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Synthetic Lethality Induced by Toxic Polyglutamine Tract II: A Survey in Drosophila

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Abstract

Mutant proteins containing an expanded polyglutamine tract induce cell death and cause neurodegenerative diseases. These toxic proteins interfere with a variety of physiological pathways, but the key interactions between the toxins and cellular factors remain unclear. To model the diseases in Drosophila, the *GMR-Gal4/UAS* gene expression system has been used extensively, which operates in the eyes. By using the system, genome-wide studies have resulted in the isolation of functionally diverse groups of Drosophila genes that interact with the disease proteins. We previously reported that coexpressing the Drosophila *Dikar* gene and an expanded polyglutamine tract by *GMR-Gal4/UAS* induced a synthetic lethality. We carried out follow-up experiments to isolate additional synthetic lethal alleles. Our data provide evidence that synthetic lethality associated with expressing an expanded polyglutamine tract is more common than thought to be and could have escaped the conventional genetic screens. Our results also suggest that 1) the gene expression system is leaky, allowing expression outside of the primary target eye cell types; 2) expressing an expanded polyglutamine tract is extremely toxic to cells; and 3) combining the leaky expression and the toxicity results in a lethal-prone condition. Thus, genetic modifications to the disease proteins' acute toxicity could frequently lead to synthetic lethality. However, synthetic lethal alleles are excluded from most conventional screens, necessitating alternative approaches such as a two-step method used in this study to isolate the modifiers. Since synthetic lethality reflects essential genetic buffering networks, studying these alleles may hold the keys to identify the critical interactions in the disease development between the toxic proteins and the physiological pathways.

Keywords

Polyglutamine Diseases, Drosophila, Genetic Screen, *GMR-Gal4/UAS* System, Synthetic Lethal,

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1. Introduction

When a protein contains a long tract of 40 or more glutamine amino acid residues, it acts as a cellular toxin, frequently resulting in cell death. In humans, a group of nine genes give rise to neurodegenerative diseases, when each is mutated to produce a toxic expanded polyglutamine tract [1]. While the fundamental questions of how an expanded polyglutamine tract leads to cellular toxicity remain unclear, studies using various genetic manipulations in model organisms have implicated an array of physiological pathways that influence or modify the detrimental effects of the expanded polyglutamine proteins [2]-[5]. For example, numerous cellular pathways have been implicated to influence Huntington's disease, including many aspects of mitochondrial functions [6], and transcriptional regulation [7]. In addition to the intrinsic toxicity of an expanded polyglutamine tract [8]-[10], studies have also shown that domains flanking the toxic polyglutamine tract in a disease protein contribute to the neurodegenerative disease development [11]-[13].

Using model organisms, large collections of modifiers have been isolated primarily by monitoring the effects of expressing expanded polyglutamine proteins in mutant backgrounds [14]-[16]. In Drosophila, the *GMR-Gal4/UAS* binary gene expression system, which operates in the eyes, is introduced as a tool to express the expanded polyglutamine proteins and to identify the modifiers [17] [18]. *GMR-Gal4/UAS* is based on a construct of *GMR* (glass multiple reporter) that carries multiple copies of an eye enhancer element to generate a conditional expression in the eyes [19] [20]. In genome-wide screens to identify modifiers, this system relied on modifications to the adult eye morphological characteristics. It is not only sensitive but also efficient in identifying the modifiers and has resulted in the identification of large groups of functionally diverse modifiers [17] [21].

Recently, we came across a synthetic lethality phenomenon resulting from coexpressing a toxic polyglutamine tract and a Drosophila gene, *Dikar*, with the *GMR-Gal4/UAS* system [22]. The synthetic lethality occurred when the simultaneous overexpression of two gene products, Dikar and an expanded polyglutamine tract, led to organismal death, whereas expressing either of them individually did not affect their viability. Since this was the first known case of synthetic lethality associated with expressing a toxic polyglutamine tract, we set out to explore the possibility that the neurotoxin-induced synthetic lethality might be a common phenomenon that has so far escaped detection. Here we describe the results from an effort to identify additional Drosophila alleles of this type of synthetic lethality. Our data suggest that the presence of a toxic polyglutamine tract induces a sensitized lethal-prone condition that could easily lead to synthetic lethality when combined with mutations.

2. Materials and Methods

2.1. Drosophila Strains and Culture

Flies were cultured on standard corn/agar media at 25˚C, unless otherwise stated. The strains and alleles used in this study were obtained from the Bloomington Drosophila Stock Center and other sources as described previously [22]. Through meiotic recombination, we generated a set of stocks, each of which contains a Chromosome 2 carrying two transgenes. These include 1) *longGMR-Gal4>UAS-httex1p-97QP-s/CyO*, 2) *longGMR-Gal4>UAS-httex1p-97QP-w/CyO*, and 3) *longGMR-Gal4>UAS-Sca3-Q78/CyO*, and 4) *shortGMR-Gal4>UAS-Sca3-Q78/CyO*.

2.2. Survival Analysis

For survival analysis, a genetic cross was set up to generate two types of progeny at equal frequency: one carrying a genotype of interest and the other, a sibling genotype, carrying a *CyO* balancer chromosome as an internal control group, similar to those described previously [22]. If the viability of a genotype is unaffected by either expressing a transgene, or by coexpressing two transgenes, the number of the progeny should be evenly divided by the two genotypes, which are recognized as carrying straight wings or carrying curly wings (determined by the dominant *Cy* mutation on the *CyO* balancer). On the other hand, if the transgenic expression causes detrimental effects on the survival of a genotype, then straight-winged progeny would be significantly lower in number

relative to the curly-winged ones. In the cases where synthetic lethality is induced, only curly-winged control progeny would be produced. Each of the crosses was repeated in vials of a triplet.

