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INTERACTIONS OF PIN AND PGP EFFLUX MECHANISMS IN POLAR AUXIN TRANSPORT

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Directional transport of the phytohormone auxin is required for the establishment and maintenance of plant polarity. The polarity of auxin transport is established primarily at the point of cellular efflux, but the activity of the efflux transporters has been difficult to characterize. Two classes of proteins have been shown to mediate auxin export: members of the PIN subfamily of major facilitator proteins and P-glycoprotein (PGP) ABC transporters. Mutations in the *PIN1* genes in Arabidopsis result in developmental defects and reduced polar auxin transport, while triple and quadruple *pin* mutations result in a loss of embryonic polarity. Defects in *PGP1*, *PGP4*, and *PGP19* result in reductions in growth and auxin transport of varying severity in Arabidopsis (*pgp1*, *pgp4*, *pgp19*), maize (*brachytic2*), and sorghum (*dwarf3*). Auxin transport defects and dwarf phenotypes are more exaggerated in *pgp1 pgp19* double mutants, suggesting overlapping function. Both PGPs and PINs have been shown to directly mediate auxin transport at the cellular level. PIN2 and PIN7 have been shown to mediate auxin transport when heterologously expressed in yeast, mammalian cells, and BY-2 cells, and *PIN1* overexpression in Arabidopsis cell cultures (but not other heterologous systems) results in increased auxin transport. Arabidopsis *pgp1*, *pgp19*, and *pgp1 pgp19* protoplasts display reductions in the transport of natural and synthetic auxins and oxidative auxin breakdown products consistent with the defects in auxin transport observed in whole plants and seedlings. Heterologous expression of PGP1 and PGP19 in yeast and mammalian cells results in increased efflux of natural and synthetic auxins, while expression of PGP4 results in auxin influx. However, heterologously expressed PGPs also transport oxidative auxin breakdown products and benzoic acid, but fail to mediate transport of mammalian multiple drug resistance substrates. These data indicate that in plants PGPs do not function as “multiple-drug resistance proteins”, but instead function as ATP-dependent hydrophobic anion transporters. Interactions with PIN proteins appear to modulate activity and enhance substrate and inhibitor specificities. PGP-PIN interactions were confirmed using yeast two-hybrid and co-immunoprecipitation assays, and PIN1 and PGP19 were found to co-localize in detergent resistant “lipid raft” membrane microdomains. PGP19 appears to stabilize PIN1 in these structures, as PIN1 was preferentially detergent-solubilized from *pgp19* membranes. Although PGP19 and PIN1 colocalize in shoot apices and the root endodermis, they do not appear to colocalize in other light-grown tissues. However, PIN1 membrane localization is more readily perturbed in vascular tissues of the root tip, suggesting an indirect effect from loss of PGP19 function. The results suggest that PINs and PGPs can function as both independent and interactive efflux mechanisms.