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Development of a RNAi-based release of insects carrying a dominant lethal (RIDL) system in *Drosophila melanogaster*

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Abstract Since the first report of the release of insects carrying a dominant lethal (RIDL) strategy, RIDL strains have been constructed in species including fruit flies and mosquitoes. However, in many insects, identification of sterile and lethal genes needed to generate a RIDL strain is limited by the lack of molecular and genetic information. Here, we created RIDL strains of *Drosophila melanogaster* using RNA interference (RNAi) of the *Pygopus* (*Pygo*) gene, a key component of the *Wingless/Wnt* signaling pathway. In two transgenic lines, XD11 and XD15, we verified lethality in the absence of tetracycline, but we were unable to demonstrate sex-specific lethality. We found that male XD15 adults maintained on medium without tetracycline had a longer lifespan than wild type. This RNAi-based RIDL strain may therefore offer the advantages of a transgene that promotes the expression of two contrary actions at different life stages: lethality in larvae and prolonged lifespan in adults, actions that could work together to provide prolonged delivery of lethality by the RIDL system. Use of RNAi can facilitate the development and application of RIDL strategies in a wide range of species.

Keywords Sterile insect technique (SIT) · RNA interference (RNAi) · Release of insects carrying a dominant lethal (RIDL) · Lethal gene

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1 Introduction

Research into the genetic manipulation of insect species for their control has increased considerably in the last decades and has been focused on increasing the sophistication of approaches to the sterile insect technique (SIT). Conventional SIT programs have achieved a number of notable successes [1–12]. However, in conventional SIT programs, male sterility is achieved by irradiation, a process associated with both risks and costs. The vigor and longevity of released sterile males are crucial to the success of any SIT strategy, yet irradiation induces chromosome fragmentation [1] that can severely impact the viability and competitiveness of sterile males. Using recombinant DNA technology, Alphey et al. [3] developed "release of insects carrying a dominant lethal" (RIDL). This technique has potential application as a stand-alone strategy for controlling pest populations [2–6] or for integration with the use of Bt crops to delay the development of Bt resistance [2–5, 7, 8].

Transgenic insect strains for use in RIDL have been constructed in many species [2, 3, 6, 8–12]. However, a lack of genomic information limits the development of the RIDL technique. There is also a potential danger of using nonspecies-specific lethality or sterility due to the possibility of losing lethal genes by genetic drift, and therefore, it may be desirable to develop more than one RIDL strain using different genetic mechanisms.

Experimental introduction of RNAi into *D. melanogaster* has been performed primarily by direct injection of dsRNA or by transforming the fly using an inverted repeat of a fragment of the target gene to produce dsRNA [13]. RNAi-based RIDL offers the potential to target disruption to a particular gene critical to a particular developmental stage.

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Here, we created RIDL strains of *D. melanogaster* using RNAi of the *Pygopus* (*Pygo*) gene, a key component of the Wingless/Wnt signaling pathway that is critical to development in *Drosophila* [14–17], and compared the lethality and lifespan of the transgenic adults grown on medium without tetracycline with that of the wild type.

2 Materials and methods

2.1 Fly culture

Flies were cultured in controlled environment chambers at 25 °C with a 14 h:12 h light-dark cycle. They were fed on media with or without tetracycline prepared according to the recipes as previously described [1, 18].

2.2 Plasmid construction, transformation, and recombination

The construct *yp1-tTA* was a gift from Dr Maxwell Scott (Institute of Molecular BioSciences, Massey University, New Zealand). The construct *tetO-Pygo*IR was modified from *tetO-hid* provided by Dr Maxwell Scott (Fig. 1) [18], using a CaSpeR-based vector W.T.P.2 *tetO-hid*, with the *hid* open reading frame (ORF) removed by restriction digestion with *Eco*RI. *hid* was replaced with a 295-bp fragment of *Pygo*IR. This fragment was placed into the construct tail-to-tail, ligated to a second intron of the gene activator *Drosophila ftz* to increase the efficiency of RNA interference, and cloned into the vector (Fig. 1).

*tetO-Pygo*IR and *yp1-tTA* were transformed into *Drosophila* strain W^{1118}. After selection of the transgenic lines, *yp1-tTA* and *tetO-Pygo*IR were recombined by crossing lines with separate insertions on the second and third chromosomes, respectively, with the line w;Sp/CyO;MKRS/TM6B. Virgin w;*tetO-Pygo*IR/CyO;*yp1-tTA*/TM6B was then crossed with male w/Y;*tetO-Pygo*IR/Sp;*yp1-tTA*/MKRS. Stocks were established by crossing virgin female and male *tetO-Pygo*IR/CyO;*yp1-tTA*/TM6B in the tetracycline-containing medium. Homozygous lines were established by crossing virgin w; *tetO-Pygo*IR/CyO;*yp1-tTA*/TM6B with male w/Y;*tetO-Pygo*IR/CyO;*yp1-tTA*/TM6B and selecting virgin female and male w; *tetO-Pygo*IR/*tetO-Pygo*IR;*yp1-tTA*/*yp1-tTA* to establish a stock.

2.3 Screening transgenic strains' response to tetracycline

Five-day-old transgenic adult flies maintained on tetracycline-containing medium were allowed to lay eggs. Within 24 h of oviposition, the eggs were collected in batches of 30, and each batch was transferred to a tube containing medium with or without tetracycline, as appropriate to the screening. Each tetracycline treatment was replicated in 5 tubes and observed. The times to pupation and eclosion were recorded, and the number of the progeny counted. The testing of 11 *yp1tTA* and *tetO-Pygo*IR strains on the survival of eggs to adult eclosion in tetracycline-containing and tetracycline-free medium was carried out in the same way.

2.4 Testing the inhibition and induction of lethality

Five-day-old adults (the parents) were allowed to lay eggs on a plate with tetracycline-containing medium on the first day. On day 2, the parents were transferred to tetracycline-free medium and allowed to lay eggs for 7 days. On day 9, the parents were transferred back to tetracycline-containing medium where they were allowed to lay eggs for a final day. Daily, from day 1 to day 9, 300 eggs were transferred into 10 tubes containing medium corresponding to that on which they had been oviposited (with or without tetracycline). The eggs and larvae were reared, and the numbers of successfully eclosed adults were counted.

2.5 Efficiency of RNAi-induced lethality

Adult transgenic males <1 day old were collected from the tetracycline-containing medium on which they were reared and transferred individually to separate tubes containing medium with or without tetracycline. Thirty males per medium were each allowed to mate with five wild-type (OR) females (5 days old) that were introduced to the tube. The flies were allowed to mate and oviposit for 5 days before the parents were removed. Thirteen days later, the numbers of eclosed adults from these 30 independent

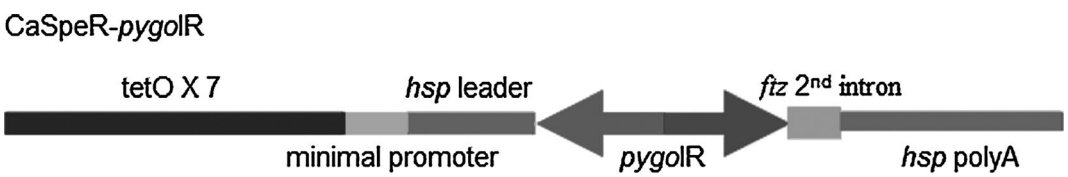

Fig. 1 Schematic representation of the *tetO-Pygo*IR construct. A 295-bp segment of the *Pygo* gene was inserted tail-to-tail and followed by a second intron of the *ftz* gene to increase the efficiency of RNA interference [19]