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High-level gene expression in *Aedes albopictus* cells using a baculovirus Hr3 enhancer and IE1 transactivator

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Abstract

Background: *Aedes aegypti* is the key vector of both the Yellow Fever and Dengue Fever viruses throughout many parts of the world. Low and variable transgene expression levels due to position effect and position effect variegation are problematic to efforts to create transgenic laboratory strains refractory to these viruses. Transformation efficiencies are also less than optimal, likely due to failure to detect expression from all integrated transgenes and potentially due to limited expression of the transposase required for transgene integration.

Results: Expression plasmids utilizing three heterologous promoters and three heterologous enhancers, in all possible combinations, were tested. The Hr3/IE1 enhancer-transactivator in combination with each of the constitutive heterologous promoters tested increased reporter gene expression significantly in transiently transfected *Aedes albopictus* C7-10 cells.

Conclusions: The addition of the Hr3 enhancer to expression cassettes and concomitant expression of the IE1 transactivator gene product is a potential method for increasing the level of transgene expression in insect systems. This mechanism could also potentially be used to increase the level of transiently-expressed transposase in order to increase the number of integration events in transposon-mediated transformation experiments.

Background

Through the efforts of many individuals in the past few years, it has become possible to genetically transform a wide variety of non-drosophilid insects of medical and agricultural importance [1]. The ability to genetically transform mosquito species allows researchers to better understand mechanisms of vector competence, design novel methods to disrupt vector-pathogen relationships and develop new insect control strategies [2-5]. New molecular methods could potentially augment continued traditional efforts to control malaria and other re-emerging arthropod-borne diseases. Similar approaches may also be used to stem the devastating infestation of eco-

nomically important crops by insecticide-resistant pest strains.

Mosquitoes transmit to humans some of the most debilitating and deadly diseases known. According to the World Health Organization, malaria alone is responsible for one million deaths annually [6]. Additionally, the transmission of yellow fever, dengue fever, West Nile virus and a variety of other encephalitis viruses permanently disrupt or end untold numbers of lives. Both anopheline [7-10] and culicine [11-17] mosquito species have been successfully transformed. In all cases, the process is labor-intensive with a few successful experiments yielding

transformation efficiencies ranging from 0.5% to 13%. These transformation efficiencies are low compared to the nearly 50% previously reported in *Drosophila* with vectors up to 8 kb in size [18]. Additionally, transgene expression in the yellow fever mosquito varies considerably both between and within families [11,12,19], likely due to differences in the transcriptional environments of specific insertion sites within the genome, such as the proximity of the transgene to enhancers or heterochromatic stretches of DNA. This phenomenon is of particular concern in the *Ae. aegypti* genome given its large size (~780 Mb) and its apparent pattern of short-period interspersion where single copy genes (1 to 2 kb) alternate with short (200–600 bp) or medium (1–4 kb) length repetitive sequences [20]. The problem is complex, however transposition has been shown to be dependent upon the amount of transiently available transposase to catalyze vector integration [21,22]. Also, the effective use of genetically-altered mosquitoes to augment current disease vector control requires the ability to create and maintain transgenic lines with consistent, predictable and high-level expression patterns of effector transgenes.

Looking to maximize the transcription of rare transgenes that do land in favorable environments and to potentially increase the levels of transiently available transposase, we tested the ability of three different enhancer elements; SV40 [23], *cop* ULR (*Drosophila*) [24] and Hr3 (*Bombyx mori* nuclear polyhedrosis virus or NPV) [25], to increase the levels of transcription from each of three heterologous promoters from the following genes: actin5C [26] and polyubiquitin (Ubi-p63E – hereafter referred to as pUb) [27] from *Drosophila* and the intermediate early gene (IE1) [28] from the *Autographa californica* multicapsid nuclear polyhedrosis virus (MNPV). Additionally, we tested the ability of the *B. mori* baculovirus IE1 gene product [29], which binds to repetitive sequences within the baculovirus homologous regions (Hrs) [30,31] and has previously been shown to function as a powerful *transactivator* in transfected lepidopteran cells [29], to yet further increase gene expression in mosquito cells.

Results

The Hr3 enhancer and the IE1 transactivator increase reporter gene activity in transiently transfected C7-10 *Aedes albopictus* cells

In transiently transfected C7-10 cells, the Act5C promoter resulted in the highest luciferase reporter activity in comparison with the remaining promoters alone (Fig. 1A). The level of measured activity directly corresponds to the amount of luciferase protein expressed by the transfected cells and thus presumably the level of transcription. Among the enhancers, Hr3 improved luciferase expression by 4-fold, 47-fold and 22-fold over the basal level expression from the Act5C, IE1 and pUb promoters

respectively; cULR improved luciferase expression over basal level from the Act5C, IE1 and pUb promoters 2-fold, 11-fold and 10-fold respectively; and eSV40 resulted in 2-fold, 8-fold and 7-fold increases respectively in luciferase expression from the Act5C, IE1 and pUb promoters (Fig. 1A). Addition of the IE1 *transactivator* unexpectedly resulted in large increases in expression from the *Renilla* control plasmid, as well as from the Hr3-containing reporter plasmids. This is seen in the apparent drop of expression indicated by the red-shaded bars in Fig. 1A. This was confirmed in several independent experiments and was seen even with decreased concentrations of the *Renilla* plasmid (see additional file 1). In order to see the relative effect of the IE1 *transactivator* on expression from the Hr3/promoter constructs, the raw firefly luciferase values were converted to a % of average pSLIE1Luc expression and plotted on a log scale (Fig. 1B). Firefly luciferase expression increased 50–200-fold over the basal level expression of all of the promoters with the addition of the IE1 *transactivator*.

