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N-*myc* suppresses major histocompatibility complex Class I gene expression through down-regulation of the p50 subunit of NF-$\varkappa$B

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In neuroblastoma, N-*myc* suppresses the expression of major histocompatibility complex (MHC) Class I antigens by reducing the binding of a nuclear factor to the enhancer-A element in the MHC Class I gene promoter. We show here that the p50 subunit of NF-$\varkappa$B is part of this complex and that expression of p50 mRNA is suppressed by N-*myc*. Transfection of a p50 expression vector in neuroblastoma cells that express N-*myc* at a high level leads to restoration of factor binding to the MHC Class I gene enhancer, restores enhancer activity and leads to re-expression of MHC Class I antigens at the cell surface. These data indicate that the p50 subunit of NF-$\varkappa$B is involved in the regulation of MHC Class I antigen expression and that N-*myc* down-regulates MHC Class I gene expression primarily through suppression of p50 expression.

Key words: MHC Class I/neuroblastoma/NF-$\varkappa$B/N-*myc*

Introduction

The expression of major histocompatibility complex (MHC) Class I genes is regulated by several enhancers in its promoter. An important enhancer is the palindromic motif 5'-GGGGATTCCCC-3', called enhancer A-region 1 (David-Watine *et al.*, 1990). Several transcription factors have been identified that bind to this enhancer in an electromobility shift assay (EMSA), among which are NF-$\varkappa$B, H2TF1, KBF1, MBP-1 and MBP-2 (Baldwin and Sharp, 1987, 1988; Israel *et al.*, 1987; Baldwin *et al.*, 1990; Fan and Maniatis, 1990; Rustgi *et al.*, 1990; van't Veer *et al.*, 1992). NF-$\varkappa$B is a constitutive factor in mature B lymphocytes, but acts as an inducible transcription factor in most other cell types (reviewed by Lenardo and Baltimore, 1989; Baeuerle, 1991; Blank *et al.*, 1992). Conversely, H2TF1 and KBF1 are constitutively active in most cell types, and are considered to be two closely related factors, which regulate basal expression of MHC Class I genes (Baldwin and Sharp, 1987; Israel *et al.*, 1987, 1989).

We have found previously that over-expression of the N-*myc* oncogene in neuroblastoma suppresses the expression of MHC Class I antigens by reducing the binding of a H2TF1-like transcription factor to the MHC Class I gene enhancer A (Bernards *et al.*, 1986; Lenardo *et al.*, 1989). The analysis of the molecular mechanism by which N-*myc* regulates the activity of the H2TF1-like transcription factor was hampered by the fact that the structure of neither this factor, nor H2TF1 had been elucidated. The structures of NF-$\varkappa$B and KBF1, on the other hand, have recently been described, revealing that NF-$\varkappa$B consists of a heterodimer of a 50 kDa (p50) and a 65 kDa (p65) *rel*-related polypeptide, whereas KBF1 consists of a homodimer of two p50 molecules (Ghosh *et al.*, 1990; Nolan *et al.*, 1991; Kieran *et al.*, 1990). p50 is synthesized as an inactive p105 precursor molecule, which is subsequently processed by proteolytic cleavage to yield the active p50 polypeptide (Blank *et al.*, 1991; Henkel *et al.*, 1992). Both NF-$\varkappa$B and KBF1 can activate transcription from the palindromic MHC enhancer motif *in vitro*, although the binding affinities of the complexes for this enhancer differ (Fujita *et al.*, 1992).

We show here that the H2TF1-like factor, which is down-regulated by N-*myc* in neuroblastoma, also contains the p50 subunit of NF-$\varkappa$B and is likely to be identical to KBF1. Introduction of a p50 expression vector into N-*myc* transfected neuroblastoma cells completely restores factor binding to the MHC enhancer and expression of MHC Class I molecules, indicating that p50 plays a major role in the regulation of expression of MHC Class I antigens and is subject to regulation by N-*myc*.

