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Determining if Polar Auxin Transport Exists in Moss Gametophytes

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Abstract

Polar auxin transport (PAT) is a directional transport process important in the function and development of sporophytic plant axes. Evolutionarily, gametophyte axes dominated the plant life cycle first, but PAT is not well understood in this developmental stage. Gaining a stronger understanding of PAT in the gametophyte may provide insight into axes evolution. Here, ^{3}H-IAA was used to examine PAT in the acrocarp species *Dicranum scoparium* and the pleurocarp species *Thuidium delicatulum* to see if any differences between growth pattern existed. Both mosses exhibited higher PAT in the acropetal directions yet a complete bidirectional flow of auxin was observed in both species. However, the different growth patterns did possess varying responses to NPA, a traditional PAT inhibitor suggesting that gametophytes may use auxin differently depending on axis orientation.

Keywords: Polar Auxin Transport, moss, orientation

1. Introduction

Land plants all depend on the regulation of five major biochemical molecules derived of several essential metabolic pathways [(1)] . These biochemical molecules, referred to as phytohormones, are gibberellin, cytokinin, etylene, abscisic acid, and auxin and are each important in controlling growth, development and arbitrating responses to biotic and abiotic environmental stressors. The auxin hormone indole-3-acetic acid (IAA) is recognized as the most widely researched and abundant auxin and plays a large role in regulating growth and development of plants during critical stages in early life including tropic responses, inhibition of abscissions, apical dominance, root/rhizome growth and stem elongation [(2)] . All plants utilize IAA typically through phloem [(3)], but auxin is the only hormone able to be unidirectionally transported by polar auxin transport. Interestingly, only land plants demonstrate PAT movement [(3)]. Evolutionarily, the earliest cell-to-cell transfer of auxin by PAT was in basal plants called bryophytes [(4)]. Therefore, auxin is regarded the *master* hormone of a plant's capacity to properly grow in an acropetal direction [(5)].

The molecular makeup of PAT systems seems to correlate with the complexity of the plant [(6)]. Basal plants display a simpler regulation of IAA [(4)] implying that these plants are more easily capable of revealing their trend between IAA regulation and subsequent developmental mechanisms relating to morphology [(6)]. Using chemiosmotics as a PAT model, H^+ gradients remain the quintessential driving force behind polar transport [(7)]. Protonated auxin ($pH \approx 5$) has been found to freely diffuse across membranes through an apoplastic route of certain simple bryophytes including sporophytic hornwort stems [(5)] or be transported via a more complex IAA influx symport protein [(6)]. Unprotonated IAA ($pH \approx 7$) is effluxed towards the cell wall [(6)]. The polarity of IAA transport is the result of the asymmetric localization of these specialized protein carriers at either side of the cell [(6)]. Although traditional convention has IAA influx directed basally to plant growth, immunolocalization studies reveal that influx carriers can sometimes be positioned on the apical tips of transporting cells [(6)]. Similarly, efflux (PIN) proteins localize on the basal ends implying the potential for cylindrical patterning of IAA.

Beginning with the isolation of the putative IAA influx protein AUX1 by Bennett *et al*. (1996), target research was conducted on detailing functionalities of the transport proteins. There have been some studies conducted on specific protein mediated PAT systems using ooctye models in an attempt to initiate particular auxin influx or efflux responses. For example, inserting a planta-abrogated AUX1 protein into the membrane of *Xenopus* oocytes resulted in reduced or eliminated AUX1 transport activity [(8)]. This demonstrates that auxin is selectively taken up by specialized plasma-membrane proteins belonging on a membrane [(8)]. Evidence supporting the proposition that specialized proteins are utilized in the cellular influx and efflux of auxin is imperative in discovering the mechanistic pathways of how different plant cells transport this important hormone.

Looking then at bryophyte PAT development might yield key information to early plant development. In the sporophyte, bryophytes used different versions of IAA transport [(5)]. For example, hornwort sporophytes move IAA with basic diffusion whereas liverwort sporophytes use a more complex method involving apolar facilitated diffusion [(5)]. Sporophytic auxin research preformed on the acrocarpus moss *Polytrichum ohioense* revealed both PAT differences in young versus old sporophytes as well as a sensitivity to different PAT inhibitors [(5)]. This depended on acropetal or basipetal flux suggesting that the sporophyte was carrying out a bidirectional transport pathway [(5)]. All three lineages of bryophyte thus exhibited different PAT methods suggesting the evolution of independently developed axial growth regulations [(5)].

