

Previews

Chilling with cephalopods: Temperature-responsive RNA editing in octopus and squid

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The molecular mechanisms that generate the developmental and physiological complexity found within cephalopods are not well understood. In this issue of *Cell*, Birk et al. and Rangan and Reck-Peterson show that cephalopods differentially edit their RNA in response to temperature changes and that this editing has consequences on protein function.

Coleoid cephalopods, which include squid, cuttlefish, and octopus, are unusual relative to their invertebrate counterparts. They have an exceptionally large nervous system, remarkable camouflaging ability, and sensory specializations, as well as extensive phenotypic plasticity in response to their environment. Little is known about the molecular underpinnings of these lineage-specific novelties and the physiological flexibility found in these animals. In this issue of *Cell*, manuscripts by Birk et al. and Rangan and Reck-Peterson explore ADAR-mediated adenosine to inosine (A-to-I) RNA editing in squid and octopus species to assess its role in temperature-related physiological plasticity.^{1,2} Using transcriptomic sequencing, extensive sequence analysis, single-molecule motility assays, and X-ray crystallography, these papers show statistically significant changes in RNA editing in response to temperature in cephalopods. They also evaluate the potential functional consequences of these protein sequence changes *in vitro*.

RNA editing is a post-transcriptional modification that alters the mRNA transcript sequence, which can result in a change in protein sequence (recoding) without altering the genome. One well-studied RNA editing mechanism within Metazoa is A-to-I editing. This is carried out by the ADAR family of adenosine deaminases on double-stranded RNA, leading the translation machinery to interpret inosine as guanosine with the potential to alter amino acid sequence. However, it is generally uncommon to find abundant

ADAR-mediated recoding of proteins in animals, with only 1,000 known recoding sites in human and *Drosophila* proteins.^{3,4} An exception to this is found within coleoid cephalopods where >50,000 proteins are recoded.⁵ Since this discovery, it has been an ongoing debate as to whether this increased incidence of RNA recoding in cephalopods is adaptive. Many hypothesized these edits are positively selected and that RNA editing may diversify the transcriptome, leading to phenotypic plasticity and the generation of complexity found within the lineage.⁵⁻⁷ However, others suggested that these changes are effectively neutral or non-adaptive.⁸

To further explore this debate, Birk et al. and Rangan and Reck-Peterson directly addressed whether RNA editing is a mechanism to achieve adaptive plasticity in response to environmental change. Marine species, including many cephalopods, need to withstand large temperature ranges as a result of seasons, oceanic thermoclines, and geographic distribution. Birk et al. exposed octopus to variable temperatures in the lab as well as caught animals found at different temperatures in the wild and assessed differences in RNA editing.¹ They show that in response to the cold, these animals more commonly edit their RNA and at a greater magnitude. These changes begin within hours of temperature change and reach a steady state within several days (Figure 1).

To assess whether this editing may be adaptive, the authors looked at two specific proteins that are recoded, Kinesin-1

and Synaptotagmin-1. These two proteins are interesting choices as they are central to paradigm-shifting discoveries made in cephalopods, including the discovery of action potentials in the giant axon of squid and the discovery of multiple universal molecular motors in cells.^{9,10} Synaptotagmin-1 is a synaptic vesicle protein that is activated by binding Ca²⁺ at the presynaptic terminal during action potential firing, leading to neurotransmitter release. Birk et al. show that temperature-dependent edits in Synaptotagmin-1 lead to conformational changes that decrease affinity to Ca²⁺, potentially accommodating longer depolarization events that are observed at lower temperatures.

Both groups assessed temperature impacts on Kinesin-1 editing. Kinesin-1 is a molecular motor that moves cargo toward the plus end of microtubules. This is important for a number of cellular functions and is essential for transporting cargo down the length of axons in the nervous system. Birk et al. show that the edited variant of octopus Kinesin-1, which is observed at lower temperatures, acts as a slower motor with shorter run lengths.

