The conserved Mynd domain of BS69 binds cellular and oncoviral proteins through a common PxLxP motif

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Running Title: BS69 interacts with a common motif in E1A,EBNA2 and MGA
Abstract

BS69 is a transcriptional co-repressor protein and a potential tumor suppressor that binds to the adenoviral oncoprotein E1A. We show that the C-terminal Mynd domain of BS69 or the closely related Mynd domains of the C. elegans proteins Bra-1 and Bra-2 bind not only to E1A but also to the Epstein-Barr virus EBNA2 oncoprotein and the Myc-related cellular protein MGA. Interaction depends on intact PxLxP motifs present in all three proteins. Moreover, viral proteins compete for binding of BS69 to MGA in a PxLxP dependent fashion. Since deletions in E1A or EBNA2 that cover the PxLxP motifs are non-transforming, our observations suggest a role of BS69 in cell growth control and are reminiscent to abrogation of Rb function by various oncoproteins.
Introduction

Oncoviral proteins of transforming DNA tumor viruses have evolved to induce viral replication in resting host cells. They do so by selective interaction with a restricted number of cellular proteins that govern the cell cycle, differentiation, or cell survival. The same cellular proteins are often mutated in various cancers demonstrating their importance in cellular growth control.

An example of such a viral oncoprotein that interferes with the function of critical growth regulators is the Epstein-Barr nuclear antigen EBNA2. EBNA2 interferes with transcriptional regulation and is required for B-cell immortalization by the Epstein-Barr virus. Association of EBNA2 with cellular transcription factors, such as CBF1/RBP-Jκ or PU-1, activates cellular and viral target genes by EBNA2-mediated recruitment of the pre-initiation complex, histone acetyltransferases, as well as the SWI/SNF remodeling complex (1,2).

Another eminent example and an invaluable tool to unravel proliferation and differentiation control is the adenoviral E1A oncoprotein. E1A is expressed early in the viral replication cycle and interferes with the function of multiple cellular proteins involved in gene regulation. E1A can immortalize rodent fibroblasts and transforms them in collaboration with E1B or with activated versions of ras (3). Comparison of E1A proteins from various viral serotypes highlights three conserved regions, named CR1, CR2 and CR3, that bind to and modulate the activities of cellular proteins implicated in cell cycle progression (the retinoblastoma protein, Rb), in chromatin remodeling (Swi/Snf complex, histone acetyltransferases) and in gene transcription (TFIID, mediator complex, various transcription factors) (3,4).

BS69 was originally identified as a nuclear protein that binds to E1A and inhibits its trans-activation potential (5). An alternatively spliced BS69 isoform, termed BRAM1, encompasses the C-terminus of BS69. BRAM1, as well as its C.elegans orthologs Bra-1
and Bra-2, were proposed to participate in BMP and TGFβ signaling pathways (6,7). The BS69 C-terminus is related to a cysteine-rich structure that has been termed Mynd domain. The Mynd domain is present in the AML1 chromosomal translocation partner ETO or in several developmentally important proteins, such as nervy and DEAF1 (8). The ETO-Mynd domain recruits a N-CoR/SMRT, sin3A, HDAC co-repressor complex and mediates gene repression (9,10). Similarly, the BS69-Mynd domain has been suggested to be involved in repression through N-CoR recruitment (11).

Here we show that the BS69 specific Mynd domain shares structural and binding features with the two C. elegans proteins Bra-1 and Bra-2. BS69-type Mynd domains bind not only to E1A, but also to the EBNA2 oncoprotein or to the Myc-related cellular transcription factor MGA. Binding depends on a common PxLxP peptide motif found in all three proteins. Moreover, E1A competes for the interaction between BS69 and MGA. Our data suggest that the respective peptide motifs in oncoviral proteins have evolved to abrogate cellular functions of BS69-type Mynd domains. The competitive binding of cellular and viral proteins is reminiscent to the abrogation of functions of Rb-type proteins.

