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*Review*

# MYC function and regulation in flies: how *Drosophila* has enlightened MYC cancer biology

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**Abstract:** Progress in our understanding of the complex signaling events driving human cancer would have been unimaginably slow without discoveries from *Drosophila* genetic studies. Significantly, many of the signaling pathways now synonymous with cancer biology were first identified as a result of elegant screens for genes fundamental to metazoan development. Indeed the name given to many core cancer-signaling cascades tells of their history as developmental patterning regulators in flies—e.g. Wingless (Wnt), Notch and Hippo. Moreover, astonishing insight has been gained into these complex signaling networks, and many other classic oncogenic signaling networks (e.g. EGFR/RAS/RAF/ERK, InR/PI3K/AKT/TOR), using sophisticated fly genetics. Of course if we are to understand how these signaling pathways drive cancer, we must determine the downstream program(s) of gene expression activated to promote the cell and tissue over growth fundamental to cancer. Here we discuss one commonality between each of these pathways: they are all implicated as upstream activators of the highly conserved MYC oncogene and transcription factor. MYC can drive all aspects of cell growth and cell cycle progression during animal development. MYC is estimated to be dysregulated in over 50% of all cancers, underscoring the importance of elucidating the signals activating MYC. We also discuss the FUBP1/FIR/FUSE system, which acts as a 'cruise control' on the MYC promoter to control RNA Polymerase II pausing and, therefore, MYC transcription in response to the developmental signaling environment. Importantly, the striking conservation between humans and flies within these major axes of MYC regulation has made *Drosophila* an extremely valuable model organism for cancer research. We therefore discuss how *Drosophila* studies have helped determine the validity of signaling pathways regulating MYC *in vivo* using sophisticated genetics, and continue to provide novel insight into cancer biology.

**Keywords:** *Drosophila*; MYC; genetics; development

## 1. The history of MYC

Since the identification of the oncogenic potential of c-MYC (MYC) in the early 1980s [1] the mammalian MYC family, which also includes MYCN, and MYCL, has been the focus of extensive investigation. Early studies demonstrated MYC drives cell cycle progression [2,3,4], which is central to MYC's capacity as an oncoprotein [5]. Despite considerable efforts using mammalian models, *Drosophila* genetic studies were first to demonstrate that the sole MYC ortholog, *dMYC* [6] drives acquisition of biomass, or cell growth, via its ability to upregulate ribosome biogenesis during animal development [7,8,9]. *dMYC* mutant flies are small, with reduced cell growth [7] and decreased rDNA transcription [9]. Conversely, increased *dMYC* is sufficient to drive increased rDNA transcription, cell growth and proliferation [7,9]. Functional conservation between fly and human MYC has been demonstrated by classic functional rescue experiments i.e. *dMYC* can transform primary mammalian cells and rescue proliferation defects in *MYC* null fibroblasts [10], whilst MYC rescues lethal *dMYC* mutations in *Drosophila* [11]. Thus, the targets and pathways impacted by MYC are conserved between flies and humans, particularly regarding the ability to drive ribosome biogenesis, cell growth and tumour progression.

Dissecting essential roles for MYC using loss of function approaches in mammals has been complicated by functional redundancy between the 3 *MYC* genes. For example, in adult bone marrow both MYC and MYCN are endogenously expressed and MYC appears to act redundantly with MYCN to achieve hematopoietic stem cell maintenance [12]. Moreover, although much has been discovered regarding the molecular and pathological mechanisms that drive cells to become malignant, dissecting the physiological/molecular mechanisms linking upstream signaling to MYC-driven cancers requires further investigation using *in vivo* genetic models. In flies the presence of a single *MYC* gene and available genetic tools have provided clear connections between developmental pathways and *dMYC* regulation. In this review, we will focus on research undertaken in *Drosophila* demonstrating how MYC is regulated during development and provide parallels with work carried out in vertebrates.

## 2. MYC function

MYC is a basic helix-loop-helix zipper (bHLHZ) transcriptional factor that heterodimerizes with a second bHLHZ protein MAX [13] to directly bind DNA sequences, called E-boxes, within the promoter regions of target genes [13-16] (reviewed in [17]). However, *in vivo* MAX loss-of-function studies in *Drosophila* have demonstrated that MYC can also elicit many of its functions independently of MAX [18]. For example, transcriptional activation of 5S rRNA and tRNA via RNA Polymerase III can be achieved in a complete loss-of-function MAX mutant background [18].