IE1 transactivator interacts with promoter sequences in addition to the Hr3 enhancer

Analysis of multiple experiments (Fig. 2, Table 1, and data not shown) revealed an interesting trend regarding the effect of the IE1 *transactivating* protein upon the promoters themselves. This effect was different for each promoter when co-transfected with an identical plasmid expressing the *Renilla* luciferase control. Addition of the IE1 *transactivator* resulted in a 17-fold increase in expression of firefly luciferase from the Act5C promoter over its basal level expression and a concomitant 30-fold increase from the *Renilla* luciferase reporter under the control of the hsp82 promoter from *Drosophila pseudobscura* [32]. When the IE1 promoter was used to drive expression of firefly luciferase, expression increased 169-fold over the basal level expression, while *Renilla* luciferase expression from the hsp82 promoter increased 138-fold. Finally, firefly luciferase expression increased 11-fold relative to basal level expression from the pUb promoter with a corresponding 202-fold increase in *Renilla* luciferase expression from the hsp82 promoter.

Discussion

Unexpectedly, the internal control for transfection and protein recovery, *Renilla* luciferase, could not reliably be used as such in the presence of the *transactivator*. The data presented reveal a differential effect of the IE1 *transactivator* (Fig. 2 and Table 1) that profoundly affects expression levels from the two luciferase plasmids in an enhancer/promoter-dependent manner. This compromises the ability to compare expression values both within an experiment where IE1 is present in some samples but not in others and between experiments where different batches of cells and assay reagents are employed. The results of a

