Search for Target Sites for Insect Control

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Peter Krell and Basil Arif provided a good introduction to the research that I am going to present today. This research falls primarily under Program 4 of the Canadian Forest Service (CFS) Genomics R&D Initiative,[1] and specifically under the project entitled "Juvenile Hormone-Based Pest Management Tools for Forest Insects." Some of the research my colleagues and I have conducted also falls under Program 1 of this initiative,[2] within the project "Structural, Functional and Comparative Genomics of Insect Pests."

Two CFS laboratories are involved in this work: my laboratory at the Laurentian Forestry Centre in Québec, Quebec, and that of Qili Feng at the Great Lakes Forestry Centre (GLFC) in Sault Ste. Marie, Ontario. The research we perform is best described as a search for molecular target sites in the context of insect pest control.

What are molecular target sites, and why search for them? In the context of insect pest control, target sites are proteins — enzymes, receptors, or carrier proteins that are specific to insects or to a group of insects — typically involved in an insect-specific process such as molt, metamorphosis, or reproduction. Because the actions of these proteins are specific, it may be possible to interfere with them using inhibitors or antagonists, with minimal effects on nontarget organisms. Alternatively, when appropriate, the genes encoding these proteins can be used to engineer more effective microbial insecticides, as shown by Dr. Arif's work on the spruce budworm. The identification and characterization of such target sites can accelerate the development of novel, target-specific forest pest control products.

In the context of target-site research, we are interested in the following proteins: enzymes, hormone receptors, and carrier proteins, all of which have a ligand (a molecule that binds to a specific site on the protein). The ligand and the associated protein "recognize" each other in a manner similar to that of a key and a lock. In the case of enzymes, the ligand is a substrate that binds to the enzyme's catalytic cavity, hence triggering a catalytic process that transforms the substrate into new products. Hormones, as ligands, also have protein receptors to which they bind in a specific manner. This binding induces conformational changes in the receptor, which in turn allows the hormone–receptor complex to bind to a specific portion of DNA often referred to as a response element. This latter binding triggers DNA transcription and a cascade of molecular events of biological significance.

To interfere with a ligand–protein interaction, it is possible to find or design a competitive inhibitor or antagonist that has sufficient similarity to the natural ligand to bind tightly to the protein's active site, without triggering the biochemical reaction normally initiated by the natural ligand. This approach is commonly used in drug discovery.

What are the steps involved in this target-site strategy to develop pest control products? First, the researcher has to identify a new target site using one of several exploratory methods — for example, by constructing expressed sequence tag (EST) libraries or by choosing a known or suspected target site candidate based on what is known of the insect's biology. Second, the researcher has to clone the relevant complementary DNA (cDNA) — that is, the gene. With the gene in hand, various avenues may be considered. It may be appropriate to use the gene to engineer a microbial agent with the goal of enhancing its insecticidal activity; in this case, the modified microbial agents will need to be assayed to determine whether the genetic manipulation confers measurable enhancement of activity. Another avenue — and this is what we are aiming at with this research — is to produce the recombinant protein and develop an in vitro assay for the high-throughput screening of potential inhibitors or antagonists. With this type of approach, it will often be relevant to determine or model the three-dimensional structure of the protein so that we can examine the size and shape of the binding site and assess the likelihood of success of an inhibitor or antagonist strategy. Such structural knowledge allows us to use computer-assisted technology to do a first screen of potential inhibitor or antagonist molecules and then to test those leads in an in vitro assay. The molecules that show the most potential for pest control in these assays could then be tested in vivo.

The two main target-site research projects funded by the CFS Genomics R&D Initiative are aimed at blocking either the biosynthesis or the action of an insect hormone known as juvenile hormone (JH). However, some of our research involves other types of target sites not related to JH.

JH plays a role in insect development, particularly in that of lepidopteran insects such as our main target pest, the eastern spruce budworm (*Choristoneura fumiferana*). Shortly after hatching, the larva starts growing and goes through a number of larval molts, during which it sheds its old exoskeleton and acquires a new, larger one which permits further growth. After these larval molts, the larva resumes growth while retaining its juvenile characteristics; this is ensured by high levels of JH. When the larva reaches the final larval stadium, levels of JH fall to undetectable levels. Under these conditions,

[1] Program 4 of the CFS Genomics R&D Initiative is the Production of Environmentally Acceptable Forest Protection Methods.

