

A storehouse for splicing proteins?

Researchers stumble on new domain within the nucleus.

While probing how *Drosophila* flight muscles specialize, Oas et al. discovered a new structure in the nucleus in which cells stow proteins (1). The structure might serve as a depot for proteins that control alternative splicing.

Drosophila muscles are fine-tuned for producing specific movements. Jump muscles give the insects their mad hops, whereas flight muscles can beat the wings 250 times per second. The two muscle types differ in their microscopic structure and in their patterns of gene expression (2). They are distinct in another way, too: thanks to alternative splicing, they produce different versions of certain proteins. Researchers think that alternative splicing is important for muscle differentiation and function. This process goes awry in the muscle-wasting disease myotonic dystrophy type 1, for instance (3). Moreover, impeding alternative splicing in the muscles of mice spurs changes in the abundance of slow-twitch fibers (4).

Drosophila flight muscles contain different types of fibers than the jump muscles, allowing researchers to investigate alternative splicing's role in fiber specialization by comparing the two muscles. Oas et al. started by searching for fly proteins that control alternative splicing in muscles. They knocked down genes whose proteins bind to RNA and gauged the effects on the animals' flying ability. Reduced expression of one protein called Arrest (or Bruno) grounded the flies and impaired alternative splicing. Knocking down *Arrest* didn't spur flight muscles to change their identity, but the altered flight muscles exhibited a pattern of alternative splicing that is usually characteristic of jump muscles. Further, although muscles lacking Arrest were in the right location and attached correctly, the myofibrils within the muscles were misshapen and displayed abnormal striation patterns.

Electron microscopy revealed additional defects. In normal flies, myofibrils



FOCAL POINT

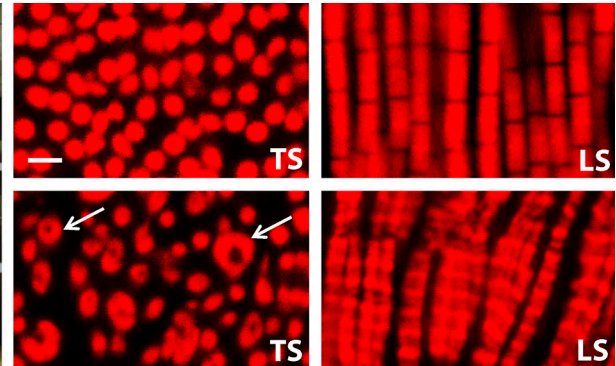


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(Left to right) Sandy Oas, Richard Cripps, and Anton Bryantsev found that the protein Arrest, which controls alternative splicing, helps spur the differentiation of *Drosophila* flight muscles. The two sets of panels show the flight muscle myofibrils end on (TS) and lengthwise (LS). In a normal fly (top panels), the myofibrils are cylindrical and show regular striations. But in a fly with reduced levels of Arrest (bottom panels), the myofibrils are misshapen and sometimes hollow (arrows).

contain filaments that are tightly packed into a hexagonal formation, but in the knockdown flies the myofibrils showed a looser arrangement. Sarcomeres, the contractile units in muscle fibers, are demarcated by dark bands called Z lines. But in flies with a deficiency of Arrest, the Z lines were often blurry or broken, and the sarcomeres were abnormally short.

To show that Arrest promotes the splicing pattern seen in flight muscles, the researchers created truncated versions of

two genes that are alternatively spliced in muscles: *sls* and *wupA*. When the researchers inserted the minigenes into cultured *Drosophila* S2 cells, which normally don't make *sls* or *wupA*, they found that the transcripts showed the splicing patterns of jump muscles. However, when

the team altered the cells to also manufacture Arrest, they produced transcripts with the characteristic splices of flight muscles.

In pupating flies whose muscles are still developing, Arrest clumped in the nuclei of the founder cells that give rise to flight muscles. "We found that Arrest wasn't in any known nuclear domain,"

says co-author Anton Bryantsev. "We concluded it was a separate, novel domain within the nucleus." The researchers named the structure the Bruno body, or B body. Another splicing factor, Muscleblind, also localized there, suggesting that storing splicing factors is the B body's task. As muscle development proceeds, Arrest spreads out within the nucleus.

The study indicates that would-be flight muscle cells cache Arrest and possibly other splicing proteins in a previously unsuspected nuclear domain. When the time is right, Arrest steers developing muscles to become flight muscles by promoting a specific splicing pattern for transcripts. Bryantsev says he and his colleagues were thrilled to discover the B body. "It's like finding a big, new mammal species in the forest next to Washington, DC." Researchers now need to work out what distinguishes the B body from the rest of the nuclear contents and how it controls dispersal of Arrest.

1. Oas, S.T., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201405058>.
2. Bryantsev, A.L., et al. 2012. *Dev. Cell.* 23:664–673.
3. Dhaenens, C.M., et al. 2011. *Biochim. Biophys. Acta.* 1812:732–742.
4. Berger, D.S., et al. 2011. *PLoS ONE.* 6:e19274.

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