2.3. Fluorescent Assay of a GFP Reporter Gene Activity

Eye discs of the wandering third instar larvae were dissected out in a phosphate-buffer saline (PBS, 130 mM NaCl, 7 mM $Na_2HPO_4 \cdot 2H_2O$, 3 mM $NaH_2PO_4 \cdot 2H_2O$, pH 7.0) and the discs were examined immediately in PBS by using an Olympus microscope. Green fluorescent images were captured using a digital camera mounted on the microscope (Spot, Diagnostic Instrument, Inc.). Using the ImageJ software (the National Institutes of Health), each color image was split into three channels and the green channel was selected for quantitative analysis. The strongest fluorescent signals from the posterior edge of each disc (the first 3 - 5 rows of the developing ommatidia) were selected as Region-Of-Interest (ROI) and measured as Mean Grey Value in ImageJ.

3. Results

When induced by the *GMR-Gal*4/*UAS* system in the eyes, *Dikar*'s expression caused compound external eye defects, including a rough surface and disrupted arrays of the bristles on the eye surface [22]. In addition, coexpressing *Dikar* and an expanded polyglutamine tract induced synthetic lethality at pupal stages. Because a conventional F1 genetic screen with a single-step approach could not be used to isolate synthetic lethal alleles, here we took a two-step approach. In the first step, *UAS*-alleles were randomly generated by *P* element insertions and were selected for their capabilities of inducing eye defects when driven by a *GMR-Gal*4 driver. In the second step, the *UAS*-alleles were individually coexpressed with a protein containing an expanded polyglutamine tract to detect synthetic lethality.

3.1. Isolation of Alleles That Disrupt Eye Development When Overexpressed

To generate alleles that could be overexpressed by using *GMR-Gal*4/*UAS*, a genetically engineered *P* element, *XP*, was used [22] [23]. Each end of the *XP* element contains an *UAS*-expression construct that is orientated in the outward direction, so it is capable of overexpressing flanking endogenous genes. Using the *XP* element to generate *UAS*-alleles that overexpress Drosophila genes is also extremely efficient, since the transposon preferentially inserts into the promoter regions of Drosophila genes [24]-[26].

We activated an *XP* element on *Chromosome* 3, *d*09108, by using a transposase source, *Delta* 2 - 3, to generate insertions randomly throughout the genome (**Figure 1**). A genetic screen was followed to identify *UAS*-alleles that disrupt eye development when overexpressed with a *Gal*4 driver, *shortGMR-Gal*4 [19] [27]. Briefly, in bottles, males carrying both *d*09108 and *Delta* 2 - 3 were crossed to females carrying the *shortGMR-Gal*4 driver (Cross 1 in **Figure 1**). Single male progeny displaying eye defects, which were detected under a dissecting microscope, were selected from the mass mating. Each of these males was a candidate carrying a newly generated *XP* element, which induces the eye defect by *shortGMR-Gal*4. Subsequently, each of the *XP* elements was mapped to the major chromosomes (Cross 2, **Figure 1**). Only the *XP* elements located on *Chromosome* 2 were retained for further investigation.

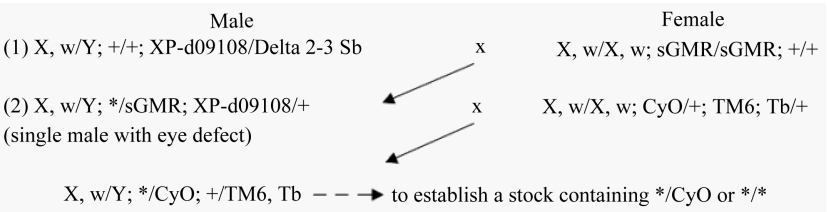

Figure 1. A scheme to isolate *UAS*-alleles that disrupt eye development when overexpressed. A *UAS*-carrying *XP* element, which is located on *Chromosome* 3, *XP-d*09108, was used as the starting element to transpose in the presence of a transposase source, *Delta* 2-3. After activating the *XP* element, single male progeny displaying eye defect, which was induced by *shortGMR-Gal*4 (*sGMR*), was recovered from the first cross as a candidate of harboring a *UAS*-allele. Insertions on *Chromosome* 2 were of interest. Such a newly inserted *XP* on *Chromosome* 2, indicated by an asterisk, was identified when no progeny with defective eyes were produced from the second cross. Subsequently, individual stocks were established to carry an *XP* insertion on *Chromosome* 2.

3.2. Eye Defects Associated with the Isolated *UAS*-Alleles

From the genetic screen in **Figure 1**, five strains carrying *XP* insertions on *Chromosome* 2 were obtained. The eye defects, which were induced by overexpressing the *UAS*-alleles using *shortGMR-Gal*4, could be roughly divided into several types, including uneven surface, oily-looking surface, eye color change, irregular distribution of the eye colors, and loss of the external bristles. The defects were often superimposed on each other, resulting in compound eye phenotypes as shown in **Figure 2(a)**.

In addition to *shortGMR-Gal*4, we examined the effect of another related *Gal*4 driver, *longGMR-Gal*4. Both of the GMR drivers contain pentamerized glass-binding sites, enabling them to express the Gal4 protein in the eyes [19] [27]. While *shortGMR-Gal*4 carries slightly shorter glass-binding core sites of 28 bp, *longGMR-Gal*4 carries 39bp glass-binding sites. *shortGMR-Gal*4 induced more severe eye phenotypes than *longGMR-Gal*4 when used in experiments to drive gene expression that caused eye defects. Using a GFP reporter gene, *UAS-2xEGFP* [20], we examined the relative expression levels of two *GMR-Gal*4 drivers in the larval eye imaginal discs. We constructed a pair of genotypes expressing GFP, *shortGMR-Gal*4>*UAS-2xEGFP* and *longGMR-Gal*4 > *UAS-2xEGFP*. The results showed that the mean GFP signal level of the *shortGMR-Gal*4>*UAS-2xEGFP* genotype was approximately 8.3 times higher than that of the *longGMR-Gal*4>*UAS-2xEGFP* genotype (59.7 ± 14.9 vs. 7.2 ± 2.5, $p < 0.001$, **Figure 3**).

