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B-*myb* rescues *ras*-induced premature senescence, which requires its transactivation domain

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Abstract

B-*myb*, a ubiquitously expressed member of the *myb* gene family, is highly regulated throughout the cell cycle and appears to be required for cell cycle progression. In contrast to its relatives A-*myb*, c-*myb*, and v-*myb*, no transforming activity of B-*myb* has been reported thus far. We report here that B-*myb* can rescue senescence induced by an activated *ras* oncogene in rodent cells in vitro. We show that transformation by B-Myb involves its ability to activate transcription. Similar to other oncogenic transcription factors, such as c-Myc and E2F, we show that B-Myb also has repression activity. We demonstrate that the C-terminus of B-Myb can function as a repressor of transcription, that B-Myb interacts with the repressor molecules BS69 and N-CoR and that the repression function, like the transactivation domain, contributes to B-*myb* transformation.

Keywords: B-myb; BS69; N-CoR; Transformation; Transcription; Repression

1. Introduction

The *myb* gene family consists of three members, A-*myb*, B-*myb*, and c-*myb*. The v-*myb* oncogene of the avian myoblastosis virus (AMV) closely resembles the c-*myb* proto-oncogene but lacks the N- and C-terminal sequences found in c-*myb*. In addition, v-*myb* has several point mutations compared to c-*myb*. C-terminal truncation enhances the c-*myb* oncogenic potential [1], a process that occurs during retroviral insertional mutagenesis in avian lymphoid and murine myeloid [2–4]. The A-*myb* and B-*myb* homologues of c-*myb* have been identified by low stringency hybridization [5] and share extensive homology in the N-terminal three tandem 50 amino acid direct repeats that specify the Myb DNA binding domain and a conserved region in the C-terminus. The expression of c-*myb* and A-*myb* is limited to immature hematopoietic cells [6,7] and male germ cells or breast epithelial cells, respectively [8,9], reviewed in Ref. [10]. B-*myb* expression is more ubiquitous and is present in virtually all proliferating cells [11,12].

B-Myb's presence in dividing cells and its activity are highly regulated at multiple levels and stages during the cell cycle. Expression of B-*myb* is down-regulated in serum starved fibroblasts and induced in late G1 upon serum stimulation in an E2F-4/p107 or p130-dependent manner [13–15]. B-Myb is thought to play an important role in G1/S transition. Over-expression of B-*myb* can bypass a p53- and $p21^{cip1}$-induced cell cycle arrest [16] and partially rescue a p107-induced cell cycle arrest [17]. Conversely, anti-

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sense repression of B-*myb* can induce a growth arrest [18]. Knockout studies have shown that a B-*myb* homozygous deletion is embryonic lethal at day 4.5–6.5, probably when maternal stores become limiting, and attempts to create an ES cell line homozygously deleted for B-*myb* failed [19], suggesting that the B-*myb* activity is essential for cell cycle progression.

B-Myb binds to the same DNA sequence as c-Myb [20,21] and B-Myb can transactivate a reporter containing promoter elements of the c-Myb target *mim*1 [22]. B-Myb and c-Myb may therefore induce expression of an overlapping set of target genes, among which are proto-oncogenes such as c-*myc*, c-*myb*, and B-*myb* themselves [21,23,24] and genes involved in proliferation such as *cdc*2 [25] and DNA polymerase α [26].

Activity of B-Myb is regulated at multiple levels and is coordinated during the cell cycle. Apart from the transcriptional up- and down-regulation by E2F and retinoblastoma family proteins, cyclin A/cdk2-mediated phosphorylation in late G1 and S phase dramatically increases B-Myb transactivation potential [27,28]. Cyclin D1 and cdk9 appear to be negative regulators of B-Myb transactivation and auto-regulation [29,30]. Similar to c-*myb*, a second transcript, lacking exon 9a, is found in a broad spectrum of cells [31]. The protein encoded by this alternatively spliced messenger of B-*myb*, lacking the exon 9a encoded domain, is transcriptionally inactive and was shown to compete for, and thereby inhibit, transactivation by full-length B-Myb [32]. Ubiquitin-conjugation and degradation of the B-Myb protein is mediated by the highly cell cycle-regulated Cdc34-$SCF^{p45Skp2}$ pathway [33], further indicating a complex, tight, and cell cycle-dependent regulation of both B-Myb abundance and activity.

Yet another level of regulation of Myb proteins can be exerted through interaction with a protein named BS69. BS69 was first identified as an interactor of the adenovirus type 5 13S E1A transforming oncogene and its binding was demonstrated to strongly inhibit E1A transactivation [34]. BS69 was subsequently shown to encode a strong repressor of transcription that can interact via a C-terminal MYND (*MY*eloid tumor gene 8 (MTG8), *N*ervy, *D*eaf-1 motif) zinc finger motif with the co-repressor N-CoR, a component of cellular complexes involved in histone deacetylation, chromatin re-modeling, and inhibition of transcription. This repression activity of BS69 could be inhibited by the adenovirus E1A [35]. A splice variant of BS69, designated BRAM1, was cloned as an interactor of the bone morphogenic receptor type 1a (BMPR1a) [36] and also binds both N-CoR and E1A. Recently, the c-Myb protein was shown to be a cellular target of BS69-mediated repression. BS69 interacts with the c-Myb negative-regulatory domain and mediates repression, which could be overcome by expression of the adenovirus E1A [37].

Surprisingly, in contrast to its c-*myb* and A-*myb* relatives [38,39], no direct involvement of B-*myb* in tumorigenesis has been reported. This is surprising, given the facts that B-*myb* expression patterns and activities in promoting cell cycle progression and its ability to override cell cycle arrests make such a role plausible. For instance, B-*myb* is highly expressed in many tumorigenic cell lines [5] and B-*myb* overexpression induces resistance to apoptosis in CTLL-2 cells [40]. Furthermore, B-*myb* expression can inhibit differentiation of monocytic cells [41] and retinoic acid-induced differentiation of neuroblastoma cells [42]. Finally, B-*myb* expression is a poor prognostic marker in neuroblastoma tumors [43].

In this study, we report that B-*myb* has transforming activity in collaboration with an activated *ras* oncogene. We show that transactivation of B-Myb is negatively regulated through interaction with the BS69 co-repressor. The relative contribution of B-Myb transactivation and repression in the oncogenic activity of B-Myb are investigated.

2. Results

2.1. Repression of B-myb transactivation by a BS69 splice variant

BS69 was recently reported to interact with the negative-regulatory domain of c-Myb, and thereby able to repress c-Myb transactivation [37]. B-*myb* is a more ubiquitously expressed family member that plays a critical role in cell cycle progression. We therefore set out to test whether BS69 could also affect B-Myb transactivation. Fig. 1A shows a schematic representation of murine B-Myb and the B-Myb mutants that have been used in this study. When co-