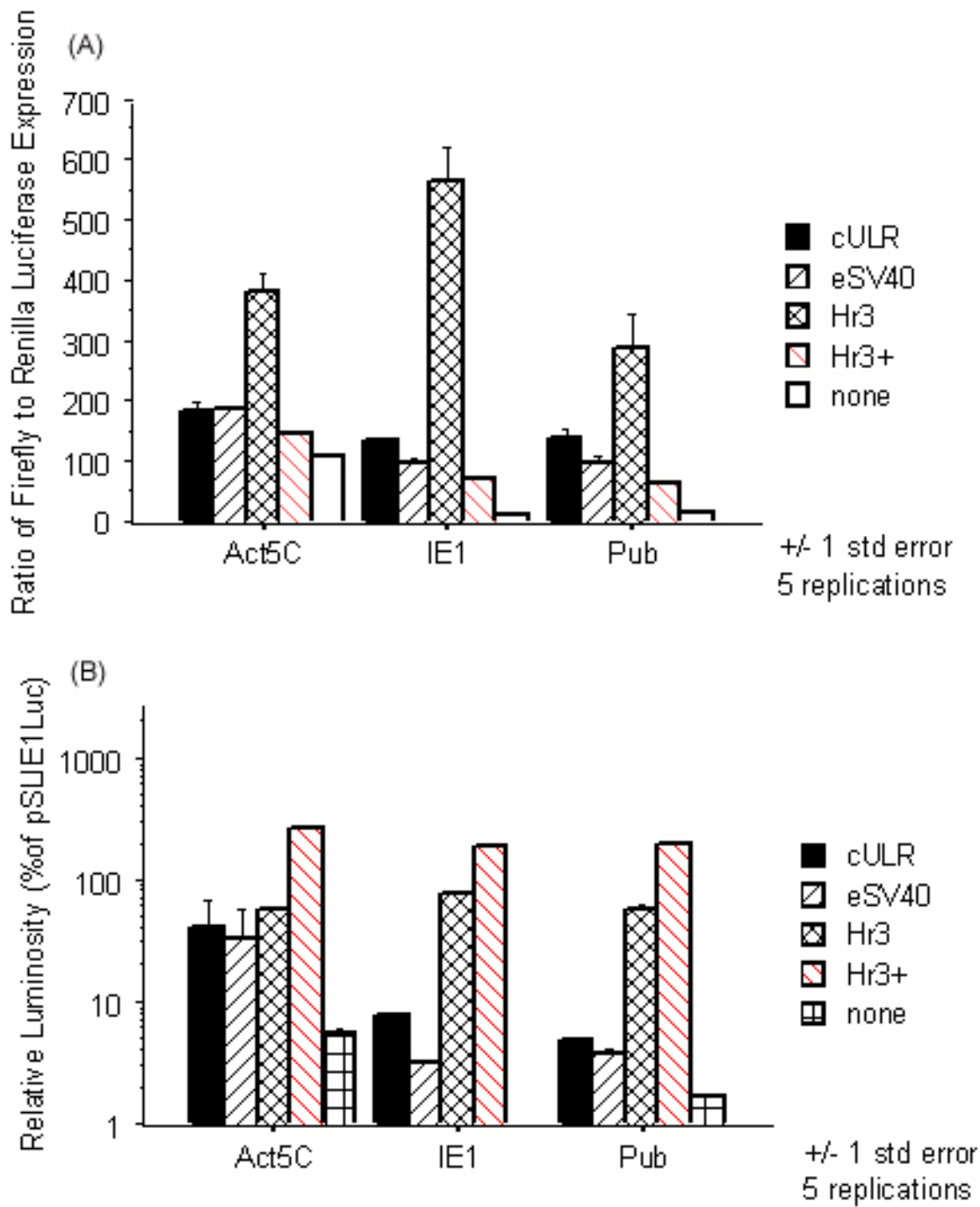


Figure 1

Firefly luciferase expression from various promoter/enhancer plasmids in *Aedes albopictus* C7-10 cells. Cells were assayed for luciferase expression 24 hrs. post-transfection. The averages of five replications are reported and error is reported as +/- 1 standard error. (A) To normalize for differences in transfection efficiency and cell cycle state within the experiment, the firefly luciferase luminescence values for each construct were divided by the corresponding *Renilla* luciferase luminescence values measured in a dual luciferase assay. Bars in red indicate the presence of the IE1 transactivator. The Hr3 enhancer clearly outperforms both the cULR and the eSV40 enhancers in combination with each of the promoters. (B) Raw firefly luciferase values are reported as a % of pSLIE1Luc expression on a log scale. The bars in red show levels of firefly luciferase expression in the presence of the IE1 transactivating protein (Hr3+). Addition of the IE1 transactivating protein (Hr3+) increased firefly luciferase expression 2.5–4-fold over all Hr3-promoter combinations alone and 50–200-fold over basal promoter expression.

