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THE EFFECT OF THE LOSS OF NONSENSE-MEDIATED MRNA DECAY ON NATURALLY OCCURING VARIANTS

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Abstract

Nonsense-mediated mRNA decay (NMD) is a biological process present in all eukaryotic organisms that functions to break down mRNAs containing nonsense mutations. In this way, the cell prevents the translation of truncated proteins, which can lead to lethality within the organism. To assess how the loss of NMD affects the viability of individuals carrying naturally occurring genetic variants in this experiment, I crossed Drosophila melanogaster (fruit fly) mutants that lack NMD pathway function to a series of lines that contain naturally occurring variants. The source of these variants is the well characterized Drosophila Genome Reference Panel (DGRP). Of the 168 DGRP lines tested, I discovered that 11 lines showed significant viability difference when NMD is inactivated. Of these 11 lines, 9 showed a significantly decreased viability, while the other two other lines had a significantly increased viability. Our major conclusion is that the majority of naturally occurring variants have no significant influence on the viability of the fly when NMD is absent. However, a small number of variants do produce a strong effect on viability, presumably because they are detrimental to the organism if not broken down by the NMD pathway

Introduction

The central dogma of biological processes is the conversion of information stored in DNA into proteins, through the processes of transcription and translation. For the proper functioning and survival of an organism, these processes must be performed with great fidelity. Nonsense-mediated mRNA decay (NMD) is a process occurring in conjunction with translation that surveilles mRNAs containing nonsense mutations, also called premature termination codons (PTCs), and causes their rapid degradation. In the absence of NMD, mRNAs containing nonsense mutations are translated into truncated proteins. These truncated proteins can cause lethality within the organism due to misfolding or the buildup of unusable proteins lacking critical domains. While this role for NMD has been clearly demonstrated in the laboratory (1), it is not yet known how the removal of the NMD pathway affects viability of organisms containing naturally occurring genetic variants.

NMD is performed by several proteins which work together to identify and break down the PTC-containing mRNAs during translation. The primary *trans*-acting proteins of the NMD machinery are called *Upf1* and *Upf2* (1). Within *Drosophila*, complete loss of *Upf1* and *Upf2* abrogates the NMD pathway and leads to organismal lethality (2). This lethality is primarily due to the misregulation of *Gadd45* expression, a naturally occurring NMD target. (2) In normal flies, *Gadd45* mRNA is constitutively degraded by the NMD pathway, but in an *Upf1* or *Upf2* mutant the *Gadd45* mRNA is overexpressed and the buildup of Gadd45 protein causes lethality (2). Loss of *Gadd45* in itself does not have an adverse effect on the viability of the fly, but can suppress the

lethality observed in *Upf1* and *Upf2* mutants. Thus, by using *Gadd45* mutants to abrogate the main effector of NMD mutant lethality, I can identify other effects of loss of NMD on viability.

My primary goal of this project was to understand how the loss of NMD affects the viability of flies contain naturally occurring genetic variants. Outside a lab environment, most organisms- including humans- contain naturally occurring variants. Variants are differences between the genomes of organisms of the same species, and potentially lead to different interactions between NMD and mRNA. Currently, NMD has only been analyzed in lab bred stocks, which due to inbreeding and lack of selective pressures, are genetically homogeneous. As a source of variation, I used the flies of the *Drosophila* Genome Reference Panel (DGRP) (3). The DGRP comprises of *Drosophila* stocks in which individual females were obtained from the wild and interbred for 20 generations to generate independent isogenic lines, each carrying a unique set of variants. Sequence analysis showed each line contains about 50,000 single nucleotide polymorphisms (SNPs) different from the *Drosophila* Reference Genome. Of those 50,000 SNPs, approximately a hundred were found to be nonsense variants and will likely be NMD substrates.

Materials and Methods

To test the viability of polymorphisms in the absence of NMD, I performed crosses between each DGRP line and a double mutant of *Upf2* and *Gadd45*. (Fig. 1) Each cross produced two different male genotypes that portrayed different phenotypic characteristics: the *Upf2/Y; Gadd45/+* flies are white eyed and represent the flies with an absence of NMD. The other genotype, *FM7i/Y; Gadd45/+*, are flies phenotypically characterized by red Bar^{-} (narrow) eyes. These are $Upf2^{+}$, so the viability is still controlled by the expression of NMD. The *Gadd45* mutant in each genetic background leads to partial rescue of NMD mutant lethality. Each cross of the lines was performed until at least 100 offspring were scored. Viability rates were determined using a ratio of collected white eyed (NMD mutant) males over the total males collected from the cross.

Figure 1:

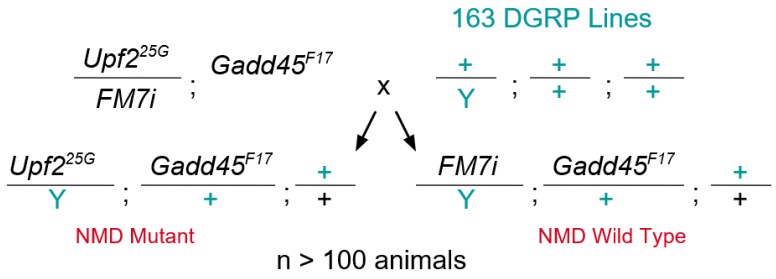

Figure 1: Cross performed between each DGRP lines and the NMD mutant. $Upf2^{25G}$ is a mutant which greatly reduces the NMD pathway function; $Gadd45^{F17}$ nullifies the expression of *Gadd45*, partially suppressing the lethality caused by losing NMD. *FM7i* is a balancer used in the cross. The black text represents the genes obtained from NMD mutant lab stock while turquoise represents the variants obtained from the DGRP lines. *Upf2* is located on the X chromosome so only hemizygous mutant males were scored.

