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Review

Drosophila Myc: A master regulator of cellular performance ☆

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ABSTRACT

The identification of the *Drosophila* homolog of the human MYC oncogene has fostered a series of studies aimed to address its functions in development and cancer biology. Due to its essential roles in many fundamental biological processes it is hard to imagine a molecular mechanism in which MYC function is not required. For this reason, the easily manipulated *Drosophila* system has greatly helped in the dissection of the genetic and molecular pathways that regulate and are regulated by MYC function. In this review, we focus on studies of MYC in the fruitfly with particular emphasis on metabolism and cell competition, highlighting the contributions of this model system in the last decade to our understanding of MYC's complex biological nature. This article is part of a Special Issue entitled: Myc proteins in cell biology and pathology.

1. Introduction

MYC is one of the most referenced molecules in biology; since its isolation in the chicken genome in early 1980s [1], plenty of literature has covered the many aspects of its function. Despite that, the last decade has witnessed its unanticipated appearance in several cellular mechanisms, demonstrating that we are still far from composing a complete picture of its biological potential. *Drosophila* Myc protein is encoded by the *dm* gene (*diminutive*, from the mutant phenotype) whose molecular characterization [2] was long anticipated by the phenotypic observation. Current technology allows the researcher to manipulate the fly genome at a level of precision that exceeds that of any other multicellular organism, and many studies in the fly aimed at investigating Myc function in different tissues and organs in both physiological and pathological conditions. In this review, we will discuss the most recent advances in MYC biology obtained in studies in *Drosophila*, focusing mainly on its roles in metabolism, tissue growth and cancer.

2. Myc family in flies: a conserved network

The phenotype of Myc mutant flies has been known since 1933 when Eleanor Nichols Skoog, while in Calvin Bridges' lab at California Institute of Technology, observed adult flies with small body size and female sterility and identified a mutation in a gene on the X chromosome that she named *diminutive* [3]. In 1996, 50 years after its phenotypic description, the search for a simple system in which to study the complexity of MYC function was finally satisfied by the discovery that *diminutive* encoded the *Drosophila* homolog of Myc [2,4]. *Drosophila* Myc protein is only 26% identical to its human counterpart, but still contains highly conserved functional domains that allowed its identification in a yeast 2-hybrid screen using human Max as a bait; subsequently, the *max* and *mnt* genes in *Drosophila* were also cloned [2]. As in mammals, the Myc/Max/Mad network also controls fundamental cellular processes in *Drosophila*, including apoptosis, tissue growth and proliferation and consists of single Myc, Max, and Mad/Mxd components [5–7].

Drosophila Myc contains several functional domains, among which is the highly conserved basic-helix-loop-helix leucine zipper domain (bHLH/LZ) present at its C-terminus that mediates Myc:Max heterodimerization [2,4]. Myc:Max heterodimers bind the E-box sequence CACGTG on target genes and activate their transcription. At its N-terminus Myc contains several conserved motifs, including the conserved Myc Boxes I and II [2,8], which are partially required for Myc transcriptional activities [8], the conserved Myc Boxes III and IV, the latter containing the acidic region, whose mutational analysis revealed for these domains a novel, conserved function in controlling

Abbreviations: ACC, acetyl CoA carboxylase; bHLH/LZ, basic helix-loop-helix leucine zipper; Brk, Brinker; CK1α, casein kinase 1α; Desat1, desaturase-1; DILPs, *Drosophila* insulin like peptides; Dlg, discs large; *dm*, *diminutive*; Dpp, decapentaplegic; FAS, fatty acid synthase; Fwe, flower; GSCs, germ stem cells; GSK3, glycogen synthase-3 kinase 3; Hpo, Hippo; InR, insulin receptor; JNK, c-Jun N-terminal kinase; Lgl, lethal giant larvae; Mlx, Max-like; *M*, *minute*; PCP, planar cell polarity; PI3K, phosphoinositide 3 kinase; Rps, ribosomal proteins; Scrib, Scribble; SPARC, *s*ecreted *p*rotein *a*cidic and *r*ich in *c*ysteine; TOR, target of rapamycin; Upd-2, unpaired-2; Wg, wingless; Wts, warts; TGA, triglyceride *a*cids; YAP, Yes-associated protein; Yki, Yorkie

