Characterization of the *Drosophila* SLOWPOKE binding protein (SLOB) promoter

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**Introduction**

*Drosophila* SLOWPOKE (SLO) is a voltage- and calcium-dependent, large conductance potassium channel important for action potential repolarization, neuronal excitability, neurotransmitter release, and hormone secretion. SLO binding protein (SLOB) binds to and modulates SLO activity. We have shown previously that modulation of SLO by SLOB has profound effects on SLO channel currents, synaptic transmission, and metabolism. Multiple isoforms of SLOB exist and are encoded by multiple transcripts; the isoforms are named based on their predicted protein molecular weights, in kilodaltons. In the *Drosophila* brain, SLOB57/51 proteins are expressed especially profoundly in insulin producing neurons of the pars intercerebralis, while SLOB71/65 proteins are enriched in the lateral neurons that participate in the generation of circadian rhythms. Here we sought to determine the transcription initiation sites in the slob gene and investigated promoter elements responsible for expression of the different slob transcripts.

**Methods**

- **Transcription start sites (TSS)** were mapped using RNA ligase mediated 5' rapid amplification of cDNA ends (RLM-RACE) (Ambion). Total RNA was extracted from heads of *y*;w*¹¹¹* flies, and RLM-RACE was performed with 4 reverse primers targeted to different regions of Slob RA/RC and Slob RB. RLM-RACE products were run on agarose gels, and individual bands were extracted. Products were then TOPO cloned and sequenced to identify TSSs.
- Luciferase reporter constructs were created by sequential cloning of regions upstream of the 2 identified TSSs and inserting sequences into the pGL4.10 luciferase reporter vector or the minimal promoter (minP) pGL4.23 vector (Promega).
- Sequences within the downstream promoter element (DPE) and motif ten structure were added to the constructs using Quickchange site-directed mutagenesis (Agilent Technologies, Huntsville, Ala) and primer sets.
- *Drosophila* S2 cells were transfected with the luciferase reporter constructs along with a vector expressing beta-galactosidase in duplicate using lipofectamine (Invitrogen). S2 cells were then used for luciferase determination, with luciferase assay performed using a luminometer. Activity of the beta-galactosidase enzyme was measured using a spectrophotometer after addition of substrate, and luciferase activity was normalized to beta-galactosidase activity to control for transfection efficiency. Fold changes were calculated by dividing normalized luciferase activity measured for the Slob RA/RC and Slob RB promoters by the luciferase activity measured in S2 cells transfected with the pGL4.10 or pGL4.23 empty vectors. Data are the mean ± SEM. *p* indicates *p* < 0.05, ** indicates *p* < 0.01, and *** indicates *p* < 0.001, one way ANOVA with Bonferroni post-hoc test or one-sample t-test.

**Conclusions**

- **TSSs for slob57/51 and slob71/65 transcripts** were determined in *y* flies. The TSSs are different from those previously identified for *y*;cn beap flies listed in FlyBase. This may be due to background differences between the 2 genotypes.
- By sequential analysis of promoter regions upstream of the 2 identified TSSs and inserting sequences into the pGL4.10 luciferase reporter vector or the minimal promoter (minP) pGL4.23 vector (Promega).

**Fig 1: RLM-RACE Products**

RLM-RACE products were separated on 1% agarose gels. Product bands were extracted, TOPO cloned, and sequenced.

**Fig 2: Two unique TSSs were identified for Slob RA/RC and Slob RB**

- **RLM-RACE Defined Start Sites:** Summary

| TSS1 | AATAGGCGAGTTGGCGAGAA |
| TSS2 | ATTACAGAGTGGAGGACGAA |

**Fig 3: The MTE and DPE core promoter elements are present in Slob RB**

Slob promoter configurations

**Fig 4: A construct containing 1 kb upstream from the Slob RA/RC TSS is sufficient for promoter activity**

**Fig 5: Transcription of Slob RB is regulated by the MTE and DPE core promoter motifs**

**Fig 6: Slob RB constructs exhibit more robust promoter activity**

**Fig 7: Promoter domains of Slob RB differentially repress or activate transcription from a minimal promoter**

**Fig 8: Mutation of hb or mir recognition sequences in Slob RB increases promoter activity**