MYC interacts with a range of chromatin modifying enzymes and transcription factors to control expression of an extensive network of downstream transcriptional targets [9,19-28]. Early studies in *Drosophila* [9,23,24] and human cells [29], revealed MYC bound 10–15% of the genome (reviewed in [30,31]). More recently, next generation sequencing (NGS), including ChIP-seq, RNA-seq and GRO-seq nuclear run-on assays, have provided deeper insight into MYC-targets [27,28,32,33]. Broadly, these studies suggest that MYC acts not so much to establish a fixed signature of gene expression, but rather enhances programs of transcriptional activity, which are based on cell type and/or developmental context [27,28]. For instance, at target promoters in

activated lymphocytes [27] increased MYC was detected on all active promoters, i.e those enriched for RNA Polymerase II (Pol II) and with active chromatin marks. Thus amplification of MYC dependent gene expression was only associated with active genes already marked as MYC targets, not additional genes. Furthermore, these studies confirmed the strong correlation between E-boxes within target promoters, and their vicinity to the transcription start site (TSS), for optimal MYC loading and amplification of active genes. Additionally, analysis from both studies indicated enhanced transcriptional elongation upon increased MYC occupancy [27,28] (reviewed in [34]). Consistent with this MYC bound sites had increased enrichment of the transcription elongation factor p-TEFb and elongating RNA Pol II [27,28]. Together, these studies suggest elevated levels of MYC in tumour cells intensifies MYCs normal function by amplifying established MYC-targets, rather than initiating new sites of gene activation.

These observations are consistent with earlier studies, which demonstrated that promoters targeted by MYC are associated with an active chromatin profile, including methylation marks at H3K4 (me1, me2, me3), acetylation of H3K27 (ac), and DNA methylation across the CpG island, a hallmark of MYC binding sites [29,35]. The demonstration that MYC binding is not detected on inactive heterochromatin [27,28,36], suggested MYC was unlikely to establish gene activation in the context of overexpression. Rather, MYC requires prior opening of chromatin for binding site recognition, consistent with the DNA amplification model. That overexpression of MYC in *Drosophila* is sufficient to drive a program of ribosome biogenesis and cell growth [7,8,9] would be expected under the amplification model, since the tissues used in these studies were all primed for cell growth (e.g. proliferating wing imaginal discs and endoreplicating salivary glands). Thus increased MYC amplifies pre-existing cell growth transcriptional networks, much as it does in a cancer setting [37,38].

## 3. MYC in Cancer

As stated above, MYC proteins drive tumourigenesis primarily by activating transcription of target genes required for growth and proliferation [31,39-43]. MYCN is predominantly expressed in the brain and amplification has been associated with neuroblastoma, glioblastoma, medulloblastoma and astrocytoma [44]. MYCL, as the name suggests, has been mostly associated with lung cancer [45]. Although MYC is frequently overexpressed in cancer via gene translocation e.g. in Burkitt's B-cell lymphoma [46,47], MYC amplification is broadly observed in many tumours including, but not limited to other leukaemias, breast, prostate, cervical, bone, brain, colon, lung and liver cancers [43,45]. Thus, all members of the MYC gene family are linked to the aetiology of a wide range of human cancers.

### MYC driven cell growth is fundamental to cancer progression

MYC drives cell growth by increasing activity from all three RNA polymerases (I, II and III) to escalate ribosome production [9,37,38,48-54]. Ribosomes form around the actively transcribing rDNA repeat genes within the nucleoli, and ribosome biogenesis is orchestrated by MYC [38]. In fact, the capacity to drive ribosome production appears to be central to MYC's powerful cell growth effects, as ribosome biogenesis is rate-limiting for progression of MYC-driven lymphoma [39,42]. The first evidence that MYC drives RNA Pol I activity and, therefore, rDNA transcription was the

observation that MYC increases expression of the homodimeric upstream activation binding factor (UBF), a crucial component of the RNA Pol I pre-initiation complex [51,55]. Subsequently, studies in both *Drosophila* [9,23] and mammals [37], revealed MYC stimulates Pol I transcriptional activity indirectly via its ability to regulate a large number of RNA polymerase II-transcribed genes.

In mammals MYC directly interacts with the Pol I recruitment complex SL-1 to assist in initiation of Pol I driven rDNA transcription to form the 47S pre-rRNA, which is processed into mature 18S, 5.8S and 28S rRNAs. In mammals [54] and flies [18,53] MYC activates RNA Pol III for 5S rRNA production for assembly of the 60S ribosomal subunit and tRNA transcription directly through an interaction with TFIIB [54]. MYC also upregulates ribosomal protein production at the level of Pol II-dependent transcription [9,37]. Thus MYC activity increases abundance of all components required to assemble the 40S and 60S ribosomal subunits. [38,56]. In addition to increasing ribosomal components, MYC also increases upstream growth signaling proteins, factors involved in ribosome assembly and processing (e.g. nucleolin and fibrillarin), components of the RNA Pol I machinery and translation factors [9,37]. Indeed evidence suggests it is MYC's role in harnessing and driving ribosome biogenesis that is central to MYC's malignant function. In particular, drug development studies have demonstrated that targeting the ribosome biogenesis program might provide a platform for therapeutic intervention of a broad range of MYC-driven cancers. Specifically, small molecule inhibition of RNA Polymerase I, the enzyme responsible for rDNA transcription, selectively kills MYC-driven lymphoma *in vivo* [38,42].

