutants in nitrate-mediated shoot growth revealed statistically significant defects only in *nlp2* and *nlp7* (*9*, *18*, *20*). To circumvent NLP redundancy and better define the overlapping or distinctive functions of NLP1-9, we conducted a genome-wide target gene survey. Each NLP was transiently expressed for 4.5 hours in transfected leaf cells from soil-grown plants for RNA sequencing (RNA-seq) analyses (*15*, *18*, *21*). Hierarchical clustering analysis of putative NLP target genes ($\log_2 \geq 1$ or ≤ -1; $P \leq 0.05$) revealed the capability of all NLPs to activate universal primary nitrate-responsive marker genes previously identified by microarray, RNA-seq, chromatin immunoprecipitation on chip (ChIP-chip), ChIP-seq, and promoter analyses (*15*, *16*, *18*, *21*, *22*) (Fig. 1, A and B, and tables S1 and S2). NLP2, NLP4, NLP7, NLP8, or NLP9 specifically activated some target genes with known functions in modulating auxin and cytokinin hormone functions, cell cycle, metabolism, peptide signaling, and shoot and root meristem activities (Fig. 1, A and B, and tables S1 and S2). NLP2 and NLP7 regulated broader nonredundant target genes with diverse functions, which might manifest as growth defects observed in *nlp2* or *nlp7* after seed germination (*9*, *18*, *20*). NLP6/7 acted predominantly as transcription activators, whereas NLP2,4,5,8,9 could activate or repress target genes. NLP1,3,6 modulated fewer target genes than other NLPs. For instance, the auxin biosynthesis gene *TAR2* was only activated by NLP2,4,5,7,8,9 (*23*). These results are consistent with NLP variants functioning combinatorially in controlling the nitrate response network and causing retarded shoot and root development in the *nlp2,4,5,6,7,8,9* septuple mutant plants grown in soil (Fig. 1C).

NRT1.1(CHL1/NPF6.3) acts as a plasma membrane nitrate transceptor that senses external nitrate in *Arabidopsis* (*10*–*12*). However, the null *chl1-5* mutant resembled wild-type (WT) plants when grown in the soil under light, whereas the *nlp2,4,5,6,7,8,9* septuple mutant failed to develop further after germination (Fig. 1C). When grown on a sterile culture medium with 0.5% sucrose and ammonium succinate, WT, *chl1-5*, and *nlp2,4,5,6,7,8,9* plants were able to develop similar shoots, albeit ones smaller than those of WT plants grown in nitrate medium (*1*, *19*, *21*). In contrast, shoot and root development of *nlp2,4,5,6,7,8,9* remained constrained in nitrate medium (Fig. 1D and fig. S1, A to F) (*20*). Transcriptome reprogramming in primary nitrate responses triggered by nitrate at 20 min ($\log_2 \geq 1$ or ≤ -1; $q \leq 0.05$) was abolished in *nlp2,4,5,6,7,8,9* (Fig. 1E). The activation of primary nitrate response genes are only partially reduced in *chl1-5* (fig. S2 and table S3). However, the uptake of nitrate showed a stronger reduction in chlorate-resistant *chl1-5* but was only moderately reduced in *nlp2,4,5,6,7,8,9* (Fig. 1F) (*6*, *24*). Chlorate could not activate primary nitrate

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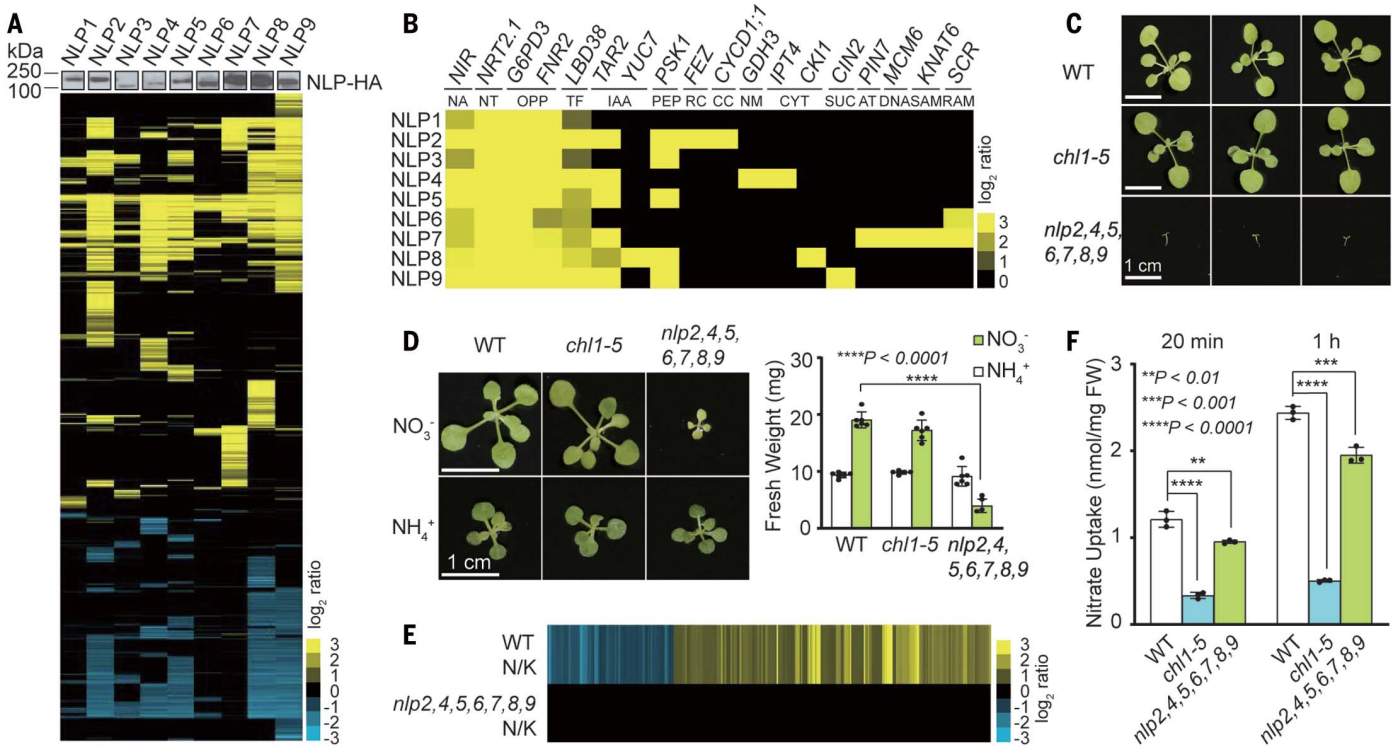

