DOCTORAL THESIS

Delineating the Genetic and Genomic Mechanisms of Hybrid Lethalities between Closely Related Caenorhabditis Nematodes

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Abstract

Evolutionary divergence and speciation are attributed, if not all, to various forms of post-zygotic hybrid incompatibility (HI), which manifests as lethality and sterility among others in hybrid progenies. Hybrid lethality, which completely prevents the gene flow within or between species, represents one of the most extreme HI forms across a wide range of phylogenetic groups. Despite a handful of hybrid lethality genes identified to date, the molecular mechanisms underlying HI remains enigmatic. We previously isolated numerous HIs between two closely related nematodes, *Caenorhabditis briggsae* and *C. nigoni*, via repeated interspecies back crosses called introgression, but the molecular mechanisms underlying the various HIs were largely unknown. To dissect the underlying molecular mechanisms of HI between the two species, two different hybrid lethality phenotypes were assessed in this thesis by a combination of genomics and genetics approaches: (1) the parent-of-origin-dependent embryonic lethality in the backcrossing hybrids; and (2) the parent-of-origin independent embryonic lethality in the F1 hybrids.

In the first case, I performed mRNA and small sequencing for the embryos of the two parental nematodes together with their hybrid F1 and reciprocally backcrossing F2 hybrids. The backcrossing hybrids fathered by *C. briggsae* (bB2) exhibited a complete breakdown whereas the F1 or the reciprocal backcrossing hybrids produced only a partial breakdown. Consistent with this, I demonstrated a differential upregulation of transposable elements (TEs) exclusively in bB2 but not in the other hybrid embryos, suggesting a possible role of the TE dysregulation in producing the more severe HI phenotypes in the bB2 embryos than in other types of hybrid embryos. In addition to TEs, dysregulation in protein-coding genes was also differentially enriched in the bB2 embryos, indicating that the bB2 embryos suffered from a higher degree of regulatory perturbations relative to other embryos that plausibly underlies its more severe HI phenotypes. I further demonstrated that the dysregulation of TEs or protein-coding genes was not directly linked to specific expression alterations of small RNAs in the hybrids, including microRNAs, 21U RNAs and 22G RNAs.

In the second case, I seek to define the molecular identity of a previously mapped HI locus that is responsible for the complete lethality in hybrid F1 embryos independent of parent-of-origin between *C. briggsae* and a *C. nigoni* line that carries a *C. briggsae* introgression. We previously mapped the locus to an interval of approximate 8Mb. However, the attempt to further reduce the interval was unsuccessful due to the recombination suppression between the two nematodes. To circumvent such suppression, a forced recombination method mediated by an artificially introduced double stranded DNA break (DSB) using CRISPR/Cas9 system was adopted, which enabled the site-directed recombination. Aided by this approach, the previous HI interval was fine mapped to a region of approximate 70 kb on the right arm of the *C. nigoni* chromosome IV, which involved only a handful of candidate genes. Nailing down the interval to a single gene is actively ongoing.

In summary, this thesis established a mechanistic link between widespread dysregulation of TEs and protein-coding genes and the asymmetrical deleterious phenotypes associated with the bB2 hybrids. In addition, a hybrid F1 embryonic lethal locus was fine mapped to a 70 kb genomic interval. These findings will not only provide new insights into the molecular mechanism of asymmetrical HI phenotypes, but also pave the way for defining the molecular identity of the HI locus between the two nematodes.