Journal of Cellular Biochemistry 67:297–303 (1997)

Retinoblastoma-Related Protein pRb2/p130 and Its Binding to the *B-myb* Promoter Increase During Human Neuroblastoma Differentiation

Giuseppe Raschellà,[1] Barbara Tanno,[1] Francesco Bonetto,[2] Roberto Amendola,[1] Tullio Battista,[2] Antonio De Luca,[3] Antonio Giordano,[3] and Marco G. Paggi[2]*

[1]Section of Toxicology and Biomedical Sciences, Ente Nuove Tecnologie Energia e Ambiente (ENEA), Rome, Italy
[2]Laboratory for Cell Metabolism and Pharmacokinetics, Center for Experimental Research, Regina Elena Cancer Institute, Rome, Italy
[3]Departments of Pathology, Anatomy, and Cell Biology, Jefferson Medical College, Philadelphia, Pennsylvania 19107

Abstract Neuroblastoma cells can undergo neural differentiation upon treatment with a variety of chemical inducers and growth factors. During this process, many cell cycle–related genes are downregulated while differentiation-specific genes are triggered. The retinoblastoma family proteins, pRb, p107, and pRb2/p130, are involved in transcriptional repression of proliferation genes, mainly through their interaction with the E2F transcription factors. We report that pRb2/p130 expression levels increased during differentiation of neuroblastoma cell line LAN-5. On the other hand, both pRb and p107 decreased and underwent progressive dephosphorylation at late differentiation times. The expression of B-myb and c-myb, two targets of the retinoblastoma family proteins, were downregulated in association with the increase of pRb2/p130, which was detected as the major component of the complex with E2F on the E2F site of the B-myb promoter in differentiated cells. Interestingly, E2F4, a preferential partner of p107 and pRb2/p130, was upregulated and underwent changes in cellular localization during differentiation. In conclusion, our data suggest a major role of pRb2/p130 in the regulation of B-myb promoter during neural differentiation despite the importance of cofactors in modulating the function of the retinoblastoma family proteins. J. Cell. Biochem. 67:297–303, 1997.

Key words: retinoblastoma family; pRb; p107; pRb2/p130; neuroblastoma; differentiation; B-myb; c-myb; E2F; promoter

In multicellular organisms, differentiation occurs through the loss of proliferation activity, which accompanies the acquisition of specialized functions. Thus, genes that contribute to cell cycle homeostasis are potentially relevant for differentiative processes as well. In vitro and in vivo studies indicate that the retinoblastoma (RB) family genes, namely *RB*, *p107*, and *Rb2/p130*, are involved in differentiation and proliferation pathways [Paggi et al., 1996]. The prototype of the *RB* family gene products is pRb, a well-known tumor suppressor [Weinberg, 1995], which shares many structural and functional similarities with p107 and pRb2/p130. All these proteins are characterized by a peculiar "pocket" structure, which mediates binding to other cellular and viral proteins [Paggi et al., 1996].

Changes in the expression levels and in the phosphorylation status of RB family proteins have been detected in the progression through the cell cycle as well as in several differentia-

Abbreviations: BSA, bovine serum albumin; DOC, deoxycholic acid, sodium salt; EDTA, ethylenediaminetetracetic acid, disodium salt; HEPES, N-[2-hydroxyethyl]piperazine-N′-2-ethanesulfonic acid; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinyldifluorene; RA, all-trans retinoic acid; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.

Contract grant sponsors: AIRC, Ministero della Sanità, CNR-ACRO, Sbarro Institute for Cancer Research and Molecular Medicine, and Council for Tobacco Research; Contract grant sponsor: NIH; Contract grant number: RO1 CA60999-01A1.