Fig. 1. Combinatorial NLP transcription factors are central to primary nitrate responses and developmental programs. (**A**) NLPs regulate both common and distinct target genes. Hierarchical clustering of RNA-seq analysis defines putative target genes of NLP1-9 by transient expression in leaf cells. NLP protein expression was determined by immunoblot analyses. (**B**) Heatmap of nitrate-responsive core genes and distinct target genes activated by NLP1-9. NA, nitrate assimilation; NT, nitrate transporter; OPP, oxidative pentose phosphate; TF, transcription factor; IAA, indole-3-acetic acid biosynthesis; PEP, peptide hormone; RC, root cap; CC, cell cycle; NM, nitrogen metabolism; CYT, cytokinin biosynthesis and signaling; SUC, sucrose invertase; AT, auxin transport; DNA, DNA synthesis; SAM, shoot apical meristem; RAM, root apical meristem. (**C**) The *nlp2,4,5,6,7,8,9* mutant abolishes shoot growth in soil. (**D**) The *nlp2,4,5,6,7,8,9* mutant displays nitrate-specific reduction in shoot and biomass. Error bars, SD; $n = 6$ biological replicates. (**E**) Primary nitrate-responsive transcriptome is abolished in *nlp2,4,5,6,7,8,9*. N, KNO_3; K, KCl. (**F**) Nitrate uptake in *nlp2,4,5,6,7,8,9*. FW, fresh weight. Error bars, SD; $n = 3$ biological replicates. Scale bars, 1 cm.

response genes (fig. S3). These findings further supported the essential role of NLPs in regulating primary nitrate responses (Fig. 1E, fig. S2, and tables S1 to S3) and plant development, as well as the existence of nitrate sensors presumably distinct from NRT1.1 (CHL1/NPF6.3) (*9*–*13*).

Nitrate derepresses NLP7 via the N terminus

The N terminus of NLPs contain the GAF-like domain and multiple conserved motifs with unknown functions (*17*). When fused to the *LexA* DNA binding domain and a nuclear localization signal, the N terminus of NLP6(1-546) is sufficient to confer nitrate response to the *8xLexA-min:GUS* reporter in nitrate-free seedlings (*15*). Single-mutant studies showed that *nlp7* but not *nlp6* exhibited overt shoot growth defects (fig. S1A). NLP6 also activated fewer target genes than did the closely related NLP7 transcription activator in transfected leaf cells (Fig. 1A and tables S1 and S2). Therefore, we examined the role of NLP7 and the functional domains of NLP7 for the activation of a synthetic nitrate-responsive reporter *4xNRE-min-LUC* in nitrate-free *Arabidopsis* leaf cells (*13*, *15*, *18*). Although *4xNRE-min-LUC* was activated by nitrate (10 mM, 2 hours) in transfected WT and *nlp6* leaf cells, its activation was diminished in *nlp7* or *nlp6,7* and abolished in *nlp2,4,5,6,7,8,9* (fig. S1G). *NRE* is evolutionarily conserved in the primary nitrate-responsive *NIR* gene promoter from *Arabidopsis*, spinach, bean, birch, and maize (*25*). These data suggested a role of NLP7 in activating *NRE*.

Full-length NLP7(1-959) conferred nitrate-specific *4xNRE-min-LUC* activation at low (0.5 mM) nitrate for 2 hours in nitrate-free leaf cells. However, the N-NLP7(1-581) domain was inactive, whereas the NLP7-C(582-959) domain was constitutively activated in the same reporter without nitrate (Fig. 2, A and B). The *Lj*NIN transcription factor from *Lotus japonicus*, which controls root nodule initiation and nitrogen fixation and shares RWP-RK DNA binding and PB1 domains with NLPs but not the N terminus (*15*, *26*), activated the *NRE*-based reporter in nitrate-free leaf cells. Analyses of the chimeric transcription factors N-NLP7-NIN-C and N-NIN-NLP7-C suggested that N-NLP7 acted as a repressor domain in full-length NLP7 and is derepressed by nitrate (Fig. 2, A and B). As the NLP7 orthologs in diverse plant species were more ancient and arose after the green algal lineage, NIN in legume species likely evolved later, having lost autorepression and nitrate responsiveness (fig. S4) (*9*, *14*, *17*, *27*).