Rangan and Reck-Peterson focus on squid microtubule motors as a target of RNA recoding and show variable editing of the Kinesin-1 transcript across tissues and temperatures. Their work highlights the complexity of the A-to-I editing regime, as it is often combinatorial, with multiple sites within a single sequence eligible for editing. They then perform *in vitro* assessment of motor function,

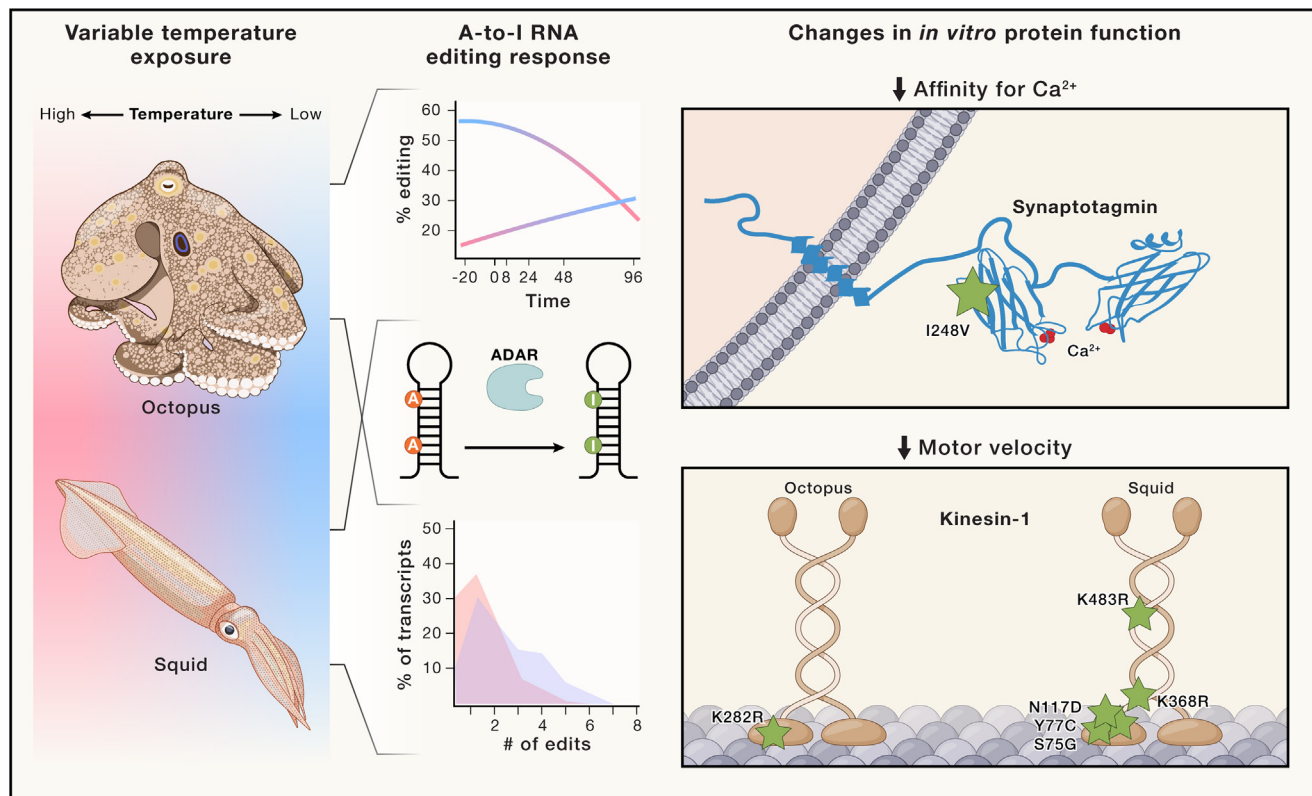


Figure 1. Cephalopods show changes in A-to-I RNA editing in response to temperature

