A conserved element in Myc that negatively regulates its proapoptotic activity

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Myc is an oncoprotein transcription factor that promotes cell proliferation and apoptosis. Analysis of highly conserved elements within vertebrate Myc proteins has been instrumental in defining the functions of the Myc protein. Here, we probe the role of a highly conserved, but little studied, element within the central region of c-Myc, termed 'Myc box III' (MbIII). We show that MbIII is important for transcriptional repression by Myc, and for transformation both *in vitro* and in a mouse model of lymphomagenesis. Curiously, disruption of MbIII decreases transformation activity by increasing the efficiency with which Myc can induce apoptosis, suggesting that MbIII is a negative regulator of programmed cell death. These findings reveal a role for MbIII in Myc biology, and establish that the oncogenic capacity of Myc is linked directly to its ability to temper the apoptotic response.

Keywords: transcription; apoptosis; Myc; transformation

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INTRODUCTION

The *c-Myc* oncogene encodes a helix–loop–helix (HLH) transcription factor that features prominently in the control of cell proliferation and in cancer. Capable of acting as both a transcriptional activator and repressor, Myc regulates many processes relevant to cell growth and proliferation (Grandori *et al*, 2000). Consistent with its potent growth-promoting properties, Myc can also stimulate apoptosis (Evan *et al*, 1992). Indeed, the ability of cells to undergo apoptosis in response to Myc is

viewed as a major cellular defence against transformation by the Myc protein (Pelengaris *et al*, 2002).

c-Myc is the defining member of a family of HLH transcription factors that have been conserved during vertebrate evolution (Grandori *et al*, 2000). Within this family, the identification and analysis of highly conserved regions has been instrumental in understanding Myc function. The transcriptional activation domain of Myc, for example, contains two highly conserved regions, termed Myc boxes (Mb) I and II, that are important for Myc activity—MbI is a common site of mutations in cancer (Bhatia *et al*, 1994) and the target of Ras-mediated signalling (Sears *et al*, 2000), whereas MbII contributes to activation of Myc target genes by recruitment of the coactivator transcription/transformation domain-associated protein (TRRAP; McMahon *et al*, 1998).

Recently, we found that a little-studied element within Myc—'Myc box III' (MbIII)—contributes to destruction of Myc by the proteasome (Herbst *et al*, 2004). MbIII is centrally located within Myc (residues 188–199) and is highly conserved among the *c-*, *N-* and *s-Myc* family members (supplementary Fig 1 online). Curiously, despite its conservation, little is known about the role of MbIII in Myc activity. As MbI and II are implicated in both Myc destruction (Flinn *et al*, 1998) and function (e.g. Sarid *et al*, 1987), we asked whether MbIII also participates in Myc's activity. Here, we present evidence that MbIII is important for Myc function and contributes to cellular transformation by downmodulating the ability of Myc to induce apoptosis.

RESULTS AND DISCUSSION

To understand how MbIII contributes to Myc function, we asked how the requirement for this element compares with that for highly conserved MbI and II in several aspects of Myc behaviour. We generated mutant Myc proteins in which we deleted MbI, MbII, or MbIII. We then compared these mutants in six measures of Myc activity: (i) anchorage-independent cell growth, (ii) alterations in cellular morphology, (iii) induction of S-phase, (iv) induction of apoptosis, (v) transcriptional regulation and (vi) ability to promote B-cell lymphomas in mice. The first five of these assays were performed with an inducible form of Myc that

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was generated by fusion to the hormone-binding domain of the oestrogen receptor (ER-Myc; Kim *et al*, 2003); we used ER-Myc to avoid potential secondary effects from constitutive overexpression of active Myc protein.

MbIII is important for transformation *in vitro*

Previous studies have demonstrated that the central region of Myc contributes to, but is not essential for, cellular transformation *in vitro* (Sarid *et al*, 1987; Stone *et al*, 1987). To revisit the role of MbIII in this process, we asked whether deletion of this element alters the ability of Myc to drive anchorage-independent cell

growth in Rat1a cells (Stone *et al*, 1987). We activated the ER-Myc fusion proteins (Fig 1A) in stably transduced Rat1a cells and measured colony formation in soft agar. As expected, ER-Myc wild type (WT) efficiently drove colony formation under these conditions (Fig 1B). This activity was reduced modestly by deletion of Mbl, and more severely by deletion of MbII. Curiously, deletion of MbIII had a significant effect on growth in soft agar, reducing the overall number of colonies by 60% and eliminating the very large colonies seen with the WT ER-Myc protein (Fig 1C). This reduction in colony formation is consistent with earlier studies (Sarid *et al*, 1987; Stone *et al*, 1987), and suggests that MbIII contributes to *in vitro* transformation by the Myc protein.

