AGES: An auxin-inducible, GAL4-compatible, gene expression system for *Drosophila*

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Abstract

The ability to control transgene expression, both spatially and temporally, is essential for studying model organisms. In *Drosophila*, spatial control is primarily provided by the GAL4/UAS system, whilst temporal control relies on a temperature-sensitive GAL80 (which inhibits GAL4) and drug-inducible systems. However, these are not ideal. Shifting temperature can impact on many physiological and behavioural traits, and the current drug-inducible systems are either leaky, toxic, incompatible with existing GAL4-driver lines, or do not generate effective levels of expression. Here we describe the Auxin-inducible Gene Expression System (AGES). AGES relies on the auxin-dependent degradation of a ubiquitously expressed GAL80, and therefore, is compatible with existing GAL4-driver lines. Water-soluble auxin is added to fly food at a low, non-lethal, concentration, which induces expression comparable to uninhibited GAL4 expression. The system works in both larvae and adults, providing a stringent, non-lethal, cost-effective, and convenient method for temporally controlling GAL4 activity in *Drosophila*.

Introduction

The ability to manipulate the expression of specific genes in model organisms has been the cornerstone of genetics research over the last 50 years. It is from such studies that geneticists have been able to elucidate fundamental biological process, such as those underlying neurodegenerative diseases (Lu and Vogel, 2009), oncogenic mechanisms (Villegas, 2019), and neural circuitry involved in memory formation (Cognigni et al., 2018).

The fruit fly, *Drosophila melanogaster*, has been at the forefront of genetics since its cultivation in the lab in the early 1900s, by geneticist Thomas Morgan (Morgan, 1910). In the 100 years since, a plethora of genetic tools have been developed for this model organism, enabling geneticists to tightly control and manipulate gene expression, allowing them to study the roles of genes in development, physiology, and behaviour. One of the most prominent developments is the GAL4/UAS system, which has been an incredibly powerful tool for the spatial control of transgenes in *Drosophila* (Brand and Perrimon, 1993). This system relies on a cloned

or endogenous promoter to drive the expression of the yeast transcription factor GAL4, which can then bind to the UAS (upstream activating sequence) sites upstream of a transgene and activate its expression. Since the development of this system, *Drosophila* researchers have created thousands of genetically distinct lines to enable expression of transgenes in specific populations of cells (for example (Jenett et al., 2012; Robie et al., 2017)). The majority of these lines are publicly available at the Bloomington Drosophila Stock Center (BDSC) and Vienna Drosophila Resource Center (VDRC).

GAL4 activity can be regulated by GAL80, a protein which antagonises GAL4 by inhibiting its activation domain (Lue et al., 1987), and thus the ability of the GAL4 to induce transcript expression (Lee and Luo, 1999; Suster et al., 2004). The generation of a fly line that ubiquitously expressed a temperature-sensitive version of GAL80 ($GAL80^{ts}$) (Matsumoto et al., 1978) was a significant advance for the field, as it allowed temporal control of GAL4-induced transgene expression (McGuire et al., 2003). Flies can be kept at temperatures permissive of transcript-expression (29°C) or temperatures that inhibit expression (18°C), offering researchers to control not only where, but when transgenes are expressed. The versatility of this system enabled researchers to determine time-specific effects of gene function, and is a system that is widely employed within the *Drosophila* community.

Although the impact of this technology on the field cannot be understated, it is becoming increasingly evident that using a temperature shift to induce gene expression is far from optimal for some experiments, especially in fields such as behaviour, neuropathy, and ageing. Flies, as ectotherms, are highly dependent on their ambient temperature, which affects a wide range of physiological and behavioural traits (Abram et al., 2017). These include sleep (Beckwith and French, 2019), territorial success (Zamudio et al., 1995), lifespan (McCabe and Partridge, 1997), development rate (Danjuma et al., 2014), immunity (Hunt et al., 2016), metabolism (Schou et al., 2017) and even their microbiome (Moghadam et al., 2018). Therefore, the use of a significant temperature shift to induce the expression is far from ideal in these contexts.

As an alternative approach, a range of drug-inducible systems have been developed, where the drug can be administered to flies in their diet to activate transcript expression (Barwell et al., 2017; Kogenaru and Isalan, 2018; Osterwalder et al., 2001; Potter et al., 2010). These systems each have advantages and disadvantages (see Table 1) and are not often compatible with the GAL4 lines, thus requiring the creation (and optimisation) of their own drivers. In addition, the systems that are compatible with existing GAL4 lines can affect the fly's physiology or survival, thereby limiting their use, and the certainty of any findings when employed.

EXPRESSION SYSTEM	REFERENCE	ADVANTAGES	DISADVANTAGES
GENESWITCH	(Osterwalder et al., 2001)	Non-toxic for adult flies	Not compatible with existing GAL4 lines Leaky expression (Poirier et al., 2008; Scialo et al., 2016) Larval exposure impacts on adult sleep patterns (Li and Stavropoulos, 2016) Drug is unsafe to handle for female researchers Drug is expensive
QF SYSTEM	(Potter et al., 2010)	Non-toxic for flies	Not compatible with existing GAL4 lines
TET-OFF GAL80	(Barwell et al., 2017)	GAL4/UAS compatible	Long induction time (> 5 days) Requires x2 copies of the GAL80 transgene Affects microbiota
DH22	(Kogenaru and Isalan, 2018)	GAL4/UAS compatible	Not compatible with existing GAL4 lines TMP drug is only dissolvable in DMSO

Table 1 – Main advantages and disadvantages of the most common drug-inducible gene expression systems currently available to the *Drosophila* community.

