



Comparative genomics and molecular characterization of the maize PIN family proteins

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A commentary on

The maize PIN gene family of auxin transporters

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Owing to the recent advances in nucleic acid sequencing technologies, there has been exponential growth in genome sequencing and annotation over the last 10 years, especially in the field of plant biology. This phenomenon spans a wide variety of clades from algae to angiosperms, both monocots and dicots, and several databases and platforms have been published to hold and mine the generated data (Goodstein et al., 2011; Rouard et al., 2011; Van Bel et al., 2011). These databases include datasets and tools that allow one to perform comparative genomic analyses. Our understanding of evolution has been greatly enriched by such genome-wide approaches, notably by the ability to compare entire gene families across species (Delseny, 2009).

Among the various aspects of plant physiology that have drawn particular attention in functional and comparative genomics approaches, hormone biosynthetic, and signaling pathways stand out as the most studied, due to their regulatory roles in plant development and growth and biotic/abiotic stress responses (Gomez-Porrás et al., 2007). Auxin, for instance, is the hormone responsible for shaping the plant, including establishing root and shoot architecture in response to developmental programs and environmental stimuli (Bohn-Courseau, 2010; Tromas and Perrot-Rechenmann, 2010). Precise regulation of plant development patterning is dependent upon the tight control of auxin repartition at both the tissue and the cellular level, which is ensured by a series of partially redundant transporters whose

sub-cellular localization and activity are under constant adjustment. To date, three protein families have been identified for auxin transport activity: the AUX1 family is involved in the import of indole-3-acetic acid (IAA), and the PIN and ABCB families mainly function as exporters (Paponov et al., 2005; Geisler and Murphy, 2006). It is generally accepted that auxin entry into the cell mediated by AUX1 participates in polarized auxin flux (Kramer, 2004; Kramer and Bennett, 2006). In addition, the free diffusion of protonated IAA through the plasmalemma accounts for some fraction of auxin entry, as it has been estimated that 17% of IAA (pKa 4.85) is protonated in the apoplastic space (pH ~ 5.5; Zazimalová et al., 2010). Once in the cytosol, however, it is exclusively present in its anionic form IAA⁻ which is unable to cross the plasma membrane. Therefore, the release of IAA and its transfer to neighboring cells is mediated solely by active auxin exporters. The ABC-type transporters ABCB1, ABCB4, ABCB14, and ABCB19 have been characterized as possessing IAA transport activity, but do not seem to contribute to polarized auxin export (Titapiwatanakun and Murphy, 2009) because they are homogeneously localized throughout the plasma membrane. On the contrary, PIN transporters can be targeted to specific regions of the plasmalemma and mediate polarized auxin flux from cell to cell (Blakeslee et al., 2005). In *Arabidopsis*, eight proteins belong to this family and share 32–85% identity at the amino acid level. Their respective roles in polarizing auxin transport rely on differences in sub-cellular localization and expression specificity. While AtPIN5 is relatively widely expressed at low levels (Krecek et al., 2009), AtPIN1, for instance, is exclusively expressed in aerial parts of the plant (Galweiler et al., 1998; Scarpella

et al., 2006). AtPIN2 (Muller et al., 1998), AtPIN3 (Friml et al., 2002b), and AtPIN4 (Friml et al., 2002a) can be detected in the root tip where they mediate tropism and root patterning, and AtPIN8 is specifically expressed in pollen (Krecek et al., 2009).

Interestingly, phylogenetic analyses of the PIN family suggested that these proteins appeared with the rise of vascular plants, while ABCB-type transporters are more ancient (Paponov et al., 2005; Zazimalova et al., 2007). Comparing AtPIN orthologs from monocots, such as rice (Wang et al., 2009), corn (Forestan et al., 2012), and sorghum (Shen et al., 2010), and dicots such as alfalfa (Schnabel and Frugoli, 2004), soybean, poplar, and *A. lyrata* (Krecek et al., 2009; Forestan and Varotto, 2010) clearly highlights a diversity of the organization of these transporters among various plant species. Particularly, the prevalence of monocot- (e.g., OsPIN10a, OsPIN10b, ZmPIN10a, ZmPIN10b, Bradi2g44990.1, Bradi2g15610.1) and dicot-specific (e.g., AtPIN3, AtPIN4, AtPIN7, POPTR0010s12320.1, POPTR0008s12830.1). PIN transporters (Forestan and Varotto, 2010) raises the issue that simple sequence comparisons may not be sufficient to identify genes that are functionally related. Molecular characterization at the single gene/transcript/protein level is therefore required to establish similarities in terms of expression profile and/or protein activity, and to accurately identify candidates for, for instance, molecular manipulation in applied research.

Forestan and coworkers performed quite an exhaustive analysis aimed at ascertaining the maize counterparts of the *Arabidopsis* and rice PIN transporters. They identified 11 potential ZmPIN and ZmPIN-like genes, in addition to the three previously identified members of this family (Carraro et al., 2006; Forestan and

Varotto, 2010), and proposed nomenclature by assigning each *ZmPIN* a name based on sequence similarity with *Arabidopsis* homologs. Next they assessed spatial and temporal expression of 14 *ZmPIN* and *ZmPIN-like* genes by RT-PCR, *in situ* hybridization, immunolocalization, and DR5-reporter lines. Their results reveal both tissue-specific (*ZmPIN1d*, *ZmPIN9*) and ubiquitous (*ZmPIN1a*, *ZmPIN1b*, *ZmPIN1c*) expression patterns, which are altered upon application of drugs (NAA, NPA) and in the *br2* mutant. Based on this molecular study, the authors also propose that *ZmPIN1a*, *ZmPIN1b*, and *ZmPIN1c*, orthologs of *AtPIN1*, could perform the functions of *AtPIN3*, *AtPIN4*, and *AtPIN7*, for which no orthologs have been identified in maize. This illustrates the necessity of performing molecular studies of individual genes in parallel with comparative functional genomic approaches. The use of a recently developed auxin reporter (Brunoud et al., 2012) and the database of auxin transport velocities (Kramer et al., 2011) should also contribute to the better characterization of auxin transport and physiology in non-model species.

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