Results

N-myc suppresses a KBF1-like factor in neuroblastoma

We have shown previously that the N-*myc* oncoprotein down-modulates the expression of MHC Class I genes in rat B104 neuroblastoma cells (Bernards *et al.*, 1986). We

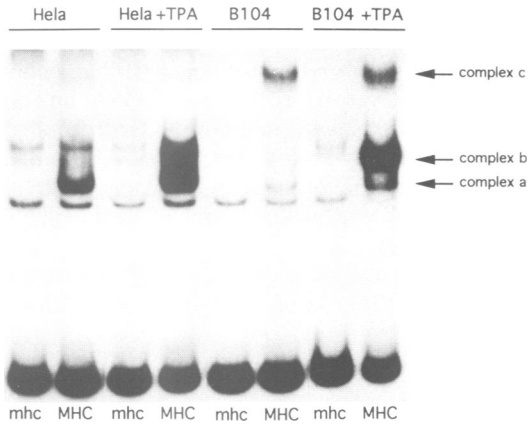

Fig. 1. Comparison of complexes bound to the MHC enhancer in HeLa and B104 cells. EMSA assay with nuclear protein extracts from human HeLa cells and rat B104 neuroblastoma cells. +TPA: cells were incubated with TPA before isolation. MHC: MHC enhancer A oligonucleotide. mhc: mutant version of the same oligonucleotide. Specific retarded complexes are indicated by arrow.

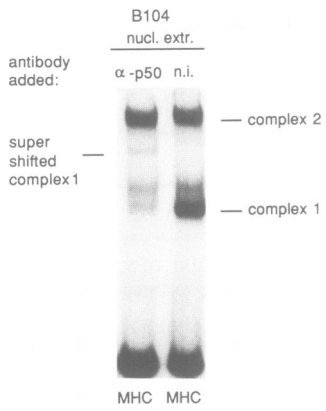

Fig. 2. Detection of p50 molecules in protein complexes bound to the MHC enhancer. EMSA assay with nuclear protein extracts from untransfected B104 neuroblastoma cells pre-incubated with anti-p50 serum (α-p50) or preimmune rabbit serum (n.i.). MHC: MHC enhancer A oligonucleotide.

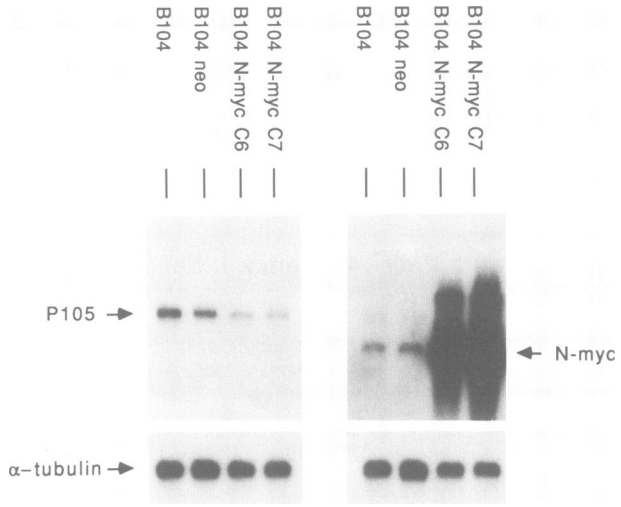

Fig. 3. Northern blot analysis of p105 expression. Total cellular RNA from B104 neuroblastoma cells, B104 neo, B104 N-*myc* C6 and B104 N-*myc* C7 transfected cells was analyzed with a p105 specific probe (left upper panel, lanes 1–4), and with a N-*myc* probe (right upper panel, lanes 5–8). As a control, both filters were reprobed with a rat α-tubulin cDNA (lower panels). p105: 4 kb mRNA, N-*myc*: 3.2 kb mRNA, and α-tubulin: 1.7 kb mRNA.

