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A TURBO **SWITCH** SPEEDS UP A **CRUCIAL CALCIUM PUMP**

On the right: Professor Poul Nissen with postdoctoral fellow Henning Tidow, PhD student Sigrid Thirup and chief technician Anna Marie Nielsen. Furthermore, people of the drug discovery team are present in this picture (from left): Jens Christian Bredahl Sørensen, Claus Olesen, Christine Juul Fælled Nielsen and Jacob Luawring.

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In plant and animal cells calmodulin-stimulated calcium pumps are key regulators of the calcium concentration inside the cells and therefore essential to life. If they fail, the intracellular calcium concentration rises pushing the cell to commit suicide. The pump contains a previously unknown third gear that makes it flush calcium out of the cell. These three stages of progressive activation enables tight control of cellular calcium and reveals new drug targets.

Calcium plays a central role in most signalling processes of life such as changes in cell activity or cell division. Calcium signalling derives from the 20.000-fold gradient between the high concentration outside the cells and the low intracellular level. For example, during signalling or under stress the calcium concentration inside the cells increases due to opening of calcium channels and triggers a corresponding reaction.

Afterwards, the intracellular concentration must be lowered again. This task is carried out by a calcium pump known as PMCA. These high-affinity pumps are situated in the cell membrane and they export calcium ions (Ca2+) from the cytoplasm to the extracellular environment. The pumps are crucial for controlling the overall balance of calcium inside cells and for local intracellular calcium ion signalling.

A regulatory switch

Export of calcium out of the cell requires a lot of energy due to the high concentration gradient. Thus, it is important that the pump is activated only when needed and cannot go in reverse. Therefore, the pump has a regulatory switch, which is actuated by the protein calmodulin. When calcium binds to calmodulin, the protein changes its shape, which enables it to dock onto the switch and activate the pump. If the intracellular calcium concentration keeps on rising, more and more pumps are turned on. However, until recently the detailed mechanism of this regulation was unknown due to the lack of structural information.

Determining the atomic structure

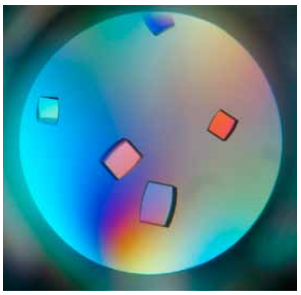
We determined the structure of a complex between the regulatory domain of the calcium pump and calmodulin by X-ray crystallography and small-angle X-ray scattering. To our great surprise, we found that the calcium pump has two binding sites for calmodulin and not just one as always thought. While the first site had been previously mapped by mutational studies the second binding site has never been discovered before. The SAXS studies confirmed this unexpected result from the crystal structure.

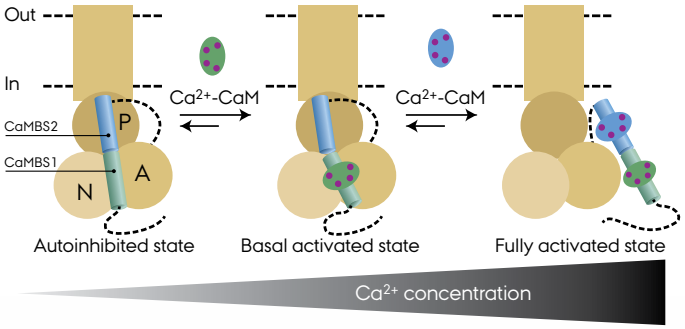
Progressive activation in three steps

The next step was to determine whether the newly discovered second binding site for calmodulin has biological significance, which was studied in yeast and the model plant thale cress. In combination with mathematical modelling the experiments revealed that pumps in which one of the two calmodulin binding sites was disabled could not reach full power.

The results show that the calcium pump is controlled in three steps: Under very low intracellular calcium concentrations the pump is completely inactive. When the concentration increases calmodulin binds to one site within the regulatory domain, which leads to moderate pumping. When the concentration rises to even higher levels calmodulin complexes bind to both sites and the pump rapidly transports large amounts of calcium out of the cell.

