

The MYC Oncogene is All About Growth

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Background

Extensive studies suggest the MYC family of proteins have the potential to control transcription of 10-15% of all genes. Yet, although MYC proteins affect multiple targets, recent studies suggest the ability to drive ribosome biogenesis and growth is critical for MYC's oncogenic properties.

Despite the finding that increased *myc* expression occurs in most human cancers, our current understanding of the transcriptional regulation of *myc* is incomplete. Here, we highlight an important mechanism for transcriptional regulation of MYC and discuss how dysregulation of MYC can lead to increased ribosome biogenesis and the overgrowth phenotypes associated with cancer (Fig. 1). We bring together findings from mammalian models with those of genetic studies using the *Drosophila* model system, which have provided insight into a mechanism critical for tight regulation of MYC transcription and shed light on how MYC proteins modulate ribosome biogenesis to control cell growth.

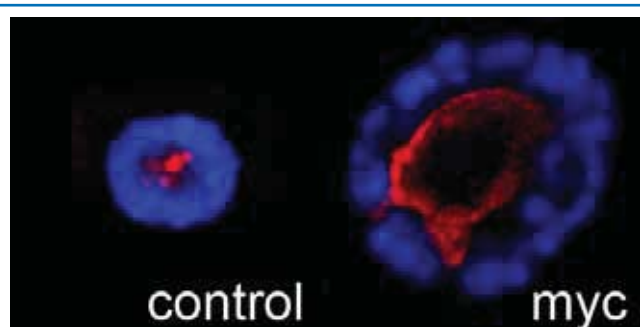


Fig. 1. rRNA levels in control and *myc* overexpressing salivary gland cells (red) with DNA stain (blue).

Keeping *myc* Transcription in Check

The role of the MYC family in human cancer has led to extensive investigation of the molecular and cellular basis of MYC's oncogenic potential. A collection of genetic experiments, microarray profiling analyses and genome binding studies have led to an understanding of the incredibly wide-ranging function of MYC proteins, which can bind to the promoters and potentially control the transcription of 10-15% of all genes (1,2). The regulatory targets of MYC include genes from virtually every biochemical and regulatory pathway in the cell, including growth, metabolism, cell cycle progression, differentiation and apoptosis (3). Small changes in MYC protein levels, either up or down, will modify these critical cellular processes, emphasising the requirement for extremely tight control of *myc* expression.

As MYC is a potent mitogen, the level of *myc* transcription must be tightly regulated. The transcription of *myc* responds to developmental signalling molecules,

which are likely to modulate the complement of a wide variety of transcription factors at the *myc* promoter. In mammals, one level of *myc* promoter regulation involves the presence of a paused, but transcriptionally engaged, RNA polymerase II (Pol II) at the start site, which must escape the promoter to allow transcript elongation. The function of the paused polymerase is potentially two-fold: 1) to allow rapid response to mitogenic signals and 2) to protect the *c-myc* promoter from unwanted activation, since the polymerase will act to block the start site and prevent pre-initiation complex formation (4,5).

Activation of transcription from the blocked *c-myc* promoter is dependent on the general transcription factor TFIID (TFIIH), the transcription initiation factor for RNA polymerase II transcribed genes. TFIIH is required for basal transcription and DNA repair, but also has a more specialised role in regulating *myc* transcription. Although TFIIH is a multi-complex protein, the key subunit for Pol II escape and the transcriptional control of *myc* is the DNA helicase, XPB (6). Interactions between TFIIH/XPB and two DNA structure-sensitive regulatory proteins called FBP and FIR control the Pol II complex movement within the promoter of the *myc* gene. This system centres round a regulatory sequence 1.2 kbp upstream of the major P2 promoter of the *myc* transcription start site known as the far upstream sequence element (FUSE). Interactions occur between the FUSE, the fuse binding protein (FBP), the fuse interacting repressor (FIR) and the XPB helicase to regulate Pol II movement. In this system, FBP and FIR act as dominant regulators of *myc* expression: FBP is a potent activator of *myc*, while FIR returns FBP-stimulated *myc* to basal levels. The antagonistic interactions between FBP and FIR are critical for tight regulation of *myc* transcription from the FUSE, and the action of both FBP and FIR is channeled through XPB. FIR is the key negative regulator within this system, which is required to neutralise FBP's stimulation of XPB and return *myc* transcription to basal levels. FIR can independently bind all the key components of this system, including the FUSE, XPB and FBP. In particular, the coordinate binding of FIR to the FUSE sequence and the XPB helicase is thought to create a loop upstream of the *c-myc* promoter to 1) tether the TFIIH complex and 2) disrupt upstream effector elements and transcription factor binding to further repress *myc* transcription (Fig. 2) (5).