found that N-*myc* suppresses the expression of MHC Class I genes by reducing the binding of an H2TF1-like factor to the enhancer A-region 1 (5′-GGGGATTCCCC-3′), in the MHC promoter, thereby inactivating this enhancer (Lenardo *et al.*, 1989). To characterize this factor further, we compared the migration of the H2TF1-like factor in B104 cells with that of factors present in HeLa nuclear extracts in an EMSA with the MHC enhancer as a probe. Complex A in Figure 1 (lane 6) represents the factor that is suppressed in N-*myc* transfected B104 neuroblastoma cells (Lenardo *et al.*, 1989). In HeLa cells the most abundant retarded complex that binds the MHC enhancer is KBF1 (lane 2) (Kieran *et al.*, 1990). This complex comigrates with the retarded complex A found in the rat B104 cells. Another transcription factor that can bind the same MHC Class I gene enhancer motif in an EMSA is the heterodimeric p50/p65 protein NF-κB. NF-κB can be induced upon treatment of cells by TPA (12-*O*-tetradecanoyl phorbol-13-acetate; Sen and Baltimore, 1986), and is indicated in Figure 1 as complex B (lanes 4 and 8). NF-κB, H2TF1 and KBF1 can be distinguished in an EMSA by virtue of their different mobilities. H2TF1 is supposed to migrate more slowly than NF-κB (Baldwin and Sharp, 1988), whereas KBF1 (p50 homodimer) migrates more quickly (Urban *et al.*, 1991). On the basis of these criteria we conclude that complex A in B104 cells behaves like KBF1 in an EMSA, and not like H2TF1. The most slowly migrating retarded complex is MBP-2 (complex C) (van't Veer *et al.*, 1992). All the complexes are specific for the MHC enhancer (MHC), since they do not bind to a mutated version of this site (lanes indicated by mhc).

p50 subunit of KBF1 is present in the KBF1-like complex

The data presented above suggest that the H2TF1-like factor, present in B104 cells, resembles KBF1. Since KBF1 is known to consist of a homodimer of two p50 molecules, we investigated whether the p50 subunit of KBF1 is indeed present in the MHC enhancer complex that is suppressed by N-*myc* in B104 cells. To determine this, we used an antibody directed against the p50 subunit of NF-κB and KBF1 in an EMSA with the palindromic MHC enhancer as a probe. Figure 2 shows that addition of an anti-mouse p50 polyclonal antiserum to a nuclear extract of rat B104 neuroblastoma cells results in a significant reduction of the binding of the KBF1-like factor (complex 1) to the MHC Class I gene enhancer and the appearance of a novel, supershifted, complex (lane 1), whereas the non-immune control serum did not alter the binding of complex 1 to the MHC enhancer (lane 2). Furthermore, the slowly migrating MBP-2 retarded complex (complex 2) was not affected by the p50 antiserum (lane 1). In a nuclear extract from TPA-treated B104 cells, the p50 antiserum recognized both the NF-κB and KBF1-like complexes, resulting in the partial disappearance and partial supershifting of these complexes in an EMSA (data not shown). Taken together these data suggest that the p50 subunit of KBF1 is present in the KBF1-like complex in B104 cells.

N-myc suppresses the expression of p105

The finding that an antibody directed against p50 supershifts the KBF1-like factor in B104 cells indicates that p50, or an immunologically closely related molecule, is part of this complex. To investigate whether the gene encoding p50 is itself subject to regulation by N-*myc*, we measured the expression of the p105 precursor of p50 in parental B104 neuroblastoma cells and in B104 N-*myc* transfectants by Northern blot analysis. Figure 3 (lanes 1–4) shows that the expression levels of p105 mRNA in two independently derived B104 N-*myc* cell lines, B104 N-*myc* C6 and B104 N-*myc* C7 (Bernards *et al.*, 1986), are less than that in the B104 parental cell line and a neomycin-transfected control cell line, B104 neo. Since the Northern blot was hybridized under high stringency and since the p50 cDNA probe detects a transcript of the predicted size (4 kb), these data strongly suggest that the expression of the p105 precursor of p50 itself, and not that of a structurally and immunologically related polypeptide, is down-regulated by N-*myc* in neuroblastoma cells.

