Nuclear translocation of proteins and the effect of phosphatidic acid

Hongyan Yao^{1,*}, Geliang Wang², and Xuemin Wang²

¹National Key Laboratory of Plant Molecular Genetics; Institute of Plant Physiology and Ecology; Chinese Academy of Sciences; Shanghai, China; ²Department of Biology; University of Missouri; St. Louis, MO USA; Donald Danforth Plant Science Center; St. Louis, MO USA

Transport of proteins containing a nuclear localization signal (NLS) into the nucleus is mediated by nuclear transport receptors called importins, typically dimers of a cargo-binding α -subunit and a β -subunit that mediates translocation through the nuclear pore complexes (NPCs). However, how proteins without canonical NLS move into the nucleus is not well understood. Recent results indicate that phospholipids, such as phosphatidic acid, play important roles in the intracellular translocation of proteins between the nucleus and cytoplasm.

The movement of proteins into the nucleus is a fundamental process critical to the basic function and regulation of eukaryotic cells. Many proteins that move into the nucleus contain a conserved short cluster of basic residues called nuclear localization sequence (NLS).^{1–4} These proteins are translocated into the nucleus through nuclear pore complexes (NPCs) by receptor-mediated import pathways.⁵ However, some proteins that move into the nucleus do not possess the apparent canonical NLS and/or they are translocated into the nucleus only under specific cues, such as stress or hormonal stimulations. In addition, some cytosolic metabolic enzymes move into the nucleus where they have regulatory functions. Recent studies indicate that phospholipid mediators, such as phosphatidic acid (PA), play important roles in regulating the nuclear movement of proteins.

importin (IMP) α/β heterodimer, where the adaptor IMP α subunit, which possesses NLS-binding site, binds the NLS, and the receptor IMP β subunit targets the IMP α /NLS-bearing protein complex to the nuclear pore in eukaryotes.^{1,6,7} This process also requires the cytoplasmic RanGDP to translocate the transport complex to NPC, and RanGTP to release IMP α /NLS-bearing protein into the nucleoplasm.^{1,8,9} Alternatively, IMP β itself or related homologs fulfills the function of both IMP α and β in binding NLSs, targeting them to the NPC.^{10–13} In the model plant *Arabidopsis thaliana*, the IMP α recognizes the NLSs and also exhibits the nuclear envelope localization typical of IMP β in other eukaryotic systems, mediates nuclear import pathway independent of IMP β or IMP α/β .¹⁴

The prototype of a monopartite NLS is the sequence motif PKKKRKV (basic residues are underlined) of the simian virus 40 (SV40) large T-antigen, whereas the prototypical bipartite NLS is that of nucleoplasmin (KRPAATKKAGQAKKKKL).^{15,16} NLSs of nuclear proteins are collected in the database of NLSdb, containing 114 experimentally determined NLSs obtained through an extensive literature search.¹⁷

Conditional Movement of Proteins Into the Nucleus

While some proteins are designated to go to the nucleus, some proteins are translocated into the nucleus only under specific conditions, such as environmental or hormonal cues. For example, phytochromes are red and far-red photoreceptors that regulate plant growth and development in response to light, and

Keywords: *Arabidopsis*, nuclear localization signal, protein nuclear import, phospholipid, phosphatidic acid

Abbreviations: NLS, nuclear localization signal; NPCs, nuclear pore complexes; PA, phosphatidic acid; PtdIns(5)P, Phosphatidylinositol-5-bisphosphate; PLD, phospholipase D.

