Analysis of phytosulphokine-alpha (PSK-α) function in *A. thaliana* development

or

Abraca-*dopsis*!

The magic of *A. thaliana*

Amanda Johnson, Ottawa University, Biology, Universität Kiel
Student # 3793130
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Summary

The fourth and final work term took place in the Botanisches Institut of Christian-Albrechts Universität in Deutschland. We researched the role of the growth factor phytosulphokine-α in *Arabidopsis thaliana* development. Four major investigations were performed. First, we determined that addition of PSK-α to the growth medium has no effect on hypocotyl length in 5-day old seedlings, grown in the light or in the dark. Using promtor-GUS lines we established the exact location of *AtPSK1,2,3,4,5* and *AtPSKR1* "expression" in 5-day-old seedlings grown in the dark. Next, seedlings were wounded and stained for promotor activity of the PSK genes. After wounding, promotor activity of *AtPSK1,2,3,4* and *AtPSKR1* increased around the wound. The final step was to localize expression of *AtPSK4* and the PSK receptors *AtPSKR1* and *AtPSKR2* in flowers using *in situ* hybridization. A protocol was established; however, no results from this final experiment were obtained.

Introduction

Plant research is relevant and cruelty-free. There is much to be learned about hormone signaling by studying Arabidopsis and rice; these are the major interests of the Plant Developmental Biology and Plant Physiology Department at Christian-Albrechts Universität.

Background on *A. thaliana*

. *A. thaliana*, or thale cress, is a small flowering plant related to mustard and cabbage. The plant contains around 26 000 genes, all of which have been sequenced. It is ideal for research because of its small size, relatively short (6 week) life cycle, and ease of transformation using *Agrobacterium tumefaciens*.

. Arabidopsis is grown at 25°C. The optimum photoperiod is 16 hours of light and 8 hours dark; however, for the majority of experiments the seeds are grown in the dark. Seeds are incubated 2 days at 4°C in the dark before being placed into the growth chamber (9).

In our lab, there are 2 ways to grow Arabidopsis: on fortified agarose in a sterile petri dish, or in a pot with soil. The former option is best when experiments require young seedlings. The latter is ideal when full grown plants are needed.

Background on Phytosulphokine-α

Phytosulfokine-α is a plant peptide growth factor represented by Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln. First discovered in conditioned medium of asparagus cell culture (11), its precursors are now known to be encoded by 5 genes: *AtPSK*1 to 5. Precursors are post-translationally sulphated on tyrosine residues to produce PSK-α (1). In Arabidopsis, PSK-α is bound by two leucine-rich receptor kinases 1 and 2, located on the plasma membrane (1).

It has been shown that PSK-α increases the formation of adventitious roots in cucumber (12), and improves chlorophyll production (13). In Arabidopsis, PSK-α increases root length in a dose-dependent manner (1). PSK receptor 1 knockouts have impaired root growth and flower development; thus, PSK could be responsible for growth and development (unpublished). Matsubayashi *et al.* showed that *AtPSK4* promotor activity is induced in wounded leaves (7). A Chinese group in collaboration with the Botanisches Institut showed increased PSK4 expression in flowers after fertilization.

This literature laid the foundation for our project. We investigated the effect of growing seedlings on agarose/basal media containing different concentrations of PSK-α; grew six P_{AtPSK}:GUS lines and stained them; grew lines, wounded and then stained them; and finally, hybridized DIG-labeled mRNA probes to sections of the tissue for immunohistochemical detection. The experimental setup and results are described on pages 3 to 5.

Experiments

1. PSK-α dose-response curve

It has previously been shown that PSK-α causes root length in Arabidopsis to increase in a dose-dependent manner (2). The first experiment involved growing wild-type Arabidopsis seedlings and measuring the length of hypocotyls. To determine whether PSK-α also causes an increase in hypocotyl length, we grew seedlings on agarose with the following concentrations of PSK-α: 0 nm, 1 nm, 3 nm, 10 nm, 30 nm, 100 nm, 300 nm, and 1000 nm. We then measured the length of the hypocotyls in 5-day old etiolated and non-etiolated seedlings. After comparing results and performing statistical analysis, it was determined that hypocotyl lengths were statistically the same, regardless of PSK-α concentration. This is not what was expected, since Arabidopsis hypocotyl length is reduced in PSKR1 knockouts (unpublished data). One explanation is that the seedlings at this stage produce sufficient PSK-α for hypocotyl growth. The additional PSK-α was not used by the plants.