Because bryophytes spend most of their life in the gametophyte stage, however, it is important to understand PAT trends during this developmental time. Inferring from past sporophyte experimentation, the rate of IAA through moss gametophytes could display different rates of PAT depending on acropetal or basipetal orientation. Possibilities in bidirectional transport and the typical tendency of axial growth found in both adult moss sporophytes and other vascular plants account for this hypothesis.

2. Methodology

2.1 Plant Habitat And Maintenance

Thuidium delicatulum and *Dicranum scoparium*, were collected in Salem, Virginia during September of 2015. The plants were collected by hand at the base ensuring to remove a layer of top soil with the rhizome system intact. Each moss specimen was stored in a Tupperware© container with roughly 10 ml of water and stored in a refrigerator. Prior to experimentation plants were removed from the refrigerator for one day to allow them to warm up and become physiologically active.

2.2 Polar Auxin Transport Protocol

Gametophyte stems were cut between 5 to 10 mm from the apical tip into 5 mm lengths with a razor blade and placed either basipetally or acropetally [(5)] between a radiolabeled 10^{-6} M agar donor block and a plain 1.8% agar (Fisher Scientific) receiver block [(5)]. In addition, more trials were run with the same radiolabeled 10^{-6} M agar donor block and with a receiver block that contained $15x10^{-5}$ M 1-N-naphthylphthalamic acid (NPA)(Pfaltz & Bauer *Inc*.) auxin inhibitor [(5)]. A small wooden cotton swab applicator was used between the agar blocks to lay the specimen on so as to support the midsection while each end of the stem was placed just up against the blocks. This was done for every specimen. Individual stems were used for 1, 3 and 5-hour time increments and all experiments were done in triplicate for a total of 18 stems per trial.

Agar blocks were removed, careful to not cross contaminate radioactivity measurements, and placed into scintillation vials containing 3 ml of EcoLite (Ecolite *Int* ™ +) scintillation liquid. Each vial was wiped with a Kimwipe (Kimtech *Inc*.) to remove fingerprints and ran through a 2800TR Tri-Carb liquid scintillation analyzer (Perkin Elmer *Inc*.) to measure disintegrations per minute (DPM). Each stem was processed for five minutes by the scintillator.

Means and basic standard errors were determined for all time points. In this way, the plain and NPA inhibited stem samples could be compared graphically. Standard deviation values were used to calculate standard error using the equation $\boldsymbol{\sigma_x = \frac{\sigma}{\sqrt{n}}}$ where $\boldsymbol{\sigma_x}$ is standard error, $\boldsymbol{\sigma}$ is standard deviation and $\boldsymbol{n}$ is averaged number of trials per experiment. At least some overlap in standard error was observed in all cases.

3. Data

Basipetal *T. delicatulum* showed gradual increase in auxin from one to three hours (0 DPM – 41,086 DPM) and then plateaued out from three to five hours (41,086 DPM – 42,661 DPM). The acropetal orientation experienced greater overall PAT (Max: Three hour DPM of 47,383) with similar trends from one to three (0 DPM – 47,383 DPM) and three to five hours (47,383 DPM – 44,748 DPM). The acropetal orientation of *T. delicatulum* experienced an overall 11% greater rate of PAT than basipetal (Figure 1A).

Basipetal and acropetal *D. scoparium* experienced the same gradual auxin increase from one to three hours (Basipetal: 0 DPM – 14,077 DPM; Acropetal: 0 DPM – 15, 095 DPM) and then a decrease from three to five hours (Basipetal: 14,077 DPM – 10,571 DPM; Acropetal: 15,095 DPM – 11,303 DPM). *D. scoparium's* acropetal orientation experienced an overall 7.2% greater rate of PAT than basipetal. Both uninhibited moss samples displayed complete bidirectional transport (Figure 2A).

The use of NPA led to a major inhibition for both basipetal and acropetal *T. delicatulum* (Figure 1B) while no clear inhibition was seen in the *D. scoparium* (Figure 2B). Basipetal *T. delicatulum* underwent a 134% decrease in DPM whereas acropetal underwent a 150% decrease. Basipetal *D. scoparium* saw a 34% increase in DPM while acropetal saw a 6.2% increase in DPM. Inhibited trials still displayed bidirectionality from both orientations in the way auxin moved over time.