**Experimental procedures**

**Plasmid constructs and transfection**

BS69, E1A and EBNA2 mutants were constructed by PCR and cloned in pcDNA3 or pGEX-4T1 vectors. DNA fragments encoding for Bra-1 (residues 82-183), Bra-2 (residues 108 to 214), RACK7 (residues 1007-1068) and ETO (residues 431 to 551) were obtained by PCR from EST clones and subcloned in pGEX-4T1. Notch1-IC and EBNA2 expression vectors, CBF1 and Gal4 responsive reporters are described in (12-14). Avian HD3 erythroblasts and QT6 fibroblasts were transfected as described in (13). Human HeLa cells were transfected with the exgen 500 reagent (Fermantas). CBF1 reporter assays were performed 24H post-transfection using 2 µg of reporter, 0,005 µg of E1A, 0,2 µg of EBNA2 and 0,2 µg of Notch1-IC and 0,2 µg of BS69 expression vectors.
Gal4 reporter assays were performed 24H post-transfection using 2 µg of reporter, 0.2 µg of Gal4 DBD, 0.2 µg of Gal4-EBNA2 and 0.05 µg of Gal4-VP16 and 0.2 µg of BS69 expression vectors. In coimmunoprecipitation experiments, QT6 cells (10^6) were transfected with 3 µg of each expression vectors and harvested 24h post-transfection. Hela cells (10^5) were transfected with 2 µg of each expression vector and harvested 48h post-transfection.

**GST-pull down assay and coimmunoprecipitation experiments**

GST-pull down assays were performed as previously described (14). Immunoprecipitation was performed in 100 mM NaCl, 20 mM Tris pH8, NP40 0.5% supplemented with protease inhibitors in experiments employing E1A and a BS69 C-terminal peptide. In experiments employing the full length BS69 protein, cells were lysed in 350 mM NaCl, 20 mM Tris pH8, 30 mM MgCl₂, NP40 1%, sonicated, cleared by centrifugation and diluted to a 20 mM Tris pH8, 100 mM NaCl, 8.5 mM MgCl₂, NP40 0.5% final concentration. Lysates were incubated with 6 µl anti-BS69 ascites at 4°C for 3h. Protein-A sepharose (Pharmacia) was added and incubated at 4°C for 1 h. Beads were washed six times in 100 mM NaCl, 20 mM Tris pH8, NP40 0.5% and proteins separated by SDS-PAGE. BS69 and EBNA2 proteins were visualized with a M2 anti-FLAG antibody (Integra Bioscience) and an anti-EBNA2 monoclonal antibody respectively (15).

**Yeast two-hybrid screen**

Yeast two hybrid screens were performed with the Matchmaker system (Clontech). A PCR fragment encoding the amino acids 411 to 561 of human BS69 was generated and subcloned in frame with the Gal4 DNA binding domain in the pGBT9 vector (Clontech). The HF7c yeast strain (Clontech) was sequentially transformed with the bait and an Epstein-Barr viral human B-cell library or a human lung library (Clontech) and plated on selective plates. Colonies were tested for β-galactosidase activity by using standard filter
assay. To control the interaction specificity, plasmids were isolated and used to transform the SFY526 yeast strain (Clontech) in combination with the bait or unrelated Gal4- fusion proteins.

**Antibodies**

A BS69 specific antibody was generated by expressing a peptide encompassing the residues 358-441 of the human BS69 protein as GST fusion protein. The fusion protein was purified by affinity chromaography on glutathion beads and an antiserum against the peptide was raised guinea pig.

