Video Article

Laser Microdissection Applied to Gene Expression Profiling of Subset of Cells from the *Drosophila* Wing Disc

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Abstract

Heterogeneous nature of tissues has proven to be a limiting factor in the amount of information that can be generated from biological samples, compromising downstream analyses. Considering the complex and dynamic cellular associations existing within many tissues, in order to recapitulate the *in vivo* interactions thorough molecular analysis one must be able to analyze specific cell populations within their native context. Laser-mediated microdissection can achieve this goal, allowing unambiguous identification and successful harvest of cells of interest under direct microscopic visualization while maintaining molecular integrity. We have applied this technology to analyse gene expression within defined areas of the developing *Drosophila* wing disc, which represents an advantageous model system to study growth control, cell differentiation and organogenesis. Larval imaginal discs are precociously subdivided into anterior and posterior, dorsal and ventral compartments by lineage restriction boundaries. Making use of the inducible GAL4-UAS binary expression system, each of these compartments can be specifically labelled in transgenic flies expressing an UAS-GFP transgene under the control of the appropriate GAL4-driver construct. In the transgenic discs, gene expression profiling of discrete subsets of cells can precisely be determined after laser-mediated microdissection, using the fluorescent GFP signal to guide laser cut.

Among the variety of downstream applications, we focused on RNA transcript profiling after localised RNA interference (RNAi). With the advent of RNAi technology, GFP labelling can be coupled with localised knockdown of a given gene, allowing to determinate the transcriptional response of a discrete cell population to the specific gene silencing. To validate this approach, we dissected equivalent areas of the disc from the posterior (labelled by GFP expression), and the anterior (unlabelled) compartment upon regional silencing in the P compartment of an otherwise ubiquitously expressed gene. RNA was extracted from microdissected silenced and unsilenced areas and comparative gene expression profiling determined by quantitative real-time RT-PCR. We show that this method can effectively be applied for accurate transcriptomics of subsets of cells within the *Drosophila* imaginal discs. Indeed, while massive disc preparation as source of RNA generally assumes cell homogeneity, it is well known that transcriptional expression can vary greatly within these structures in consequence of positional information. Using localized fluorescent GFP signal to guide laser cut, more accurate transcriptional analyses can be performed and profitably applied to disparate applications, including transcript profiling of distinct cell lineages within their native context.

Protocol

Part 1. Preparation of *Drosophila* Imaginal Wing Discs Subjected to Specific and Localized RNAi.

As biological material, we used *Drosophila* imaginal wing discs obtained from transgenic larvae subjected to the localized and specific gene silencing by means of the GAL4/UAS system [1]. This larval progeny orginates from a genetic cross involving two parental lines: one carrying the GAL4 driver, the second the UAS responder. In addition to express GAL4 in a specific temporal and/or spatial pattern, the driver line carries an UAS-GFP reporter that allows to visualize the GAL4 expression domain. The UAS responder line expresses, under the control of the UAS sequence, an hairpin silencing RNA (hpRNA) able to specifically target the selected gene of interest (call it gene X) [2]. Once expressed in the cell, the hpRNA X is cleaved to generate small interfering RNA (siRNA) able to specifically silence the selected gene. In the larval progeny, that carries both driver and responder transgenes, the UAS-silencing construct is only transcribed in those cells expressing the GAL4 protein, and thus is able to induce a spatially restricted silencing of the selected gene X.

Among the variety of possible applications, we applied this approach to silence a selected gene of our interest (gene X) under the control of the *engrailed*-GAL4 (*en*) driver, which can trigger specific silencing in the posterior compartment of the wing disc [3], whose territory was marked by the expression of the UAS-GFP transgene.

The silenced imaginal wing discs were hand-dissected, taking care to eliminate surrounding contaminating tissues, especially the adhering tracheas. Each isolated wing disc was then quickly and gently transferred to a previously treated, RNase free dissection frame for immediate laser microdissection of selected areas.

Part 2. Laser microdissection of selected areas from the silenced (posterior) and unsilenced (anterior) compartments of the wing disc.

Once the wing disc was on the membrane, laser microdissection of specific areas from silenced (posterior; GFP-labelled) and unsilenced (anterior; GFP-unlabelled) compartments was achieved. This was performed by drawing an area that could easily fit in both, the GFP-positive and GFP-negative sides of the disc. Under the microdissector, we first focused the laser beam on the GFP-positive tissue, making a cut along the selected perimeter. The microdissector proceeds with cutting at the selected speed and power, but it is possible to refine the cutting manually,

until the selected area of tissue falls in the tube underneath. This tube contains a small volume of the TRI Reagent, so that the GFP-positive tissue is ready for subsequent RNA extraction.

We subsequently moved the cutting area to the opposite, GFP-negative side of the wing disc, in order to dissect an equivalent territory from the unsilenced, GFP-negative tissue. Once again, after the automatic cut, we proceeded to refining the cut manually. Once the cut was done, also the GFP-negative tissue was recovered in the TRI Reagent, ready for RNA extraction.

Part 3. RNA Extraction and Transcription Profiling from Microdissected Tissue Areas.

We spun the tubes to detach the liquid from the cap and added TRI Reagent up to 1 ml, then performed RNA extraction following the standard TRI Reagent protocol. To collect enough material, we repeated the cutting procedure on 100 imaginal wing discs. Starting from 100 areas of about 5000 μm^2 each, our yield was of 1.5 µg of RNA. Accuracy of the manual dissection was then controlled by checking GFP expression in the silenced and unsilenced samples by RT-PCR, using Super Script III (Invitrogen) to synthesize cDNA with Random Hexamers. Subsequent quantitative analyses focused to determine the expression levels of the silenced gene X, together with those of putative targets of its regulatory pathway, were performed by Real Time RT-PCR using Master Mix (Invitrogen).

Discussion

With respect to their basal expression levels achieved in the anterior/posterior compartments of unsilenced wing discs, the activity of the selected gene X was found reduced to 40% upon silencing. In contrast, one of its putative targets (genes Y) was found significantly up-regulated (7 fold-increase), leading us to validate the hypothesis that it was negatively regulated by gene X.

We conclude that the above described experimental approach can successfully be applied to the validation of putative targets of diverse regulatory pathways.

In addition, we surmise that improvement in the yield of RNA extraction could rapidly allow characterization of localized transcription profiling by microarray analysis. This will open the possibility to achieve a more detailed view of important biological processes. For examples, the localized formation of morphogenetic gradients [4, 5] or the transcriptional switch underlining the process of cell competition [6], where loosing and winner cell clones can be visualized by differential GFP expression, could be more specifically addressed. Whatever the application, this approach can help to define the transcriptional response of different cellular territories in their native tissue context.

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