Breaking the code for nuclear domains: the case of B-bodies

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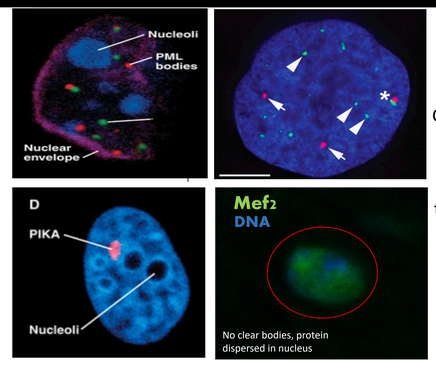
ABSTRACT

The eukaryotic nucleus is a busy place with a high concentration of proteins performing diverse functions. Despite the crowded environment and absence of internal membranes, nuclear organization remains structured via distinct areas that selectively recruit and release proteins – nuclear domains. How nuclear domains are formed and maintained is currently not well understood.

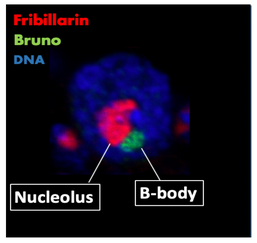
B-bodies are a newly discovered type of nuclear domains that can be found in the indirect flight muscles of Drosophila; their principle resident is the RNA-binding protein Bruno. This protein has 3 RNA-recognizing motifs (RRM1,2,3) as well as two disordered regions. B-bodies are highly dynamic domains which appearance changes during myogenesis. We used ectopic expression of GFP-tagged Bruno mutants to identify critical regions in the primary sequence that are required for B-body targeting.

Our results indicate that a functional RRM2 domain is required for Bruno trafficking to B-bodies. In contrast, the functionality of RRM3 domain is dispensable in this regard. We speculate that RRM2 domain may be necessary to interact with yet unknown structural RNA molecule(s) in order to retain Bruno in B-bodies.

1. INTRODUCTION



Examples of nuclear domains include: Cajal bodies, nucleoli, PML bodies and PIKA domains. Nuclear domain proteins are typically RNA binding proteins, so consequently Mef2 cannot form nuclear domains.

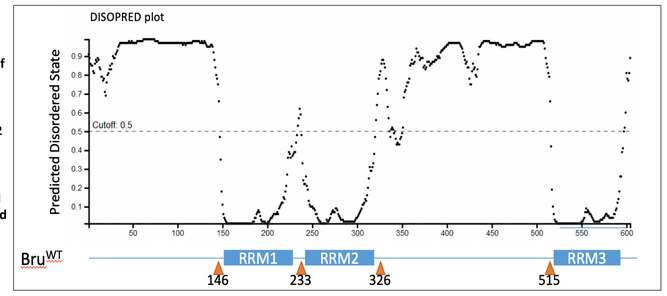


The B-body is a novel nuclear domain.

Novel Nuclear Domain

- **Discovered in 2014***
- **Located in the nuclei of flight muscles of Drosophila Melanogaster.**
- **Composed of at least 2 RNA-binding proteins regulating alternative pre-mRNA splicing: Bruno and Musclebind**
- **Dynamic formation and disassembly**

*tOas et al., *Journal of Cell Biology* (2014): 895-908.



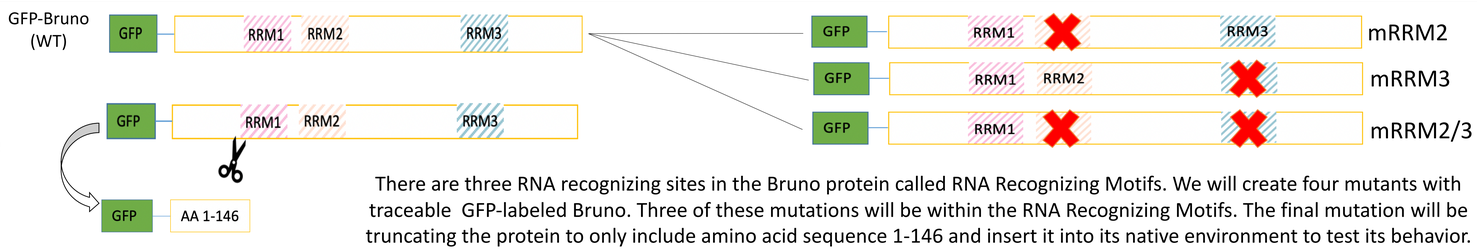
The wild type protein Bruno contains two distinct disorder regions and three ordered regions. The ordered regions are RNA recognizing motifs.

2. RESEARCH QUESTIONS

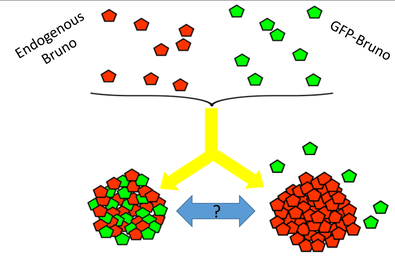
What parts of the Bruno protein are necessary to form B-Bodies within the nucleus?