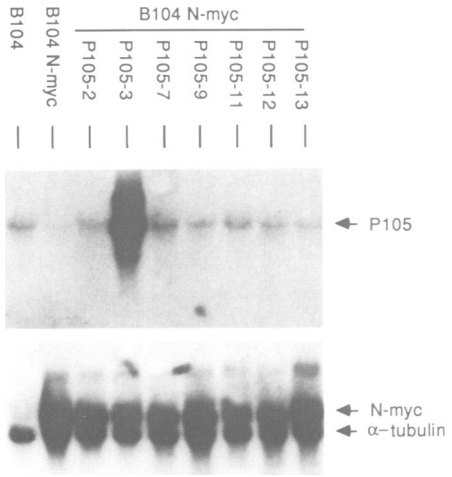

Fig. 4. Northern blot analysis of p105-transfected neuroblastoma cells. Total cellular RNA from seven p105 transfected B104 N-*myc* cell lines (lanes labeled B104 N-*myc* p105-2, -3, -7, -9, -11, -12 and -13), the parental B104 N-*myc* cells (B104 N-*myc*) and the untransfected B104 cells (B104) was probed for p105 expression (**upper panel**). As a control the filter was reprobed with a rat α-tubulin cDNA and an N-*myc* fragment (**lower panel**). mRNAs are 4 kb (p105), 3.2 kb (N-*myc*) and 1.7 kb (α-tubulin).

Transfection of p105

The data presented above suggested that N-*myc* reduces KBF1-like binding activity, at least in part, by reducing the expression of the p50 subunit. To investigate this further, we co-transfected a p105 expression plasmid into the B104 N-*myc* C7 cells with the dominant selectable marker pSV2gpt. Mycophenolic acid-resistant transfectants were selected and analyzed for the expression of p105 mRNA by Northern blot analysis. Figure 4 (upper panel) shows that all seven stable p105 transfectants of B104 N-*myc* C7 express p105 mRNA. One transfectant, B104 N-*myc* p105-3, expressed a very high level of p105, whereas the six others had levels comparable to those in the parental B104 cell line (Figure 4). No alteration in the expression level of N-*myc* was found in the p105 transfectants (Figure 4, lower panel).

Analysis of p105 transfectants

Next, we investigated whether reintroduction of p105 expression in the B104 N-*myc* C7 cells would lead to the restoration of MHC enhancer binding activity in these cells. The p105 precursor protein has to be processed to yield the active DNA-binding p50 molecule (Ghosh *et al.*, 1990; Kieran *et al.*, 1990). We made whole cell protein extracts from each of the p105-transfected cell lines as well as from control B104 and B104 N-*myc* C7 cells. These extracts were subsequently used in an EMSA with the palindromic MHC enhancer as a probe (Figure 5, lanes labeled MHC). As a control, the assay was also performed with a mutant version of this motif (lanes labeled mhc). Figure 5 shows that all p105 transfectants of the B104 N-*myc* C7 cell line re-expressed a factor that comigrates with the KBF1-like retarded complex of the parental B104 cell line. The complex only binds to the wild-type MHC enhancer probe, and the highest level is seen in the B104 N-*myc* p105 transfectant that expressed the highest level of p105 mRNA (B104 N-*myc* p105-3). We conclude that transfection of p105 in N-*myc*-expressing neuroblastoma cells is sufficient to restore KBF1-like binding activity. The finding that transfection of p105 restores KBF1-like binding activity also indicates that N-*myc* suppresses this binding activity in neuroblastoma cells primarily by reducing the expression of the p50 subunit of NF-κB.

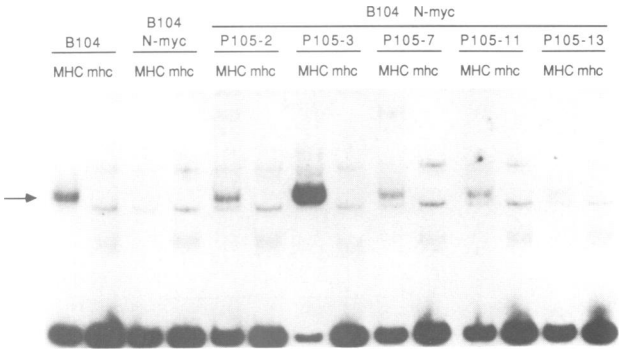

Fig. 5. EMSA assay with p105-transfected neuroblastoma cells. Whole cell protein extracts were prepared from five p105-transfected B104 N-*myc* cell lines (lanes labeled B104 N-*myc* p105-2, -3, -7, -11 and -13), their parental B104 N-*myc* cells (B104 N-*myc*) and the untransfected B104 cells (B104). The extracts were incubated with the MHC enhancer A wild-type oligonucleotide (MHC) or a mutated version (mhc). The position of the KBF1-like retarded complex is indicated.