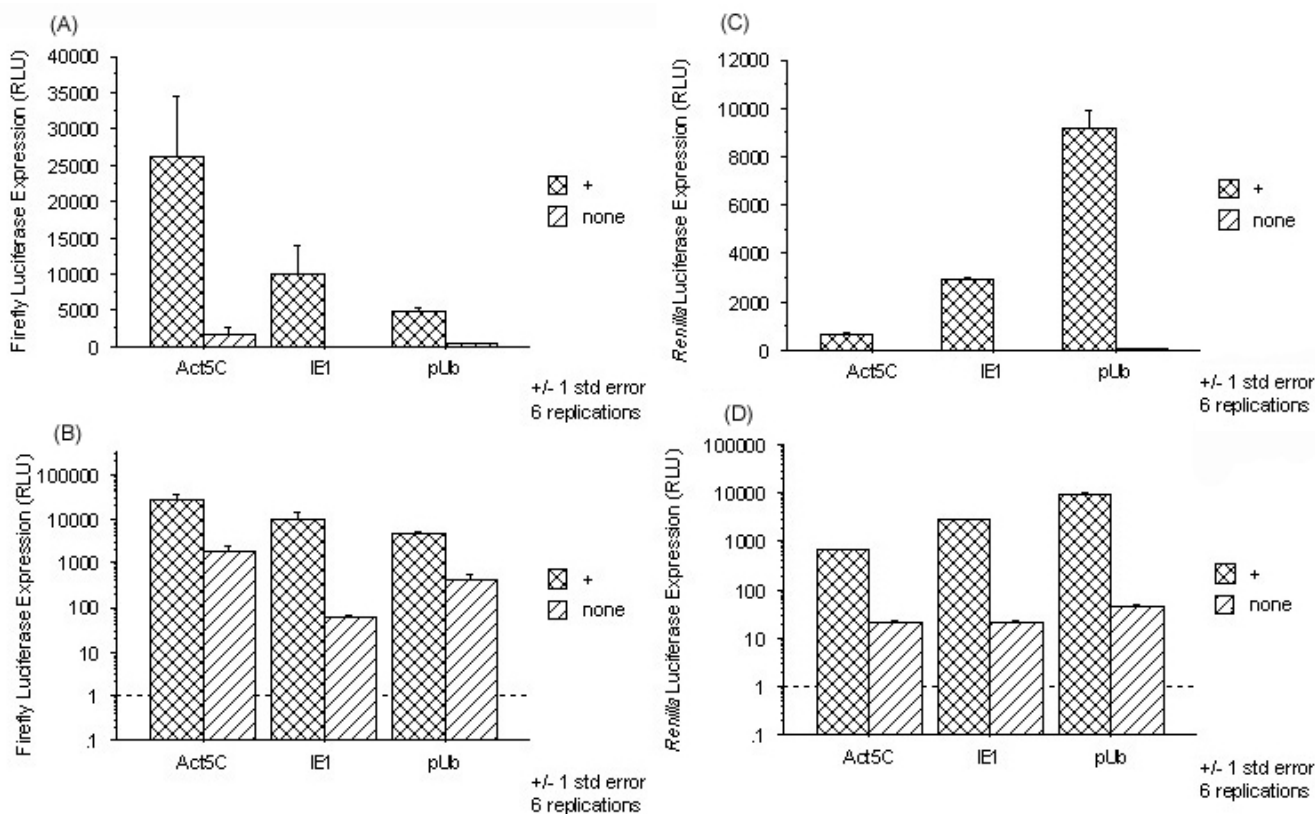


Figure 2

Differential effect of the IE1 *transactivator* on the transcription levels from various promoters. Each promoter-Luc construct was co-transfected with psp82*Renilla*Luc, both in the absence and presence of the IE1 *transactivator*, and assayed for both firefly and *Renilla* luciferase expression, 24 hrs. post-transfection. One experiment with six replicates was performed with the same batch of cells, DNA/liposome complexes and luciferase reagents. Error is reported as +/- 1 standard error. Each set of data is plotted both on a linear and a log scale. (A) Addition of the *transactivator* (+) caused a 16-fold increase in firefly luciferase expression from the Act5C promoter, a 169-fold increase in expression from the IE1 promoter and an 11-fold increase in expression from the pUb promoter. (B) The same data as shown in (A) but plotted on a log scale. (C) Addition of the *transactivator* resulted in different levels of expression from the psp82*Renilla*Luc construct depending upon which promoter was used to drive expression of the firefly luciferase construct. (D) The same data as shown in (C) but plotted on a log scale.

single experiment involving the *transactivator* are reported here, however additional experiments show similar results. The ratio of firefly to *Renilla* luciferase is reported for all promoter/enhancer combinations to allow accurate comparison of the three promoters alone and in combination with each of the three enhancers. It should be noted that the addition of the *transactivator* does significantly increase firefly luciferase expression from all three promoters with the Hr3 enhancer sequence, though this is masked by the simultaneous increase in expression from the *Renilla* luciferase control plasmid.

The IE1 *transactivator* is clearly interacting with the promoters in *trans*, even in the absence of the Hr3 enhancer

element (Fig. 2, Table 1 and data not shown). This observation agrees with previously published data that the cytoplasmic A3 actin gene promoter of *B. mori* was upregulated as much as one hundred-fold by the co-transfection of a plasmid encoding the *B. mori* IE1 gene product (BmIE1) [29]. When the Hr3 enhancer is present, there is a cooperative effect, and luciferase expression increases as much as 200-fold (Fig. 1) over that of the promoter alone. This cooperativity is consistent with results obtained with Hr3-enhanced CAT expression cassettes driven by the *B. mori* cytoplasmic actin gene promoter co-expressed with the BmIE1 protein in lepidopteran cell lines *Bm5* and *Sf21* (an increase of up to three orders of magnitude) [29].

Table 1: Change in Basal Luciferase Expression from Promoters with Addition of the IE1 Transactivator

Promoter	Firefly Luciferase	Renilla Luciferase	Ratio
Act5C	↑ 17 x	↑ 30 x	↓ 1.9 x
IE1	↑ 169 x	↑ 138 x	↑ 1.2 x
pUb	↑ 11 x	↑ 202 x	↓ 18.3 x

This summary of the data presented in Fig. 2 shows the fold change of firefly luciferase expression from each basal promoter following addition of the IE1 transactivator, the fold-increase in expression from the control *Renilla* luciferase plasmid under control of the hsp82 promoter and the overall change in ratio.

The significant differences seen in expression from each of the promoters tested (Fig. 2 and Table 1) reveal that not all promoters are affected in the same manner, nor is the co-transfected plasmid. The presence of the Hr3 enhancer region upstream of the promoter driving expression of the IE1 transactivator protein, results in high levels of IE1 protein from a relatively low amount of plasmid DNA. Despite this abundance of IE1 protein, it appears that transcription from the pUb promoter, in the absence of the Hr3 enhancer, increases only 11-fold (Fig. 2A and Table 1), while transcription from the hsp82 promoter driving *Renilla* luciferase expression is exceptionally high (Fig. 2B and Table 1). The simplest explanation is that the IE1 protein has different affinities for binding sites on the various promoters and/or the IE1 protein is sequestering necessary basal transcription factors. It has also been observed that some viral promoters, IE-0, IE-2 and PE-38, are inhibited by IE1 expression [33-35]. Clearly, the actions of the IE1 transactivator in this study are consistent with its ability to bind Hrs [36-38]. In addition, the protein has two independent functional acidic activation domains and two potential positively-charged inhibitory domains [39,40], consistent with its observed ability to both enhance and inhibit expression from different promoters. Also, lower concentrations of the plasmid bearing the IE1 gene sequence in these transient assays result in greater increases in luciferase expression (see additional file 2). This observation is consistent with the mechanism of negative regulation by the IE1 protein previously proposed [35] where the cooperative binding of the Hrs occurs at a lower concentration than that required for binding to the half sequence regions (Hs) present in negatively regulated promoters. It is also consistent with the presence of the Hr3 enhancer sequence on the plasmid producing the IE1 transactivating protein, which results in up-regulation of IE1 transcription, consequently reducing the number of plasmid copies needed to produce optimal protein levels. When this experiment was repeated using pUb to drive *Renilla* luciferase expression (data not shown and additional file 3), significant differences between promoters were also observed, though not the same differences described above with the hsp82-*Renilla* expression