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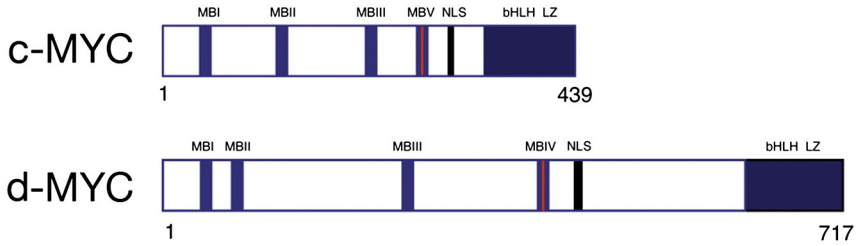

Fig. 1. Myc proteins. Schematic representation of Myc proteins. *Drosophila* Myc is longer than its vertebrate counterpart but the functional domains are conserved. In the draw we have outlined the relative position of the conserved Myc Boxes I–IV, the nuclear localization sequence (NLS), the acidic box (in red) within the sequence of MBIV and at the C-terminus the basic helix-loop-helix leucin zipper (bHLH LZ) DNA-binding domain.

Myc protein stability (Fig. 1) [8,9]. *Drosophila* Mnt is similar to both mammalian Mnt and Mad, and forms heterodimers via its bHLH/LZ domain with the cognate domain of Max [2,10]. Mnt:Max heterodimers repress transcription using the functional "Sin3 binding domain" (SID) at their N-termini to mediate the transcriptional co-repressor activity of Sin3 and histone deacetylase activity (HDACs) [11,12]. Finally, Max is the most conserved member of the *Drosophila* Myc network, being 42% identical to human Max in overall amino acid sequence [2]. Mnt:Max heterodimers bind the same E-boxes as Myc:Max heterodimers *in vitro* and can repress the transcription of Myc:Max targets to antagonize Myc function [10,13]. Interestingly, flies lacking Max show weaker phenotypes than *dm* mutants, suggesting that Myc may have functions independent of Max or Mnt in flies [14]. Experiments where *Drosophila myc* cDNA was used to rescue proliferation defects of mouse embryonic fibroblasts from *c-myc* mutant mice demonstrated that *Drosophila* and vertebrate Myc can functionally substitute for each other [15]. Moreover, *Drosophila myc* cDNA was able to induce transformation of rat embryonic fibroblasts when expressed together with an activated form of Ras (Ras^{V12}) [4]. In addition, developmental defects of *Drosophila* dm^{PG45} hypomorphic mutants were rescued by expression of the human *c-myc* cDNA [16]. These results indicate that many of Myc's functions are conserved from insects to mammals.

An important mammalian Myc-related complex is the Mondo-Max-like (Mlx) protein family, a bHLH/LZ network that works in parallel to the Myc–Max–Mnt node to control glucose and glutamine metabolism. Members of the Mondo-Mlx family bind to carbohydrate response elements (ChoRE) that contain similar E-boxes (CAAGTG) to those of the Myc–Max–Mnt family [17]. In *Drosophila* the product of the *Mio* gene, the single ortholog of the human *Mondo A* and *B* genes, binds to the Bigmax protein, the fly ortholog of human Mlx [17].

Competition between human Mnt monomers and heterodimers of Mnt and Mlx, a member of Mondo-Max-like protein network, to repress the transcription of ChoRE genes [18], suggests the presence of common targets for the two transcriptional networks in regulating metabolic pathways relevant to metabolism and growth (see Section 5).