**Results**

**Adenoviral E1A proteins bind to BS69-type Mynd domains**

The human BS69 protein displays signatures of proteins that are involved in chromatin and gene regulation such as PHD zinc-finger (16), bromo (17), PWWP(18) and Mynd domains (8) (Figure 1A). The carboxy-terminal third of BS69 corresponds to the splice variant BRAM1 and was described as a target of E1A (5). To determine the interaction between BS69 and E1A more precisely, we expressed BS69 deletion mutants as GST-fusion proteins in bacteria (Figure 1A) and examined their interaction with in vitro translated 13SE1A. The BS69 Mynd domain was found to be required and sufficient for E1A binding (Figure 1B). Mutation of a single conserved cysteine residue in the BS69 Mynd domain (cysteine 532 to serine, Figure 1A) abrogated E1A binding (Figures 1B and C). BRAM1 orthologs have also been identified in C. elegans as Bra-1 and Bra-2 that are implicated in TGFβ/BMP signaling (6,7). Whereas the Mynd domains of Bra-1 and Bra-2 also bind to E1A, the Mynd domains of RACK7 (KIAA1125) and of ETO (9,10), respectively failed to do so (Figure 1E). These data suggest that BS69-type Mynd domains are specific and conserved targets of E1A.
The Epstein-Barr viral protein EBNA2 binds to BS69-type Mynd domains

To identify additional proteins that interact with BS69-type Mynd domains, we performed yeast-two-hybrid screens of an Epstein-Barr virus immortalized B-cell library, using the BS69 Mynd domain as bait. Eight His+, β-Gal+ clones were isolated from $1 \times 10^6$ independent transformants. All eight clones encoded different C-terminal portions of the Epstein-Barr viral protein EBNA2. Figure 1B shows that similar to E1A, binding of EBNA2 is restricted to and depends on the integrity of the BS69-Mynd domain (Figure 1B). Fibroblasts were co-transfected with BS69 and EBNA2 expression vectors to examine whether EBNA2 also binds to BS69 in cells. As shown in Figure 1D, BS69 immunoprecipitates EBNA2. Similarly to E1A, EBNA2 also binds to Bra-1 and Bra-2 but not to ETO or RACK7 (Figure 1E).

BS69 binds to a PxLxP peptide motif present in both viral proteins

Binding of two different oncoviral proteins to the BS69 Mynd domain prompted a search for a common target site. A series of oncoprotein mutants was generated and examined for interaction with the BS69 Mynd domain (Figure 2). Amino acid (aa) residues 86 to 128 in E1A (Figure 2A) and two independent protein parts in EBNA2 (aa 379 to 408 and aa 409 to 439, Figure 2B) were identified. Comparison of the corresponding amino acid sequences revealed a common pentamer peptide motif, PxLxP (Figure 2C). To determine whether this motif is involved in binding to BS69, the central invariable leucine residue was mutated into an alanine in E1A. As shown in Figure 3A, the resulting 12S and 13S E1A (L115A E1A) mutant proteins failed to interact with BS69. Similarly, EBNA2, mutated within both PxLxP motifs (EBNA2 L385/439A), failed to interact with BS69 (Figure 3B). Mutation of a single motif in EBNA2 (EBNA2L385A and EBNA2 L439A) however did not abrogate BS69 binding, suggesting redundancy of EBNA2 PxLxP motifs (data not shown). These data suggest that both oncoviral proteins interact specifically with the BS69 specific Mynd domain via a common structural motif.
**E1A competes the interaction between BS69 and MGA**

We searched for cellular proteins that interact with the BS69 Mynd domain in a lung library using the yeast-two-hybrid assay. From one million transformants two lacZ positive clones displayed very fast staining kinetics and were therefore further examined. Sequence analysis revealed that both clones were identical and encompassed amino acid residues 2566 to 2975 of the Myc-related protein MGA (19). Sequence analysis revealed that PxLxP motifs are present in duplicate in the MGA C-terminus (Figure 2C). Deletion analysis, as shown in Figure 3C, confirmed that the PxLxP motifs in MGA are essential for binding to the BS69 Mynd domain.