We then examined the eye defects induced by overexpressing the five *UAS*-strains with either *shortGMR-Gal*4 or *longGMR-Gal*4. The results showed that the degree to which eye defects could be induced by the two drivers is correlated with the level of the drivers' strength. Overall, using the stronger *shortGMR-Gal*4 driver induced more severe eye defects than the *longGMR-Gal*4 driver (**Figure 2(a)** and **Figure 2(b)**). For example, the eyes of a genotype carrying *UAS*-8 showed rougher, or bumpier, surfaces when driven by *shortGMR-Gal*4 than *longGMR-Gal*4. The eye surface of the genotype expressing *UAS*-22 displayed a severer loss of the bristles when driven by *shortGMR-Gal*4 than *longGMR-Gal*4. In fact, even in a wild type background, the *shortGMR-Gal*4 driver by itself induced a defect on the eye surface seen as unevenly distributed bristles under a higher magnification, while the eye of the *longGMR-Gal*4 appeared mostly normal (WT, **Figure 2(a)** and **Figure 2(b)**).

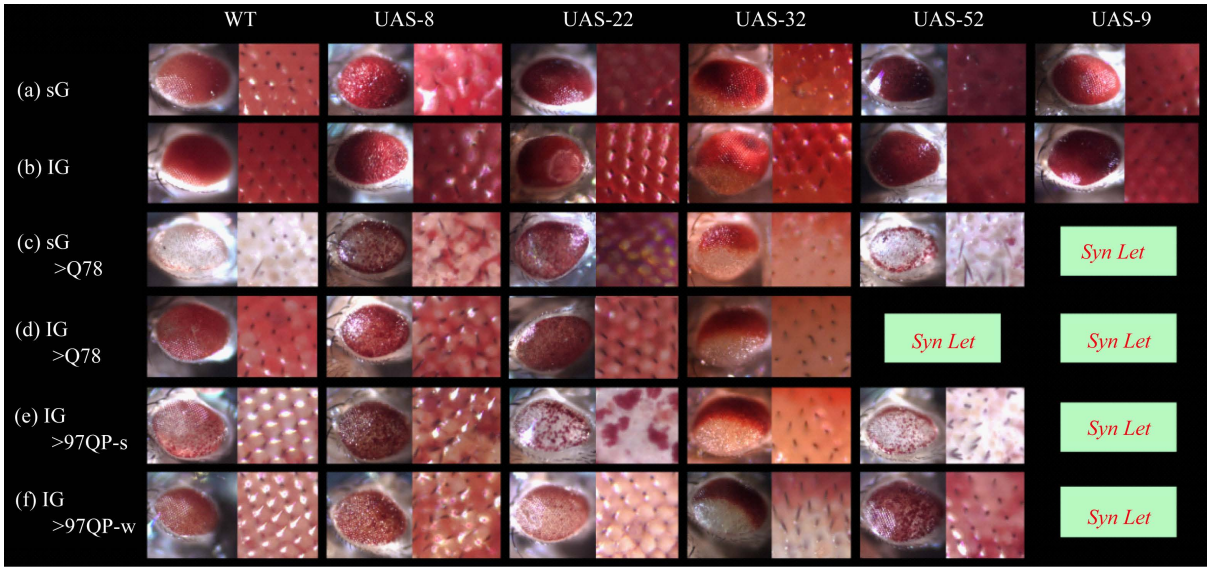

Figure 2. Eye defects associated with five *UAS*-alleles. (a) Expression of *UAS*-alleles with *shortGMR-Gal*4 (*sG*). (b) Expression of *UAS*-alleles with *longGMR-Gal*4 (*lG*). (c) Coexpression of each *UAS*-allele and *UAS-Sca*3-*Q*78 with *shortGMR-Gal*4. *sG>Q*78, *shortGMR-Gal*4 drives *UAS-Sca*3-*Q*78. (d) Coexpression of each *UAS*-allele and *UAS-Sca*3-*Q*78 with *longGMR-Gal*4. *lG>Q*78 indicates that *longGMR-Gal*4 drives *UAS-Sca*3-*Q*78. (e) Coexpression of each *UAS*-allele and *UAS-httex*1*p*-97*QP-s* with *longGMR-Gal*4. *lG*>97*QP-s* indicates that *longGMR-Gal*4 drives *UAS-httex*1*p*-97*QP-s*. (f) Coexpression of each *UAS*-allele and *UAS-httex*1*p*-97*QP-w* with *longGMR-Gal*4. *lG*>97*QP-w* indicates that *longGMR-Gal*4 drives *UAS-httex*1*p*-97*QP-w*. *UAS-httex*1*p*-97*QP-s* is a stronger allele than that of *UAS-httex*1*p*-97*QP-w* [22]. *Syn Let* indicates a synthetic lethal genotype.