Table I. Transient transfection of MHC−CAT plasmid into neuroblastoma cells

Cell lines	pActinCAT (c.p.m.)	pMHCCAT	
		c.p.m.	Fold induction
B104	26 007	7001	17.8
B104 N-myc	24 715	394	1.0
B104 N-myc p105-2	25 116	2253	5.7
B104 N-myc p105-3	23 213	1663	4.2
B104 N-myc p105-9	32 089	3187	8.1

Untransfected B104 cells, B104 N-*myc* cells and three p105-transfected B104 N-*myc* cell lines were transiently transfected with CAT reporter constructs as indicated and a β-galactosidase control plasmid. The mean of the CAT activity of two independent transfection assays of one representative experiment is given as c.p.m. [^{14}C]chloramphenicol substrate conversion. The ratio of CAT activity on the MHC reporter construct of cell lines over conversion in B104 N-*myc* cells is given.

p105 transfection restores MHC enhancer activity

We have shown previously that down-modulation of factor binding to the MHC enhancer by N-*myc* was accompanied by an almost complete inactivation of this enhancer (Lenardo *et al.*, 1989). It was therefore of interest to test whether the introduction of the p105 expression plasmid in B104 N-*myc* C7 cells would result in restoration of the activity of this enhancer. To investigate this, we used a plasmid that harbored three copies of the MHC enhancer A-region 1 upstream of an enhancerless core promoter, linked to the chloramphenicol acetyl transferase (CAT) gene. This plasmid was transfected into three of the B104 N-*myc* p105 cell lines and, as a control, into the B104 and B104 N-*myc* C7 cell lines. Forty-eight hours after transfection, cells were harvested and analyzed for CAT activity. Five independent transfection experiments were performed with the three p105 transfected cell lines and two control cell lines. Table I shows the results of one of these experiments, which is representative for all five experiments. The mean values of two duplicate transfections for each cell line are given. As can be seen from this table, the MHC enhancer−CAT construct