*Correspondence to: Marco G. Paggi, Laboratory for Cell Metabolism and Pharmacokinetics, Center for Experimental Research, Regina Elena Cancer Institute, Via delle Messi d'Oro 156, 00158 Rome, Italy. E-mail: paggi@ifo.it

Received 1 August 1997; Accepted 4 August 1997

tion systems [Sidle et al., 1996; Herwig and Strauss, 1997]. Since RB family proteins are often expressed simultaneously and recognize, although with different affinities, the same cellular targets, it is challenging to single out the functional role of each protein of this family during multistep events, such as differentiation. During the cell cycle, the activity of RB family proteins is regulated by cyclin-dependent kinases [MacLachlan et al., 1995] and modulated by interaction with different members of the E2F family [Moberg et al., 1996; Beijersbergen and Bernards, 1996]. The results, obtained by single [Lee et al., 1992; Jacks et al., 1992] and double [Cobrinik et al., 1996] knockout experiments of the *RB* family genes, indicate their nonoverlapping functions. Recent data suggest a cooperative effect of *p107* and *Rb2/p130* in controlling several gene targets [Hurford et al., 1997].

Several cell cycle–related genes are known to be controlled by the RB family proteins [Mudryj et al., 1990; Lam and Watson, 1993; Lam et al., 1995], among them B-myb and c-myb. B-myb is particularly interesting, since its downmodulation is a necessary prerequisite for the establishment of a differentiated phenotype in several cellular types [Raschellà et al., 1995; Bies et al., 1996]. Its promoter contains an E2F site [Lam and Watson, 1993; Lam et al., 1995] which is not occupied in cycling cells where B-myb is expressed [Zwicker et al., 1996]. On the contrary, the E2F site appears to have an important role in the negative regulation of *B-myb* promoter through the formation of E2F complexes with RB family proteins [Sala et al., 1996]. Transcriptional repression seems to be the main mechanism acting in the control of *B-myb* expression during neuroblastoma cell differentiation [Raschellà et al., 1996].

Neuroblastoma (NB), the most common solid tumor of childhood, arises from embryonic neural crest cells [Helman et al., 1987]. In vitro NB cell lines often maintain the differentiation potential of embryonic neuroblasts [Tsokos et al., 1996], thus representing a good homogeneous cellular context for investigating gene modulations occurring during differentiation.

In order to follow the changes in expression level and in DNA binding activity of RB family proteins and of their E2F partners during neural differentiation, we used the LAN-5 human NB cell line [Seeger et al., 1982]. Our results pointed out the role of pRb2/p130 in binding *B-myb* promoter and the changes of expression level of E2F4 as well as its cellular localization in differentiated cells.

METHODS

Cell Line

The human NB cell line LAN-5 was maintained in culture and induced to differentiate with RA (Sigma-Aldrich, Milan, Italy) as described [Raschellà et al., 1992, 1995].

Western Blot Analysis

Cell lysis, SDS-PAGE (5×10^5 cells/lane), and protein transfer to a PVDF membrane were done as described [Baldi et al., 1995]. pRb was detected using the G3-245 monoclonal antibody (PharMingen, San Diego, CA); p107 levels were determined using a rabbit polyclonal antibody (sc-318; Santa Cruz Biotechnology, Inc. Santa Cruz, CA); polyclonal antibodies for pRb2/p130 and B-myb detection were described elsewhere [Pertile et al., 1995; Raschellà et al., 1996]; the polyclonal antibody to c-myb was C-19 (Santa Cruz Biotechnology); polyclonal antibodies to E2F1 and E2F4 were C-20 and C-108 (Santa Cruz Biotechnology); anti-HSP70 (Ab1) monoclonal antibody was purchased from Oncogene Science Inc. (Cambridge, MA). Bands were detected using the ECL reagents (Amersham, Milan, Italy) for chemiluminescence and quantified using the ImageQuant 3.3 software (Molecular Dynamics, Sunnyvale, CA).

Electrophoretic Mobility Shift Assays (EMSA)

Total cell lysates and EMSAs were done as described [Lam and Watson, 1993]. Briefly, 15 µg of total extract was incubated in a volume of 20 µl containing 20 mM HEPES (pH 7.9), 20 mM NaCl, 2 mM $MgCl_2$, 10% glycerol, 1 mM PMSF, 20 µg DNAse-free BSA, 2 µg sonicated salmon sperm DNA, and 200 pg ^{32}P-labeled double-stranded oligonucleotide spanning the E2F site in the human B-myb promoter [Lam et al., 1995]. Incubation was carried out for 20 min on ice. When indicated, DOC was added to the mix after 10 min of incubation on ice. DOC concentrations were 0.2, 0.6, and 1.2%. In some experiments, a pRb2/p130-specific polyclonal antibody [Pertile et al., 1995] was added to the mix at a 1:3 dilution in TNE 1X (10 mM Tris-HCl, pH 8.1, 1 mM EDTA, 100 mM NaCl). In control reactions, nonimmune antibody at the same dilution was added.

