Identification of candidate downstream genes for the homeodomain transcription factor Labial in Drosophila through oligonucleotide-array transcript imaging

ABSTRACT

Our results identify a number of novel candidate downstream target genes for Labial, suggesting that this homeoprotein differentially regulates a limited and distinct set of embryonically expressed Drosophila genes.

INTRODUCTION

Background The homeotic/Hox genes encode a network of evolutionarily conserved homeodomain transcription factors that are involved in the specification of segmental identity along the anterior-posterior body axis of animals as diverse as insects and vertebrates. In Drosophila, these genes are arranged on the chromosome in two gene clusters known as the Antennapedia and Bithorax complexes. There is a correlation between the relative position of the Hox genes within the cluster and their spatial and temporal expression pattern in the body in that genes located towards the 3' end are expressed more anterior and earlier than genes located towards the 5' end (spatial and temporal colinearity). Given their central role in developmental processes, it has been proposed that the homeoproteins do not act directly to specify morphological differences but rather control a battery of subordinate genes encoding cellular functions directly required in differentiation. In search of these subordinate genes, various strategies such as enhancer trapping, immunoprecipitation of chromatin fragments, subtractive hybridization, selection for binding sites in yeast, and heat-shock-induced overexpression have been used.Only a small number of target genes of homeoproteins have been identified to date, however; most of these encode either transcription factors or cell-signaling molecules. In contrast to these results, recent studies suggest that homeoproteins may bind at significant levels to the majority of genes in the Drosophila embryo and regulate a large number of downstream genes. Here we focus on the homeotic gene labial (lab) in the Drosophila embryo. It is the most proximal gene within the Drosophila Antennapedia complex; it encodes an Antennapedia-like Q50 homeodomain transcription factor and is one of the most anteriorly expressed homeotic genes along the anterior-posterior body axis. Genetic studies have demonstrated that lab is required for proper head formation and for the specification of cellular identity in the midgut as well as in the embryonic brain. The lab gene and its vertebrate Hox1 orthologs are among the best-characterized examples of evolutionary conservation of structure, expression and function of Hox genes in animal development. To address the question of which and how many downstream genes are under control of lab, we used a combination of in vivo overexpression techniques and quantitative transcript imaging with oligonucleotide arrays. By using transgenic flies carrying the lab gene under the control of a heat-inducible promoter, we ubiquitously overexpressed lab following heat-shock treatment in Drosophila embryos. We then used high-density oligonucleotide arrays representing 1,513 identified Drosophila genes for large-scale detection and quantification of induced gene expression. We find significant changes in gene expression for 96 identified genes following lab overexpression. Quantitative reverse-transcriptase PCR on a selection of these genes verified the differential expression levels in response to heat-shock-induced overexpression of lab. Our findings identify a number of novel candidate downstream genes for lab and thus show that oligonucleotide arrays are powerful tools for analyzing, at a genome-wide level, the number, identity

and quantitative expression level of genes in the Drosophila embryo.

CONCLUSION

Conclusions Taken together, our results identify a large number of novel candidate downstream genes of the homeodomain transcription factor Lab. To our knowledge, most of these 96 identified and sequenced genes have not been previously shown to be lab targets. At present, we do not know which genes are direct targets (regulated directly by Lab protein binding to DNA regulatory sequences) or indirect targets of lab gene action. Furthermore, our results demonstrate that oligonucleotide arrays are useful tools for analyzing, at a genome-wide level, the number, identity and quantitative expression levels of candidate downstream genes differentially regulated in vivo by developmental control genes. This confirms the general utility of microarrays for studying diverse molecular and cellular processes in Drosophila. Considering the evolutionary conservation of gene structure, expression and function, we propose that these results obtained in Drosophila will also be valid for lab orthologs in other animals, including vertebrates. It will now be important to determine which of the detected candidate downstream genes in Drosophila are direct targets and how they exert the developmental genetic programs imposed by lab gene action.