

A peptide to treat Huntington's disease

Dr Florence Maschat is studying Huntington's disease, using both *Drosophila* and mouse models. Here, she discusses her discovery of a peptide that can protect against the damaging effects of the disease and her hopes for its clinical application



What are the main research objectives of your group?

For the past 10 years our group has been interested in understanding the role of the Engrailed homeoprotein in neurogenesis. Engrailed is involved in different neurodegenerative diseases, including Parkinson's. We found that Engrailed in *Drosophila* directly activates Huntingtin, whose human homologue is involved in Huntington's disease. This led to our study of this devastating disease.

Can you provide some background to Huntington's disease (HD)?

HD is a rare genetic neurodegenerative disease. Symptoms usually appear in patients when they reach their 40s. There are motor, cognitive and psychiatric symptoms, and sometimes

dementia. The emergence of symptoms is slow, but once they appear the disease progresses quickly. We know that HD results from the abnormal expansion of a repetitive glutamine (Q) domain, which is localised at the N-terminus of the Huntingtin protein. When the polyQ repeat contains more than 35 Q's, the protein forms aggregates. This aggregation is associated with neuron degeneration, particularly affecting the striatum and leading to a decrease in brain volume. There is a clear correlation between polyQ length and symptom onset.

What is the Huntingtin protein and what is its role in HD?

The exact roles of the Huntingtin protein (Htt) are unknown, but they seem to be manifold. We know it interacts with several others and plays important roles in vesicle trafficking along axons. In the context of disease, numerous factors (including transcription factors) become trapped in the aggregates, which has dramatic effects on cell survival.

It is important to study the normal functions of the protein to better understand what is happening in disease. We found that the *Drosophila* Huntingtin protein may share biological properties with its human homologue. Therefore, we can use the genetic tools offered by *Drosophila* to study the functions of the human Huntingtin.

Could you elucidate the shortcomings of existing HD treatments?

The major therapeutics currently in use are designed to improve the primary

symptomatology of the condition and are mainly psychiatric agents to control motor and cognitive symptoms. Several drugs for other neurodegenerative diseases, for example targeting the glutamatergic system (memantine and latrepirdine) or antioxidants (CO-Q10), are currently in clinical trials for HD. However, there is a lack of medication specifically targeting the cell toxicity induced in HD. Most of the research to find a cure focuses on reducing the expression of the mutated allele, or reducing the aggregation of the polyQ stretch which is present in other neurodegenerative diseases.

However, our project was based on the observation that the ratio between normal and mutated proteins is crucial in HD. Therefore, increasing normal Huntingtin may be beneficial for cells expressing polyQ-hHtt.

You were able to identify the protective 23aa peptide (P42). What does P42 do and what are the implications of this finding?

P42 is a part of the Huntingtin protein. Our work in *Drosophila* has shown that P42 is protective of different phenotypes induced by

A novel therapy for neurodegeneration

Research at the **Institut de Génomique Fonctionnelle** has led to the discovery of P42, a peptide with potential use in the treatment of Huntington's disease. With clinical trials in the pipeline, this peptide could provide a much needed boost to the current therapeutics landscape

HUNTINGTON'S DISEASE (HD) is an inherited neurodegenerative disease caused by a mutation in the Huntingtin gene. HD is one of nine polyglutamine (polyQ) diseases, so called because they are caused by the abnormal repetition of the CAG codon, which codes for the amino acid glutamine (Q). Dr Florence Maschat, leader of the 'From Huntingtin functions to potential treatments' project at the Institut de Génomique Fonctionnelle, Montpellier, France, recognises the shared features of these diseases: "The presence of abnormal expanded polyQ drives the formation of aggregates. As a result, neural cells suffer stress, leading to cellular dysfunction," she explains. HD is caused by expansion of the polyglutamine region in the N-terminus of the Huntingtin protein (polyQ-Htt). When the repeat exceeds 35 Q, an abnormal Htt protein forms toxic aggregates and drives neurodegeneration.

on the mechanisms of disease pathogenesis. Animal models have demonstrated that polyQ-induced neurodegenerative disease is likely to involve transcriptional dysregulation, protein misfolding and impaired intracellular transport. Lastly, the majority of research focuses on finding a treatment to alleviate HD's symptoms. Given the multiple pathological mechanisms of the disease, it is unlikely that the sole use of a drug that targets just one factor will be effective. Many drugs have proven ineffective in clinical trials and there is a need for a medication that specifically targets polyQ-Htt.

