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Report

High-Affinity Auxin Transport by the AUX1 Influx Carrier Protein

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Summary

In plants, auxin is a key regulator of development and is unique among plant hormones in that its function requires polarized transport between neighboring cells to form concentration gradients across various plant tissues [1–5]. Although putative auxin-influx [6, 7] and -efflux [8–15] transporters have been identified by using molecular genetic approaches, a detailed functional understanding for many of these transporters remains undetermined. Here we present the functional characterization of the auxin-influx carrier AUX1. Upon expression of AUX1 in *Xenopus* oocytes, saturable, pH-dependent uptake of ^{3}H-IAA was measured. Mutations in *AUX1* that abrogate physiological responses to IAA in planta resulted in loss or reduction of ^{3}H-IAA uptake in AUX1-expressing oocytes. AUX1-mediated uptake of ^{3}H-IAA was reduced by the IAA analogs 2,4-D and 1-NOA, but not by other auxin analogs. The measured K_m for AUX1-mediated uptake of ^{3}H-IAA was at concentrations at which physiological responses are observed for exogenously added IAA and 2,4-D. This is the first report demonstrating detailed functional characteristics of a plant auxin-influx transporter. This biochemical characterization provides new insights and a novel tool for studying auxin entry into cells and its pivotal roles in plant growth and development.

Results and Discussion

Although molecular genetic studies have identified both putative auxin-influx and putative auxin-efflux carriers, the biochemical characterization of their activities remains largely undetermined. We therefore initiated studies to specifically determine whether AUX1 transports IAA and other auxin forms by using a *Xenopus* oocyte expression system. Because EYFP-AUX1 fusions have been shown to complement the *aux1-22* phenotypes in planta [16], we used a EYFP-AUX1 fusion to show that AUX1 was properly targeted to the plasma membranes when expressed in *Xenopus* oocytes (Figure 1A). Oocytes expressing untagged *AUX1* took up significantly higher amounts of ^{3}H-IAA as compared to water-injected controls (Figure 1B). Transport of ^{3}H-IAA in *AUX1*-expressing oocytes was saturable, with a K_m value of ~800 nM (Figure 1C). These observations provided a direct estimation for the affinity of AUX1 for IAA. A previous report calculated the K_m of auxin transport in plant suspension-culture cells by mathematically adjusting for the anionic form of IAA and found that carrier-mediated auxin uptake was between 1 and 5 μM [3].

To confirm that the saturable transport of ^{3}H-IAA in AUX1-expressing oocytes was due to the activity of this protein, we created point mutations in the AUX1 protein. These mutations had been identified and partially characterized in planta as failing to respond to exogenously added IAA to the same degree as *aux1* null mutants [17]. To reduce the possibility that mutated forms of AUX1 would not be properly trafficked to the plasma membrane, we selected three AUX1 mutants (*aux1-7*, *aux1-102*, and *aux1-117*) that contain single amino acid substitutions in flexible loop regions of the AUX1 protein (Figure 2A). After confirming that the YFP-tagged forms of these mutants were properly sorted to the plasma membrane of injected oocytes (see Figure S1 in the Supplemental Data available online), we tested their ability to transport added ^{3}H-IAA. ^{3}H-IAA uptake was completely abolished in oocytes expressing either *aux1-7* or *aux1-117* mutants, but oocytes expressing *aux1-102* displayed a ~60% reduction in ^{3}H-IAA transport activity (Figure 2B). Furthermore, the expression of the double mutant in oocytes containing both *aux1-102* and *aux1-117* substitutions abolished uptake activity (Figure 2B). Taken together, the observations that *aux1-7* and *aux1-117* abolish and that *aux1-102* modulates uptake of ^{3}H-IAA provide additional support for the conclusion that expression of a functional AUX1 transporter protein in oocytes is required for the increased uptake of auxin that we measured in *Xenopus* oocytes.