The auxin-degron system, first identified in *Arabidopsis*, involves the auxin-dependent ubiquitination, and subsequent degradation, of proteins which are tagged with a specific auxin-inducible degron (AID) sequence (Dharmasiri et al., 2005; Dharmasiri and Estelle, 2002; Li et al., 2019). Its natural function allows for the prompt elimination of Aux/IAA transcription factors in plants and has been adapted for use in other species, most notably *C. elegans*, to artificially target proteins for rapid degradation (Zhang et al., 2015). In this system, the F-box protein TIR1, an auxin-receptor, binds with conserved proteins Skp1 and Cullin to form a Skp1–Cul1–F-box (SCF) E3 ligase complex which ubiquitinates proteins tagged with an AID sequence in an auxin-dependent manner. The specificity and versatility of the system has made it an effective tool in multiple systems mice (Yesbolatova et al., 2020), and human cell culture (Li et al., 2019). It has also been employed in flies to induce the degradation of specific *Drosophila* proteins (Trost et al., 2016).

Here, we apply the auxin-degron system to enable both temporal and spatial control of transgene expression when combined with the GAL4/UAS system in *Drosophila*. This was achieved by creating a ubiquitously expressed GAL80 fused with AID tags, such that GAL80 is degraded in the presence of the auxin phytohormone (Figure 1). We demonstrate that this system works in both larvae (5 mM auxin) and adult flies (10 mM), which are concentrations that do not affect fly survival or development. This Auxin-inducible Gene Expression System (AGES) is safe to handle, cheap, easy to prepare, provides tuneable and stringent (i.e. not leaky) expression, and most importantly is compatible with the existing GAL4-driver lines developed by the *Drosophila* community.

Results

Generation of an auxin-degradable GAL80 *Drosophila* line

To generate an auxin inducible system for control of GAL4 activity in *Drosophila,* we designed a transgene that would ubiquitously express two proteins, TIR1, and AID-tagged GAL80. The T2A sequence between TIR1 and AID-tagged GAL80 is a "cleavage" element that is known to work efficiently in *Drosophila* cell culture (Daniels et al., 2014). The *AtTIR1-T2A-AID-GAL80-AID* sequence was codon optimized, synthesized, and cloned downstream of the *αTub84B* promoter in *pattB* (Figure 1 and Figure 1 – figure supplement 1). The AID tag is from the minimal degron of *IAA17* (Morawska and Ulrich, 2013), whose fusion to luciferase resulted in a fusion protein with short half-life in plants (~ 10 min) (Dreher et al., 2006). The *αTub84B* (tubulin) promoter drives expression of the bicistronic sequence in all cells, all of the time, and (in the absence of auxin) will inhibit any GAL4 activity. When present, auxin will tether the TIR1 to the AID sequences, triggering the degradation of GAL80 and the release of GAL4 inhibition (Figure 1).

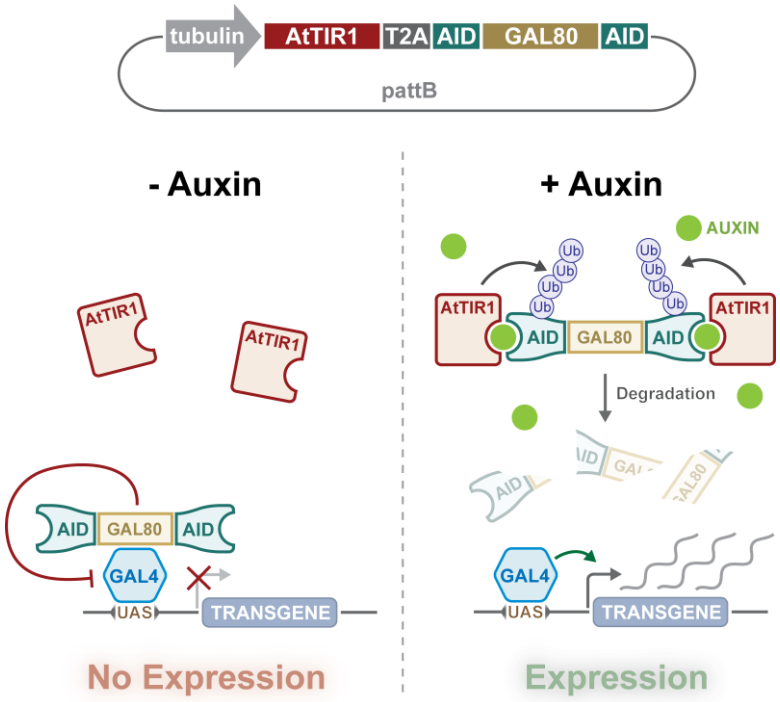

Figure 1. Design and action of the AGES system. A tubulin promoter ubiquitously expresses the bicistronic TIR1 and AGES cassette. AID-tagged GAL80 is cleaved from the TIR1 due to the presence of the T2A sequence. In the absence of auxin, GAL80 can inhibit GAL4 activity, whilst the presence of auxin induces degradation of GAL80, allowing GAL4 to drive the expression of UAS-transgene(s).