This work elucidates a new therapeutic strategy for HD, and couples a powerful peptide with an effective delivery method

The pathway of discovery that led to P42 began when Maschat identified the targets of the Engrailed homeoprotein in *Drosophila*, one of which is the *Drosophila* Huntingtin. Having tested the action of Engrailed in a model of HD, Maschat found that it was protective. "This was the starting point of our work in HD, since it suggested that the ratio between normal and mutant Huntingtin was important to the disease," she reveals. This important finding led the team to question which part of the wild-type Htt is important for its protective effect.

AN INTRINSIC PEPTIDE

Based on this observation, the team screened for peptides derived from Htt which could prevent polyQ-Htt aggregation in HeLa cells. Human Htt was divided into four peptides (P1 – P4) and each was tested for their protective effect. P4 was found to be the only peptide able to effectively block aggregation. A further division of P4 based on homologies between Human and *Drosophila* showed that only P42, a 23 aa peptide within P4, had retained the protective properties of the larger fragment.

P42 lies between proteolytic sites crucial to the pathogenesis of HD. The N-terminal fragments produced by proteolysis can

the mutant polyQ-Htt. In particular, we found the peptide is protective of eye degeneration induced in different models of HD. It suppresses eye degeneration in *Drosophila* expressing human exon1 with expanded polyQ, but has no effect in other polyQ-induced diseases. These genetic data indicate that if P42 acts directly on polyQ-Htt it may act through exon1, not the polyQ domain. Our preliminary data confirm the interaction of P42 with the first 17aa (N17 domain) of Huntington, a domain which is required for the first step of aggregation.

How do you plan to use P42 therapeutically?

It was very important to find non-invasive administration methods for P42 in mammals. A major limitation of peptides for therapeutic use, aside from the difficulties of administration, is their short half-life. We were very aware that we may need to perform repeated administrations of P42 because of this, which is why we tried to find strategies for oral administration. Our results in HeLa cells and *Drosophila* are preliminary to more extensive preclinical and clinical studies.

We have achieved the first step of these preclinical studies by confirming the protective properties of P42 in a mouse model of HD. They will also require P42 to be tested in another HD mouse model, or preferably in a non-rodent model, before beginning a clinical trial.

Symptoms usually develop between the ages of 30-50 and can vary between individuals, even within the same family, but progress in a formulaic manner. Early symptoms comprise minor changes to mood, but as the disease advances, difficulties with coordination develop, along with mental health difficulties and a decline in mental ability. Complications reduce life expectancy to around 20 years following the appearance of symptoms. There is no cure for HD; existing drugs can only relieve symptoms and the disease eventually necessitates full-time care.

Although HD is rare – five to 10 cases per 100,000 persons – its consequences are devastating and there is a clear need for more effective therapeutics. Although there have been a number of groundbreaking discoveries in HD in recent years, Maschat's project is set apart from other research in the field by the fact it is based on increasing the function of wild-type Huntingtin (Htt). Her work has led to the discovery of P42, a peptide that can protect against aggregation in HD.

PATHWAY TO DISCOVERY

The search for a cure focuses on three key areas, the first of which is the normal function of Htt. Htt is known to play diverse roles in axonal trafficking, cytoskeletal organisation and intracellular transport. However, the extent to which these functions are affected by HD is poorly understood. The second area of interest centres

INTELLIGENCE

A HUNTINGTIN PEPTIDE INHIBITS POLYQ-HUNTINGTIN ASSOCIATED DEFECTS

OBJECTIVES

To explore Huntington's disease: Neurophysiopathology studies will be pursued from *Drosophila* to mice. Identifying the normal functions of Huntingtin is an important challenge to better understand the disease. To this end, studies in *Drosophila* are particularly suitable. Better exploring the abilities of P42 or derivatives to fight against this disease is also one of the challenges.

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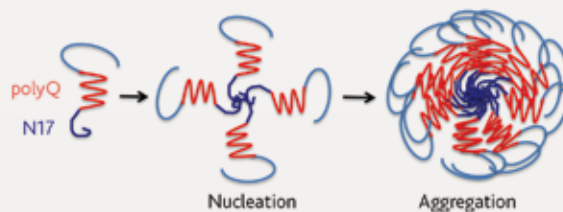
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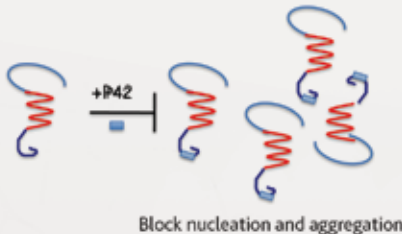
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DR FLORENCE MASCHAT has always worked on *Drosophila*. She obtained her PhD in Paris VII University working in Jean-Antoine Lepesant's laboratory and then started working on Engrailed homeoprotein during her postdoc in Thomas Kornberg's laboratory at University of California San Francisco (UCSF). Moving to Montpellier – the Institut de Génétique Humaine – she developed chromatin immunoprecipitation technique using UV crosslinking, which allowed to identify direct Engrailed target genes, among which is huntingtin. She therefore started to work on Huntington's disease, first using fly models. More recently, she moved to the Institut de Génétique Fonctionnelle, which is more dedicated to neurodevelopment and to the study of neurodegenerative diseases. In this new environment, she could also develop studies on mice.

