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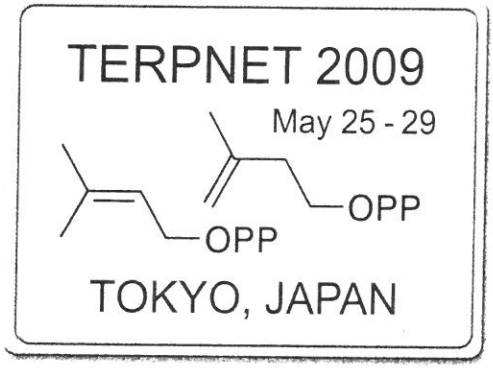

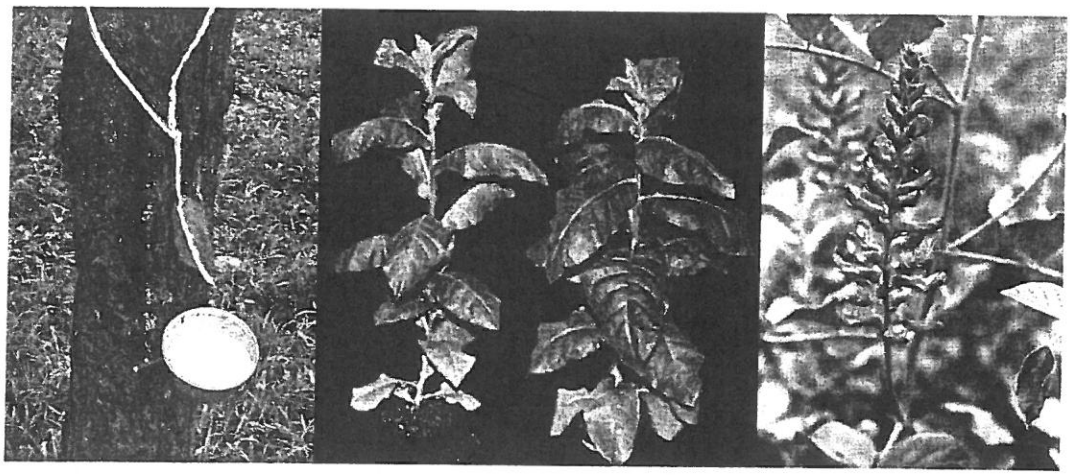

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Uncovering the Thapsigargin Biosynthesis Pathway

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A Drug in Short Supply

Prostate cancer is one of the most prevalent forms of cancer in western countries, and currently accounts for over 13% of all cancer cases in men in Denmark. Prostate cancer cells tend to divide slowly, making them a poor target for most current chemotherapeutic agents. One promising new drug for the treatment of prostate cancer is thapsigargin, a secondary metabolite from the Apiacieae, *Thapsia garganica*. Thapsigargin is highly cytotoxic to all human cells, but by covalently linking thapsigargin to a short peptide sequence, a prodrug has been developed which is only activated in the presence of prostate specific antigen (PSA). In this way it is hoped that thapsigargin toxicity can be selectively targeted against prostate cancer cells.

Figure 1, *Thapsia garganica*

Unfortunately, wild *Thapsia garganica* plants *are* the only current source of thapsigargin, and it is a species that is notoriously difficult to cultivate. Furthermore, at least 43 steps are required to chemically synthesize the drug *in vitro*, making it timely and expensive. Thus, if the future supply of thapsigargin is to be met a more efficient method of production is required.

The Plan – from *Thapsia* to moss

Given the requirement for an inexpensive and stable supply of the drug thapsigargin, we propose to identify the enzymes involved in its biochemical synthesis pathway in *Thapsia*. Indentification of the enzymes involved in synthesizing thapsigargin opens up the possibility of transferring some, or all, of this metabolic pathway into a different host. Production of thapsigargin, or a precursor thereof, in a heterologous system that can be easily cultivated would ensure a stable and inexpensive supply of the drug. The system we have chosen as a heterologous host in which to introduce thapsigargin biosynthesis is the moss, *Physcomitrella patens*. *Physcomitrellla* is a hardy land plant that can be maintained on a simple mineral media, grown easily under controlled conditions, and cultured in liquid media in a "biofermenter". Genetic modification of *Physcomitrella* is also relatively simple, because it is able to undergo homologous recombination, and also because it can be maintained indefinately as a haploid gametophyte.

