Mutation Notes

Drosophila melanogaster P{GAL4-Hsp70.PB} transposon insertion on 3rd chromosome creates mutations in *mth* and *Ptpmeg* genes.

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The third chromosome P element insertion P{w^{+mC}=GAL4-Hsp70.PB}89-2-1 (Bloomington stock number 1799) (Brand, Manoukian, and Perrimon, 1994), hereafter referred to as P{*hs-GAL4*}89, is being used as a heat-inducible GAL4 driver for the ubiquitous, conditional activation of UAS responders (Armstrong *et al*., 2002; Chanut *et al*., 2002; Kozlova and Thummel, 2002; Liu and Lehmann, 2008; Roman, He, and Davis, 2000; Seong, Ogashiwa, Matsuo, Fuyama, and Aigaki, 2001). To determine whether the P insertion in the P{*hs-GAL4*}89 line may potentially interfere with the expression of a gene or genes located at the genomic integration site, we amplified flanking genomic DNA by inverse PCR. Genomic DNA isolated from P{*hs-GAL4*}89 flies was digested with *Hpa*I, which cuts in the polylinker of the pCaSpeR3 transformation vector from which the P{*hs-GAL4*}89 element was derived (Brand *et al*., 1994) and in flanking genomic DNA. Restriction fragments were ligated under dilute conditions to favor circularization of DNAs. The portion of the polylinker that remained attached to genomic DNA after *Hpa*I digestion was then cut by digestion with *Sal*I to generate linearized DNAs with P-element-derived ends. Linearized DNA was then amplified by PCR and sequenced using primers 5'-GGATCCCCGGGCGAG-3' and 5'-CCTGCAGCCCAAGCTT-3'.

PCR yielded a single product of about 0.8 kb. Sequencing of the PCR product indicated that the transposon resides in the *Ptpmeg* gene, located 543 bp upstream of the transcription start of transcripts RI and RJ, and within a large intron that is specific for transcripts RH and RK (Figure 1). This intron harbors three other genes, *methuselah* (*mth*) and *methuselah-like 9* and *10* (*mthl9*, *mthl10*), that are transcribed in the opposite direction to *Ptpmeg*. The P{*hs-GAL4*}89 element is inserted after position +38 downstream of the putative transcription start site of the *mth* gene (Figure 1B). Consistent with this observation, microarray data indicate that *mth* RNA was 30-fold reduced in salivary glands of homozygous P{*hs-GAL4*}89 animals compared to control animals without the element (Liu and Lehmann, 2008). These findings are surprising, because *mth* is considered to be an essential gene. Homozygous *mth* null mutants die during the embryonic stage (Ja, Carvalho, Madrigal, Roberts, and Benzer, 2009). Despite the strong reduction, *mth* RNA was still detectable in homozygous animals (Liu and Lehmann, 2008), suggesting that basal expression of *mth* does not require sequences located immediately upstream of the transcription start site. It appears that a minimal amount of *mth* RNA sufficient for survival can be produced from an internal promoter. This interpretation is supported by the report of a positive transcriptional control element located +28 to +217 relative to the transcription start site of *mth*. A reporter gene that carries this element, but entirely lacks upstream sequences of *mth*, indeed exhibits basal transcriptional activity (H. Kim, Kim, Lee, Yang, and Han, 2006).

Finally, we wondered whether the GAL4 gene of the transposon is transcribed in the same direction as *mth* or *Ptpmeg*. The inverse PCR results had indicated that the P transposon is inserted in the same 5'-3' orientation as Ptpmeg. We determined the orientation of hs-GAL4 within the transposon by PCR with the help of primer 5'-CAGACACTTGGCGCACTTCGGT-3', which hybridizes within the GAL4 gene, and primers Casper1 (5'-GATCCCCGGGCGAGCTCGAAT-3') and Casper2 (5'-AACGCTACAAACGGTGGCGA-3'), which hybridize with vector sequences. Only Casper2, which was designed to give a PCR product if hs-GAL4 was oriented the same way as vector and Ptpmeg, but not Casper1, yielded a product. Thus, hs-GAL4 is transcribed in the same direction as Ptpmeg (Figure 1B).

