Coupling clearing and Hybridization Chain Reaction approaches to investigate gene expression in organs inside whole-mount intact insect.

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Abstract

Detecting RNA molecules within their natural environment inside intact arthropods has long been challenging, particularly in small organisms covered by a tanned and pigmented cuticle. Here, we have developed a methodology that enables high-resolution analysis of the spatial distribution of transcripts of interest without having to dissect tiny organs or tissues, thereby preserving their integrity. We have combined an *in situ* amplification approach based on Hybridization Chain Reaction, which enhances the signal-to-noise ratio, and a clearing approach that allows the visualization of inner organs beneath the cuticle. We have implemented this methodology for the first time in Hemiptera, mapping two salivary aphid (*Acyrthosiphon pisum*) transcripts, the effector c002 and the salivary sheath protein SHP. With a multiplex approach, we could simultaneously detect different mRNAs in whole-mount pea aphid head-thorax samples and show that they were distributed in distinct secretory cells of salivary glands.

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