

The use of branched DNA ISH (RNAscope™) to characterize the systemic spread of RNAi effects throughout the body of western corn rootworm larvae

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Western corn rootworm (WCR) is a significant corn pest in the United States, causing an estimated 1 billion dollars in damage and treatment costs every year. RNAi-mediated gene knockdown is one of the more recent methods being developed to control agricultural insect pests. It is now well established that WCR is susceptible to RNAi through ingestion of targeted dsRNA [1]. These dsRNA molecules are taken up by the insect gut epithelial cells and processed into siRNAs. These siRNAs bind to endogenous mRNAs, accelerating their breakdown and decreasing translation into protein. If the genes targeted by these dsRNAs encode proteins critical for the insect's health and viability, then these dsRNAs can be lethal.

The effects of dsRNA injected into WCR have been shown to spread from the site of injection to the gut [2]. However, the systemic spread of RNAi-mediated gene knockdown in WCR has not yet been directly demonstrated. New, high-sensitivity in situ hybridization (ISH) methods, such as RNAscope ISH, allow the detection and localization of single mRNA molecules in tissue sections [3]. The purpose of this study was to assess whether RNAscope ISH could be used to characterize the spread of the RNAi-mediated knockdown of vATPaseC mRNA through the gut and other body tissues of WCR.

WCR were reared on artificial diet until the 3rd instar stage, then moved into individual wells containing food overlaid with a 184-mer dsRNA targeted to vATPaseC. Insects were collected at 0, 16, and 48 hours post-treatment and processed for paraffin embedding. Paraffin sections were cut 7 µm thick, collected on Superfrost Plus slides (Fisher Scientific), air-dried overnight, and baked for 1 hour at 60°C. LR White sections were trimmed and faced with a Diatome Ultratrim diamond knife, sectioned 500 nm thick, and stained with toluidine blue at 60°C. Slides were probed for vATPaseC mRNA using RNAscope™ VS (Advanced Cell Diagnostics, Haywood, CA) on a Ventana Discovery Ultra (Roche Diagnostics, Indianapolis, IN) automated slide staining system using the manufacturer's recommended times and conditions.

Untreated WCR larvae have a large number of vATPaseC mRNA molecules in the cells of the midgut. These mRNAs are also present in many of the other cell types of the larva, including muscle cells and fat body cells (Fig. 2). After 16 hours of feeding on diet containing vATPaseC dsRNA, the cells of the gut show a reduction in the prevalence of vATPaseC mRNAs. Distal regions of the fat body show a similar reduction in these mRNAs. At 48 hours post-treatment, the cells of the gut show a dramatic loss of vATPaseC mRNAs. The distal fat body also shows a nearly complete loss of these mRNAs at the 48 hour timepoint. Larvae fed with the same amount of a nonhomologous dsRNA show no change in vATPaseC mRNA levels in either the gut or other body tissues.

The loss of ATPaseC mRNAs from gut tissues and tissues spatially separated from the gut was demonstrated using RNAscope ISH in WCR. These results show that RNAi-mediated gene knockdown is systemic in WCR. This successful characterization of RNAi effects in WCR by RNAscope will likely be adaptable to the characterization of RNAi effects on other genes in WCR and in other organisms.

References

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 [3] F Wang *et al.*, *Journal of Molecular Diagnostics* **14** (2012), p. 22.