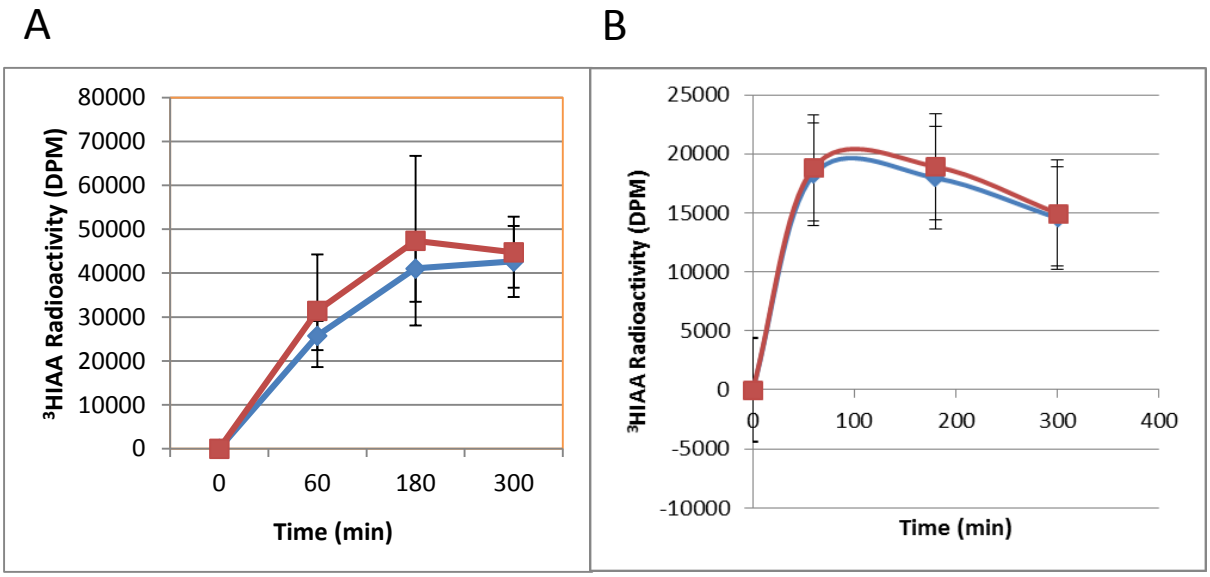

Figure 1. *Thuidium delicatulum* transport of auxin (A. uninhibited and B. with $15*10^{-5}$ M NPA inhibition) over 5 hours in basipetal (blue) and acropetal (red) directions. Both graphs suggest that *T. delicatulum* moves auxin actively in PAT and that this process is able to be inhibited.

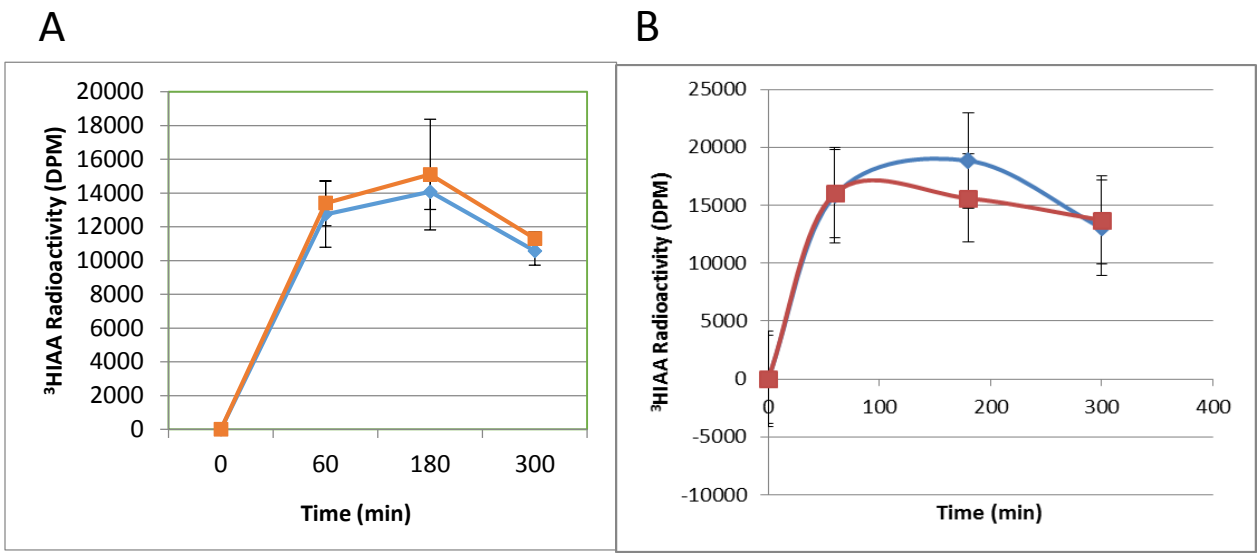

Figure 2. *Dicranum scoparium* transport of auxin (A. uninhibited and B. with $15*10^{-5}$ M NPA inhibition) over 5 hours in basipetal (blue) and acropetal (red) directions. Both graphs suggest that *D. scoparium* can move auxin bidirectionally but it is not inhibited by traditional PAT inhibitors.