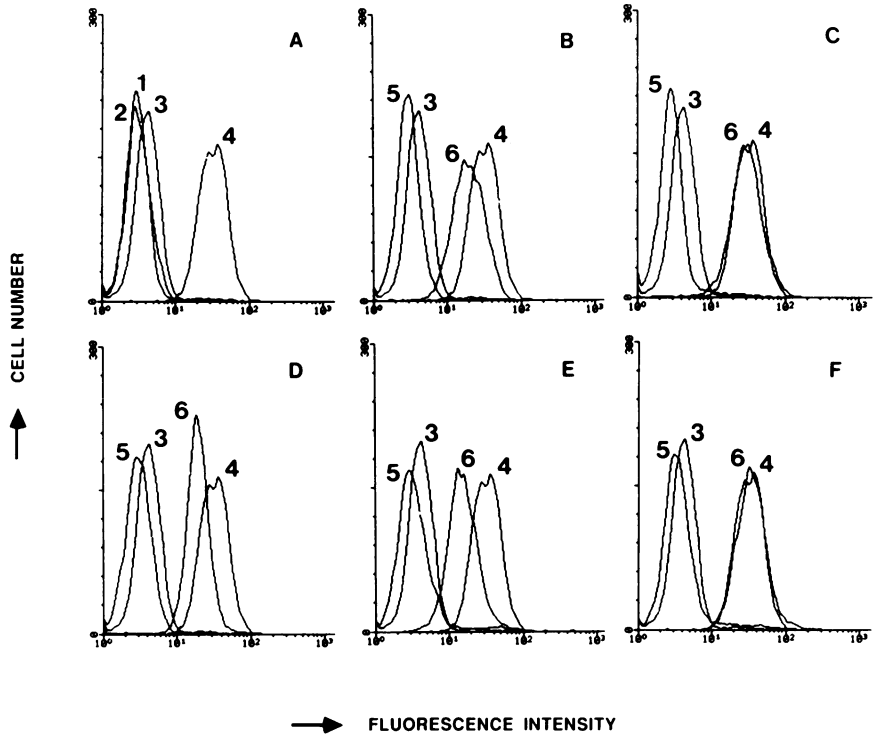

Fig. 6. Cytofluorimetric analysis of MHC Class I cell surface expression of p105 transfected cells. B104 cells and transfected derivatives were stained with the mouse monoclonal antibody OXP 01, which reacts with rat MHC Class I antigens, and a fluoresceinated sheep anti-mouse IgG secondary antibody. Cell surface fluorescence of 5×10^3 cells was measured using a cytofluorograph. Graphs 2, 4 and 6: staining with primary and secondary antibody. Control, graphs 1, 3 and 5: staining with the secondary antibody only. The cell lines analyzed are: panel **A**: graphs 1 and 2, B104 N-*myc* cells; 3 and 4, untransfected B104 cells. In panels **B**–**F**, graphs 5 and 6 represent five p105 transfected B104 N-*myc* cell lines (panel B: B104 N-*myc* p105-2; panel C: B104 N-*myc* p105-3; panel D: B104 N-*myc* p105-7; panel E: B104 N-*myc* p105-9; panel F: B104 N-*myc* p105-11) and are compared with untransfected B104 cells, graphs 2 and 4.

is 4- to 8-fold more active in the p105-transfected cell lines than in the parental B104 N-*myc* C7 cell line. No significant CAT activity was detected when the same cell lines were transfected with a plasmid that harbored three copies of a mutant version of the MHC enhancer linked to the core promoter–CAT gene, indicating that the observed effects of p105 transfection were mediated through the MHC enhancer motif present in the transfected plasmid (data not shown). Compared with the other p105 transfectants, the MHC Class I gene enhancer was only weakly activated in the p105-3 transfectant (Table I). This transfectant expressed the highest level of p50 mRNA and consequently had the highest level of factor binding to the MHC enhancer (Figure 5). The relatively low activity of the MHC enhancer in this transfectant is therefore most probably due to 'self-squelching' caused by depletion of necessary co-factors by binding to the overexpressed p50 protein.

Transfection of an actin promoter–CAT plasmid invariably gave equally high levels of CAT activity in all cell lines (Table I). Taken together, these data indicate that transfection of p105 in N-*myc*-expressing neuroblastoma cells restores activity of the MHC Class I enhancer A.

MHC Class I cell surface expression restored in p105 transfectants

To investigate whether transfection of p105 resulted in the re-expression of MHC Class I polypeptides at the cell surface of B104 N-*myc* C7 cells, five different p105 transfectants were stained with a mouse monoclonal antibody directed against rat MHC Class I molecules and with a fluorescein-coupled secondary antibody directed against mouse immunoglobulins. Cell surface fluorescence of the cell lines was measured by flow cytometry. Figure 6A shows a control flow analysis of MHC Class I antigen expression of the parental B104 neuroblastoma cells and the N-*myc*-transfected B104 N-*myc* C7 cells. It shows that parental B104 cells (graph 4) express high levels of cell surface MHC Class I antigens, whereas the B104 N-*myc* C7 cell line (graph 2) had hardly any detectable cell surface MHC Class I expression. As a control, both cell lines were incubated only with the fluorescein-coupled second antibody to determine the level of non-specific staining (panel A, graphs 1 and 3). Next, we analyzed the five p105-transfected cell lines for cell surface MHC Class I expression. Panels B, C, D, E and F show that all p105 transfectants have significantly higher levels of MHC Class I expression than the parental B104 N-*myc* C7 cells (panels B–F, graphs indicated with a 6). The level of MHC Class I expression in two p105 transfectants is only slightly lower than that of the B104 cell line (panels D and E, graphs 4 and 6). This is consistent with our earlier finding that N-*myc* acts on two different sites in the MHC Class I gene promoter, one of which is the MHC enhancer A (Lenardo *et al.*, 1989).

A B104 N-*myc* C7 cell line stably transfected with pSV2gpt and a protein kinase C expression plasmid (Bernards, 1991) did not shown any increased level of cell surface MHC Class I expression (data not shown). We conclude that introduction of a p105 expression plasmid into B104 N-*myc* cells almost completely reverts the N-*myc*-mediated down-modulation of MHC Class I expression.