AGES allows induction of GAL4 activity in adult flies

We combined the Ages system with *c564-GAL4* to see if we could get inducible expression in fat body. A nuclear localised GFP (nls-GFP) was used as a reporter of GAL4 activity. We first examined adult female flies that were placed on food containing different concentrations of water-soluble auxin (K-NAA). A negative control consisting of fly food without auxin (0 mM auxin) was included to investigate whether there was any leaky GAL4 activity in absence of auxin. After 24 hours, GFP fluorescence in the abdomen were assayed. Note that there is some naturally occurring autofluorescence in the fly abdomen. At 5 mM and 10 mM, GFP fluorescence is clearly detectable in the abdomen of live female flies (Figure 2A). Based on this assay, 10 mM

for 24 hours results in ~60% of the GFP fluorescence levels seen in the positive control (Figure 2B). At 1 mM there is no detectable GFP above background fluorescence, and the levels are indistinguishable from both the 0 mM and the negative control (lacking both the GAL4 driver and the *UAS-nls-GFP*). To provide an alternative and more direct assay of transgene induction, quantitative PCR (qPCR) was used to measure the levels of *GFP* mRNA (Figure 2C). Here 5 mM and 10 mM show levels of mRNA equivalent to the positive control, while 0 mM showed no significant difference when compared with the negative control. Shorter exposure times of adults to auxin containing food were tested (data not shown), however, 24 hours is the minimal amount of time required for proper induction of GAL4 activity.

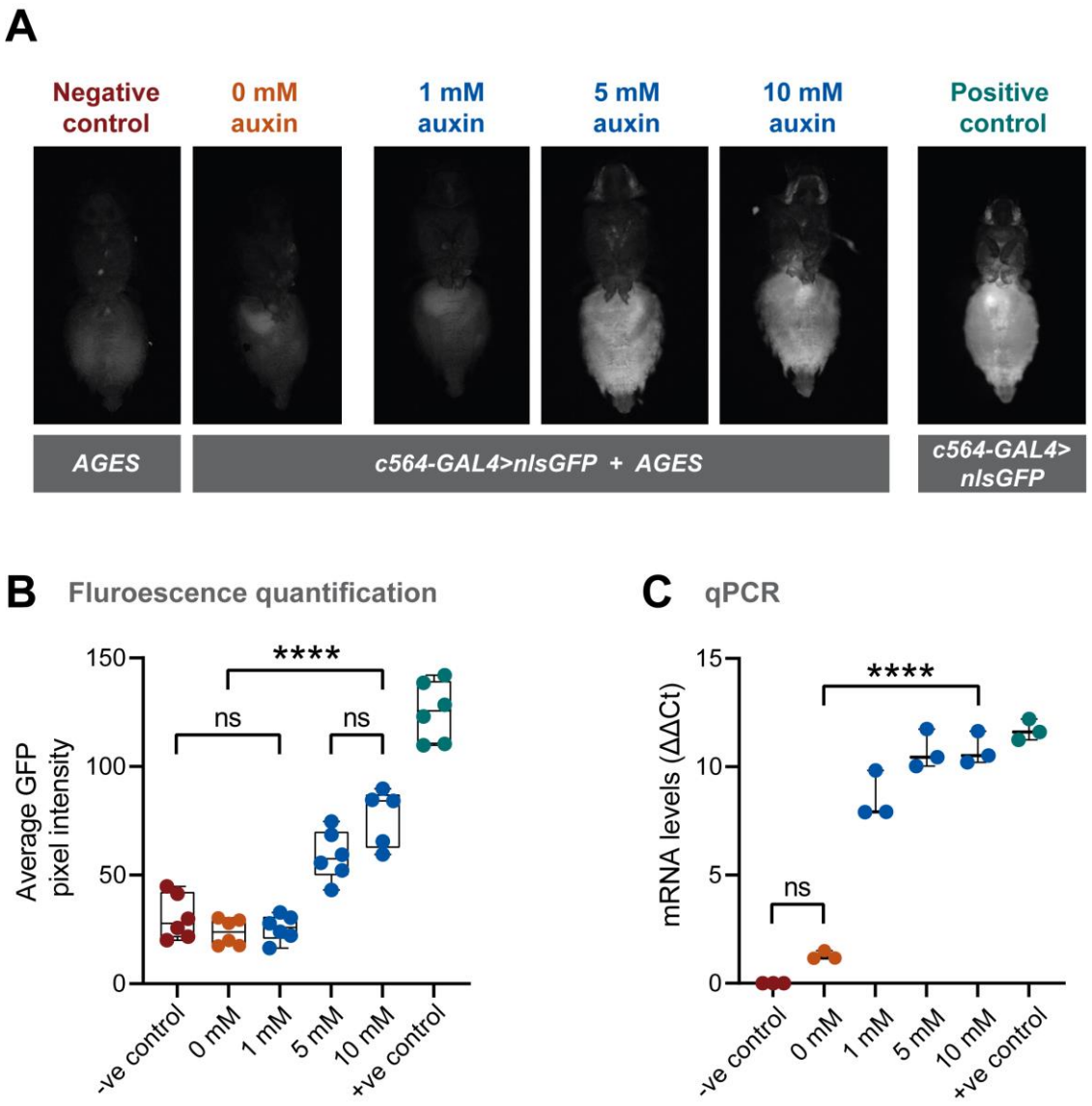

Figure 2. AGES effectively induces GAL4 activity in *Drosophila* adults. A) Ventral images of live females that express GAL4 in fat body tissue. Ingestion of food containing auxin (>= 5 mM for 24 hours) induces GAL4 activity and the expression of GFP. B) Quantification of GFP levels. Pixel intensity thresholding was performed to isolate abdomens as regions of interest. The average pixel intensities were quantified and analysed using one-way ANOVA (****, $p < 0.0001$). C) qPCR data for *GFP* mRNA levels using different concentrations of auxin. Values were normalised to housekeeping gene *RpL4* (*Ribosomal Protein L4*) and relative expression levels (compared to the negative control) were calculated using the ΔΔCt method. Y-axis displaying ΔΔCt values and statistics done using one-way ANOVA (****, $p < 0.0001$).

