Genetic mapping of the \( p47^{L.3.2} \) mutation in \textit{Drosophila melanogaster}

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Abstract

An EMS-based forward genetic screen was conducted in an apoptotic null background to identify genetic aberrations that contribute to regulation of cell growth in \textit{Drosophila melanogaster}. The current work maps the genomic location of one of the identified mutants, \textit{L.3.2}. Genetic crosses conducted through the Fly-CURE consortium determined that the gene locus for the \textit{L.3.2} mutation is \textit{p47} on chromosome 2R.
Figure 1.

The Dark	extsuperscript{82}, p47	extsuperscript{L.3.2} mutant results in abnormal cellular structure and hair growth: A.) Lateral view of FRT42D, Dark	extsuperscript{82} mosaic control eye. Eye tissue from control mosaic (FRT42D, Dark	extsuperscript{82}) display a slight R\textgreater{}W phenotype as previously identified (Akdemir et al, 2006). The Dark mosaic eyes have a regular eye shape and size. B.) Lateral view of eyes from mosaic FRT42D, Dark	extsuperscript{82}, p47	extsuperscript{L.3.2}. Dark	extsuperscript{82}, p47	extsuperscript{L.3.2} mosaic eyes displayed W\textgreater{}R phenotypes. These also depicted a strong rough eye phenotype and overall reduction in eye size. All eye images captured at 40x. C.) A screenshot of the JBrowse genome browser accessed through FlyBase.org, highlighting the region that failed to complement, via crosses to Df(2R)ED1715 (BDSC 8931) and Df(2R)ED1673 (BDSC 9062); 2R:7,395,885.. 7,489,834. Subsequent single allele complementation mapping demonstrated that the allele, p47	extsuperscript{L.3.2} (Larkin, 2021) failed to complement (red asterisk).
An ethyl methanesulfonate (EMS) based genetic screen was conducted in *Drosophila* aimed at identifying regulators of cell growth, cell division, and development. EMS mutations were induced in *FRT42D, Dark* <sup>82</sup> flies, which are null for *Dark* activity (<sup>Dark</sup><sup>82</sup>) and possess a mw<sup>+</sup> cassette. Conducting the screen in a *Dark* null mosaic background blocks apoptosis, facilitating identification of a broader range of genetic contributors to cell growth and development (Akdemir et al, 2006, Kagey et al., 2012). Mutagenized flies (*FRT42D,Dark*<sup>82</sup>,L.3.2) were crossed to *FRT42D; Ey-Flp* flies to yield mosaic clones of homozygosity in eye tissue. Control *FRT42D,Dark*<sup>82</sup> x *FRT42D; Ey-Flp* crosses result in eyes with a fairly normal developmental patterning and a slight increase in the ratio of red:white pigmentation to about 60:40 (Figure 1A). Progeny that were mosaic for *FRT42D,Dark*<sup>82</sup>,L.3.2 yielded progeny with a rough eye pattern depicting disrupted ommatidial structure and greater white:red pigmentation, shifting from the *Dark*<sup>82</sup> mosaic control (Figure 1B).

In order to identify the gene locus responsible for the rough phenotype observed in the L.3.2 mosaic flies, *FRT42D,Dark*<sup>82</sup>,L.3.2 virgin female flies were crossed in serial to males from the Bloomington Stock Center 2R deficiency kit (Cook et. al., 2012). Complementation crosses were conducted by undergraduate students at the following Fly-CURE consortium institutions: University of Detroit Mercy, Nevada State University, and Ohio Northern University. From these crosses, Df(2R)ED1715 (BDSC 8931) and Df(2R)ED1673 (BDSC 9062) failed to complement, narrowing the gene region to 2R:7,395,885..7,489,834 (Fig. 1C, Table 1). Single gene mutants of alleles from this narrowed down region were then crossed to *FRT42D,Dark*<sup>82</sup>,L.3.2 flies. Crosses between Mi[MIC]p47<sup>M113219</sup>/SM6a (BDSC 58033) x *FRT42D,Dark*<sup>82</sup>,L.3.2 failed to complement, while crosses to three adjacent genes, *Inos* (P[SUPor-P]Inos<sup>KG07679</sup>/CyO (BDSC14921)), *Aldh* (P[EPgy2]Aldh-III<sup>KY12056</sup>/CyO (BDSC 20339), and *wec* (P[iaclW]wec<sup>k08815</sup> /CyO (BDSC 10818)), resulted in complementation (Table 1). This data confirms L.3.2 as a novel lethal allele of p47, p47<sup>L.3.2</sup>.