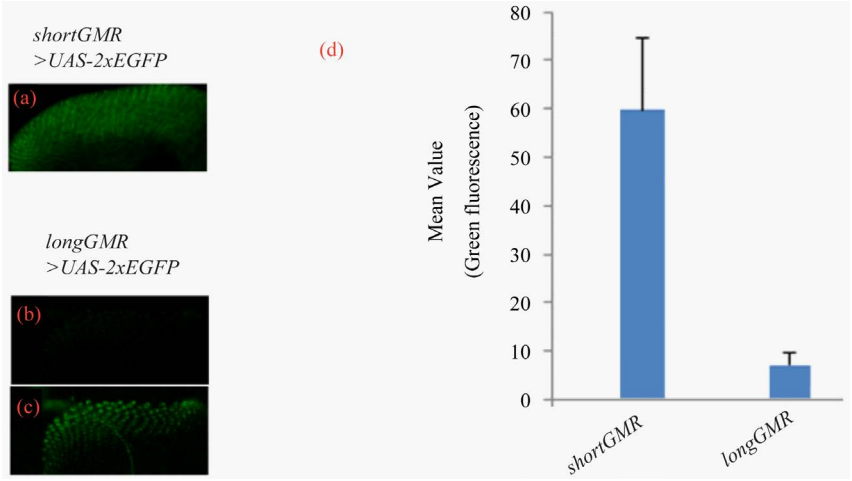

Figure 3. Activity of the *shortGMR-Gal*4 and *longGMR-Gal*4 drivers in eye imaginal discs. (a) (b) A GFP reporter gene, *UAS-2xEGFP*, was used to examine activities of two *GMR-Gal*4 drivers. Representative images were taken from late third instar larval imaginal eye discs of two genotypes, *shortGMR-Gal*4>*UAS-2xEGFP* and *longGMR-Gal*4>*UAS-2xEGFP*. The signals from expressing *UAS-2xEGFP* were captured under identical exposure conditions (Panel (a) and (b)) to allow a direct comparison of the driving strengths between *shortGMR-Gal*4 and *longGMR-Gal*4. (c) The image was identical to that in Panel B, except that it was digitally enhanced by increasing the brightness level. (d) A quantitative analysis of the EGFP reporter signals. The green fluorescent intensity (Mean value) from the *shortGMR-Gal*4>*UAS-2xEGFP* genotype (20 discs) was significantly stronger than that of *longGMR-Gal*4>*UAS-2xEGFP* (22 discs) ($p < 0.001$, two-tailed Student's *t*-test). The Mean Grey Value of the fluorescent strength for each disc, which was measured using ImageJ (see Methods), was used to calculate the mean value for each of the genotypes.

3.3. Expressing the *UAS*-Alleles by Two *GMR-Gal*4 Drivers Showed Very Little Negative Effects on Survival

To ask if expression of the *UAS*-alleles affects viability in Drosophila, we expressed the individual *UAS*-alleles with *shortGMR-Gal*4 and assayed for their survival. To determine the survival rate, a genetic cross was used to produce a pair of the sibling genotypes. One genotype expressed the *UAS*-allele, while the other was a wild type control containing a balancer chromosome, *CyO*. If a *UAS*-allele expression induced no detrimental effect on viability, the two sibling genotypes would be produced at the same frequency. On the other hand, if the expression caused a viability problem, the control genotype would be produced at a higher frequency than the sibling genotype expressing the *UAS*-allele. For all of the *UAS*-alleles, the results show that progeny of the genotype expressing an individual *UAS*-allele were produced either at a frequency comparable to the internal wild type control (approximately 50%), or at a high frequency than the control sibling genotype ($p > 0.05$, **Figure 4(a)**). Similar results were obtained when the *longGMR-Gal*4 driver was used to express the individual *UAS*-alleles ($p > 0.05$, **Figure 4(b)**). Thus, none of these alleles, when driven by either of the *GMR-Gal*4 drivers, were detrimental to viability.

3.4. Coexpressing the *UAS*-Alleles with an Expanded Polyglutamine Tract Induced Survival Problems including Synthetic Lethality

The *UAS*-alleles were individually coexpressed with a transgene encoding a polyglutamine tract consisting of 97 glutamine residues, *UAS-httex*1*p*-97*QP-s* [22]. These tests revealed that some of the transgenes when coexpressed were detrimental to the survival, giving rise to reduced viability and even synthetic lethality. Shown in **Figure 5(a)**, coexpressing*UAS*-22 and *UAS-httex*1*p*-97*QP-s* with *longGMR-Gal*4 induced a decreased survival rate, from 52.7% of the control to 44.2% ($p = 0.024$), while coexpressing*UAS*-52 and *UAS-httex*1*p*-97*QP-s* caused a sharp decline on the survival rate to only 16.6% ($p = 0.003$). The strongest effect from the coexpressing tests was a synthetic lethality phenotype that was induced by coexpressing*UAS*-9 and *UAS-httex*1*p*-97*QP-s* with

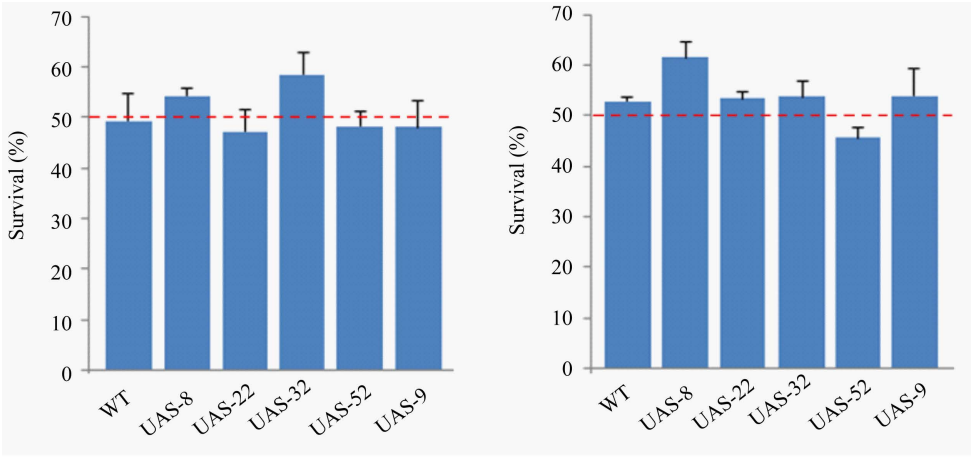

Figure 4. Survival rates of five genotypes expressing *UAS*-alleles with either of the *GMR-Gal*4 drivers. (a) Expression driven by *shortGMR-Gal*4. (b) Expression driven by *longGMR-Gal*4. Each genotype in the figure was generated from a cross, along with another sibling genotype containing a *CyO* balancer. Thus, from each cross, the genotype that expressed a transgene accounted for approximately 50% of the total progeny (red dashed line) when the overexpression did not weaken the survival. However, when a detrimental effect was induced by expressing the *UAS*-gene, the Cy^+ progeny should account for significantly less than 50% of the total progeny from the cross. WT, a wild type control.