Further to MYC's oncogenic potential, MYC couples growth with cell cycle progression by promoting G1 progression. MYC drives S phase by directly upregulating the S phase cyclins, Cyclin D and Cyclin E [24]. The upregulation of G1 cyclins leads to phosphorylation and inactivation of the key inhibitor of S phase progression, Rbf, a member of the *Drosophila* Retinoblastoma family. Inhibition of Rbf results in release of the E2F1 transcription factor from the inhibitory complex with Rbf, permitting upregulation of E2F1 dependent S phase genes [57,58]. Like MYC, early in G1 phase, dMYC activates genes required for DNA replication and progression through S phase [7,9,59].

The ability of MYC to amplify active gene expression further stresses the importance of maintaining tight control of MYC activity. Consequently, several mechanisms act to ensure MYC is tightly regulated at the level of mRNA transcription [60,61], mRNA stability [62-65], and protein turnover [66,67]. In addition, to allow organ and tissue growth in response to nutrients and other external cues, the levels of MYC need to be responsive to growth and developmental signals [68]. Despite the large number of transcriptional targets and the associated oncogenic potential of MYC dysregulation, how MYC transcription is regulated in response to developmental growth signals is largely unknown. As most signaling pathways and core components of the transcriptional machinery required for development are well characterized and conserved between *Drosophila* and humans [68,69,70], fly genetic models remain an excellent means of rapidly identifying and dissecting pathways impacting MYC activity.

## 4. MYC regulation

### FUBP1-FUSE-FIR activate MYC transcription

Multiple upstream signaling pathways funnel into *MYC* transcription by modulating the

complement of transcription factors and enhancers on the *MYC* promoter [60,71,72,73]. Previous reviews have focused on canonical transcription factors (e.g. E2F, B-catenin/TCF, Smad3, STAT3) in MYC transcription [60], here we will focus on two non-canonical *MYC* regulators (FUBP1 and FIR), which we believe will be required for "fine tuning" activated *MYC* transcription in response to multiple inputs from the extracellular signaling environment.

The FUSE binding protein (FBP/FUBP1) and the FBP Interacting Repressor (FIR) bind the Far Upstream Sequence Element (FUSE) region of singled stranded DNA in the "activated" *MYC* promoter (Figure 1) [71,72,74,75]. We predict that the FUBP1/FIR/FUSE system acts as a "cruise-control" by controlling RNA Polymerase II pausing and, therefore, *MYC* transcription in response to the developmental signaling environment [73]. Specifically, mammalian tissue culture studies suggests that RNA Pol II release and *MYC* transcription in response to serum stimulation requires interactions between the XPB helicase component of the Transcription Factor IIH (TFIIH) complex and FUBP1 and FIR complexes [76,77]. In response to binding of chromatin remodeling complexes and canonical *MYC* transcription factors, TFIIH/XPB stimulates *MYC* promoter melting, which is required for physical interaction between FUBP1 and FIR at the single stranded FUSE [76,77]. The "pulse" of *MYC* transcription stimulated by serum in culture correlates with decreased enrichment for RNA Pol II across the *MYC* Transcription Start Site (TSS) and sequential recruitment of FUBP1 and FIR to the FUSE element [77]. Enrichment for FUBP1 is detected prior to a peak in *MYC* mRNA expression, consistent with FUBP1 promoting release of paused RNA Pol II to activate *MYC* transcription. Conversely, FIR is detected at FUSE subsequent to the peak in *MYC* mRNA and is proposed to inactivate RNA Pol II thereby returning *MYC* transcription to basal levels (Figure 1) [77]. Consistent with a repressive function of FIR, loss-of-function FIR mutations are associated with increased *MYC* mRNA levels in colorectal cancer [78].