According to the chemiosmotic model of auxin transport in plants, uptake of IAA from the extracellular space to the cytoplasm can occur by two mechanisms, diffusion of the protonated form of IAA across the plasma membrane and carrier-mediated uptake of deprotonated IAA [3, 18, 19]. If the increased ^{3}H-IAA uptake observed in oocytes expressing *AUX1* represents carrier-mediated uptake of the deprotonated form, we would expect to observe changes in rates of ^{3}H-IAA uptake in water-injected oocytes as compared to *AUX1*-expressing oocytes in different pH conditions. At low pH, when ^{3}H-IAA is predominantly protonated, uptake rates of ^{3}H-IAA were high in both *AUX1*-expressing and water-injected oocytes as a result of the increased diffusion of protonated IAA across the plasma membrane (Figure 3A). In water-injected oocytes, ^{3}H-IAA uptake was rapidly reduced to levels <20% of maximal uptake (Figure 3A, open circles) at higher pH conditions in which

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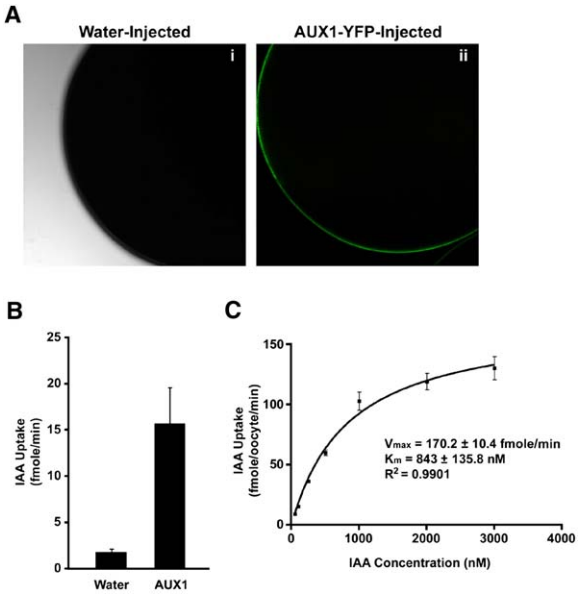

Figure 1. AUXI Transports IAA into *Xenopus* Oocytes

(A) EYFP-AUX1 is properly targeted to the plasma membrane in *Xenopus laevis* oocytes. In panel (A_i), water-injected oocytes in both bright-field and confocal images are shown. In panel (A_{ii}), a confocal image of a *Xenopus* oocyte expressing the EYFP-AUX1 fusion protein (green) indicates localization to the plasma membrane.

(B) *Xenopus* oocytes expressing the AUX1 transporter display increased IAA uptake. Uptake studies of ^{3}H-IAA into *Xenopus* oocytes injected with water or *AUX1*-cRNA at pH 6.4 were performed. The oocytes injected with *AUX1* cRNA displayed >10 times increase of ^{3}H-IAA uptake in comparison to water-injected controls. Values indicated represent the mean ± standard deviation (SD); n = 8 oocytes.

(C) Kinetic analysis of IAA uptake by AUX1. Uptake studies of ^{3}H-IAA into *Xenopus* oocytes injected with *AUX1*-cRNA at pH 6.4 were performed. Mean IAA uptake rates at indicated concentration are shown ± SD; n = 8 for each concentration (experiments were repeated on oocytes from four different frogs and showed similar results).

