

“Mega”- an ENU-induced hypomorphic allele of c-myb reveals an essential role for transactivation by myb in the regulation of multiple hematopoietic branch points.

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GNF is conducting a genome wide scan in mice to discover genes important in a variety of phenotypes and diseases, including development, neurology, metabolism and immunology. Mutant mice are created by treating animals with the mutagen ethylnitrosourea (ENU). Offspring of mutant animals are screened using a variety of assays and animals with abnormal phenotypes are bred to establish heritability of the new trait. Mutations are mapped by breeding to a different strain of mice and using Single Nucleotide Polymorphisms (SNPs) that differ between strains to identify the chromosomal region that associates with the phenotype of interest. Using a panel of assays including flow cytometry, hematology, antibody responses and transplant rejection we have identified more than 50 putative mutants, established the heritability of 16 phenotypes, obtained map positions for 8 mutants, and identified the mutated gene for two lines.

One of these lines, “mega”, displays a recessive phenotype which includes elevated myeloid cells, reduced numbers of B cells and elevated numbers of platelets (thrombocythemia). Using an F2 intercross with 129/J, the mutation was mapped to a region on mouse chromosome 10 between 15 and 32 Mb with a LRS score >30. This region contains approximately 120 genes including the gene for c-myb at 18.05 Mb. c-myb is known to be involved in myeloid transformation. Sequencing of this gene revealed a single base change resulting in an amino acid substitution in the transactivation domain at amino acid 303 (M303V). Transfection of the mutant transactivation domain fused to the GAL4 DNA binding domain shows that this mutation results in a hypomorphic myb allele with a 50% decrease in the transactivation potential of the mutant protein. In contrast to the phenotype of myb null animals, which are embryonic lethal due to a failure to transition from embryonic to fetal hematopoiesis, mice harboring the M303V mutation are viable and produce mutant offspring at the expected frequency. Through investigations of our hypomorphic allele, we have identified several points where c-myb appears to play important roles in hematopoietic development. This work highlights the utility of ENU mutagenesis screens to generate mutant mouse models for important genes where no genetic models previously existed, especially for those genes that are lethal when knocked out by traditional approaches.