Laminaria digitata

Credit photograph: http://www.hippocratus.com/pages/detail_plante.asp?ID=lam002

Why

These algae are the earth's **iodine** reservoir and are suspected to have a great ecological importance concerning the cycle of iodine. Whereas our thyroid gland can concentrate the outside iodine 7 times, these algae can concentrate it 30000 times!

This chemical compound is suspected to play a capital role in ecology. In fact, it is implied in cloud formation but also in ozone destruction!

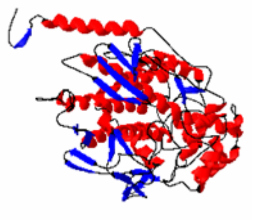
Iodine was discovered at the beginning of the 19th century in these brown algae and they have therefore been exploited since then as a source of iodine which also has an important role in human health (in growth for example).

The haloperoxidases, and especially iodoperoxidases, are key enzymes involved in iodine uptake and the emission of halogenated compounds (=iodine, bromine and chlorine).

Moreover, the halogen mechanism seems to be part of original mechanisms of the alga's **defence** against pathogens and **stress** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=339) making the study of haloperoxidases a very interesting challenge.

The questions scientists are asking themselves are: How are these enzymes working? What is the role of iodine in this alga? Why does it concentrate it at so high levels?

On the other hand, this alga is also being studied by scientists specialized in **population genetics** as they constitute real forests under the sea surface which are the equivalent of our oak forests on land. In fact, each individual is very old and represent a source of protection, freshness, humidity or food for other organisms such as shellfishes.



Structural modelisation of an iodoperoxydase in *L. digitata*
Credit picture: Catherine Leblanc

How

Therefore, scientists wanted to characterize these haloperoxidases. They purified the different proteins from algae and sequenced them thanks to **mass spectrometry**. They were therefore able to guess the sequence of the corresponding genes and made degenerated **primers**. These primers were used to clone the genes by doing a **PCR** (Polymerase Chain Reaction).

Scientists are now interested in the physiological characteristics of these enzymes and are trying to understand when these proteins are fabricated (=synthesized) in the cell, what is the chain reaction that leads to their activation and are, therefore developing **functional genomics** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=322) in this purpose.

With the protein sequence obtained after purification, scientists have studied their structures by comparison with other known haloperoxidases through database research.

Another developed approach to understand the role of the haloperoxidases is to look at the level of expression of each gene in different conditions thanks to the **macroarray** technique (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=325), and for that it is necessary to characterize a maximum of corresponding DNA sequences.

To have more information about the characterization of these haloperoxidases, see the read more section.

The different conditions which are being looked at are linked to **stress** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=339) and **defence** responses: pathogen attack, high light exposure, immersion, presence of oxidative species, etc.

An example of the conditions that are tested is the application of natural defence stimulators like oligoalginates on the alga. Oligoalginates are small molecules that come from the alga's cell walls and that are liberated when there a pathogen is degrading the alga (considered as an external attack). The alga has the capacity of detecting these fragments and activates its defence reaction in consequence. When put in contact with the alga, these small molecules simulate the attack of an exterior pathogen. This perception of the attack causes ionic exchanges through the membrane and an oxidative burst which is a general phenomenon in all eukaryotes. Then there is a cascade of signalisation inside the cell which leads to the activation of certain genes.

Until now, the genes for which the transcription is activated in the defence reaction had not been identified; but, thanks to the **macroarray** technique (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=325), RNAs coming from stressed algae have been hybridized and the up-regulated ones are being identified.

Having identified those genes, scientists hope to find specific molecular markers of defence responses and among them certain coding for enzymes belonging to the haloperoxidase family in order to confirm their essential role in the defence reaction.

