Video Article

RNAi Interference by dsRNA Injection into *Drosophila* Embryos

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Abstract

Genetic screening is one of the most powerful methods available for gaining insights into complex biological process¹. Over the years many improvements and tools for genetic manipulation have become available in *Drosophila*². Soon after the initial discovery by Frie and Mello³ that double stranded RNA can be used to knockdown the activity of individual genes in *Caenorhabditis elegans*, RNA interference (RNAi) was shown to provide a powerful reverse genetic approach to analyze gene functions in *Drosophila* organ development^{4,5}.

Many organs, including lung, kidney, liver, and vascular system, are composed of branched tubular networks that transport vital fluids or gases^{6,7}. The analysis of *Drosophila* tracheal formation provides an excellent model system to study the morphogenesis of other tubular organs⁸. The Berkeley *Drosophila* genome project has revealed hundreds of genes that are expressed in the tracheal system. To study the molecular and cellular mechanism of tube formation, the challenge is to understand the roles of these genes in tracheal development. Here, we described a detailed method of dsRNA injection into *Drosophila* embryo to knockdown individual gene expression. We successfully knocked down endogenous dysfusion(dys) gene expression by dsRNA injection. Dys is a bHLH-PAS protein expressed in tracheal fusion cells, and it is required for tracheal branch fusion^{9,10}. dys-RNAi completely eliminated dys expression and resulted in tracheal fusion defect. This relatively simple method provides a tool to identify genes required for tissue and organ development in *Drosophila*.

Protocol

1. Embryo Collection

1. Set up cages at 25°C using 2-4 day-old w¹¹¹⁸ flies. Grape juice plates are changed every hour during the day to synchronize the egg collection over 1-2 day period before collection
2. Collect embryos for 1 hr at 25°C
3. Cut a rectangular piece of grape juice agar, cut lightly in the middle with a razor blade, leave a line in the agar
4. Use a metal probe to transfer embryos from grape juice plate to your piece of grape juice agar, line them up in a straight line with the embryo's longer axis at 45 degree angle to the line in the middle of the agar. Get a straight line of about 50 embryos. Make sure all the embryos point in the same direction, with posterior end pointing away from the line.
5. Cut a rectangular piece of double stick tape, and place it to a 18x18mm cover slip.
6. Pick up embryos with double stick tape on the cover slip
7. Place the cover slip to a glass slide, dry the embryo in air for about 10 min.
8. Cover the embryo with a thin layer of 700 Halocarbon oil, now it's ready for injection.

2. Pulling Needle

Make microinjection needles by pulling glass capillary tubes. We use a needle puller from World Precision Instrument (Model PUL-1). The needle puller program specifications are: heat: 1, delay 4.

3. Filling the Needles

Back-load needles using the Eppendorf p20 pipette fitted with Eppendorf Microloader tips, normally load 1.5-2 µL dsRNA

4. Break the Needles

1. Before breaking the needle, place a coverslip onto a slide.
2. Prepare a slide with a drop of oil in the middle of the slide
3. Turn on the Picospritzer III picopump to adjust the air pressure
4. Break the filled needle against the edge of a cover slip, creating a sharp point.
5. When the tip is broken, step on the foot pedal of Picospritzer III picopump, some dsRNA will leak out of the needle tip.
6. Take out the slide with the coverslip.
7. Insert the needle under the oil drop in the middle of the slide prepared before.
8. Adjust the setting on the Picospritzer III picopump to get 100-200pl dsRNA when you step on the foot pedal. dsRNA can be seen as a small droplet.

5. Injection

1. Bring the needle tip to the posterior portion of the first blastoderm embryo into the same focal plane and inject the embryo off center around 30-50% egg length.
2. After you step on the foot pedal of the Picospritzer III picopump, a small amount of dsRNA will appear as a small dot in the injection site.

6. Phenotypic Analysis

1. After injection, place the slide into a covered moist chamber (plastic Petri dish) at 18°C until the embryos develop to the desired embryonic stage.
2. Use probe to remove embryos from the double sided tape and push them to the edge of the cover slip.
Wash the embryos off the slide into a collection vial with nylon mesh at the bottom using heptane, wash 3 times with PBT.
Dechorionate embryos in 50% bleach for 5 min, and wash 3 times with PBT.
Transfer embryos from the collection vial to a piece of nylon mesh, and then dip the nylon mesh into the fixative solution (5ml heptane, 4.5ml PBS, 0.5ml 37% formaldehyde) to release embryos. Fix embryos in the fixative solution for 30min.
Devitellinize embryos with methanol, transfer embryos to an eppendorf tube, and immunostain the embryos using standard protocols.
3. Embryos can also develop to appropriate larval stage.
4. Prepare a slide with a drop of Halocarbon 700 oil in the middle
5. Transfer one larvae to the slide, put a cover slip on the top
6. Observe larvae under bright field microscope.

7. Representative Results:

An example of dsRNA knock down *dys* gene in *Drosophila* embryo is shown in Fig1. GFP-dsRNA injected embryos are the negative control. *Dys* is a bHLH PAS transcription factor expressed in tracheal fusion cells. GFP-dsRNA injected embryos show normal tracheal fusion, and *Dys* protein is present in fusion cells (Figure 1A). On the other hand, *dys*-dsRNA injected embryos show failed branch fusion, and *Dys* proteins are not present in fusion cells (Figure 1B). When dsRNA injected embryos develop to larval stage, the *dys*-dsRNA injected larvae showed failed branch fusion (Fig.1D) compared to GFP- dsRNA injected negative control (Figure 1C). These results showed effective knock down of endogenous *dys* gene expression by dsRNA injection into *Drosophila* embryos

Figure 1. Removal of *dys* function by *dys*-RNAi reveals tracheal fusion defects. Blastoderm embryos (w^{1118}) were injected with either GFP-dsRNA (negative control) or *dys*-dsRNA and assayed for tracheal defects. (A) Stage 16 embryo injected with GFP-dsRNA and stained with α -Dys and MAb 2A12 that stains the tracheal lumen. Prominently shown is the lateral trunk, which has fused. Arrows point to lateral trunk Dys-positive fusion cells. (B) Stage 16 embryo injected with *dys*-dsRNA and stained with α -Dys and MAb 2A12. The embryo showed a lack of lateral trunk fusion (the asterisk indicates the normal location of a lateral-trunk fusion in control embryos). No Dys-positive cells were observed, indicating that the *dys*-RNA effectively abolished Dys protein. (C to D) Second-instar larvae with either GFP-dsRNA or *dys*-dsRNA were examined by bright-field microscopy for tracheal defects. (C) Sites of fusion are indicated by an asterisk in GFP-dsRNA injected control larvae. (D) The lateral trunk failed to fuse in the *dys*-RNA injected larvae, (*) indicates the normal location of a lateral trunk fusion.

Discussion

The dsRNA injection method present here enables a very sensitive and rapid analysis of gene function in *Drosophila* tracheal development. This method can potentially be applied to analyze gene function for other tissue and organ development.

Disclosures

No conflicts of interest declared.

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