Adult males eat less than females (Wong et al., 2009), therefore, this will impact on auxin ingestion and possibly on transgene induction. As with females, we examined both GFP fluorescence levels in the abdomen, and GFP mRNA levels in male flies (Figure 2 – figure supplement 1). Although reduced compared to females, there is still a robust induction of GFP expression.

For AGES to work with GAL4 lines expressed in the central nervous system, auxin must be able to pass the selectively-permeable glial membrane, akin to the mammalian blood-brain barrier (Limmer et al., 2014). To test this, we used *elav-GAL4* (expressed in all neurons) and examined the expression of GFP in the adult brain. Similar to the fat body *c564-GAL4* driver, we observe robust expression of GFP, when flies are fed auxin food (10 mM) for 24 hours (Figure 3). An anti-Elav antibody was included as a counterstain. Furthermore, this demonstrates that AGES works in the central nervous system and that auxin can cross the blood brain barrier in *Drosophila*.

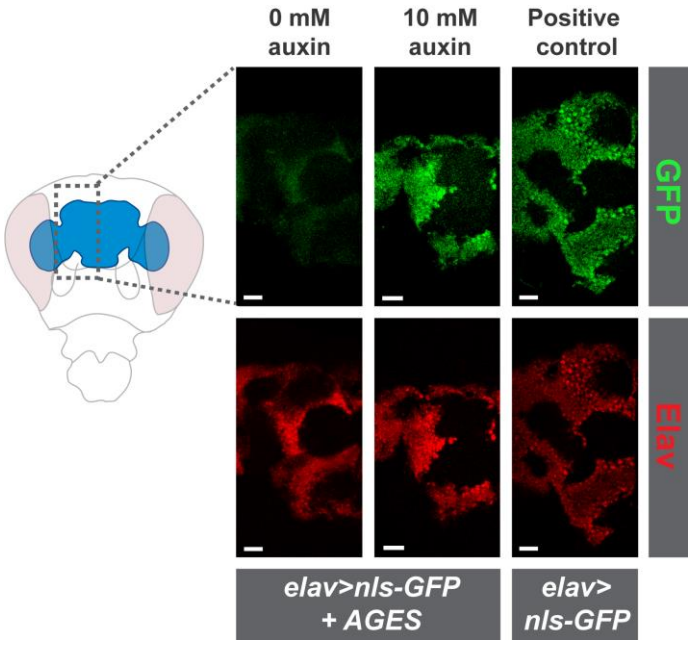

Figure 3. AGES allows induction of GAL4 activity in the *Drosophila* brain. Confocal images of adult brains stained with anti-GFP and anti-Elav. Adult were fed food containing 5 mM auxin for 24 hours. Scale bars represent 20 µm.

AGES allows induction of GAL4 activity in *Drosophila* larvae

In addition to the need for temporal control of GAL4 activity in the adult, researchers might also want to induce expression at specific points during larval development. To test if AGES works in larvae, we used the *c564-GAL4* fat body driver and tested multiple auxin concentrations (Figure 4A). Here we observed that expression could be induced at lower concentrations of auxin. When using food containing no auxin, there is no detectable GFP, whereas with 0.1 mM auxin, GFP is expressed, although at lower levels than with higher concentrations of auxin (Figure 4A). Also, at 0.1 mM, expression is not ubiquitous within the fat body tissue

143 (Figure 4B). Strong expression is observed for concentrations of 1 mM and above (Figure 4A). Larvae require
144 at least 10 hours of exposure to auxin food before reaching high levels of expression (Figure 4C).
145

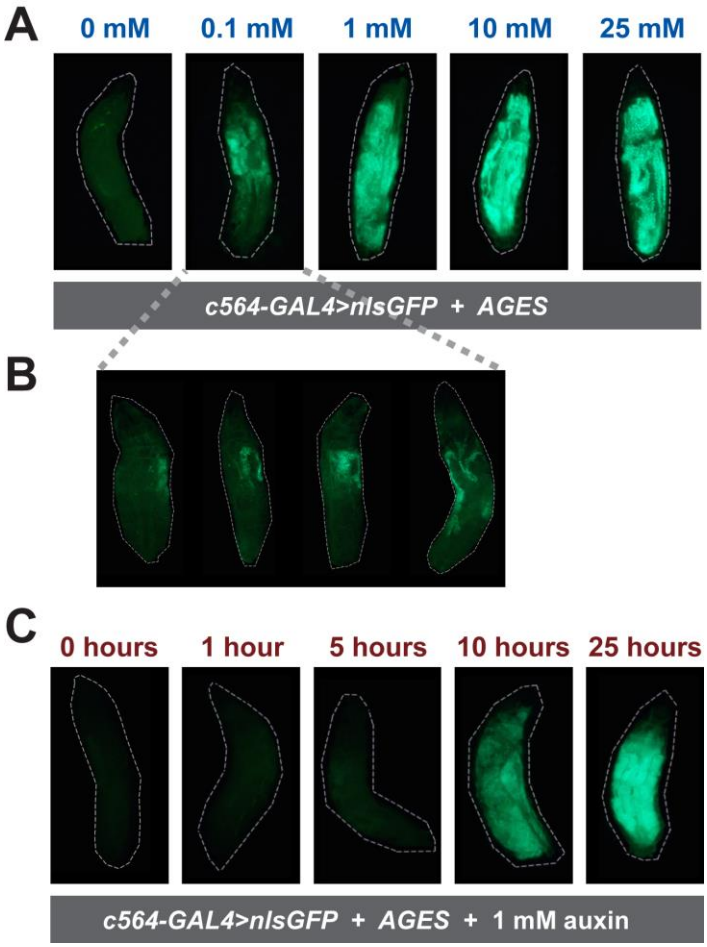

Figure 4. AGES allows induction of GAL4 activity in the *Drosophila* larvae. Bright field images of 3rd instar larvae. A) GFP fluorescence in fat body tissue after induction (for 24 hours) on food with different concentrations of auxin. B) Sporadic GFP expression when using 0.1 mM auxin). C) Representative images of larvae fed on 1 mM auxin and imaged at various time intervals.