*Correspondence to: Hongyan Yao; Email: hyiao@sibs.ac.cn

Submitted: 08/26/2014

Revised: 09/04/2014

Accepted: 09/11/2014

<http://dx.doi.org/10.4161/15592324.2014.977711>

Addendum to: Yao H, Wang G, Guo L, Wang X. Phosphatidic acid interacts with a MYB transcription factor and regulates its nuclear localization and function in *Arabidopsis*. The Plant Cell 2013; 25: 5030–42. doi: 10.1105/tpc.113.120162

Receptor-mediated Pathways for Protein Import to the Nucleus

The classic receptor-mediated import pathways involve recognition by the

their nuclear movement upon light activation is regarded as a key regulatory step in phytochrome signaling.¹⁸⁻²⁰ Phytochromes exist in 2 photo-interconvertible conformational states: a biologically inactive Pr form and a biologically active Pfr form. Phytochrome-B (PhyB) is the prominent photo-stable phytochrome involved in red-light-sensing and shade-avoidance responses.²¹ PhyB contains NLS, but in the Pr form, the NLS in the C-terminal PAS-related domain (PRD) is masked through direct interactions with the phyB's N-terminal bilin lyase domain (BLD) and PHY subdomains of the N terminus. In the Pfr form, structural rearrangements in BLD and PHY lead to weaker interactions with the PRD, allowing the exposure of the NLS for translocation.²²

On the other hand, the photoreceptor phytochrome-A (phyA) does not contain a specific NLS. The nuclear import of light-dependent phyA depends on 2 specific plant proteins, namely FAR-RED ELONGATED HYPOCOTYL1 (FHY1) and FHY1-LIKE (FHL) in planta, and the import is mediated by the conformer-(Pfr>Pr) dependent interaction of these proteins.²³⁻²⁵ PhyA regulates germination and seedling establishment by mediating very low fluence (VLFR) and far-red high irradiance (FR-HIR) responses in Arabidopsis. In darkness, phyA is a soluble cytosolic protein in the biologically inactive Pr form, but upon light, a portion of phyA forms into biologically active Pfr and then rapidly moves to nucleus.^{20,26,27}

The concomitant nuclear translocation is another pathway for proteins with or without canonical NLS for movement to the nucleus. Arabidopsis transcription factors, E2F and DP, are not predominantly localized to the nucleus, and the complete nuclear localization of some members is driven by the co-expression of their partner proteins. The nuclear localization of AtE2F1/3 and AtDPa depends on their interaction and occurs concomitantly in a cooperative manner.²⁸ The GFP fusion protein with single AtE2F1, AtE2F3, or AtDPa did not exhibit an exclusive nuclear localization except when AtE2F1 or AtE2F3 was co-expressed with AtDPa, indicating that neither E2F1/3 nor DPa has an autonomous activity for nuclear

localization.²⁸ Even the addition of the SV40 NLS to AtE2F3 did not cause the nuclear localization, whereas it improved the efficiency of the nuclear localization when DPa was co-expressed.²⁸ The concomitant nuclear translocation conferred by the DPa interaction would be an effective dimerization partner of both AtE2F1 and AtE2F3.²⁸

Another case for concomitant nuclear translocation is the CCAAT-box binding nuclear factor Y (NF-Y) that consists of 3 different subunits, A, B, and C. All Arabidopsis NF-YBs lack NLS and their transport into the nucleus is proposed to be dependent on their interaction with NF-YC, while NF-YA and NF-YC are imported into the nucleus independently.²⁹ Interaction of the representatives of the NF-Y subunits, NF-YB10 and NF-YC9, in the cytoplasm is essential for the translocation of NF-YB into the nucleus and follows a piggyback mechanism.²⁹

Hexokinases (HXKs) function as glucose sensors and the catalytic and sensory functions of Arabidopsis HXK1 (AtHXK1) can be uncoupled.³⁰ The AtHXK1-GFP fusion protein was found to be in mitochondria, but a small amount of AtHXK1 is also associated with nuclei.^{31,32} Nuclear AtHXK1 controls the expression of specific photosynthetic genes as a transcriptional co-repressor by creating a nuclear complex core with the vacuolar H⁺-ATPase B1 and the 19S regulatory particle of proteasome subunit.^{31,33} Nuclear AtHXK1 also enhances the degradation of ETHYLENE-INSENSITIVE3 (EIN3), a key transcriptional regulator in ethylene signaling to mediate glucose signaling into or out of the nucleus and cross talk with ethylene signaling.³² In ethylene signaling, ETHYLENE-INSENSITIVE2 (EIN2) functions upstream EIN3. EIN2 is localized at the ER membrane.³⁴ The ethylene signal promotes the cleavage of the carboxyl end of EIN2 (CEND) from ER-located EIN2, and facilitates its nuclear localization to stabilize EIN3 protein.³⁵