4. Discussion

The gametophytes of the bryophytes *T. delicatulum* and *D. scoparium* each displayed the ability to undergo PAT in a bidirectional pattern when sampled ≈ 10 mm from the apical meristem. This bidirectionality appears to be different from past Poli *et al.* (2003, 2014) studies on *Polytrichum ohioense* where a strongly unidirectional PAT trend was observed within a particular developmental timeframe in the plant's life. Methodology conducted in the preceding experiment was done with relative accordance to Poli *et al.* (2003) protocols conducted on the bryophyte sporophyte. As such, data collected here is comparable in regards to land bryophyte gametophyte and sporophyte data. Overlap in standard error (Figures 1 and 2) suggests no statistically significant differences in auxin's bidirectional flux between acropetal or basipetal orientations. These data suggest that both specimen, and consequential growth habits, expressed PAT as seen from their bidirectional, arched flow of auxin over a five-hour period. This contrast from unidirectionality observed in the sporophyte of *P. ohioense*, Poli *et al.* 2003, to the bidirectionality observed in the gametophytes of this experiment implies bryophyte growth may be more limited in its temporally dominant, zygote-producing form as opposed to the sporophyte form. More interestingly, is that the moss gametophytes studied here have different growth orientations; *T. delicatulum* is pleurocarpus and *D. scoparium* is acrocarpus, and their auxin movement is not equally inhibited. Could this be representative of unique evolutionary needs from growing horizontally or vertically?

Speculation as to why different moss orientations seem to require unique growth and subsequent auxin regulation can be made as a result of these data. The ability of the pleurocarpus species *T. delicatulum* to undergo PAT inhibition implies that, much like bacterial growth within the constraints of a culture dish, there exists a potential culmination point where the plant regulates its auxin bidirectionally so as to slow or cease its growth. This process could be the result of contact inhibition with competing obstacles along a surface or possibly an evolutionary requirement to maintain a manageable, monolayer of stalks. It would then stand to reason that lack of PAT inhibition observed in acrocarpus *D. scoparium* is due to the species ability to grow vertically, free of most spatial constraints. Further inhibitory testing is required to say for certain.

It is known for certain, however, that polar auxin transport patterns do appear to have developed independently among plant sporophytes. The cells involved express several classes of auxin influx and efflux support proteins [(3)] as demonstrated through some bryophytes lineages utilizing different levels of complexity in their hormone transport. While many reputed PAT influx and efflux proteins have been isolated through genetic molecular processes, a universal functional understanding of some of these transport proteins has yet to be determined [(8)]. Therefore, further exploration of physiological movement of auxins should be useful. Understanding the differences between

gametophytic and sporophytic auxin transport utilization is critical for a stronger understanding of axis evolution during land plant expansion across the earth.

Recent studies have shed some light onto issues involving understanding PAT protein mechanisms. In part, it is due to potential sub-functionalization of proteins as was found in some members of the AUX/LAX family of PAT genes [(9)]. Such evolutionary mutations could be the result of neo-functionalization (the acquiring of new functionality) or pseudogenization (a loss of previous functionality) of PAT genes [(9)]. Even in cases where major PAT proteins have been identified and recognized to preform specialized functions, as is the case with PIN proteins, much still remains a mystery [(10)]. The many factors that contribute to the exact expression patterns of a plant's proteins, as well as how their intracellular distribution is affected via environmental signaling such as tropism, still remains largely unknown [(10)].

To ensure that the trend difference between acrocarpus and pleurocarpus moss gametophytes is true, additional species of each must be tested. In addition, the polar auxin transport inhibitors, naphthoxyacetic acid (NOA) and 2,3,5-triiodobenzoic acid (TIBA) should also be explored. Using additional inhibitors may provide additional insight into a true mechanism of auxin transport through moss gametophyte axes.

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