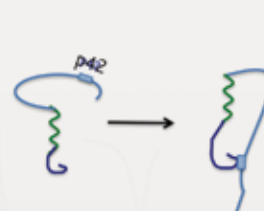


Model for the protective role of P42



Block nucleation and aggregation

Hypothesis for normal conditions



Interaction of P42 with N-terminal part of Htt

Model for P42 action in preventing the aggregation process.

aggregate with themselves, and other proteins, to form cytoplasmic and nuclear inclusions. These inclusions have a range of pathogenic consequences for cells, including transcriptional dysregulation, mitochondrial impairment and cell death. It is possible that P42 may slow Htt cleavage, thus delaying Htt proteolysis.

PROTECTIVE EFFECTS

Following the identification of P42, the researchers sought to ascertain its precise beneficial effects. They first eliminated the possibility that it affects polyQ-Htt expression. This meant P42 was either acting indirectly, through protein partners of polyQ-Htt, or by direct interaction with polyQ-Htt. The fact that P42 was not protective for other polyQ toxic proteins strongly suggested that P42 was not interacting with the polyQ region. Using co-immunoprecipitation in combination with bimolecular fluorescence complementation (BiFC) assays, the researchers were able to verify that P42 interacts with exon 1, in particular with an N-terminal region of 17 aa's (N17), of Htt.

The researchers used a *Drosophila* model of HD, and HeLa cells, to test the protective properties of the peptide on aggregation. P42 was able to prevent polyQ-Htt-induced aggregation in both. The aggregation process requires a nucleation step, during which the N17 domains adopt a conformation in which they are able to interact with themselves, accelerating aggregation. By targeting exon1, P42 may be able to impact nucleation, therefore preventing the formation of polyQ-Htt aggregates.

The precise role of aggregates in cell toxicity remains contentious, so the team also assessed the impact of P42 on cellular and functional phenotypes. These included eye degeneration, impairment of vesicular axonal trafficking and physiological behaviour in *Drosophila*. P42 was able to completely reverse some defects, while others were only partially rescued. We suspect that P42 might be able to block the evolution of the disease.

PROMISING TREATMENT

In order to use P42 therapeutically, it must be able to cross the blood-brain barrier. To this end, the team fused P42 to the protein transduction domain (PTD) of the TAT protein. This 11 aa domain enables P42 to enter cells by crossing the cell membrane. In the future, other PTDs such as Penetratin could be tested for increased activity and/or lower toxicity. To perform oral administration and keep the peptide intact until cellular entry, the researchers used a nanostructure-based drug delivery system called Aonys®, which contains P42-TAT in a water-in-oil microemulsion.

After testing the fusion proteins in *Drosophila*, the researchers tested mice models of HD, treated with P42-TAT or placebo (empty micelles). The effects of both pre- and post-symptomatic treatments were analysed on a range of defects. In the P42-TAT treated mice, there was recovery of weight and behavioural phenotypes. Brain sections showed that P42 effectively diffused to different parts of the brain and cellular compartments, as well as a recovery of brain volume and reduction in aggregates in the P42 treated animals.

These results demonstrate the highly protective properties of P42 on polyQ-Htt aggregation and the resultant cellular and behavioural dysfunctions. This work elucidates a new therapeutic strategy for HD, and couples a powerful peptide with an effective delivery method. Maschat is enthusiastic about the future of P42: "An important advantage of P42 is that it specifically targets the Htt protein, making it a potential tool to fight HD. An important issue for us now is to improve P42's efficiency," she forecasts. The next step is more extensive preclinical and clinical studies on P42. The team may also consider combinatorial therapies, using P42 along with other drugs interfering with other stages of the disease, to increase the efficiency of treatment. Although there remains work to be done, this exciting discovery should give hope to HD sufferers everywhere.