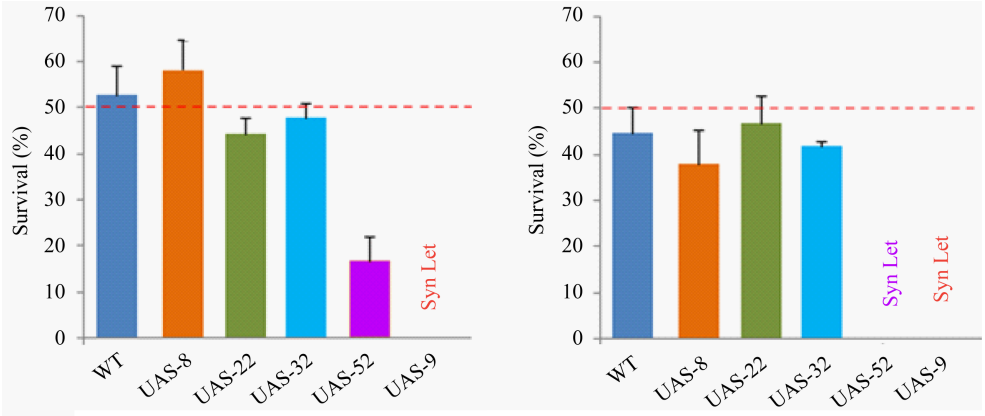

Figure 5. Survival rates of genotypes coexpressing the *UAS*-alleles and two expanded polyglutamine proteins with the *longGMR-Gal*4 driver. (a) Coexpression of each *UAS*-allele and *UAS-httex*1*p*-97*QP-s* with *longGMR-Gal*4; (b) Coexpression of each *UAS*-allele and *UAS-Sca*3-*Q*78 with *longGMR-Gal*4. WT, a wild type control. Syn Let, a synthetic lethal phenotype.

*longGMR-Gal*4. We also coexpressed the *UAS*-alleles with another transgene, *UAS-Sca*3-*Q*78, which encodes a polyglutamine tract consisting of 78 glutamine residues [18]. Data from this later coexpression series showed that *UAS*-9, or *UAS*-52, when coexpressed with *UAS-Sca*3-*Q*78, each generated a synthetic lethality (**Figure 5(b)**).

The *UAS-httex*1*p*-97*QP-s* and *UAS-Sca*3-*Q*78 transgenes do not share any homologous sequences, despite of each encoding an expanded polyglutamine tract. This is because the expanded polyglutamine tracts are encoded in two different ways. While alternate CAA and CAG codons were incorporated in *UAS-httex*1*p*-97*QP*, only the CAG repeats are present in *UAS-Sca*3-*Q*78 [18] [22] [28]. In addition to the differential codon usages, the encoding protein domains flanking the tracts are also unrelated between *httex*1*p*-97*QP* and *Sca*3-*Q*78. Since the only obvious similarity between the transgenes is that each of them encodes a toxic polyglutamine tract, the

synthetic lethality from both of the coexpression tests was most likely mediated through the expanded polyglutamine tracts. To test this possibility, an additional coexpression experiment was carried out by using a third transgene encoding only an expanded polyglutamine tract without flanking domains, *UAS*-41*Q* [29]. Coexpressing*UAS*-9 and *UAS*-41*Q* with *longGMR-Gal*4 also induced synthetic lethality, providing further supporting evidence that the observed synthetic lethality is mediated through the neurotoxic polyglutamine tract.

As a control, we also examined *UAS-Sca*3-*Q*27, which encodes a non-toxic tract of 27 glutamine residues. It did not cause viability issues when coexpressed with *UAS*-9 by *longGMR-Gal*4. Taken together, these results indicated that the synthetic lethality of *UAS*-9 was mediated through a toxic polyglutamine tract.

3.5. Survival Rate and the *GMR-GAL4*/*UAS* Activity

The *UAS*-alleles were isolated for their capabilities of inducing eye defects when overexpressed. Overall, the severity of these eye defects was correlated with the strength of the *GMR-Gal*4 drivers (**Figure 2(a)** and **Figure 2(b)**). To investigate if the strength of the *Gal*4 drivers in the eyes was also correlated with the survival rate of a genotype that coexpresses an *UAS*-allele and an expanded polyglutamine tract, we used the pair of *GMR-Gal*4 drivers, *longGMR-Gal*4 and *shortGMR-Gal*4. The results in **Figure 6** show the survival rates of five genotypes coexpressing the *UAS*-alleles individually with *UAS-Sca*3-*Q*78 by using either *longGMR-Gal*4, or *shortGMR-Gal*4. Expressing *UAS-Sca*3-*Q*78 in a wild type background by the stronger *shortGMR-Gal*4 driver appeared to induce a slightly lower survival rate than that of *longGMR-Gal*4 (0.449 ± 0.047 vs. 0.366 ± 0.031, p = 0.05). Coexpressing*UAS-Sca*3-*Q*78 and *UAS*-8 by *shortGMR-Gal*4 may also slightly reduce the survival rate (0.391 ± 0.062 vs. 0.312 ± 0.018, p = 0.08). However, coexpressing*UAS*-22 and *UAS-Sca*3-*Q*78 by *shortGMR-Gal*4 induced a significantly lower survival rate than that of *longGMR-Gal*4 (0.466 ± 0.050 vs. 0.213 ± 0.016, p = 0.01). Thus, the results from coexpressing each of these *UAS*-alleles and an expanded polyglutamine protein by *shortGMR-Gal*4 showed a tendency of lowering the survival rates by a stronger *Gal*4 driver in the eyes than a weaker one.