RWP-RK transcription factors are specific to vascular plants, green algae, and slime molds (*17*). Although human cells can transport and reduce nitrate to support an alternative plant diet–based NO pathway (*28*), humans have no orthologs for NRT1.1 transceptor or NLPs. We asked whether NLP7 could activate a synthetic version of the nitrate-responsive reporter *Human-4xNRE-min-LUC* with a mammalian minimal promoter and *SV40* terminator in heterologous 293T human cells (Fig. 2C). As in nitrate-free *Arabidopsis* leaf cells, *Human-4xNRE-min-LUC* was activated by NLP7 only in the presence of nitrate (Fig. 2D). Thus, NLP7 alone may function both as a nitrate sensor and a transcription activator in the heterologous human cell system.

Nitrate directly binds to NLP7

To test the hypothesis that NLP7 is both a ligand-dependent transcription activator and an intracellular nitrate sensor, we measured

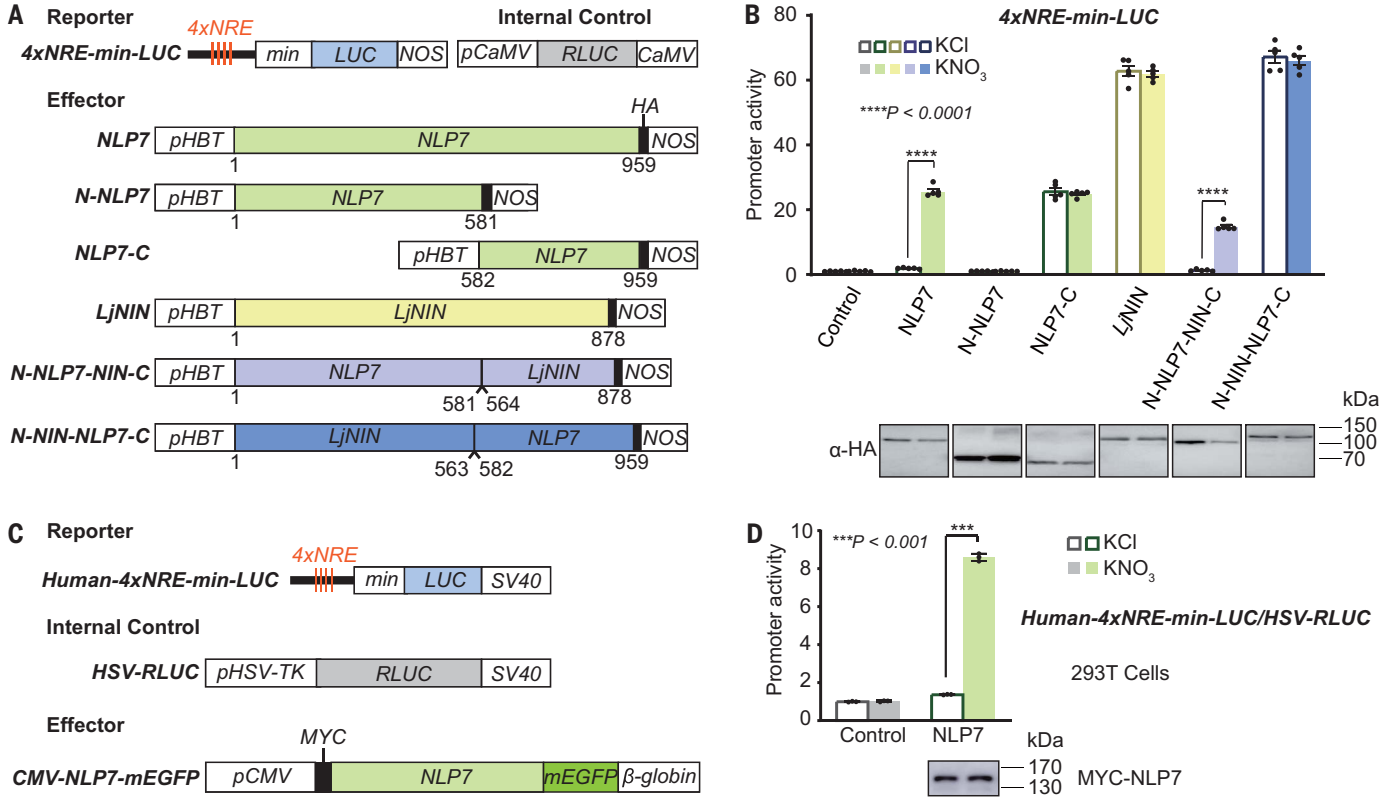

Fig. 2. Nitrate derepresses NLP7. (**A**) Reporters and effectors for transient assays in leaf cells. The nitrate-responsive reporter (*4xNRE-min-LUC*) contains four copies of nitrate-responsive element (*NRE*) and the *35S* minimal promoter (*min*) fused to firefly luciferase gene (*LUC*) and *NOS* terminator (*NOS*). The *35S* cauliflower mosaic virus promoter controls the *Renilla* luciferase gene (*pCaMV-RLUC*) as the internal control. The effector expression is controlled by a constitutive *HBT* promoter (*19*). Numbering shows the amino acid positions. (**B**) Analyses of NLP-NIN chimeras reveal derepression of NLP7 by nitrate. Transactivation assays of *NRE-LUC* were carried out in *nlp2,4,5,6,7,8,9* leaf cells by expressing effectors for 4 hours before induction (0.5 mM KNO_3 for 2 hours). Luciferase activity was normalized to *pCaMV-RLUC* activity. Error bars, SD; *n* = 5 biological replicates. Effector protein expression was determined by immunoblot analyses. (**C**) Reporters and effector for transient assays in 293T human cells. The nitrate-responsive *Human-4xNRE-min-LUC* reporter contains the *SV40* terminator. The herpes simplex virus (*HSV*) promoter–driven *RLUC* (*pHSV-RLUC*) is an internal control. The *MYC-NLP7-mGFP* gene is controlled by a human cytomegalovirus (*CMV*) promoter with the β-*globin* gene terminator. (**D**) *NLP7* activates *Human-4xNRE-min-LUC* in response to nitrate in heterologous 293T human cells. Luciferase activity was normalized to *pHSV-RLUC* activity (10 mM KNO_3 for 2 hours). Error bars, SD; *n* = 3 biological replicates.