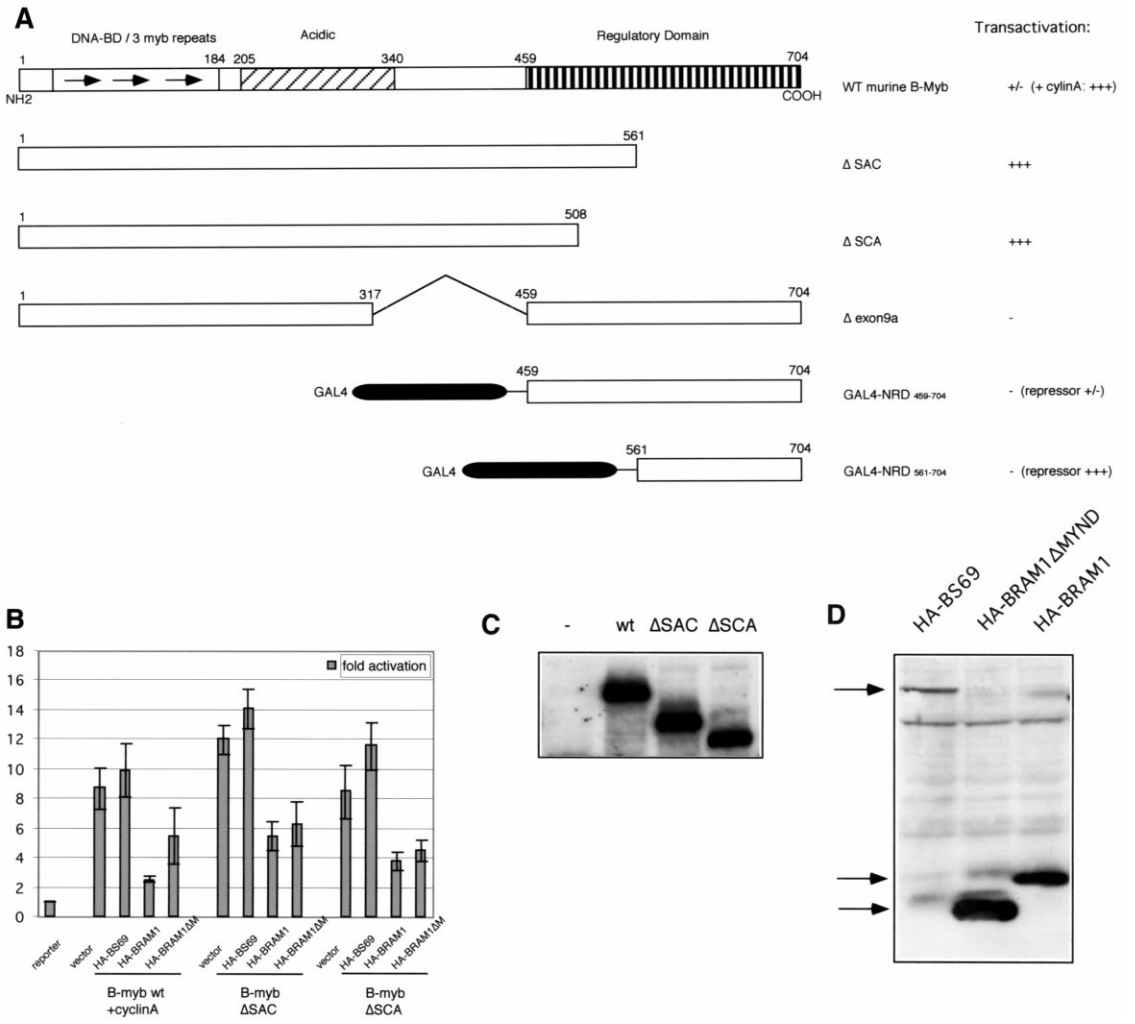


Fig. 1. Repression of B-*myb* transactivation by a BS69 splice variant. (A) Schematic representation of murine B-Myb protein and the mutants derived of it that were used in this study. (B) U2OS cells were transfected with the indicated plasmids: 3 μg luciferase reporter, 0.5 μg of CMV (cytomegalo virus, from which promoter is derived) β-gal, 2 μg pRcCMV-cyclin A, 3 μg of pcDNA3.1-HA-B-Myb, and 5 μg of BS69/BRAM1 constructs. BRAM1ΔM indicates the MYND domain deletion mutant of BRAM1. Fold activation of the 3 × A(mim)TK luciferase myb reporter was determined 2 days after transfection and was arbitrarily set at one. All luciferase activities were normalized to β-galactosidase internal control. The error bars indicate the standard deviation as determined from three independent (duplicate) experiments. Expression levels of B-Myb (weight + cyclin A) in the presence of BRAM1 (C) and BS69/BRAM1 levels in the presence of B-Myb weight + cyclin A (D) were analyzed on Western blot.

transfected with cyclin A, full-length B-Myb is able to activate an Myb-responsive reporter gene (Fig. 1B). Transactivation of B-Myb could be effectively repressed by co-expression of BRAM1, a splice variant of BS69, but not by a mutant of BRAM-1 that lacks the C-terminal MYND domain (BRAM1ΔM), which is required for interaction with the co-repressor N-CoR [35]. This effect of BRAM-1 was not due to effects of BRAM-1 on the CMV promoter that drives B-*myb* expression, as CMV-BRAM-1 and CMV-BRAM1ΔM were expressed at comparable levels (Fig. 1D). No significant repression

of B-Myb transactivation was seen by full-length BS69, probably because it is expressed at much lower levels than BRAM1 (Fig. 1D). As BS69 was reported to interact with the C-terminal negative-regulatory domain of c-Myb, we tested whether BS69 or BRAM1 would be able to repress transactivation of C-terminally truncated, and thereby hyper-activated, mutants of B-Myb. Fig. 1C shows that these truncated mutants were expressed to similar levels compared to wild-type murine B-Myb. As expected, these mutants are more potent in activating the *myb* reporter than wild-type B-Myb, even in the absence of cyclin A over-expression [27]. However, transactivation of these C-terminally truncated B-Myb proteins was inhibited less efficiently (two–three-fold) by BRAM1 as compared to wild-type B-Myb. We conclude that B-Myb transactivation is negatively regulated by BRAM1 and that the carboxyl-terminus of B-Myb contributes to efficient repression by BRAM1.

2.2. Direct interactions with B-myb and BS69/N-CoR

To address whether the effect of BS69 on B-Myb was the result of a direct physical interaction between these two proteins, we performed co-immunoprecipitation experiments. We used a GAL4 DNA Binding Domain fusion of BS69 in these experiments, because it is expressed at higher levels than HA-tagged BS69. Fig. 2A shows that in an immunoprecipitation for GAL4-BS69, using an anti-GAL4 antibody, B-Myb could be detected, whereas the truncated mutants ΔSAC and ΔSCA interacted less efficiently with BS69. A naturally occurring splice variant of B-*myb*, that lacks the exon 9a, but does contain an intact C-terminus, was at least as potent in interacting with BS69 as full-length B-Myb. The reverse experiment further substantiated the interaction of BS69 and B-Myb. In an immunoprecipitation using a polyclonal anti-serum raised against B-Myb, GAL4-BS69 could be detected (Fig. 2B). Again, a C-terminally truncated mutant of B-Myb interacted less avidly with BS69, whereas the Δ9a mutant again showed high affinity for BS69. We conclude from these experiments that B-Myb interacts with the repressor BS69. The carboxyl-terminus of B-myb, but not the transactivation domain encoded by exon 9a, contributes to efficient binding to BS69 and BRAM1, but it is not the sole determinant.

BS69 is reported to repress transcription in part via recruitment of the co-repressor N-CoR through its C-terminal MYND finger [35]. We therefore tested whether B-Myb could also be detected in the N-CoR immunoprecipitates. Fig. 2B shows that upon over-expression in U2OS cells, all B-Myb mutants tested were readily detectable in a FLAG-tagged N-CoR immunoprecipitation, indicating that negative regulation of B-Myb transactivation may involve N-CoR/HDAC-containing complexes. The observation that all B-Myb mutants interact with N-CoR with equal affinity, together with our finding that co-expression of BS69 or BRAM1 did not enhance binding (data not shown), suggests that B-Myb may also interact with N-CoR independently of BS69. Addition of cyclin A in the transfections did not alter the observed interactions of B-Myb with BS69, BRAM1, or N-CoR (data not shown).

2.3. The B-Myb C terminal-regulatory domain represses transcription

To further pinpoint the interaction of BS69 and BRAM1 with B-Myb and to clarify a potential role of the B-Myb C-terminal-regulatory domain in this interaction, we fused parts of the B-Myb C-terminus to the DNA binding domain (amino acids 1–147) of the yeast transcription factor GAL4 (Fig. 1A). GAL4-B-$Myb_{459–704}$ comprises the C-terminal domain as present in the B-Myb splice variant Δ9a. GAL4-B-$Myb_{561–704}$ consists of the sequences which are deleted in the transcriptionally highly active B-MybΔSAC mutant. To investigate whether the C-terminal-regulatory domain inhibits B-Myb transactivation, we tested whether these GAL4 fusion proteins could repress promoter activity when targeted to an active promoter harboring GAL4 DNA binding sites. Upon co-transfection with this GAL4-responsive luciferase reporter, which exhibits a basal level of activity in U2OS cells, GAL4-B-$Myb_{561–704}$ could potently inhibit the basal activity of this reporter in a dose-dependent fashion (Fig. 3A). The slightly longer fusion protein (amino acids 459–704 of B-Myb) was less able to inhibit transcription in this assay. This may indicate that the larger fusion protein also contains sequences involved in activation of transcription, or may be folded incorrectly. Differ-