However, coexpressing other *UAS*-alleles with *UAS-Sca*3-*Q*78 by *shortGMR-Gal*4 resulted in increased survival rates than that of *longGMR-Gal*4 (**Figure 6**). When *UAS*-32 and *UAS-Sca*3-*Q*78 were coexpressed by *shortGMR-Gal*4, the survival rate was significantly higher than that of the *longGMR-Gal*4 (0.424 ± 0.009 vs. 0.528 ± 0.034, p = 0.027). Furthermore, when *UAS*-52 was coexpressed with *UAS-Sca*3-*Q*78 by *shortGMR-Gal*4, a significant fraction of the genotype survived to adulthood (21.2%), while the coexpression by *longGMR-Gal*4 induced a synthetic lethality (p = 0.002, **Figure 6**). Synthetic lethality was seen when *UAS*-9 was coexpressed with *UAS-Sca*3-*Q*78 by using either *longGMR-Gal*4 or *shortGMR-Gal*4.

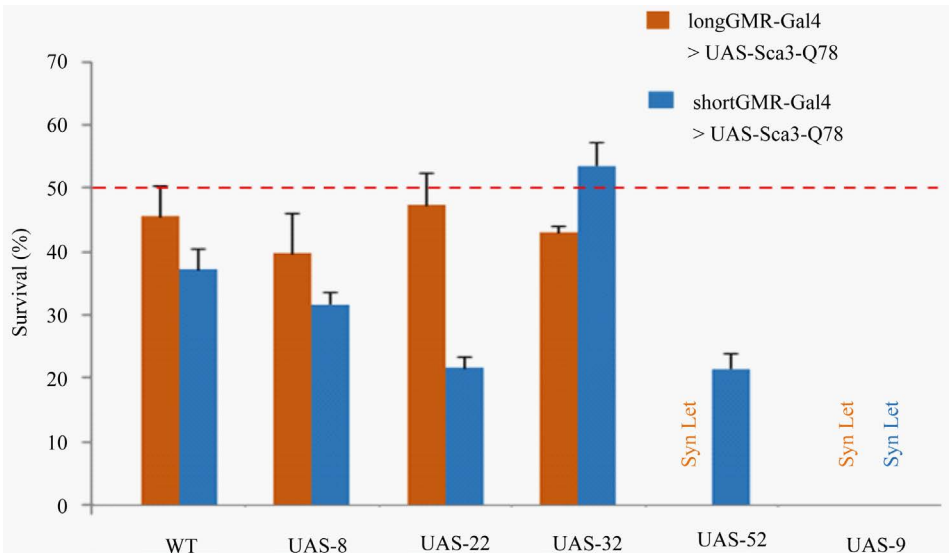

Figure 6. Survival rates of genotypes coexpressing the UAS alleles and *UAS*-Sca-Q78 with either the *longGMR-Gal*4 or *shortGMR-Gal*4 driver. WT, a wild type control; Syn Let, a synthetic lethal phenotype.

3.6. Survival Rates and the Strength of Alleles Encoding an Expanded Polyglutamine Protein

In addition to examining the effects on survival rate from drivers, we also studied the effects from two alleles that express an extended polyglutamine tract, *UAS-httex1p-97QP-w* (an eye weak allele) and *UAS-httex1p-97QP-s* (an eye strong allele) [22]. When driven by *longGMR-Gal4*, *UAS-httex1p-97QP-w* caused slightly less color loss than that of *UAS-httex1p-97QP-s* (**Figure 2**). As shown in **Figure 7**, coexpressing all five *UAS*-alleles individually with *UAS-httex1p-97QP-w* had nearly the same effects on the survival rates as those of *UAS-httex1p-97QP-s* ($p > 0.05$). An exception was *UAS-Dikar* that was isolated in our previous experiments [22]. While coexpressing *UAS-Dikar* and *UAS-httex1p-97QP-s* induced a synthetic lethal phenotype, the vast majority of the genotype coexpressing *UAS-Dikar* and *UAS-httex1p-97QP-w* survived to adulthood (**Figure 7**, $p < 0.001$). Thus, *UAS-Dikar* was the only allele that displayed a correlation of its responses to *UAS-polyQ* alleles' strength in the eyes.

3.7. Survival Rates and Temperature

Temperature is known to affect the activity of the *Gal4/UAS* system in Drosophila, with a range from 29˚C (strong expression output) to 18˚C (weak expression output) [30]. Using a GFP reporter gene, we examined the expression levels of the *longGMR-Gal4>UAS-2xEGFP* genotype grown at 23˚C and 25˚C. The results showed that the relative GFP signal intensity of the larval eye discs grown at 25˚C is approximately 84% higher than that at 23˚C (24.5 ± 7.9 vs. 13.3 ± 4.3, $p = 0.002$).

We then asked if the survival rates of the genotypes coexpressing the *UAS*-strains and an expanded polyglutamine protein also show a temperature dependency. As shown in **Figure 8(a)**, at 25˚C, *UAS*-52 caused severe viability problems when it was coexpressed with the *UAS-httex1p-97QP-w* allele with *longGMR-Gal4*. At 23˚C, a significantly less severe effect on the survival rate was observed (0.461 ± 0.046 at 25˚C vs. 0.166 ± 0.032 at 23˚C, $p < 0.001$). A more striking effect of the temperature shift was seen for *UAS*-9. While its coexpression at 25˚C with *UAS-httex1p-97QP-w* induced synthetic lethality, more than 50% of the coexpressing genotype survived to adulthood at 23˚C (0.274/0.5 vs. 0, $p < 0.001$, **Figure 8(a)**).