Our previous study of Half pint (Hfp), the *Drosophila* ortholog of FIR, demonstrated Hfp can also behave as a *myc* repressor and tumour suppressor protein (7). More recently, we have developed *Drosophila* models to demonstrate that regulation of *myc* transcription requires interaction between Hfp and the *Drosophila* homolog of the XPB helicase subunit of TFIIH, Haywire (Hay) (8).

Our studies provide *in vivo* evidence that Hfp binds to the *Drosophila myc* (*dmyc*) promoter and that repression of *dmyc* transcription requires Hfp. Using *Drosophila* models to generate GFP-marked Hfp-RNAi cells, we demonstrated that loss of Hfp results in ectopic activation of the *myc* promoter and a cell overgrowth phenotype (Fig. 3). These data suggest that the loss-of-function FIR mutants described in colorectal cancer may be sufficient to increase *myc* expression, drive increased ribosome biogenesis and increase growth, which could lead to cancer initiation and progression. Further support for conservation of the proposed FIR and XPB mechanism for *myc* control in *Drosophila* is provided by genetic epistasis experiments demonstrating that the repression of *dmyc* by Hfp occurs in a manner dependent on Hay, as the increase in *dmyc* transcription and cell growth associated with loss of Hfp is dependent on the presence of Hay (8).

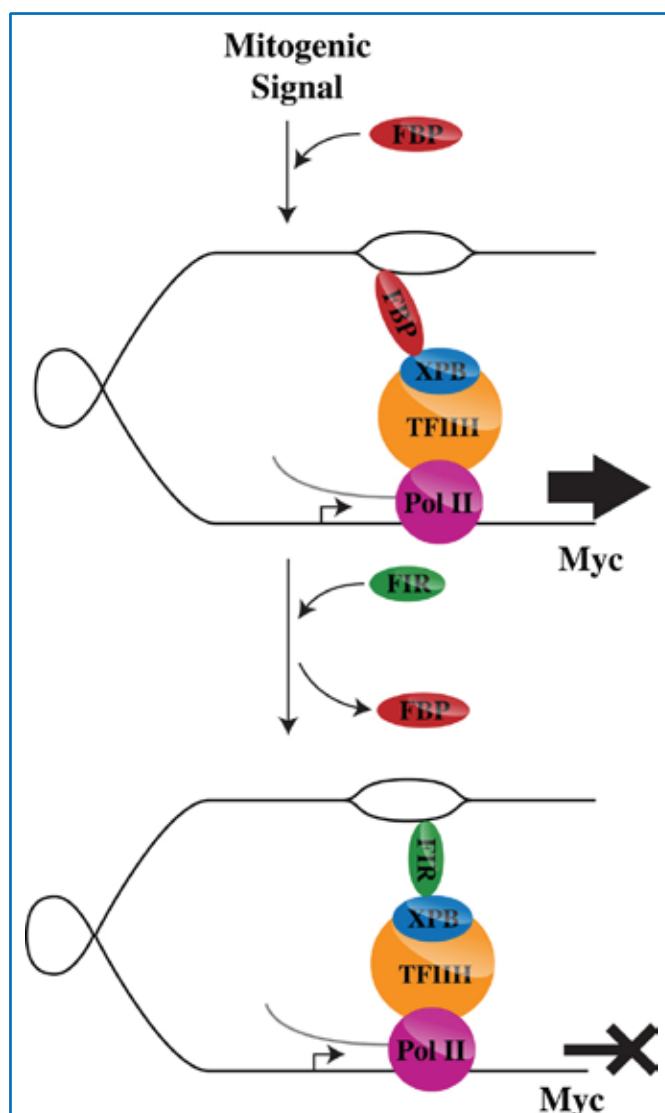


Fig. 2. Model for regulation of the pulse of *myc* transcription.

