Trafficking and localization of *KNOTTED1* related mRNAs in shoot meristems

Munenori Kitagawa, Xiaosa Xu & David Jackson

To cite this article: Munenori Kitagawa, Xiaosa Xu & David Jackson (2022) Trafficking and localization of *KNOTTED1* related mRNAs in shoot meristems, Communicative & Integrative Biology, 15:1, 158-163, DOI: 10.1080/19420889.2022.2095125

To link to this article: [https://doi.org/10.1080/19420889.2022.2095125](https://doi.org/10.1080/19420889.2022.2095125)
**ABSTRACT**

Multicellular organisms use transcripts and proteins as signaling molecules for cell-to-cell communication. Maize KNOTTED1 (KN1) was the first homeodomain transcription factor identified in plants, and functions in maintaining shoot stem cells. KN1 acts non-cell autonomously, and both its messenger RNA (mRNA) and protein traffic between cells through intercellular nanochannels called plasmodesmata. KN1 protein and mRNA trafficking are regulated by a chaperonin subunit and a catalytic subunit of the RNA exosome, respectively. These studies suggest that the function of KN1 in stem cell regulation requires the cell-to-cell transport of both its protein and mRNA. However, in situ hybridization experiments published 25 years ago suggested that KN1 mRNA was missing from the epidermal (L1) layer of shoot meristems, suggesting that only the KN1 protein could traffic. Here, we show evidence that KN1 mRNA is present at a low level in L1 cells of maize meristems, supporting an idea that both KN1 protein and mRNA traffic to the L1 layer. We also summarize mRNA expression patterns of KN1 homologs in diverse angiosperm species, and discuss KN1 trafficking mechanisms.

Cell-to-cell communication is essential for determining cell fates, and is the basis for multicellular development. For example, stem cells divide to self-renew and produce cells destined to differentiate, and many forms of cell-to-cell communication regulate their identity and proliferation [1,2]. Plants use multiple types of cell-to-cell signaling, including secreted ligands and receptors, as well as direct transfer of molecules through plasmodesmata, membrane-lined nanochannels that penetrate the cell wall [3-5]. Plasmodesmal signaling is critical for maintaining plant stem cell niches, or meristems [6-8]. Several transcription factors, including homeodomain factors, act as non-cell-autonomous signals by trafficking through plasmodesmata [9].

Maize KNOTTED1 (KN1) was the first homeodomain transcription factor identified in plants, and the first transcription factor found to traffic via plasmodesmata [10,11]. KN1 homologs, so-called class I KN1-like homeobox (KNOX I) genes, are conserved in all taxa in the plant kingdom [12,13]. The primary function of KNOX I genes is to maintain the pool of stem cells in shoot meristems, as shown by the loss of meristems in maize kn1 mutants [14-16]. This function, as well as cell-to-cell mobility, is conserved widely, for example, in the KN1 homolog SHOOT MERISTEMLESS (STM) in *Arabidopsis* [17-19]. While transcription factor protein trafficking is broadly documented, the function of class I KNOX genes requires trafficking of both their protein and mRNA [7,8,19]. Regulators of class I KNOX protein and mRNA trafficking, such as chaperonins and an RNA exosome subunit, respectively, and additional mobile transcription factors, such as WUSCHEL and SHORT-ROOT, have been identified [7,8,20-22].

In addition to short-range cell-to-cell trafficking, proteins and mRNAs are also selectively transported systemically between plant organs via the phloem. Regulatory factors and protein/RNA motifs and modifications important for this long-range transport have also been identified [23,24]. Thus, cell-to-cell signaling using proteins and mRNAs is a rapidly developing field, and although significant progress has been made in understanding its mechanisms, there are still many open questions.

