Aim 2: P42, a therapeutic peptide against Huntington's disease
(Project leader: F. Maschat)

Several target genes of Engrailed homeopotein known for their role in neuronal development have been isolated in the laboratory. Among these targets is the *huntingtin* gene encoding a protein whose human counterpart is involved in Huntington's disease, a rare neurodegenerative disease with a prevalence of 1.3 out of 10 000 people in Europe. There is currently no cure for this disease. This disease results from the abnormal expansion of a domain polyQ content in the N-terminal part of human Huntingtin protein (Htt). When this domain exceeds 35Q, polyQ-Htt protein forms aggregates and degeneration of striatal neurons is observed. Several studies, including ours, have identified an influence of the normal function of Htt in the disease (Mugat et al., 2008). Indeed, we showed that activation of normal Huntingtin (*via* Engrailed, or by other mechanisms) allow saving phenotypes induced by the mutant protein polyQ-Htt.

We searched within the Htt protein, which part was responsible for this protective effect. This analysis allowed us to isolate a 23 aa peptide (P42) that prevents aggregation of polyQ-Htt (see Figure). We confirmed the protective role of P42 *in vivo* in entire organisms, first in Drosophila, where P42 is able to save the formation of aggregates and various phenotypes induced by expression of the mutant protein polyQ-Htt (Arribat, Bonneaud et al., 2013; Article acclaimed in International Innovation).