Next, we determined whether E1A proteins compete for the interaction between BS69 and MGA. A GST-BS69 protein construct was pre-incubated with total cellular extracts of mock-transfected or E1A-transfected QT6 fibroblasts, and tested for its ability to pull-down radiolabelled MGA protein. As shown in Figure 3D, binding of either 12SE1A or 13SE1A proteins to BS69 abrogated the binding of MGA whereas E1A mutants that fail to bind to BS69 (12SE1AL115A and 13SE1AL115A) do not compete for BS69-MGA interaction.

**E1A and EBNA2 neutralize BS69 functions**

E1A has previously been shown to neutralize the repressing function of BS69 (11). Repressor activity of BS69 can be determined in an assay where reporter expression is driven by the oncoviral protein (5). To examine whether E1A and EBNA2 similarly affect BS69 function, we took advantage of the fact that both oncoviral proteins activate reporter gene expression through the CBF1/RPB-Jκ transcription factor (14,15,20-22) - a downstream target of Notch signaling (23,24). As shown in Figure 4A, expression of BS69 strongly reduces reporter activation through E1A or EBNA2 but did not affect reporter activation through the active form of Notch1 (23,24). Furthermore, E1A or EBNA2 mutants that failed to bind BS69 were also not suppressed by BS69. To validate the inhibitory
effect of BS69 on EBNA2 trans-activation, EBNA2 was fused to the DNA binding domain of the Gal4 transcription factor (Gal-), and the activity of the resulting fusion protein (Gal-EBNA2) was examined on an appropriate reporter. As shown in Figure 4B, BS69 expression strongly reduces reporter activation through the Gal-EBNA2 fusion protein yet not of a Gal-VP16 control. These data show that specific interaction between BS69 and its target proteins is a prerequisite for BS69 mediated gene repression. The results also suggest that the inhibitory function of BS69 is specifically neutralized by E1A and EBNA2.

**Discussion**

BS69 mediates transcriptional inhibition and represents a potential tumor suppressor protein (11). For gene repression, BS69 essentially requires the function of its C-terminal Mynd domain (5,11) that also represents a conserved target of different viral oncoproteins. We found that BS69 type-Mynd domains interact through conserved PxLxP peptide motifs with the oncoviral proteins E1A and EBNA2 and with the cellular MGA protein. MGA is a member of the Myc-Max network of bHLH-ZIP transcription factors that contains a T-box domain and presumably links cell proliferation control to mesoderm specification during embryogenesis (19). Our data also show that E1A competes the interaction between BS69 and MGA.

Whereas the C. elegans Bra-1 and Bra-2 Mynd domains shared the same binding specificities as BS69, the related Mynd domains of RACK7 and ETO proteins failed to do so, suggesting that various types of Mynd domains mediate highly specific protein interactions. Comparison of the BS69, RACK7, and ETO Mynd domains revealed differences in amino acids and cysteine spacing that might account for distinct protein interactions (Figure 1A). In agreement with this notion is the observation that the interaction between E1A or EBNA2 with BS69 is abrogated by a point mutation of a single cysteine residue (C523S) in the Mynd domain.

PxLxP motifs were identified as essential components of the BS69 Mynd targets.
The first PxLxP target was found in E1A between conserved regions CR1 and CR2 (Figures 2A and 3A). Others had previously suggested a BS69 interaction site located between CR1 and CR2 of E1A in addition to binding to CR3 (5). In our hands however, CR3 poorly contributes to BS69 binding. This conclusion is based on the observations that comparable BS69 immuno-precipitation was obtained with 12S and 13SE1A (Figure 3A) and that weak residual binding of the PxLxP mutant 13SE1AL115A was only observed under low stringent binding conditions (data not shown). Moreover, two PxLxP motifs in EBNA2, located in regions CR7 and CR8, were also identified as critical for BS69 binding (Figures 2B and 3B). Mutations of the PxLxP motifs in either E1A or EBNA2 however, did not inhibit binding to and trans-activation through CBF1 (Figure 4 and data not shown). Furthermore, E1A PxLxP mutants specifically disrupt the interaction with BS69 without affecting the ability of E1A to associate with Rb (data not shown). Thus, we conclude that PxLxP motifs are indispensable for BS69-type Mynd domain interactions.