2. P_{AtPSK}:GUS analysis

The second set of experiments involved P_{AtPSK}:GUS lines. These are transgenic plants produced by transfecting wild-type Arabidopsis with *Agrobacterium tumifaciens*. Agrobacteria contain plasmids with the respective PSK gene whose promotor has been tagged with beta-Glucoronidase. Thus, whenever the specific promotor is active in the P_{AtPSK}:GUS plant, the enzyme is produced and accumulates. Promotor activity is detected by incubating tissue in solution containing the substrate, X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid) (10). GUS staining is useful because gene expression is often controlled at the transcriptional level. In these cases, the more promotor activity, the darker blue the stained tissue, and the higher the gene expression.

We performed GUS analyses of promotor activity in *AtPSK1,2,3,4,5* and *AtPSKR1* in hypocotyls of 5-day-old etiolated seedlings. Location of GUS staining is as follows: *AtPSK1*, none; *AtPSK2*, root, hypocotyl, cotyledon; *AtPSK3*, root, hypocotyl, cotyledon; *AtPSK4*, root, hypocotyl, cotyledon; *AtPSK5*, root, hypocotyl, cotyledon; and *AtPSKR1*, root, cotyledon.

GUS-stained hypocotyls were sectioned to determine where the promotors were active. Following staining, seedlings were fixed and embedded in the plastic-resin Technovit®. Using a Microtome sectioner, blocks were sliced into ribbons 20 µm thick and mounted on glass slides. In all cases, staining occurred in the central cylinder of the hypocotyl, and not in the epidermis, outer cortex, inner cortex, or the endodermis. *AtPSK* gene promotor activity occurred in the central cylinder because this is where growth is initiated (4).

3. PSK promotor activity after wounding

Transcription of some genes in Arabidopsis is induced in response to wounding. Matsubayashi showed that wounding of a mature leaf induced promotor activity for PSK4 (7). We grew 5-day old seedlings on agarose/basal media in the dark. We wounded them with tweezers or a needle and waited 10 hours before GUS-staining. Upon observation under a microscope, some seedlings were darkly stained around the wound, indicative of higher promotor activity for the respective PSK gene. From these experiments, we saw that transcription of *AtPSK1,2,3,4* and *AtPSKR1* is induced after wounding.

4. Detection of PSK gene expression by *in situ* hybridization in Arabidopsis

Our next step in studying PSK-a genes is to quantify gene expression via *in situ* hybridization (ISH). The difference between ISH and promotor GUS analysis is, ISH measures mRNA within the tissue, while GUS-staining only indicates gene promotor activity. The first step was to produce DIG-labelled RNA probes. We began by making probes for *AtPSK4, AtPSKR1*, and *AtPSKR2*. Genomic DNA was amplified using PCR.

The resulting DNA fragment was cloned into pGEM-T Easy, then sense and antisense probes were generated via *in vitro* transcription. Fertilized flowers were prepared by removing sepals, petals and stamen, and then brushing the carpel with a mature anther one day before collection and fixation. Unfertilized flowers also had sepals, petals and stamen removed, and were collected from the plant the following day. Both fertilized and unfertilized Arabidopsis flowers were fixed in Paraplast and sliced into 20 μm sections. The probe was hybridized overnight to the tissue, and then sections were washed and stained.

Since ISH is such a lengthy procedure, we only had time to do one experiment. We used a sense probe as the control and antisense probe as experimental. Problematically, most of the sections came off the slides during the numerous washes. At the end of the experiment only the control (sense) slides had sections. Thus, it could not be concluded whether *AtPSK4* is overexpressed in the ovary following fertilization.

The Place

Kiel is the capital of Schleswig-Holstein in northern Germany. Located on the Baltic sea, it is known for its sailing ships. When the Olympics took place in Germany, all sailing competitions were held at the Kiel-Schilksee. Every summer the world's largest sailing competition takes place in Kiel during Kieler Woche. With a population of 230 000, Kiel is comfortably small and very scenic.