Post-translational modifications of proteins under stress conditions also facilitate protein nuclear translocation. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been largely reported not only to be a ubiquitous enzyme involved

in glycolysis, but also behaves as a moonlighting (namely nuclear localization and DNA binding) protein in both animal and plant cells. Redox modifications of GAPDH cysteine regulate its catalytic activity, and also confer its non-metabolic function. S-nitrosylated modifications of GAPDH have a profound effect on interaction with other proteins and eventually change its subcellular localization, which can transmit an apoptotic stimulus in animal cells.³⁶⁻³⁹ The acetylation of 3 lysine residues was also proposed for the nuclear translocation of GAPDH after the apoptotic stimulus in animal cells.⁴⁰ Plant cytosolic GAPDHs, referred as GAPCs, can be translocated to the nucleus under particular, oxidizing conditions for multifunctional properties.⁴¹⁻⁴³ GAPC accumulates in nuclei upon stress in plants, and has a motif with some similarity to NLS at the N-terminus with 6 basic amino acids (MADKKIRIGINGFGRIGRLVAR), which overlaps with the NAD-binding domain (GXGXXG).⁴⁴ Their interaction between oxidized GAPC and PLD- δ did not show the role for oxidized GAPC's translocation. In Arabidopsis leaves, oxidized GAPC interacts with PLD δ and promote PA production and stomatal closure.⁴⁵ PA also binds to oxidized GAPC and promotes its proteolysis, and the GAPC2 and its cleavage product are present both in the cytosol and the nucleus.⁴¹ However, it is unclear whether the GAPC's NLS has the nuclear localization function.

Lipid Effects on Proteins Movement Into the Nucleus

In addition to protein-protein interaction and posttranslational modifications, lipid protein interactions are involved in mediating the intracellular translocation between the nucleus and cytoplasm. Phosphatidylinositol-5-bisphosphate [PtdIns(5)P] promotes protein translocation to the nucleus⁴⁶ and also from the nucleus to the cytoplasm.⁴⁷⁻⁴⁹ For example, in *Arabidopsis*, PtdIns(5)P promotes translocation of an Arabidopsis trithorax homolog (ATX1), a histone 3 lysine 4 (H3K4me3) trimethyltransferase, from the nucleus to the cytoplasm.⁴⁷

PA is a lipid mediator involved in various cellular processes, and one of the key mechanisms of action by PA is its direct interaction with proteins. The PA binding can regulate the target protein activity and intracellular location. PA can promote or inhibit protein localization to the nucleus, depending on the specific protein. For example, PA in the endoplasmic reticulum (ER) binds to the transcriptional repressor Opi1p (OVERPRODUCER OF INOSITOL PROTEIN 1) and keeps it out of the nucleus in yeast⁵⁰ (Fig. 1). This PA tethering relieves the transcriptional suppression so membrane lipid synthesis will increase. The *de novo* lipid biosynthesis will consume PA, which releases Opi1p that enters the nucleus to suppress lipid production^{50,51}. In plants, PA tethers ABSCISIC ACID INSENSITIVE 1 (ABI1) to the plasma membrane, decreasing the translocation of ABI1 into the nucleus where it binds to ARABIDOPSIS THALIANA HOMEBOX PROTEIN 6 (ATHB6), a transcription factor that negatively regulates ABA responses^{52,53} (Fig. 1).

PA can directly interact with the MYB transcription factor WEREWOLF (WER) that does not have a detectable nuclear

localization signal based on its amino acid sequence.^{54,55} The interaction is necessary for WER's nuclear localization⁵⁵ (Fig. 1). The PA interaction may tether WER to the nuclear membrane and this tethering is necessary for the localization of the proteins from the cytosol to the nucleus. These studies also propose that PA exists in nuclear membrane.