Indirect Immunofluorescence

Cells were grown in Labtek chamber slides (Nunc Inc., Naperville, IL) for the appropriate time, rinsed twice with PBS, fixed for 10 min in cold (−20°C) aceton:methanol (67:33 v/v), and incubated with a polyclonal antibody to E2F4 (C-108) diluted 1:20, as previously described [Negroni et al., 1991].

RESULTS

Detection of RB and Myb Proteins During NB Differentiation

The expression of the RB family and of B-myb and c-myb gene products was determined during LAN-5 human NB differentiation. Western blot analysis was carried out at the time points after RA induction indicated in Figure 1A. The levels of expression of pRb and p107, after an initial increase at early differentiation times (2–8 h), decreased at late times (3–10 days). In addition, the migration pattern of pRb and p107 varied during the differentiative pathway. In fact, a progressive prevalence of a faster migrating, dephosphorylated band was detectable in both cases, being for pRb the only form detectable after 10 days of RA induction. Unlike pRb and p107, the levels of expression of pRb2/p130 markedly increased throughout the differentiation process. Both c-myb and B-myb proteins, during differentiation, showed a marked downregulative trend, particularly evident for c-myb. The expression levels of the analyzed proteins were quantified, normalized against HSP70 expression, and plotted (Fig. 1B). Interestingly, the sharp decrease of myb gene products paralleled the dephosphorylation of the RB family proteins and the increase of pRb2/p130.

pRb2/p130 Binding to the E2F Site of the *B-myb* Promoter During Differentiation

The results of our Western blot analysis, together with the presence of E2F sites on the *B-myb* and *c-myb* promoters [Lam and Watson, 1993; Lam et al., 1995; Mudryj et al., 1990], were suggestive of a possible relationship between the expression of the RB and the myb proteins. We carried out a series of EMSAs on LAN-5 cell lysates, using a synthetic oligodeoxynucleotide spanning the E2F site in the promoter region of the human *B-myb* gene [Lam et al., 1995] (Fig. 2A). Two bands (dubbed *a* and *b*) were specifically competed by an excess of unlabeled oligodeoxynucleotide. The overall pattern in EMSA did not change during differentiation (not shown). In order to evaluate the presence of protein-protein-DNA interactions, a similar EMSA was carried out in the presence of increasing amounts of DOC, which has the ability to dissociate protein-protein bonds maintaining, up to a certain concentration, protein-DNA interactions [Moberg et al., 1992]. The specific band *a* disappeared at low DOC concentrations, suggesting that protein-protein interactions