Conservation of PxLxP motifs in various viral serotypes suggests that the motifs mediate important onco-protein functions. This notion is supported by the fact that others have shown that deletions covering CR7 or CR8 in EBNA2 and deletion of sequences comprising the BS69 binding sites in E1A abrogate the transformation potential of the respective onco-proteins (25,26). A question that emerges is whether the PxLxP sequence would be sufficient for BS69 interaction. Data base searches identified many proteins that contain PxLxP motifs. One of them is the human TATA-box binding protein, TBP, that contains PxLxP at its C-terminus. TBP, however, failed to bind to BS69 (data not shown). This suggests that additional structural constraints are required for specific BS69-Mynd domain/ PxLxP interaction that await further analysis. Nevertheless, we believe that the remarkable conservation of protein sequence and interaction specificity across species is intriguing and will help to elucidate Mynd functions in development and tumorigenesis.

Data presented here also include identification of MGA as a cellular binding partner.
for BS69. E1A competed with MGA for BS69 binding and the interaction of MGA with
BS69 depended on PxLxP motifs in the C-terminus of MGA. The competitive interaction
between BS69 and a critical cellular protein with two entirely different viral oncoproteins via
identical protein motifs is reminiscent to the interaction between cellular pocket binding
proteins of the Rb-family with several DNA tumor-viral oncoproteins through LxCxE motifs
(27-29). In keeping with that idea it has been speculated that BS69 is a tumor suppressor
that is frequently deleted in cancers and leukemia and that inhibits transcription through
proto-oncogene products (11). Of particular interest is also the genetic connection between
the TGF\(\beta\) /BMP pathways and Bra-1/Bra-2 (6,7). Our data show that the C.elegans Bra-
1/Bra-2 Mynd domains display the same binding specificity as BS69. Since Bra-1 has
been implicated in TGF\(\beta\) signaling it will be interesting to define whether E1A and EBNA2
may disrupt TFG\(\beta\) signaling and alter cell proliferation and differentiation through abrogating
functions of BS69-type Mynd domains.

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References
1. Rowe, D. T. (1999) Front Biosci 4, D346-71.
2. Bornkamm, G. W., and Hammerschmidt, W. (2001) Philos Trans R Soc Lond B Biol
Sciences 356(1408), 437-59.
3. Flint, J., and Shenk, T. (1997) Annu Rev Genet 31, 177-212
4. Boyer, T. G., Martin, M. E., Lees, E., Ricciardi, R. P., and Berk, A. J. (1999) Nature
399(6733), 276-9
5. Hateboer, G., Gennissen, A., Ramos, Y. F., Kerkhoven, R. M., Sonntag-Buck, V., Stunnenberg, H. G., and Bernards, R. (1995) *Embo J* **14**(13), 3159-69

6. Morita, K., Shimizu, M., Shibuya, H., and Ueno, N. (2001) *Proc Natl Acad Sci U S A* **98**(11), 6284-8.

7. Kurozumi, K., Nishita, M., Yamaguchi, K., Fujita, T., Ueno, N., and Shibuya, H. (1998) *Genes Cells* **3**(4), 257-64

8. Gross, C. T., and McGinnis, W. (1996) *Embo J* **15**(8), 1961-70

9. Wang, J., Hoshino, T., Redner, R. L., Kajigaya, S., and Liu, J. M. (1998) *Proc Natl Acad Sci U S A* **95**(18), 10860-5.

10. Lutterbach, B., Sun, D., Schuetz, J., and Hiebert, S. W. (1998) *Mol Cell Biol* **18**(6), 3604-11

11. Masselink, H., and Bernards, R. (2000) *Oncogene* **19**(12), 1538-46

12. Zimber-Strobl, U., Kremmer, E., Grasser, F., Marschall, G., Laux, G., and Bornkamm, G. W. (1993) *Embo J* **12**(1), 167-75.