MbIII is dispensable for many aspects of Myc function

To understand why the Δ MbIII mutant is defective in transformation, we probed its activity in several assays. We first asked whether this protein could rescue morphological defects associated with deletion of the *c-Myc* gene. We expressed the ER-Myc fusion proteins (Fig 2A) in *myc*-null Ho15.19 cells (Mateyak *et al*, 1997) and scored their morphology. As reported (Mateyak *et al*, 1997), WT Myc restored the fibroblast morphology of these cells, converting them from a flat, phase-dull, appearance (Fig 2, Vect.) to a spindle-shaped, phase-bright, morphology (Fig 2B, WT). Deletion of MbII blocked this activity. Interestingly, however, deletion of either Mbl or III had little effect in this assay—both the Δ Mbl and Δ MbIII mutants rescued the fibroblast appearance of these cells. The ability of these proteins to rescue Ho15.19 morphology demonstrates that Mbl and III are dispensable for this activity, and reveals that the Δ Mbl and Δ MbIII Myc mutants retain at least some aspect of WT Myc function.

To determine whether MbIII is important for the ability of Myc to stimulate S-phase entry (Eilers *et al*, 1989), we activated ER-Myc proteins in serum-starved, density-arrested, Ho15.19 cells, and measured S-phase by bromodeoxyuridine (BrdU) incorporation (Fig 2C). WT ER-Myc stimulated a significant level (12%) of QJ/S-phase entry in these cells. Consistent with previous reports (Spotts *et al*, 1997), deletion of MbII disrupted this activity. Remarkably, both the Δ Mbl and Δ MbIII mutants retained WT levels of S-phase-promoting activity. These results further support the idea that these proteins retain biological activity and demonstrate that the conserved Mbl and III elements are required for only a subset of Myc functions.

MbIII is required for transcriptional repression

We next compared the contribution of Mbl and III to transcriptional regulation. We generated clonal cell lines expressing either WT ER-Myc, or the Δ Mbl or Δ MbIII mutants. We activated the ER-Myc fusion proteins, and measured transcript levels from four target genes that are activated by Myc (Fig 2D), and four that are repressed (Fig 2E). As expected, activation of WT ER-Myc in these cells led to an induction of AHCY, Cad, Cdc2 and Rcl transcript levels. Comparison with the mutant proteins revealed that deletion of either Mbl or III reduced (but did not eliminate) gene activation. The only exception to this trend was the *Cdc2* gene, the induction of which was disrupted by deletion of either Mbl or III. Thus, Mbl and III seem to contribute to full transcriptional activation by Myc, but are not universally required for this process.

Analysis of Myc-repressed genes (Fig 2E) revealed a very different pattern of behaviour. Deletion of Mbl blocked transcriptional

Fig 1 Myc box III is important for transformation *in vitro*. Rat1a cells were transduced with retroviruses expressing the indicated wild-type (WT) or mutant ER-Myc protein levels. (A) Steady-state ER-Myc protein levels were determined by western blotting. (B) Cells were plated in soft agar in the presence of 4HT, and the number of colonies for each construct determined 21 days after seeding. Error bars depict standard error of the mean (s.e.m.; $n = 3$). (C) Morphology of colonies analysed in (B).

downregulation of the *gadd45* and *Gas1* genes, but had no effect on repression of p21 or p15. Deletion of MblIII, in contrast, eliminated transcriptional repression by Myc at all genes examined. On the basis of this result, we conclude that MblIII is essential for the ability of Myc to repress target gene transcription, whereas Mbl is required for repression at only a subset of Myc target genes.

