genetic and epigenetic components that constitute the complex molecular network underlying stem cell biology.

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Huntingtin aggregates ask to be eaten

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A new study identifies a protective role for cellular aggregates in Huntington disease by showing that aggregates promote the clearance of mutant protein by activating autophagy through the inhibition of mTOR. This challenges the common view that they are possibly innocuous but probably harmful to the host cell.

A common thread connecting various neurodegenerative diseases is the accumulation of insoluble protein aggregates in and around neurons. In Huntington disease, these aggregates almost certainly result from the expansion of glutamine repeats in the huntingtin protein, perhaps altering its structure and enabling it to form cellular aggregates. Although there is considerable disagreement as to whether the aggregates are harmful to cells, mounting evidence suggests that clearing them through the autophagy pathway can reduce cell death[1]. Autophagy is a conserved process in plant, fungal and animal cells and is generally thought to recycle cytoplasmic components when cells are starved for nutrients and, under harsher conditions, act as an alternate mechanism for programmed cell death[2]. Research has traditionally focused on its destructive role, but there is an increasing awareness that it has a protective function in human diseases such as cancer[3]. In this issue, Brinda Ravikumar and colleagues (page 585) report that cells containing huntingtin aggregates protect themselves by clearing mutant protein through the autophagic pathway[4]. The aggregates themselves seem to have a key role in inducing autophagy by sequestering and suppressing mTOR, a negative regulator of the autophagic pathway.

When mutant huntingtin is expressed, the first 100–150 residues of the protein, including the polyglutamine repeats, are cleaved off and act as the toxic entity[5]. It is unclear how the fragments cause disease, but there is a well-established correlation between the length of the polyglutamine repeats and disease progression, including the formation of insoluble aggregates. The debate over whether the aggregates themselves are toxic is based largely on the observation that cell death in the brain does not always correlate with the presence of aggregates[6]. There is a growing agreement, however, that aggregates are present but are too small to be detected by commonly used microscopy or filtration techniques[6]. Recent *in vitro* experiments also support the idea that they are toxic, as injecting aggregates directly into cells recapitulates the effects of expressing mutant huntingtin[7].

Consuming aggregates

There is suggestive evidence that autophagy acts as a protective mechanism by degrading mutant huntingtin. Although huntingtin aggregates are resistant to cytosolic proteases, they do not seem to be permanent, as their presence depends on the continued expression of the mutated gene[6,8]. Mutant huntingtin can be taken up and degraded by autophagic vacuoles[6,9]. Additionally, treating cells with rapamycin, an FDA-approved immunosuppressant that also induces autophagy by inhibiting mTOR, substantially enhanced the clearance of aggregates and