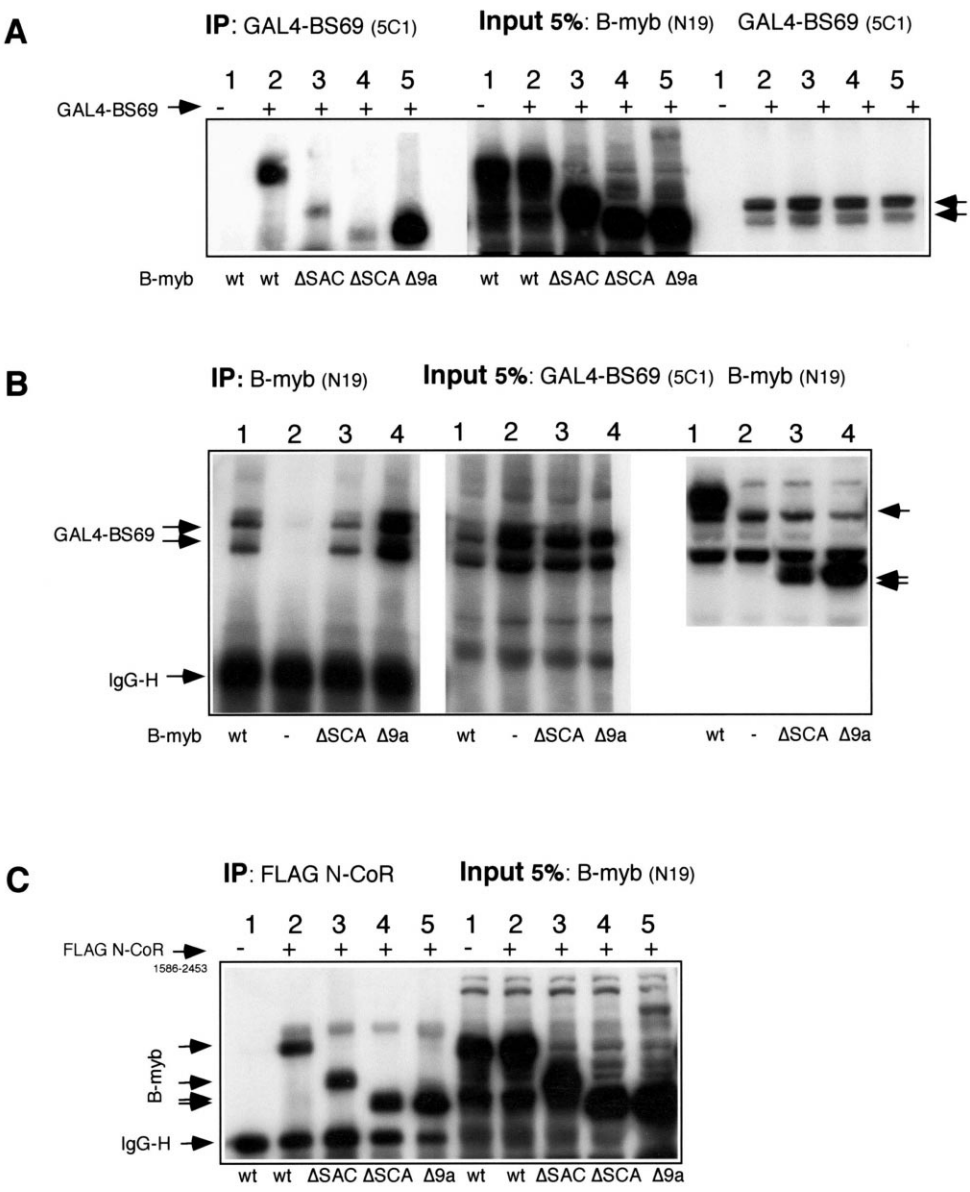


Fig. 2. Physical interaction of B-Myb with BS69 and N-CoR. (A) U2OS cells were transfected with 10 μg pRcCMV-GAL4-BS69 and 10 μg of pcDNA3.1-B-myb construct. Forty-eight hours post-transfection, cells were lysed and immunoprecipitation was carried out using the anti-GAL4 monoclonal 5C1. Immunopreciptated complexes were separated on 10% SDS-PAGE and probed with an anti-B-Myb polyclonal antiserum. Note co-immunoprecipitation of B-Myb in lanes 2–5 with GAL4-BS69, but not in the control without BS69 (lane 1). Input material, 5%, was also separated on gel and shows expression of the transfected Myb and BS69 constructs (right panels). (B) Transfections as in panel (A), here immunoprecipitated using the anti-B-Myb polyclonal anti-serum and the Western blot probed with an anti-GAL4 monoclonal antibody, showing co-precipitation of GAL4-BS69 with weight, ΔSCA, and Δ9a B-Myb, but not in the control without B-Myb expression (lane 2). Input material is shown in the middle and right panels. (C) U2OS cells were transfected with 10 μg pcDNA-2 × FLAG-NCoR (amino acids 1586–2453) and 10 μg pcDNA3.1 HA- B-*myb*. Immunoprecipitation was carried out using M2 anti-FLAG monoclonal antibodies. Lanes 2–5 show co-precipitation of all four B-Myb proteins with N-CoR, but not when N-CoR was omitted from the transfection (lane 1).

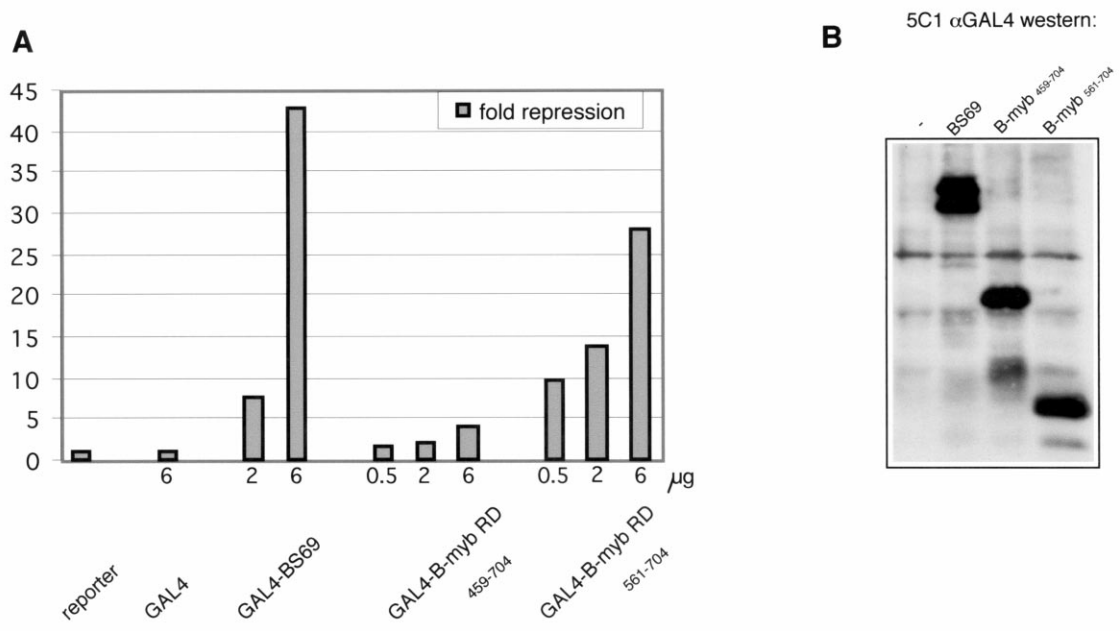


Fig. 3. The B-Myb C-terminal domain represses transcription. (A) U2OS cells were transfected with 3 μg of 5× Gal4-TK-luciferase reporter, 0.5 μg CMV β-gal and the indicated amount of GAL4-fusion proteins. Luciferase and β-gal activities were determined 2.5 days post-transfection and the fold repression compared to normalized reporter activity with GAL4 alone was calculated. (B) Expression levels of the GAL4-B-Myb-regulatory domain fusions and GAL4-BS69 on Western blot probed with the anti-GAL4 5C1 antibody, showing similar expression levels of GAL4 fusion proteins in U2OS cells.

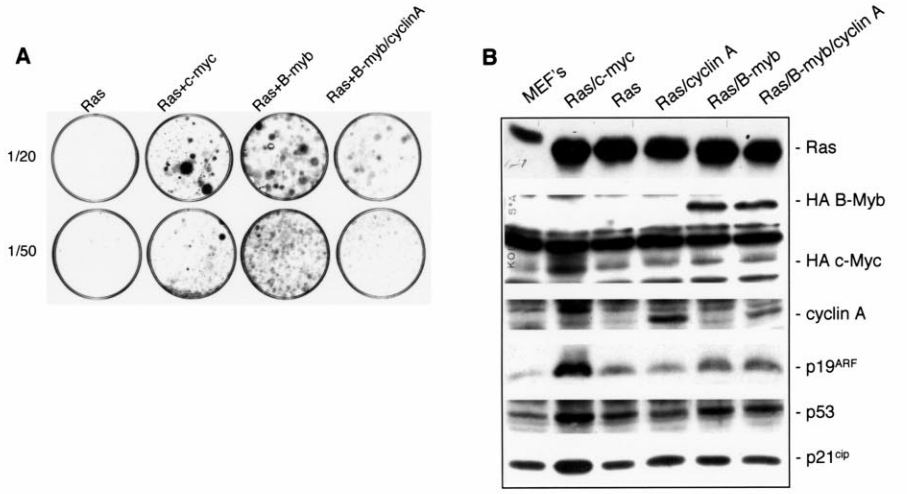
ences in expression levels could not account for the striking difference in repression activity (Fig. 3B). Attempts to detect interaction between these GAL4-regulatory domain fusions with either BS69, BRAM1, or N-CoR using immunoprecipitations of transfected proteins failed repeatedly. This suggests that the B-Myb C-terminal-regulatory domain is necessary for repression, but it is not sufficient for detection of stable interactions with repression complexes containing N-CoR and/or BS69.