Deletion of MblIII promotes apoptosis by Myc

We next examined the ability of the ER-Myc fusion proteins to stimulate apoptosis. Stably transduced Ho15.19 cells were treated with increasing concentrations of the DNA-damaging drug etoposide, and cellular viability was determined. As expected, WT Myc stimulated cell death in response to etoposide (Fig 3A; compare 'WT' and 'Vect.'). Loss of Mbl had little, if any, effect on the ability of Myc to induce apoptosis in this assay, whereas deletion of MblII completely disrupted this activity. Unexpectedly, deletion of MblIII significantly enhanced the ability of Myc to drive cell death: not only did the Δ MblIII deletion promote a higher rate of spontaneous apoptosis, but it also enhanced the efficiency of cell killing by etoposide by ~ 8 -fold. This result demonstrates that the proapoptotic activity of Myc can be enhanced by deletion of MblIII, and suggests that this element normally acts to attenuate the ability of Myc to induce apoptosis.

To determine whether the enhanced ability of the Δ MblIII deletion mutant to stimulate apoptosis is responsible for the decreased transforming potential of this protein, we asked if we could rescue the transformation defect by blocking apoptosis. We ectopically expressed the antiapoptotic protein Bcl-2 (Vaux *et al*, 1988), together with the Myc mutants, in Rat1a cells and repeated the transformation assay (Fig 3B). Remarkably, Bcl-2 expression completely rescued the transformation defects associated with loss of MblIII. This effect was specific to the Δ MblIII deletion mutant, because defects associated with loss of Mbl or II were not suppressed by Bcl-2 expression. These effects are unlikely to be due to secondary effects on the stability of Myc mutants, because Bcl-2 does not affect the rate of Myc proteolysis (supplementary Fig 2 online). The ability to rescue the transformation defects of the Δ MblIII mutant by expression of Bcl-2 strongly suggests that

◀ **Fig 2** | MblIII is dispensable for many aspects of Myc function. *myc*-null cells were transduced with retroviruses expressing the indicated WT and mutant ER-Myc proteins, and treated with 4HT. (A) Steady-state ER-Myc protein levels were determined by western blotting. (B) Cellular morphology was visualized by phase-contrast microscopy. (C) Transduced Ho15.19 cells were driven to quiescence by serum starvation and density arrest. Quiescent cells were then treated with 4HT in the presence of BrdU. After 18 h, BrdU-positive cells were detected by indirect immunofluorescence and nuclei counterstained with 4'-6 diamidino-2-phenylindole (DAPI). Error bars depict s.e.m. ($n = 5$). (D) Activation of Myc target genes was measured by driving clonal populations of transduced Ho15.19 cells to quiescence by serum starvation and density arrest, treatment with 4HT, isolation of RNA and analysis of indicated transcript levels by reverse transcription, real-time PCR. 'Relative RNA level' was calculated by normalization to the signal for β -actin in each sample, and setting the vector value to 1. (E) Repression of Myc target genes was determined as described in (D). Error bars depict s.e.m. ($n = 3$).

Fig 3 | Loss of MbIII stimulates Myc's ability to induce apoptosis. (A) Transduced Ho15.19 cells, expressing the indicated ER-Myc proteins, were treated with increasing amounts of etoposide. At 24 h after exposure to the drug, cellular viability was determined by trypan blue exclusion. Error bars depict s.e.m. ($n = 4$). Annexin V staining confirmed that cell death occurred through apoptosis (data not shown). (B) Rat1a cells were transduced with retroviruses expressing the indicated WT or mutant ER-Myc proteins, either in the presence or absence of ectopic Bcl-2 expression. Cells were plated in soft agar in the presence of 4HT, and colonies scored as in Fig 2. Error bars depict s.e.m. ($n = 3$). (C) MEFs, infected with the indicated MSCV-based vectors, were treated with increasing amounts of etoposide. At 24 h after exposure to the drug, cellular viability was determined by trypan blue exclusion. Error bars depict s.e.m. ($n = 6$). (D) Steady-state levels of Myc, p53, Arf and actin in infected MEFs in the absence (–) or presence (+) of 20 μ M etoposide.

MbIII contributes to transformation by downmodulating Myc's proapoptotic ability.

Ho15.19 cells are an immortalized cell line. To determine if MbIII has a similar role in primary cells, we repeated the apoptosis assay in mouse embryo fibroblasts (MEFs; Fig 3C). This experiment confirmed that the Δ MbIII mutant has significantly enhanced proapoptotic activity in MEFs, despite being expressed at lower levels than the WT protein (Fig 3D). Interestingly, both the Δ MbI and Δ MbII mutants showed increased proapoptotic activity in MEFs, compared with Ho15.19 cells. For Δ MbII, this could be accounted for by significantly higher levels of the mutant protein (Fig 3D, lane 7). The Δ MbI mutant, however, was expressed at lower levels than WT Myc, suggesting that, like Δ MbIII, this mutant may have intrinsically higher apoptotic abilities. Note that

treatment of cells with etoposide resulted in a marked reduction in the steady-state level of all Myc proteins (Fig 3D, even-numbered lanes), suggesting that Myc protein itself may be downregulated during the apoptotic programme.