146

147 **Effects of auxin on development and adult survival**

148 For a universally applicable drug-inducible system, the drug should not impact on development or mortality
149 of the flies. We tested whether continuous exposure to different concentrations of auxin would impact on
150 developmental timing. Time to pupation (from egg-laying) is unaffected for concentrations up to 5 mM,
151 however, 10 mM caused a delay of approximately one day (Figure 5A). 10 mM auxin causes some
152 developmental delay, although it has no impact on the survival of the flies through these developmental
153 stages (compared to a no auxin control) (Figure 5B). These data indicate that 10 mM auxin should be avoided
154 for larval induction, however, this is not an issue as > 1 mM is sufficient for robust induction of expression
155 (Figure 4A) and 5 mM does not cause any developmental delay. Concentrations of 5 mM and 10 mM auxin
156 can induce expression in adults (Figure 2 and Figure 2 – figure supplement 1). Survival assays on both male
157 and female adults show that concentrations of 5 mM and 10 mM have no effect on survival and lifespan
158 (Figure 5C and 5D). Therefore, in summary, 5 mM auxin is optimal for use in larvae, whilst 10 mM auxin is
159 best for use in adults.

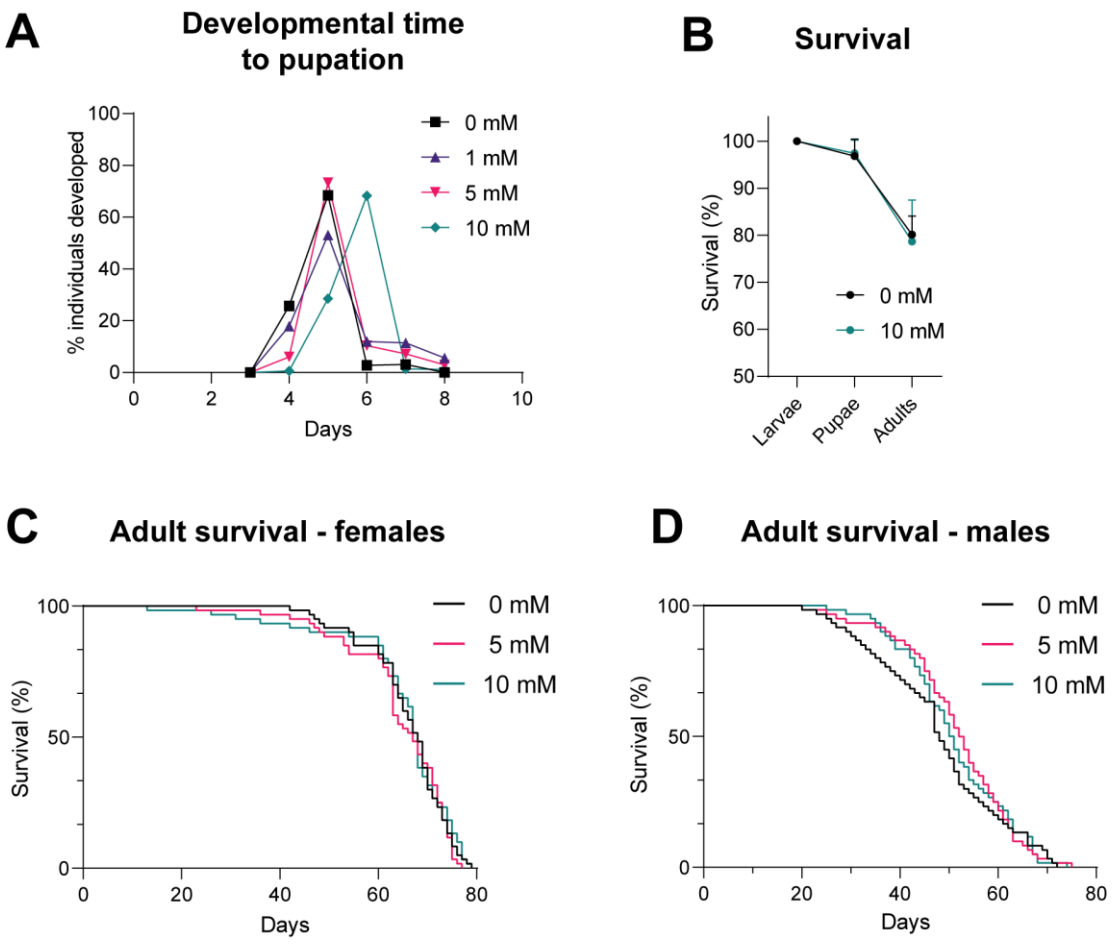

Figure 5. Impact of auxin on developmental timing and survival. A) Time taken from egg-laying to pupation. B) Survival across developmental stages (larval to pupal and pupal to adult) with 10 mM auxin. C) Survival of adult females during continuous exposure to auxin. D) Survival of adult males during continuous exposure to auxin. Logrank test and weighted Gehan-Breslow-Wilcoxon model (ns) were used for the adult survival assays.