Based on the genetic data presented, we determined that the *L.3.2* phenotype genetically mapped to the gene *p47*. This p47<sup>L.3.2</sup> allele is located at 2R:7,465,018..7,466,759 (Figure 1C). p47 is known to regulate the ATPase activity of p97, a member of the AAA+ superfamily of ATPases (Meyer et. al., 1998). Molecular interactions between p97 and p47 are implicated in a variety of cellular processes, including ER and golgi membrane fusion (Kondo et. al., 1997; Uchiyama and Kondo, 2005), mitotic spindle disassembly (Cao et. al., 2003) nuclear envelope formation (Hetzer et. al., 2001), and autophagosome biogenesis (Krick et. al., 2010). Evidence for a role of p47 in regulating cell growth comes from studies of the human p47 ortholog, NSFL1C. Studies in the MCF10AT breast cancer model show that NSFL1C is a target of epidermal growth factor signaling (Chen et. al., 2007), while recent work in a human adult T-cell leukemia/lymphoma (ATLL) cell line demonstrates that inhibition of autophagy-mediated p47 degradation ultimately leads to restoration of caspase-3 mediated apoptosis and decreased rates of cell growth (Fauzi et. al., 2021). The connection between loss of p47 and apoptosis induction, may provide insight as to why p47 was missed in the initial round of Flp/FRT screens, and suggests p47 may be a conditional growth suppressor. Finally, the rough eye phenotype observed in the p47<sup>L.3.2</sup> mutant flies agrees with early work on p47 in flies demonstrating that a gain-of-function mutation in p47 disrupts rhodopsin processing, suggesting a role for p47 in proper eye development (Sang and Ready, 2002). Further functional characterization of p47 protein activity in *Drosophila* will be required to delineate its role in eye development.

### Table 1. Complementation results with BDSC 2R deficiency lines. Additional deficiency lines not found in the 2R kit and individual alleles crossed to *FRT42D, Dark*<sup>82</sup>, L.3.2 flies are also displayed. Deficiency mapping narrowed down the region of the L.3.2 mutation to 2R:7,395,885..7,489,834, which contains the gene p47. Crosses of *FRT42D,Dark*<sup>82</sup>,L.3.2 x y<sup>1,w*;MI[MIC]p47<sup>M113219</sup>/SM6a* failed to complement, establishing p47 as the location of the L.3.2 mutation.

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>BDSC Stock #</th>
<th>Region</th>
<th>Complementation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df (2R)ED1715</td>
<td>8931</td>
<td>2R:7,326,951..7,916,923</td>
<td>Fail to Complement</td>
</tr>
<tr>
<td>Df (2R)ED1725</td>
<td>8941</td>
<td>2R:7,613,924..8,156,045</td>
<td>Fail to Complement</td>
</tr>
<tr>
<td>Df (2R)ED1673</td>
<td>9062</td>
<td>2R:6,985,802..7,533,553</td>
<td>Fail to Complement</td>
</tr>
</tbody>
</table>
### Additional Deficiency Lines

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>BDSC Stock #</th>
<th>Region</th>
<th>Complementation Result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df (2R)BSC264</td>
<td>23163</td>
<td>2R:7,395,885..7,489,834</td>
<td>Fail to Complement</td>
</tr>
<tr>
<td>Df (2R)BSC263</td>
<td>23162</td>
<td>2R:7,146,864..7,447,410</td>
<td>Complement</td>
</tr>
</tbody>
</table>

### Single Genes Tested Within Fail to Complement Region

<table>
<thead>
<tr>
<th>Gene</th>
<th>BDSC Stock #</th>
<th>Allele</th>
<th>Complementation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>p47</td>
<td>58033</td>
<td>Mi{MIC}p47^{M113219}, 2R:7464671..7467108</td>
<td>Fail to Complement</td>
</tr>
<tr>
<td>Inos</td>
<td>14921</td>
<td>P{SUPor-P}Inos^{KG07679}, 2R:7454134..7459668</td>
<td>Complement</td>
</tr>
<tr>
<td>Wech</td>
<td>10818</td>
<td>P{lacW}wech^{K08815}, 2R:7476659..7492017</td>
<td>Complement</td>
</tr>
<tr>
<td>Aldh-III</td>
<td>20339</td>
<td>P{EPgy2}Aldh-III^{EY12056}, 2R:7464829..7479226</td>
<td>Complement</td>
</tr>
</tbody>
</table>

* The Fly-CURE consortium employs the following guidelines for determination of genetic complementation indicated in this table. 1. Crosses designated as 'Complement' resulted in at least 10 straight wing progeny when scored. 2. Crosses designated as 'Fail to Complement', resulted in at least 100 curly wing progeny and 0 straight wing progeny when scored.

### Reagents

- w\(^-\); FRT42D, Dark\(^{82}\)/CyO (Akdemir et al., 2006)
- w\(^-\); FRT42D Dark\(^{82}\), p47\(^L.3.2\)/CyO (this study)
- w\(^-\); FRT42D; Ey-Flp (BDSC 8211)

Bloomington Drosophila Stock Center 2R Deficiency Kit (Cook et al., 2012)

**Additional Bloomington Stocks (See Table 1 for complete list of stock numbers)**

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**References**


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