*Drosophila* Hfp is functionally homologous to mammalian FIR, being implicated in the repression of *MYC* transcription in *Drosophila*. Specifically, Hfp behaves as a tumour suppressor, as loss of Hfp results in overproliferation and tissue overgrowth [79]. RNA interference to deplete Hfp results in increased *dMYC* expression and cell growth [80]. *Drosophila* studies have also demonstrated enrichment for Hfp on the *dMYC* promoter and that Hfp is essential for repression of *dMYC* expression *in vivo*. Moreover, Hfp-dependent repression requires interaction between Hfp and the *Drosophila* homolog of the XPB DNA helicase component of TFIIH, Haywire [80], demonstrating the mechanism proposed for transcriptional repression of *MYC* mediated by FIR-XPB-RNA Pol II is conserved in flies. Given FIR and Hfp both act as transcriptional repressors of *MYC* [77,80], we predict that the homolog of FUBP1, Psi, will also activate *dMYC* transcription *in vivo*. Furthermore, we predict that FUBP1-stimulated and FIR-mediated repression of *MYC* transcription will be responsive to developmental pathways patterning MYC (Wg/Wnt, Notch and BMP/TGF-B/Dpp) and growth regulating pathways (EGFR/RAS/MAPK and PI3K/AKT/TOR), discussed in the context of MYC below.

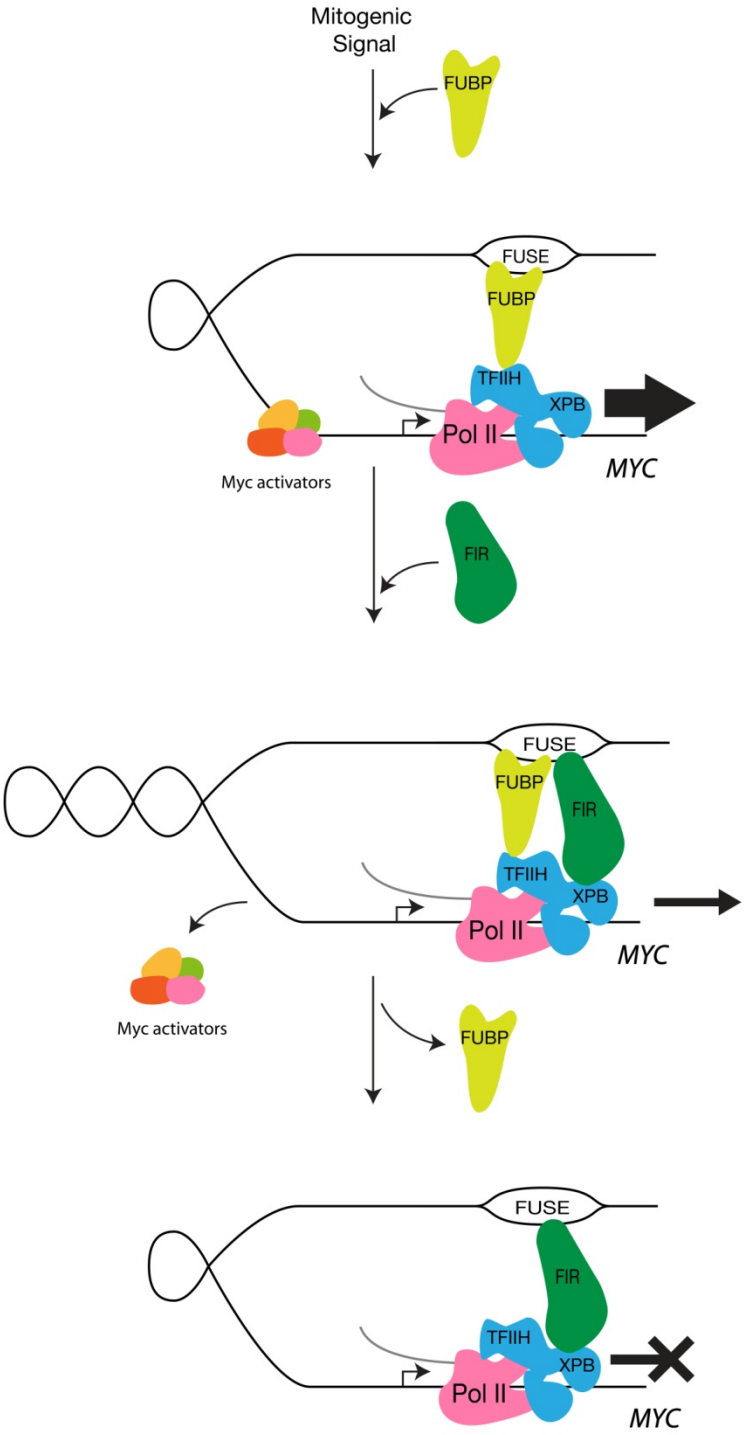


**Figure 1. The FUSE binding protein (FBP/FUBP) and the FBP Interacting Repressor (FIR) bind the Far Upstream Sequence Element (FUSE) region of singled stranded DNA in the "activated" MYC promoter. Prior to the peak in MYC expression, FUBP is recruited promoting release of poised RNA Pol II to activate MYC transcription. Subsequently, FIR is recruited to the MYC promoter following to the peak in MYC and is proposed to inactivate Pol II to return MYC transcription to basal levels. Thus the FUSE/FUBP/FIR axis has been speculated to be a "cruise-control" for controlling stimulated MYC transcription.**