To probe the mechanism through which the Δ MbIII mutant signals apoptosis, we examined activation of the Arf–p53 pathway in transduced MEFs (Fig 3D). Although Arf levels were not altered significantly in this assay, p53 was potentially induced by the presence of all Myc proteins (compare lanes 1 and 2 with lanes 3–10), and further induced by etoposide treatment. Importantly, all mutant Myc proteins induced p53 to a level comparable with that of WT Myc. These findings suggest that the enhanced proapoptotic function of the Δ MbI and III mutants is mediated by induction of a p53-independent pathway.

MbIII contributes to lymphomagenesis by Myc *in vivo*

To ask whether the function of MbIII is important for tumour development *in vivo*, we developed a system to study the effects of Myc overexpression within the haematopoietic compartment of mice (Fig 4A). Haematopoietic stem cells (HSCs) were isolated from fetal livers of $p53^{+/-}$ mice and transduced with recombinant mouse stem-cell viruses (MSCVs) expressing various Myc proteins, together with the green fluorescent protein (GFP; Schmitt *et al*, 2000). These modified $p53^{+/-}$ stem cells were then used to reconstitute the haematopoietic compartment of lethally irradiated recipient mice. Recipients were monitored for lymphoma development and dissemination by palpation of the lymph nodes,

blood smears, and by whole-body fluorescent imaging of GFP-expressing tumour cells (Schmitt *et al*, 2002).

Mice transplanted with HSCs expressing WT Myc developed large disseminated GFP-positive lymphomas (Fig 4B), with accompanying leukaemia, and were killed at morbidity at an average of 49.6 ± 8.8 days after stem-cell transplantation (data not shown). This disease was completely penetrant in recipient animals, with no survival past 9 weeks (Fig 4C). Immunophenotyping for both lymphoid and myeloid cell surface markers showed these tumours to be pre-B-cell lymphomas (B220+/IgM-; data not shown). Consistent with the requirement for MbII in all aspects of Myc activity, mice reconstituted with HSCs

Fig 4 | An anti-apoptotic function for Myc is important for lymphomagenesis *in vivo*. (A) Protocol for adoptive transfer experiments using HSC-expressing mutant Myc proteins. (B) *In vivo* GFP imaging and haematoxylin & eosin-stained blood smears showing lymphomas/leukaemias in mice reconstituted with transduced $p53^{+/-}$ HSCs. Arrows indicate the GFP-positive bone marrow of ΔMbI recipients. (C) Survival of mice reconstituted with $p53^{+/-}$ stem cells expressing indicated Myc mutants. Data are presented in a Kaplan-Meier (KM) format. (D) KM curve showing the percentage of mouse survival in mice reconstituted with WT stem cells co-infected with retroviral vectors expressing Bcl-2 and the indicated Myc mutants.

expressing the Δ MbII Myc mutant remained healthy and tumour-free throughout the course of the analysis (~ 200 days; Fig 4C).

Analysis of mice reconstituted with HSCs expressing the Δ MbI mutant revealed a very different pattern of tumour development. Unlike the large disseminated lymphomas seen in WT Myc recipients, Δ MbI recipients developed GFP+ leukaemia with minimal lymph node involvement (Fig 4B). This tumour phenotype appeared with complete penetrance in recipient animals, but also correlated with a significantly longer overall survival time relative to WT Myc recipients (90.6 ± 12.2 days, $P < 0.0001$; Fig 4C). GFP imaging of Δ MbI recipients before killing showed an almost complete replacement of bone marrow with tumour cells. Curiously, staining for cell surface markers on these bone marrow-derived tumour cells revealed the tumours to be of T-cell origin (B220–/Thy1+; Fig 4B, lower panel). Thus, deletion of MbI markedly alters the type of tumour initiated by Myc in this system, converting Myc from an inducer of B-cell lymphoma to an inducer of T-cell leukaemia.