Mitogenic signals stimulate recruitment of transcription factors and promoter unwinding. As a consequence, FUSE melting allows recruitment of FBP, Pol II release and transcript elongation. Additional promoter unwinding results in the DNA conformation that allows FIR binding, the tethering of Pol II and repression of *myc* transcription.

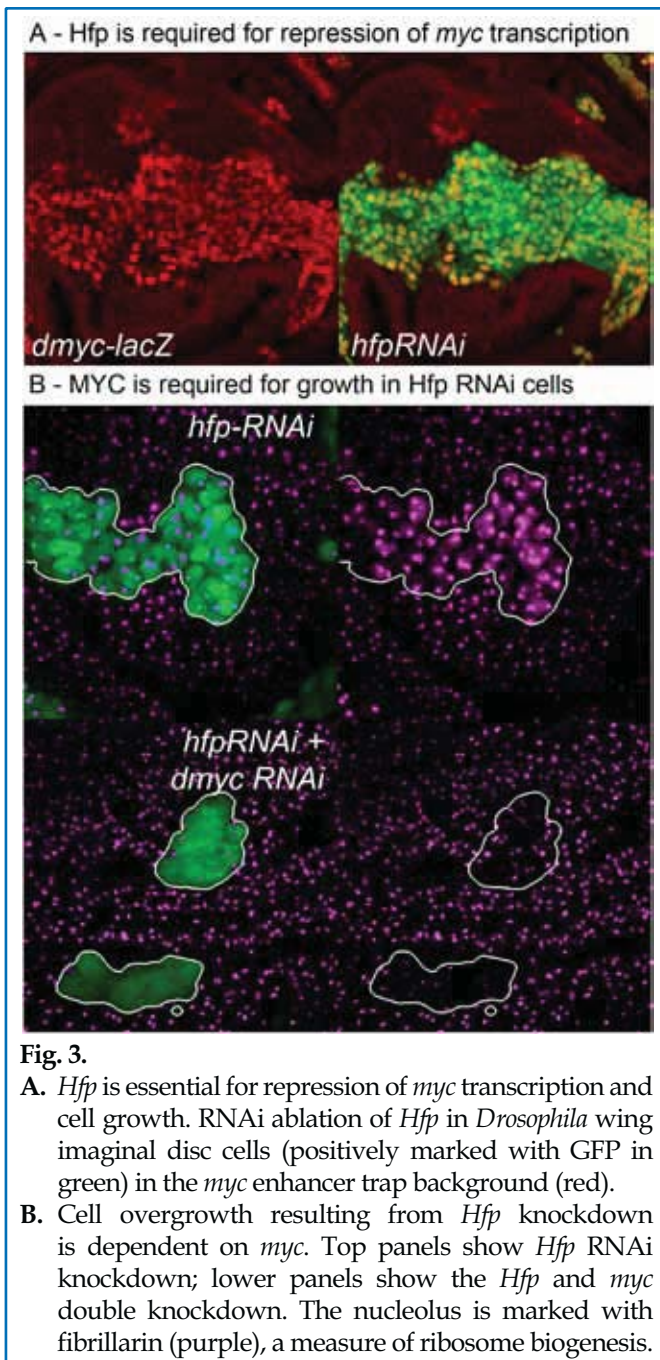
Consistent with roles in regulating *c-myc* transcription, *FIR* mutations have been correlated with colorectal cancer (9) and *XPB* has been linked with the human disease xeroderma pigmentosum (4,10), but potential changes to *myc* transcription and cell growth phenotypes in this disease have been difficult to study in mammals. For example, although *FIR* mutations correlate with colorectal cancer incidence (9), it is unknown whether deregulated *FIR* is the cause of either 1) the increased *c-myc* expression and/or 2) the overgrowth phenotypes associated with these cancers, which occurs in a dMyc-dependent manner. We have shown that the increased ribosome biogenesis and cell and tissue growth resulting from loss of Hfp is suppressed by the *dmyc* RNAi (Fig. 3), which suggests cell overgrowth occurs in a dMyc-dependent manner.