2.4. B-myb can overcome ras^{V12}-induced premature senescence

By analogy to the effects of the adenovirus E1A on the cellular transcription factor E2F, which is released by E1A from pocket protein repression, E1A may also act to prevent binding of BS69 to cell cycle regulators such as B-Myb, thereby causing de-repression of its activity. However, we have never observed a significant cell cycle effect upon over-expression of BS69 or BRAM1 in any cell line tested. Since we show here that BS69 acts as a repressor of B-Myb transactivation, one possible explanation for this failure to detect cell cycle effects of BS69 would be that B-Myb is not rate-limiting for cell cycle progression in many cell types. We therefore attempted to generate a cell line that depends for its proliferation on B-Myb activity. Our strategy was to generate cells that are transformed as a result of B-*myb* expression.

Resistance of primary rodent cells to transformation by a *ras* oncogene is due, at least in part, to the induction of replicative senescence. This process requires induction of $p19^{ARF}$ and *p*53, as mutation of either of these genes allows escape from replicative senescence and causes oncogenic transformation [44]. Likewise, co-expression of c-*myc* or E1A allows escape from *ras*-induced senescence and these genes collaborate with *ras* in oncogenic transformation [45,46]. We therefore asked whether B-*myb* could also act to prevent *ras*-induced senescence.

To investigate this, low passage primary mouse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Ras
Ras+c-myc
Ras+B-myb
Ras+B-myb/cyclinA
1/20
1/50
B
MEF's
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Ras
Ras/cyclin A
Ras/B-myb
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- Ras
- HA B-Myb
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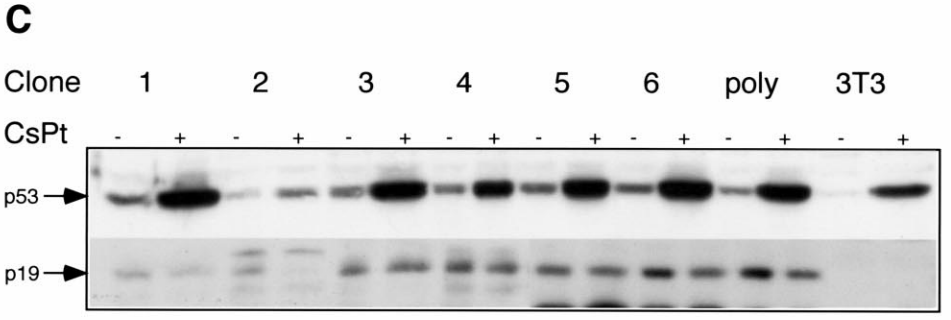
- cyclin A
- p19ARF
- p53
- p21cip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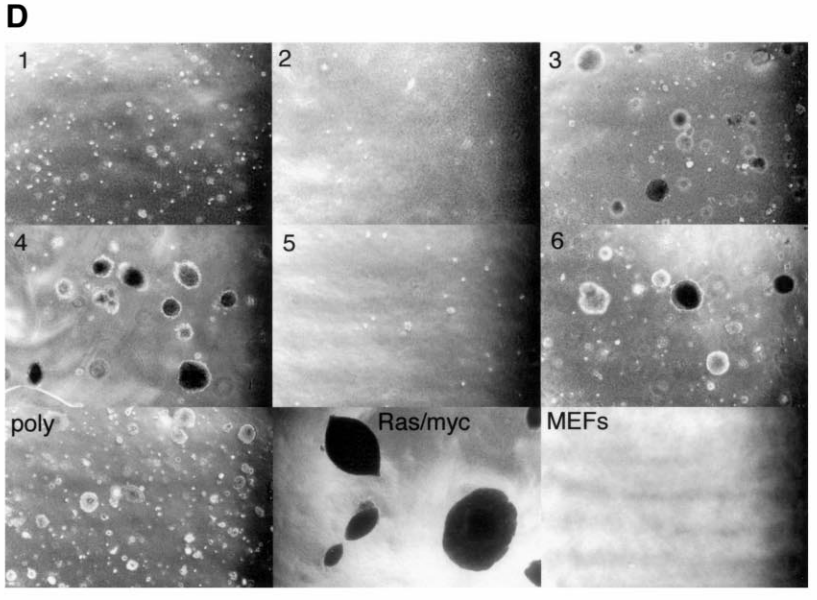
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Ras/myc
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embryonic fibroblasts (MEFs) were infected with retroviruses expressing the *ras*^{V12} oncogene and murine B-*myb*. We also asked whether co-expression of *cyclin A*, which is required to stimulate B-Myb transactivation, could stimulate a potential cooperation between B-*myb* and *ras* in this assay. As a positive control the c-*myc* oncogene was included. Fig. 4A shows clearly that over-expression of B-*myb* allowed cells to escape from *ras*^{V12}-induced senescence as a large number of slow-growing colonies appeared in the dishes. Co-expression of *cyclin A* did not stimulate, but rather decreased, the ability of B-*myb* to overcome *ras*-induced premature senescence in the MEFs. This may be due to the ability of cyclin A to induce apoptosis [47,48].

Western blot analysis was carried out on polyclonal pools of infected MEFs to confirm expression of the transduced genes (Fig. 4B). Furthermore, we wished to determine whether the *p19*^{ARF}-or *p53* genes had been inactivated in the B-*myb* plus *ras*-transformed cells, as loss of either of these two genes is known to allow escape from *ras*-induced senescence. Fig. 4B shows no dramatic increase in the p53 protein stability, which is indicative of mutational inactivation. Furthermore, we did not observe a loss of p21^{cip1} expression, another trait of cells with mutated p53. Finally, no loss of p19^{ARF} expression was seen in B-*myb* + *ras* transduced cells, indicating that also this gene is still intact. To further explore the characteristics of the B-*myb* + *ras*-transformed cells, we isolated independent single clones and tested those also for the presence of functional p53. To do this, we asked whether p53 protein was stabilized in these clones after induction of DNA damage by *cis*-platinum. Fig. 4C shows that all six B-*myb* + *ras* clones tested, although different in morphology and growth rate, responded with an increase in the p53 protein abundance after DNA damaging treatment, indicating that the *p53* gene was not mutated. Furthermore, all clones expressed p19^{ARF} (Fig. 4C), even though these cell lines were passaged for several weeks. To distinguish between immortalization and full transformation we analyzed anchorage-independent growth of the six clones. The soft agar assay showed a heterogeneous picture; only half of the clones were readily able to form colonies in soft agar, as was the polyclonal population, whereas other clones were clearly defective for growth in soft agar. We conclude that B-*myb* allows escape from *ras*^{V12}-induced replicative senescence in primary rodent fibroblasts, without a requirement for inactivation of the p19^{ARF}-p53 pathway. Additional genetic lesions or epigenetic events may subsequently lead to a more 'aggressive' phenotype and allow anchorage-independent growth, as observed for other oncogenes.

2.5. B-myb transactivation is involved in cooperation with *ras*^{V12}

To explore which functions of B-Myb contribute to the collaboration with an activated *ras* allele in transformation, we generated retroviruses expressing mutants of murine B-Myb that are transcriptionally activated (ΔSAC and ΔSCA) or transcriptionally inactive (B-MybΔ9a), and infected these together with a *ras*^{V12} retrovirus in the MEFs. The results, shown in Fig. 5A, indicate that both activated B-*myb* truncation mutants are still active in the *ras* co-transformation, but have a reduced activity compared to full-length B-*myb*. The transcriptionally inactive splice variant Δ9a is clearly unable to cooperate with *ras*^{V12} in transformation, strongly suggesting requirement for B-Myb transactivation in co-transformation with *ras*. Expression levels of the different B-

Fig. 4. B-*myb* overcomes Ras-induced senescence. (A) MEFs were infected twice in an 8 h interval with the indicated combinations of pBabepuro-Ras, pLZRS-zeo-myc, pLZRS-HA-B-myb, and pLZRS-cyclinA, and selected with 2 μg/ml of puromycin for 2 days. After selection the cells were re-plated at low concentrations and colonies were fixed and stained after 4 weeks. Compared to the *ras* + c-*myc* control, slightly more *ras* + B-*myb* colonies were observed but these were slow-growing and of lower cell density. (B) Western blot analysis of polyclonal pools of retrovirally transduced MEFs showing expression of the proteins encoded by the retroviral vectors and expression of components of the p19^{ARF}/p53/p21^{cip1} pathway, which is highly up-regulated in the c-*myc* expressing clone (lane 3), but not in B-*myb* expressing MEFs (lanes 5 and 6). (C) Western blots showing expression and stabilization of p53 after DNA damage in six *ras* + B-*myb* transduced monoclonal lines. p19^{ARF} expression is present in all clones except in the NIH-3T3 negative control. (D) Anchorage-independent growth in soft agar of *ras* + B-*myb* transduced and independent clones 1–6 and a polyclonal population *ras* + B-*myb* cells, compared to *ras* + c-*myc* transduced and wild-type MEFs.