13. Kowenz-Leutz, E., Twamley, G., Ansieau, S., and Leutz, A. (1994) *Genes Dev* **8**(22), 2781-91

14. Ansieau, S., Strobl, L. J., and Leutz, A. (2001) *Genes Dev* **15**(4), 380-5.

15. Zimber-Strobl, U., Strobl, L. J., Meitinger, C., Hinrichs, R., Sakai, T., Furukawa, T., Honjo, T., and Bornkamm, G. W. (1994) *Embo J* **13**(20), 4973-82

16. Aasland, R., Gibson, T. J., and Stewart, A. F. (1995) *Trends Biochem Sci* **20**(2), 56-9

17. Jeanmougin, F., Wurtz, J. M., Le Douarin, B., Chambon, P., and Losson, R. (1997) *Trends Biochem Sci* **22**(5), 151-3

18. Stec, I., Nagl, S. B., van Ommen, G. J., and den Dunnen, J. T. (2000) *FEBS Lett* **473**(1), 1-5

19. Hurlin, P. J., Steingrimsson, E., Copeland, N. G., Jenkins, N. A., and Eisenman, R. N. (1999) *Embo J* **18**(24), 7019-28.
20. Waltzer, L., Logeaut, F., Brou, C., Israel, A., Sergeant, A., and Manet, E. (1994) *Embo J* **13**(23), 5633-8.

21. Henkel, T., Ling, P. D., Hayward, S. D., and Peterson, M. G. (1994) *Science* **265**(5168), 92-5

22. Grossman, S. R., Johanssen, E., Tong, X., Yalamanchili, R., and Kieff, E. (1994) *Proc Natl Acad Sci U S A* **91**(16), 7568-72

23. Egan, S. E., St-Pierre, B., and Leow, C. C. (1998) *Curr Top Microbiol Immunol* **228**, 273-324

24. Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999) *Science* **284**(5415), 770-6

25. Cohen, J. I., Wang, F., and Kieff, E. (1991) *J Virol* **65**(5), 2545-54

26. Bautista, D. S., Hitt, M., McGrory, J., and Graham, F. L. (1991) *Virology* **182**(2), 578-96

27. Zalvide, J., Stubdal, H., and DeCaprio, J. A. (1998) *Mol Cell Biol* **18**(3), 1408-15.

28. Lee, J. O., Russo, A. A., and Pavletich, N. P. (1998) *Nature* **391**(6670), 859-65.

29. Figge, J., Webster, T., Smith, T. F., and Paucha, E. (1988) *J Virol* **62**(5), 1814-8.
Figure Legends

Figure 1: E1A and EBNA2 bind to the BS69 Mynd domain.

A. Top: schematic representation of the two BS69 and BRAM1 proteins and of the BS69 deletion mutants expressed as GST-fusion proteins and used in E1A and EBNA2 binding assays (Figure 1B). PHD: Plant Homeodomain zinc-finger structure, Br: bromodomain, PWWP: PWWP domain, Md: Mynd domain. Respective positions in the proteins are indicated. The star indicates the mutation C523S.

Bottom: Alignment of the Mynd domain sequences of the human BS69 (hBS69 aa 516-561, AN#X86098), the Drosophila BS69 (dBS69 aa 563-603, AN#AAF58494), the Bra-2 (aa 144-189, AN#AF003389), the Bra-1 (aa 117-163, AN#Z70208), the human RACK7 (hRACK7 aa 1021-1066, AN#AB032951), the Drosophila RACK7 (dRACK7 aa 1392-1437, AN#AAF57177) and the human ETO (hETO aa 477-525, AN#X70990) proteins.

B. The Mynd domain of BS69 interacts with E1A and EBNA2 in vitro. Binding of the 13SE1A and EBNA2 proteins to various BS69 deletion mutants (depicted in Figure 1A), expressed as GST-fusion proteins or to the GST moiety (G-) as control. Input 10%.