Studies of the temporal association of activators and repressors on the *myc* promoter revealed that binding of *FIR* is one of the key events at the *myc* promoter in the early response to mitogens, which is required for returning transcription to basal levels. The dynamics of *myc* promoter binding by *FIR* is reciprocal to *myc* expression, with the level of bound *FIR* increasing with a coordinate decrease in *myc* transcription. Although *in vitro* mammalian studies have shown that the response of *c-myc* to serum is defective in *FIR* loss-of-function and *XPB* patient cells (5), the upstream factors in serum mediating *c-myc* repression via *XPB* and *FIR* have not been identified. In *Drosophila*, we have shown that Hfp protein levels are regulated, in part, by Wingless (*Wg*) (7). Thus, Hfp may provide an important connection between *Wg* signalling, the regulation of *dmyc* transcription and cell growth. As close connections have been made between the Wnt pathway, Myc regulation and colorectal cancer, the identification of a mutant splice form of *FIR* in primary colorectal cancer tissue, which is unable to repress *myc* transcription and potentially promotes tumour development by sustaining high levels of MYC (9), may be of great interest. We predict that cross-talk between a specific complement of growth signals, including *Wg*, will tightly regulate *myc* transcription and growth via Hfp and Hay, which are likely to be relevant to the processes involved in the deregulation of MYC during human malignancy.

Thus analogous systems are required for transcriptional regulation of the *myc* oncogene and *dmyc*. Our future studies aim to understand the developmental regulation of these proteins in *Drosophila* and will, therefore, be informative in understanding the regulation of *myc* by the homologous proteins in mammals. These novel insights into the molecular mechanisms required for controlling *myc* transcription, which are likely to be important for understanding *FIR* and *XPB* related cancers.

MYC Drives all Aspects of Cell Growth

Studies carried out in mammals and the *Drosophila* model have shown that MYC is essential for ribosome biogenesis and, therefore, the accumulation of cellular mass or cell growth (Fig. 1). Importantly, although MYC proteins affect multiple targets, the ability to drive ribosome biogenesis and growth appears critical for MYC's oncogenic properties during lymphoma (11,12).



The ability of MYC to drive production of rRNA has been shown to be central to its powerful cell growth effects, as ribosome biogenesis is well known to be rate-limiting for cell growth. Both mammalian and *Drosophila* MYC are extremely efficient at activating transcription via RNA polymerases I (1,2) and III (13,14). Furthermore, *Drosophila* genetic experiments have confirmed that MYC can stimulate expression of the Pol I factor TIF-IA (2) and, in mammals, another Pol I factor, UBF (15). MYC most likely regulates Pol III targets via interaction with the Pol III factor TFIIIB-component, Brf (13,14). The *Drosophila* studies further demonstrated that the Pol III functions of MYC are independent of MAX, suggesting that MYC can activate Pol III genes via direct interaction with Brf (14). In line with this, mammalian *myc* mutants lacking DNA binding function can rescue much of the growth defect in *myc* (-/-) primary fibroblasts, suggesting MAX-independent functions for MYC (16).

In *Drosophila*, recent work revealed that MYC is an important mediator of PI3K/TOR-dependent regulation of ribosome biogenesis (17). Using a functional genomics approach, the PI3K/TOR target FOXO was shown to downregulate MYC to reduce translation when muscle tissues were nutrient deprived. Not only did FOXO regulate *myc* mRNA levels, but direct regulation was suggested by identification of FOXO binding sites in the *myc* promoter (17). *dMyc* has also been shown to regulate the transcription of TORC1 targets, whereby chromatin immunoprecipitation experiments confirmed MYC protein enrichment in the promoter regions of TORC1 target genes (17). The amount of MYC protein binding could be increased or decreased with the addition of insulin or rapamycin, respectively, suggesting TORC1 controls MYC activity by managing the amount of MYC protein bound to the promoters of TORC1 targets. Thus, this study (17) incorporates *Drosophila* MYC into the PI3K/TOR pathway, which complements MYC's role in the control of ribosome synthesis gene expression.

Deregulation of translational control is an emerging mechanism for cancer formation, and recent evidence strongly suggests that MYC-dependent oncogenic signalling may monopolise the translational machinery to elicit cooperative effects on cell growth, cell cycle progression, and genome instability as a mechanism for cancer initiation. Studies in *Eμ-Myc* mice, which are predisposed to developing B-cell lymphomas, in a ribosomal protein mutant background (*RpL24^{+/-}* or *RpL38^{+/-}*) resulted in the restoration of increased protein synthesis and cell division back down to normal levels, the re-establishment of the apoptotic response, and the delayed onset of lymphomas (11,18). Thus, genetic tools for restoration of aberrant increases in protein synthesis control are now available, which will enable the dissection of important mechanisms in cancer that rely on the translational machinery (18).

