BREAKTHROUGH ON THE RESEARCH FRONT

From zebrafish to human

Our body functions thanks to proteins whose synthesis is directed by our genes. Each protein plays a well defined role. Gene mutations prevent the corresponding protein from fulfilling its function and may lead to diseases. This is the case for Hereditary Spastic Paraplegia (HSP), a group of monogenic neurodegenerative diseases.
SPG3A-HSP is one of the most frequently mutated genes in autosomal dominant HSP (and the major form of early onset HSP): 10% of cases.

Dr Jamilé HAZAN's team has demonstrated that in the zebrafish atlastin, the protein encoded by the *SPG3A* gene, controls the architecture of spinal motor neurons and thus the mobility of the larvae during embryonic development of this small fish.

Published in the prestigious journal, *Nature Neuroscience*, this article provides fundamental information about the physiopathology of our diseases on a molecular level.
Fassier C *et al. Nature NeuroScience* (2010)

Coralie Fassier, the first author of this article, received the *ASL-HSP France* Research Prize in 2009 for the creation and characterization of the first HSP mouse model linked to mutations in the spastin gene (SPG4), which is the gene the most frequently altered in our diseases. Our Association has also provided Jamilé Hazan's team with the funding necessary to achieve this published research project in 2009 and 2010.
ASL-HSP France is proud and happy to have contributed to this high-quality piece of work, which is now being continued.

The work of Jamilé HAZAN's team

When a gene is identified as responsible for a genetic disease, it is essential to understand the biological function of the protein it encodes.

- 21 genes responsible for Hereditary Spastic Paraplegia (HSP) have been identified to date (SPG3A/atlastin, SPG4/spastin, SPG11, etc…). For our purposes, it is essential to discover the physiological role played by these HSP proteins.
- These research issues will help to:
    1. understand the molecular mechanisms involved in our diseases,
    2. develop a therapeutic strategy aimed at re-establishing the function of these proteins.

- During embryogenesis, in humans and in animal models, the formation of the nervous system follows a specific program using proteins which are homologous between the different species.
- In the embryo, neurons are born and develop under the action of specific molecules using a communication network inside the cell called "signaling or molecular pathways". Several research programmes focus on analyzing the role of the proteins involved in these "pathways" particularly those that are implicated in the occurrence of neurodegenerative disorders.
- For HSP, studying the role of a protein in a simple organism (such as the zebrafish) may clarify the function that this given protein plays during development and maintenance of the corticospinal tracts in humans.

- Once the experimental model and the gene have been chosen, the researchers can opt for one of the two following approaches:

First approach

→ Create a transgenic organism (worm, fly, fish, mouse) with a specifically modified gene which will lead to the production of an abnormal protein or to the lack of this protein, and which will be passed onto subsequent generations.

→ The resulting defects in the biology of these model organisms can then be studied and compared to healthy organisms.

Second approach

→ Manipulate a normal organism using specific reagents to target the gene of interest before studying the behavior and biology of the modified organism.

- Whatever the approach is, the strategy applied is that of "reverse genetics" leading to a "gain" or "loss" of function of the studied gene: inhibiting its expression, or conversely overexpressing it.

Procedures for studying the role of the SPG3A gene in the zebrafish

- Dr Jamilé Hazan's team[1] has first selected the atlastin protein encoded by the gene SPG3A which is a major gene for early-onset pure HSP, which first symptoms occur very early in childhood (§ Summary of information). SPG3A mutations are responsible for more than 10% of autosomal dominant HSP.
- They have chosen to study the function of atlastin in the zebrafish because this small fish presents many advantages including the fact that it is a vertebrate species whose embryos/larvae are transparent during development. The zebrafish develops over 5 days, embryos are produced in large quantities by a couple of fish and its nervous system is easier to characterize on a morphological level than that of mammals.

- Coralie Fassier and Jamilé Hazan proceeded in two steps:
  - 1st step: Injection of reagents in zebrafish embryos, leading to either a significant depletion (loss) or an excess (gain) of atlastin,
  - 2nd step: observe the motor behavior of the "baby fish (or larvae)" that start swimming from the second day after fertilization and study the associated cellular/neuronal modifications.

SPG3 and the BMP pathway: a strong link, the results speak from themselves.