Discussion

The GAL4/UAS/GAL80^{ts} systems enable *Drosophila* researchers to control the expression of transgenes both spatially and temporally. However, the impact of the required temperature shift on physiology and behaviour to activate/deactivate the system, can affect the experimental results and subsequent conclusions (Abram et al., 2017). Drug inducible systems have been developed as an alternative to the GAL80^{ts} method, however, these are not ideal, limited by issues such as leaky expression, long induction times or incompatibility with existing GAL4 lines. Here we present AGES, an auxin-inducible gene expression system, which eliminates the need for temperature shifts to manipulate temporal expression, and does not possess any of the drawbacks of the current drug-inducible systems.

A significant benefit of AGES over existing systems is its compatibility with existing GAL4-driver lines. Currently, the most prevalent drug-inducible expression systems, i.e. the GeneSwitch (Osterwalder et al.,

173 2001) and the Q-system (Potter et al., 2010), require the generation of their own driver lines. AGES uses a
174 GAL80 fusion protein, which allows the use of the system alongside the majority of existing GAL4-driver lines,
175 including split-GAL4 lines (Luan et al., 2006; Pfeiffer et al., 2010), whereby suppression of the GAL4-UAS
176 activation is achieved in all cells where auxin is absent. As with all GAL80-based methods of GAL4 control,
177 the GAL4 line must contain the standard GAL4 activation domain (rather than a VP16 or p65 domain) for
178 GAL80 to be affective. We have demonstrated the system using two driver lines, *c564-GAL4* and *elav-GAL4*,
179 which are utilised for fat body and neuron-specific expression respectively. Two different lines were chosen
180 to assess whether the auxin hormone used (K-NAA) could freely access various cell-types within the fruit fly,
181 particularly the central nervous system where auxin must pass the selectively-permeable glial membrane,
182 akin to the mammalian blood-brain barrier (Limmer et al., 2014). Our results demonstrate that feeding flies
183 auxin-containing food (5 mM) for 24 hours is sufficient to drive expression when using both *c564-GAL4* and
184 *elav-GAL4*. Therefore, given that auxin can transverse the blood-brain barrier, this system should, in theory,
185 be compatible with all other GAL4 lines.

186 Successful gene driver systems require negligible expression when un-induced. While some current systems
187 are known to exhibit leaky expression (Scialo et al., 2016), AGES demonstrates an almost complete repression
188 of GAL4 activity when un-induced. Using GFP fluorescence as a readout, 0 mM auxin does not show any
189 greater signal than the negative control (Figure 4A). Also, the level of *GFP* mRNA without auxin showed no
190 significant difference when compared to the negative control. The AID tags on GAL80 allow the rapid, and
191 tuneable, degradation of the GAL80 protein, providing users with the option to determine the strength of
192 expression by adjusting the concentration of auxin in the food, or by limiting the exposure time of the flies
193 to auxin.

194 AGES is also a system that is safe for both humans and flies alike. Auxin hormone carries no hazardous
195 concerns for human handling, and no lethal effects were identified for the effective concentrations of 10 mM
196 or below for both adult and larval stages (Figure 5). Continuous exposure of larvae to 10 mM auxin does
197 result in some developmental delay; however, it does not affect survival. Higher concentrations of auxin
198 result in increased mortality rates (data not shown) and should be avoided. Users are encouraged to consider
199 this if attempting to optimise exposure conditions for specific experiments, or GAL4 driver lines that have
200 not been assessed in this paper. Overall, based on our data, we recommend the use of 5 mM auxin and 10
201 mM for optimal induction in larvae and adults, respectively.

202 It is also paramount that inducible systems are easy and cheap to apply, to facilitate their practical use in
203 diverse experimental settings. While expenses of the GeneSwitch system average £0.31 (GBP) per vial, AGES
204 costs ~120x less at £0.0025 per vial (authors' estimate). Furthermore, this water-soluble auxin (K-NAA) can
205 be easily added to the food immediately before the food is poured into vials or bottles, requiring no additional
206 safety measures, enabling ease of production. In our experience, auxin-containing food can be stored 4 ^{o}C

for up to 4 weeks where the hormone's potency still persists. Further studies are required to assess the stability and effectiveness of auxin when stored for longer periods of time, or in differing conditions.

Despite the various advantages that AGES offers the fly community, there are still limitations to consider. These include the level of expression obtained and the response time of the system (24 hours) and that induced levels are not always at the maximum levels obtained without the system. For example, as male adults eat less than their female counterparts, lower expression is observed in males (Figure S2). However, as GAL4 induced expression is inherently strong, reduced transgene expression for most experiments is unlikely to be an issue. Furthermore, a 24 hour induction time, although not instant, is both practical and convenient for researchers performing experiments in the lab.

The sensitivity of the system is directly related to the amount of auxin present within the tissue, and thus concerns are raised regarding the fly's age and sex, as these factors impact feeding rates (Carey et al., 2006; Wong et al., 2009). As all experimental adult flies were aged to 5 days post-eclosion, the effects of age on the system are yet to be determined, although lower expression is to be expected in older flies. To address these concerns, alterations to the auxin application could be made, such as a longer exposure (> 24 hours), which as we have shown does not impact on survival (Figure 5D and 5E).