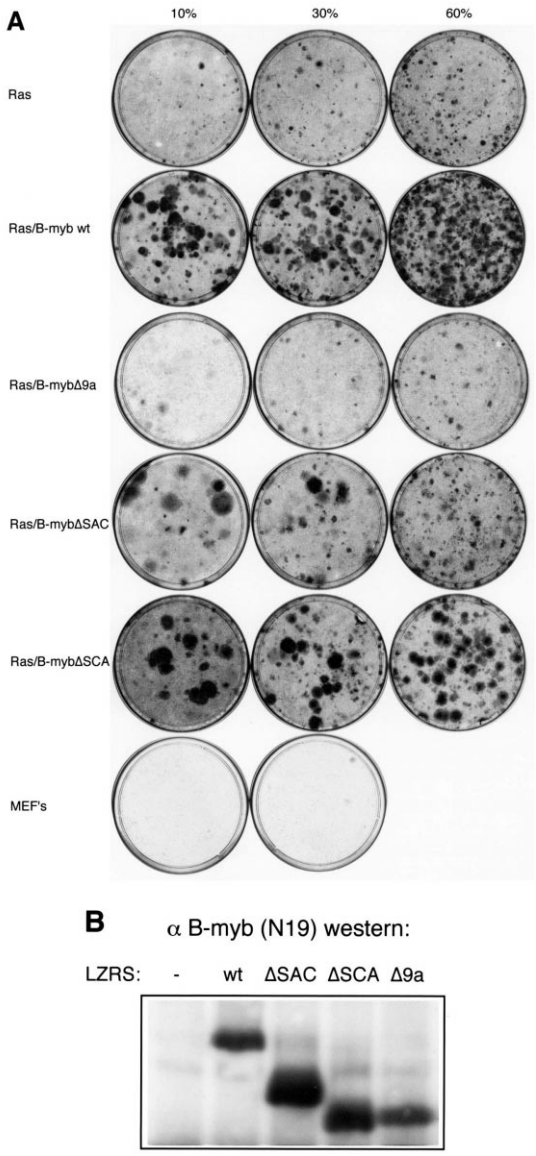

Fig. 5. B-Myb transactivation is required for cooperation with Ras^{V12}. (A) Subconfluent MEFs were infected twice with combinations of pBabepuro-Ras^{V12} and pLZRS-HA-B-myb and B-myb mutants as indicated, split 1:3 and selected for puromycin resistance (2 μg/ml) for 3 days, subsequently seeded in 10 cm dishes in three densities and grown for 25 days in the presence of 1 μg puromycin per ml. (B) Western blot probed with N19 anti-B-Myb antibody, showing comparable levels of retroviral B-myb expression in infected NIH-3T3 fibroblasts for LZRS-B-myb and B-mybΔ9a. The less potently transforming B-mybΔSAC and ΔSCA proteins were expressed to slightly higher levels.

myb mutants used were compared on western blot after infection of NIH-3T3 mouse fibroblasts. The activated B-Myb mutants were expressed to slightly higher levels than wild type B-Myb and the splice variant Δ9a, ruling out the possibility that differences in protein abundance or retroviral titers contributed to the observed effects (Fig. 5B). We conclude that B-Myb transformation potential depends on the presence of the exon 9a encoded transactivation domain and is decreased by truncation of the C-terminal repression domain.

2.6. Growth inhibition of B-Myb + Ras-transformed cells by modulators of B-Myb transactivation

Transactivation clearly contributes to B-Myb's ability to rescue cells from *ras*-induced senescence. We therefore asked whether over-expression of negative regulators of B-Myb transactivation, such as p107, BS69, or BRAM1 could inhibit the growth of B-*myb* + *ras*-transformed MEFs. Fig. 6A shows that transfection with a *BS*69 expression vector did not inhibit colony outgrowth in *ras* + B-*myb* cells. Stable expression of p107, another interactor and repressor of B-Myb transactivation [13,24] completely blocked colony outgrowth. However, *p107* is a potent inhibitor of proliferation in many cell lines [49] and is known to inhibit other critical cell cycle regulators, such as c-Myc and E2F [50,51]. Since *p107* also inhibited proliferation in *ras* + 13S *E1A*-transformed control MEFs and in *ras* + c-*myc*-transformed MEFs to some extent, we cannot rule out that *p*107 inhibits B-*myb* + *ras*-transformed cells in a B-*myb*-independent fashion.

To rule out the possibility that the low expression levels of BS69 would prevent detection of a clear effect on cell proliferation, we also tested the BRAM1 splice variant and a BRAM1 mutant lacking the MYND domain required for N-CoR interaction (Fig. 6B). We further tested whether activation of B-Myb by cyclin A would yield an increase in colony outgrowth. Compared to the GFP-Neo control, no significant effect on transformation was observed by any of the genes used, except for *p107*. We conclude that BS69 or BRAM1 over-expression does not interfere with the proliferation of B-*myb* + *ras*-transformed cells in vitro.

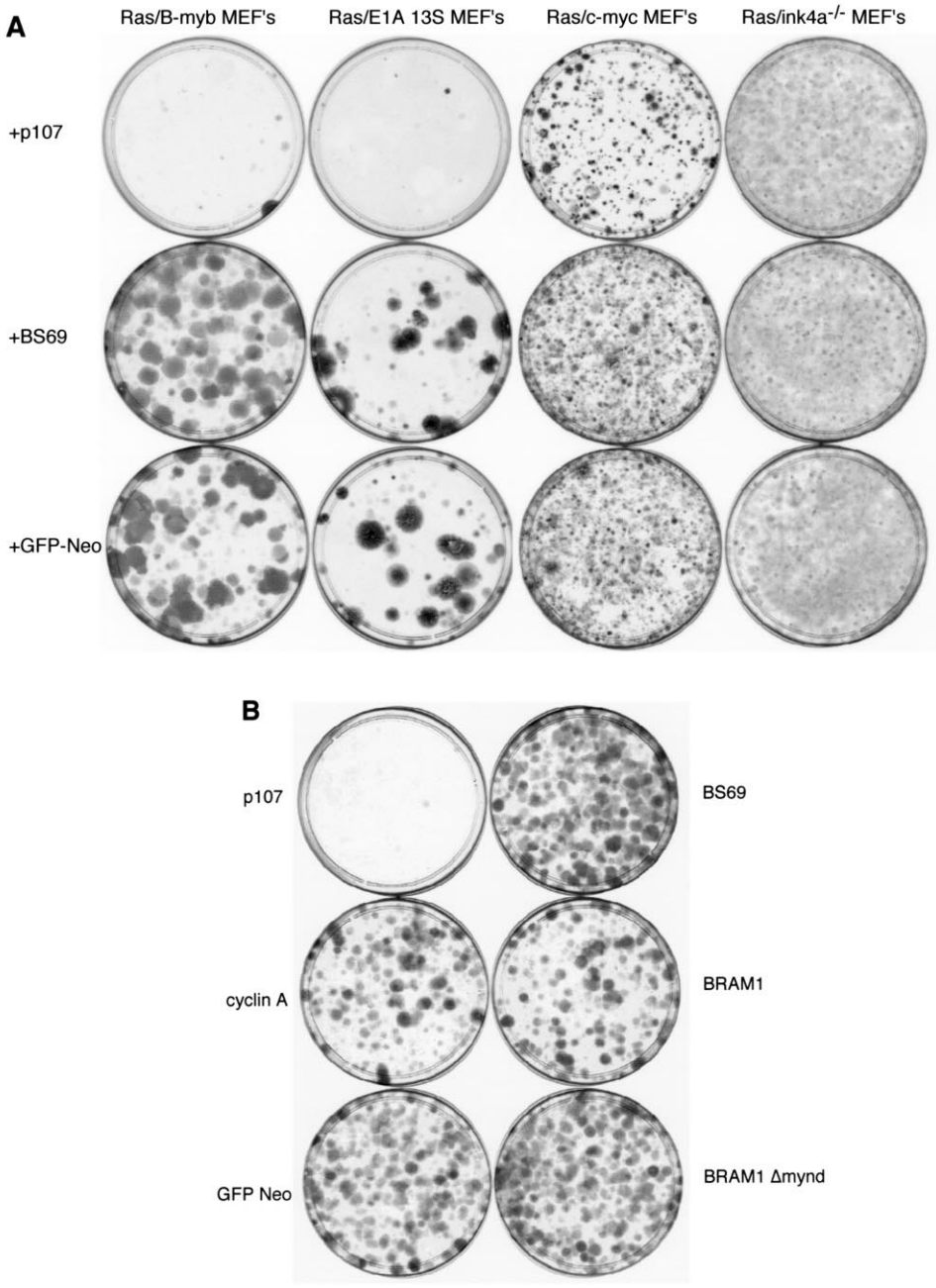

Fig. 6. Growth inhibition of *ras* + B-*myb*-transformed MEFs by modulators of B-*Myb* transactivation. (A) Polyclonal pools of transduced MEFs were plated at low density and transfected with 10 μg pRcCMV-HA-p107, pRcCMV-BS69-HA, and pRcCMV-GFP in the indicated combinations and selected for G418 (0.5 mg/ml) resistance for 2 weeks, followed by fixation and staining. Only *p107* inhibits colony outgrowth in both *ras* + B-*myb* and *ras* + *E1A* transduced MEFs. (B) A polyclonal pool of *ras* + B-*myb* transduced MEFs were seeded and transfected and G418 selected as in (A), colony outgrowth shows a clear inhibition by *p107*, but not by other genes tested.