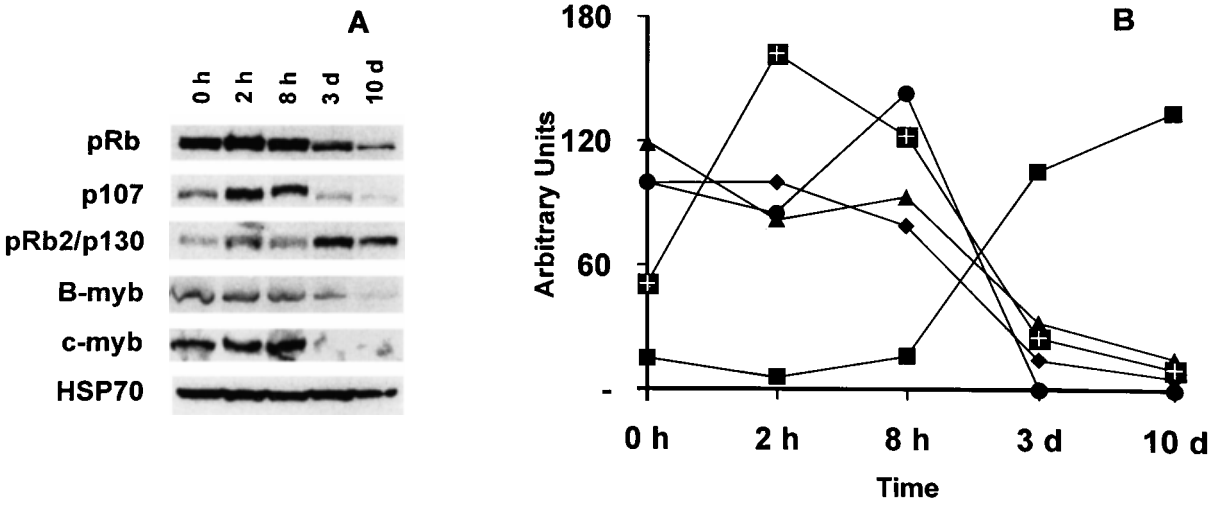

Fig. 1. Expression levels of RB family proteins, B-myb, and c-myb during NB differentiation. **A:** NB cell line LAN-5 was induced to differentiation with RA, and cells were harvested at the indicated times. h, hours; d, days. Western blot analysis was done after protein separation by 6.5% SDS-PAGE. **B:** The blots in A were quantified by densitometric scanning, normalized against the content of HSP70, and plotted. Diamonds, pRb; crossed squares, p107; solid squares, pRb2/p130; triangles, B-myb; circles, c-myb. Time values on the abscissa are not drawn in scale.

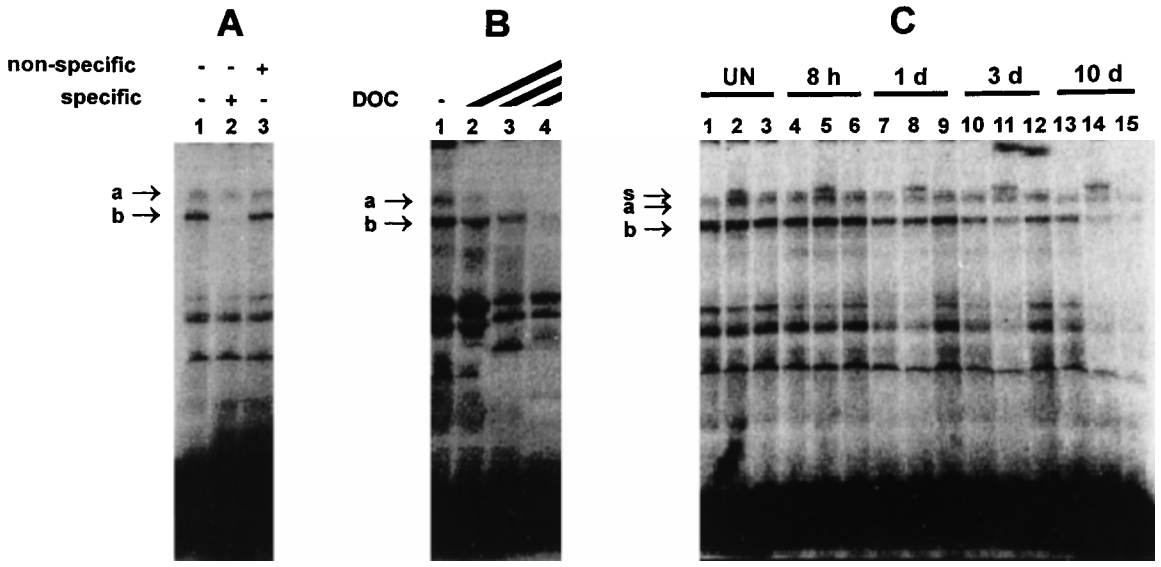

Fig. 2. Characterization of E2f binding complexes on the B-*myb* promoter during NB differentiation. Cellular extracts were prepared from uninduced and RA-treated LAN-5 cells. h, hours; d, days. EMSAs were carried out as described in Materials and Methods. **A:** EMSA using uninduced cellular extract in the absence (**lane 1**) and in the presence of specific (**lane 2**) and nonspecific (**lane 3**) competitor. Specific bands are indicated as *a* and *b*. **B:** EMSA using uninduced cell extract in the absence and in the presence of increasing amounts of DOC (0.2, 0.6, and 1.2%). **C:** EMSA with uninduced and with RA-treated cellular extracts in the absence (**lanes 1,4,7,10,13**) or in the presence of a specific antibody to pRb2/p130 (**lanes 2,5,8,11,14**). Control nonimmune antibody was used in **lanes 3,6,9,12,15.**