Discussions on Myc function generally refer to the activities exerted by components of the Myc/Max/Mnt network. However, in this review we will focus primarily on the function of the most investigated member of the network: Myc, and its role in controlling growth and cell competition.

3. Myc controls growth and size

Overexpression of Myc in cells of the imaginal discs (larval organs, composed of diploid epithelial cells, which give rise to the adult appendages and part of the body wall) induces growth by accelerating mass accumulation and the rate of the G1/S transition of the cell cycle. However, the cells are unable to proliferate faster because their entry into the M-phase is limited by availability of String/CDC25, which is developmentally regulated. This results in larger than normal cells [19]. Conversely, *dm* mutant disc cells slow entry into G1-phase and exhibit slower overall cellular division rates. Animals carrying weak *dm* alleles (dm^1, dm^{P0}) are developmentally delayed, yielding adult flies with small but normally proportioned body size and small bristles [19], while dm^4 null animals die as larvae early in development [20]. Conversely, Myc over-expression results in flies that are about 16% larger than normal [21]. Animals mutant for *mnt*, the fly homolog of the human *mad* gene, are viable and show a growth phenotype similar to Myc over-expression, as cells mutant for *mnt* are larger and *mnt* adults are heavier than wild-type [10]. The *mnt* phenotype was partially rescued by reducing the *dm* gene dosage, indicating that *mnt* and *myc* exert opposite control on animal growth [13]. Surprisingly, analysis of *max* null mutants revealed that development of *max* null animals takes longer than *myc* null or *myc:mnt* double mutant animals, but the flies die as "pharate" (differentiated but non-viable) adults. This suggested the existence of Max-independent Myc activities, perhaps substituted by Mlx, that allow flies to survive longer than dm^4 null animals [14].

Drosophila Myc influences the expression of a large number of genes that are involved in diverse cellular processes; however a preeminent group of Myc-activated targets encodes for proteins involved in ribosome biogenesis, translation, and metabolism [22–24]. Analysis of the target genes demonstrated that the majority of them encode for RNA-binding proteins, rRNA processing and ribosomal proteins, which all contribute to ribosome biogenesis [22,25–27]. Myc function in ribosome biogenesis is conserved in vertebrates, where c-Myc activates the expression of the RNA helicase DDX18 (in flies encoded by the *pitchoune* gene) [28], and the nucleolar proteins Nucleolin and Nol12 (encoded in flies by the *modulo* and *viriato* genes, respectively) [29,30]. Numerous studies in both flies and vertebrates revealed that Myc also contributes to ribosome biogenesis by directly activating RNA polymerases I and III [14,24,31]. A common co-activator for RNA polymerase III is Brf11, which acts also downstream of nutrients and of the TOR (target of rapamycin) pathway to induce organismal growth [32]. Many of Myc target genes, members of the chromatin remodeling complexes, are also repressed by Mnt, consistent with the antagonistic roles for Myc and Mnt in promoting and suppressing cellular growth [13]. Indeed, Myc was shown to be in a complex with the transcriptional cofactors Tip48 (Reptin) and Tip49 (Pontin) [25], components of chromatin remodeling complexes [33]. While most of the functions of mammalian Tip48 and Tip49 are associated with cellular growth and cancer progression [34], in flies, genetic interaction experiments with Myc revealed an unexpected function for Pontin and Reptin to repress gene transcription *in vivo* [25]. This function is conserved also in *Xenopus*, where Myc function is required together with Pontin and Reptin to repress Miz-1 transcriptional activity [35]. Myc regulation of growth is mediated also by direct binding to protein members of complexes that control histone modification or to enzymes that favor transcription. Myc binds to members of the Trithorax-group such as the histonemethyltransferase Ash2 ("Absent, small, or homeotic discs 2"; the homolog of vertebrate ASH2L), the ATPase Brahma (the homolog of human hBrm and Brg1) and finally the histone-demethylase Lid

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