Results

To assess the viability of variants within the DGRP lines, I took advantage of a mutation of *Upf2* called $Upf2^{25G}$. $Upf2^{25G}$ is a hypomorphic mutation which greatly reduces the NMD pathway within the fly, but allows some survival, and thus allows the assessment of the change of viability. As a hemizygote male (*Upf2* is on the X chromosome), $Upf2^{25G}$ has about a 15% viability. When heterozygous, a null mutation of *Gadd45* increases $Upf2^{25G}$ viability to ~45%, thus allowing me to more efficiently detect reduction or increase of viability.

I counted at least 100 animals from each cross, allowing for the detection of small (up to ~20%) changes in viability. For each line, the data was used to determine which of the 168 lines crossed possessed a significant change in survival. Using binomial statistical analysis, I determined that only 11 lines had significant viability differences from the average 46% viability rate. I found a large majority of the flies clustered around the average viability suggesting that most truncated proteins produced by losing NMD do not cause overt lethality (Fig. 2).

Figure 2:

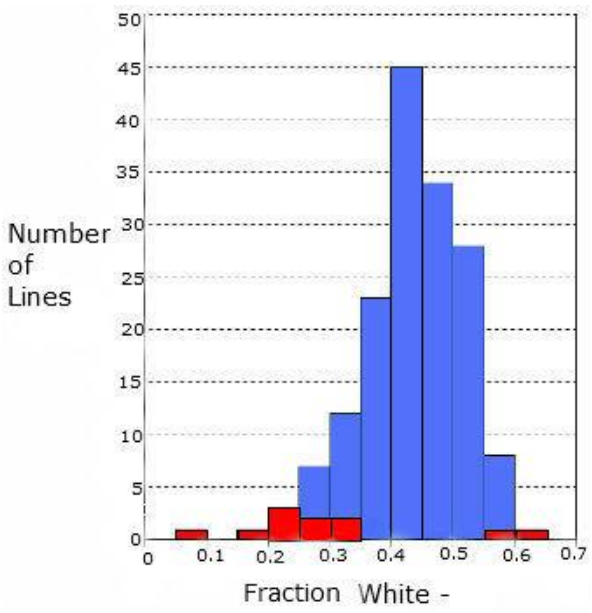

Figure 2: Blue bars on the graph represent the number of lines associated with each given viability rates. The red bars represent the lines with significantly lower or higher viability in comparison to the average viability. It can be seen that most lines cluster around the average viability of 46%, showing that most naturally occurring variants do not cause lethality within organisms lacking the NMD pathway.

11 lines show significant deviation from the average; 9 lines had decreased viability, with two of these lines showing a very strong decreased viability. The other two significant lines were found to have an increase in viability, one a strongly so (Fig. 2).

Discussion

Before this study, a possible requirement for NMD in repressing potentially deleterious naturally occurring variants had not been experimentally tested. My results reveal for the first time that most naturally occurring variants do not lead to dominant detrimental effects on viability within organisms lacking NMD pathway function. Thus, in contrast to previous assumptions, (4), I conclude NMD is unlikely to play a major role in shaping the genome evolution in the fly.

One caveat to my experimental design is that variants in the RAL lines I used are known to be homozygous viable, due to the 50 generations of interbreeding used to create the lines. Thus, it is possible that recessive lethal alleles (not present in RAL lines) can be converted to lethal dominant mutations when the NMD pathway is lost. To test this possibility, I will examine variants obtained from naturally occurring heterozygous flies to test the true effects of the loss of NMD pathway function on the viability rates of naturally occurring variants.

While most lines do not possess a change in viability rates, a small subset of lines do show a strong decrease in viability, in an NMD mutant background, thus providing a basis for the narrowing which genes are mutated in such a way that loss of NMD causes them to become detrimental. I will use genetic mapping and candidate gene approaches to identify the variants in these lines and their location on the chromosome that lead to this affect in the change in viability of the flies. The genetic mapping will help identify the gene family or class of gene product possibly responsible for the observed viability difference found in the significant lines. This mapping will also show if a particular gene family is responsible for every change in viability found in the significant lines, or if each line contains a unique set that causes viability differences. Since every DGRP lines has been sequenced, I can also use the extracted list from the database to identify all stop codon encoded genes present in each significant line. Private variants are polymorphisms unique one individual line. Since few lines lead to changes in viability such variants will be a primary focus, since they are the best candidates for specifically affecting the viability of the significant line. Since most of the lines in my screen had the expected viability rates, private variants are most likely the cause for the viability rate differences observed in the significant lines.

Finally, inhibition of NMD has been proposed as a potential treatment for alieving genetic disease (5). My results suggest that removal of the NMD pathway is compatible with viability even in heterogeneous genetic backgrounds such as is observed in human populations.

References

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