Comparison of the efficiency of TIR1 transgenes to provoke auxin induced LAG-1 degradation in *Caenorhabditis elegans* germline stem cells

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**Figure 1. Comparison of TIR1 transgene mediated degradation in *C. elegans* germline stem cells by qRT-PCR:** (A) Schematic showing four distinct TIR1 transgenes driven by different promoters, indicated by the gene name followed by “p”, for promoter. mRuby and BFP are fluorescent proteins, F2A is a self-cleavable linker. Not shown is the substrate, LAG-1::degron. (B) Schematic illustrating sample collection for qRT-PCR analysis. The animals were dissected and the extruded gonads collected for RNA purification and then qRT-PCR analysis. (C & D) The relative mRNA level of LAG-1 dependent transcriptional targets *lst-1* (C) and *sygl-1* (D) from dissected gonad samples of the indicated genotype and treatment. q175 is a *glp-1* null allele, where the mRNA level of *sygl-1* and *lst-1* were normalized by setting their levels to one for this genotype. ar202 is a *glp-1* gain of function allele. Results are from three biological replicates, except for *sun-
In the C. elegans germline, GLP-1 Notch signaling employs transcriptional targets, lst-1 and sygl-1, to maintain the germline stem cell (GSC) fate (Kershner et al. 2014; Chen et al. 2020). In the nucleus, the transcription factor LAG-1 and GLP-1(INTRA) form a complex that activates transcription of targets (Lee et al., 2016; Chen et al., 2020). LAG-1 is widely expressed and is also required for LIN-12 Notch signaling in somatic cells (Greenwald and Kovall 2013). Utilizing the AID system, we recently demonstrated that LAG-1 functions germline-autonomously to promote the GSC fate (Chen et al. 2020).

We have previously shown that the LAG-1 protein levels are reduced to background using the gld-1p::TIR1 transgene (Chen et al. 2020). However, the low level of endogenous LAG-1 in the germline stem cell region, with or without auxin treatment, makes quantitative protein level comparisons challenging. Here, we measured the level of mRNA for the LAG-1 transcriptional targets lst-1 and sygl-1 by quantitative real-time PCR (qRT-PCR) from dissected gonads (Fig. 1B), as an indirect but more sensitive output for measuring degradation efficiency of LAG-1::degron by different TIR1 transgenes. Since LAG-1 degradation in the wild type germline caused the GSCs to enter meiosis, the qRT-PCR experiments were conducted in the gld-2 gld-1 double mutant background (see complete genotype in the Reagent section). GLD-1 and GLD-2 are the two major pathways that promote GSCs to enter meiosis. In gld-2 gld-1 double mutants the mitotically cycling germ cells fail to enter meiosis and results in a germline tumor, irrespective of GLP-1 signaling or LAG-1 function (Kershner et al. 2014; Chen et al. 2020). In addition, the glp-1 gain of function allele ar202 was included in the gld-2 gld-1 double mutant background, allowing the transcription of lst-1 and sygl-1 mRNA to occur throughout the germline, thus increasing signal-to-noise ratio. The auxin treatment and qRT-PCR experiments were conducted as previously described (Chen et al. 2020). Starvation synchronized L1 animals were grown on NGM plates supplemented with or without 1 mM auxin for 48 hours at 25 °C. After auxin treatment, ~50 adult animals were dissected to isolate the gonads (Fig. 1B, Chen et al. 2020). The relative mRNA levels of lst-1 and sygl-1 are presented in Figure 1C & D.

One potential issue for the AID system is auxin independent TIR1 degradation of the target proteins. With the lag-1::degron allele, we have previously shown that both LAG-1 protein accumulation and progenitor zone size are largely normal in the presence of gld-1p::TIR1, without auxin (Chen et al. 2020). Here we compared the mRNA levels of lst-1 and sygl-1 from three different strains grown on NGM plates without auxin (Fig. 1C & D). The data showed that both lst-1 and sygl-1 mRNA level were not significantly different among animals without auxin regardless of which TIR transgene they carried (Fig. 1C & D), indicating that auxin independent TIR1 degradation of LAG-1 did not occur. We found that all four TIR1s can degrade LAG-1 sufficiently to generate Glp-1 like sterile animals in otherwise wild type background. In the tumorous germaries, the levels of lst-1 and sygl-1 mRNA were reduced in auxin treated animals, also supporting the conclusion that the AID system is knocking down LAG-1 protein. Importantly, we observed that lst-1 and sygl-1 mRNA level was lower in the gld-1p::TIR1 and pie-1p::TIR1 backgrounds compared to that in the sun-1p::TIR1 and mex-5p::TIR1 backgrounds, after auxin treatment (Fig. 1). When compared to glp-1(q175) null background, we noticed that the average expression level of lst-1 and sygl-1 mRNA was slightly higher in gld-1p::TIR1, pie-1p::TIR1, and glp-1p::TIR1 + pie-1p::TIR1 background after auxin treatment. Statistical analysis indicates the lst-1 mRNA level was indistinguishable compared to glp-1 null germline, while the sygl-1 mRNA level is slightly but significantly higher than that in glp-1 null germlines. Together, we concluded that gld-1p::TIR1 and pie-1p::TIR1 are more efficient in degrading LAG-1 in the germline stem cell region.

It is not known what molecular feature(s) of the TIR1 transgenes determined high efficiency degradation of LAG-1::degron in germline stem cells, as the transgenes differ not only in the promoter, but also in the fluorescent protein tag, and in the 3’ UTR. For example, the mex-5p and sun-1p transgenes contain fluorescent protein BFP with a C-terminal degron tag, separated by a self-cleavable linker F2A, a design that allows a direct functional check of TIR1 activity through loss of BFP fluorescence. In contrast, pie-1p::TIR1 and glp-1p::TIR1 have mRuby fused to TIR1 without the degron motif. It is possible that reduced efficiency of mex-5p and sun-1p transgenes, rather than a consequence of
different promoters, was due to competition of TIR1 for BFP::degron versus LAG-1::degron. Finally, we recommend testing multiple TIR1 transgenes to determine which one is optimal for the protein, cell type and timing of interest.

Reagents

The following strains are used in this study:

**BS3879:**

- glp-1(q175)/hT2::gfp [bli-4(e937) let-?(q782) qIs48] (III)

**BS4310:**

- glp-1(ar202)/hT2::gfp [bli-4(e937) let-?(q782) qIs48] (III)

**BS5629:**

- glp-1(ar202)/hT2::gfp [bli-4(e937) let-?(q782) qIs48] (III)

**BS5631:**

- lag-1(oz536oz537[lag-1::degron::3xHA]) (IV)

**BS5630:**

- lag-1(oz536oz537[lag-1::degron::3xHA]) (IV)

**BS7023:**

- wrdSi8 [mex-5p::TIR1::F2A::mTagBFP2::degron-NLS::tbb-2 3’UTR] (II)

**BS7024:**

- cpIs103 [sun-1p::TIR1::F2A::mTagBFP2::degron-NLS::tbb-2 3’UTR] (II)

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References

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