Previous studies suggested that KN1 protein and mRNA interact as they traffic between cells, perhaps by forming a ribonucleoprotein (RNP) complex [11,25,26]. If KN1 and STM traffic as RNPs, they may need to streamline their shape to pass through the tiny plasmodesmatal pores. Chaperones and RNA helicases may be involved in this process [27,28]. This process may also involve RNA-binding proteins that function as carriers, and their receptors, as well as actin and myosin that can alter plasmodesmal pore size [27,29,30]. In our recent study, we found that a catalytic subunit of the RNA exosome, *Arabidopsis*...
Ribosomal RNA-Processing Protein 44A (AtRRP44A), controls KN1 and STM mRNA trafficking between cells [8]. AtRRP44A is predominantly nuclear, but when levels in the cytoplasm are enhanced by the addition of a nuclear export sequence, it has a capacity to localize to plasmodesmata. These findings suggest that AtRRP44A is involved in the plasmodesmata targeting of class I KNOX RNPs, the conversion of RNPs to a mobile form, or the trafficking through plasmodesmata. In support of these ideas, we found that KN1 mRNAs localize to cytoplasmic puncta that move dynamically around the cytoplasm, and transiently interact with plasmodesmata [8]. This interaction could allow KN1 mRNA to traffic through plasmodesmata to neighboring cells. However, how KN1 mRNA is targeted to plasmodesmata is unknown. The mRNA of another mobile factor, FLOWERING LOCUS T, is tethered to endosomes and recruited to plasmodesmata via microtubules and actin [31]. Since STM is also associated with endosomes and microtubule-associated proteins [20,21], it may be targeted to plasmodesmata by a similar mechanism.

The trafficking of KN1 and STM proteins and RNAs has been studied mostly in Arabidopsis and tobacco leaves, but how they traffic in the shoot meristem, where their function is less well understood. However, mutants that reduce KN1/STM protein or mRNA trafficking in the leaf, such as chaperonin or RNA exosome subunits mutants, significantly affected meristem development [7,8,19], suggesting their trafficking in the meristem is important for normal development. Angiosperm shoot meristems have a layered structure, where an outer epidermal L1 layer covers inner layers. Despite multiple reports of KN1 and STM mRNA trafficking, the original report of KN1 trafficking presented contradictory results, as KN1 mRNA was detected in the inner meristem layers but absent from the L1, whereas KN1 protein was detected throughout all meristem layers [32,33]. This difference in localization led to the prediction, and later demonstration, that KN1 protein can traffic from the inner meristem layers to the L1 [11]. However, the original report and several others suggested that KN1 traffics with its mRNA as an RNP [8,25]. Homeodomain proteins are known for their DNA binding activity, but their specific mRNA binding has also been demonstrated in flies [34,35]. However, if KN1 mRNA can traffic, and KN1 protein and mRNA can form an RNP, it is puzzling that KN1 mRNA is not detected in the L1 layer of the maize shoot meristem. One possible explanation is that KN1 RNPs traffic between cells in the inner meristem layers, but only KN1 protein traffics to L1 [36], however, this seems unlikely. Another possibility is that KN1 mRNA does traffic to the L1, but its levels are too low to be detected by in situ hybridization. Even a few KN1 mRNA molecules in the L1 could be amplified by multiple rounds of translation to produce abundant protein levels [37,38]. Indeed, we present evidence here that this is likely to be the case.

Recently, single-cell mRNA sequencing (scRNA-seq) has provided unprecedented resolution in plant expression studies [39–41]. In a scRNA-seq experiment of developing maize ears, we found multiple distinct cellular clusters representing known cell types and domains, and indeed we found KN1 transcripts in meristem L1 cells [42,43] (Figure 1(a)). However, these transcripts could be background noise or sporadic expressions captured in the scRNA-seq experiments. A recent laser microdissection (LCM) RNA-seq experiment also detected KN1 transcripts in L1 cells of the shoot meristem. The KN1 mRNA levels in the L1 were about one tenth of those in the L2, but much higher than in leaf primordia, where STM expression is repressed [44]. To support these findings, we performed KN1 in situ hybridization [32] using a longer detection period. Indeed, we detected weak KN1 mRNA in situ signal in L1 cells (Figure 1(b)). While we cannot rule out the possibility that this signal is from diffusion of the alkaline phosphatase reaction product, the combined evidence of scRNA-seq, LCM and mRNA in situ hybridization supports the idea that a small amount of KN1 mRNA traffics from the inner meristem layers to the L1.