plasmid. Finally, it should be noted that each of the enhancers alone also differentially affected the expression from each promoter. These data collectively highlight the value of evaluating the effects of new promoter/enhancer/transactivator combinations on the expression of a reporter gene within a related cell line, prior to investing significant time and effort in the creation of transgenic lines. Though cell lines do not completely mimic the cellular and nuclear environment of an entire organism, they can yield significant insight into both the potential interaction between regulatory elements driving transgene expression and the potential impact of unknown endogenous transacting factors.

Conclusions

Clearly, we have shown that the baculovirus homologous region, Hr3, along with the IE1 transactivating protein, significantly increases transgene expression from each of the three heterologous, constitutive promoters tested in mosquito cells. Some concern does exist that endogenous promoters might be down-regulated by the presence of the IE1 protein, and that available host cell transcription factors might be sequestered by complexes stabilized by the IE1 transactivator, however a lower concentration of IE1 transactivator would likely mitigate these effects. Preliminary transposition assays confirm the ability of the Hr3 enhancer/IE1 transactivator combination to function in syncytial preblastoderm mosquito embryos and to significantly increase observed transposition frequencies when used to drive transposase expression (Coates, *et al.*, unpublished data). Use of tissue-specific promoters/enhancers and/or inducible expression may effectively reduce any potential fitness load imposed by interactions of the ie1 protein with endogenous regulatory elements. Perhaps the most promising application is the use of HR/IE1 in helper plasmids transiently expressing transposase in an attempt to increase the number of stable transgene integration events by increasing the amount of available transposase, particularly if germline-specific promoters were used to express the transactivator protein. The HR/IE1 strategy is a promising tool for high-level transgene expression and/or increased transposition frequency in

culicine mosquitoes and possibly other insect species as well.

Methods

Construction of the luciferase expression plasmids

A 2.7-kb *HindIII-SalI* fragment from pGL2-Basic (Promega), containing the firefly luciferase coding region and the SV40 poly-Adenylation signal, was inserted into the corresponding sites of pBCKS+ (Stratagene) to create pBCLuc. A 2.7-kb *SmaI-SalI* fragment from pBCLuc was inserted into the *SmaI-SalI* sites of pSLfa1180fa [41] to create pSLLuc. The *Drosophila* Actin5C promoter was excised from pHermesA5CEGFP [13] by *PstI* and *BamHI* digestion and inserted into the corresponding sites of PSLuc to create PSLAct5CLuc. The *SacII* site was removed from pIE1-3 (Novagen) and then the 657-bp *EcoRI-BamHI* fragment containing the AcMNPV IE1 promoter was inserted into the corresponding sites of pSLLuc to create pSLIE1Luc. A 2-kb *KpnI-BamHI* fragment from pB [pUB-nls-EGFP] [42] containing the *Drosophila* polyubiquitin promoter was inserted into the corresponding sites of pSLLuc to create pSLpUbLuc. The *copia* ULR was amplified by polymerase chain reaction (PCR) from *copia* LTR-ULR-CAT [43] using the primers 5'-AAGCTTGGGCCAGTCCATGCCTA-3' and 5'-CCGCGGATACGTTTACGCTTGTC-3', cleaved by digestion with *HindIII* and *SacII* and inserted into the corresponding sites of pBCKS+ to create pBCcULR. The *HindIII-SacII* fragment from this plasmid was then inserted into the corresponding sites of pSLAct5CLuc and pSLIE1Luc to create pSLcULRAct5CLuc and pSLcULRIE1Luc. pBCcULR was digested with *HindIII* and the site filled with the Klenow fragment of DNA polymerase I (Promega), then digested with *SacII* and ligated into pSLpUbLuc which had been cut with *NotI* and the site filled with Klenow fragment, then cut with *SacII* to create pSLcULRpUbLuc. The SV40 enhancer region from pRL-SV40 (Promega) was PCR-amplified using the primers 5'-AAGCTTCTGAGGCGGAAAGAACCA-3' and 5'-CCGCGGAAAATTAGCCAGCCATGG-3', digested with *HindIII* and *SacII* and inserted into the corresponding sites of pBCKS+ to make pBCeSV40. The *HindIII-SacII* fragment of pBCeSV40 was then inserted into the corresponding sites of pSLAct5CLuc and pSLIE1Luc to create pSLeSV40Act5CLuc and pSLeSV40IE1Luc. pBCeSV40 was digested with *HindIII*, the site filled with Klenow fragment, then digested with *SacII* and ligated to pSLpUbLuc digested with *NotI*, the site filled with Klenow fragment and subsequently digested with *SacII* to produce pSLeSV40pUbLuc. A 1.2-kb *PstI-BamHI* fragment containing the *B. mori* NPV Hr3 enhancer from p153 [25] was inserted into the corresponding sites of pBCKS+ to create pBCHr3. The *PstI-BamHI* fragment of pBCHr3 was inserted into the corresponding sites of pSLAct5CLuc to create pSLHr3Act5CLuc. The *HindIII-SacII* fragment of pBCHr3