Discussion

We have shown previously that transfection of an N-*myc* expression vector in rat B104 neuroblastoma cells leads to loss of factor binding to the MHC Class I gene enhancer and down-regulation of MHC Class I antigen expression (Bernards *et al.*, 1986; Lenardo *et al.*, 1989). We show here that this factor comigrates with the p50 homodimer, KBF1, which is present in HeLa cell extracts. We also show that the p50 subunit of NF-$\varkappa$B is part of this transcription factor complex in B104 cells, and that the gene encoding p50 is down-modulated by N-*myc* in neuroblastoma cells. Introduction of a plasmid that directs the synthesis of the p50 subunit of NF-$\varkappa$B in N-*myc*-transfected neuroblastoma cells restores binding activity and causes a significant increase in the level of cell surface MHC Class I antigen expression. Our data thus suggest that N-*myc* suppresses MHC Class I antigen expression to a significant extent by reducing the expression of the p50 subunit of NF-$\varkappa$B.

The question to which we have only found a partial answer concerns the exact structure of the KBF1-like complex in B104 neuroblastoma cells. The facts that the complex comigrates with KBF1 present in HeLa cells, and can be super-shifted with an antibody to p50, strongly suggests that it contains p50, or an immunologically closely related polypeptide. That p50 itself is present in the KBF1-like complex is strongly suggested by the finding that N-*myc* suppresses the expression of p50 mRNA (Figure 3). We cannot exclude the possibility, however, that the KBF1-like complex consists of a heterodimer between p50 and a second, *rel*-like polypeptide. One such polypeptide, p49, was recently identified (Schmid *et al.*, 1991). Although p49 is not expressed in B104 neuroblastoma cells (data not shown), we cannot exclude the possibility that other *rel*-like proteins are expressed in these cells. A more likely possibility, which we favor at present, is that this complex is indeed a homodimer of two p50 molecules. We favor this model for two reasons. First, the finding that a 3- to 4-fold reduction in p50 mRNA (Figure 3) leads to a 10- to 15-fold reduction in enhancer binding (Figure 5) is more consistent with the complex being a homodimer of p50 rather than a heterodimer. Secondly, the finding that the amount of binding activity in p105 transfectants is proportional to the amount of p105 mRNA (Figures 4 and 5) also suggests a homodimeric structure. An additional argument for the factor that is present in B104 cells being KBF1, is the fact that KBF1 binds the NF-$\varkappa$B enhancer motif in an EMSA with low affinity (Macchi *et al.*, 1989; Kieran *et al.*, 1990), as does the KBF1-like factor present in the B104 cells (Bernards, 1991). Thus, it is very likely that the factor present in the B104 neuroblastoma cells, which is subject to N-*myc* suppression, is indeed KBF1.

Our data also suggest that p50 itself is a key player in the regulation of MHC Class I antigen expression. This is in apparent contradiction with the work of others, who were unable to show *in vivo* transactivation by p50 molecules of CAT reporter constructs (Kieran *et al.*,1990; Hémar *et al.*, 1991; Schmid *et al.*, 1991; Schmitz and Baeuerle, 1991; Bours *et al.*, 1992; Ryseck *et al.*, 1992). However, in most of these studies, non-palindromic NF-$\varkappa$B-like motifs, and not the palindromic MHC enhancer A itself were used in transient transfection experiments with p105 or p50. Recent *in vitro* transcription experiments show that a p50 homodimer can only activate transcription efficiently from the palindromic MHC enhancer, and that this is dependent on the conformation of the p50 homodimer (Fujita *et al.*, 1992). The apparent discrepancy between our data and those of Kieran *et al.* (1990) on transactivation by p50 of the palindromic MHC enhancer may be explained by the use of stable p105 transfectants versus transient transfection. Our unpublished data also indicate that transient transfection of p105 in B104 N-*myc* C7 cells fails to activate the MHC Class I gene enhancer. This could be caused by slow processing of the inactive p105 precursor molecule to the active DNA-binding p50 molecule.

The notion that the p50 subunit of NF-$\varkappa$B is involved in activation of the expression of MHC Class I antigens is supported by several other lines of experimentation. First, A.Israel and colleagues showed that a dominant-negative mutant of p50 lacking the DNA-binding region resulted in down-regulation of MHC Class I molecules, probably due to sequestering the endogenous wild-type p50 molecules in a complex that can no longer bind DNA (Logeat *et al.*, 1991). Moreover, deficiency of KBF1 and NF-$\varkappa$B binding to the MHC enhancer A correlates with the absence of Class I mRNA in a number of human tumor cell lines (Blanchet *et al.*, 1992).

