How does STING-mediated inflammation protect against neurodegeneration.

In which low levels of inflammation might inflammatory drugs have a lower than average benefit?

The parkin gene, suggests that these particular cytokines were also elevated in disease-free relatives of people who have Parkinson's disease. Sliter and colleagues' study of these relatives, who have Parkinson's disease, found that the bloodstream level of inflammation-driving molecules called cytokines was much higher in disease-free relatives of people who have Parkinson's disease, who lack STING, as well as PINK1 or parkin, the expression of inflammatory cytokines did not increase as a result of mitochondrial stress. This indicated that STING is required to drive the inflammation mediated by this type of stress (Fig. 1). Moreover, an absence of STING prevented the movement defects and neuronal losses that usually occur in old mutator mice that lack STING, as well as PINK1 or parkin, which are elevated above normal in old mutator mice that lack parkin. The authors found that the bloodstream levels of the inflammatory cytokines IL-6, IL-1β and CCL2, which are elevated above normal in old mutator mice that lack parkin, are also higher than normal in people with Parkinson's disease who have mutations in both copies of the parkin gene. However, the authors observed that these cytokines were also elevated in disease-free relatives of people who have Parkinson's disease. Sliter and colleagues' study of these relatives, who have a mutation in only one of their two copies of the parkin gene, suggests that these particular cytokine alterations are not sufficient to cause the disease. Interestingly, people who receive long-term treatment with non-steroidal anti-inflammatory drugs have a lower than average risk of developing Parkinson's disease.

This observation is consistent with a model in which low levels of inflammation might protect against neurodegeneration.

The exciting results reported by Sliter and colleagues raise many important questions. How does STING-mediated inflammation cause neuronal death? Why are dopamine-secreting neuronal cells specifically affected? Is STING-dependent inflammation linked to other abnormalities associated with neurodegeneration, such as the formation of protein aggregates?

However, before these questions can be answered in the context of human disease, a crucial consideration is how well these mice provide a model of human Parkinson's disease. Further insights might come from using other systems, such as rats or fruit flies (Drosophila), which better mimic the types of change that occur in human Parkinson's disease. Finally, given that impaired mitochondrial function and inflammation are common features of several neurodegenerative disorders, it is tempting to speculate that STING-dependent inflammation might contribute in a similar way to other neurodegenerative conditions, such as Alzheimer's disease. It would be interesting to test this idea in experiments.

Sliter and colleagues' work might lead to studies in human cells that provide fresh insights into treating Parkinson's disease. Perhaps drugs that selectively inhibit STING-dependent inflammation will one day be used to treat or prevent disease — if it's possible to control any detrimental side effects on the immune system that might arise from targeting inflammation in this way. Will techniques such as monitoring the level of mitochondrial DNA in the bloodstream to detect abnormal mitophagy, or tracking the expression of STING-dependent cytokines, enable early diagnosis or make it possible to assess a person's risk of developing Parkinson's disease before symptoms appear? Sliter and colleagues' work points to new avenues of investigation in the efforts to improve the treatment options for Parkinson's disease.

**Proteins assemble as they are being made**

An investigation finds that most protein complexes in yeast cells assemble before the subunits have fully formed. This mechanism might prevent the formation of toxic protein aggregates. **See Letter p.268**

**Molecular Biology**

Most cellular processes are carried out by proteins, which generally assemble into heteromeric complexes — those composed of two or more distinct subunits. Although it was thought for many years that protein subunits diffuse freely in the cell and form complexes through random collisions, this seems unlikely, given that the cellular environment is extremely crowded. On page 268, Shiber et al.1 provide *in vivo* evidence that, in eukaryotic organisms (which include animals, plants and fungi), most protein complexes in the cytoplasm are assembled co-translationally — that is, assembly occurs while at least one of the subunits is still being synthesized by the cell's ribosome machinery.