## 5. Signaling inputs to MYC

### *5.1. The WNT and Notch developmental pathways*

In mammals, MYC is a target of Wnt/Wg signaling in both normal intestine and in colorectal carcinomas [81,82,83]. *In vivo*, Wnt signalling is required for *MYC* expression and proliferation of intestinal crypt stem cells in mice [84,85]. MYC can prevent cell cycle arrest normally induced by blocking the Wnt pathway *in vitro* [86] and MYC is required for Wnt-dependent tumourigenesis [87]. Therefore, in the context of the intestinal crypt, MYC normally acts downstream of Wnt to drive proliferation and chronic Wnt activity is required for tumourigenesis. In mammals, the Wnt-responsive transcription factor LEF/TCF, TCF4 binds and directly regulates *MYC* [81,86,87,88], however, to date evidence for direct TCF binding to the *dMYC* promoter has not been reported for *Drosophila*.

Intriguingly, accumulated evidence suggests Wnt proteins behave as tumour suppressors in certain contexts, being required to inhibit cell transformation by growth inhibition and differentiation [89-93]. In *Drosophila* the primordial Wnt family member Wingless/Wg differentially regulates proliferation in the wing imaginal disc. In the wing pouch, Wg inhibits cell division and drives differentiation across the dorsal-ventral boundary by down regulating *MYC* [7], whilst in the neighbouring hinge tissue at the same developmental time point Wg promotes proliferation [94], however whether this is mediated by MYC requires further investigation. Similar differential effects of Wnt have been reported in the intestinal crypt, where Paneth cells require Wnt signals to achieve terminal differentiation. Thus Wnt signals in the crypt can separately drive stem cell proliferation and paneth cell differentiation [95,96]. Together the fly and mammalian studies demonstrate that Wg/Wnt can behave as either a tumour suppressor or oncogene within the same tissue/organ, and cell fate will almost certainly depend on the local signaling environment. The fact that both gene programs are deregulated simultaneously in invasive colorectal cancer [97] reinforces the importance of understanding the diverse biological effects of the Wnt pathway on *MYC* regulation *in vivo*, using developmental models.

Like Wnt, Notch signaling has been implicated in many developmental processes such as cell fate specification, stem cell maintenance and initiation of differentiation [98]. Similar to Wnt/Wg the effects of Notch signaling on cell proliferation are context dependent [99,100]. Crosstalk between Wg and Notch signaling is key to cell cycle arrest and differentiation across the dorsal-ventral boundary of the wing imaginal disc, where activation of Notch is required for the expression of Wg [101]. Notch also acts downstream of Wg to repress MYC [102,103], however, the molecular mechanism(s) for altered MYC protein abundance are yet to be elucidated. As mentioned above, Hfp is the *Drosophila* FIR homolog and an essential repressor of *dMYC* transcription [79,80]. Although upstream factors regulating mammalian FIR have not been reported, in *Drosophila* Wg signaling regulates Hfp in the wing imaginal disc. Specifically, activation of the Wg pathway results in upregulation of Hfp, while cells with reduced levels of Wg signaling show reduced Hfp protein [79]. Thus, Hfp may provide a connection between Wg signaling and the regulation of *dMYC* transcription in *Drosophila*. Moreover, identification of FIR mutant isoforms unable to repress *MYC* in colorectal cancer [78], suggest dysregulation of Wnt signaling might further promote colorectal cancer by disruption of FIR-mediated *MYC* repression.

*5.2. TGFB/BMP pathway*

The TGFβ/BMP superfamily of growth factor signals can behave as tumour-suppressors or oncogenes depending on the tissue microenvironment [104]. Initial studies suggested TGFβ represses MYC expression [105] via a downstream transcriptional regulatory complex including Smad3, Smad4, the repressor E2F4/5, and p107 [106]. In *Drosophila* lymph glands, the BMP ortholog Dpp also exhibits MYC-dependent tumour suppressor behaviour [107]. Downregulation of Dpp/BMP signaling drives over proliferation and prevents differentiation of precursor cells into mature blood cells, and MYC knockdown abrogated overproliferation caused by Dpp/BMP inhibition, suggesting MYC is the major mediator of Dpp/BMP pathway driven progenitor proliferation in the lymph gland.