was inserted into the corresponding sites of pSLIE1Luc to create pSLHr3IE1Luc. The *EcoRV-SacII* fragment of pBCHr3 was ligated to pSLpUbLuc digested with *NotI*, the site filled with Klenow fragment, and then digested with *SacII* to create pSLHr3pUbLuc. pHP82*Renilla*Luc was created by inserting a 1-kb *KpnI-BamHI* fragment from pKHP82 [44] into the corresponding sites of pBCKS+ and then inserting the *KpnI-PstI* fragment from this plasmid into the corresponding sites of pRL-SV40. ppUb*Renilla*Luc was created by first digesting pSLpUbLuc with *NotI*, filling in the site with Klenow fragment, then digesting with *PstI* to produce a 2-kb fragment which was ligated to pRL-SV40 prepared by digestion with *BglII*, the site filled with Klenow fragment and then digested with *PstI*.

Cell cultures and transfections

Aedes albopictus C7-10 cells were maintained at 25°C with 5% CO₂ in Eagle's media plus 5% fetal calf serum with the following additions per liter: 10 mL 10% (wt/vol) D(+)-glucose, 10 mL 200 mM L-glutamine, 10 mL MEM vitamin solution, 20 mL MEM non-essential amino acids, 10 mL Penicillin/Streptomycin (10,000 U/mL), 29.3 mL sodium bicarbonate (7.5% w/v) [45]. 400 µL of cells at a density of 2 × 10⁶ cells/mL were seeded into 24-well microtiter plates and incubated at 25°C for 24 hrs. Cells were transfected with 0.4 µg total DNA and 0.8 µL LipofectAMINE 2000 (Life Technologies) in 10 µL serum-free, antibiotic-free media. pHP82*Renilla*Luc and the firefly constructs were transfected at a 1:2 ratio. The IE1 transactivator plasmid [25] was present as 1/10 of the total DNA.

Luciferase assays

Transfected cells were assayed 24 hrs. post-transfection using a Turner Designs 20/20 luminometer and a Dual Luciferase Assay (Promega). The manufacturer's passive lysis protocol was followed. In addition, cell lysates were snap frozen in liquid nitrogen immediately after lysis to minimize luciferase protein degradation. All samples were diluted 20-fold in 1 × PLB (passive lysis buffer) in order to obtain a reading within the range of the luminometer.

Authors' contributions

CG carried out the studies described in this paper and drafted the manuscript. CC originated the study, participated in its design and planning and edited the manuscript. Both authors read and approved the final manuscript.

Additional material

Additional File 1

The IE1 transactivator significantly affects expression from the Renilla luciferase control plasmid. Cells were transfected as detailed in the methods with 0.27 µg Hr3Act5CLuc (firefly) plasmid, 0.04 µg IE1 transactivator plasmid and the indicated amount of Renilla luciferase control plasmid. Because this is a control plasmid with no Hr3 enhancer element present, one would expect the expression levels to parallel those shown in the absence of the IE1 transactivator (solid bars).

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Additional File 2

Lower concentrations of the IE1 transactivator result in greater expression from both the Hr3Actin5C (firefly luciferase) and the hsp82 (Renilla luciferase) promoters. Cells were transfected as detailed in the methods with 0.27 µg Hr3Act5CLuc (firefly) plasmid, 0.14 µg hsp82RenillaLuc plasmid and the indicated amount of IE1 transactivator plasmid.

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Additional File 3

The hsp82 versus the pUb promoter for Renilla luciferase expression. Cells were transfected as detailed in the methods with the only difference being the promoter used to drive expression of the Renilla luciferase control plasmid. The pUb promoter seems to be upregulated more than the hsp82 promoter in the presence of the transactivator. Interestingly, the corresponding expression from the Hr3Act5C firefly luciferase plasmid is less when the pUb Renilla plasmid is co-transfected. Other differences were seen when this experiment was repeated with Hr3IE1 and Hr3pUb firefly luciferase plasmids. Clearly, the IE1 transactivator binds sequences other than the Hr3 enhancer sequence in eukaryotic promoters and the effect is dependent upon the combination of promoters present.