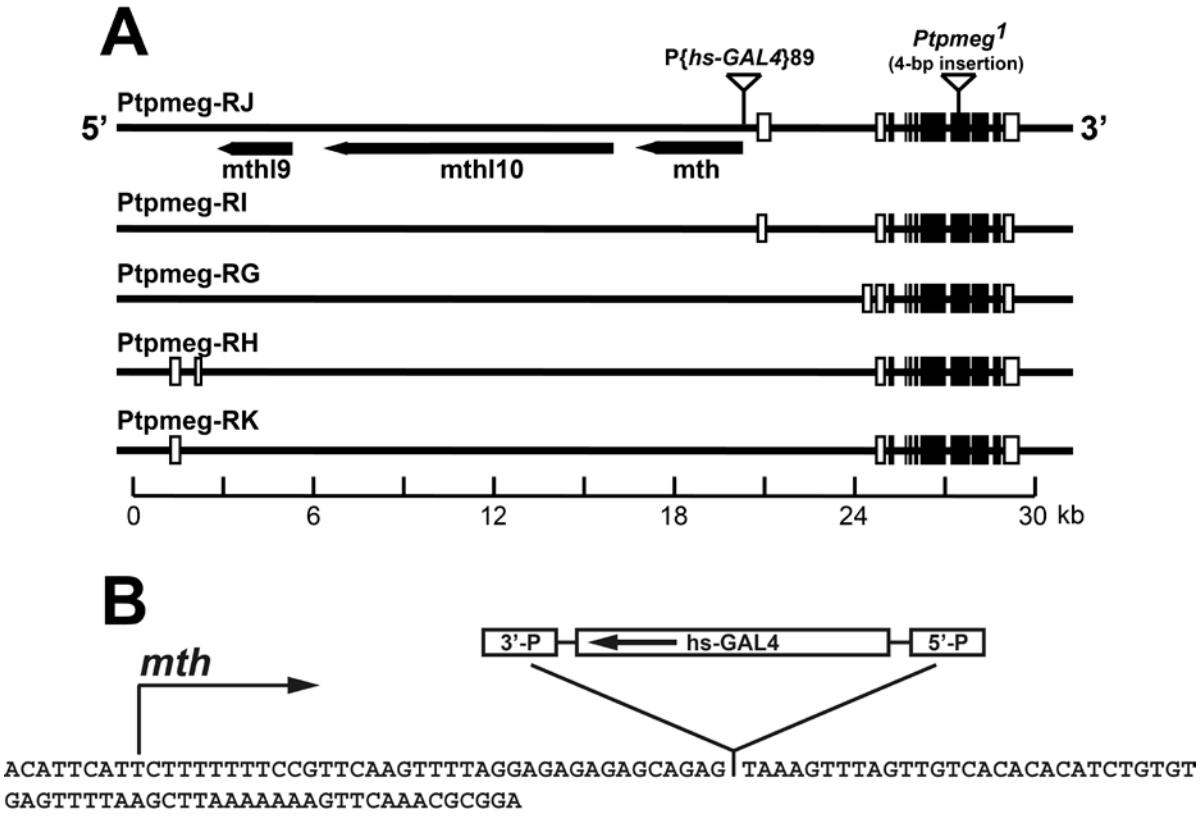

Figure 1. Genomic location and orientation of hs-GAL4 in the P{*hs-GAL4*}89 line. (A) Genomic organization at the P(*hs-GAL4*}89 integration site. *Ptpmeg* is transcribed from left to right, whereas the intronic genes, *mth*, *mthl10*, and *mthl9*, are transcribed from right to left. (B) P element and hs-GAL4 transgene are located in the indicated orientations within the non-coding 5'-exon of *mth* close to the putative transcription start site predicted by Flybase.

Acknowledgments: This work was supported by NIH grant 1R15GM104836-01.

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