were involved in its composition. On the other hand, band *b* disappeared only at high DOC concentrations, (1.2%), which was suggestive of direct protein-DNA interaction (Fig. 2B). The same pattern was detected throughout differentiation (not shown). Since our Western blot analysis indicated an increase in the levels of pRb2/p130 during LAN-5 differentiation, we reasoned that pRb2/p130 could be a component of one of the complexes formed on the E2F site of the B-myb promoter. Thus, we carried out an EMSA incubating the LAN-5 cell lysates, at different times of differentiation, with a specific antibody to pRb2/p130 protein (Fig. 2C). Band *b* was supershifted (dubbed *s*) by the antibody, indicating that pRb2/p130 was part of the complex. Interestingly, the supershifted band *s* increased during differentiation to become prevalent at late times (10 days in Fig. 3C).

Modulation of E2F4 Expression During Differentiation

Since pRb2/p130 and p107 bind preferentially to E2F4 and pRb to E2F1, E2F2, and E2F3 [Paggi et al., 1996], we carried out Western blot experiments to detect the levels of E2F1 and E2F4 proteins during differentiation of LAN-5 cells. Figure 3A shows that the expression of E2F1 remained substantially unchanged. On the other hand, the levels of E2F4 were low in cells in basal growth conditions and increased dramatically at early times of differentiation, to remain high up to 10 days. The expression levels of the analyzed proteins were quantified, normalized against HSP70 expression, and plotted (Fig. 3B).

Cellular Localization of E2F4 During NB Differentiation

Recently, it has been reported that the cellular localization of E2F4 changes during the cell cycle [Lindeman et al., 1997]. Since differentiated NB cells are mostly in G_0/G_1 phase [Negroni et al., 1991] (data not shown), we detected by indirect immunofluorescence E2F4 localization in LAN-5 cells before and 10 days after induction of differentiation. In cycling undifferentiated cells (Fig. 4A,B), E2F4 was detected as a spotty fluorescence both in the nucleus and in the cytoplasm. In differentiated cells (Fig. 4C,D), fluorescence was more intense and localized in the nucleus and in the perinuclear region, while it was completely negative along neurites (Fig. 4D, arrow).

DISCUSSION

Human NB cell lines represent a useful in vitro model to study the molecular mechanisms