nitrate binding to NLP7 using microscale thermophoresis (MST) and surface plasmon resonance (SPR) (*29*, *30*). We expressed and purified GST-NLP7-8xHIS fusion protein from *Escherichia coli* (fig. S5A) and performed binding experiments by MST at different nitrate concentrations (Fig. 3A). Similar to NreA, which can bind nitrate with a dissociation constant (K_d) of 22 μM, NLP7 (not HIS-GST) selectively recognized nitrate (Fig. 3A) with a K_d of 52 ± 20 μM but not its structural analog chlorate or anion phosphate (Fig. 3, A and D, and fig. S5, B to D and F). Because the N terminus of NLP7 is crucial for nitrate responsiveness, we also expressed and purified the N terminus NLP7(1-581)–based GST-N-NLP7-8xHIS fusion protein and carried out the MST assay. Nitrate could bind to N-NLP7 with a K_d of 86 ± 38 μM but not chlorate or phosphate (Fig. 3, B and E, and fig. S5E). To confirm the interaction between NLP7 and nitrate, we performed alternative binding experiments using SPR. Purified GST-N-NLP7-8xHIS protein was immobilized on a sensor chip and different concentrations of nitrate, chlorate, or phosphate were monitored by SPR with an average K_d of 72 ± 34 μM only for nitrate (Fig. 3, C and F, and fig. S5, G to I).

A genetically encoded fluorescent biosensor visualizes nitrate in plants

Ligand–sensor interaction may trigger a conformational change in the sensor protein. We generated a genetically encoded fluorescent biosensor, mCitrine-NLP7, similar to the single fluorescent protein–based glucose biosensor Green Glifon (*31*). We hypothesized that the nitrate-bound split mCitrine-NLP7 nitrate biosensor (sCiNiS) would reconstitute mCitrine to emit fluorescent signals (*31*). The predicted nuclear localization signal ($_{630}$RRKKK$_{638}$) of NLP7 was mutated to AAAAA to avoid competition with the endogenous NLP7, which is retained in the nucleus after nitrate induction for transcriptional activation (Fig. 4A and fig. S6, A and B) (*16*, *18*). Quantitative confocal imaging of the cytoplasmic nitrate by sCiNiS was carried out in mesophyll cells of cotyledons and columella cells of root tips in transgenic plants (Fig. 4, B and C). The reconstituted mCitrine fluorescent signal was detected within 5 min after nitrate (10 mM), but not KCl, induction in both mesophyll cells and primary root tip cells at single-cell resolution in intact sCiNiS transgenic seedlings that developed normally (Fig. 4, B and C, and fig. S6C). Soil nitrate concentrations can vary from the micromolar to the millimolar range (*6*). We tested different nitrate concentrations using nitrate-free transgenic seedlings and showed that the sCiNiS biosensor detected a range of nitrate concentrations from 100 μM to 10 mM in single mesophyll cells in intact plants, consistent with a sensitive and specific nitrate binding K_d of 52 μM for NLP7 in vitro (Fig. 3A and fig. S6D).