C. A single point mutation in the Mynd domain abrogates BS69 binding to E1A. QT6 fibroblasts were transfected with E1A and/or FLAG-tagged BS69 cDNA expression vectors as indicated. Cellular protein complexes were immuno-precipitated with M73 anti-E1A antibody. Co-immuno-precipitated (CoIP) and ectopic expression of BS69 were revealed by immuno-blotting using an anti-FLAG M2 antibody.

D. BS69 binds to EBNA2 in cells. HeLa fibroblasts were transfected with EBNA2 and FLAG-tagged BS69 cDNA expression vectors as indicated. Cellular protein complexes were immuno-precipitated with a polyclonal anti-BS69 antibody. Co-immuno-precipitated
(CoIP) and ectopic expression of EBNA2 and BS69 were revealed by immuno-blotting using monoclonal anti EBNA2 and anti-FLAG M2 antibodies, respectively.

E. E1A and EBNA2 bind specifically to the BS69-type Mynd domain. Binding of E1A and EBNA2 to Bra-1, Bra-2, RACK7 and ETO Mynd domains expressed as GST-fusion proteins or to the GST moiety (G-) as control.

**Figure 2: Mapping of the BS69 interaction site in E1A and EBNA2 proteins.**

A and B. Binding of various E1A (panel A) or EBNA2 (panel B) mutants (as depicted on the right) to BS69 residues 411 to 561 expressed as GST-fusion protein (G-∆ 410) or to the GST moiety (G-). Input 10%. Numbers above and below E1A and EBNA2 proteins indicate conserved regions (CR) and amino acids, respectively of the oncoproteins.

C. Identification of PxLxP motifs in E1A, EBNA2 (CR7 and CR8) and MGA sequences.

Ad: Adenovirus, EBV: Epstein-Barr virus, HPV: Herpes Papio Virus.

**Figure 3: The BS69 Mynd domain targets a PxLxP peptide present in E1A, EBNA2 and MGA.**

A. Disruption of the PxLxP motif in E1A abrogates binding to BS69. QT6 fibroblasts were transfected with various E1A and/or FLAG-tagged BS69 (residues 411-561) cDNA expression vectors, as indicated. Cellular protein complexes were immuno-precipitated with an anti-E1A monoclonal antibody. Co-immuno-precipitated (CoIP) and ectopic expression of BS69 were revealed by immuno-blotting using an anti-FLAG M2 antibody.

B. Disruption of both PxLxP motifs in EBNA2 abrogates binding to BS69. In vitro radiolabelled EBNA2 proteins were incubated with total cellular extracts from mock-transfected (-) or from HeLa fibroblasts transfected with a FLAG tagged BS69 C terminal peptide (residues 411-561). Cellular protein complexes were immuno-precipitated with a monoclonal anti-FLAG antibody. Co-immuno-precipitated (CoIP) EBNA2 and BS69 were
revealed by autoradiography and by immuno-blotting using a monoclonal anti-HA antibody, respectively.

C. Deletion of both PxLxP motifs in MGA abrogates binding to BS69. Binding of MGA peptides (as depicted on the top) to BS69 expressed as GST fusion protein (G-∆ 410) or to the GST moiety (G-) as control. Input 10%.

D. E1A and MGA compete for binding to BS69. GST (G-) or GST-BS69 (G-∆ 410) loaded glutathione beads were pre-incubated with total cellular extracts of mock-transfected or E1A-transfected QT6 fibroblasts and tested for their ability to pull-down an in vitro radiolabelled MGA protein. Top: Binding of MGA. Bottom: E1A expression revealed by immuno-blotting using a monoclonal M73 anti-E1A antibody. Input 10%.

Figure 4: BS69 interferes with transcriptional activation in a PxLxP dependent fashion.

A. Comparison of the activation of a reporter harboring multimerized CBF1-responsive elements (as depicted on the top) through 13SE1A, 13SE1AL115A, EBNA2, EBNA2 L385/439A, Notch1-IC. in the absence or presence of BS69 (∆ 410). Bars indicate repression (-fold) induced by BS69.