It is also interesting to compare expression patterns of KN1 and STM homologs in diverse angiosperm species. Expression varies significantly between species and meristem stages, suggesting interesting hypotheses about the regulation of trafficking of KN1/STM-related transcripts. In maize, KN1 mRNA appears to be restricted to the inner meristem layers in both vegetative and inflorescence stages, and is mostly undetectable in the L1 layer [32] except as described above. Similar patterns are seen in other species, including in brachypodium spikelet and floral meristems and wheat vegetative meristems [45,46]. In some species, however, expression is clearly observed in the L1 layer at particular stages of development. For example, mRNA of the rice KN1 ortholog ORYZA SATIVA HOMEBOX1 (OSH1) localizes to the inner meristem layers of vegetative and inflorescence meristems, but is also observed in the L1 meristem layer in spikelet and early stage flower meristems. However, expression is once again restricted to the inner meristem layers in the late stage
Figure 1. KN1 mRNAs are detected at low levels in L1 (epidermal) cells of maize meristems. (a) Single-cell RNA sequencing [42] indicates that KN1 transcripts are abundant in meristem (clusters 9, 10, and 11), vasculature (clusters 4, 5, and 12), and ground tissue (clusters 1 and 8), but also present at low levels in meristem L1 cells (cluster 6, asterisks). (b) Over-exposure of a KN1 mRNA in situ hybridization shows a weak signal in the L1 (pink) and a strong signal in the inner meristem layers (dark blue) in a maize ear spikelet pair meristem. (c-g) Rice OSH1 mRNA is absent from the L1 layer of the vegetative shoot apical meristem (SAM) (c) but observed in some L1 cells in the inflorescence meristem (im) (d), and is throughout the L1 in the spikelet meristem (sm) (e) and floret meristem (fm) (f), then is again restricted to the inner layers in the later stage fm (g). P0 and P1, plastochron 0 and 1; rg, rudimentary glume; sl, sterile lemma; ca, carpel. (h) mRNA in situ hybridization showing STM mRNA in the entire vegetative shoot meristem including L1 layer in Arabidopsis. The data used for panel A is from [42]. Panel C, D, E, F-G, and H used images from [48,54–56] and [8] with permission, respectively. Scale bars = 50 µm.
flower meristems [47,48] (Figure 1(c–g)). In tomato and tobacco, KN1 ortholog mRNAs are also restricted to the inner cell layers in vegetative meristems, but are clearly detected in the L1 layer at the reproductive stages [49–51]. Thus, localization of KN1 homolog transcripts is often excluded from the L1 layer in vegetative stages, but found in the L1 layer in later stages. A different situation is observed for Arabidopsis STM, where its mRNA is not detected in the L1 in early embryo stages, but is detected there in later embryo and seedling and reproductive stages [17] (Figure 1(h)). What causes these changes in mRNA localization between species and meristem stages? One possibility is that KNOX I gene transcription switches between layers depending on the species and/or developmental stage. However, another possibility is that the mobility of KNOX I mRNA between cell layers is differentially regulated. In support of this idea, the permeability and number of plasmodesmata change dynamically during meristem transitions [52], and this might affect selective transport of specific transcripts. A better understanding of these processes could enable manipulation of KNOX expression and localization to fine-tune meristem activity, and improve plant growth and crop yields.

Acknowledgments

We thanks Dr. Byoung Il Je for sharing the KN1 probes [53]. We also thank Dr. Thu Tran for assisting in editing the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research is supported by the National Science Foundation (IOS 1930101)

ORCID

Munenori Kitagawa @ http://orcid.org/0000-0002-2766-3889
Xiaoa Xu @ http://orcid.org/0000-0002-3452-6751
David Jackson @ http://orcid.org/0000-0002-4269-7649