With Who

Structural biology and chemistry have been developed within the **Station Biologique de Roscoff** (http://www.sb-roscoff.fr/); however collaborations concerning the chemical analysis of the halogenated compounds are needed. Thanks to their algal model, they have integrated a national program called "toxicologie nucléaire environnementale" (=environmental nuclear toxicology, web site: http://www-dsv.cea.fr/content/cea/p_prog/p_pour_nucl/p_tn/) which is interested in iodine fluxes in the environment and therefore the impact on mammals too. They are associated with laboratories (CEA: http://www.cea.fr/, INSERM: http://www.inserm.fr/fr/home.html) which are working specifically on mammals and the concentration of iodine in the thyroid gland. Although these systems of concentration are completely different in algae and mammals, there are common interests such as iodine chemistry and chemical analysis of halogenated compounds.

Concerning the **physiological aspect of the study**, they are collaborating with biogeochemists from France and England who are interested by the cycle of iodine from the marine environment to the atmosphere and are trying to answer questions of ecological nature such as when is the alga liberating iodine and what is the real impact of algal iodine emission on the environment (local formation of clouds, impact of climate and ozone destruction).

Perspectives

Scientists hope to soon have **functional genomic** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=322) tools for their research. They're counting on ***Ectocarpus siliculosus*** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=313) to become a model they will be able to use to do **mutagenesis** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=323) and study haloperoxidases and more generally the genes involved in defence responses and more specifically on haloperoxidases proteins involved in the halogen metabolism. They also would like to develop **mass spectrometry** and **crystallography** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=328) to be able to characterize these enzymes structurally. They believe they'll have much more information about how these enzymes work and when they are activated in the future.

There are many potential applications associated with these studies:

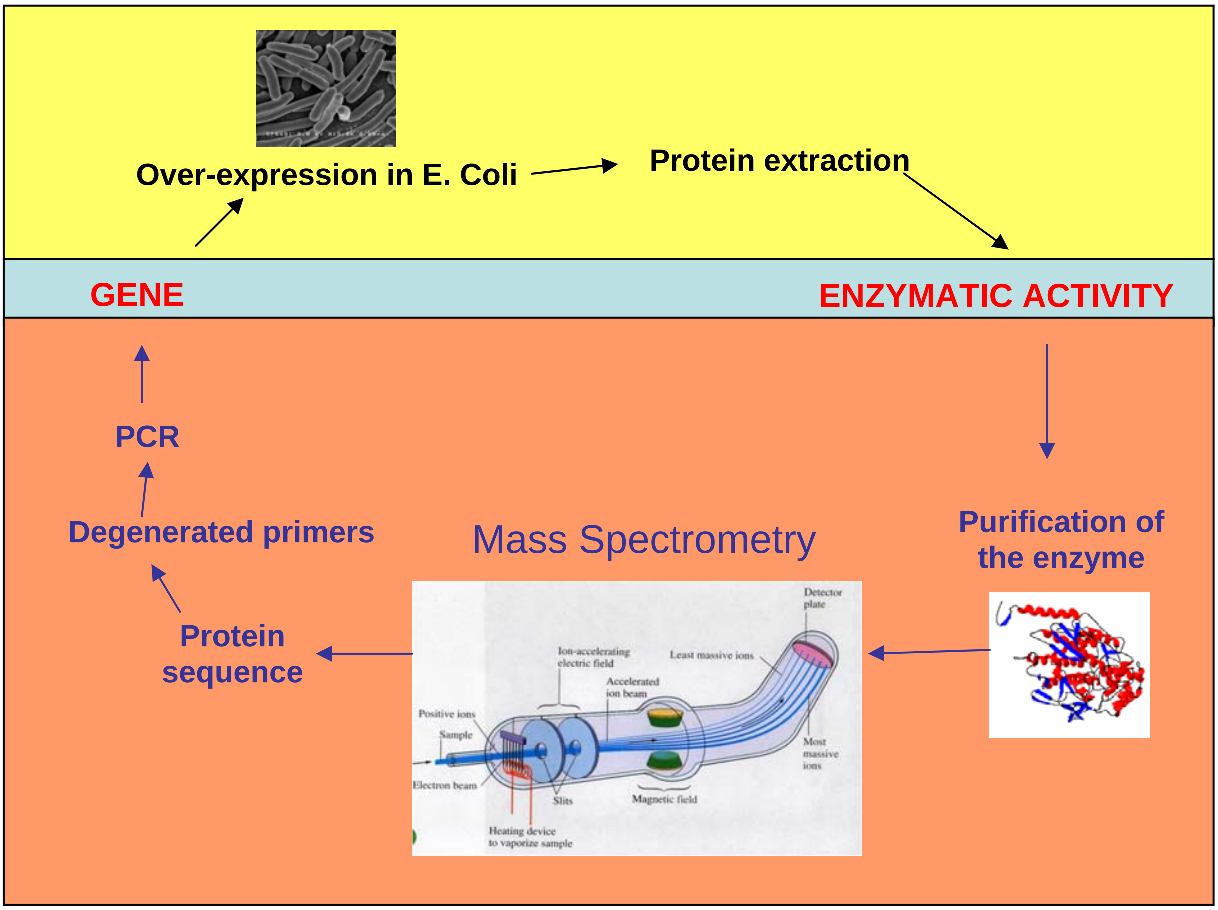
1) **Ecologically**, having gathered these data on defence response (activation of specific genes) could be of great importance. If they know a panel of genes which are usually activated in defence reaction, they will be able to go on the field to sample bits of algae and evaluate their biological health. This would mean collaborating with the specialists in **population genetic**.