Conversely, TGFβ has been implicated in driving an epithelial-mesenchymal transition (EMT) and melanoma metastasis [108]. Specifically, TGFβ treatment indirectly increases phosphorylation of the known oncogene AKT1, which in turn results in MYC phosphorylation, increased protein stability and MYC accumulation (Figure 2, discussed further below) [108]. In the *Drosophila* wing disc Dpp/BMP also drives MYC-dependent cell proliferation [109,110,111]. Specifically, Dpp represses transcription at the *brinker* locus (*brk*) via its downstream transcriptional effector p-Mad. Brk is a transcriptional repressor, which normally limits *MYC* expression in the wing [111]. The net effect is that Dpp/BMP signaling upregulates *MYC* at the level of transcription to drive cell growth in the wing. Therefore, using different molecular mechanisms to target MYC, the TGFβ/BMP pathway can be oncogenic in some contexts and a tumour suppressor in others (Figure 2).

*5.3. The Hippo pathway*

The Hippo tumour suppressor pathway is a central controller of organ size [112,113], which not surprisingly has been implicated in MYC regulation in flies [114] and mammals [115]. Hippo inactivation allows downstream pathway component Yorkie (Yki; YAP in vertebrates) to enter the nucleus and, in conjunction with transcription factors such as Scalloped (Sd; TEAD/TEF in vertebrates), activates the expression of genes that promote cellular growth and proliferation and prevent apoptosis [116]. In *Drosophila*, MYC transcription is upregulated *in vivo* in response to inactivation of the Hpo pathway or ectopic expression of Yki [114]. Conversely, reducing Yki activity reduces *dMYC* transcription. ChIP analysis revealed enrichment for both Sd and Yki proximal to the transcription start site of the *dMYC* gene, suggesting that *dMYC* is a direct target of Yki/Sd transcriptional complex (Figure 2) [114]. Furthermore, Yki protein levels were elevated in cells with reduced dMYC function, whilst overexpression of dMYC resulted in lower Yki levels, suggesting a regulatory feedback mechanism might exist between MYC and Yki to maintain organ size. Consistent with this a second study demonstrated that Yki-driven overproliferation and competitive activity requires MYC in wing imaginal disc clones [117]. In mammals, MYC is transcriptionally activated in livers from mice expressing YAP, raising the possibility that part of YAP/Yki's functions are regulated by MYC [116,118]. However, it remains to be determined whether a regulatory feedback relationship between YAP and MYC also exists in mammals.

More recently, studies in mammalian tissue culture revealed that over activation of YAP globally suppresses miRNAs, some of which are normally associated with translational repression of *MYC* [115]. Hippo signaling is, therefore, likely to exploit both transcriptional and miRNA-mediated

