MOBILE ELEMENTS

piRNAs make sense of retroviral invaders

Antisense Piwi-interacting RNAs (piRNAs) protect the germline genome from insertion-mediated mutation and instability by silencing established transposons. However, what prevents invasion by mobile elements in the first place has remained largely unknown. Now, a study in *Cell* shows that production of sense-strand piRNAs may have a pivotal role in this process.

An epidemic of KORV-A, a gammaretrovirus that infects the soma and germline of wild koalas, provided an ideal opportunity to study the germline response to retroviral invasion. Analysis of piRNAs mapping to established retrotransposons in the koala germline genome revealed similar levels of sense- and antisense-strand piRNAs. This result is consistent with the mechanism used to silence established transposons in the germline of other mammals, in which sense-strand piRNAs template ping-pong amplification of target-silencing antisense piRNAs. By contrast, predominantly sense-strand piRNAs mapped to KORV-A, suggesting that different mechanisms are used to suppress new versus established retroviral germline insertions.

The KORV-A provirus produces two transcripts: a spliced transcript that encodes the Env protein and an unspliced transcript that encodes the viral genome and the Gag and Pol proteins. Analysis of the distribution of KORV-A piRNAs at introns, exons, splice sites and exon–exon boundaries strongly suggests that the piRNA machinery preferentially processes and cuts unspliced KORV-A transcripts into sense-strand piRNAs. Removal of these transcripts (and the functions they encode) suppresses the ability of the virus to replicate and transpose.

The authors propose that this sense-strand piRNA-mediated suppression is analogous to the innate phase of the cellular immune response. During initial retroviral infection, a 'pattern' specific to unspliced proviral transcripts triggers an innate genome immune response. This response rids the germ cells of viral transcripts by processing them into sense-strand piRNAs and protects the genome from mass invasion.

Credit: Gerry Pearce/Alamy

Moving forwards, it will be important to identify the precise nature of the pattern that activates the innate genome immune system. The authors speculate that persistent introns or poorly translated transcripts could be the trigger, but further studies will be needed to test these hypotheses.

Dorothy Clyde

ORIGINAL ARTICLE Yu, T. et al. The piRNA response to retroviral invasion of the Koala genome. *Cell* https://doi.org/10.1016/j.cell.2019.09.002 (2019)

Credit: Martin Shields/Alamy

EVOLUTIONARY GENETICS

An adaptive walk in the park

Direct evidence that specific alleles are adaptive requires the functional connection of genotype, phenotype and fitness. Using CRISPR–Cas9 genome editing, a recent *Nature* study retraces the convergent evolution of resistance to cardiac glycosides in insects, pinpointing the adaptive consequences of individual alleles.

Cardiac glycosides are highly toxic plant secondary metabolites that act by inhibiting the sodium–potassium ATPase. Nonetheless, more than 100 insect species across six orders have evolved parallel amino acid substitutions in the α-subunit (ATPα) that enable them to feed from these plants and in some cases sequester the toxins.

Using maximum likelihood ancestral state reconstruction across a species phylogeny, the authors identified three amino acid residues (at sites 111, 119 and 122) within ATPα that frequently underwent substitutions associated with cardiac glycoside specialization. Comparison of a random permutation null model with the mutational order across 21 specialized lineages revealed that the order of substitutions was unlikely to be random.

Next, the team focused on the monarch lineage (Danainae), which includes butterfly species that do not feed on cardiac glycoside-producing plants and those that sequester the toxins, such as the monarch butterfly (*Danaus plexippus*). They generated *Drosophila* lines with precise substitutions at ATPα residues 111, 119 and 122 to reconstruct four genotypes that have evolved sequentially in the monarch lineage (Q111L, A119S, L111V and, lastly, N122H).

The Q111L substitution only mildly increased survival of knock-in fly lines fed the cardiac glycoside ouabain at lower concentrations, with no effects seen at higher concentrations. By contrast, Q111L flies that also harboured the A119S substitution showed an increase in survival also at higher ouabain concentrations, as did those with the subsequent L111V mutation. In flies with the 'monarch' genotype (that is, all four substitutions), even the highest ouabain concentrations had no impact on survival. Each consecutive substitution had a neutral-to-positive effect on target-site insensitivity, as determined by sodium pump enzymatic assays using head extracts, with 'monarch flies' exhibiting the same insensitivity to ouabain as monarch butterflies.

Finally, the authors generated single-substitution knock-in lines for N122H, often the last mutation to evolve, and for A119S, a substitution also present in non-specialized insects. These lines helped decipher the roles of antagonistic pleiotropy and epistasis of individual substitutions, which shape the resistance-conferring mutational path. For example, A119S was found to counter the pleiotropic 'costs' of substitutions at sites 111 and 122 throughout the adaptive walk.

Taken together, this study functionally validates the adaptive alleles underlying cardiac glycoside specialization in the monarch butterfly, providing "in vivo validation of a multi-step adaptive walk in a multicellular organism".

Linda Koch

ORIGINAL ARTICLE Karageorgi, M. et al. Genome editing retraces the evolution of toxin resistance in the monarch butterfly. *Nature* https://doi.org/10.1038/s41586-019-1610-8 (2019)