Conclusions

The MYC oncoprotein is upregulated in most human cancers, with dysregulation leading to cancer initiation and malignancy. MYC has potential as a drug target in cancer therapy, as inhibiting MYC can halt tumour cell growth and proliferation. In particular, the finding that MYC-dependent growth depends on ribosome biogenesis presents a potentially powerful drug therapy whereby inhibition of ribosome biogenesis could be used to specifically target tumours with elevated MYC. The promise of treating a key oncogene such as MYC with anti-cancer agents has only become feasible through our increased understanding of MYC biology using the combination of genomic approaches, genetic models developed in *Drosophila*, mouse models and analysis of human cancer cells.

References

1. Grandori, C., Gomez-Roman, N., Felton-Edkins, Z.A., Ngouenet, C., Galloway, D.A., Eisenman, R.N., and White, R.J. (2005) *Nat. Cell Biol.* 7, 311-318
2. Grewal, S.S., Li, L., Orian, A., Eisenman, R.N., and Edgar, B.A. (2005) *Nat. Cell Biol.* 7, 295-302

3. Siddall, N.A., Lin, J.I., Hime, G.R., and Quinn, L.M. (2009) *Curr. Drug Targets* **10**, 590-601
4. Liu, J., Kouzine, F., Nie, Z., Chung, H.J., Elisha-Feil, Z., Weber, A., Zhao, K., and Levens, D. (2006) *EMBO J.* **25**, 2119-2130
5. Levens, D. (2008) *J. Natl. Cancer Inst. Monogr.* **39**, 41-43
6. Liu, J., He, L., Collins, I., Ge, H., Libutti, D., Li, J., Egly, J.M., and Levens, D. (2000) *Mol. Cell* **5**, 331-341
7. Quinn, L.M., Dickins, R.A., Coombe, M., Hime, G.R., Bowtell, D.D., and Richardson, H. (2004) *Development* **131**, 1411-1423
8. Mitchell, N.C., Johanson, T. M., Cranna, N.J., Er, A.L., Richardson, H.E., Hannan, R.D., and Quinn, L.M. (2010) *Development* **137**, 2875-2884
9. Matsushita, K., Tomonaga, T., Shimada, H., Shioya, A., Higashi, M., Matsubara, H., Harigaya, K., Nomura, F., Libutti, D., Levens, D., and Ochiai, T. (2006) *Cancer Res.* **66**, 1409-1417
10. Liu, J., Akoulitchev, S., Weber, A., Ge, H., Chuikov, S., Libutti, D., Wang, X.W., Conaway, J. W., Harris, C.C., Conaway, R.C., Reinberg, D., and Levens, D. (2001) *Cell* **104**, 353-363
11. Barna, M., Pusic, A., Zollo, O., Costa, M., Kondrashov, N., Rego, E., Rao, P.H., and Ruggero, D. (2008) *Nature* **456**, 971-975
12. Ruggero, D., Montanaro, L., Ma, L., Xu, W., Londei, P., Cordon-Cardo, C., and Pandolfi, P.P. (2004) *Nat. Med.* **10**, 484-486
13. Gomez-Roman, N., Grandori, C., Eisenman, R.N., and White, R.J. (2003) *Nature* **421**, 290-294
14. Steiger, D., Furrer, M., Schwinkendorf, D., and Gallant, P. (2008) *Nat. Genet.* **40**, 1084-1091
15. Poortinga, G., Hannan, K.M., Snelling, H., Walkley, C.R., Jenkins, A., Sharkey, K., Wall, M., Brandenburger, Y., Palatsides, M., Pearson, R.B., McArthur, G.A., and Hannan, R.D. (2004) *EMBO J.* **23**, 3325-3335
16. Cowling, V.H., and Cole, M.D. (2007) *Mol. Cell. Biol.* **27**, 2059-2073
17. Teleman, A.A., Hietakangas, V., Sayadian, A.C., and Cohen, S.M. (2008) *Cell Metab.* **7**, 21-32
18. Ruggero, D. (2009) *Cancer Res* **69**, 8839-8843