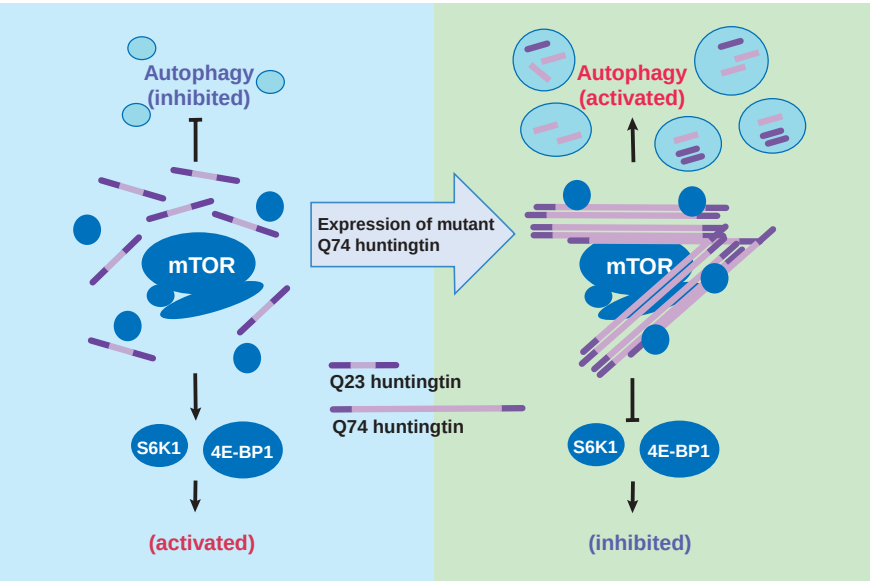

Figure 1 The mTOR pathway stimulates cellular growth in reponse to nutrients and growth factors by enhancing protein synthesis and inhibiting autophagy. The pathway functions normally in cells expressing the Gln_{23} huntingtin fragment (Q23) but is inhibited by the aggregates that form in cells expressing the Gln_{74} fragment (Q74).

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reduced cell death in a heterologous system[1]. Together, these observations suggest that the cell's inability to efficiently degrade the mutant protein compounds its harmful effects over time. Indeed, neuronal cells are probably more sensitive to any harmful effects because they accumulate mutant protein more quickly than cells that dilute it by proliferating.

A paradox complicated understanding the molecular details of how rapamycin-induced autophagy might prevent cells from dying. When the authors transfected cells with mutant huntingtin and treated them with rapamycin 9 h later, they found the expected reduction in aggregate formation and cell death. If they waited until 33 h after transfection, however, treatment with rapamycin could no longer suppress either aggregate formation or death. Rapamycin acts by inhibiting the mTOR kinase, which forms the core of a nutrient- and growth factor–sensitive complex that controls protein synthesis and inhibits autophagy[10]. The authors surmise that rapamycin cannot protect the cells that express huntingtin at the later time points because, after prolonged huntingtin expression, the mTOR pathway is already fully inhibited.

Aggregates inactivate mTOR

The authors next examined whether the aggregates might sequester and inhibit mTOR. There is a precedent for this idea in the repeated observation that aggregates containing huntingtin sequester and inactivate various essential proteins, including proteasome components, heat-shock proteins and transcriptional regulators[6]. Inhibition of the mTOR pathway is consistent with the reduction in size of neurons containing aggregates. In a heterologous system, the expression of mutant huntingtin containing a repeat of 74 glutamines (Gln_{74}) tagged with green fluorescent protein led to the formation of large, easily visualized aggregates, whereas expression of huntingtin with only 23 glutamines (Gln_{23}) was diffusely distributed in the cells. Endogenous mTOR colocalized with the aggregates and coimmunoprecipitated with Gln_{74} huntingtin but not with Gln_{23} huntingtin (**Fig. 1**).

To test whether Gln_{74} aggregates inhibit the mTOR pathway, the authors assessed the phosphorylation states of S6K1 and 4E-BP1, sensitive indicators of mTOR activity. The pathway was only mildly suppressed in cells containing aggregates relative to the strong inhibition caused by rapamycin or an RNAi-mediated knockdown of mTOR[11]. This is somewhat puzzling as it suggests that even in cells with Gln_{74} aggregates, rapamycin further inactivates the pathway and induces autophagy. mTOR might regulate the two markers and the autophagic pathway through separate mechanisms, and therefore, their phosphorylation states might not accurately reflect the level of autophagy. The authors also show that an overactivated mTOR pathway markedly increases aggregate formation and cell death. This observation provides evidence that the mTOR pathway has a role in the cellular pathogenesis of Huntington disease.

That aggregates can have a protective role in Huntington disease is notable and contrasts with the more common belief that they are toxic. The authors have shown that a deregulated mTOR pathway can affect disease. This pathway controls the fundamental process of cell growth and is involved in a diverse set of pathological conditions that includes several types of cancer, vessel restenosis and host rejection of organ transplants. Should further studies clarify its role in Huntington disease, these findings would reinforce the idea that the deregulation of a basic cellular process is a common occurrence in many human diseases.

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