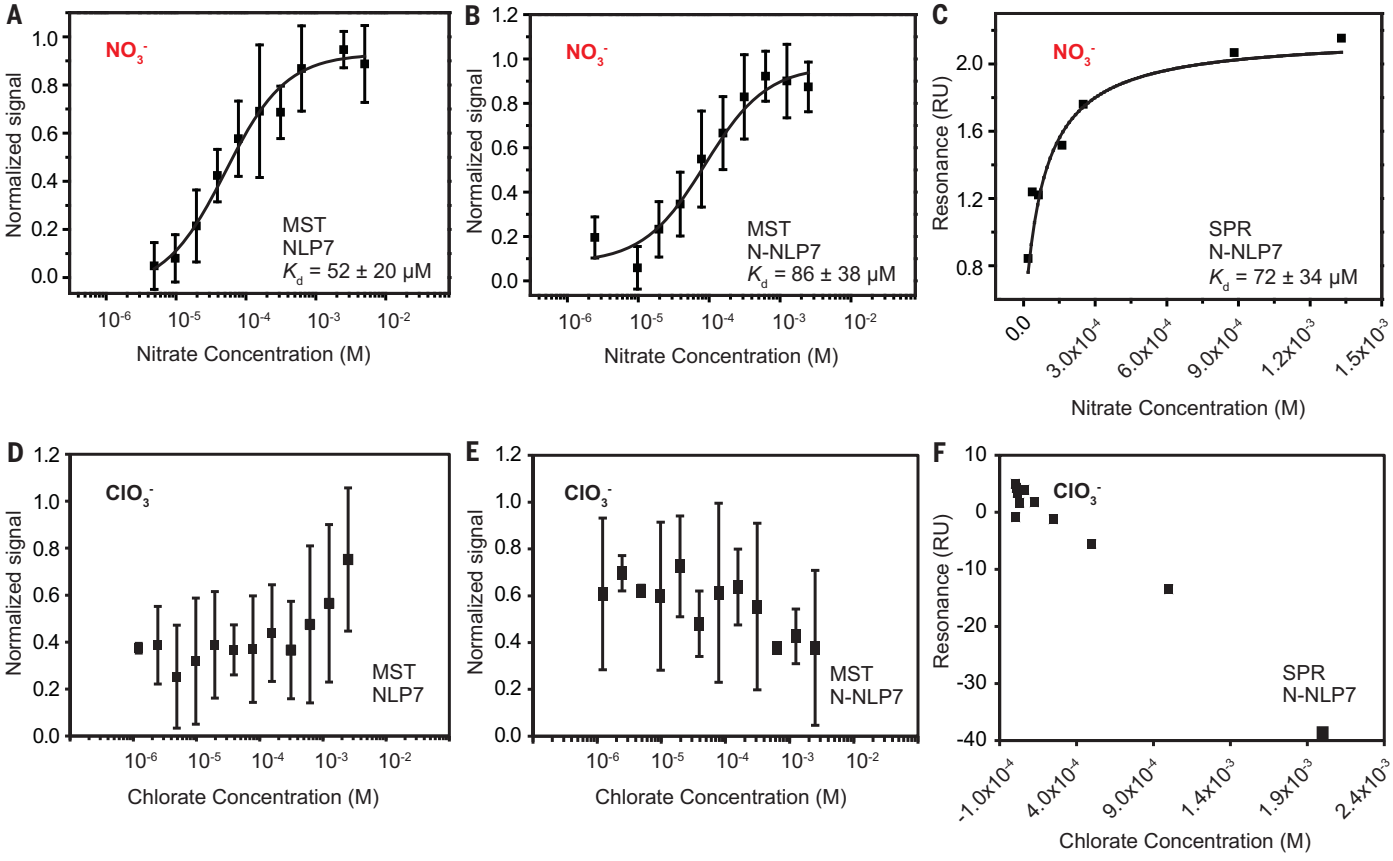

Fig. 3. Nitrate directly binds to NLP7. (**A**) NO_3^- binding to full-length NLP7, as measured by microscale thermophoresis (MST). Dissociation constant (K_d) = 52 ± 20 μM. Error bar, SD; n = 3. (**B**) NO_3^- binding to N-NLP7. K_d = 86 ± 38 μM. Error bar, SD; n = 3 independent thermophoresis measurements. (**C**) Analysis of NO_3^-–N-NLP7 interaction, as measured by surface plasmon resonance (SPR). The result is a representative of three independent experiments. The K_d is the average of three independent experiments. RU, resonance units. (**D**) ClO_3^- does not bind to full-length NLP7. Error bars, SD; n = 3 independent thermophoresis measurements. (**E**) ClO_3^- does not bind to N-NLP7. Error bars, SD; n = 3 independent thermophoresis measurements. (**F**) SPR analysis of ClO_3^-–N-NLP7 interaction. The result is a representative of three independent experiments.

Evolutionary conservation of nitrate sensors in plants

Architectures of nitrate-binding sites in bacteria and cyanobacteria proteins have been solved by crystal structure analyses of cytoplasmic NreA of *Staphylococci carnosus*, periplasmic NarX of *E. coli*, and periplasmic NrtA of *Synechocystis* sp. These nitrate-binding proteins support anaerobic respiration and nitrate transport. Their crystal structures are available in the Protein Data Bank (PDB IDs 4IUK, 3EZH, and 2G29, respectively) (*21*). We found that seven of eight critical residues in the nitrate-binding pocket of the NreA nitrate sensor could be aligned to the conserved motifs next to the putative GAF-like domain in NLP1-9 (*17*, *21*) (Fig. 5, A and B, and fig. S7A). The protein structure of the nitrate-binding domain in NLP7 predicted by the artificial intelligence programs AlphaFold2 and RoseTTAfold (*32*, *33*) also resembled that in NreA (Fig. 5C).