Interestingly, analysis of mice reconstituted with HSCs expressing the Δ MbIII mutant showed a tumour phenotype that was significantly less severe than WT Myc recipients. Whereas tumorigenesis induced by WT Myc was completely penetrant, only 3 out of 7 recipients of Δ MbIII-infected HSCs developed pre-B-cell lymphomas (with accompanying leukaemia). Moreover, the Δ MbIII recipients that developed lymphomas showed a delayed onset of disease and prolonged survival (86.3 ± 16.3 days, $P < 0.001$). Therefore, as we saw *in vitro*, MbIII has an important role in promoting transformation by Myc *in vivo*.

Finally, we repeated the *in vivo* analysis using HSCs that overexpressed Bcl-2 (Fig 4D). This analysis gave a striking result: In the presence of overexpressed Bcl-2, mice reconstituted with HSCs expressing the WT, Δ MbI and Δ MbIII forms of Myc showed rapid, and similar, patterns of disease onset and progression. Tumorigenesis initiated by all three forms of Myc was completely penetrant, and all recipient mice were dead 50 days after reconstitution. The average survival of these three sets of mice was similar— 32.7 ± 3.7 days for WT, 35.3 ± 4.1 days for Δ MbI ($P = 0.58$) and 39.0 ± 8.2 days for Δ MbIII ($P = 0.12$). Crucially, all three sets of mice developed aggressive, highly disseminated, B-cell lymphomas (data not shown), revealing that blocking apoptosis can rescue not only the efficiency of tumorigenesis, but also the type of tumour, that is induced by the Δ MbI and Δ MbIII deletion mutants.

CONCLUSION

The data presented here demonstrate that the ability of Myc to attenuate its proapoptotic ability is important for regulating oncogenic transformation both *in vitro* and *in vivo*. Analysis in the mouse lymphomagenesis model reveals at least two ways in which modulation of the apoptotic response by Myc is important. For MbI, this function appears to regulate tumour type; the ability of Bcl-2 to switch the tumour type promoted by the Δ MbI mutant from T-cell leukaemia back to B-cell lymphoma is consistent with a model in which MbI downmodulates apoptosis specifically in B cells. For MbIII, this function regulates tumour penetrance and progression, consistent with a model in which MbIII functions broadly to attenuate Myc's apoptotic ability. As the Δ MbIII mutant retains many Myc activities, with the exception of transcriptional repression, we speculate that downmodulation of the apoptotic

response may be related to repression of specific proapoptotic target genes. Regardless of the mechanism, however, these data establish that negative regulation of the apoptotic response is a process through which Myc promotes tumorigenesis and reveal that Myc can differentially regulate apoptosis in different cellular compartments. The anti-apoptotic function of MbIII underscores the complexity of cellular transformation by the Myc protein, and suggests a strategy for selectively inducing apoptosis in cancer cells by interfering with MbIII activity.

METHODS

Antibodies. Antibodies used in this study were: α -Myc N-262 (Santa Cruz, CA, USA), α -p53 (p53-505 NovoCastra, Newcastle upon Tyne, UK), α -p19ARF (ab14244 Abcam, Cambridge, MA, USA) and α -actin (AC-15 Sigma, St Louis, MO, USA).

Plasmid DNA manipulations. WT and mutant Myc coding sequences (Herbst et al, 2004) were transferred into the retroviral vector pBabeHygroFER (Reilly & Herr, 2002) or into an MSCV retroviral vector upstream of IRES-GFP (Schmitt et al, 2002).

Mammalian cell culture. Rat1, Rat1a and Ho15.19 cells were transduced by retroviral gene transfer to express the appropriate pBabeHygroFER–Myc expression construct, or vector control. MEFs were retrovirally transduced with the appropriate MSCV vector. Determination of S-phase in retrovirally transduced Ho15.19 cells was performed as described (Kim et al, 2003). Analysis of cDNA levels for AHCY, Cad, Cdc2, Rcl, gadd45, gas1, p21 and p15 in clonal Rat1 cells expressing the appropriate ER–Myc proteins was performed as described (Kim et al, 2003). All transformation and apoptosis assays have been performed a minimum of three times, with the exception of the MEF apoptosis assays—which have been independently performed twice, using different batches of MEFs.