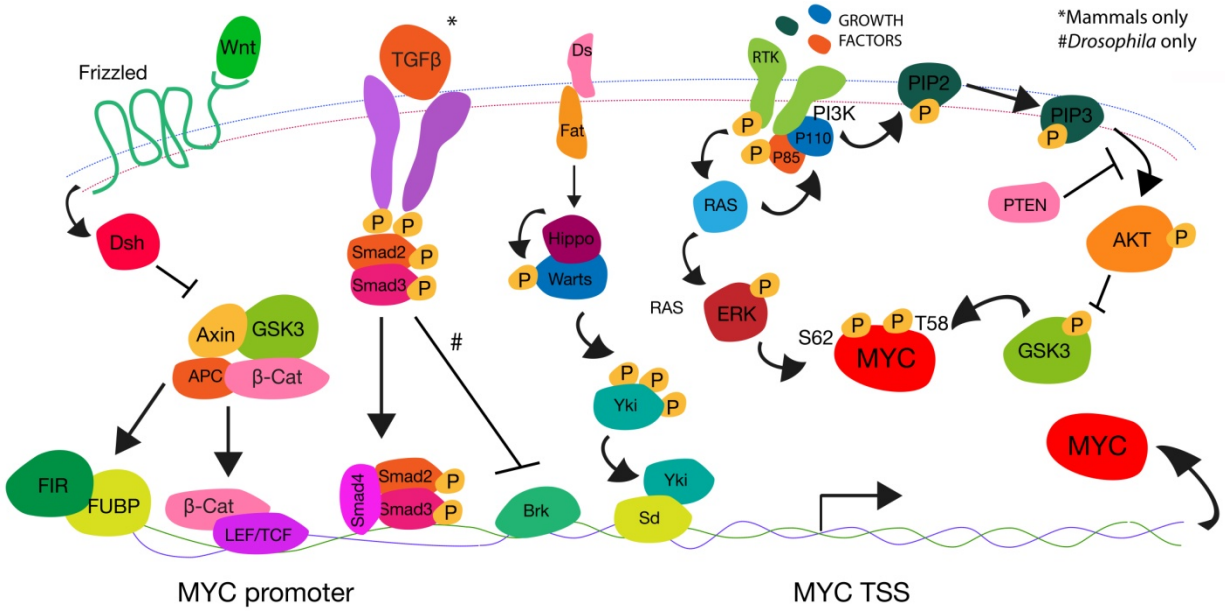


**Figure 2. Signaling inputs to MYC. WNT/Wg signaling drives relocation of β-catenin to the nucleus, which can activate MYC expression via canonical transcription factors TCF/ LEF. Alternatively, Wnt/Wg signaling can repress MYC, via the ssDNA binding MYC-repressor FIR. TGFβ/BMP can repress MYC via the Smad transcription factor complex, which alternatively can activate MYC by repressing the transcriptional repressor Brk. Inactivation of Hippo signaling drives MYC expression by phosphorylating Yki to activate its interaction with the transcriptional coactivator Sd. The RAS and PI3K pathways modulate MYC protein stability. ERK stabilises MYC via phosphorylation of Ser62. Decreased activity of the PI3K pathway leads to increased GSK3β-driven phosphorylation of Thr58, which targets MYC for turnover. All pathways are conserved between mammals and *Drosophila*. * Indicts pathway components only elucidated in mammals, # Indicts pathway components only determined in *Drosophila*.**

silencing mechanisms to modulate MYC abundance, and therefore, organ growth. Thus determining whether the transcriptional mechanisms uncovered using *Drosophila* translate into mammalian systems and, moreover, whether Hippo-dependent MYC regulation is disrupted in cancer is critical to gaining a full understanding of MYC dysregulation in tumourigenesis.

### *5.4. RAS/RAF/MAPK, PI3K/AKT/TOR and WNT/GSK3β regulate MYC protein stability*

Signaling downstream of the EGF Receptor (EGFR) can proceed through the RAS/RAF/MAPK and/or PI3K/AKT/TOR pathway, and activation of either pathway is strongly implicated in many cancers. Interactions between these mitogenic signaling pathways are required for ribosome biogenesis, growth and proliferation in flies and mammals [119-127]. Gain of PI3K/AKT/TOR signaling drives a range of cancers, with many pathway components implicated as oncogenes or tumour suppressors [128]. The PI3K pathway can signal in part through the AKT/TOR pathway to increase protein translation and cell growth [121,122,125] and nutrient-dependent TOR signalling

controls ribosomal RNA (rRNA) transcription, via the conserved RNA Pol I initiation factor, TIF-IA [123].

In *Drosophila*, MYC not only controls expression of ribosome synthesis genes, but also mediates signalling via the InR/PI3K/AKT/TOR signalling pathway to further drive ribosome biogenesis [122,129,130]. Imaginal disc cells lacking TSC activity (i.e with increased TOR signaling) overproliferate in a MYC-dependent manner [131]. Moreover, in mammalian MYC-driven lymphoma mouse models (E*μ-Myc*) the AKT/TOR pathway is required for maximal MYC-dependent ribosome biogenesis [132]. Specifically, inhibition of the AKT/TOR pathway leads to lymphoma cell death via apoptosis, which suggests therapeutic strategies targeting AKT/TOR will be viable for treatment of MYC-driven malignancies.

The importance of rapid MYC turnover to normal growth control is highlighted by its short half-life (around 30 min) [133,134] and is evidenced by the detection of stabilising mutations in transforming retroviruses and Burkitt's lymphoma [135]. Early *in vitro* mammalian cell culture studies also demonstrated co-operative "oncogenesis" between EGFR/RAS and MYC [136], which may occur, at least in part, via the ability of the RAS pathway to stabilise MYC and further increase MYC protein levels [62,63,64,137,138]. *Drosophila* studies suggest that the RAS pathway will also likely regulate MYC turnover *in vivo,* as RAS activation in wing imaginal discs promotes cell growth by increasing MYC protein levels, and RAS is required to maintain endogenous levels of MYC [120].