2) Concerning **health**, parallels with the thyroid are of great importance. For instance, it is known that when there is an infection, enzymes which are called myeloperoxidases (which are in fact, bromoperoxidases a type of haloperoxidases) will halogenate certain cyclic molecules which will become chemo-attractants for leucocytes. Leucocytes are actors of great importance in the immune response. Therefore, if scientists are able to find enzymes which are able to halogenate the substrate in a precise spots, they could be used to produce drugs. In order to go forward concerning this idea, over expression needs to be realised in a **genetic model** (http://www.marine-genomics-europe.org/index2.php?rub=c&pid=334) organism.

3) There is also a potential **economical** application concerning the chemistry industry in general. As far as the used water treatment, for instance, javel water, HOCl, is being used but it also needs to be eliminated. These enzymes could be used to purify the water for its HOCl.
Finally, halogenated compounds are very reactive and have many different applications in chemistry making them key molecules to study.

Read more:

Cloning these enzymes wasn't such an easy task: Scientists started by getting interested to the **RNA**s extracted from the alga in order to find RNA messengers coding for these enzymes specifically. As these enzymes weren't known at the beginning, they had to be purified in order to sequence fragments of them by **mass spectrometry** (see **proteomics** at: http://www.marine-genomics-europe.org/index2.php?rub=c&pid=327). Thanks to these bits of sequences, they were able to go back to the gene coding for these enzymes by making degenerated primers. These primers were made by translating the genetic universal code backwards in order to get a DNA sequence from a protein sequence. As explained in the link to the **PCR** explanation (http://users.ugent.be/~avierstr/principles/pcr.html), these primers are essential for cloning the DNA sequence.



Technical pathways for discovering the gene sequence and the enzymatic activity.
The pathway used in these studies is in orange.

Usually scientists go from the genetic sequence to the enzymatic activity going through over expression in *Escherichia coli* and protein extraction. In the case of ***Laminaria digitata***, the genes coding for the protein of interest, the alloperoxidases, were identified going from the enzymatic activity to the gene sequence. This was done using **mass spectrometry** to determine the protein sequence. By translating the protein sequence into the genetic sequence according to the universal genetic code, degenerated primers corresponding to the gene were designed. These were then used to amplify the gene sequence thanks to the **PCR** technique.

See diagram of the Mass Spectrometer at:
http://library.tedankara.k12.tr/chemistry/vol2/the%20structure%20of%20the%20atom/z19.htm