References

[1] Fouracre JP, Poethig RS. Lonely at the top? Regulation of shoot apical meristem activity by intrinsic and extrinsic factors. Curr Opin Plant Biol. 2020;58:17–24.
[2] Kitagawa M, Jackson D. Control of meristem size. Annu Rev Plant Biol. 2019;70(1):269–291.
[3] Li ZP, Patelini A, Glavier M, et al. Intercellular trafficking via plasmodesmata: molecular layers of complexity. Cell Mol Life Sci. 2021;78(3):799–816.
[4] Peters WS, Jensen KH, Stone HA, et al. Plasmodesmata and the problems with size: interpreting the confusion. J Plant Physiol. 2021;257:153341.
[5] Sankoh AF, Burch-Smith TM. Approaches for investigating plasmodesmata and effective communication. Curr Opin Plant Biol. 2021;64:102143.
[6] Daum G, Medzhiradszky A, Suzuki T, et al. A mechanistic framework for noncell autonomous stem cell induction in arabidopsis. Proc Nat Acad Sci. 2014;111(40):14619–14624.
[7] Xu XM, Wang J, Xuan Z, et al. Chaperonins facilitate KNOTTED1 cell-to-cell trafficking and stem cell function. Science. 2011;333(6046):1141–1144.
[8] Kitagawa M, Wu P, Balkunde R, et al. An RNA exosome subunit mediates cell-to-cell trafficking of a homeobox mRNA via plasmodesmata. Science. 2022;375(6577):177–182.
[9] Fuchs M, Lohmann JU. Aiming for the top: non-cell autonomous control of shoot stem cells in arabidopsis. J Plant Res. 2020;133(3):297–309.
[10] Vollbrecht E, Veit B, Sinha N, et al. The developmental gene KNOTTED-1 is a member of a maize homeobox gene family. Nature. 1991;350(6315):241–243.
[11] Lucas WJ, Bouché-Pillon S, Jackson DP, et al. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. Science. 1995;270(5244):1980–1983.
[12] Wilhelmsson PK, Mühlich C, Ullrich KK, et al. Comprehensive genome-wide classification reveals that many plant-specific transcription factors evolved in streptophyte algae. Genome Biol Evol. 2017;9(12):3384–3397.
[13] Szövényi P, Waller M, Kirbis A. Evolution of the plant body plan. Curr Top Dev Biol. 2019;131:1–34.
[14] Kerstetter RA, Laudencia-Chingcuanco D, Smith LG, et al. Loss-of-function mutations in the maize homeobox gene, knotted1, are defective in shoot meristem maintenance. Development. 1997;124(16):3045–3054.
[15] Vollbrecht E, Reiser L, Hake S. Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, knotted1. Development. 2000;127(14):3161–3172.
[16] Bolduc N, Tyers RG, Freeling M, et al. Unequal redundancy in maize knotted1 homeobox genes. Plant Physiol. 2014;164(1):229–238.
[17] Long JA, Moan EI, Medford JJ, et al. A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of arabidopsis. Nature. 1996;379(6560):66–69.
[18] Kim J-Y, Yuan Z, Jackson D. Developmental regulation and significance of KNOX protein trafficking in arabidopsis. Development. 2003;130(18):4351–4362.
[19] Balkunde R, Kitagawa M, Xu XM, et al. SHOOT MERISTEMLESS trafficking controls auxillary meristem
formation, meristem size and organ boundaries in arabidopsis. Plant J. 2017;90(3):435–446.

[20] Winter N, Kollwigg G, Zhang S, et al. MPB2C, a microtubule-associated protein, regulates non-cell-autonomy of the homeodomain protein KNOTTED1. Plant Cell. 2007;19(10):3001–3018.

[21] Liu L, Li C, Song S, et al. FTIP-dependent STM trafficking regulates shoot meristem development in arabidopsis. Cell Rep. 2018;23(6):1879–1890.

[22] Gundu S, Tabassum N, Blilou I. Moving with purpose and direction: transcription factor movement and cell fate determination revisited. Curr Opin Plant Biol. 2020;57:124–132.

[23] Kehr J, Morris RJ, and Krager F. Long-distance transported RNAs: from identity to function. Annu Rev Plant Biol. 2021 73 :457–474.

[24] Wang T, Li XJ, Zhang XJ, et al. RNA motifs and modification involve in RNA long-distance transport in plants. Front Cell Dev Biol. 2021;9:651278.

[25] Kim JY, Rim Y, Wang L, et al. A novel cell-to-cell trafficking assay indicates that the KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. Genes Dev. 2005;19(7):788–793.

[26] Dubnau J, Struhl G. RNA recognition and translational regulation by a homeodomain protein. Nature. 1996;379(6667):694–699.

[27] Krager F, Monzer J, Shash K, et al. Cell-to-cell transport of proteins: requirement for unfolding and characterization of binding to a putative plasmodesmal receptor. Plant J. 1998;15(3):367–381.

[28] Reagan BC, Ganusova EE, Fernandez JC, et al. RNA on the move: the plasmodesmata perspective. Plant Sci. 2018;275:1–10.

[29] Diao M, Huang S. An update on the role of the actin cytoskeleton in plasmodesmata: a focus on formins. Front Plant Sci. 2021;12:647123.

[30] Yan Y, Ham B-K, Chong YH, et al. A plant small RNA-binding protein 1 family mediates cell-to-cell trafficking of RNAI signals. Mol Plant. 2020;13(2):321–335.

[31] Luo K-R, Huang N-C, and Chang Y-H, et al. Arabidopsis cyclophilins direct plasmodesmata-targeting of mobile mRNA via organelle hitchhiking. Res Square. 2022. doi:10.21203/rs.3.rs-1088339/v1.

[32] Jackson D, Veit B, Hake S. Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. Development. 1994;120(2):405–413.

[33] Smith LG, Hake S. The initiation and determination of leaves. Plant Cell. 1992;4(9):1017–1022.

[34] Chan S-K, Struhl G. Sequence-specific RNA binding by bicoid. Nature. 1997;388(6643):634.

