

Promoter architecture and sex-specific gene expression in the microcrustacean *Daphnia pulex* revealed by large-scale profiling of 5'-mRNA ends

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ABSTRACT

Large-scale identification of transcription start sites (TSSs) using 5'-mRNA profiling has yielded insight into promoter location, architecture, and regulation for limited number of major model organisms. However, much less is known about transcription initiation and *cis*-regulatory elements across a wider spectrum of Metazoa. To broaden our understanding of core promoter structure in species from metazoan clades with currently scant genome data, we sought to characterize the landscape of *cis*-regulatory elements in the microcrustacean *Daphnia pulex*, an important model organism for studies in ecology, toxicology, and genetics. We performed CAGE (Cap Analysis of Gene Expression) from total RNA derived from three separate developmental states: sexual females, asexual females, and males, reflecting distinct sexes and modes of reproduction. We mapped over 120 million CAGE reads to the *D. pulex* genome and generated a “Daphnia Promoter Atlas” containing 12,662 unique promoters and 10,665 consensus promoters active in all three states. Analysis of initiation sites showed the expected enrichment of the CA-dinucleotide at TSSs (associated with Initiator-motif containing promoters) but also significant over-representation of GN-dinucleotides. Overall, our data suggest that *D. pulex* initiation sites are among the most GC-rich yet observed for any metazoan. Computational *de novo* motif discovery around CAGE-identified TSSs revealed eight putative core promoter elements, including the canonical TATA (TATAWAA) and Initiator (CAGWY) motifs, as well as statistically significant motifs with no obvious orthologs in other metazoans. Analysis of the differentially-expressed genes suggests that a significant number of cell cycle genes (each with net negative regulatory effects on meiosis) are up-regulated in asexual females, providing a glimpse of the molecular events that underpin the facultative parthenogenesis in *D. pulex*. Taken together, this work provides the first picture of transcription initiation and promoter architecture within Crustacea. Our Daphnia Promoter Atlas provides a basis for future study among *Daphnia* spp. as well as for comparative genomic analyses of transcriptional control in diverse metazoans.

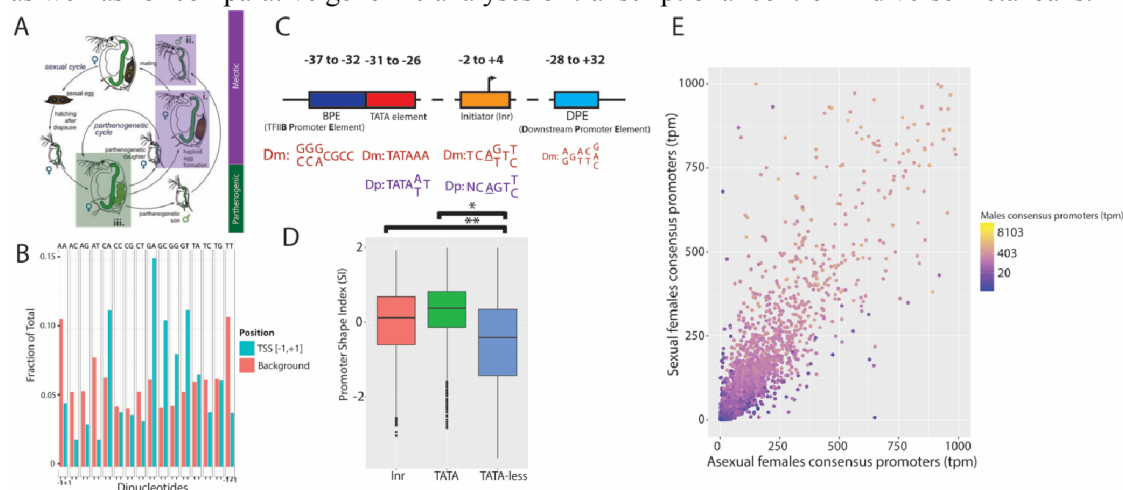


Figure 1: Promoter architecture and sex-specific gene regulation in the microcrustacean *D. pulex*. A. Illustration of the developmental stages (states) included in this study, which reflect meiotic and parthenogenic modes of reproduction. B. Analysis of identified TSS positions indicates enrichment of CA and GN dinucleotides relative to background. C. Model comparing canonical core promoter motif sequences between *D. melanogaster* (red) and *D. pulex*, the latter identified by this study. D. *D. pulex* promoters containing Initiator (Inr) and TATA elements have a more peaked shape than do TATA-less promoters (* and **; $p < 0.001$). E. Comparison of consensus promoter ($n=10,580$) activity (as measured by normalized CAGE tags) across three states in *D. pulex*. Activity of male promoters is presented according to a color gradient (shown at right).