To functionally define the essential residues for nitrate binding in NLP7, we carried out alanine (A) scanning mutagenesis of eight putative nitrate-binding residues defined in the nitrate-NreA crystal form (*21*) and examined the nitrate response of mutated NLP7 in nitrate-free leaf cells. NLP7 mutations of four residues, Trp^{395}→Ala (W395A), H404A, L406A, or Y436A, significantly decreased nitrate-induced *4xNRE-min-LUC* activity at low (0.5 mM) nitrate for 2 hours. Because H404, L406, and Y436 are conserved in NLP2,4,5,6,8,9 with similar structures in the nitrate-binding domain (fig. S7, A and B), we next generated and analyzed double (HL/AA) and triple (HLY/AAA) mutants of NLP7, which abolished nitrate-induced *4xNRE-min-LUC* activity (Fig. 5D). The HLY nitrate-binding residues are also conserved among NLP7 orthologs within the structurally similar nitrate sensor domain in crop plants including rapeseed *Bna*NLP7, soybean *Gm*NLP6, maize *Zm*NLP6, wheat *Ta*NLP7, and rice *Os*NLP3 (Fig. 5E and fig. S7, C and D). We propose that NLP7 and its orthologs may serve as nitrate sensors conserved in photosynthetic plants preserved from charophytes to angiosperms, including eudicots and monocots but not chlorophytes (fig. S7, C and D).

To demonstrate the function of the nitrate-binding domain in NLP7, we examined the ability of NLP7 and NLP7(HLY/AAA) to activate the nitrate-responsive reporter *Human-4xNRE-min-LUC* in the heterologous 293T cell system. Compared with NLP7, NLP7(HLY/AAA) abrogated the LUC activity induced by nitrate-bound and activated NLP7 (Fig. 5F). Similar to NLP7, *Os*NLP3 as the rice NLP7 ortholog activated the nitrate-dependent *Human-4xNRE-min-LUC* activity, which was eliminated with *Os*NLP3(HLY/AAA, mutations in H392A, L394A, and Y424A) (Fig. 5F). To verify that the residues H404, L406, and Y436 are required for nitrate binding in the NLP7 sensor domain, we performed MST assays and SPR analyses using purified NLP7 (HLY/AAA) or N-NLP7(HLY/AAA, 1-581) protein (Fig. 5, G to I, and fig. S5A). Mutation of these three amino acids impeded nitrate binding in vitro. Furthermore, NLP7-HA but not NLP7(HLY/AAA)-HA complemented shoot growth and biomass phenotypes (Fig. 5J and fig. S8A) or activation of nitrate-responsive genes for nitrate transport, assimilation, and

