Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 315 (2008) 303–316
DEVELOPMENTAL BIOLOGY
www.elsevier.com/developmentalbiology

Drosophila growth and development in the absence of dMyc and dMnt

Sarah B. Pierce [a], Cynthia Yost [a], Sarah A.R. Anderson [a], Erin M. Flynn [a], Jeffrey Delrow [b], Robert N. Eisenman [a,*]

[a] *Division of Basic Sciences, Fred Hutchinson Cancer Research Center, MS A2-025, POB 19024, Seattle, WA 98109-1024, USA*
[b] *Genomics Resource, Fred Hutchinson Cancer Research Center, Seattle WA, USA*

Received for publication 8 October 2007; revised 3 December 2007; accepted 11 December 2007
Available online 27 December 2007

Abstract

Myc oncoproteins are essential regulators of the growth and proliferation of mammalian cells. In *Drosophila* the single ortholog of Myc (dMyc), encoded by the *dm* gene, influences organismal size and the growth of both mitotic and endoreplicating cells. A null mutation in *dm* results in attenuated endoreplication and growth arrest early in larval development. *Drosophila* also contains a single ortholog of the mammalian Mad/Mnt transcriptional repressor proteins (dMnt), which is thought to antagonize dMyc function. Here we show that animals lacking both dMyc and dMnt display increased viability and grow significantly larger and develop further than dMyc single mutants. We observe increased endoreplication and growth of larval tissues in these double mutants and disproportionate growth of the imaginal discs. Gene expression analysis indicates that loss of dMyc leads to decreased expression of genes required for ribosome biogenesis and protein synthesis. The additional loss of dMnt partially rescues expression of a small number of dMyc and dMnt genes that are primarily involved in rRNA synthesis and processing. Our results indicate that dMnt repression is normally overridden by dMyc activation during larval development. Therefore the severity of the *dm* null phenotype is likely due to unopposed repression by dMnt on a subset of genes critical for cell and organismal growth. Surprisingly, considerable growth and development can occur in the absence of both dMyc and dMnt.

Keywords: *Drosophila*; Myc; Mnt; Growth; Transcription; Larval development; Ribosome biogenesis

Introduction

Throughout evolution, biological systems have employed molecular antagonism as a means of maintaining highly regulated and robust control over biochemical reactions, signal transduction pathways, and transcriptional networks (Gerhart and Kirschner, 1997). At the level of transcriptional control there are a number of well documented examples of transcriptional activators and repressors whose mutually antagonistic behavior at specific promoters serves to determine the rate of transcription and the temporal response to signaling (for review, see Barolo and Posakony, 2002). An interesting case of transcriptional antagonism is provided by the Max transcription factor network, a molecular module comprised of a group of basic-helix–loop–helix–leucine zipper (bHLHZ) transcription factors, all of which form individual heterodimers with the small bHLHZ protein Max. The Max network encompasses the functions of the Myc oncoprotein family and its antagonists, the Mxd family of proteins (for reviews, see Eisenman, 2006; Grandori et al., 2000; Luscher, 2001; Oster et al., 2002).

In vertebrates the expression of Myc family proteins (c-, N-, L-Myc) is induced and maintained in response to a wide range of growth and proliferative signals (Liu and Levens, 2006). Heterodimerization of Myc with Max is obligatory for binding to the E-box sequence, CACGTG, leading to modest levels of transcriptional activation of genes proximal to Myc–Max binding sites. Such activation occurs through recruitment of multiple complexes that modify chromatin and/or stimulate RNA polymerase activity (for reviews, see Adhikary and Eilers, 2005; Amati et al., 2001; Cole and Nikiforov, 2006). Moreover Myc can act to repress transcription by forming an inhibitory complex with Miz-1, a BTB-POZ domain activator (Adhikary et al., 2005; Staller et al., 2001; for review, see Kleine-Kohlbrecher et al., 2006).

* Corresponding author. Fax: +1 206 667 6522.
E-mail address: eisenman@fhcrc.org (R.N. Eisenman).

0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2007.12.026

A distinct group of bHLHZ proteins, the Mxd family (Mxd1–4 and Mnt, previously known as the Mad family), whose members also dimerize with Max and recognize E-box sites in DNA, acts as antagonists of Myc function. Mxd proteins repress transcription through their association with the mSin3 co-repressor complex, which contains histone deacetylase (HDAC) activity (for reviews, see Hooker and Hurlin, 2006; Rottmann and Luscher, 2006). Several lines of evidence indicate that Mxd downregulates genes that are normally activated by Myc and that the cellular proliferation and growth promoting activities induced by Myc are inhibited by Mxd overexpression (Amati and Land, 1994; Iritani et al., 2002; Roussel et al., 1996). These findings are consistent with the idea that the HDAC activity evinced upon Mxd–Max binding would reverse the HAT-induced histone acetylation resulting from Myc–Max binding. In general *mxd* gene expression is induced during terminal differentiation and cell cycle arrest, periods when Myc expression is normally downregulated, suggesting that Mxd proteins may initiate a silencing pathway for Myc target genes involved in cell proliferation and growth (Hooker and Hurlin, 2006; Rottmann and Luscher, 2006). This would imply that downregulation of Myc is not sufficient for target gene silencing. Indeed Mxd1 loss of function, especially in the context of $p27^{Kip1}$ deletion, has been shown to impede differentiation of granulocytes and hematopoietic stem cells (McArthur et al., 2002; Walkley et al., 2005). However, not all Mxd family proteins have expression patterns related to growth arrest. The Mnt protein is expressed in quiescent and differentiating cells but is also readily detected, along with Myc, in actively proliferating cells (Hurlin et al., 1997). The simultaneous presence of Myc and Mnt is thought to reflect a balanced and dynamic regulation of histone acetylation and transcription at E-box binding sites.