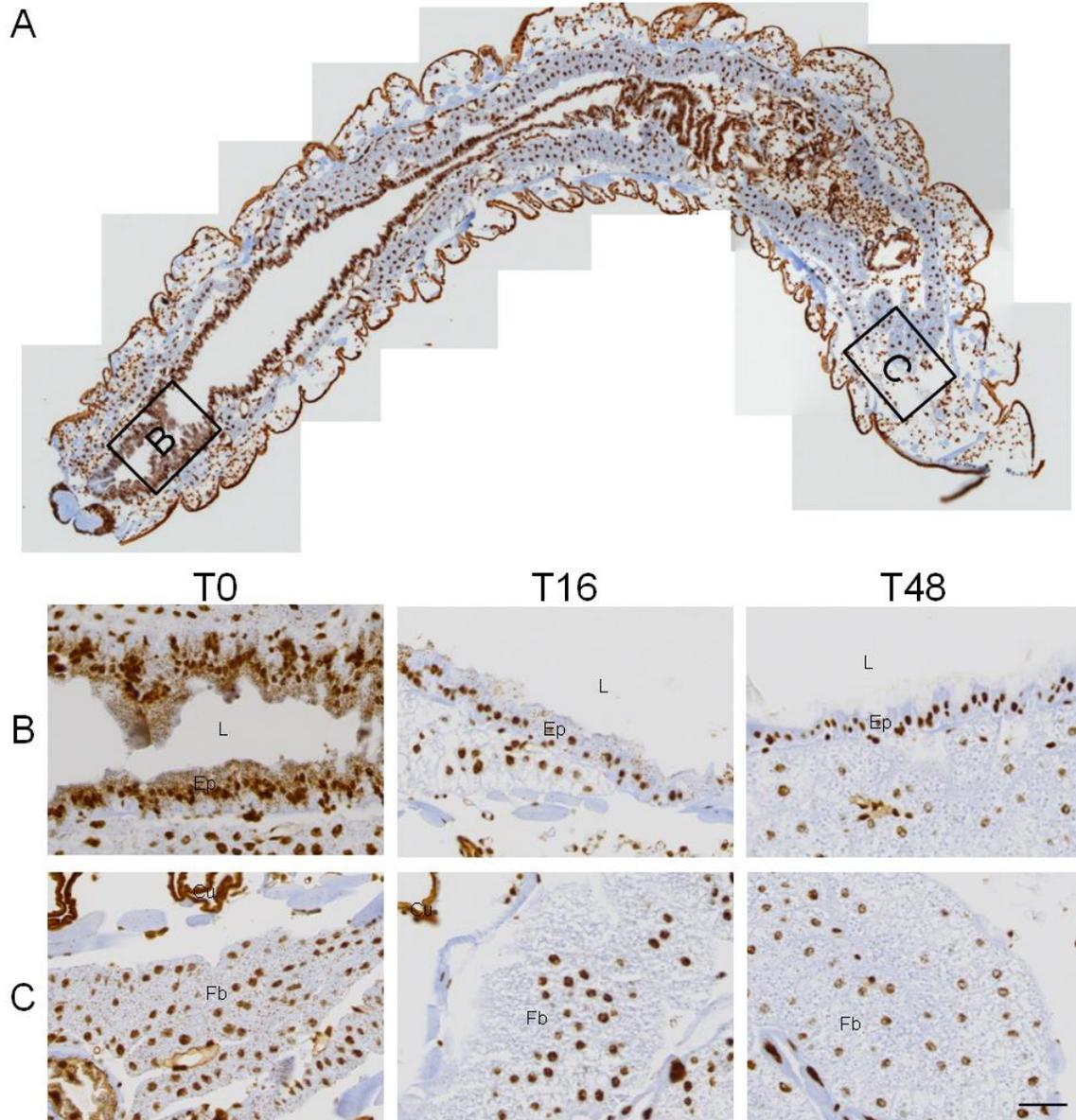


Figure 1. Visualization of vATPaseC mRNA in WCR larva: direct evidence for systemic spread of RNAi effects. Western corn rootworm larvae were fed vATPaseC dsRNA (A) and samples were collected at several time points and processed for RNAscope *in situ* hybridization, which detects individual mRNAs (small brown spots). In the cells lining the gut (region B), a dramatic loss of mRNAs was seen. Interestingly, a similar pattern of mRNA loss can be seen in other body cells (region C), demonstrating that RNAi effects spread systemically throughout the body in WCR. (L=gut lumen, Ep=gut epithelium, Cu=outer cuticle, Fb=fat body; scale bar = 50 microns).