Christian-Albrechts Universität has a student body of 20 000 and is known for its natural sciences, engineering, agriculture, economics and educational science. The biology department features a botanical institute. Not many people can say they work beside a botanical garden. Nevertheless, such was the case with my research in Kiel. My building overlooked vast colourful gardens, a pond, and greenhouses with thousands of different species of plants.

The Experience

Initially, I expected Deutschland to be just like Canada, except for the language. I soon discovered that Deutschland is similar to Canada in some ways, but very different in others.

My first thrill was driving on the Autobahn, which has no speed limit. The trees were much bigger than in Ontario. There were many windmills and fields of rapeseed. I was brought to my Studentendorf ("student housing") where I met my roommate, Julia. We became friends right away, and the first weekend she showed me the center of Kiel and introduced me to more people. I was very happy she spoke English!

On my first day of work, my supervisor and I discussed project possibilities. I was eager to begin, because everything we talked about sounded very interesting. We ate lunch in the Mensa. German food is amazing compared to North American food! Freshly prepared hot meals with fresh bread and salad. For dessert there was always some kind of joghurt-pudding. Chocolate comes in all brands and flavours. After lunch we sat and drank coffee in the lunch room. It was a nice break that I always looked forward to.

Then came the Europameisterschaft 2008 soccer championship. The university set up a huge screen for public viewings. EM2008 Deutschland paraphernalia was everywhere! Even Kinder chocolate bars came with collectable stickers featuring members of Deutschland's team. We bet on the score of each game and on who would win. The fans were incredible! Everytime Deutschland won, the streets were filled with people waving flags and singing "Lo Lo Los gehts". It was a huge party.

Meanwhile, I met Archana, Ryan, Dylan and Sam, the other RISE exchange students. We traveled together on weekends and usually met up during the week for dinner or bowling. In total there were about 300 North American exchange students in Germany. Throughout the summer I traveled to Berlin, Hamburg, Dresden, Prague, Heidelberg, Munich, and Vienna. Many fun times were had.

The last week of June was Kieler Woche—the biggest summer festival in northern Europe. Sailing competitions, concerts, rides, Bratwurst, beer, and a lot of people. One night my roommate and I saw the German film "Keinohrhasen" at an outdoor cinema.   Another night a group of us watched Germany win against Turkey in the semi finals of EM2008. During the Windjammer Parade, the Kieler Fjord was full of ships. We watched them from the beach.

I had a basic knowledge of the German language before coming to Kiel. Over the summer I learned a lot by listening to people.   At work most people spoke to me in English. At my Studentendorf, my roommates and I spoke German. When I was out and about (at the Supermarkt or Hauptbahnhof, for example) I tried to speak German.

Every weekday I worked from 9 am until about 6 pm. Lab meetings were on Thursday morning. I had the opportunity to give a 30-minute talk during one of the lab meetings. Monday and Tuesday there were seminars, sometimes in Deutsch and sometimes in English. These covered a wide range of scientific topics, from physics to marine biology. On Fridays afternoons, I sometimes attended a seminar class taught by Bianka.

The work I did in the lab varied during the course of the summer. I started out preparing agarose/basal media plates and planting seeds. When the seeds were 5 days old I took pictures and made measurements. Next I grew P:GUS seedlings, stained them, sectioned them, and took pictures. For the last month or so my work involved molecular biology: cloning, *in vitro* transcription, and *in situ* hybridization.   I consider myself privileged to have done so many different things in such a short time span. There was always something for me to do, so I was never bored. I felt as though my project was important.

I was thoroughly impressed with the communication and co-operation of the people I worked with. In the 3 months that I worked there we had BBQs, went to a Polterabend (engagement party), had a potluck breakfast, and watched the olympics during work hours.   Manuela was awarded her PhD during the summer, and a hilarious graduation ritual and celebration followed.   When someone had a birthday, they would bring in a cake and we would all eat it together. Working in the lab at the University of Kiel was a positive experience.

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Dem Deutschen Volke


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