Dopamine helps worms deal with stress

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To maintain protein homeostasis, animals have developed stress response pathways such as the ubiquitin proteasome system (UPS). Joshi and colleagues have demonstrated that in *Caenorhabditis elegans*, dopamine release from neurons acts on receptors in the epithelia to modulate protein turnover, by controlling the expression of regulators of the xenobiotic stress response. Dopamine receptor mutants challenged with pathogenic bacteria were defective in protein turnover and were also more sensitive to infection thus highlighting a role for monoamine signalling in innate immunity and stress responses.

See also: **KK Joshi *et al*** (September 2016)

Protein homeostasis, or proteostasis, is the process by which an organism regulates its cellular proteome. The proteome is in a constant state of flux due to perturbations from the environment, such as extremes of temperature, toxins and pathogen exposure. To maintain proteostasis when confronted with these environmental challenges, animals have evolved cellular stress response pathways to either guide proper folding, or to remove damaged or incorrectly folded proteins (Vilchez *et al*, 2014). The ubiquitin proteasome system (UPS), by which proteins are flagged for removal by the covalent addition of chains of ubiquitin (Ub) and degraded via the 26S proteasome, is critical in mediating these processes. This cellular stress response pathway is tightly regulated within individual cells (Labbadia & Morimoto, 2015), but it is unclear how multicellular organisms are able to coordinate such pathways over multiple tissues.

The recent work of Joshi *et al* (2016) reveals that in *Caenorhabditis elegans*, a neuronal signal modulates proteostasis in

tissues such as the intestine and hypodermis, which act as the first line of defence against xenobiotic stressors. The authors used transgenic lines expressing an unstable protein Ub^{G76V}-GFP in epithelial tissues to screen for neuronal proteins (Sieburth *et al*, 2005) that influence Ub^{G76V}-GFP stability. Ub^{G76V}-GFP mimics a mono-ubiquitinated protein upon which additional Ubs are later added to flag for proteolysis by the 26S proteasome. A defect in protein turnover machinery would be reflected by increased levels of Ub^{G76V}-GFP. Several genes involved in dopamine signalling were identified in this screen, including the D1-like dopamine (DA) receptors DOP-1 and DOP-4 and the tyrosine hydroxylase CAT-2, which is required for DA synthesis. Mutations in these genes resulted in elevated Ub^{G76V}-GFP, indicating that DA signalling acts via D1-like DA receptors (DARs) to promote degradation of unstable proteins in the epithelia.

DOP-1 is expressed in several neurons, but is also found in the intestine and hypodermis (Tsalik *et al*, 2003). Therefore, DA could influence proteostasis either directly in epithelial tissues or indirectly through neurons that subsequently signal to the epithelia. To test this, the authors generated transgenic lines in which DOP-1 expression was restricted to the intestine. Intestine-specific DOP-1 rescued Ub^{G76V}-GFP to wild-type levels, showing that DA directly acts onto epithelial DARs to modulate protein turnover.

How does DA signalling affect the UPS? Joshi *et al* (2016) demonstrated by Western blotting that mutants of DA signalling showed a decrease in non-, mono- and di-ubiquitinated species of Ub^{G76V}-GFP. DA signalling mutants could, hence, be defective either in clearing these substrates or in extension of a poly-Ub chain. To investigate whether proteasome function was impaired

by defective DA signalling, the authors checked by fluorescence spectroscopy for changes in the levels of a fluorescent chymotryptic substrate Suc-Leu-Leu-Val-Tyr-AMC, when proteasomal subunits were knocked down by RNAi. Interestingly, there was no substantial difference in proteasome activity in DA signalling mutants compared to wild type, suggesting that protein turnover defects in these mutants are due to impaired poly-ubiquitination. Consistent with this, knockdown of proteasome subunit *pbs-5* further enhanced the stability of Ub^{G76V}-GFP in DA signalling mutants.