In summary, AGES offers the fly community a cheap, safe, and easy system for temporal transgene expression using existing GAL4 lines. Furthermore, it does not require a shift in temperature changes for induction of gene expression. It is a particularly promising tool for research fields where researchers want to avoid using temperature shifts (e.g. aging, behavioural genetics and neuropathology), and will undoubtedly have wide-ranging benefits for multiple fields of study.

Materials and Methods

Generation of *AGES* line

The *AtTIR1-T2A-AID-GAL80-AID* sequence was synthesized by Twist Biosciences (twistbioscience.com) (Figure 1 – Supplement 1). The *GAL80* sequence (from *S. cerevisiae*) and the *AtTIR1* sequence (Zhang et al., 2015) were codon optimized for *Drosophila* for more effective translation. The *αTub84B* promoter was amplified from a *tubulin-eGFP* plasmid (gift from M. Dionne) using the following primers: tub_FWD: GATATCAAGCTTGCACAGGTCC and tub-RV: GTACCTTCACGCTGTGGATGAGG. The *αTub84B* and *AtTIR1-T2A-AID-GAL80-AID* sequences were cloned into *pattB* (Bischof et al., 2013) using Gibson assembly (Gibson et al., 2009). Successful clones were sequenced verified. Annotated sequence is in Figure 1 – figure supplement 2. Microinjection was performed by *Cambridge Fly Facility* using the VK00040 line, which has an *attB* site at location att3B (87B10) on chromosomal arm 3R. Injected adult males were collected and mated to w^{1118} virgin females to identify transgenics (orange eyes). *tub-TIR1-T2A-AID-GAL80-AID* will be available at Bloomington Drosophila Stock Centre or, alternatively, from the corresponding authors.

241 **Fly stocks and food**

242 In this study *c564-GAL4, UAS-nls-GFP / Cyo-Actin-GAL4-GFP* flies were used to perform fluorescent reporter
243 experiments in the adult and larval fat body, and for quantification of *GFP* mRNA expression. Moreover, these
244 flies were used in adult survival assays. *CantonS* flies were used in the developmental survival and
245 developmental timeline experiments. For fluorescent reporter experiments in the adult brain we used
246 *elavGAL4, UAS-nls-GFP*. Flies were kept at 25 °C on and standard *Drosophila* food (recipe in supplementary
247 material) was supplemented (just before being aliquoted into vials/bottles/plates) with auxin (K-NAA (1-
248 Naphthaleneacetic acid potassium salt) available from Phytotech (#N610) or Glentham Life Sciences
249 (GK2088)) at varying concentrations.

250 **Immunohistochemistry**

251 Adult brains were dissected in 1x PBS and fixed for 25 minutes at room temperature in 4% formaldehyde
252 (methanol free) in 0.3% Triton X-100 PBS (PBST). They were washed four times for 1 hour with 0.3% PBST.
253 Normal Goat Serum (2 % in PBST) was used for tissue blocking (RT, 15 min- 1 hour) and subsequent overnight
254 primary antibody incubation. All tissue washes were done in PBST.

255 Primary antibodies used were chicken anti-GFP (Abcam #13970, 1:2000), and rat anti-Elav 1:500
256 (Developmental Studies Hybridoma Bank, DSHB). Secondary antibodies used include Alexa Fluor® 488 and
257 545 at concentration 1:200 (Life technologies) and tissue was incubated for 1.5 hours at room temperature.
258 Tissue was mounted on standard glass slides in Vectashield Mounting medium (Vector Laboratories). Brains
259 were imaged using a Zeiss LSM 510 microscope. Analysis of acquired images was done using Fiji (Schindelin
260 et al., 2012).

261 **Imaging and Image Analysis**

262 Live imaging of GFP in adult flies and larvae was performed using a Nikon SMZ 1500 microscope. In larvae, to
263 identify earliest auxin-induction effects, animals were then placed on 1 mM auxin- supplemented food and
264 imaged at 0, 1, 5, 10 and 25 hour intervals. Adults were placed on food containing varying auxin
265 concentrations for 24 hours prior to imaging.
266
267 Live GFP levels were quantified in 6 animals per condition (including negative and positive controls). Binary
268 images were created by using a threshold (Outsu's thresholding) and the Wand Tool in Fiji was used to trace
269 the GFP-positive area as region of interest (ROI). The ROI corresponded to abdomens in adults or whole
270 larvae. Mean pixel intensity per ROI was calculated using the Measure plugin in Fiji for a total of 6 biological
271 replicates. Statistical significance analysed using ONE-way ANOVA. Statistical tests and plots were performed
272 using the software GraphPad Prism version 9 for Windows.

273

274 **Developmental and Survival Assays**

275 All animals were kept at 25 °C. To determine the effects of auxin on larval development, 10 mated Canton-S
276 flies were allowed to lay eggs on fly food containing 0, 1, 5, or 10 mM of auxin for a maximum of 24 hours,
277 or until ~50 eggs were counted. 5 replicates were obtained for each concentration, except for 0 mM where
278 4 replicates were used. Daily emergence of L3 larvae, and presence of pupae were recorded to determine
279 the time required for egg-to-L3, and egg-to-pupae development for each concentration.

280 Adult survival assays were performed on *c564-GAL4, UAS-nls-GFP / AGES* flies on three replicates, with 20
281 flies per replicate. Male and female animals were separated 5 days post-eclosion. Each replicate was
282 continuously exposed to 0 mM, 5mM and 10 mM Auxin food. Death was scored daily until all flies were
283 deceased. Statistical tests and plots were performed using the software GraphPad Prism version 9 for
284 Windows.