3. Discussion

In this report, we show that B-*myb* can rescue premature senescence induced by an activated *ras* oncogene in primary rodent fibroblasts, a property that is shared by a number of established oncogenes such as c-*myc* and adenovirus E1A. As a transactivation-defective mutant of B-Myb was defective in the *ras* co-transformation, this activity of the B-Myb protein appears to involve its transcription activation function. However, we also found that C-terminal deletion mutants of B-Myb, which are constitutively active in transactivation, have a decreased activity in *ras* co-transformation assays, indicating a positive role for the B-Myb C-terminus in transformation as well. This is in contrast to c-*myb* transformation, which is clearly enhanced by truncation of the C-terminus, as observed in the highly related viral oncogene v-*myb*. Furthermore, the C-terminus of the c-*myb* proto-oncogene is found inactivated by retroviral insertion mutagenesis in the avian lymphoid and murine myeloid malignancies [38,52] and a truncation in human leukemia was recently observed [53]. Finally, full-length c-Myb is less efficient in inducing colony formation in primary hematopoietic cells than the C-terminally truncated c-Myb [38].

In contrast to c-Myb, our data indicate that inactivation of the carboxyl-terminus of B-Myb does not enhance co-transformation with *ras*. This may indicate that the C-terminus of B-Myb has other activities than only acting to restrain the activity of the transactivation domain and suggests that this regulatory domain can play a positive role in growth regulation by B-Myb. Although, the B-Myb and c-Myb carboxyl termini share a function in negative regulation of their transactivation domains, the sequence conservation is limited to a small region [5]. Furthermore, activation by cyclin A-dependent phosphorylation of the C-terminus is not reported for c-Myb.

Our present data indicate that, apart from being able to stimulate transcription, B-Myb also has transcription repression activity, which is, at least in part, localized in the carboxyl-terminal domain. As this domain is involved in efficient transformation by B-*myb*, our data indicate that both transactivation and repression by B-Myb contribute to efficient collaboration with *ras* in transformation of MEFs, even though the former appears to be more critically required than the latter. A dual activation/repression activity has also been seen in other oncogenic transcription factors. For example, c-Myc harbors N-terminal transactivation and repression domains, both of which are required for oncogenesis, reviewed in [54,55]. Furthermore, repression by E2F may contribute to its oncogenic action, as repression-defective mutants of this protein can confer resistance to anti-proliferative signals [56].

Modulation of B-Myb transactivation by the C-terminus is an important aspect of the many mechanisms that operate to regulate the B-Myb activity. Additional levels of B-Myb regulation take place at the transcriptional level, via E2F transcription factors, by post-translational modifications by cyclinA/cdk2, and by protein degradation via the ubiquitin pathway and Cdc34-SCF (Skp Cullin F-box protein complex) complex. How the Myb transactivation domain negatively regulated by the C-terminus remains unknown. A direct interaction of the C-terminus and DNA binding domain has been observed in c-Myb [57,58]. However, the C-terminus can also inhibit transactivation of the central activation domain in heterologous DNA binding domain fusions, implying a functional interaction with the transactivation domain and C-terminal negative-regulatory domain in c-Myb [59,60]. The recent identification of BS69 as a negative regulator of transcription that binds to the negative-regulatory domain of c-Myb indicates that the C-terminus may play an active role in repressing the transactivation of the Myb proteins. Our present data indicate that B-Myb is also repressed by BS69 and interacts with both BS69 and the BS69 interacting co-repressor N-CoR. Taken together with our observation that the C-terminus of B-Myb can potently inhibit transcription, this may indicate that regulation of Myb transactivation through repression by its C-terminus is an active process that may involve interaction with N-CoR/HDAC-containing complexes, rather than passive shielding of the central transactivation domain. Interestingly, a number of c-Myb target genes are repressed upon over-expression of c-*myb* [61–64], indicating that c-Myb can also act as a negative regulator of transcription in the context of certain promoters.

Earlier studies suggested that the B-Myb C-terminus enhanced transactivation when fused to the c-Myb DNA binding and transactivation domain [65]. The domain swaps used in this study include B-Myb

sequences from amino acids 260 to 704, which includes activating sequences such as the acidic region and the domain encoded by exon 9a, which is shown to be essential for B-Myb transactivation [30].

We have not seen a direct interaction between the B-Myb C-terminal-regulatory domain and BS69. However, we did observe a strongly decreased affinity of the C-terminally truncated B-Myb mutants for BS69, suggesting that the C-terminus of B-Myb is necessary, but not sufficient, for interaction with co-repressor molecules. The interaction of B-Myb and N-CoR was equally strong for all mutants tested and may therefore be direct and independent of BS69. It is not uncommon for large N-CoR-containing repressor complexes to have both direct and indirect interaction domains with their targets, which may also explain our inability to pinpoint the exact binding sites on B-Myb for N-CoR and BS69.

We show that mouse embryo fibroblasts transformed by expression of *ras* and B-*myb* form colonies that are slow-growing, have a low cell density, and are heterogeneous in morphology and have a variable ability to grow in soft agar. Expression levels of *ras* and B-*myb* were similar in morphologically distinct clones and could therefore not account for the observed differences (data not shown). We propose that B-*myb* expression is a first event that allows the cells to bypass the *ras* senescence checkpoint and that further passaging allows cells to acquire more genetic or epigenetic alterations that allow faster proliferation and anchorage-independent growth, as is also observed for many established oncogenes. In agreement with this, continuous passaging led to changes in morphology and increased growth rates of these clonal cell lines (data not shown). Co-expression of the BS69/BRAM1 negative modulator of B-Myb transactivation does not have a significant effect on the proliferation of B-*myb*-transformed MEFs. This may be caused by the fact that BS69 does not completely inhibit B-Myb transactivation, leaving the cells with enough expression of B-Myb downstream target genes to divide. Alternatively, other genetic and/or epigenetic events that the cells may have acquired after rescue of *ras*-induced senescence by B-*myb* may explain the lack of inhibitory activity.

An important question is the potential role of B-*myb* in human cancer. B-*myb* expression patterns and activities in promoting cell cycle progression and override of cell cycle arrests make such a role plausible. A number of publications have indeed suggested that B-*myb* might play a role in the onset and promotion of tumor formation [41–43,66]. Potent viral oncogenes, such as HPV *E7* [67] and adenovirus *E1A*, can target B-*myb* activation at several levels. Firstly, at the transcriptional level through de-regulation of pocket proteins and release of E2F activity. Secondly, at the regulatory level through induction of cyclinA/cdk2 activity, which enhances the B-Myb transactivation. Finally, through sequestration of the inhibitors of Myb transactivation, BS69 and p107. Another issue that remains unanswered at present concerns the cellular targets of B-Myb that are responsible for the observed oncogenic activity of B-*myb*. The reagents and cell lines described here, combined with the use of DNA microarray technology, may provide new insights in this matter.

4. Materials and methods

4.1. Plasmids and cloning

Plasmids encoding BS69 and derived mutants, FLAG-N-CoR, 5× TK-luc and CMV β-gal have been described [35]. pcDNA3-HA-B-myb was obtained from Roger Watson, HA-B-mybΔSAC and ΔSCA were derived by polymerase chain reaction (PCR), B-myb Δ9a by splicing, overlapping PCR fragments and cloned BamHI-NotI into pcDNA3.1 and pLZRS. pLZRS-cyclin A was cloned using the EcoRI-NotI fragment from pCMV-cyclinA. pBabe-puro ras^{V12} was obtained from Maarten van Lohuizen and LZRS zeo-c-*myc* from Thijn Brummelkamp at NKI.