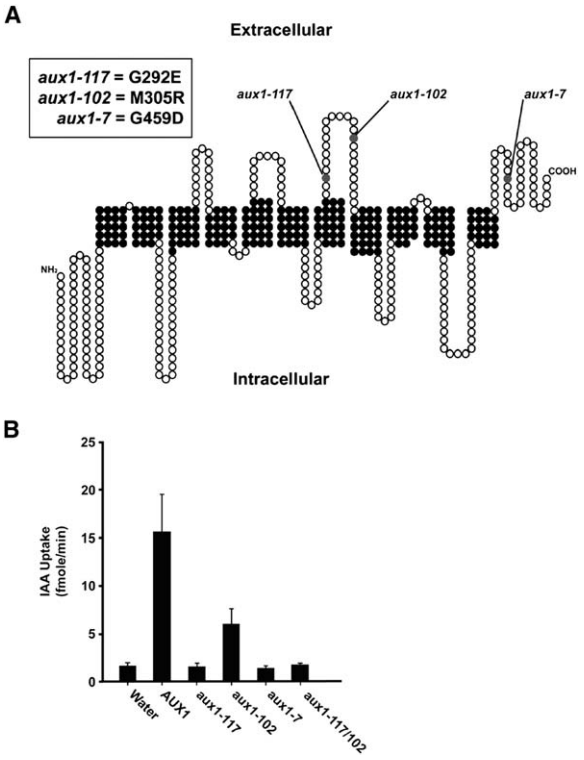

Figure 2. Mutations in AUX1 Inhibit IAA Uptake into Oocytes

(A) A schematic diagram illustrating the membrane topology of AUX1. Black circles represent AUX1 amino acid residues predicted to represent transmembrane α helices. Open circles represent amino acid residues predicted to be intra-α-helical flexible loops that are located either intracellularly or extracellularly. Point mutations analyzed in this study are indicated by gray circles, and details of these mutations are presented in the inset.

(B) AUX1 mutants reduce or abolish IAA uptake. Uptake studies of ^{3}H-IAA into *Xenopus* oocytes expressing *AUX1* cRNA or *AUX1* cRNA containing the indicated point mutations alter or abolish auxin uptake. Mean IAA uptake rates for the different point mutations were calculated ± SD; n = 8.

the majority of IAA is deprotonated (pKa = 4.7). In contrast, *AUX1*-expressing oocytes maintained higher rates of ^{3}H-IAA uptake at >80% of maximal uptake until pH $\geq$ 6.5 (Figure 3A, filled circles). Although we cannot exclude that higher pH may also affect activity of the AUX1 transporter itself, at pH 6.5 the ratio of deprotonated IAA to protonated IAA is ~100:1; therefore, the majority of ^{3}H-IAA uptake at this pH should occur via a carrier-mediated transport of the ^{3}H-IAA anion. Measurements of extracellular pH in planta under specific conditions range from 5.3 to 5.7 [20, 21]. Even under these conditions, the predicted ratios of deprotonated IAA to protonated IAA would be ~4–10:1. Carrier-mediated uptake of ^{3}H-IAA, defined as the difference between ^{3}H-IAA uptake in *AUX1*-expressing oocytes minus ^{3}H-IAA uptake in water-injected oocytes, displayed maximal activity between pH 6.0 and 6.5 (Figure 3A, triangles). In an earlier study, maximal carrier-mediated IAA uptake activity in suspension cells was estimated to occur at pH 5.9 [3]. Importantly, in this study the use of a heterologous oocyte expression system provided the ability to measure the specific stimulation of IAA uptake induced by *AUX1* expression under different pH conditions without additional auxin carriers being present in the membrane. The observation that increased external pH up to 7.0 did not reduce rates of IAA uptake into *AUX1*-expressing oocytes in the same manner that was observed in the water-injected oocytes strongly suggests that AUX1 is transporting IAA in its anionic form.

In whole-plant tissues and in plant cells, it is difficult to assess the specific effects of auxin analogs on auxin transporters because there are multiple mechanisms for auxin uptake and efflux operating in the same membrane. The ability to measure specific uptake of ^{3}H-IAA in *AUX1*-expressing oocytes provided a powerful tool for detailed examination of how specific auxin analogs modulate *AUX1*-mediated IAA transport across the plasma membrane. First, uptake of ^{3}H-IAA in *AUX1*-expressing oocytes was effectively competed by addition of unlabeled IAA (Figure 3B). The addition of the auxin analog 2,4-dichlorophenoxyacetic acid (2,4-D) competitively inhibited uptake of ^{3}H-IAA in *AUX1*-expressing oocytes (Figure 3B). In contrast, the addition of the lipophilic auxin analog 1-naphthaleneacetic acid (NAA) did not decrease ^{3}H-IAA uptake in *AUX1*-expressing oocytes (Figure 3B). These results are consistent with whole-plant studies because, whereas *aux1* mutant plants are less sensitive to both IAA and 2,4-D, *aux1*