SYNAPTIC PHYSIOLOGY

A rapid response to RA

Retinoic acid (RA) is known to have many roles in neural development, but its effects at the developing synapse are relatively unexplored.

In a study reported in the *Journal of Cell Science*, Liao and colleagues investigated how RA influences neurotransmitter release at embryonic neuromuscular synapses, and they found that it enhances acetylcholine (ACh) release by means of a novel mechanism that does not require protein synthesis or transcriptional changes.

The authors applied RA to nerve-muscle cultures from *Xenopus laevis* embryos, and measured spontaneous synaptic currents (SSCs) by taking whole-cell recordings from the postsynaptic muscle cells. They showed that RA increased the frequency of these currents, indicating an increase in either neurotransmitter release from the presynaptic terminal, or the sensitivity of the postsynaptic cell to ACh. They ruled out the possibility of an increase in postsynaptic sensitivity by showing that there was no change in the SSC amplitude distribution, and they concluded that RA was acting primarily on the presynaptic cell.

So, how does RA enhance ACh release at the neuromuscular synapse?

As RA is lipophilic, it might simply disrupt the presynaptic membrane. However, Liao *et al.* showed that all-*trans* retinol, which is more disruptive to the membrane than RA, had no effect on SSC frequency. The rapid onset and decay of the RA-induced enhancement was incompatible with an event that required protein synthesis or transcriptional changes, and it was unaffected by inhibitors of protein synthesis, such as anisomycin or cycloheximide. The authors speculate that RA modulates the activity of components of the secretory machinery through a more direct mechanism, possibly involving post-translational modification.

Interestingly, an agonist of the RA receptor RAR β — one of the nuclear receptors that usually mediates RA's effects on transcription — also enhanced ACh release at the neuromuscular synapse. Therefore,

HUMAN GENETICS

Drawn into bad company

Polyglutamine (polyQ) repeat expansions are involved in several human disorders, perhaps the most famous among them being Huntington's disease (HD). The HD gene was cloned in 1993, and subsequently, much effort has gone into the molecular characterization of the disease. Two significant new strides in this direction have just been made — Schaffar *et al.* show that transcription factors drawn into the bad company of mutant huntingtin (HTT) cannot bind their DNA targets, whereas Choo *et al.* find that HTT associates with the mitochondrial outer membrane and that mutant HTT alters mitochondrial permeability.

Expanded tracts of polyQ might cause disease through negative interactions with the ubiquitin-proteasome system or with important proteins, such as TATA-box-binding proteins (TBP) and transcriptional co-activator CREB-binding proteins (CBP), which also contain polyQ repeats. Schaffar *et al.* used an *in vitro* system, mouse neuroblastoma cells and a yeast model to focus on the second hypothesis.

By using a combination of mutant and wild-type HTT and TBP with or without polyQ tracts, they showed that TBP binds to

HTT only when the polyQ tracts are present. Not surprisingly, aggregated TBPs cannot bind their targets. Mutant HTT is able to exert this toxic effect on transcription factors even before the formation of the multiprotein aggregates, which are also known as inclusion bodies and which are a hallmark of the disease at the cellular level. The authors also show *in vitro* and in mouse neuroblastoma cells that insoluble pre-existing aggregates of HTT cannot interact with TBP; only the newly synthesized, soluble HTT can.

Toxic forms of polyQ only form after a conformational change in mutant HTT — Schaffar *et al.* show that the non-toxic glutathione S-transferase (GST)-tagged HTT becomes toxic when the GST is cleaved. Drawing a parallel with the *in vivo* situation, they suggest that similar to the GST tag in the experimental setting, the rest of the HTT protein shields the polyQ repeats from adopting the toxic conformation. They propose that this shielding process might account for the late onset of the disease.

The late onset of the disease might also involve Hsp70/Hsp40 chaperones, which also

prevent HTT polyQ tracts from adopting the toxic conformation. These chaperones might also protect polyQ tracts on other proteins, such as TBPs, and so prevent the formation of complexes between these proteins and HTT. The authors suggest that as age takes its toll on the efficiency of the chaperone system, the symptoms of disease begin to appear.

HD has also been associated with mitochondrial dysfunction and this aspect of the disease was the focus of the study by Choo *et al.* Not only is the wild-type and mutant HTT associated with the outer mitochondrial membrane, but, as the authors show in a mouse knock-in HD model, the expanded polyQ tract causes mitochondrial membranes to be leaky, leading to what is known as mitochondrial permeability transition — an important contributor to apoptosis and necrosis.

The gene that is responsible for HD was cloned more than 10 years ago and although we still do not know what HTT, we are coming ever closer to understanding how expanded polyQ repeats cause this and other polyQ-associated diseases.

Magdalena Skipper, Editor,
Nature Reviews Genetics

References and links

ORIGINAL RESEARCH PAPERS Schaffar, G. *et al.* Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol. Cell* **15**, 95–105 (2004) | Choo, Y. S. *et al.* Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. *Hum. Mol. Genet.* **13**, 1407–1420 (2004)