The different effects of PA are likely to be determined by the location and source of PA on the membranes, as well as other factors associated with the PA-binding proteins. In the PA-Opi1p interaction, PA on the ER is metabolized for *de novo* lipid biosynthesis^{50,51}, whereas in the PA-WER interaction, phospholipase D (PLD) contributes to the production of PA⁵⁵. The spatial and temporal production of PA by PLD may result in the different effects of PA on the nuclear localization of its target proteins, and PA produced by nucleus-associated PLD may regulate WER's nuclear localization.

The *Arabidopsis* genome has PLDs, PLD α (3), β (2), γ (3), δ , ϵ , ζ (2), with different reaction requirements.⁵⁶ The subcellular localization for some PLDs is still unknown. PLD γ was detected in the nuclear fraction from subcellular fractions

of *Arabidopsis* leaves,⁵⁷ and PLD β 1 is predicted to localize in the nucleus (<http://www.arabidopsis.org/>).

The nucleus-associated PLDs may contribute to the PA production on and in the nucleus. In addition, PA can be produced by other reactions, such as the activation of phospholipase C followed by diacylglycerol kinases. Further studies are needed to determine how PA mediates the nuclear localization of proteins and whether the PA-protein interactions constitute a mechanism for the nuclear translocation of proteins that lack NLS.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Jennifer Potratz for critically reading the manuscript.

Funding

The work was supported as part of the Center for Advanced Biofuels Systems (CABS), an Energy Frontier Research Center funded by the US. Department of Energy, Office of Science, Basic Energy Sciences under Award DE-SC0001295 and supported by the National Science Foundation (NSF) under Award IOS-0818740. HY acknowledges support by Chinese Scholarship Council.

References

- Adam SA, Gerace L. Cytosolic proteins that specifically bind nuclear location signals are receptors for nuclear import. *Cell* 1991; 66:837-47; PMID:1653647
- Adam SA, Sterne-Marr R, Gerace L. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J Cell Biol* 1990; 111:807-16; PMID:2391365
- Hahn S, Maurer P, Caesar S, Schlenstedt G. Classical NLS proteins from *Saccharomyces cerevisiae*. *J Mol Biol* 2008; 379:678-94; PMID:18485366
- Hall NM, Hereford L, Herskowitz I. Targeting of *E. coli* beta-galactosidase to the nucleus in yeast. *Cell* 1984; 36:1057-65; PMID:6323016
- Kalderon D, Richardson WD, Markham AF, Smith, A. E. Sequence requirements for nuclear location of simian virus 40 large-T antigen. *Nature* 1984; 311:33-8; PMID:6088992
- Adam EJH, Adam SA. Identification of cytosolic factors required for nuclear location sequence-mediated binding to the nuclear envelope. *J Cell Biol* 1994; 125:547-55; PMID:8175880
- Gorlich D, Prehn S, Laskey RA, Hartmann E. Isolation of a protein that is essential for the first step of nuclear protein import. *Cell* 1994; 79:767-78; PMID:8001116

Figure 1. Phospholipid effects on protein intracellular translocation between nucleus and cytoplasm reported in plant and yeast. In plant and yeast, phosphatidic acid (PA) can promote or inhibit protein localization to the nucleus, depending on the specific protein. In *Arabidopsis*, PA can directly interact with a MYB transcription factor WEREWOLF (WER), and that the interaction is necessary for WER's nuclear localization to regulate the root hair development. PA tethers its binding protein ABI1 to the plasma membrane, resulting in prevention of ABA INSENSITIVE 1 (ABI1) from translocation into the nucleus where it binds to ARABIDOPSIS THALIANA HOMEBOX PROTEIN 6 (ATHB6), a transcription factor that negatively regulates ABA responses. In *Saccharomyces cerevisiae*, PA in the endoplasmic reticulum (ER) binds to the transcriptional repressor OVERPRODUCER OF INOSITOL PROTEIN 1 (Opi1p) and keeps it out of the nucleus to regulate phospholipid metabolism. Another phospholipid in *Arabidopsis*, phosphatidylinositol-5-bisphosphate [PtdIns(5)P] promotes translocation of an *Arabidopsis* trithorax homolog (ATX1), a histone 3 lysine 4 (H3K4me3) trimethyltransferase, from the nucleus to the cytoplasm to regulate gene expression.

