AMPK-mediated control of P-bodies as a novel mechanism of gene expression control in peripheral sensory neurons

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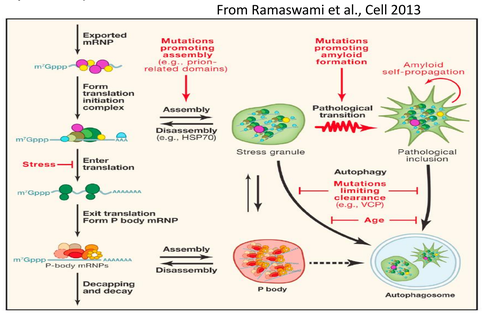
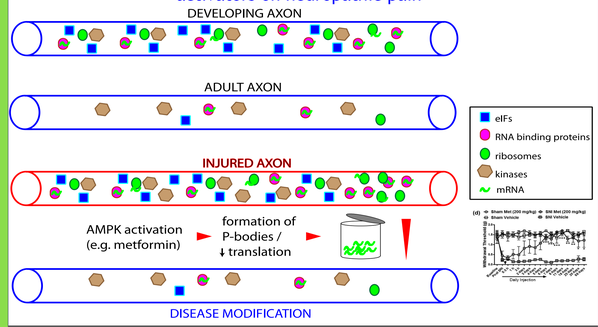
ABSTRACT

Changes in gene expression have long been recognized as a central mechanism for altered sensitivity and excitability of nociceptors. We, and others, have focused on translation control, in particular local, activity-dependent translation control as a novel means to modulate gene expression in response to injury. In this context, an increase in local translation, downstream of extracellular signal regulated kinase (ERK) and/or mechanistic target of rapamycin complex 1 (mTORC1) activation leads to an enhancement of pain sensitivity and an increase in measures of excitability. A possible mechanism to mitigate these effects is activation of adenosine monophosphate activated protein kinase (AMPK) because signaling via this kinase leads to inhibition of ERK and mTORC1 signaling to translation machinery. In addition to these effects, inhibition of translation via AMPK may also lead to changes in mRNA turnover. We have tested that hypothesis here examining major sites of mRNA repression and decay in cells, called P bodies, upon AMPK activation in trigeminal (TG) and dorsal root ganglion (DRG) neurons. We find that translation (using the sunset technique) and P body formation are reciprocally regulated upon pharmacological activation of AMPK in TG and DRG neurons. While AMPK activation leads to a decrease in puromycin incorporation into nascently synthesized peptides, it also causes a robust increase in P bodies (as revealed by rck/p54-positive puncta) suggesting mRNA sequestration from translation machinery and potentially mRNA degradation because P bodies are major sites for mRNA decapping in cells. We are currently exploring whether AMPK activation in vivo leads to enhanced P body formation in DRG neurons and whether injury alters P body dynamics. Our findings enhance our understanding of gene expression regulation in the peripheral nervous system and suggest a potential role for P bodies in pain plasticity.

From Ramaswami et al., Cell 2013



General scheme describing how AMPK-mediated modulation of P bodies may be involved in the disease modifying effects of AMPK activators on neuropathic pain

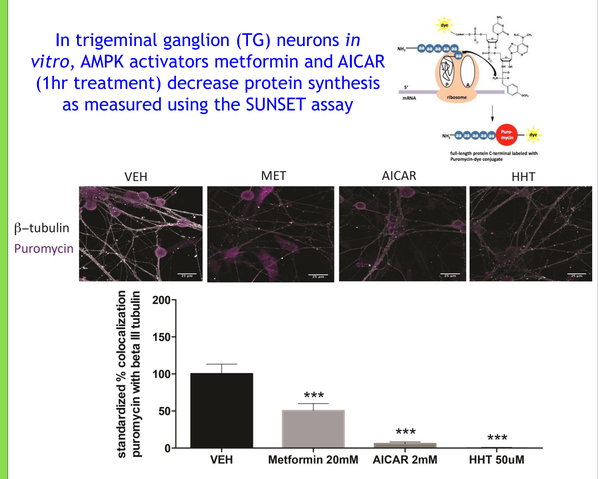


HYPOTHESIS

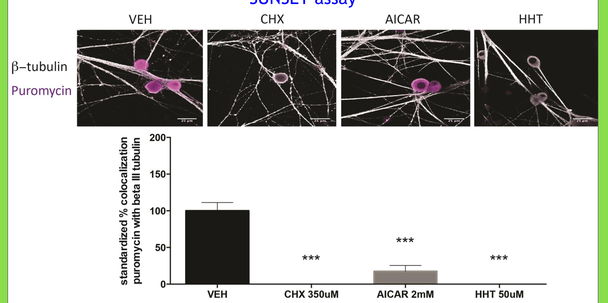
AMPK activation stimulates P body formation in TG and DRG neurons and reciprocally decreases protein synthesis

RESULTS

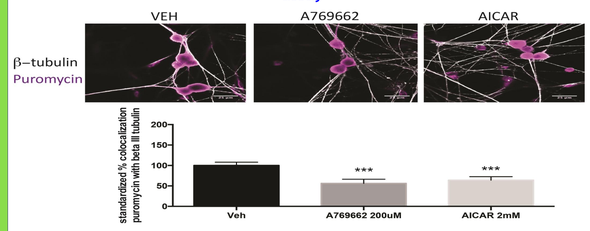
In trigeminal ganglion (TG) neurons *in vitro*, AMPK activators metformin and AICAR (1hr treatment) decrease protein synthesis as measured using the SUNSET assay