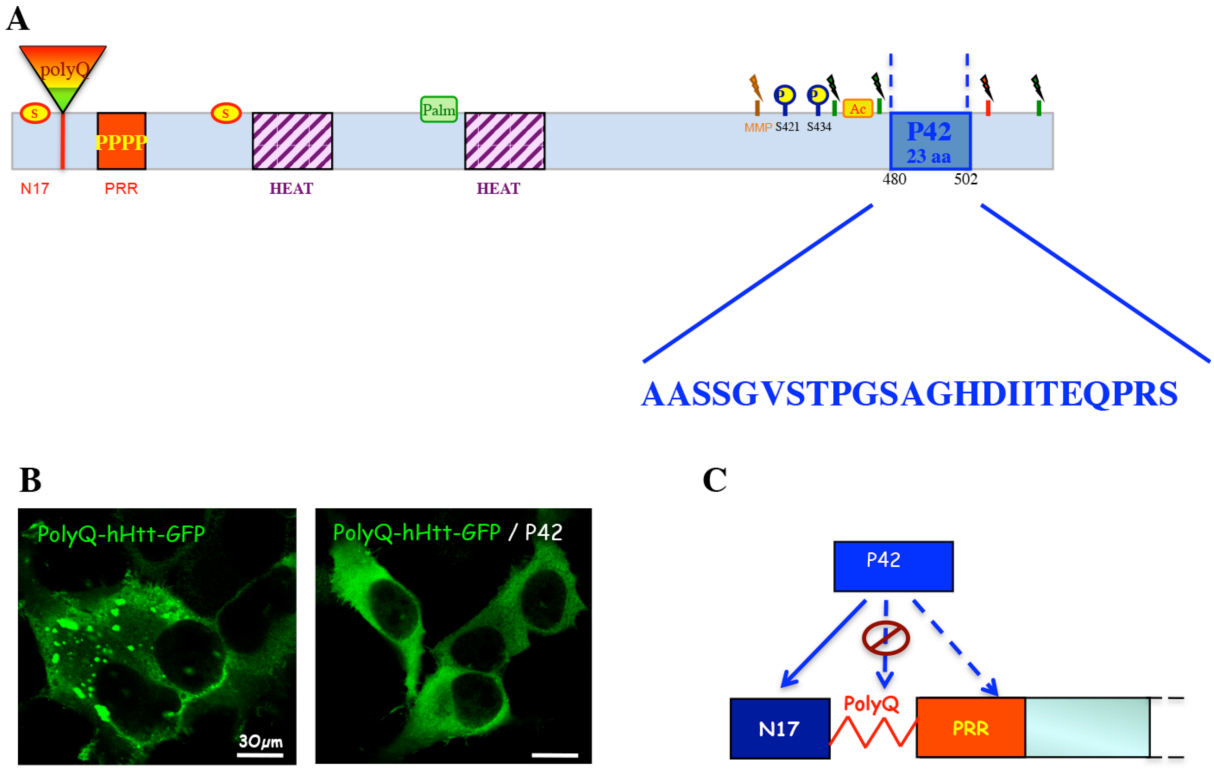

Figure: A- Location of P42 peptide within the 548 aa N-terminal part of human Huntingtin (hHtt) protein.The amino acid sequence of P42 is shown. B- Cultured HeLa cells transfected with polyQ-hHtt-GFP presenting cytoplasmic aggregates; co-transfection with polyQ-hHtt-GFP and P42 prevents aggregate formation (Arribat et al., 2013). C- Possible mechanism of action of P42 is an interaction with the Htt protein. Co-immunoprecipitation and BiFC experiments confirmed a direct interaction of P42 with N17.

Unlike other strategies adopted so far in the field, this peptide does not directly target the polyQ mutation present in the Huntingtin protein, but specifically acts on the mutant Huntingtin interacting with the first 17 aa, which are involved in the early stages aggregation.

To test the protective effect of P42 in a mouse model of this disease (model R6 / 2), we have combined two complementary technologies: i) firstly we merged the HIV TAT P42 area to ensure its diffusion and passage across the blood-brain barrier; ii) secondly to better protect our peptide degradation and to make daily treatment, we used a new oral spray process after dilution of the peptide in microemulsions water-in-oil (Aonys® technology developed by a private company Medesis Pharma-Baillargues, with which we work). We were able, by mass spectrometry techniques on brain sections (Maldi linear ion trap spectrometer), the distribution of P42-TAT by Aonys® compared to more conventional delivery techniques. This allowed us to verify that P42-TAT was well found in the brain after oral administration of Aonys® type of microemulsion. Using this technique original administration, non-invasive, we could show the protective effect of P42 on the R6 / 2 mice (Arribat et al., 2014), during pre-symptomatic treatment, but also post- symptomatic, suggesting that P42 could be used to prevent but also treat the symptoms of the disease.

Current projects:

P42 part of endogenous Htt, the current goal is to analyze the physiological role of this peptide in order to better understand his work as a protective agent. A second component is to improve the stability of P42 therapeutic purposes. For this purpose the dual therapy involving P42 and other protective molecules will also be tested.

Our data showed that P42 has the ability not only to reduce the negative impact of the expression of the mutant protein polyQ-Htt (for example preventing its aggregation) but also by increasing the function of the normal Htt protein, to which he belongs.

Related publications :

- Paucard*, A., Couly* S., Bonneaud, N., Maurice, T., Benigno, L., Jourdan, C., Vignes, M. and Maschat, F. “Improvement of BDNF signalling by P42 peptide in Huntington's disease.” *Submitted. *co authors.*
- Marelli, C., Maschat, F. (2016). “P42: a novel and promising peptide-based therapy for Huntington’s disease”. *Orphanet Journal of Rare Diseases*. 11:24. DOI: 10.1186/s13023-016-0405-3
- Arribat, Y., Talmat-Amar, Y., Paucard, A., Lesport, P., Bauer, C., Bec, N., Parmentier, ML., Benigno, L., Larroque, C., Maurel, P., Maschat, F. (2014) Systemic delivery of P42 peptide: a new weapon to fight Huntington's disease. *Acta Neuropathologica Communications* (2):86-103.
- Arribat*, Y., Bonneaud*, N., Talmat-Amar, Y., Layalle, S., Parmentier, M. L., Maschat, F. (2013) A Huntingtin Peptide Inhibits PolyQ-Huntingtin Associated Defects. *PLoS One* 8(7): e68775. **co authors.*
- Mugat, B., Parmentier, M-L, Bonneaud, N., Chan H.O.E, Maschat, F. (2008) Protective role of Engrailed in a *Drosophila* model of Huntington’s disease. *Human Molecular Genetics*, 17(22), 3601- 3616; doi:10.1093/hmg/ddn255.