In mammalian cell culture systems, RAS-driven MAP kinase activity mediates phosphorylation of MYC (Ser62), which stabilizes MYC and is also required for subsequent phosphorylation of MYC (Thr58) by GSK3β and ubiquitination-mediated MYC degradation [135,137,139,140,141]. In the context of MYC-driven S phase progression, this fits with the observation that early in G1 phase RAS-mediated activation of the PI3K/AKT pathway phosphorylates and inhibits GSK3β, which will stabilise MYC. Later in G1 phase, AKT activity will decline to activate GSK3β, which in turn will phosphorylate MYC on Thr58, to drive MYC turnover [142]. This sets up the potential for cross talk between the PI3K/AKT pathway and the WNT pathway in the regulation of MYC. Indeed, the AXIN1 tumour suppressor, a negative regulator of the WNT pathway, provides a scaffold (that includes GSK3β, Pin1, and PP2A-B56α), which drives MYC turnover [146]. Specifically, knockdown of AXIN decreases the abundance of MYC in the AXIN/GSK3β/Pin1/PP2A-B56α degradation complex, which is associated with decreased MYC-Thr58 and enhanced MYC-Ser62 phosphorylation, thus stabilising MYC [146]. Conversely, AXIN over expression drives MYC turnover. Thus, canonical WNT signaling has the potential to increase MYC at the level of transcription [81,86,87,88] and protein stability [146].

In mammals, phosphorylation of MYC at Thr58 by GSK3β, the recognition site for the F-box component of the SCF-ubiquitin ligase (Fbw7/hCDC4), drives MYC turnover via the proteasome pathway [67,143]. A putative conserved domain similar to Thr58 of MYC as also been identified in flies [144] and the *Drosophila* Fbw7/hCDC4 homolog Archipelago (Ago) drives dMYC degradation [145]. In addition, the InR/TOR pathway induces dMYC accumulation in *Drosophila* S2 cells, and this correlates with decreased GSK3β suggesting that nutrient pathways also converge at GSK3β to control MYC stability in flies [130].

## 6. MYC in cell competition: implications from fly studies to cancer

Animal development not only requires coordinate regulation of cell growth, proliferation and cell survival, but also depends on competitive cell-cell interactions [147]. Pioneering studies in *Drosophila* revealed cell competition is dictated by dMYC abundance i.e. cells with relatively higher levels of dMYC out-compete their neighbours, which are eliminated via apoptosis [148,149]. Interestingly, cell competition was not observed for other growth regulators including PI3K/Dp110 or Cyclin D [148].

The question remains how these intriguing discoveries made a decade ago in *Drosophila* relate to mammalian biology and, in particular, whether MYC directs cell competition in the context of cancer [150,151,152]? There is some evidence that MYC-dependent cell competition does occur in mammalian tissues, as conditional deletion of MYC from intestinal cells leads to regeneration from the wild-type tissue and elimination of the *MYC* depleted cells [87]. Furthermore, in line with MYC-driven cancer being dependent on ribosome biogenesis [42], *Drosophila* studies have shown that competition was eliminated by reduced translational capacity (via heterozygosity for ribosomal protein RpL19) [149].

Together the *Drosophila* studies predict elevated MYC expression drives tumour progression, which is a feature of most cancers, by enabling tumour cells to out-compete their "weaker" neighbours with lower MYC abundance. Determining how tumour cells compete with the surrounding normal tissues will therefore be of critical importance to our understanding of how cancer progresses to more lethal stages. Elegant *Drosophila* co-culture studies suggest cells with higher levels of dMYC achieve competition via secreted soluble factors [153]. Thus, identification of the secreted factors driving MYC-dependent competition has fantastic therapeutic potential, particularly the possibility of using drugs to inhibit the activity of these secreted factors to prevent the loss of healthy normal cells and, therefore, reduce the progression of MYC-driven tumours.

## 7. Conclusion

MYC is upregulated in most human cancers, with mis-regulation leading to cancer initiation and malignancy. This review has highlighted the commonality between the function and regulation of MYC between Drosophila and mammals. The picture that is emerging is a complex array of inputs from major developmental signaling pathways (e.g. Wnt, Notch, TGF/BMP, Hippo) and established oncogenic networks (e.g. EGFR/RAS/RAF/ERK, InR/PI3K/AKT/TOR), which act at multiple levels (e.g. transcription, translation and protein stability) to modulate MYC abundance, the key determinant of cell and tissue growth. The knowledge we have gained from Drosophila is, therefore, not only useful for understanding fly growth and development, but also translates to the study of MYC in mammals. Indeed, it was in flies that MYC's capacity to drive ribosome biogenesis was first reported and, recently, pre-clinical mammalian drug discovery trails have demonstrated efficacy for small molecules inhibiting MYC driven ribosome biogenesis [42], [154]. Thus Drosophila studies have defined the physiological function of MYC and indubitably hastened the progress of the MYC field to a point where therapies targeting MYC-driven cancers are plausible.

## Conflict of Interest

Authors declare no conflicts of interest in this paper.

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