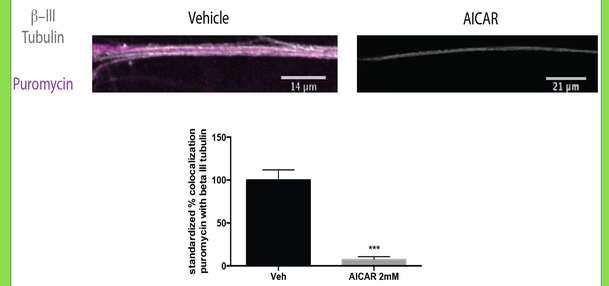
In dorsal root ganglion (DRG) neurons *in vitro*, the AMPK activator AICAR and ribosome inhibitors cyclohexamide and homoharringtonine (1hr treatment) decrease protein synthesis as measured using the SUNSET assay



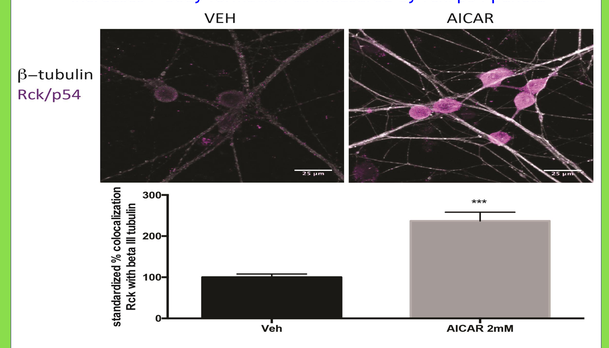
In DRG neurons *in vitro*, the AMPK activators AICAR and A769662 (1hr treatment) decrease protein synthesis as measured using the SUNSET assay



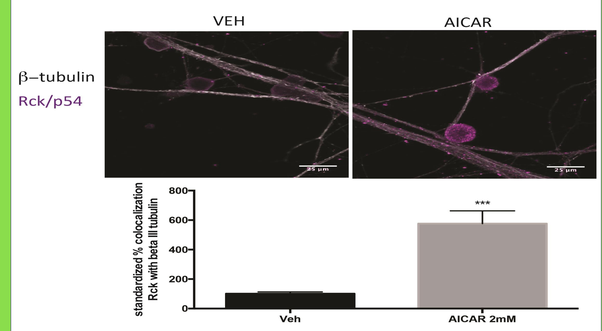
In DRG axons in vitro, the AMPK activator AICAR (1hr treatment) decreases protein synthesis as measured using the SUNSET assay



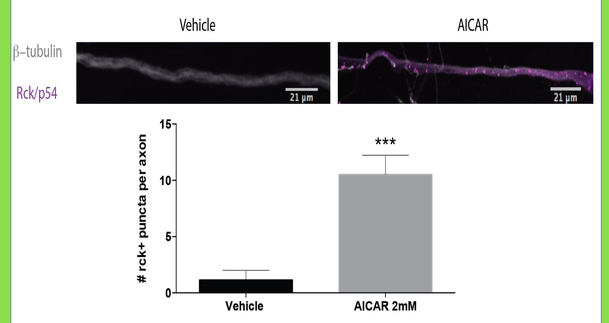
In TG neurons *in vitro*, the AMPK activator AICAR (1hr treatment) increases P body formation as measured by rck/p54 puncta



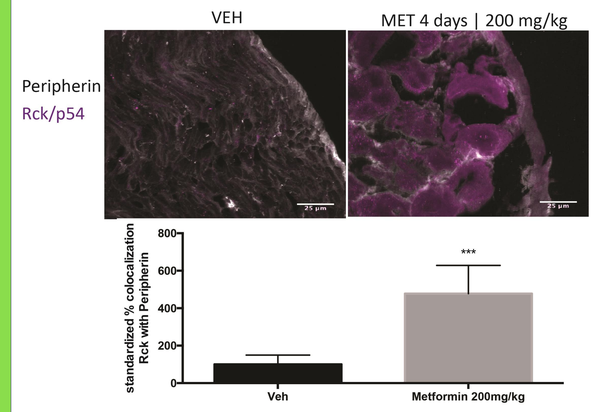
In DRG neurons *in vitro*, the AMPK activator AICAR (1hr treatment) increases P body formation as measured by rck/p54 puncta



In DRG axons in vitro, AMPK activator AICAR (1hr treatment) increases P body formation as measured by rck/p54 puncta



Daily treatment with metformin (200mg/kg, IP) induces P body formation in DRG neurons *in vivo*



FUTURE DIRECTIONS

1. Assess whether *in vivo* treatment with metformin or other AMPK activators modulates P body formation in DRG axons
2. Investigate changes in P body dynamics in DRG neurons following inflammation or nerve injury
3. Assess whether AMPK activators change P body dynamics following nerve injury
4. Investigate possible links between AMPK regulation of P bodies and AMPK disease modifying effects on neuropathic pain (see scheme)

CONCLUSIONS

- AMPK activators decrease nascent protein synthesis in DRG and TG neurons
- AMPK activators robustly stimulate P body formation in DRG and TG neurons including in their axons
- *In vivo* treatment with metformin induces P body formation in DRG neurons
- AMPK regulation of P body formation and protein synthesis appear to be reciprocally linked

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ACKNOWLEDGEMENTS

This work was supported by grants from NIH (TJP) and from American Pain Society (OKM)