8. Rexach M, Blobel G. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell* 1996; 83:683-92; PMID:8521485
9. Weis K, Dingwall C, Lamond AI. Characterization of the nuclear protein import mechanism using Ran mutants with altered nucleotide binding specificities. *EMBO J* 1996; 15:7120-8; PMID:9003787
10. Aitchison JD, Blobel G, Rout MP. Kap104p: A karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science* 1996; 274:624-7; PMID:8849456
11. Pollard VW, Michael WM, Nakielný S, Siomi MC, Wang F, Dreyfuss G. A novel receptor-mediated nuclear protein import pathway. *Cell* 1996; 86:985-94; PMID:8808633
12. Rout MP, Blobel G, Aitchison JD. A distinct nuclear import pathway used by ribosomal proteins. *Cell* 1997; 89:715-25; PMID:9182759
13. Tiganis T, Flint AJ, Adam SA, Tonks NK. Association of the T-cell protein tyrosine phosphatase with nuclear import factor p97. *J Biol Chem* 1997; 272:21548-57; PMID:9261175
14. Hubner S, Smith HMS, Hu W, Chan CK, Rihs HP, Paschali BM, Raikhel NV, Jans DA. Plant importin alpha binds nuclear localization sequences with high affinity and can mediate nuclear import independent of importin beta. *J Biol Chem* 1999; 274:22610-7; PMID:10428841
15. Kalderon D, Roberts BL, Richardson WD, Smith AE. A short amino acid sequence able to specify nuclear location. *Cell* 1984; 39:499-509; PMID:6096007
16. Robbins J, Dilworth SM, Laskey RA, Dingwall C. Two interdependent basic domains in nucleoplasmic nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* 1991; 64:615-23; PMID:1991323
17. Nair R, Carter P, Rost B. NLSdb: database of nuclear localization signals. *Nucl Acids Res* 2003; 31:397-9; PMID:12520032
18. Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. *Annu Rev Genet* 2004; 38:87-117; PMID:15568973; <http://dx.doi.org/10.1146/annurev.genet.38.072902.092259>
19. Nagatani A. Light-regulated nuclear localization of phytochromes. *Curr Opin Plant Biol* 2004; 7:708-11; PMID:15491920
20. Nagy F, Schafer E. Phytochromes control photomorphogenesis by differentially regulated, interacting signaling pathways in higher plants. *Annu Rev Plant Biol* 2002; 53:329-55; PMID:12221979; <http://dx.doi.org/10.1146/annurev.arplant.53.100301.135302>
21. Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D. Phytochromes: photosensory perception and signal transduction. *Science* 1995; 268:675-80; PMID:7732376; <http://dx.doi.org/10.1126/science.7732376>
22. Chen M, Tao Y, Lim J, Shaw A, Chory J. Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. *Curr Biol* 2005; 15: 637-42; PMID:15823535; <http://dx.doi.org/10.1016/j.cub.2005.02.028>
23. Hiltbrunner A, Tscheuschler A, Viczian A, Kunkel T, Kircher S, Schafer E. FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor. *Plant Cell Physiol* 2006; 47:1023-34; PMID:16861711
24. Hiltbrunner A, Viczian A, Bury E, Tscheuschler A, Kircher S, Toth R, et al. Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. *Curr Biol* 2005; 15:2125-33; PMID:16332538
25. Pfeiffer A, Kunkel T, Hiltbrunner A, Neuhaus G, Wolf I, Speth V, et al. A cell-free system for light dependent nuclear import of phytochrome. *Plant J* 2009; 57:680-9; PMID:18980642
26. Kim L, Kircher S, Toth R, Adam E, Schafer E, Nagy F. Light induced nuclear import of phytochrome-A:GFP fusion proteins is differentially regulated in transgenic tobacco and Arabidopsis. *Plant J* 2000; 22:125-33; PMID:10792828
27. Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, Schafer E. Light quality-dependent import of the plant photoreceptors phytochrome A and B. *Plant Cell* 1999; 11:1445-56; PMID:10449579; <http://dx.doi.org/10.1105/tpc.11.8.1445>
28. Kosugi S, Ohashi Y. Interaction of the Arabidopsis E2F and DP proteins confers their concomitant nuclear translocation and transactivation. *Plant Physiol* 2002; 128:833-43; PMID:11891240; <http://dx.doi.org/10.1104/pp.010642>
29. Hackenberg D, Wu Y, Voigt A, Adams R, Schramm P, Grimm B. Studies on differential nuclear translocation mechanism and assembly of the three subunits of the Arabidopsis thaliana transcription factor NF-Y. *Mol Plant* 2012; 5:876-88. PMID:22199235; <http://dx.doi.org/10.1093/mp/ssl107>
30. Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, et al. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light and hormonal signaling. *Science* 2003; 300:332-6; PMID:12690200; <http://dx.doi.org/10.1126/science.1080585>
31. Cho YH, Yoo SD, Sheen J. Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell* 2006; 127:579-89; PMID:17081979
32. Yanagisawa S, Yoo SD, Sheen J. Differential regulation of EIN3 stability by glucose and ethylene signaling in plants. *Nature* 2003; 425:521-5; PMID:14523448
33. Chen JG. Sweet sensor, surprising partners. *Sci STKE* 2007; 373:pe7; PMID:17299203
34. Bisson MM, Bleckmann A, Allekotte S, Groth G. EIN2, the central regulator of ethylene signalling, is localized at the ER membrane where it interacts with the ethylene receptor ETR1. *Biochem J* 2009; 424:1-6; PMID:19769567
35. Wen X, Zhang C, Ji Y, Zhao Q, He W, An F, Jiang L, Guo H. Activation of ethylene signaling is mediated by nuclear translocation of the cleaved EIN2 carboxyl terminus. *Cell Res* 2012; 22:1613-6; PMID:23070300
36. Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y, Takahashi M, Cheah JH, Tankou SK, Hester LD, et al. S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol* 2005; 7:665-74; PMID:15951807
37. Kornberg MD, Sen N, Hara MR, Juluri KR, Nguyen JV, Snowman AM, Law L, Hester LD, Snyder SH. GAPDH mediates nitrosylation of nuclear proteins. *Nat Cell Biol* 2010; 12:1094-100; PMID:20972425
38. Nakamura T, Lipton SA. Emerging role of protein-protein transnitrosylation in cell signaling pathways. *Antioxid Redox Signal* 2013; 18:239-49; PMID:22657837; <http://dx.doi.org/10.1089/ars.2012.4703>
39. Sen N, Hara MR, Ahmad AS, Cascio MB, Kamiya A, Ehmsen JT, Agrawal N, Hester L, Doré S, Snyder SH, et al. GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. *Neuron* 2009; 63:81-91; PMID:19607794; .
40. Ventura M, Mateo F, Serratos J, Salaet I, Carujo S, Bachs O, Pujol MJ. Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase is regulated by acetylation. *Int J Biochem Cell Biol* 2010; 42:1672-80; PMID:20601085; <http://dx.doi.org/10.1016/j.biocel.2010.06.014>
41. Kim SC, Guo L, Wang X. Phosphatidic acid binds to cytosolic glyceraldehyde-3-phosphate dehydrogenase and promotes its cleavage in Arabidopsis. *J Biol Chem* 2013; 288:11834-44; PMID:23504314
42. Vescovi M, Zaffagnini M, Festa M, Trost P, LoSchiavo F, Costa A. Nuclear accumulation of cytosolic glyceraldehyde-3-phosphatedehydrogenase in cadmium-stressed Arabidopsis roots. *Plant Physiol* 2013; 162:333-46; PMID:23569110