What is the minimal amino acid sequence within the Bruno protein that is sufficient in forming B-Bodies?

3. METHODS

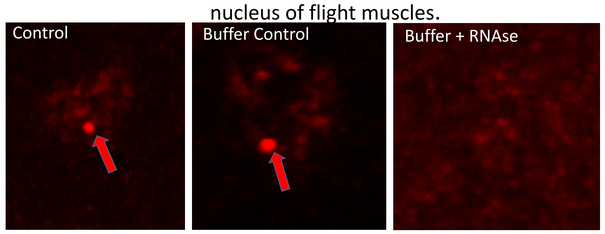


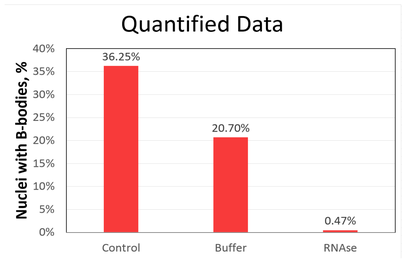
There are three RNA recognizing sites in the Bruno protein called RNA Recognizing Motifs. We will create four mutants with traceable GFP-labeled Bruno. Three of these mutations will be within the RNA Recognizing Motifs. The final mutation will be truncating the protein to only include amino acid sequence 1-146 and insert it into its native environment to test its behavior.



4. RESULTS

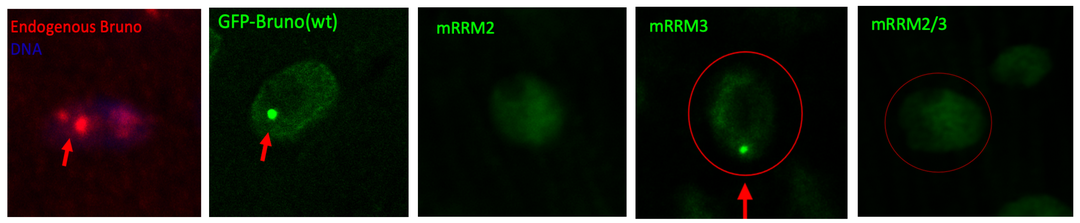
RNA is needed to maintain the integrity of the B-body in the nucleus of flight muscles.



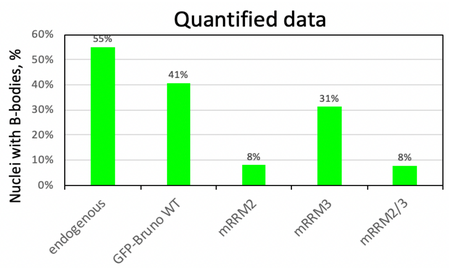


The endogenous Bruno was treated in three separate groups; control, buffer control and buffer+RNAse. The distribution of endogenous Bruno (red) was stained with anti-Bruno and was studied using immunofluorescences. Red arrows indicate B-bodies.

RNA binding motif, RRM2, is necessary for the formation of B-bodies in the nucleus of flight muscles.

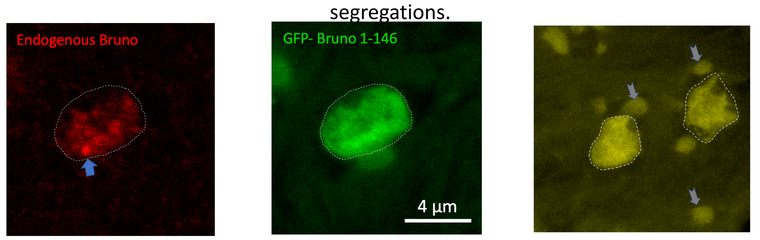


Bruno variant	B-body visual quality
GFP- wt	+++
GFP-mRRM2	+/-
GFP-mRRM3	+++
GFP-mRRM2/3	+/-



Distribution of endogenous Bruno protein (red) and GFP-Bruno proteins (green) was studied by immunofluorescence using anti-Bruno and anti-GFP antibodies, respectively. Arrows indicate B-bodies.

Bruno 1-146 does not accumulate in b-bodies, but it does form non-specific segregations.



Arrow indicates accumulations of the Bruno protein. The distribution of the endogenous Bruno protein (red) and GFP-Bruno mutant (green) were studied with immunofluorescence using anti-Bruno and anti-GFP antibodies.

5. CONCLUSIONS

Formation of B-bodies depends on the presence of RNA molecules.

RNA-binding ability of Bruno protein is necessary to accumulate in B-bodies.

RRM2 domain is responsible for driving Bruno into B-bodies

N-terminal unstructured region in necessary to segregate Bruno from intracellular environment

Apparently, a combination of unstructured region and RNA-binding domain is required to accumulate Bruno in B-bodies

6. FUTURE DIRECTIONS

In the future we will begin creating new mutants by adding the RRM's to the first unstructured region.

7. ACKNOWLEDGEMENTS

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