Determination of apoptosis. Retrovirally transduced Ho15.19 cells were seeded in six-well dishes (2×10^5 cells per dish) in phenol red-free Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum (CS) and $2 \mu\text{M}$ 4 hydroxytamoxifen (4HT). After 24 h, cells were treated with increasing amounts of etoposide and incubated for a further 24 h. Adherent and nonadherent cells were collected, and cellular viability determined by trypan blue exclusion. Apoptosis assays in MEFs were performed similarly.

Anchorage-independently growth. Retrovirally transduced Rat1a cells were seeded onto 6 cm dishes in soft agar supplemented with $2 \mu\text{M}$ 4HT. Cells were refed every 7 days. The number of colonies (> 100 cells each) for each construct was determined 21 days after seeding, and normalized to the number of colonies for WT Myc.

In vivo analysis of Myc mutants. Isolation, infection and transplantation of HSCs was performed as described (Schmitt et al, 2002). HSCs were obtained from $p53^{+/-}$ C57BL/6 mouse (Jackson Laboratory, Bar Harbor, ME, USA) E13.5–15.5 fetal livers. Reconstituted animals were monitored for illness by lymph node palpation, by monitoring overall morbidity, and by whole-body fluorescence imaging (Schmitt et al, 2002). The onset of lymph node hyperplasia was assessed by regular palpation of the axillary, brachial and inguinal lymph nodes. Overall survival was defined as the time from stem-cell reconstitution until the animal reached a terminal stage and had to be killed.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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REFERENCES

- Bhatia K, Spangler G, Gaidano G, Hamdy N, Dalla-Favera R, Magrath I (1994) Mutations in the coding region of c-myc occur frequently in acquired immunodeficiency syndrome-associated lymphomas. *Blood* **84**: 883–888
- Eilers M, Picard D, Yamamoto KR, Bishop JM (1989) Chimaeras of myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells. *Nature* **340**: 66–68
- Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC (1992) Induction of apoptosis in fibroblasts by c-myc protein. *Cell* **69**: 119–128
- Flinn EM, Busch CMC, Wright APH (1998) Myc boxes, which are conserved in myc family proteins, are signals for protein degradation via the proteasome. *Mol Cell Biol* **18**: 5961–5969
- Grandori C, Cowley SM, James LP, Eisenman RN (2000) The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu Rev Cell Dev Biol* **16**: 653–699
- Herbst A, Salghetti SE, Kim SY, Tansey WP (2004) Multiple cell-type-specific elements regulate Myc protein stability. *Oncogene* **23**: 9448–9456
- Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP (2003) Skp2 regulates Myc protein stability and activity. *Mol Cell* **11**: 1177–1188
- Mateyak MK, Obaya AJ, Adachi S, Sedivy JM (1997) Phenotypes of c-Myc-deficient rat fibroblasts isolated by targeted homologous recombination. *Cell Growth Differ* **8**: 1039–1048
- McMahon SB, Van Buskirk HA, Dugan KA, Copeland TD, Cole MD (1998) The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell* **94**: 363–374
- Pelengaris S, Khan M, Evan G (2002) c-MYC: more than just a matter of life and death. *Nat Rev Cancer* **2**: 764–776
- Reilly PT, Herr W (2002) Spontaneous reversion of tsBN67 cell proliferation and cytokinesis defects in the absence of HCF-1 function. *Exp Cell Res* **277**: 119–130
- Sarid J, Halazonetis TD, Murphy W, Leder P (1987) Evolutionarily conserved regions of the human c-myc protein can be uncoupled from transforming activity. *Proc Natl Acad Sci USA* **84**: 170–173
- Schmitt CA, Rosenthal CT, Lowe SW (2000) Genetic analysis of chemoresistance in primary murine lymphomas. *Nat Med* **6**: 1029–1035
- Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW (2002) A senescence program controlled by p53 and p16(INK4a) contributes to the outcome of cancer therapy. *Cell* **109**: 335–346
- Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR (2000) Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* **14**: 2501–2514
- Spotts GD, Patel SV, Xiao Q, Hann SR (1997) Identification of downstream-initiated c-Myc proteins which are dominant-negative inhibitors of transactivation by full-length c-Myc proteins. *Mol Cell Biol* **17**: 1459–1468
- Stone J, de Lange T, Ramsay G, Jakobovits E, Bishop JM, Varmus H, Lee W (1987) Definition of regions in human c-myc that are involved in transformation and nuclear localization. *Mol Cell Biol* **7**: 1697–1709
- Vaux DL, Cory S, Adams JM (1988) Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**: 440–442