4.2. Retroviral infections

Phoenix cells were transfected via the standard calcium phosphate precipitation method and helper-free retrovirus was obtained from transfected 10 cm dishes 36, 46 and 60 h after transfection, and stored at −80°C. Low-passage MEFs (FVB strain) were infected twice with retrovirus with an interval of 8 h. Twenty-four hours after infection, the cells were split 1:3 and selected in 2 μg puromycin per ml for 2–3 days. After this, cells were re-plated in several dilutions in medium containing 1 μg of puro-

mycin per ml After colony formation (3–4 weeks), cells were fixed in methanol/acetone and stained with coomassie brilliant blue.

4.3. Cell culture and transfections

U2OS, NIH-3T3, and Phoenix cells were obtained from ATCC (American Type Culture Collection) and maintained in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FCS. MEFs were isolated from 12 to 14 day old FVB embryos and maintained in DMEM supplemented with 10% NCS. Soft agar assays were done in normal medium containing 0.5% agarose, plating cells at 10 000, 30 000, and 100 000 per well in duplicate in a six-well plate with a bottom layer of 1% agarose, colonies were photographed and counted after 2 weeks. Transfections were done using standard calcium phosphate/HBS (Hepes Buffered Saline) precipitation method. Luciferase and β-galactosidase assays were performed as described [35].

4.4. Antibodies, immunoprecipitations and Western blotting

Anti-HA (12CA5) and anti-GAL4 DBD (5C1) mouse monoclonals were obtained from tissue culture supernatant, anti-cyclin A (BF683), anti-p53 rabbit polyclonal (FL393), anti-$p21^{CIP}$ mouse monoclonal (F5) from Santa Cruz, anti-FLAG M2 mouse monoclonal from Sigma, anti-$p19^{ARF}$ (R562) from Abcam, and anti-Ras (R02120) from Transduction Laboratories. Immunoprecipitations and Western blotting were performed in ELB buffer as described [35].

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References

[1] T.J. Gonda, C. Buckmaster, R.G. Ramsay, Activation of c-myb by carboxy-terminal truncation: relationship to transformation of murine haemopoietic cells in vitro, EMBO J. 8 (1989) 1777–1783.

[2] M.R. Kanter, R.E. Smith, W.S. Hayward, Rapid induction of B-cell lymphomas: insertional activation of c-myb by avian leukosis virus, J. Virol. 62 (1988) 1423–1432.

[3] E. Pizer, E.H. Humphries, RAV-1 insertional mutagenesis: disruption of the c-myb locus and development of avian B-cell lymphomas, J. Virol. 63 (1989) 1630–1640.

[4] Y. Weinstein, J.L. Cleveland, D.S. Askew, U.R. Rapp, J.N. Ihle, Insertion and truncation of c-myb by murine leukemia virus in a myeloid cell line derived from cultures of normal hematopoietic cells, J. Virol. 61 (1987) 2339–2343.

[5] N. Nomura, M. Takahashi, M. Matsui, S. Ishii, T. Date, S. Sasamoto, R. Ishizaki, Isolation of human cDNA clones of myb-related genes, A-myb and B-myb, Nucleic Acids Res. 16 (1988) 11075–11089.

[6] E.H. Westin, R.C. Gallo, S.K. Arya, A. Eva, L.M. Souza, M.A. Baluda, S.A. Aaronson, F. Wong-Staal, Differential expression of the amv gene in human hematopoietic cells, Proc. Natl Acad. Sci. USA 79 (1982) 2194–2198.

[7] E.H. Westin, F. Wong-Staal, E.P. Gelmann, R. Dalla-Favera, T.S. Papas, J.A. Lautenberger, A. Eva, E.P. Reddy, S.R. Tronick, S.A. Aaronson, R.C. Gallo, Expression of cellular homologues of retroviral onc genes in human hematopoietic cells, Proc. Natl Acad. Sci. USA 79 (1982) 2490–2494.

[8] A. Toscani, R.V. Mettus, R. Coupland, H. Simpkins, J. Litvin, J. Orth, K.S. Hatton, E.P. Reddy, Arrest of spermatogenesis and defective breast development in mice lacking A-myb, Nature 386 (1997) 713–717.

[9] R.V. Mettus, J. Litvin, A. Wali, A. Toscani, K. Latham, K. Hatton, E.P. Reddy, Murine A-myb: evidence for differential splicing and tissue-specific expression, Oncogene 9 (1994) 3077–3086.

[10] I.H. Oh, E.P. Reddy, The myb gene family in cell growth, differentiation and apoptosis, Oncogene 18 (1999) 3017–3033.

[11] E.W. Lam, C. Robinson, R.J. Watson, Characterization and cell cycle-regulated expression of mouse B-myb, Oncogene 7 (1992) 1885–1890.

[12] J. Sitzmann, K. Noben-Trauth, H. Kamano, K.H. Klempnauer, Expression of B-Myb during mouse embryogenesis, Oncogene 12 (1996) 1889–1894.

[13] A. Sala, I. Casella, T. Bellon, B. Calabretta, R.J. Watson, C. Peschle, B-myb promotes S phase and is a downstream target of the negative regulator p107 in human cells, J. Biol. Chem. 271 (1996) 9363–9367.

[14] J. Zwicker, N. Liu, K. Engeland, F.C. Lucibello, R. Muller, Cell cycle regulation of E2F site occupation in vivo, Science 271 (1996) 1595–1597.

[15] J.D. Bennett, P.G. Farlie, R.J. Watson, E2F binding is required but not sufficient for repression of B-myb transcription in quiescent fibroblasts, Oncogene 13 (1996) 1073–1082.

[16] D. Lin, M. Fiscella, P.M. O'Connor, J. Jackman, M. Chen, L.L. Luo, A. Sala, S. Travali, E. Appella, W.E. Mercer, Constitutive expression of B-myb can bypass p53-induced Waf1/Cip1-mediated G1 arrest, Proc. Natl Acad. Sci. USA 91 (1994) 10079–10083.