[2] Program 1 of the CFS Genomics R&D Initiative is Forest Tree Production and Protection Systems.

secretion of the molting hormone ecdysone triggers metamorphosis and the pupal molt, which eventually gives rise to the moth.

It has long been known that if JH level can be artificially reduced early in larval life, this will cause precocious metamorphosis, early cessation of feeding, and a possible failure to complete development to the adult stage, as the pupa typically will not be viable. Our laboratories are examining two of the strategies for inducing precocious metamorphosis of the spruce budworm larvae: (1) blocking JH production using an inhibitor of a JH biosynthetic enzyme, and (2) blocking JH action by preventing binding to its receptor. Both strategies would have the same result.

There are six known forms of the sesquiterpenoid JH (Figure 1), the most common and simplest one being JH III, which is the only JH produced by most insects. Lepidoptera, however, produce four additional forms of this hormone, recognizable by the presence of one to three ethyl branches on the hormone's farnesyl backbone. We have hypothesized that one or more of the enzymes involved in the biosynthesis of these lepidopteran forms of JH have modifications in their catalytic cavity to accommodate these bulkier compounds.

We have recently shown that the Lepidoptera produce two very distinct farnesyl diphosphate synthase (FPPS) homologs. This enzyme is involved in the formation of the farnesyl carbon skeleton of JH and in other metabolic pathways. We have

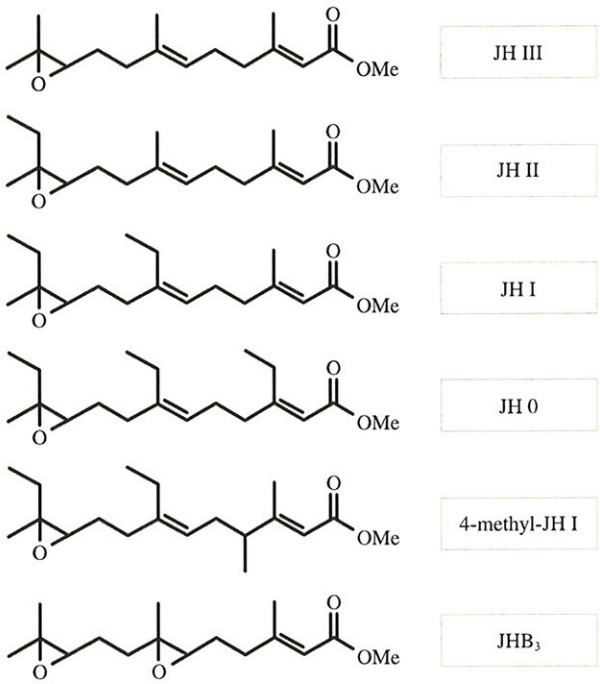

Figure 1. The six known forms of juvenile hormone. JH II, JH I, JH 0, and 4-methyl JH I have so far been found only in the Lepidoptera. (O=oxygen; Me=methyl.)

cloned FPPS cDNAs from lepidopteran representatives, including the spruce budworm, as well as from a number of non-lepidopteran insects. Through extensive amino acid sequence comparison, we showed that one of the two lepidopteran FPPSs (type I) displays several unique amino acid substitutions within its catalytic cavity. For example, we found four substitutions within a specific motif of this protein, which is well conserved among all living organisms, except the Lepidoptera. We used homology modeling and docking simulations to examine the potential impact of these substitutions on the volume of the catalytic cavity and found that there is actually an increase in the volume of the catalytic cavity of this enzyme relative to "conventional" FPPSs. In comparison, the second lepidopteran FPPS type has fewer substitutions within its active site and in this respect appears more "conventional" than the type-I protein, although unique in other respects. It is still not clear which of these two FPPS types is involved in JH biosynthesis, but we know that the expression of the type-II enzyme is essentially confined to the JH-producing glands, whereas that of the type-I enzyme is more ubiquitous (Cusson et al.). Whichever of these two FPPS homologs is involved in JH biosynthesis, the substitutions identified in each could facilitate the production of the ethyl-substituted precursor of JH. As a consequence, it would become possible to find an inhibitor that is specific to this particular catalytic cavity without affecting conventional FPPSs, making it a Lepidoptera-specific compound. We are now in the process of developing in vitro assays to test these hypotheses.