43. Waver I, Bucholc M, Astier J, Anielska-Mazur A, Dahan J, Kulik A, Wyslouche-Cieszyńska A, Zareba
- Kozioł M, Krzywinska E, Dadlez M, et al. Regulation of Nicotianatabacum osmotic stress-activated protein kinase and its cellular partner GAPDH by nitric oxide in response to salinity. *Biochem J* 2010; 429:73-83; PMID:20397974
44. Holtgrete S, Gohlke J, Starmann J, Druce S, Klocke S, Altmann B, Wojtera J, Lindermayr C, Scheibe R. Regulation of plant cytosolic glyceraldehyde-3-phosphate dehydrogenase isoforms by thiol modifications. *Physiol Plant* 2008; 133:211-28; PMID:18298409
45. Guo L, Devaiah SP, Narasimhan R, Pan X, Zhang Y, Zhang W, Wang X. Cytosolic glyceraldehyde-3-phosphate dehydrogenases interact with phospholipase Dδ to transduce hydrogen peroxide signals in the Arabidopsis response to stress. *Plant Cell* 2012; 24:2200-12; PMID:22589465
46. Gozani O, Karuman P, Jones DR, Ivanov D, Cha J, Lugovskoy AA, Baird CL, Zhu H, Field SJ, Lessnick SL, Villaseñor J, et al. The PHD finger of the chromatin-associated protein ING2 functions as a nuclear phosphoinositide receptor. *Cell* 2003; 114:99-111; PMID:12859901
47. Alvarez-Venegas R, Sadler M, Hlavacka A, Baluska F, Xia Y, Lu G, Firsov A, Sarath G, Moriyama H, Dubrovsky JG, et al. The Arabidopsis homolog of triphorax, ATX1, binds phosphatidylinositol 5-phosphate, and the two regulate a common set of target genes. *Proc Natl Acad Sci USA* 2006; 103:6049-54; PMID:16585509
48. Dieck CB, Boss WF, Perera IY. A role for phosphoinositides in regulating plant nuclear functions. *Front Plant Sci* 2012; 3:50; PMID:22645589
49. Viiri KM, Jänis J, Siggers T, Heinonen TY, Valjakka J, Bulky ML, Mäki M, Lohi O. DNA-binding and -bending activities of SAP30L and SAP30 are mediated by a zinc-dependent module and monophosphoinositides. *Mol Cell Biol* 2009; 29:342-56; PMID:19015240
50. Loewen CJ, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, Henry SA, Levine TP. Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. *Science* 2004; 304:1644-7; PMID:15192221
51. Carman GM, Henry SA. Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. *J Biol Chem* 2007; 282:37293-7; PMID:17981800
52. Himmelbach A, Hoffmann T, Leube M, Hohener B, Grill E. Homeodomain protein ATHB6 is a target of the protein phosphatase ABI1 and regulates hormone responses in Arabidopsis. *EMBO J* 2002; 21:3029-38; PMID:12065416
53. Zhang W, Qin C, Zhao J, Wang X. Phospholipase D alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. *Proc Natl Acad Sci USA* 2004; 101:9508-13; PMID:15197253
54. Ryu KH, Kang YH, Park YH, Hwang I, Schiefelbein J, Lee MM. The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis. *Development* 2005; 132:4765-75; PMID:16207757
55. Yao H, Wang G, Guo L, Wang X. Phosphatidic acid interacts with a MYB transcription factor and regulates its nuclear localization and function in Arabidopsis. *Plant Cell* 2013; 25:5030-42; PMID:24368785
56. Qin C, Wang X. The Arabidopsis phospholipase D family. Characterization of a calcium-independent and phosphatidylcholine-selective PLDzeta 1 with distinct regulatory domains. *Plant Physiol* 2002; 128:1057-68; PMID:11891260; <http://dx.doi.org/10.1104/pp.010928>
57. Fan L, Zheng S, Cui D, Wang X. Subcellular distribution and tissue expression of phospholipase Dα, Dβ, and Dγ in Arabidopsis. *Plant Physiol* 1999; 119:1371-8. PMID:10198096; <http://dx.doi.org/10.1104/pp.119.4.1371>