# Mesoderm tissue development in *Drosophila melanogaster*

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## Abstract

Embryonic mesoderm develops into the skeletal muscles and the visceral muscles that protect organ tissue. Two genes, *CG11148* and *CG7224*, are thought to be present in the mesoderm of *Drosophila melanogaster*. By studying defects caused by lack of the gene, we were able to confirm the presence of *CG11148* and *CG7224* in the embryonic mesoderm. Using staining techniques, we discovered the embryos had problems with correctly forming visceral mesoderm. We also uncovered that the adult mutants for *CG11148* have defects in the adult wing formation. The data shows that both genes impact the development of the mesoderm and are good candidates for future research on *Drosophila* development.

## Background

Studying genetics has become a critical area of research as scientists look to gain an insight into human development. Since using human embryos is not a viable option, researchers use model organisms to understand embryonic development. One of the common species used is the fruit fly, *Drosophila melanogaster*. Its usefulness as a model organism comes not only from its short generation time and ease to maintain, but also because the same pathways that govern major pattern development of human embryos are also found in the developing fly.

In embryonic development of humans, flies, and other triploblastic organisms, there are three tissue types that the cells differentiate into, endoderm, ectoderm, and mesoderm. The endoderm matures into the digestive system and other internal structures. The ectoderm becomes the skin as well as the nervous system. The mesoderm forms the skeletal muscles and muscles surrounding the organs. Muscle development begins as a single layer of cells that quickly differentiates into two different types of cells.[1] The cells closest to the body wall are the somatic mesoderm or skeletal muscles and the cells inside the body become the visceral mesoderm and line the internal organs. The two types of mesoderm continue to replicate and eventually fuse together to make up the musculature of the fly larvae.

We are interested in understanding the development of the muscles. Two genes, *CG11148* and *CG7224* have been noted in studies that are related to muscle development. The gene for *CG11148* is found on the fourth (dot) chromosome and there are 5 known isoforms for the protein, averaging about 1530 amino acids long. CG7224 is a much shorter protein composed of only 118 amino acids. Scherzer and colleagues performed a large-scale analysis of genes that showed changes in regulation during the modeling of Parkinson's disease in *Drosophila*.[2] Parkinson's disease causes degeneration of neural cells and problems with muscle function. *CG11148* and *CG7224* were both screened in the study, but neither was studied in depth. Research by Zun and colleagues identified an upregulation of *CG7224* in flies used to model Parkinson's.[3] Junion et al showed that *CG7224* had an increased upregulation in a test to identify cis regulatory molecule for

Drosophila myocyte enhancer factor 2 (Dmef-2), an important factor in the differentiation of muscle cells.[4] These two studies suggest that *CG7224* and *CG11148* may be involved in the role Parkinson's plays in the muscle cells.

Since there was no specific information about these two genes and their potential role in the mesoderm, we examined the function of these genes in *Drosophila*.

## Materials and Methods

### Fly Lines

Fly stocks for *CG11148*, *CG7224*, Df(4)38 and Df(4)G were purchased through Bloomington Stock Center. The *CG11148* flies are homozygous viable. *CG7224* flies are not fully viable and thus kept heterozygous by using a balancer.

### Embryo collection

Fly embryos were collected on grape agar plates then dechlorinated in 50% bleach before fixation in a 1:1 mixture of formaldehyde (37%) in 1X PBS with 0.05M EGTA, and heptane. After fixation for 30-40 minutes, methanol was added to help remove the vitella membrane followed by washes with ethanol before storage at −20°C.

### Immunostaining

Stored embryos were warmed to room temperature before beginning the staining process. After washes in PTX (PBS + 0.1% Trition), the embryos were rotated in a 5% Normal Goat Serum (NGS) / PBT (PTX + 2% Bovine Serum Albumen) solution for 1-2 hours. Primary antibodies were added and rotated at 4°C overnight. Primary antibodies included Fasiclin III (1:10), fork head (1:500), and elav (1:10). Primary antibodies were removed and saved for use again, and then the embryos were washed with 1X PTX, 6x over 30 minutes. Secondary antibodies were added, either biotinylated goat anti-rabbit IgG or biotinylated goat anit-mouse IgG. Embryos were rotated in the secondary for about 1 hour, washed, incubated in Vectastain, and then stained using diaminobenzidine (DAB). The embryos were stored in methyl salicylate to allow for better visualization.