Variable temperature exposures in squid and octopus lead to changes in A-to-I RNA editing and recoding of proteins. These changes are facilitated by the family of ADAR enzymes changing adenine to inosine, which is read during protein translation as guanine. Percent RNA editing in octopus increases when temperature decreases. Number of edits per transcript increases in squid when temperature is low. Proteins Synaptotagmin-1 and Kinesin-1 both show functional changes in edited proteins compared to wild type. Green stars denote the location of recoded protein edits, annotated with the amino acid change and location in the sequence. Edits in Synaptotagmin-1 showed a decreased affinity for Ca^{2+} . Edits in Kinesin-1 protein discovered in both octopus and squid shared slower motor velocity. They also showed variable changes in run distance and landing rate.

which varies as a result of combinatorial edits. While the Kinesin-1 recoding sites are not conserved between squid and octopus, most of the Kinesin-1 variants found in squid also showed slower motor velocity than the unedited variant. They also find that the edited motors have longer run distances and better landing rates. The edited Kinesin-1 variants found in squid and octopus share a slower velocity but other changes in function are species specific.

Finally, Ragan and Reck-Peterson used these findings to inform experimental assessment of Kinesin-1 and Dynein-1 function in humans and yeast. They show that conserved amino acids that are preferentially recoded in cephalopods in response to temperature are good targets for investigation in human and yeast proteins, as experiments show that motor function is similarly impacted. These in-

sights highlight the power of evolutionary and comparative approaches to illuminate unintuitive and impactful biology.

These manuscripts are the first evidence that RNA editing in cephalopods changes in response to external stimuli and suggests that editing may be important for organismal plasticity. This is necessary evidence to support an adaptive hypothesis. However, many exciting questions are left to explore. The first question is how these edits impact function *in vivo*. With Crispr/Cas9-targeted mutagenesis now available in cephalopods and the possibility of transgenesis within reach, functional analysis of ADAR proteins and specific edits will be possible.¹¹ Usually both wild-type and edited transcripts co-exist, as editing rarely occurs at 100% efficiency, so it will be interesting to understand if these changes have an impact on phenotype.

Considering both incidence and magnitude of RNA editing in conjunction with this newly discovered acute environmental response, there remains room for expanded statistical analysis of this phenomena as well. Previous evolutionary analyses are reliant upon likely incomplete datasets. Pairing new sequencing data, mindful of environmental stimuli and ecology, with expanded theoretical approaches, addressing sequence evolution and selection, may help elucidate how a minimally targeted process can influence phenotype in an adaptive manner. Finally, this work will benefit from greater investment in the study of other molluscs and species within the Lophotrochozoa, both ecologically and molecularly. It is difficult to put these findings in context when so little is known about the molecular biology and physiology of non-model organisms.

It is an exciting idea that cephalopods may build themselves using fundamentally different molecular tools than those we know so well. Understanding this unique usage of RNA editing may give us the opportunity to employ it for our own purposes. There is still much to learn about these processes, and the debate about RNA editing's adaptive importance is likely to continue. However, this work clearly highlights the mysteries remaining within the expanse of biodiversity. Using modern methods in neglected systems is exposing completely unknown biology that has the potential to change our lives, and the thrill is that we have only barely touched the surface.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Medication effects on the gut microbiome in allo-HCT

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Decreased gut microbiome diversity has been associated with negative outcome in allogeneic hematopoietic stem cell transfer (HCT). A study published in this issue of *Cell* identifies associations between non-antibiotic drug administration, microbiome state transitions, and response to HCT, highlighting the potential impact of such drugs on microbiome and HCT outcome.

Allogeneic hematopoietic stem cell transfer (allo-HCT) is the transfer of hematopoietic stem cells from a healthy donor to reconstitute the recipient patient's immune system. Allo-HCT can be a curative treatment for

patients with hematological malignancies, likely because the transplanted cells recognize the tumor cells as non-self and eliminate them (graft-vs.-tumor). However, those undergoing allo-HCT can face

serious side effects including graft-vs.-host disease (GVHD), where the grafted cells attack the patient's healthy tissues.

Previously, the composition of the gut microbiome has been shown to influence