[17] A. Sala, A. De Luca, A. Giordano, C. Peschle, The retinoblas-

toma family member p107 binds to B-MYB and suppresses its autoregulatory activity, J. Biol. Chem. 271 (1996) 28738–28740.
[18] A. Sala, B. Calabretta, Regulation of BALB/c 3T3 fibroblast proliferation by B-myb is accompanied by selective activation of cdc2 and cyclin D1 expression, Proc. Natl Acad. Sci. USA. 89 (1992) 10415–10419.
[19] Y. Tanaka, N.P. Patestos, T. Maekawa, S. Ishii, B-myb is required for inner cell mass formation at an early stage of development, J. Biol. Chem. 274 (1999) 28067–28070.
[20] G. Mizuguchi, H. Nakagoshi, T. Nagase, N. Nomura, T. Date, Y. Ueno, S. Ishii, DNA binding activity and transcriptional activator function of the human B-myb protein compared with c-MYB, J Biol. Chem. 265 (1990) 9280–9284.
[21] H. Nakagoshi, C. Kanei-Ishii, T. Sawazaki, G. Mizuguchi, S. Ishii, Transcriptional activation of the c-myc gene by the c-myb and B-myb gene products, Oncogene 7 (1992) 1233–1240.
[22] S.A. Ness, A. Marknell, T. Graf, The v-myb oncogene product binds to and activates the promyelocyte-specific mim-1 gene, Cell 59 (1989) 1115–1125.
[23] H. Nakagoshi, Y. Takemoto, S. Ishii, Functional domains of the human B-myb gene product, J. Biol. Chem. 268 (1993) 14161–14167.
[24] A. Sala, B. Saitta, P. De Luca, M.N. Cervellera, I. Casella, R.E. Lewis, R. Watson, C. Peschle, B-MYB transactivates its own promoter through SPI-binding sites, Oncogene 18 (1999) 1333–1339.
[25] Y. Furukawa, H. Piwnica-Worms, T.J. Ernst, Y. Kanakura, J.D. Griffin, cdc2 gene expression at the G1 to S transition in human T lymphocytes, Science 250 (1990) 805–808.
[26] D. Venturelli, S. Travali, B. Calabretta, Inhibition of T-cell proliferation by a MYB antisense oligomer is accompanied by selective down-regulation of DNA polymerase alpha expression, Proc. Natl Acad. Sci. USA 87 (1990) 5963–5967.
[27] S. Lane, P. Farlie, R. Watson, B-Myb function can be markedly enhanced by cyclin A-dependent kinase and protein truncation, Oncogene 14 (1997) 2445–2453.
[28] A. Sala, M. Kundu, I. Casella, A. Engelhard, B. Calabretta, L. Grasso, M.G. Paggi, A. Giordano, R.J. Watson, K. Khalili, C. Peschle, Activation of human B-MYB by cyclins, Proc. Natl Acad. Sci. USA 94 (1997) 532–536.
[29] G. De Falco, L. Bagella, P.P. Claudio, A. De Luca, Y. Fu, B. Calabretta, A. Sala, A. Giordano, Physical interaction between CDK9 and B-Myb results in suppression of B-MYB by gene regulation, Oncogene 19 (2000) 373–379.
[30] S. Horstmann, S. Ferrari, K.H. Klempnauer, Regulation of B-Myb activity by cyclin D1, Oncogene 19 (2000) 298–306.
[31 H. Kamano, B. Burk, K. Noben-Trauth, K.H. Klempnauer, Differential splicing of the mouse B-myb gene, Oncogene 11 (1995) 2575–2582.
[32] S. Horstmann, S. Ferrari, K.H. Klempnauer, An alternatively spliced isoform of B-Myb is a transcriptional inhibitor, Oncogene 19 (2000) 5428–5434.
[33] S. Charrasse, I. Carena, V. Brondani, K.H. Klempnauer, S. Ferrari, Degradation of B-Myb by ubiquitin-mediated proteolysis: involvement of the Cdc34-SCF(p45Skp2) pathway, Oncogene 19 (2000) 2986–2995.
[34] G. Hateboer, A. Gennissen, Y.F. Ramos, R.M. Kerkhoven, V. Sonntag-Buck, H.G. Stunnenberg, R. Bernards, BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation, EMBO J. 14 (1995) 3159–3169.
[35] H. Masselink, R. Bernards, The adenovirus E1A binding protein BS69 is a corepressor of transcription through recruitment of N-CoR, Oncogene 19 (2000) 1538–1546.
[36] K. Kurozumi, M. Nishita, K. Yamaguchi, T. Fujita, N. Ueno, H. Shibuya, BRAM1, a BMP receptor-associated molecule involved in BMP signalling, Genes Cells 3 (1998) 257–264.
[37] N.E. Ladendorff, S. Wu, J.S. Lipsick, BS69, an adenovirus E1A-associated protein, inhibits the transcriptional activity of c-Myb, Oncogene 20 (2001) 125–132.
[38] P. Ferrao, E.M. Macmillan, L.K. Ashman, T.J. Gonda, Enforced expression of full length c-Myb leads to density-dependent transformation of murine haemopoietic cells, Oncogene 11 (1995) 1631–1638.
[39] S.E. DeRocco, R. Iozzo, X.P. Ma, R. Schwarting, D. Peterson, B. Calabretta, Ectopic expression of A-myb in transgenic mice causes follicular hyperplasia and enhanced B lymphocyte proliferation, Proc. Natl Acad. Sci. USA 94 (1997) 3240–3244.
[40] E. Grassilli, P. Salomoni, D. Perrotti, C. Franceschi, B. Calabretta, Resistance to apoptosis in CTLL-2 cells overexpressing B-Myb is associated with B-Myb-dependent bcl-2 induction, Cancer Res. 59 (1999) 2451–2456.
[41] J. Bies, B. Hoffman, A. Amanullah, T. Giese, L. Wolff, B-Myb prevents growth arrest associated with terminal differentiation of monocytic cells, Oncogene 12 (1996) 355–363.
[42] G. Raschella, A. Negroni, S. Pucci, R. Amendola, S. Valeri, B. Calabretta, B-myb transcriptional regulation and mRNA stability during differentiation of neuroblastoma cells, Exp. Cell Res. 222 (1996) 395–399.
[43] G. Raschella, V. Cesi, R. Amendola, A. Negroni, B. Tanno, P. Altavista, G.P. Tonini, B. De Bernardi, B. Calabretta, Expression of B-myb in neuroblastoma tumors is a poor prognostic factor independent from MYCN amplification, Cancer Res. 59 (1999) 3365–3368.
[44] M. Serrano, A.W. Lin, M.E. McCurrach, D. Beach, S.W. Lowe, Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a, Cell 88 (1997) 593–602.
[45] H. Land, L.F. Parada, R.A. Weinberg, Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes, Nature 304 (1983) 596–602.
[46] H.E. Ruley, Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture, Nature 304 (1983) 602–606.
[47] A.T. Hoang, K.J. Cohen, J.F. Barrett, D.A. Bergstrom, C.V. Dang, Participation of cyclin A in Myc-induced apoptosis, Proc. Natl Acad. Sci. USA 91 (1994) 6875–6879.
[48] W. Meikrantz, S. Gisselbrecht, S.W. Tam, R. Schlegel, Activation of cyclin A-dependent protein kinases during apoptosis, Proc. Natl Acad. Sci. USA 91 (1994) 3754–3758.
[49] L. Zhu, S. van den Heuvel, K. Helin, A. Fattaey, M. Ewen, D. Livingston, N. Dyson, E. Harlow, Inhibition of cell prolifera-

tion by p107, a relative of the retinoblastoma protein, Genes Dev. 7 (1993) 1111–1125.
[50] D. Ginsberg, G. Vairo, T. Chittenden, Z.X. Xiao, G. Xu, K.L. Wydner, J.A. DeCaprio, J.B. Lawrence, D.M. Livingston, E2F-4, a new member of the E2F transcription factor family, interacts with p107, Genes Dev. 8 (1994) 2665–2679.
[51] R.L. Beijersbergen, E.M. Hijmans, L. Zhu, R. Bernards, Interaction of c-Myc with the pRb-related protein p107 results in inhibition of c-Myc-mediated transactivation, EMBO J. 13 (1994) 4080–4086.
[52] G.L. Shen-Ong, M. Potter, J.F. Mushinski, S. Lavu, E.P. Reddy, Activation of the c-myb locus by viral insertional mutagenesis in plasmacytoid lymphosarcomas, Science 226 (1984) 1077–1080.
[53] A. Tomita, M. Towatari, S. Tsuzuki, F. Hayakawa, H. Kosugi, K. Tamai, T. Miyazaki, T. Kinoshita, H. Saito, c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300, Oncogene 19 (2000) 444–451.
[54] L.M. Facchini, L.Z. Penn, The molecular role of Myc in growth and transformation: recent discoveries lead to new insights, Faseb J. 12 (1998) 633–651.
[55] G.F. Claassen, S.R. Hann, Myc-mediated transformation: the repression connection, Oncogene 18 (1999) 2925–2933.
[56] H.S. Zhang, A.A. Postigo, D.C. Dean, Active transcriptional repression by the Rb-E2F complex mediates G1 arrest triggered by p16INK4a, TGFbeta, and contact inhibition, Cell 97 (1999) 53–61.
[57] A.B. Dash, F.C. Orrico, S.A. Ness, The EVES motif mediates both intermolecular and intramolecular regulation of c-Myb, Genes Dev. 10 (1996) 1858–1869.
[58] A. Kiewitz, H. Wolfes, Mapping of protein-protein interactions between c-myb and its coactivator CBP by a new phage display technique, FEBS Lett. 415 (1997) 258–262.
[59] F. Kalkbrenner, S. Guehmann, K. Moelling, Transcriptional activation by human c-myb and v-myb genes, Oncogene 5 (1990) 657–661.
[60] J.W. Dubendorff, L.J. Whittaker, J.T. Eltman, J.S. Lipsick, Carboxy-terminal elements of c-Myb negatively regulate transcriptional activation in cis and in trans, Genes Dev. 6 (1992) 2524–2535.
[61] B. Ganter, J.S. Lipsick, Myb binding sites within the N-ras promoter repress transcription, Oncogene 15 (1997) 193–202.
[62] G. Mizuguchi, C. Kanei-Ishii, T. Takahashi, T. Yasukawa, T. Nagase, M. Horikoshi, T. Yamamoto, S. Ishii, c-Myb repression of c-erbB-2 transcription by direct binding to the c-erbB-2 promoter, J. Biol. Chem. 270 (1995) 9384–9389.
[63] H. Nakagoshi, T. Nagase, Y. Ueno, S. Ishii, Transcriptional trans-repression by the c-myb proto-oncogene product, Nucleic Acids Res. 17 (1989) 7315–7324.
[64] G.R. Vandenbark, Y. Chen, E. Friday, K. Pavlik, B. Anthony, C. deCastro, R.E. Kaufman, Complex regulation of human c-kit transcription by promoter repressors, activators, and specific myb elements, Cell Growth Differ. 7 (1996) 1383–1392.
[65] I.H. Oh, E.P. Reddy, The C-terminal domain of B-Myb acts as a positive regulator of transcription and modulates its biological functions, Mol Cell Biol. 18 (1998) 499–511.
[66] J. Bies, L. Wolff, Acceleration of apoptosis in transforming growth factor beta 1-treated M1 cells ectopically expressing B-myb, Cancer Res. 55 (1995) 501–504.
[67] E.W. Lam, J.D. Morris, R. Davies, T. Crook, R.J. Watson, K.H. Vousden, HPV16 E7 oncoprotein deregulates B-myb expression: correlation with targeting of p107/E2F complexes, EMBO J. 13 (1994) 871–878.