The ABC of ABC-transport in the hyperthermophilic archaeon Pyrococcus furiosus
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Archaea and hyperthermophiles

Living organisms of our earth can be divided into two groups, the prokaryotes and the eukaryotes. Eukaryotic cells have a nucleus, a special compartment in the cell, where the genetic material, the DNA is located. The DNA in the prokaryotic cell is floating freely in the cell. The eukaryotes, that is where we belong to, together with animals, plants and fungi. Bacteria and archaea belong to the prokaryotes. Archaea resemble bacteria but in certain features they resemble more the eukaryotes. That is the reason why they form a distinct group within the prokaryotes.

Why are archaea so interesting? Arehae are found and thrive in environments where a human being would not survive. The (hyper)thermophilic archaea grow best at temperatures between 55 and 113 °C. Psychrophiles grow at low temperatures, like on Antarctica, barofiles only survive under high pressure, acidophiles at acidic environments (pH 0.5 to 4), while alkaliphiles prefer a basic (soap-like) environment (pH 8 to 11.5). And last but nor least, halophiles only survive at very high salt concentrations, as found for instance in the Dead Sea. Of course there are also archaea which only can grow at a combination of extreme conditions, like an acido thermophile.

Archaea which survive and grow under extreme conditions are interesting because these organisms can grow there where we can not survive. This feature opens possibilities for industry. Enzymes and other proteins isolated from extremophiles are very stble. The industry uses these extremely stable enzymes to be able to have processes done in a more efficient and environmentally clean way. But also for fundamental research there is enough to discover on extremophiles. Why can these organisms live under these extreme conditions? When an egg is boiled, the egg solidifies because the proteins in the egg can not stand the high temperature and aggregate. A hyperthermophilic archaeon, however, thrives at these high temperatures and its proteins function perfectly under these circumstances. This seems strange at first, because the building blocks of the archaenal cell are the same as of all other cells. It is the combination, the order, of the building blocks that determines whether a protein is stable under these extreme conditions.

My research focussed on a hyperthermophilic archaean. Hyperthermophiles are found at places where the surrounds are heated by volcanic activity. Many hyperthermophiles are found in the (deep)sea. At cracks in the crust of the earth the hot lava flows into the sea. The lava is very hot, around 400 °C. The seawater is cold, around 4 °C. Where
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the hot lava contacts the seawater, a sort or
towers appear from which black ‘smoke’
seems to escape. These ‘smoking’ towers
are called Black-smokers. Because of the
combination of cold seawater and hot lava,
a large temperature gradient exists around
these black-smokers and the minerals
which are in soluble in the hot water
precipitate as a black rain. In the direct
surroundings of a black smoker, several
different organisms are found which have
adapted to these high temperatures, like
tube-worms. Although these organisms are
found close to the black-smoker, they can
only survive because of the high
temperature gradient. Tube-worms for
instance can grow at temperatures of
maximally 40 °C, while certain
hyperthermophilic archaebacteria still survive at
113 °C. These hot-springs are not only
found in the deepsea but also a few meters
below the sea level. Hyperthermophilic
archaebacteria are also found at geysers and in
hot-springs, for instance in Yellowstone
Parc in the United States.

My research

The archaeon which I studied during
my research, Pyrococcus furiosus, was
found in Italy in geothermally heated
sediments of a hot-spring in the sea, close
to Volcano Island. This organism was
described in 1987. P. furiosus is a
hyperthermophilic archaeon which only
feels happy at temperatures between 70 and
105 °C. Humans need oxygen from the air
to survive, P. furiosus is an anaerobic
organism and can only survive when there
is no oxygen in its environment. Seen
through a microscope, the organism looks
like a little ball with a tail. The tail is used
for movement. The name Pyrococcus
furiosus says something about its looks and
its growth. Literally it means furious fire-
ball. P. furiosus is a very fast growing
organism and doubles itself every 30
minutes under ideal conditions.

Archaebacteria are freely present in the
seawater but can only grow at high
temperatures. The moment archaebacteria are in
an area with the right temperature, the
organisms have to find food. The food of
P. furiosus consists of proteins and sugars.
These nutrients first need to be taken up by
the cell before they can be used for growth
and maintenance. Around the cell is a fatty
layer, the membrane, which is
impermeable. Therefore, the cell needs
small channels in this membrane for
nutrients to enter the cell and for waste
products to leave the cell. The channels are
formed by special proteins, called transport
proteins. During my research a number of
these transport proteins were studied.

P. furiosus can grow on several
different sugars, for instance maltose,
cellobiose and starch. When I started my
research, already something was known
about how P. furiosus can utilize sugars for
energy, the so-called metabolism. But it
was not known how these sugars enter the
cell. That was the most important question
during my research. We found that P.
furiosus uses a different transport protein
for each sugar. For the most important sugars we identified three different transport proteins, which all are members of the family of ABC-transporters. ABC stands for ATP-binding cassette. The ABC-transporters belong to a large family of transport proteins and are found in all bacteria, archaea and eukaryotes, among which humans. ATP, adenosine triphosphate, is the form of energy that is used by these transport proteins to transport their substrate over the membrane. The ATP-binding cassette is that part of the transport protein which binds ATP. This part is similar for all ABC-transport proteins which belong to this large family. An ABC-transport protein complex consists of a so-called binding protein, two proteins which together form the channel in the membrane, and two proteins on the inside of the cell which form the driving force of the transporter by supplying the transporter with energy. The binding protein is present on the outside of the cell and catches sugars from the environment, keeps them and transfers them to the two proteins which form the channel in the membrane. At the expense of ATP the sugars are then led through the channel and enter the cell. In the cell the sugars are broken down, metabolised, and new energy is formed for growth and maintainance.

Because *P. furiosus* originates from the open sea and not from a closed system like for instance a lake, the organism needs to be able to immediately take up sugars, as fast as possible and as much as possible, otherwise the sugars disappear again out of reach. The binding protein of *P. furiosus* is capable of binding these sugars very efficiently already at very low concentrations. Interesting is that this binding is done much more efficiently than with binding proteins from organisms which live at 37 °C. *P. furiosus* has to be able to react quickly on the presence of a particular sugar. It is not ideal for an organism to continuously have all transport proteins present and maintain them because this costs a lot of energy and building blocks. We found that *P. furiosus* only makes those transport proteins which it needs at that moment. So, when *P. furiosus* grows on cellobiose, only the ABC-transport system for cellobiose transport is present. During my research I transferred the genes encoding the different binding proteins to the bacterium *E. coli*, resulting in *E. coli* making the binding proteins. This made it possible to determine which gene encoded for which sugar binding protein. We also purified the different binding proteins from all other proteins, to be able to study them more accurately without the possibly interfering influence of other proteins. Using these experiments we could determine which sugars are bound by the binding proteins and which not. Surprisingly, a number of sugars were bound by the binding proteins from which was previously not known that *P. furiosus* could grow on them. These sugars turned out also to be grow substrates for *P. furiosus*.

The complete genetic information, the so-called genome sequence, of *P. furiosus*
and two other Pyrococcus species, *P. abyssi* and *P. horikoshii*, is known. We examined these genome sequences to check which binding proteins were present and which were absent. Although the three species are related, they do not share all the different binding proteins. Remarkable was the finding that *P. abyssi* and *P. horikoshii* do have binding proteins for sugars while these two species are unable to grow on sugars. Most likely other proteins are missing which are necessary for growth on sugars.

My research led to a greater understanding in the mechanisms of sugar transport in *P. furiosus*. A more complete picture is now present on sugar metabolism in *P. furiosus* and related species.