It is difficult to assess if there is a correlation between the survival rates and the compound eye defects from the coexpression experiments, since expressing each of these *UAS*-alleles alone in the eyes causes various eye defects (**Figure 2**). Moreover, at 25˚C, some of the coexpressing genotypes were synthetic lethal and thus their eye phenotypes remain unknown. *UAS*-9 is an extreme case, because, at 25˚C, coexpressing*UAS*-9 resulted in

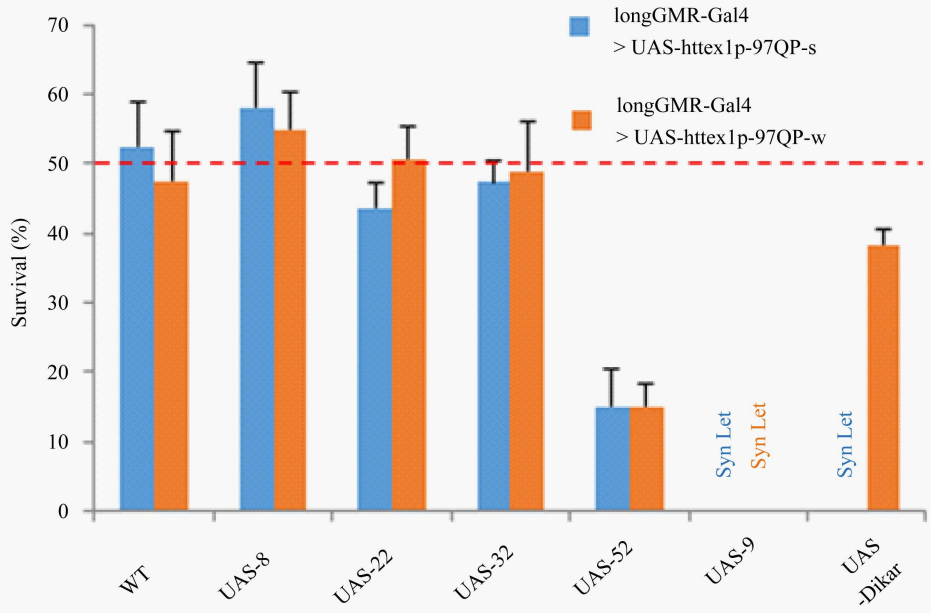

Figure 7. Survival rates of genotypes coexpressing a *UAS*-allele and either *UAS-httex1p-97QP-s*, or *UAS-httex1p-97QP-s*, with *longGMR-Gal4*. WT, a wild type control; Syn Let, a synthetic lethal phenotype.

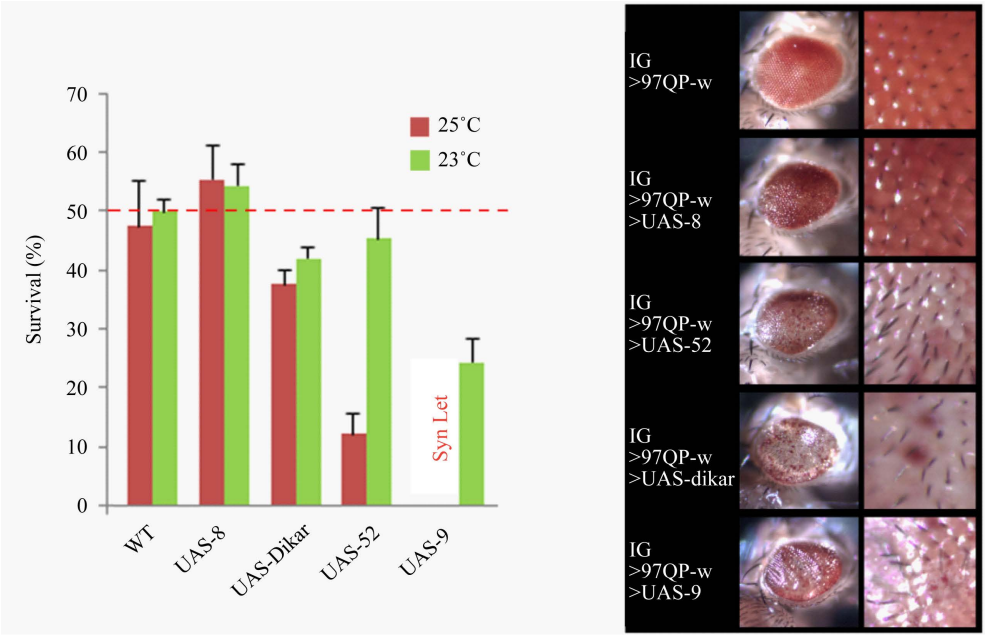

Figure 8. Survival and eye defects of genotypes coexpressing the *UAS*-alleles and *UAS-httex*1*p*-97*QP-w* with *longGMR-Gal*4 at 25˚C and 23˚C. (a) Survival of genotypes coexpressing the *UAS*-alleles and *UAS-httex*1*p*-97*QP-w* with *longGMR-Gal*4; (b) Adult eye defects resulting from coexpressing a *UAS*-allele and *UAS-httex*1*p*-97*QP-w* with *longGMR-Gal*4 at 23˚C. WT, a wild type control; Syn Let, a synthetic lethal phenotype; lG, *longGMR-Gal*4; *UAS*-97*QP-w*, *UAS-httex*1*p*-97*QP-w*.

synthetic lethality in all of the tests (**Figure 2**). At 23˚C, the surviving adults of coexpressing*UAS*-9 and *UAS-httex*1*p*-97*QP-w* with *longGMR-Gal*4 provided an opportunity to reveal the coexpressing eye defects. As shown in **Figure 8(b)**, the adult eyes of this genotype displayed loss of eye color, missing bristles, and an uneven eye surface. In addition, the eye surface was often collapsed, indicating structural damages underneath. Thus, it appears that the *UAS*-9 allele, which induced synthetic lethality under all of the tested coexpression conditionsat 25˚C, caused the most severe eye damage when coexpressed with *UAS-httex*1*p*-97*QP-w* by *long-GMR-Gal*4 at 23˚C.