Dopamine modulates cellular physiology via transcriptional and post-transcriptional means (Cadet *et al*, 2010). To investigate how DA signalling affects proteostasis, Joshi *et al* (2016) performed RNA-seq to compare the mRNA profile of *dop-1* mutants versus wild type. Strikingly, the set of genes down-regulated in *dop-1* was strongly enriched for detoxification genes such as CYP (cytochrome P) genes and UGTs (glycosyl transferase), showing that DOP-1 signalling promotes the response to xenobiotic stressors. Heat-shock chaperone proteins were also upregulated in *dop-1* mutants, even at lower temperatures, suggesting that DA signalling prevents a chaperone response in normal conditions and is consistent with impaired proteostasis in these mutants.

But does an impaired xenobiotic response cause defective protein turnover? Joshi and colleagues tested this by RNAi-mediated knockdown of CYPs and UGTs and subsequently assayed for turnover of Ub^{G76V}-GFP. Animals with reduced levels of these enzymes showed elevated Ub^{G76V}-GFP levels compared with controls, showing that turnover of this protein can be affected by the xenobiotic response. Further, the authors tested Ub^{G76V}-GFP stability in mutants of the master regulators of the stress response,

Figure 1. A model for DA signalling and modulation of proteostasis.

DA neurons sense when animals crawl into the bacterial lawn and release DA that can bind to DOP-1 on epithelial cells. This activates the expression of xenobiotic detoxification genes in the epithelia, which promotes the removal of ROS and toxins that can otherwise damage proteins and alter proteostasis.

such as SKN-1/Nrf2 (An & Blackwell, 2003), DAF-16/FOXO (Murphy *et al*, 2003), CRH-1/CREB, the GATA transcription factor ELT-3, NHR-28 (Nuclear Hormone Receptor) and PQM-1 (ZnF/leucine-zipper factor). Mutants of these master regulators all showed increased stability of Ub^{G76V}-GFP, demonstrating that the xenobiotic response is required for proper proteostasis.

Dopamine is released from a small number of mechanosensory neurons—the ADEs, CEPs and PDEs—for example in response to shear stress on the body wall of animals entering food (bacterial) lawns (Sawin *et al*, 2000). Given that xenobiotic detoxification genes are upregulated by DA signalling, the authors postulated that DA-mediated responses via the mechanosensory detection of food sources are required for innate immunity against bacterial infection. Interestingly, mutants defective in mechanosensation, including alleles of *trp-4*, the mechanosensory channel in the dopamine-releasing neurons, also display reduced Ub^{G76V}-GFP turnover in the epithelia. Similarly, genetic ablation of the dopamine neurons using a *trp-4(d)* transgene led to increased epithelial Ub^{G76V}-GFP stability. Therefore, DA release from mechanosensory neurons appears to be responsible for regulating protein turnover in the epithelia.

Does exposure to pathogenic bacteria result in altered proteostasis in a DA-dependent manner? Mutations in xenobiotic master regulators impair innate immunity against pathogenic bacteria such as *Pseudomonas aeruginosa* strain PA14. Joshi

et al (2016) showed that animals grown on PA14 showed higher turnover of Ub^{G76V}-GFP compared with animals grown on the standard OP50 feeding strain, and this effect was blocked by mutations in *dop-1*. Indeed, like mutants of xenobiotic stress response regulators, *dop-1* mutants were more sensitive to PA14 exposure.

The work of Joshi and colleagues reveals a novel neuromodulator-based mechanism by which animals act to protect proteostasis in response to infection and other xenobiotic stressors. Further questions can now be pursued: Does infection cause defective proteostasis, and is this what makes animals succumb to infection? Also, are there implications for human disease? Parkinson's disease (PD) is a neurodegenerative condition in which DA neurons are particularly susceptible, and is characterised by aggregated proteins and defective proteostasis. Loss of DA signalling in PD has both neuronal and non-neuronal effects (Stayte & Vissel, 2014). Closer investigation of DA-mediated proteostasis regulation could therefore have implications for our understanding of innate immunity and tissue non-autonomous DA-dependent pathology.

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