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References

- Robinson Alan S., Franz Gerald, Atkinson Peter W.: **Insect transgenesis and its potential role in agriculture and human health.** *Insect Biochem Mol Biol* 2004, **34**:113-120.
- Collins FH, James AA: **Genetic modification of mosquitoes.** *Sci Med* 1996:52-61.
- Beerntsen BT, James AA, Christensen BM: **Genetics of mosquito vector competence.** *Microbiol Mol Biol Rev* 2000, **64**:115-137.
- Blair CD, Adelman ZN, Olson KE: **Molecular strategies for interrupting arthropod-borne virus transmission by mosquitoes.** *Clin Microbiol Rev* 2000, **13**:651-661.
- Alphey L, Andreasen M: **Dominant lethality and insect population control.** *Mol Biochem Parasitol* 2002, **121**:173-178.
- WHO: **Malaria in Africa Infosheet.** [http://www.rbm.who.int/cmc_upload/0/000/015/370/RBMInfosheet_3.htm].
- Catteruccia F, Nolan T, Loukeris TG, Blass C, Savakis C, Kafatos FC, Crisanti A: **Stable germline transformation of the malaria mosquito *Anopheles stephensi*.** *Nature* 2000, **405**:959-962.
- Grossman GL, Rafferty CS, Clayton JR, Stevens TK, Mukabayire O, Benedict MQ: **Germline transformation of the malaria vector, *Anopheles gambiae*, with the piggyBac transposable element.** *Insect Mol Biol* 2001, **10**:597-604.
- Nolan Tony, Bower Tom M., Brown Anthony E., Crisanti Andrea, Catteruccia Flaminia: **piggyBac-mediated germline transformation of the malaria mosquito *Anopheles stephensi* using the red fluorescent protein dsRED as a selectable marker.** *J Biol Chem* 2002, **277**:8759-8762.
- Perera OP, Harrell RA, Handler AM: **Germ-line transformation of the South American malaria vector, *Anopheles albimanus*, with a piggyBac/EGFP transposon vector is routine and highly efficient.** *Insect Mol Biol* 2002, **11**:291-297.
- Jasinskiene N, Coates CJ, Benedict MQ, A.J. Cornel, Salazar-Rafferty C, James AA, Collins FH: **Stable, transposon-mediated transformation of the yellow fever mosquito, *Aedes aegypti*, using the Hermes element from the house fly.** *Proc Natl Acad Sci USA* 1998, **95**:3743-3747.
- Coates CJ, Jasinskiene N, James AA: **Mariner transposition and transformation of the yellow fever mosquito, *Aedes aegypti*.** *Proc Natl Acad Sci USA* 1998, **95**:3748-3751.
- Pinkerton AC, Michel K, O'Brochta DA, Atkinson PVW: **Green fluorescent protein as a genetic marker in transgenic *Aedes aegypti*.** *Insect Mol Biol* 2000, **9**:1-10.
- Kokoza V, Ahmed A, Wimmer EA, Raikhel AS: **Efficient transformation of the yellow fever mosquito *Aedes aegypti* using the piggyBac transposable element vector pBac[3xP3-EGFP afm].** *Insect Biochem Mol Biol* 2001, **31**:1137-1143.
- Allen Margaret L., O'Brochta David A., Atkinson Peter W., Levesque Cynthia S.: **Stable, Germ-Line Transformation of *Culex quinquefasciatus* (Diptera: Culicidae).** *J Med Entomol* 2001, **38**:701-710.
- Lobo NF, Hua-Van A, Li X, Nolen BM, Fraser Jr MJ: **Germ line transformation of the yellow fever mosquito, *Aedes aegypti*, mediated by transpositional insertion of a piggyBac vector.** *Insect Mol Biol* 2002, **11**:133-139.
- Ito Junitsu, Ghosh Anil, Moreira Luciano A., Wimmer Ernst A., Jacobs-Lorena Marcelo: **Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite.** *Nature* 2002, **417**:452-455.
- Spradling AC: **P element-mediated transformation.** *Drosophila: A Practical Approach* Edited by: Roberts DB. Oxford, IRL Press; 1986:175-197.
- Coates Craig J., Jasinskiene Nijole, Pott Gregory B., James Anthony A.: **Promoter-directed expression of recombinant fire-fly luciferase in the salivary glands of Hermes-transformed *Aedes aegypti*.** *Gene* 1999, **226**:317-325.
- Knudson DL, Zheng L, Gordon SW, Brown SE, Kafatos FC: **Genome Organization of Vectors.** *The Biology of Disease Vectors* Edited by: Beatty BJ and Marquardt WC. Fort Collins, CO, University Press of Colorado; 1996:175-214.
- Kapetanaki Maria G., Loukeris Thanasis G., Livadaras Ioannis, Savakis Charalambos: **High frequencies of Minos transposon mobilization are obtained in insects by using in vitro synthesized mRNA as a source of transposase.** *Nucl Acids Res* 2002, **30**:3333-3340.
- Davidson Ann E., Balciunas Darius, Mohn Deanna, Shaffer Jennifer, Hermanson Spencer, Sivasubbu Sridhar, Cliff M. Pat, Hackett Perry B., Ekker Stephen C.: **Efficient gene delivery and gene expression in zebrafish using the Sleeping Beauty transposon.** *Dev Biol* 2003, **263**:191-202.
- Moreau P, Hen R, Wasyluk B, Everett R, Gaub MP, Chambon P: **The SV40 72 base pair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants.** *Nucl Acids Res* 1981, **9**:6047-6068.
- Matyunina LV, Jordan IK, McDonald JF: **Naturally occurring variation in copia expression is due to both element (cis) and host (trans) regulatory variation.** *Proc Natl Acad Sci USA* 1996, **93**:7097-7102.
- Lu M, Farrell PJ, Johnson R, Iatrou K: **A baculovirus (*Bombyx mori* nuclear polyhedrosis virus) repeat element functions as**