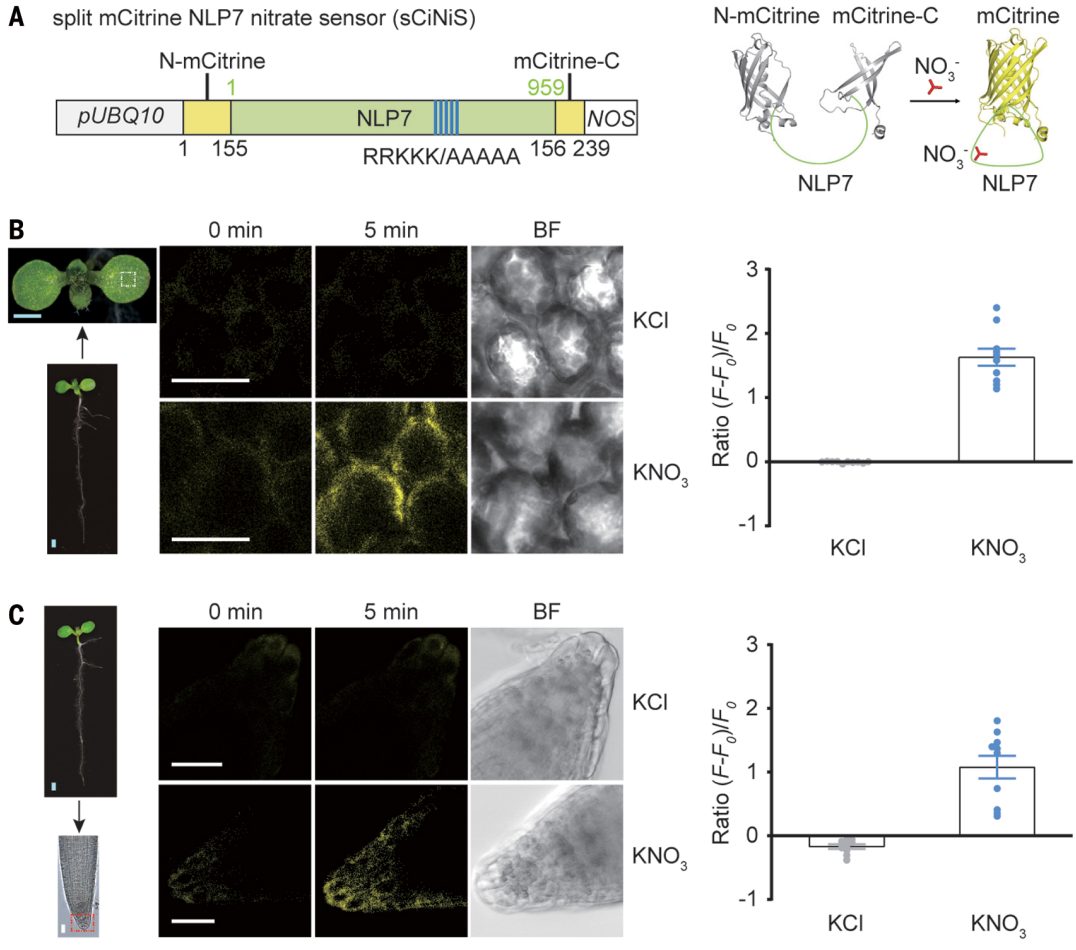

Fig. 4. The genetically encoded biosensor detects intracellular nitrate in transgenic shoots and roots. (**A**) A schematic representation of domain structure and a model of the nitrate biosensor. Nitrate triggers a conformational change of the split mCitrine-NLP7 nitrate biosensor (sCiNiS) and reconstitutes mCitrine to emit fluorescent signals. The predicted nuclear localization signal ($_{630}$RRKKK$_{638}$) of NLP7 was mutated to AAAAA to avoid competition with the nitrate-induced endogenous NLP7 in the nucleus. (**B**) Imaging the cytoplasmic nitrate by sCiNiS in mesophyll cells of cotyledons. Dashed white line indicates the imaging site. Images are representative of 10 cotyledons. Blue scale bar, 1 mm. White scale bar, 30 μm. (**C**) Imaging the cytoplasmic nitrate by sCiNiS in root tips. Dashed red line indicates the imaging site. Images are representative of 10 root tips, KCl or KNO_3 (10 mM). $(F - F_0)/F_0$, the relative fluorescence intensity. Error bars, SD; n = 10 biological replicates.

transcription in *nlp7* (fig. S8B). The data support the role of these residues in NLP7 to function as a dual master transcription activator and nitrate sensor central to transcriptional reprogramming and plant development.

Discussion

Nitrate is an essential nutrient for photosynthetic plant growth in most soils and controls metabolic and developmental processes pivotal to plant vegetative and reproductive development. However, nitrogen fertilizer is energy intensive to produce and causes pollution; furthermore, its overuse in agriculture to boost crop yields has led to environmentally disastrous eutrophication worldwide. Global and regional studies indicate declining nitrogen availability on Earth (*1*–*7*). Improved plant nitrogen use efficiency could contribute to sustainable agriculture and ecosystem preservation.

Our data identified NLP2,4,5,6,7,8,9 as transcription factors that initiate signaling for nitrate-mediated transcriptome reprogramming and coordinate transport, metabolism, hormone, signaling, transcription, and root-shoot developmental programs (*5*–*7*, *18*). With genetic, genomic, cellular, imaging, biochemical, and structural analyses, we discovered that NLP7 has dual regulatory modes as a transcription activator and an intracellular nitrate sensor. Nitrate binding triggers a conformational change and transcriptional derepression of NLP7, acting simultaneously and synergistically with CPK-dependent NLP7 (S205) phosphorylation for nuclear retention (*16*, *18*).

The sequence and structure of the nitrate-binding pocket of NLP7 is evolutionarily conserved and found in bacteria nitrate sensor NreA proteins (*34*), as well as in other *Arabidopsis* NLPs and the NLP7 orthologs of land plants and freshwater green algae in charophytes. We speculate that NLP7 orthologs and other NLPs, but not NINs, in diverse plant species also act as sensor-transcription factors to initiate primary nitrate responses regulating overlapping and distinctive target genes for a range of physiological functions in the nutrient-regulatory networks (*5*–*8*, *18*). The functions of NRT1.1(CHL1/NPF6.3) as an extracellular nitrate transceptor and transporter (*10*–*12*) seem distinct when plants are grown in soil or NO_3^- medium. The development of the genetically encoded fluorescent nitrate biosensor sCiNiS enables sensitive and specific monitoring of single-cell nitrate signals in whole seedlings, which can now support real-time live imaging and elucidate how plants dynamically translocate nitrate between tissues or cell types.

Our results reveal a regulatory mechanism that photosynthetic plants use to sense inorganic nitrogen, which then activates plant signaling networks and growth responses. Our insights may suggest avenues through which to enhance nitrogen utilization efficiency in crops, reduce fertilizer and energy inputs, and mitigate climate change arising from the emission of greenhouse gases in the interest of supporting more-sustainable agriculture (*1*, *5*, *6*, *35*).