[35] Carnesecchi J, Boumpas P, van Nierop Y, et al. The Hox transcription factor Ultrabithorax binds RNA and regulates co-transcriptional splicing through an interaction with RNA polymerase II. Nucleic Acids Res. 2022;50(2):763–783.

[36] Roberts AG, Oparaika KJ. Plasmodesmata and the control of symplastic transport. Plant Cell Environ. 2003;26(1):103–124.

[37] Kennell D, Riezman H. Transcription and translation initiation frequencies of the Escherichia coli lac operon. J Mol Biol. 1977;114(1):1–21.

[38] Goldberg I, Paulsson J, Zawilski SM, et al. Real-time kinetics of gene activity in individual bacteria. Cell. 2005;123(6):1025–1036.

[39] Denyer T, Timmermans MCP. Crafting a blueprint for single-cell RNA sequencing. Trends Plant Sci. 2022;27(1):92–103.

[40] Shojaee A, Saavedra M, and Huang SSC. Potentials of single-cell genomes in deciphering cellular phenotypes. Curr Opin Plant Biol. 2021 63 ;102059.

[41] Seyfferth C, Renema J, Wendrich JR, et al. Advances and opportunities in single-cell transcriptomics for plant research. Annu Rev Plant Biol. 2021;72(1):847–866.

[42] Xu XS, Crow M, Rice BR, et al. Single-cell RNA sequencing of developing maize ears facilitates functional analysis and trait candidate gene discovery. Dev Cell. 2021;56(4):557–568.

[43] Satterlee JW, Strable J, Scanlon MJ. Plant stem-cell organization and differentiation at single-cell resolution. Proc Nat Acad Sci. 2020;117(52):33689–33699.

[44] Knauer S, Javelle M, Li L, et al. A high-resolution gene expression atlas links dedicated meristem genes to key architectural traits. Genome Res. 2019;29 (12):1962–1973.

[45] Derbyshire P, Byrne ME. More spikelets is required for spikelet fate in the inflorescence of brachypodium. Plant Physiol. 2013;161(3):1291–1302.

[46] Morimoto R, Kosugi T, Nakamura C, et al. Intragenic diversity and functional conservation of the three homologous loci of the KN1-type homeobox gene W KnoxI in common wheat. Plant Mol Biol. 2005;57(6):907–924.

[47] Sentoku N, Sato Y, Kurata N, et al. Regional expression of the rice KN1 -type homeobox gene family during embryo, shoot, and flower development. Plant Cell. 1999;11(9):1651–1663.

[48] Tanaka W, Ohsomi S, and Kawakami N, et al. Flower meristem maintenance by TILLERS ABSENT 1 is essential for ovule development in rice. Development. 2021;148(24):dev199932.

[49] Tamaoki M, Kusaba S, Kano-Murakami Y, et al. Ectopic expression of a tobacco homeobox gene, NTH15, dramatically alters leaf morphology and hormone levels in transgenic tobacco. Plant Cell Physiol. 1997;38(8):917–927.

[50] Nishimura A, Tamaoki M, Sato Y, et al. The expression of tobacco knotted1-type class 1 homeobox genes correspond to regions predicted by the cytostitutional zonation model. Plant J. 1999;18(4):337–347.

[51] Parnis A, Cohen O, Gutfinger T, et al. The dominant developmental mutants of tomato, mouse-ear and curly, are associated with distinct modes of abnormal transcriptional regulation of a knotted gene. Plant Cell. 1997;9(12):2143–2158.

[52] Sager R, Lee J-Y. Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses. J Exp Bot. 2014;65(22):6337–6358.
[53] Je BI, Gruel J, Lee YK, et al. Signaling from maize organ primordia via FASCIATED EAR3 regulates stem cell proliferation and yield traits. Nat Genet. 2016;48(7):785–791.

[54] Ohmori Y, Tanaka W, and Kojima M, et al. WUSCHEL-RELATED HOMEobox4 Is involved in meristem maintenance and is negatively regulated by the CLE Gene FCP1 in Rice. Plant Cell. 2013;25(1):229–241.

[55] Suzaki T, Toriba T, Fujimoto M, et al. Conservation and diversification of meristem maintenance mechanism in Oryza sativa: function of the FLORAL ORGAN NUMBER2 gene. Plant Cell Physiol. 2006;47(12):1591–1602.

[56] Tanaka W, Ohmori Y, and Ushijima T, et al. Axillary meristem formation in rice requires the WUSCHEL ortholog TILLERS ABSENT1. Plant Cell. 2015;27(4):1173–1184.