The identification of dMyc, dMax, and dMnt in *Drosophila* and the absence of any paralogs have greatly facilitated genetic analyses of these proteins and their functions (for recent reviews, see de la Cova and Johnston, 2006; Gallant, 2006). Many crucial properties of the Max network have been conserved in flies, including heterodimerization of dMyc and dMnt with dMax, E-box recognition, transcription activation by dMyc-dMax, and Sin3 binding and repression by dMnt–dMax (Gallant et al., 1996; Loo et al., 2005). Furthermore dMyc can co-transform murine fibroblasts and rescue proliferation of c-Myc deficient mammalian cells while c-Myc can rescue lethal mutations of dMyc in *Drosophila* (Benassayag et al., 2005; Schreiber-Agus et al., 1997; Trumpp et al., 2001). An important conclusion from the *Drosophila* studies is that dMyc regulates cell and organismal size. Hypomorphic mutants of *dm* (*diminutive*, the gene encoding dMyc) are viable yet smaller and are comprised of smaller cells (Johnston et al., 1999) whereas a null mutation (dm^4) leads to lethality due to arrested growth at an early larval stage, an effect closely linked to a dramatic failure in the growth of endoreplicating cells (Pierce et al., 2004). Mutation in the C-terminal bHLHZ region of dMyc also led to a profound decrease in the growth and endoreplication of germline and somatic cells in the ovary (Maines et al., 2004). By contrast tissue-specific overexpression of dMyc results in larger than normal cells in both mitotic and endoreplicating tissues, while widespread dMyc overexpression produces larger flies (Pierce et al., 2004; Johnston et al., 1999). Analysis of clones in the wing disc shows that cells overexpressing dMyc increased in size at a faster rate than wild-type cells while their division time was unaffected, indicating that dMyc predominantly influences cellular growth rate. The notion that Myc regulates cell growth is reinforced by results of many expression profiling studies showing that a significant fraction of genes whose transcription is altered by Myc in *Drosophila* and mammalian cells are involved in ribosome biogenesis, protein translation, and metabolism (Coller et al., 2000; Hulf et al., 2005; Li et al., 2005; O'Connell et al., 2003; Orian et al., 2003; Schlosser et al., 2005). Moreover Myc has been shown to stimulate transcription of ribosomal RNA encoding genes by direct binding to rDNA promoters in mammalian cells or by enhancing expression of RNA polymerase I components in *Drosophila* (Arabi et al., 2005; Grandori et al., 2005; Grewal et al., 2005).

Taken together with evidence that c-Myc activates RNA polymerase III transcription of tRNAs and 5S ribosomal RNA, the studies described above indicate that Myc functions in both flies and vertebrates as a general transcriptional regulator of cell growth through stimulation of all three RNA polymerases. In this context it is interesting to consider the role of dMnt in growth control. Previous work has shown that overexpression of Mxd1 (Mad1) or dMnt attenuates rRNA transcription and results in smaller cells, suggesting an important regulatory role in growth (Iritani et al., 2002; Loo et al., 2005; Orian et al., 2005; Poortinga et al., 2004). However a null mutation in *dmnt* ($dmnt^1$) produced a surprisingly mild phenotype. While the $dmnt^1$ adult flies showed increased weight, larger cells, and decreased lifespan compared to controls, they were viable and fertile, with no detectable developmental delays (Loo et al., 2005). This contrasts sharply with the lethal consequences of *dm* loss of function. In order to further explore the consequences of antagonism between dMyc and dMnt we have now examined the effects of *dmnt* mutation in a *dm* null background.

Materials and methods

Fly strains

The dm^4dmnt^1 line was generated by recombining dm^4 (Pierce et al., 2004) and $dmnt^1$ (Loo et al., 2005) X chromosomes and screening for recombinants by PCR. As controls we used precise excision lines isolated in the generation of dm^4 or $dmnt^1$.

For all experiments other than mitotic clone analysis, mutant and control X chromosomes were balanced with FM7i, Act-GFP and non-GFP mutant or control hemizygous males were analyzed.

For the mitotic clone experiments, ywnlsGFPFRT19A;70FLP70I-SceI/TM6B was constructed by recombining ywnlsGFPFRT18E (Davis et al., 1995) with FRT19A (Xu and Rubin, 1993) and crossing ywnlsGFPFRT19A to 70FLP70I-SceI/TM6B (Rong and Golic, 2000). dm^4, $dmnt^1$, and dm^4dmnt^1 were recombined with FRT19A.

Flies and larvae were grown at 25 °C, unless otherwise noted.

Larval growth assays

For larval growth assays and analysis of larval tissues, eggs were collected onto grape juice agar plates and larvae of the appropriate genotype were