The development of organisms under the control of SIRT1

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The Notch signalling pathway is one of the major signalling pathways involved in the development of living organisms. Its regulation is essential to ensure that every individual is born with "everything as and where it should be". In collaboration with researchers from the Goethe University in Frankfurt, Franck Dequiedt has revealed a new control mechanism in the Notch signalling pathway.

By definition, an organism is a collection of elements composing a functional structure. In biology, an organism refers to all the cells, tissues and organs that comprise an individual. It is the interaction between these multiple elements, but also the adaptation of these elements to external stimuli, that enable organisms to live and survive. Although highly complex and still far from being fully known and understood, the development of higher organisms relies on several important signalling pathways. Theses pathways consist of a series of molecular events triggered by a stimulus and enabling the transmission of a signal within a cell or from one cell to another.

Of particular importance among these pathways is the Notch cascade.

"It is pathways such as Notch, Homeobox or Wnt that determine into which animal an organism is going to develop. They play many different roles and are involved in organogenesis during several stages of development", explains Franck Dequiedt, a "research associate" of the FNRS at the Faculty of Agro-Bio Tech in Gembloux and GIGA at ULg. But how are just a few signalling pathways at the origin of the great diversity of organisms that exist on earth? "For such biodiversity to be possible, these pathways must be flexible and adaptative to the variations of the environment", continues Franck Dequiedt. Hence the interest in understanding how these pathways are modulated and controlled.

Ubiquitin, not the only commander on board

Over the past few years, there has been a new boom in research on the Notch signalling pathway. Scientists have discovered that this pathway is involved in the specification of stem cells; in addition, a deregulation in this pathway can lead to the appearance of cancer. In brief, the Notch pathway functions as follows: Notch is a transmembrane receptor that is activated by two major families of ligands. These ligands bind to the extracellular part of the Notch receptor, thus causing a portion in the intracellular part of the receptor to
be cleaved off. This part, known as the NICD (*Notch intracellular domain*), migrates from the cytoplasm to the nucleus, where it activates the expression of the genes involved developmental programs.

During its migration from the membrane to the cell nucleus, the stability of NICD is tightly controlled, i.e. the cell may or may not degrade it. "This *protein* can be marked by another protein, ubiquitin, which indicates to a proteasome, a cellular machinery specialized in degradation of proteins, to degrade the NICD by proteolysis. This allows the cell to control the intensity and the duration of the Notch signal", Franck Dequiedt points out.

In collaboration with a team from the Institute of Cardiovascular Regeneration and the Department of Cardiology at the *Goethe University* in Frankfurt, directed by Michael Potente, Franck Dequiedt has revealed another pathway enabling the cell to control the levels of the NICD and therefore the strength and the duration of the Notch signal. The results of this study have been published in the journal *Nature* (1).

**When SIRT1 gets involved**

"*We have worked together on this study because it concerns the "meeting" of two important proteins: Notch and SIRT1*", explains the FNRS researcher. "*In our laboratory, we are working on enzymes called "histone deacetylases" and SIRT1 belongs to this superfamily of enzymes*", specifies Franck Dequiedt. Thus, SIRT1 removes the acetyl groups (CH3-CO-) which are grafted onto proteins. A protein has different properties depending on whether or not it is acetylated. "*For the first time, we have shown that NICD is an acetylated protein that is deacetylated by SIRT1*", reveals Franck Dequiedt. Subsequently, when the SIRT1 levels are modified, this automatically affects the levels of NICD inside the cell. "*The more NICD is acetylated, the more stable it is and vice versa*", the scientist explains. "*We can therefore say that SIRT1 indirectly controls the stability of NICD*".

