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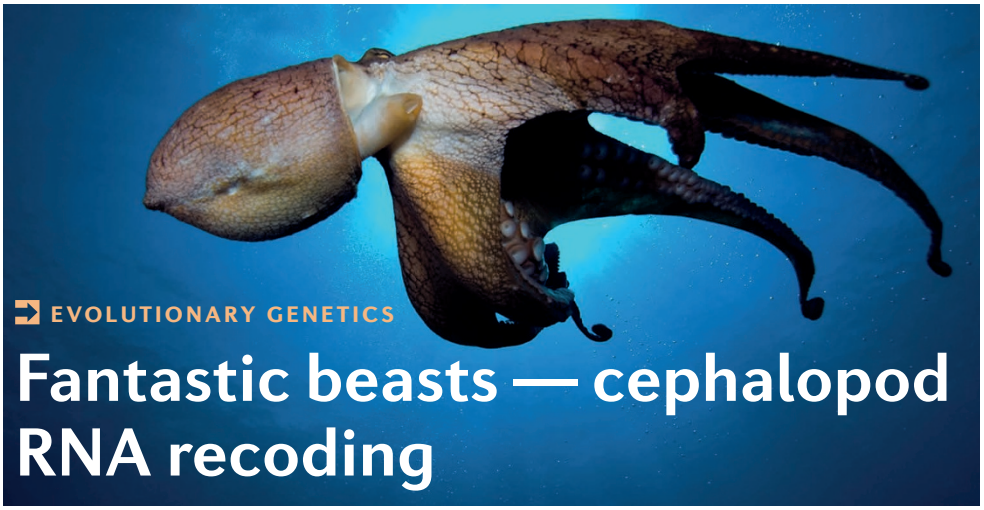

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EVOLUTIONARY GENETICS

Fantastic beasts — cephalopod RNA recoding

> cephalopods diversify their neuronal proteome with RNA editing

Post-transcriptional modifications, such as mRNA editing, have the potential to diversify the proteome. Most animals do not show much mRNA editing that results in a codon change (recoding). However, squid (a member of the cephalopod taxa) were recently shown to have high levels of recoding, but it was not known if this translates into functional diversification of the proteome. Now, Liscovitch-Brauer *et al.* show that cephalopods diversify their neuronal proteome with RNA editing, and that this limits genome evolution.

A common form of RNA editing in animals is adenosine (A) deamination to inosine (I) by ADAR (adenosine deaminases acting on RNA) enzymes. The authors assessed recoding by using RNA sequencing (RNA-seq) data to assemble a transcriptome for several species, and then comparing this to known DNA and RNA sequences. Systematic mismatches were used to identify A-to-I RNA editing events. Four species of coleoid cephalopod (two species of octopus, a squid and a cuttlefish) were compared to more primitive species (a nautiloid and a mollusc). For the squid, octopus and cuttlefish species, 80,000–130,000 A-to-I editing events were obtained. By contrast, the few mismatches seen in the nautiloid and mollusc were attributed to background noise. The full genome for *Octopus bimaculoides* was also used to assess RNA editing, and showed a substantial overlap with A-to-I sites identified by the reference-genome-free approach.

Interestingly, RNA editing in *O. bimaculoides* was twofold higher in neuronal tissue than in non-neuronal tissue. In addition, mass-spectrometry-based proteomic analysis of squid neuronal samples validated 432 protein recoding sites. These data suggest that the coleoid cephalopod lineage has evolved with extensive neuronal recoding resulting from RNA editing.

In mammals, RNA editing in coding regions is negatively correlated with gene importance and recoding sites are rarely conserved. However, 1,146 recoding sites, in 443 proteins, were shared across the four coleoid cephalopod species in this analysis. Sequence analysis identified the non-synonymous (recoding) sites, and found that 65% of edits in cephalopod genomes are non-synonymous. Furthermore, the non-synonymous to synonymous ratio for different sites across cephalopod genomes increases at highly edited sites, and increases even further at conserved sites, which is indicative of positive selection. Thus, highly edited, conserved sites in cephalopod RNA are almost all recoding.

To test whether recoding events can affect protein function, cephalopod Kv2 potassium channels, which have conserved recoding sites in their mRNA, were functionally evaluated. Unedited and edited channels were expressed in *Xenopus laevis* oocytes and investigated for their opening, closing and inactivation kinetics using the cut-open oocyte Vaseline gap voltage-clamp technique. The edited channels showed clear differences in their functional kinetics, which varied across species. As these channels are important for central nervous system functionality, these data suggest that RNA recoding could influence neurophysiology in cephalopods.

To edit RNA, ADAR enzymes require double stranded RNA (dsRNA) structures, and maintaining these requires sequence conservation. Interestingly, sequence analysis showed that genomic variability is decreased in the regions surrounding recoding and non-recoding RNA edit sites. Thus, transcriptome plasticity conferred by RNA editing comes at the cost of decreased genome evolution. Future research could investigate whether RNA recoding in the nervous system of the coleoid cephalopod lineage is linked to the complex behaviours exhibited by these animals.

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ORIGINAL ARTICLE Liscovitch-Brauer, N. *et al.* Trade-off between transcriptome plasticity and genome evolution in cephalopods. *Cell* **169**, 191–202.e11 (2017)