In related studies we have found that N-*myc* alters the expression of protein kinase C (PKC) isoforms in neuroblastoma (Bernards, 1991). As a result of this, NF-$\varkappa$B can no longer be activated by PKC in N-*myc*-transfected neuroblastoma cells. Restoration of PKC signaling by transfection of a PKC expression vector led to restoration of NF-$\varkappa$B inducibility (Bernards, 1991), although the total amount of NF-$\varkappa$B present in the B104 N-*myc* cells was lower than in the untransfected B104 cells, consistent with the down-regulation of one of its subunits. It thus appears that N-*myc* inhibits KBF1 activity by reducing p50 gene expression and inhibits NF-$\varkappa$B activity at two levels: inhibition of PKC signal transduction and inhibition of expression of one of the subunits of NF-$\varkappa$B. The identification of a second, independent, mechanism of inhibition of NF-$\varkappa$B activity by N-*myc* suggests that inhibition of NF-$\varkappa$B activity is an important aspect of N-*myc* action in neuroblastoma cells. Also, the fact that N-*myc* inhibits two transcription factors that both can bind to the same DNA sequence motif raises the possibility that this enhancer motif may control the expression of a number of genes (one of which are the MHC Class I antigens) that together act to suppress tumorigenicity of cells. Inactivation of these genes by N-*myc* may be one of the mechanisms by which N-*myc* enhances tumorigenicity of neuroblastoma tumor cells.

Materials and methods

Cell culture and transfections

Untransfected HeLa and B104 cells were treated for 3 h with TPA (100 ng/ml) before isolation (Bernards, 1991). Transfections were performed using the standard calcium phosphate precipitation technique (Graham and van der Eb, 1973). Stable transfectants of B104 N-*myc* C7 cells (Bernards *et al.*, 1986) were made by transfecting 100 mm plates with 20 μg of a mouse p105 full length cDNA expression vector under the control of a CMV enhancer (gift of S.Ghosh) together with 2 μg of the selectable marker pSV2gpt plasmid. Mycophenolic acid resistant colonies were isolated (Bernards, 1991). For transient transfection assays, 100 mm plates of neuroblastoma cells were transfected with 10 μg of CAT reporter plasmid and 10 μg of β-galactosidase control plasmid.

Electrophoresis mobility shift assay

The preparation of nuclear or whole cell protein extracts from cell lines, and gel electrophoresis were carried out as described by Bernards (1991).

The oligonucleotides used in the binding reaction were MHC (wild-type): TGGGGATTCCCCA, and mhc (mutant): TGCGGATTCCCGA, and were labeled as described by Bernards (1991). In supershift experiments, 10 μg of protein extract were pre-incubated for 2 h at 4°C with 2 μl of p50 polyclonal antiserum or non-immune serum (gift of S.Ghosh).

Northern blot analysis

Total cellular RNA was extracted from cell lines and 20 μg of RNA was electrophoresed through a 1% agarose–formaldehyde gel. After transfer of nitrocellulose, filters were hybridized in 5 × SSPE/50% formamide with insert fragments of N-*myc*, α-tubulin and of the p105 cDNA [p50 clone 3, 4 kb *Not*I fragment (Ghosh *et al.*, 1990)] plasmids as described by Bernards *et al.* (1986).

CAT assays

Forty-eight hours after transfection, protein extracts were made as described previously (Lenardo *et al.*, 1989) and CAT activity was determined using the phase extraction assay for CAT activity (Seed and Sheen, 1988). Reporter plasmids used were pMHCfosCATΔ65 (Lenardo *et al.*, 1989), J32 (harbouring two mutated κB sites, Pierce *et al.*, 1988) and pActinCAT. Cells were co-transfected with pRSVβgal (Lenardo *et al.*, 1989), and β-galactosidase activity was determined (Hall *et al.*, 1983) to control for transfection efficiency. Transfection efficiencies differed by <20%.

Immunofluorescence

Cell surface expression of MHC Class I antigens was determined using the mouse monoclonal antibody to rat MHC Class I molecules, OXP 01 (Serotec, Bicester, UK), and quantified using a cytofluorograph as described by Bernards *et al.* (1986).

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