- During development of the zebrafish, when atlastin is no longer expressed, the larvae lose their mobility. This effect is preceded by an abnormal architecture of the motor neurons and is associated with activation of the BMP (Bone Morphogenetic Protein) signaling pathway (see inset).
- Conversely, the overexpression of atlastin leads to the inhibition of the BMP pathway.
- At this level of their discovery, the researchers have revealed a potential link between atlastin and a BMP receptor (BMPR1) in zebrafish spinal motor neurons. By cultivating zebrafish spinal neurons *in vitro*, they show that atlastin co-localizes with BMPR1 within vesicles located all along the axons.
- Finally, the ultimate proof of a functional interaction between atlastin and the BMP pathway *in vivo*: the inhibition of the BMP pathway in atlastin-depleted zebrafish is sufficient to restore their mobility as well as the normal architecture of their motor neurons.

- This amazing study in physiopathology[2] revealed that during vertebrate development, atlastin controls the mobility of the larvae and the architecture of spinal motor neurons by inhibiting the BMP pathway.
- Before this analysis has shown the link between an HSP protein and the BMP pathway *in vivo* in a vertebrate model organism, other teams studying neuronal cells *in vitro* revealed that the proteins involved in other forms of HSP (spastin/SPG4, NIPA1/SPG6 and Spartin/SPG20) may also inhibit the BMP pathway[3]. The same link has been equally suggested for other neurodegenerative disease such as Amyotrophic Lateral Sclerosis or Spinal Muscular Atrophy.
- A misregulation of the BMP pathway, even minor, could be a common mechanism to different neurological disorders, and particularly to various form of HSP. A recent experiment performed on rats which underwent spinal cord section underpins this concept since an intrathecal injection of a BMP antagonist improved the locomotor performance of the operated animals and the regeneration of their corticospinal tracts[4].
- Undoubtedly, this work provides important pieces of information about the mechanisms leading to the diseases we are interested in. Silencing the BMP pathway in the human spinal cord is not currently feasible and the researchers are now trying to understand the regulation of the BMP pathway activity, which might open up novel therapeutic approaches for HSP.

Whatever the case, the BMP pathway has serious potential…

References

1- Fassier C *et al* Zebrafish atlastin controls motility and spinal motor axon architecture via inhibition of the BMP pathway. *Nature Neuroscience* 2010; 13**:** 1380-7.
2- Physiopathology: study of the mechanisms responsible for the symptoms of a disease.
3- Tsang HT *et al.* The hereditary spastic paraplegia NIPA1, spastin and spartin are inhibitors of mammalian BMP signaling. *Hum/Mol. Genet*. 2009 ; **18** : 3805-21.
4- Matsuura I *et al.* BMP inhibition enhances axonal growth and functional recovery after spinal cord injury. *J.Neurochem.*2008; **105:** 1471-79.

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INTERVIEW with Dr Jamilé HAZAN & Dr Coralie FASSIER

***Spastic* to Jamilé Hazan (JH):**
For the first time in vertebrates, you have demonstrated *in vivo* the role of a protein which is deficient in some HSP patients. Since you have discovered SPG4/spastin which is more frequently mutated in HSP than SPG3A/atlastin, why did not you start with SPG4 rather than SPG3?
JH:
-I would just like to remind you that I also discovered SPG3A locus and that we are interested in all SPG genes! That said, SPG3A is still the second most frequently mutated gene in HSP. More importantly, SPG3A mutations lead to juvenile-onset pure HSP, which suggested a potential effect of its deficiency during development, and that is not the case for SPG4 which is mainly related to adult-forms of HSP. Moreover, since SPG3A is expressed very early during embryonic development, it was a prime candidate to study the effect of its alteration during fish development.

Spastic
- Can you, please, explain us why you were interested in studying the role of SPG3/atlastin on zebrafish embryos, I mean during embryonic development, and not on adult zebrafish?

JH
The idea is simple! If a deficient or an abnormal protein leads to axonal degeneration of motor neurons in humans, it is of interest to see whether this protein is crucial for their formation and whether its alteration during development causes a fragility of certain types of neurons and more specifically the vulnerability of some synaptic connections, that will lead to their degeneration later in life and potentially cause the disease.
Consequently, studying the role of a protein during the formation of the nervous system may allow us to understand how an alteration of this protein can cause neuronal degeneration, since several molecular mechanisms involved in the development of the nervous system are similarly implicated in its degeneration.
Furthermore, the zebrafish is a very good model to study the development of the nervous system since it has been extensively characterized by imaging techniques (due to the transparency of zebrafish embryos) and using large collections of mutants affecting different areas of the brain and of the spinal cord as well as distinct populations of neurons.

Spastic
- But is atlastin expressed in adulthood? And can we consider our diseases as disorders, which may result from a deficiency in the prenatal formation of the pyramidal system?