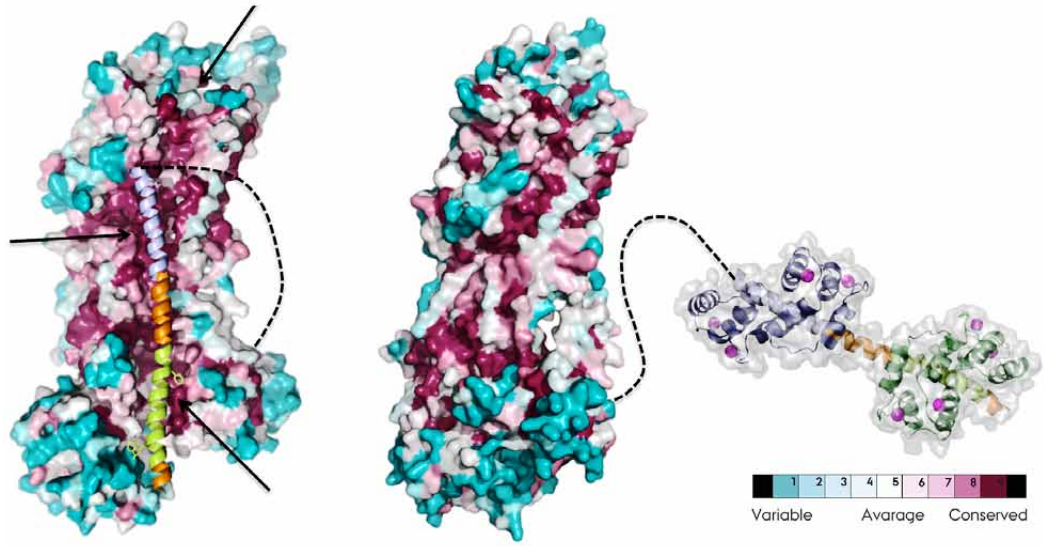
In conclusion, we have developed a general structural model for calmodulin-mediated regulation of PMCA calcium pumps involving a turbo switch. This mechanism allows for a stringent and highly responsive control of the intracellular calcium concentration in plant and animal cells. This discovery improves our understanding of a fundamental biological mechanism in all higher organisms. In the future the discovery could allow for better treatments of certain diseases in which the calcium balance is disturbed. An example is chronic kidney disease.





Left: We used crystals of the regulatory domain of calcium pumps from thale cress (*A. thaliana*) in complex with calmodulin and Ca^{+2} for structure determination. The crystals were obtained by the sitting-drop vapour diffusion method and grew to dimensions of up to 0.7 x 0.35 x 0.2 mm.

Right: The schematic shows the three stages that enable the calcium pump to respond progressively to rising intracellular calcium concentrations: The inactive stage. The basal activated stage with one calmodulin molecule bound to the pump. The fully activated stagewith two calmodulin molecules attached to the pump. The dotted lines represent the cell membrane in which the calcium pump is situated.



Structural models of the calcium pump during its inactive stage and fully activated stage. Drug sites of interest are marked with arrows.

New targets for chemotherapeutics

The SERCA calcium pump is related to the PMCA calcium pump and it is present in all higher cells, where it pumps calcium from the cytoplasm into internal stores, e.g. when muscles are relaxed.

This calcium pump has been extensively studied in our lab with structure determination of several high-resolution crystals structures in different functional stages as well as in complexes with inhibitors and regulatory proteins.

These structures do not only enable detailed insight into the functional reaction cycle of ion pumps like PMCA and SERCA, but also provide a basis for drug discovery. If drugs can be targeted specifically towards tumours the calcium pumps of the cancer cells are promising targets for chemotherapeutics since the cells commit suicide by apoptosis if the pumps are blocked.

Bacterial ion pumps and new antibiotics

Bacteria also possess calcium pumps as revealed by the increasing number of sequenced bacterial genomes. Their functions are still poorly understood, but a calcium pump from *Streptococcus pneumonia* has been shown to be vital for the survival of the pathogen in the infected host. Similarly, bacterial copper pumps from the same protein family are critically important for pathogenic organisms.

These bacterial ion pumps are attractive targets for new antibiotics. Their activity is easily monitored in high-throughput assays that facilitate powerful drug discovery programs in combination with structural studies.