B. Comparison of the activation of a reporter harboring multimerized Gal4-responsive elements as depicted on the top through a Gal4DBD-EBNA2 (Gal-EBNA2) or through a Gal4-VP16 (Gal-VP16) fusion protein in absence or in presence of a BS69Ct peptide (∆ 410). Bars indicate the repression (-fold) induced by BS69 expression, as in A.
Figure 1, Ansieau & Leutz

A.

|       | PHD | Br | PWWP | Mynd |
|-------|-----|----|------|------|
| 1     | 63  | 105| 146  | 190  | 243  | 279  | 412  | 518  | 561  |
|       |     |    |      |      |      |      | BS69 |      |      |
|       |     |    |      |      |      | 147  | 153  | 196  |      |
|       |     |    |      |      |      |      | BS69 |      |      |

B.

|       | 13SE1A | EBNA2 |
|-------|---------|--------|
| Input | [image] | [image] |
| G     | [image] | [image] |
| Δ410  | [image] | [image] |
| Δ459  | [image] | [image] |
| Δ469  | [image] | [image] |
| Δ503  | [image] | [image] |
| Δ410ΔMd | [image] | [image] |
| Δ410mut | [image] | [image] |

C.

|       | 13SE1A | Δ410 | Δ410mut |
|-------|--------|------|---------|
| BS69  | [image] | [image] | [image] |
| E1A   | [image] | [image] | [image] |

D.

|       | BS69 | EBNA2 |
|-------|------|-------|
| Co-IP | [image] | [image] |
| EBNA2 | [image] | [image] |
| BS69  | [image] | [image] |

E.

|       | E1A | EBNA2 |
|-------|-----|-------|
| input | [image] | [image] |
| G     | [image] | [image] |
| G-Bra-1 | [image] | [image] |
| G-Bra-2 | [image] | [image] |
| G-RACK7 | [image] | [image] |
| G-ETO | [image] | [image] |
A. CR 1 2 3 13SE1A 1 289
   12SE1A
   Δ 1/36
   Δ CR1
   Δ 86/128
   Δ CR2
   Δ 191/218
   Δ 239/253

B. CR 1 2 3 4 56 7 8 9 13SE1A 1 487
   FL EBNA2
   Δ 365/487
   Δ 1/365
   Δ 379/439
   Δ 409/439
   Δ 379/408

C.

|              | M | P | N | L | V | P |
|--------------|---|---|---|---|---|---|
| E1A (Ad5, 112/7) |   |   |   |   |   |   |
| E1A (Ad12, 112/7) |   |   |   |   |   |   |
| EBNA2 (EBV A, 382/7) |   |   |   |   |   |   |
| EBNA2 (EBV B, 349/54) |   |   |   |   |   |   |
| EBNA2 (HVP, 420/5) |   |   |   |   |   |   |
| EBNA2 (EBV A, 436/41) |   |   |   |   |   |   |
| E1A (Ad5, 112/7) |   |   |   |   |   |   |
| E1A (Ad12, 112/7) |   |   |   |   |   |   |
| EBNA2 (EBV A, 382/7) |   |   |   |   |   |   |
| EBNA2 (EBV B, 349/54) |   |   |   |   |   |   |
| EBNA2 (HVP, 420/5) |   |   |   |   |   |   |
| EBNA2 (EBV A, 436/41) |   |   |   |   |   |   |
| hMGA (2927-32) |   |   |   |   |   |   |
| hMGA (2952-7) |   |   |   |   |   |   |
Figure 4, Ansieau & Leutz

A. CBF1-βglobin-luc

B. Gal4-Tk luc

Fold Repression

13SE1A 13SE1AL115A EBNA2 EBNA2 L385/439A Notch1-IC

Gal-VP16
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Stephane Ansieau and Achim Leutz

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