### Insitu hybridization

First, the embryos were allowed to rotate in Xylene for a minimum of 2 hours. After rinsing in ethanol, then methanol, the embryos were rinsed in 2:1:1 methanol, 10% formaldehyde, and PBTw (PBS + 0.1% Tween-20). Next, the embryos were fixed for 20-30 minutes in a 1:1 solution of PBTw and formaldehyde. After removing the fixative with washes of PBTw, the embryos were incubated with Proteinase K titrated to 4 ug/ml for 5-6 minutes. Quick rinses with PBTw followed by washes in 0.1M Triethanolamine-HCl (TEA), then TEA with acetic anhydride to stop the reaction of the Proteinase K. Embryos were post fixed in 1:1 PBTw and 10% formaldehyde before being introduced into the hybridization buffer (Hyb). Hybridization occurred overnight in a mix of the probe solution in hyb a water bath at 65°C. The following morning, the embryos were washed of the probe solution, then slowly moved from the hyb into 2X SSC + 0.3% CHAPS. Once in the SSC with CHAPS, the embryos were treated with 20 ug/ml RNase A in 2X SSC at 37°C. After washes in SSC with CHAPS to remove the RNase A, the

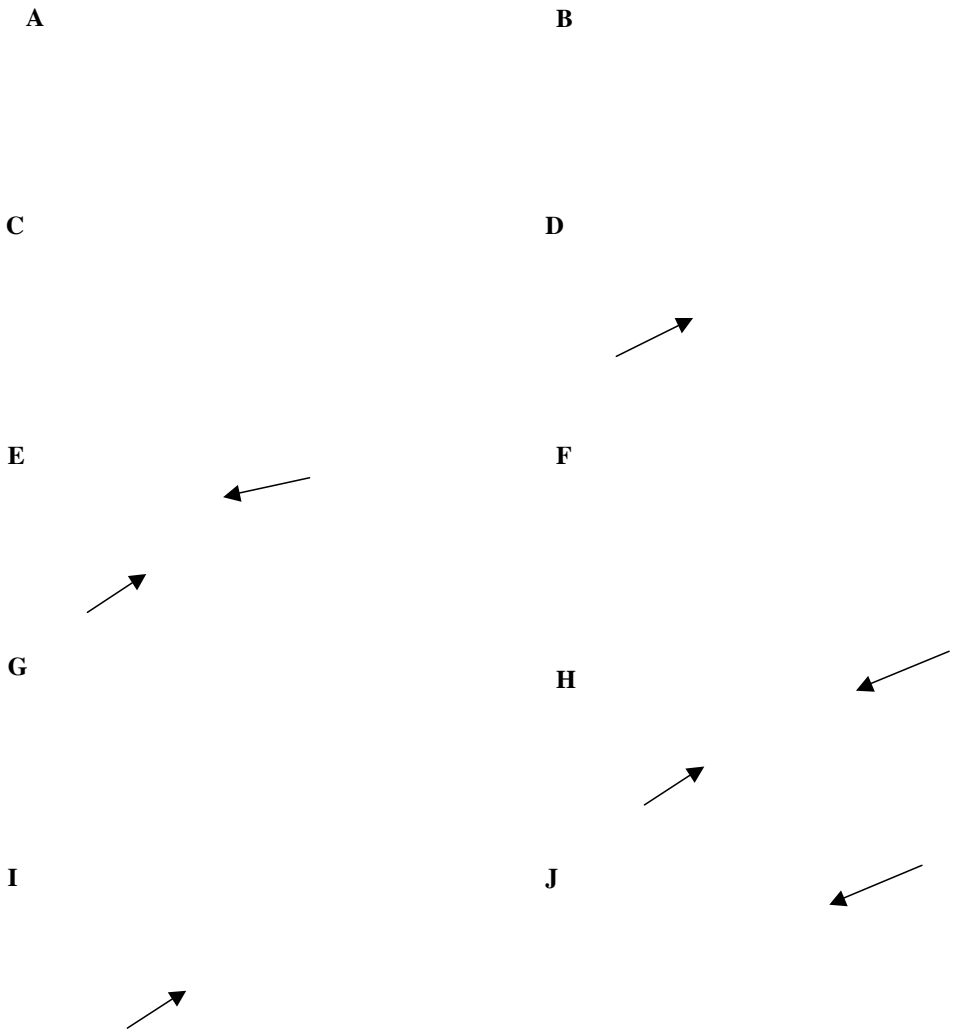


Figure 3: Insitu expression pattern for *CG11148*. A: Early Embryo with maternal expression. B: Stage 5-6. Decreased expression in embryo. C: Stage 7-8. Little expression in the embryo. D: Stage 9-10. Some expression in the gut and mesoderm (black arrows). E: Expression in the mesoderm and hindgut. F: Stage 13. Minimal expression throughout the embryo. Lateral view. G: Stage 13. dorsal ventral view. H: Stage 14. Increased expression in mesoderm and gut. Lateral view. I: Stage 14. dorsal ventral view. J: Stage 15-16. Clear expression in gut lining, somatic mesoderm, and head region