JH & Coralie Fassier
- First question: Yes, the protein is expressed in adult neurons. However, we do not yet know how its level of expression varies depending on time and location, in the different populations of neurons. We also do not know when and how potential variations in the levels of altered atlastin can cause the specific degeneration of certain types of neurons…
Second question: For mutations in SPG3/atlastin, the relationship with embryogenesis is particularly obvious since it is involved in juvenile-onset forms of HSP, i.e. during childhood. And once again, studying a potential deficit during development helps to anticipate what will happen with a lesion in adults.

Spastic
- Moving onto the interaction between atlastin and BMP signaling that your work has shown. Since atlastin clearly inhibits BMP signaling, does it mean that BMP signaling is probably too "high" in patients with mutations in SPG3A and is it thus possible to consider a therapy, which can reduce this level in the adult spinal cord?

JH & Coralie Fassier
- Theoretically yes, but the BMP pathway is far too important at many different levels to switch it off. Bluntly, this manipulation would be more toxic for the patients than beneficial. For the moment, we need to understand the specific regulation of the BMP pathway and especially to characterize its targets and downstream molecular mechanisms in the zebrafish as well as in higher vertebrate models, before thinking of potential therapeutic applications.

Spastic
- On behalf of *ASL-HSP France and EURO-HSP*, thank you for working on our diseases with so much commitment and unwavering relentlessness. Thank you especially for your findings!
Your discoveries give us hope.

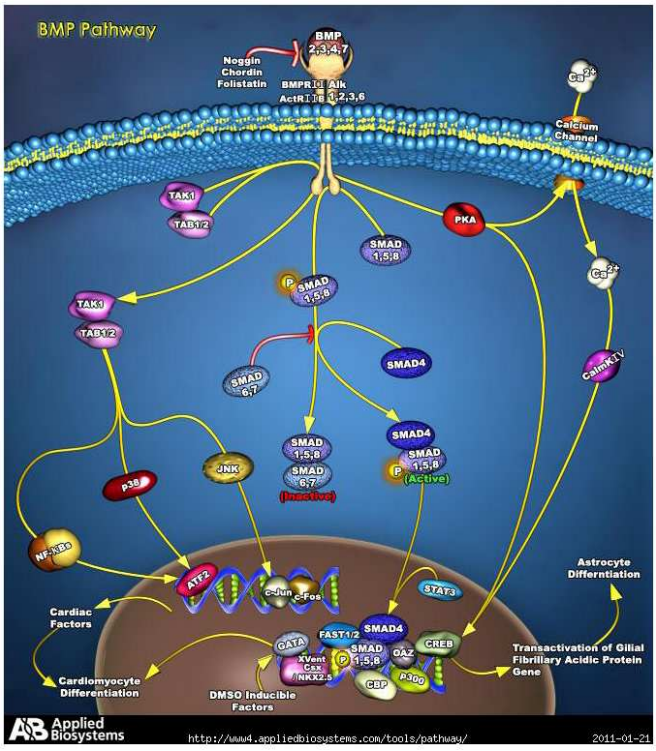
Inset

Signaling pathways or "maps" of cellular traffic

Motor neurons which conducts impulse from the brain to the extremity of the spinal cord should follow a precise pattern of differentiation and establish exquisite connections during embryonic development: after being differentiated into a neuron, the neuronal cell should extend its axon towards precise targets, either other neurons or specific muscles in a meticulous manner. These axon guidance steps are carried out in a very strict spatio-temporal order by integrating information provided by the surrounding tissue. This information is transmitted to the neuronal cell via signaling pathways. These molecular pathways are established during embryonic development (prenatal period). After birth, these pathways will continue to be used by the different types of cells to communicate with their neighboring cells.

In general, a pathway connects the cell surface (i.e., the membrane) to its nucleus. When a signaling molecule binds to its receptor localized on the cell membrane, it starts a molecular pathway which generates the sequential activation of a large number of molecules within the cell. This pathway makes its way across the cytoplasm to inform the nucleus and induce the appropriate response to this event by activating or silencing the expression of a set of given genes. For each signaling molecule, there is a receptor and an associated pathway. Throughout its life, every cell must respond to multiple external stimuli, by triggering a given molecular pathway. Some pathways are identical in different types of cells whilst others are specific to a precise cell type (blood cells, cells from the epithelium, muscle, bone, neuron…). In summary, each cell should permanently adapt its behavior to various stimuli by using a complex network of pathways, comparable to the network of London underground.

For example, the Bone Morphogenetic Proteins (BMP) pathway is activated when a BMP molecule, binds to a specific receptor called BMPR.



BMP pathway