For the past few years, Qili Feng's group has been working on isolating a JH receptor. JH is an elusive molecule, and the challenge of cloning it has been taken up by several competitive laboratories. To achieve their goal, Dr. Feng and his team are using an original strategy based on the observation that JH can activate its own hydrolysis into an inactive form, JH acid. This hydrolysis is effected by a specific enzyme called juvenile hormone esterase (JHE), which is instrumental in preparing the insect for metamorphosis. JH can very rapidly activate transcription of the JHE gene; researchers believe that it does so by binding to its receptor, with the receptor–hormone complex subsequently binding to a response element within the promoter[3] of the JHE gene, which in turn activates JHE transcription. Qili Feng's group has identified the 30-base-pair response element within this promoter (Kethidi et al. 2004). They have made constructs of this promoter with luciferase, a reporter gene (or genetic marker), and transformed a culture of spruce budworm cells. When these cells are treated with JH, the reporter gene is induced in a measurable way, thereby providing a cell-based assay system. This system could be used to screen antagonists of JH, which would presumably prevent binding to the JH receptor and thus, JH activity. Using the response element DNA as a probe, they were able to identify a nuclear protein that binds

[3] A DNA site to which RNA polymerase binds and initiates transcription.

specifically to it and that is therefore a candidate JH receptor. Efforts are currently being made to purify this protein.

I now wish to turn your attention to the enzyme chitinase, which is involved in the breakdown of chitin, a homopolymer of *N*-acetylglucosamine and a major constituent of the insect cuticle secreted by the epidermis. Chitinase expression is induced by the secretion of the molting hormone ecdysone just before the molt. It helps break down the old cuticle when it is being shed. In this particular case, Qili Feng's group used a chitinase cDNA to modify *Autographa californica* nucleopolyhedrovirus, or AcMNPV (Zheng et al. 2002), so that it can be used as a viral insecticide against a number of insect pests. They have assayed this modified virus and compared it with the parent virus. The results indicate that the chitinase gene is an excellent candidate for the genetic improvement of baculoviruses.

What is the significance of this research for Canada? First, it helps put Canada on the leading edge of genomics-based pest control research and product development. Second, this research aims at broadening control options for forest pests by identifying effective and environmentally safe products. Indeed, there are currently very few options for the control of insect pests such as the spruce budworm. Also, some of the target-site proteins cloned at the GLFC are being used to engineer more effective viral insecticides aimed at the spruce budworm and other pests. As other speakers at this symposium have pointed out, some of the discoveries made through this research may affect other areas — for instance, the development of gene switches using an ecdysone receptor [see Krell, this publication]. It seems quite possible that researchers could also use JH receptors in similar gene switches, given that JH is not found in humans. Finally, the two laboratories involved in this research have significantly contributed to the training of highly qualified personnel in the area of insect biotechnology in the last five years, including eight postdoctoral fellows and numerous students and technicians.

Our research collaborators include Indiana University–Purdue University at Indianapolis; the University of Kentucky; the Gembloux Agricultural University in Belgium; the Highthroughput Factory at the RIKEN Harima Institute in Japan, where we have a collaborator helping out on homology modeling; the Southwest Agricultural University in China; and Peter Krell at the University of Guelph, Ontario.

The main sources of funding for our research have been the CFS Genomics R&D Initiative and Genome Canada, through the Ontario Genomics Institute. We have also received funds from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Biocontrol Network, the CFS, and the Ministère des Relations internationales du Québec.

In terms of future directions for our research, a number of possibilities can now be explored, but I think that efforts should be focused on the most promising target sites, while exploring new leads provided by ongoing microarray work. Indeed, the recently constructed spruce budworm EST libraries and the sequences of viral genomes will provide us with new tools for the development of DNA chips, or microarrays, that will help us identify genes that are potentially targeted by viral pathogens. We also need to bolster CFS expertise in structural bioinformatics and protein biochemistry to speed up the product development phase of this research. Lastly, industrial partnerships will have to be sought once in vitro and cell-based assays have reached the point where they can be used for screening compounds.

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