285 **RNA extraction and RT qPCR**

286 In order to quantify whether levels of *GFP* mRNA expression changed in the presence of Auxin, the *c564-*
287 *GAL4, UAS-nls-GFP/ AGES* flies were placed on 0 mM, 5 mM and 10 mM auxin-supplemented food for 24 hrs.
288 The *c564-GAL4, UAS-nls-GFP/ +* flies were used as a positive control whilst the *AGES* flies were used as a
289 negative control (lacking both the GAL4 driver and the *UAS-nls-GFP*). The 0 mM was included to investigate
290 whether there was any leaky GAL4 activity in absence of auxin.

291 Total RNA was extracted from whole adult flies, 3 per replicate per condition, using a standard TRIzol ®
292 extraction protocol (Scientific, 2016). DNA was degraded using RNAse-free DNAse (Thermo Scientific) and
293 cDNA synthesis was performed using the iScript™ cDNA synthesis kit (Bio-Rad), following manufacturer's
294 instructions. Real time quantitative PCR was performed with iTaq™ Universal SYBR® Green Supermix (Bio-
295 Rad) using the StepOnePlus™ Real-Time PCR System (Applied Biosystems).

296 The gene *RpL4* (*Ribosomal Protein L4*) was used as reference gene. The *GFP* mRNA expression levels were
297 calculated using the ΔΔCt method (ΔCt = Ct [mean Ct of reference gene] – Ct [target]; ΔΔCt = ΔCt [target] –
298 mean ΔCt [control]) (Livak and Schmittgen, 2001). Target refers to the auxin concentrations (0mM, 5mM,
299 10mM) and control refers to the *AGES* flies. Primers used: RpL4_FW - 5'-TCCACCTTGAAGAAGGGCTA-3',
300 RpL4_RV – 5'-TTGCGGATCTCCTCAGACTT-3', GFP_FW – 5'-GAGCTGTACAAGAGCAGGCA-3', GFP_RV – 5'-
301 GTTGACGGCGTTTTCGTTCA-3'.

302

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Figure Legends

Figure 1. Design and action of the AGES system. A tubulin promoter ubiquitously expresses the bicistronic TIR1 and AGES cassette. AID-tagged GAL80 is cleaved from the TIR1 due to the presence of the T2A sequence. In the absence of auxin, GAL80 can inhibit GAL4 activity, whilst the presence of auxin induces degradation of GAL80, allowing GAL4 to drive the expression of UAS-transgene(s).

Figure 2. AGES effectively induces GAL4 activity in *Drosophila* adults. A) Ventral images of live females that express GAL4 in fat body tissue. Ingestion of food containing auxin (>= 5 mM for 24 hours) induces GAL4 activity and the expression of GFP. B) Quantification of GFP levels. Pixel intensity thresholding was performed to isolate abdomens as regions of interest. The average pixel intensities were quantified and analysed using one-way ANOVA (****, $p < 0.0001$). C) qPCR data for *GFP* mRNA levels using different concentrations of auxin. Values were normalised to housekeeping gene RpL4 (Ribosomal Protein L4) and relative expression levels (compared to the negative control) were calculated using the ΔΔCt method. Y-axis displaying ΔΔCt values and statistics done using one-way ANOVA (****, $p < 0.0001$).

Figure 3. AGES allows induction of GAL4 activity in the *Drosophila* brain. Confocal images of adult brains stained with anti-GFP and anti-Elav. Scale bars represent 20 µm.

Figure 4. AGES allows induction of GAL4 activity in the *Drosophila* larvae. Bright field images of 3rd instar larvae. A) GFP fluorescence in fat body tissue after induction (for 24 hours) on food with different concentrations of auxin. B) Sporadic GFP expression when using 0.1 mM auxin). C) Representative images of larvae fed on 1 mM auxin and imaged at various time intervals.

Figure 5. Impact of auxin on developmental timing and survival. A) Time taken from egg-laying to pupation. B) Survival across developmental stages (larval to pupal and pupal to adult) with 10 mM auxin. C) Survival of adult females during continuous exposure to auxin. D) Survival of adult males during continuous exposure to auxin. Logrank test and weighted Gehan-Breslow-Wilcoxon model (ns) were used for the adult survival assays.

335 **Figure 1 – figure supplement 1.** Plasmid map of the AGES plasmid. Map of the *pattB-tubP-AtTIR1-T2A-*
336 *miniAID-Gal80-miniAID-SV40* plasmid (generated using Snapgene). The full sequence is in Figure 1 – figure
337 supplement 2.

338 **Figure 1 – figure supplement 2.**

339 Genbank (.gb) sequence file for *pattB-tubP-AtTIR1-T2A-miniAID-Gal80-miniAID-SV40*

340 **Figure 2 – figure supplement 1.**

341 AGES effectively induces GAL4 activity in *Drosophila* adult males. A) Ventral images of live males that express
342 GAL4 in fat body tissue. Ingestion of food containing auxin (24 hours) induces GAL4 activity and the
343 expression of GFP. B) Quantification of GFP levels. Pixel intensity thresholding was performed to isolate
344 abdomens as regions of interest. The average pixel intensity intensities were quantified and analysed using
345 Ordinary one-way ANOVA (**, $p < 0.01$, ****, $p < 0.0001$). C) qPCR data for *GFP* mRNA levels using different
346 concentrations of auxin. Values were normalised to housekeeping gene *RpL4* (*Ribosomal Protein L4*) and
347 relative expression levels were calculated using the ΔΔCt method. Y-axis displaying ΔΔCt values and statistics
348 done using Ordinary one-way ANOVA (**, $p < 0.01$).

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351 ## References

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