- a powerful constitutive enhancer in transfected insect cells. *J Biol Chem* 1997, **272**:30724-30728.
26. Thummel CS, Boulet AM, Lipshitz HD: **Vectors for Drosophila P-element-mediated transformation and tissue culture transfection.** *Gene* 1988, **74**:445-456.
 27. Lee HS, Simon JA, Lis JT: **Structure and expression of ubiquitin genes of Drosophila melanogaster.** *Mol Cell Biol* 1988, **8**:4727-4735.
 28. Pullen SS, Friesen PD: **Early transcription of the IE-1 transregulator gene of Autographa californica nuclear polyhedrosis virus is regulated by DNA sequences within its 5' noncoding leader region.** *J Virol* 1995, **69**:156-165.
 29. Lu Maolong, Johnson Russell R., Iatrou Kostas: **Trans-activation of a cell housekeeping gene promoter by the ie1 gene product of baculoviruses.** *Virology* 1996, **218**:103-113.
 30. Guarino LA, Summers MD: **Interspersed homologous DNA of Autographa californica nuclear polyhedrosis virus enhances delayed-early gene expression.** *J Virol* 1986, **60**:215-223.
 31. Kremer A, Knebel-Mordsdorf D: **The early baculovirus he65 promoter: On the mechanism of transcriptional activation by IE1.** *Virology* 1998, **249**:336-351.
 32. Blackman RK, Meselson M: **Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the Drosophila hsp82 gene.** *J Mol Biol* 1986, **188**:499-515.
 33. Carson DD, Summers MD, Guarino LA: **Transient expression of the Autographa californica nuclear polyhedrosis virus intermediate-early gene, IE-N, is regulated by three viral elements.** *J Virol* 1991, **65**:945-951.
 34. Kovacs GR, Guarino LA, Summers MD: **Novel regulatory properties of the ie1 and ie0 transactivators encoded by the baculovirus Autographa californica multicapsid nuclear polyhedrosis virus.** *J Virol* 1991, **65**:5281-5288.
 35. Leisy DJ, Rasmussen C, Owusu EO, Rohrmann GF: **A mechanism for negative gene regulation in Autographa californica multinucleocapsid nuclear polyhedrosis virus.** *J Virol* 1997, **71**:5088-5094.
 36. Guarino LA, Dong W: **Expression of an enhancer-binding protein in insect cells transfected with the Autographa californica nuclear polyhedrosis virus IE1 gene.** *J Virol* 1991, **65**.
 37. Guarino LA, Dong W: **Functional dissection of the Autographa californica nuclear polyhedrosis enhancer element hr5.** *Virology* 1994, **200**:328-335.
 38. Rodems SM, Friesen PD: **Transcriptional enhancer activity of hr5 requires dual-palindrome half sites that mediate binding of a dimeric form of the baculovirus transregulator ie1.** *J Virol* 1995, **69**:5368-5375.
 39. Choi J, Guarino LA: **The baculovirus transactivator ie1 binds to viral enhancer elements in the absence of insect cell factors.** *J Virol* 1995, **69**:4548-4551.
 40. Slack JM, Blissard GW: **Identification of two independent transcriptional activation domains in the Autographa californica multicapsid nuclear polyhedrosis virus ie1 protein.** *J Virol* 1997, **71**:9579-9587.
 41. Horn C, Wimmer EA: **A versatile vector set for animal transgenesis.** *Dev Genes Evol* 2000, **210**:630-637.
 42. Handler AM, Harrell R. A. 2nd: **Transformation of the Caribbean fruit fly, Anastrepha suspensa, with a piggyBac vector marked with polyubiquitin-regulated GFP.** *Insect Biochem Mol Biol* 2001, **31**:199-205.
 43. Wilson Susanne, Matyunina Lilya V., McDonald John F.: **An enhancer region within the copia untranslated leader contains binding sites for Drosophila regulatory proteins.** *Gene* 1998, **209**:239-246.
 44. Coates CJ, Howells AJ, O'Brochta DA, Atkinson PW: **The 5' regulatory region from the Drosophila pseudobscura hsp82 gene results in a high level of reporter gene expression in Lucilia cuprina embryos.** *Gene* 1996, **175**:199-201.
 45. Shih KM, Gerenday A, Fallon AM: **Culture of mosquito cells in Eagle's medium.** *In Vitro Cell Dev Biol-Animal* 1998, **34**:629-630.

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