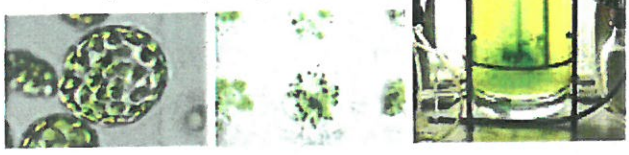
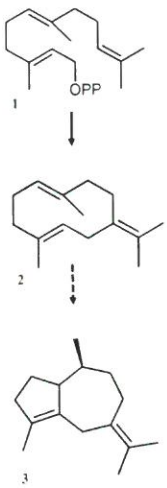


Figure 2, *Physcomitrella patens* in the form of (a) protoplasts (from which the cell wall has been chemically removed) that are able to be genetically transformed. (b) Protonema and gametophores growing on a mineral agar plate, and (c) immature protonemal tissue being grown in liquid media in a biofermenter.

Proposed Enzymes Involved



Nothing is currently known about the enzymes responsible for synthesizing thapsigargin in *Thapsia*. However, using knowledge about the chemical structure of thapsigargin (5) has enabled us to propose a metabolic pathway with a number intermediates. In this hypothetical pathway, we are able to postulate that specific metabolic steps are carried out by enzymes of a particular family. For example, the 15 member sesquiterpene that forms the carbon skeleton of thapsigargin is likely to be formed by the conversion of farnesyl pyrophosphate (1) to germacrene B (2) by a sesquiterpene synthase. The cyclisation of the 5 and 7 carbon rings may be carried out by this same enzyme, or by a separate cyclase. Later in the pathway, the closure of the lactone ring (4) could be accomplished by a Cyp71

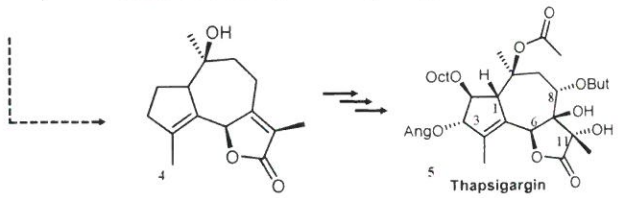


Figure 3. Selected steps from the proposed biosynthetic pathway of thapsigargin (5). The precursor, farnesyl pyrophospate (1) is present in all plants. This can be converted to germacrene B (2), in which the 15 member carbon ring undergoes further cyclisation (3). A lactone ring is created on C7 and C8 (4) by a lactone cyclase, examples of which exist in the cyp71 family of P450s.

How do we find the genes?

100 µg of total RNA was isolated from *Thapsia garganica* roots and sent for pyrosequencing. From 295 000 sequenced reads, averaging 201 bp, almost 40 000 contigs were assembled. These contigs consist of full and partial mRNA sequences, and represent a significant portion of the transcribed genome of *Thapsia garganica* at the time of RNA isolation. Creating a BLAST database containing these sequences allows us to search the *Thapsia* transcriptome for genes encoding proteins with predicted function, including sesquiterpene synthases and cytochrome P450s.

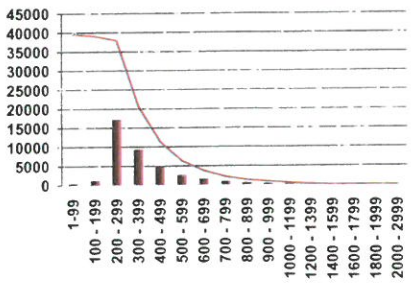


Figure 3. The number and length of contigs obtained from pyrosequencing of the *Thapsia garganica* genome. Bars show the number of contigs sequenced within each size range, and the solid line indicates the total number of contigs received of that length and above.

Progress: To date, two full length sesquiterpene synthases, and two cyp71 have been identified in *Thapsia garganica*, and are now undergoing biochemical characterization.