Another observation made by the researchers in Liège: there is competition between ubiquitination and acetylation. "*If we promote acetylation of NICD by reducing the levels of SIRT1, we impair its ubiquitination, which leads to an increased stability of the NICD*", explains Franck Dequiedt. And conversely: the more SIRT1, the more unstable the NICD will be. Its action on the expression of the genes involved in the development will therefore be reduced. In short, if there are high levels of SIRT1 in the cell, this causes a reduction in the intensity and duration of the Notch signal, whereas if these levels are low, the signal will last longer..
Notch and the formation of new vessels

In order to provide more concrete proof of the link between SIRT1 and the Notch signalling pathway, the researchers chose to demonstrate that these mechanisms function within the vascular system, one of the major systems controlled by the Notch signalling pathway. "We have been working on what is known as ‘tip cell differentiation’", specifies Franck Dequiedt. New vascular branches emerge from existing vascular trunks. "A cell will sprout, send out filipodia and will thus be the source of a new vascular branch that will grow in a directional manner", explains the researcher. Within this small new growing vessel, not all the cells are equal. There are tip cells and stalk cells. "At the tip of the growing vessel, a cell directs the vessel's growth and the others follow. The tip cell sprouts and creates new branches while the stalk cells form the trunk of the new vessel", continues Franck Dequiedt. But how does that relate to the Notch signalling pathway? The cellular specification of tip cells/stalk cells depends precisely on this signalling pathway! The tip cell makes the DLL4 protein (Delta-like 4 protein) which is a Notch receptor ligand. Once released by the tip cell, this protein activates the Notch receptors of the other cells, thus telling them that they must behave like stalk cells. As a result, a reduction in the Notch pathway increases the number of cells behaving like tip cells, which in turn increases the number of branches resulting in a denser vascular network.

Focus on the retina of mice and on the tail of zebrafish

Franck Dequiedt and his colleagues in Germany were able to observe the effects of a modulation in the Notch signal via SIRT1 on the branching of the vascular system, in vitro, but also in vivo. "When we create a culture of endothelial cells on a substrate, we can see if they form a branched vascular network depending on the strength of the Notch signal", points out Franck Dequiedt. "In vivo, we can also observe this in the development of the vascular system in the post-natal mouse retina", he continues. Since the pattern formed by this vascular system is very reproducible from one mouse to the next, it is easy to observe the differences caused by deregulations of the Notch signalling pathway.

"In mice where we specifically inactivated SIRT1 in the endothelial cells, we observed a far simpler vascular network in the retina with far less branching and budding",
explains the scientist. By activating SIRT1, the researchers favoured the acetylation of the NICD and hence its stability. As a result, the intensity and duration of the Notch signal is far greater and therefore, there is a decrease in the number of tip cells ready to develop into new vascular branches...

The same effect was observed in the development of the vascular network in the trunk of the zebrafish. "The animals we use are transparent at the start of their life and are therefore the ideal model to observe
modifications in their development. When we inhibit SIRT1, the growth of the vascular network in their trunk region is reduced", says Franck Dequiedt. These experiments therefore confirm the action of the SIRT1 protein on the Notch signalling pathway during angiogenesis. What about the effect of the histone deacetylase in the development of other tissues under the influence of the Notch signal? "We have already observed the same regulation in the development of muscular cells", Franck Dequiedt replies. The mechanism controlling the intensity and duration of the Notch signal by SIRT1, recently described in the journal Nature, is thus a mechanism that can be potentially applied in all tissues whose development relies on the Notch signalling pathway.

(1). Virginia Guarani, Gianluca Deflorian, Claudio A. Franco, Marcus Krüger, Li-Kun Phng, Katie Bentley, Louise Toussaint, Franck Dequiedt, Raul Mostoslavsky, Mirko H.H. Schmidt, Barbara Zimmermann, Ralf P. Brandes, Marina Mione, Christoph H. Westphal1, Thomas Braun, Andreas M. Zeiher, Holger Gerhardt, Stefanie Dimmeler, Michael Potente. Acetylation-dependent regulation of endothelial Notch signalling by the SIRT1 deacetylase. DOI: 10.1038/nature09917.