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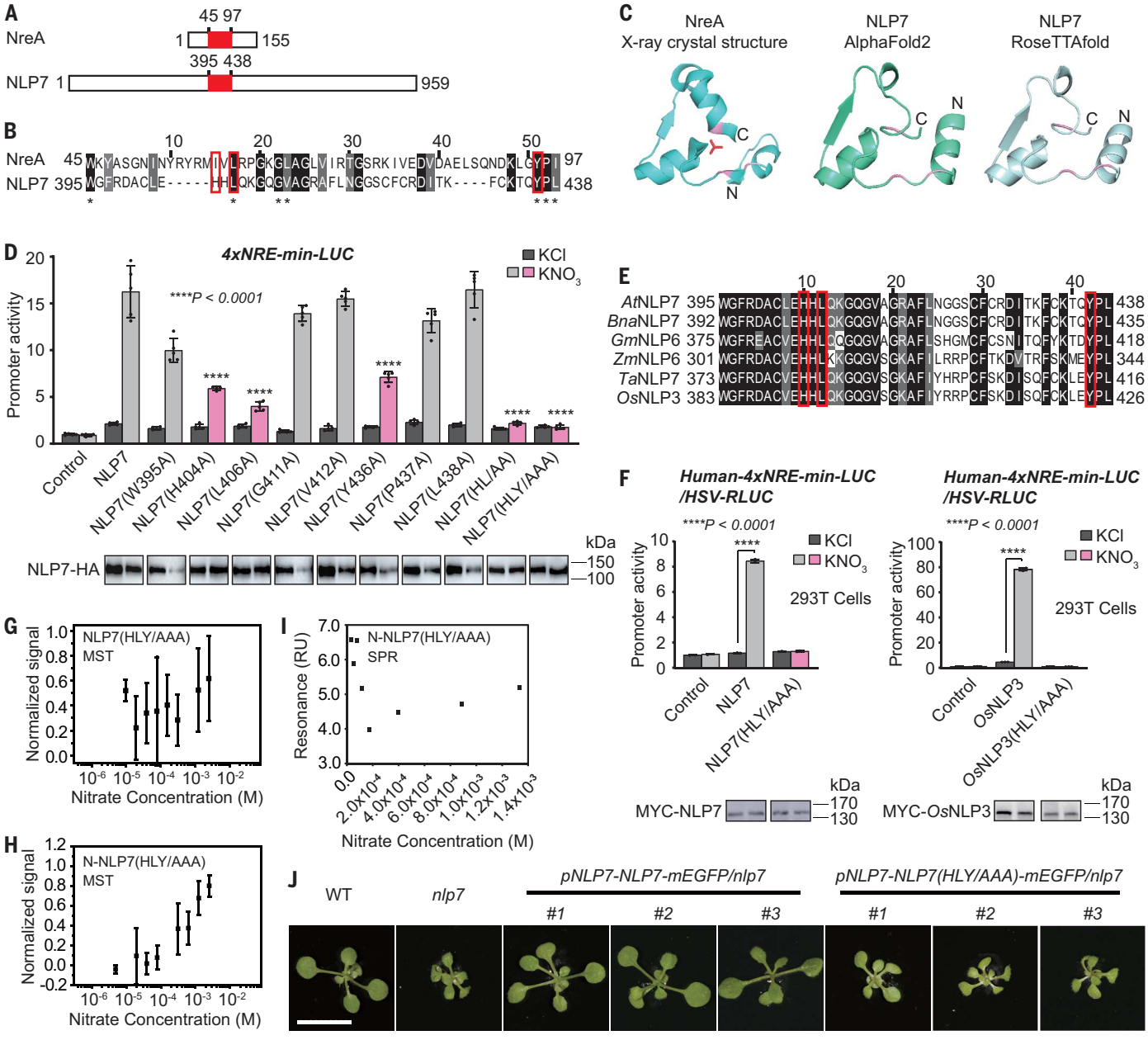

Fig. 5. The NLP7 sensor domain resembles NreA with conserved residues for nitrate perception and signaling. (**A**) The nitrate-binding domain of the bacterial nitrate sensor NreA shares homology to a region of NLP7. The homologies are in red. Numbering refers to amino acid positions. (**B**) Sequence alignment of the nitrate-binding domain of NreA and NLP7. Black box indicates conserved residue. Gray box indicates semiconserved residue. Three essential nitrate-binding residues are outlined in red. Asterisks mark the conserved residues in the nitrate-binding pocket of NreA. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**C**) Comparison of the crystal structure of the NreA nitrate-binding domain and the predicted structure of NLP7. Red stick: nitrate; Pink: NreA(I59, L61, Y95) or NLP7(H404, L406, Y436). (**D**) Nitrate-binding mutant screens by transient assays in leaf cells. Transactivation of *4xNRE-min-LUC* by NLP7 or nitrate-binding mutants was analyzed in *nlp2,4,5,6,7,8,9* leaf protoplasts (0.5 mM KNO_3 for 2 hours). Error bars, SD; $n = 5$ biological replicates. (**E**) The key nitrate-binding residues are conserved in NLPs from major crops. Critical nitrate-binding residues are outlined in red. (**F**) Functional conservation of NLP7 and rice *Os*NLP3 for nitrate perception and transcription activation in heterologous 293T human cells. The nitrate-binding residues of NLP7(H404, L406, Y436) or *Os*NLP3(H392, L394, Y424) are essential for nitrate activation of *Human-4xNRE-min-LUC*. Error bars, SD; $n = 3$ biological replicates. (**G** to **I**) Mutant full-length NLP7(HLY/AAA) and N-NLP7(HYL/AAA) abolish nitrate binding. Error bars, SD; $n = 3$ independent thermophoresis measurements. The result is a representative of three independent experiments in SPR assays. (**J**) *pNLP7-NLP7-GFP* but not *pNLP7-NLP7(HLY/AAA)-GFP* complements the *nlp7-1* mutant. Scale bar, 1 cm.

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SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.add1104
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MDAR Reproducibility Checklist

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NIN-like protein 7 transcription factor is a plant nitrate sensor

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Transcription-activating nitrate sensor

Plants depend on nitrogen, responding with changes in growth and metabolism when nitrogen supplies change. Indeed, nitrogen fertilizer underlies a good deal of agricultural crop productivity. Studying a family of seven similar genes, Liu *et al*. identified the nitrate sensor in the small mustard plant *Arabidopsis thaliana*. The protein's nitrate-binding pocket resembles that found in bacteria nitrate sensors. Conformation change upon nitrate binding allows the protein to then function as a transcriptional activator, triggering the plant's responses to nitrogen availability. —PJH