The study of co-translational protein-complex formation *in vivo* was challenging until a technique known as ribosome profiling was developed2 in 2009. This technique allows the positions of ribosomes on messenger RNAs to be determined by sequencing RNA fragments, and is usually used to monitor translation — the process in which the ribosome decodes mRNA and uses it as a template for protein synthesis. Shiber et al. used a modified protocol called selective ribosome profiling3, which isolates ribosomes that are synthesizing nascent protein chains already interacting with another protein. Subsequent sequencing of the corresponding RNA fragments reveals the mRNAs that encode the interacting nascent chains. The sequencing also identifies the protein domains involved in the interaction, because only ribosomes bound
to nascent chains that contain fully exposed interaction domains will be isolated by selective ribosome profiling.

The authors investigated the prevalence of co-translational protein–complex assembly for 12 stable, well-characterized heteromeric complexes in the yeast *Saccharomyces cerevisiae*. They found that 9 of the 12 complexes assembled in a co-translational manner. Although it had been suspected that some protein complexes are assembled co-translationally, the finding that this process is widespread is surprising. Notably, the three protein complexes that did not seem to do this use dedicated chaperone proteins to assist in assembly. Given that the major function of chaperones is to prevent misfolding and random aggregation of proteins during protein folding, the researchers hypothesized that co-translational protein–complex assembly might serve a similar purpose.

Shiber et al. went on to find that six of the nine complexes that assemble co-translationally have a directional assembly mode: one of the subunits must be fully folded before it engages the nascent chain of a second subunit, but the fully folded second subunit cannot engage the nascent chain of the first subunit. This means that the second subunit must always participate in co-translational assembly as a nascent chain. Intriguingly, when the authors studied yeast strains that had been engineered not to produce the fully folded subunit, they observed that the nascent chain of the second subunit forms aggregates. This indicates that co-translational assembly does indeed prevent the formation of potentially toxic protein aggregates.

Although the authors convincingly show that co-translational protein–complex assembly is widespread, it is unclear how the subunits are brought into proximity to enable complex formation. There are two plausible, broad models, which are not mutually exclusive.

In the first model, the two mRNAs that encode the subunits are translated near to each other. This proximity could be achieved by an RNA-binding protein that bridges the two mRNAs (Fig. 1a). Such a scenario has been suggested for the assembly of heteromeric ion channels. However, a genome-wide analysis of human RNA–protein interactions found that it is rare for two different mRNAs to be bridged by an RNA-binding protein. But a physical linkage is not necessarily needed to bring two mRNAs close together: mRNAs that share certain sequence motifs could co-localize in RNA bodies known as granules (Fig. 1b), thereby allowing them to be translated at defined subcellular locations.

In the second model, the two mRNAs are not close to each other. Instead, an RNA-binding protein recruits a fully folded protein subunit to an untranslated region (the 3’ UTR) of an mRNA that encodes the second subunit, thus allowing the folded subunit to interact with the second subunit as the latter is translated. In mammalian cells, 3’ UTRs of mRNAs have been shown to recruit proteins that then interact with the mRNAs’ nascent or newly made proteins (Fig. 1c). And in budding yeast, proteins recruited to mRNAs undergoing translation have been observed to be temporarily deposited on the ribosome before being transferred to the nascent protein chain (Fig. 1d).

Recruitment of interacting proteins by 3’ UTRs is reminiscent of co-translational protein–complex formation in bacteria, in which protein subunits are often encoded by a cluster of neighboring genes (an operon) that are expressed as a group. However, in bacteria, the only fully folded protein that can interact with nascent proteins is one of the encoded subunits, whereas the use of the 3’ UTR in eukaryotes might allow several different interactors to be recruited to nascent proteins—thus enabling a variety of protein complexes to be assembled co-translationally.

More experiments are needed to work out how interacting protein subunits are brought together and how mis-assembly of complexes is prevented. Nevertheless, Shiber et al. have demonstrated that protein–complex formation often relies on recruitment mechanisms, rather than diffusion, to achieve specific protein interactions. Their findings add to an increasing number of in vivo observations suggesting that most cellular processes are interconnected: mRNAs not only encode proteins, but also increase the specificity of protein–complex formation by assisting the compartmentalization of proteins in the cytoplasm, and by regulating localized translation. Finally, it remains to be seen whether the majority of stable protein complexes in mammalian cells are also assembled co-translationally—but it seems likely that they are.

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