

Supplemental data to Orian et al., “Genomic Binding by the Drosophila Myc, Max, Mad/Mnt Transcription Factor Network”
(Genes & Development 2003)

Abstract

The Myc/Max/Mad transcription factor network is critically involved in cell behavior, however there is relatively little information on its genomic binding sites. We have employed the DamID method (Steensel and Henikoff 2000; van Steensel et al. 2001) to carry out global genomic mapping of the Drosophila Myc, Max, and Mad/Mnt proteins. Each was tethered to *E. coli* DNA adenine-methyltransferase (Dam) permitting methylation proximal to *in vivo* binding sites in Kc cells. Microarray analyses of methylated DNA fragments reveals binding to multiple loci on all major Drosophila chromosomes. This approach also reveals dynamic interactions among network members as we find that increased levels of dMax influence the extent of dMyc, but not dMnt, binding. Computer analysis using the REDUCE algorithm (Bussemaker et al. 2001) demonstrates that binding regions correlate with the presence of E-boxes, CG repeats and other sequence motifs. The surprisingly large number of directly bound loci (~15% of coding regions) suggests that the network interacts widely with the genome. Furthermore, we employ microarray expression analysis to demonstrate that hundreds of DamID binding loci correspond to genes whose expression is directly regulated by dMyc in larvae. These results suggest that a fundamental aspect of Max network function involves widespread binding and regulation of gene expression.

A. This web page provides links to DamID binding data, gene names, REDUCE regression analysis, gene expression data, and primary data sets used in the accompanying publication.

The following data sets can be found:

(All are in Microsoft Excel format, and contain a legend introduction.)

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| S1 Loci&Target.xls | Complete list of binding loci and network gene expression target (including gene name chromosomal location, and position). |
| S2 GO_list.xls | Gene Ontology classification for molecular function/localization of annotated genes |
| S5 MycMaxMnt_Reduce.xls | Complete REDUCE results for dMyc, dMax, dMnt |

Access to primary data:

Primary Data can be accessed upon request

Request e-mail should be sent to: Dr. Amir Orian (Oryan) aoryan@fhcrc.org

Dr. Robert Eisenman Eisenman@fhcrc.org

S3 DamRawData DamID primary-data: Spreadsheets for each of the proteins (dMyc, dMnt, dMax) DamID primary binding data

S4 Expres_RawData Gene expression, primary data for larval dmyc expression arrays (7h and 14h spreadsheets)

The files contain the ID of each listed gene according to reference 28 of the manuscript (Rubin, G.M. et al. A Drosophila complementary DNA resource. Science 287, 2222-2224 (2000)). These ID's can be searched via flybase (<http://flybase.bio.indiana.edu>).

In the event you encounter difficulties or have further questions please contact Amir Oryan (aoryan@fhcrc.org) or Robert Eisenman (eisenman@fhcrc.org).

B. PCR primer sets used in ChIP assay (Fig5)

bic E box flanking primers;

Right primer (start -50) 5'- CATGGCGATTTCTTTTGACAT-3'

Left primer (ends +70) 5'- TCAGTAAAACGCCAATCGAA-3'

kis (control) primers

Right primer (start +13) 5'- GCC GCG GTG TAA AGT TGT AA-3'

Left primer (ends +210) 5'- TTA TCA TTC ATT GCG CCT CA-3'