4. Discussion

The Drosophila eye consists of approximately 750 reiterated units, referred to as the ommatidia. Some mutations cause minor perturbation to the eye formation, which could be seen as slightly disordered arrays of the ommatidia, or a rough eye, directly under a dissecting microscope. Thus, the Drosophila eye is highly sensitive to mutations and the corresponding phenotypes are readily visible. The sensitivity and efficiency of using the eye as a tool to monitor genetic modifications have made the eye a favorite model in investigations of genetic interactions. Furthermore, since adult flies with severe eye defects, which were induced by mutations such as *eya* (*eyes absent*) were viable [31], the eye model is largely regarded as unbiased in genetic screens, allowing the isolation of both weak and strong mutations that influence eye formation.

To investigate gene activity and genetic pathways, expressing genes in the developing Drosophila eyes by using *GMR-Gal4/UAS* has been employed more frequently than any other expression systems [30] [32]. This system has facilitated the identification and characterization of large collections of genes and genetic pathways that interact with the expanded polyglutamine proteins [29] [33]-[36]. However, despite the fact that *GMR-Gal4/UAS* operates predominantly in the eyes, the system triggers “leaky” expression in tissue types other than in the eyes. For example, the transcription factor that regulates *GMR-Gal*4, the glass protein, has been found in a number of non-eye tissue types [37]-[39]. Moreover, the *glass* gene is essential for the larval development, because loss-of-function *glass* mutants cause developmental failure at larval stages [38] [40]-[42]. In addition, the isolation of the Drosophila synthetic lethal *Dikar* gene [22] and additional synthetic lethal alleles described in this report

have raised a possibility that *GMR-Gal4/UAS* is biased against the isolation of strongest interacting modifiers, *i.e.*, the synthetic lethal alleles.

Due to its innate toxic nature, an expanded polyglutamine tract may generate a lethal-prone condition, frequently giving rise to synthetic lethality when the genetic background is modified. It is possible that the *GMR-Gal4* drivers operate in a non-eye cell type that is extremely sensitive to the neural toxin. Thus, the seemingly trivial yet toxic expression could lead to a near-catastrophic condition to the cells that are essential for viability, though the leaky non-eye expression is insignificant when compared to that of the primary activity in the eyes. Consistent with this hypothesis, our results indicate that the synthetic lethality phenomenon is unrelated to the primary *GMR-Gal4*'s activity in the eyes. First, Expressing *UAS*-9 in the eyes caused eye defects that are compatible to those of several other *UAS*-strains, such as *UAS*-8. *UAS*-9 induced synthetic lethality in all of the coexpression assays, while *UAS*-8 had very little, if any, effects in the assays. Second, though the *shortGMR-Gal4* activity is more than 8 times higher than that of *longGMR-Gal4* in the developing eyes, its effects on survival was weaker than *longGMR-Gal4* in a set of the coexpressing assays involving *UAS*-52 and *UAS-Sca3-Q78* (21.2% survival rate vs. a synthetic lethality) (**Figure 6**).

This survey showed that *GMR-Gal4* would allow the strongest modifiers, *i.e.*, the synthetic-lethal alleles, to escape detections in the conventional genetic screens. For example, *UAS*-52 would likely have escaped the detections, since the assays mostly generated coexpression genotypes that were either synthetic lethal or low in viability. Another example is *UAS-Dikar*, which could have gone unnoticed during detection as, coexpressing*UAS-Dikar* and an expanded polyglutamine tract nearly always resulted in synthetic lethality [22], it would have had almost certainly escaped the detections. *UAS*-9 is an extreme example, which would certainly be excluded from the conventional screens. The genotypes coexpressing*UAS*-9 and any one of the four *UAS*-polyglutamine transgenes (*UAS-httex1p-97QP-w*, *UAS-httex1p-97QP-s*, *UAS-Sca3-Q78*, or *UAS-41Q*) with either of the two *GMR-Gal4* drivers (*longGMR-Gal4* or *shortGMR-Gal4*) were synthetic lethal. Surviving progeny of a coexpression genotype, *longGMR-Gal4>UAS-9 + UAS-httex1p-97QP-w*, were seen only after the rearing temperature was lowered to 23˚C from 25˚C, a condition that reduced the *Gal4/UAS* activity by roughly 50% and presumably lowered the activity of the cell death pathways, too.

In spite of having established many models to develop therapeutic interventions for the expanded polyglutamine diseases, the critical targets of the treatments including the major cellular components, or physiological pathways, remain elusive [3] [6] [7] [16] [43] [44]. Synthetic lethality reveals an essential genetic interaction that provides a buffering mechanism [45]. The synthetic-lethal alleles likely belong to interacting genes that are not only stronger than the known modifiers, but also act in the same biochemical pathway in which the expanded polyglutamine tract induces cellular toxicity, or in a parallel buffering pathway that operates in the same physiological process [46]-[48]. It is also possible that these alleles interact with an expanded polyglutamine tract at early stages of the model disease development, which could disrupt multiple physiological processes and amplify the toxicity. Since results from studying stronger modifiers, essential interactions, and earlier players in the disease development may be used to better target the diseases, isolation and characterization of the synthetic-lethal alleles could play pivotal roles in developing therapeutic strategies. In addition to the synthetic lethality associated with expressing a toxic polyglutamine tract, similar synthetic lethality may also be commonly associated with expressing other acute toxic disease proteins in model organisms, since it is still difficult for the current heterologous gene expression technology to precisely target a specific cell type without the leaky side effects. To identify the synthetic-lethal alleles, however, exploring alternative approaches to the conventional F1 genetic screens, such as the two-step design used in this study, would be necessary.

5. Conclusion

Our results suggest that the toxic polyglutamine tract induces an acute condition that could easily leads to cell death when strong interacting factors are present. The synthetic lethal alleles may be more common that thought, and might have escaped the conventional genetic detection methods, due the synthetic lethality. Future studies into the mechanistic aspects of the synthetic lethal alleles will provide insight into the critical physiological pathways where the synthetic lethal alleles interact with the expanded polyglutamine proteins to cause neurodegenerative cell death.

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