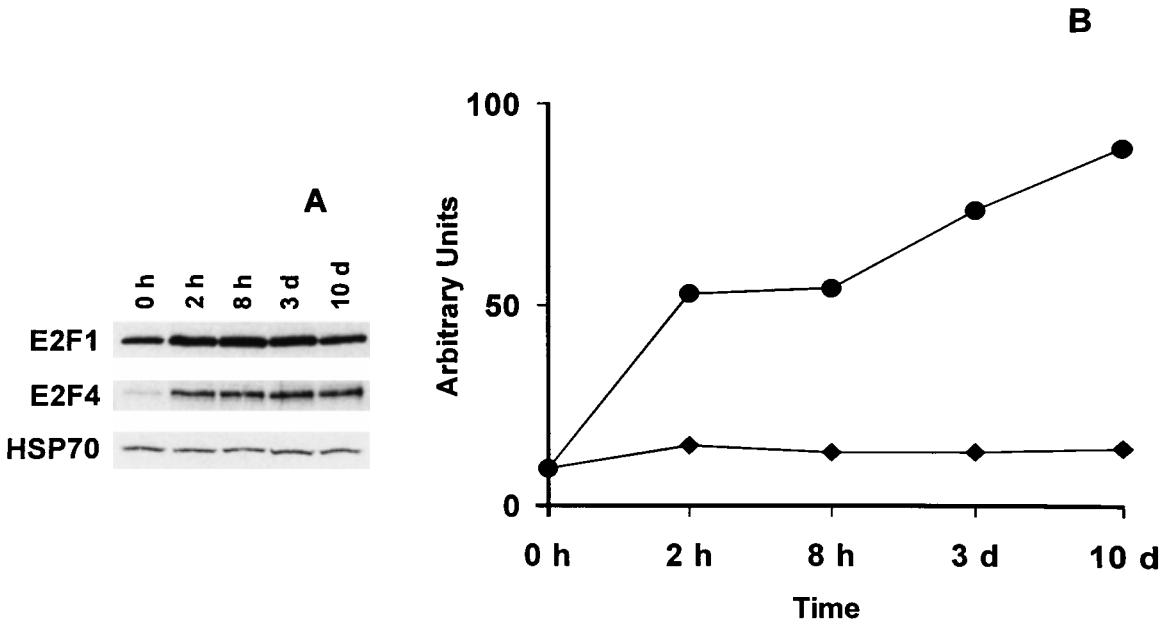

Fig. 3. Expression levels of E2F1 and E2F4 proteins during NB differentiation. h, hours; d, days. **A:** LAN-5 cells were treated as described in Fig. 1, and Western blot analysis was done after protein separation by 10% SDS-PAGE. **B:** The blots in A were quantified by densitometric scanning, normalized against the content of HSP70, and plotted. Diamonds, E2F1; circles, E2F4. Time values on the abscissa are not drawn in scale.

underlying the differentiative processes of neuroectodermal cells [Tsokos et al., 1996] and possibly to find targets which can be used for the differentiation therapy of this neoplasia [Lotan et al., 1990]. In this report, we describe the increase of pRb2/p130 during neuronal differentiation of NB cells and the parallel decrease of B-myb and c-myb. Previous experiments showed that the negative regulation of both c-myb and B-myb take place at transcriptional level [Thiele et al., 1988; Raschellà et al., 1996]. Transcriptional repression of the *B-myb* gene occurs, at least in part, through an E2F site in the promoter region when RB family proteins form complexes with E2F factors bound to DNA [Zwicker et al., 1996]. Although binding of RB family proteins to E2F on the E2F site of the *B-myb* promoter does not seem sufficient, it is necessary to exert optimal transcriptional repression [Bennett et al., 1996]. Our EMSA experiments detected an E2F-pRb2/p130 complex on the E2F site of the human *B-myb* promoter, which became prevalent at late times of neuronal differentiation. Since RB family proteins repress transcription when associated with E2F factors bound to E2F sites [Beijersbergen and Bernards, 1996], it is likely that, in the RB family, pRb2/p130 is the major repressor of *B-myb* transcription in fully differentiated NB cells. It is interesting to compare our data with a recent finding in the adipose tissue in which increased levels of pRb2/p130 and a major E2F-pRb2/p130 complex are detected, in growth-arrested postconfluent preadipocytes and in fully differentiated adipocytes, to drop during the clonal expansion phase, when p107 levels increase [Richon et al., 1997]. Thus, in adipocyte differentiation, the kinetics of E2F-pRb2/p130 complex formation is biphasic and linked to the proliferative state. In NB cells, where the differentiation pathway proceeds together with the loss of proliferation capability, we found that the formation of the E2F-pRb2/p130 complex on the E2F site in the *B-myb* promoter steadily increased during differentiation, paralleling the increase in the levels of pRb2/p130. Analyzing the protein levels of E2F1 and E2F4, which are the partners of pRb and p107 and/or pRb2/p130, respectively, [Paggi et al., 1996], we found that E2F1 did not undergo significant modulation during differentiation, while E2F4 expression levels markedly increased soon after differentiation induction. Accordingly, we demonstrated an increased amount of E2F-pRb2/p130 complex on the E2F site in the *B-myb* promoter during neuronal differentiation, since pRb2/p130 associates preferentially with E2F4 [Beijersbergen and Bernards, 1996]. Our data

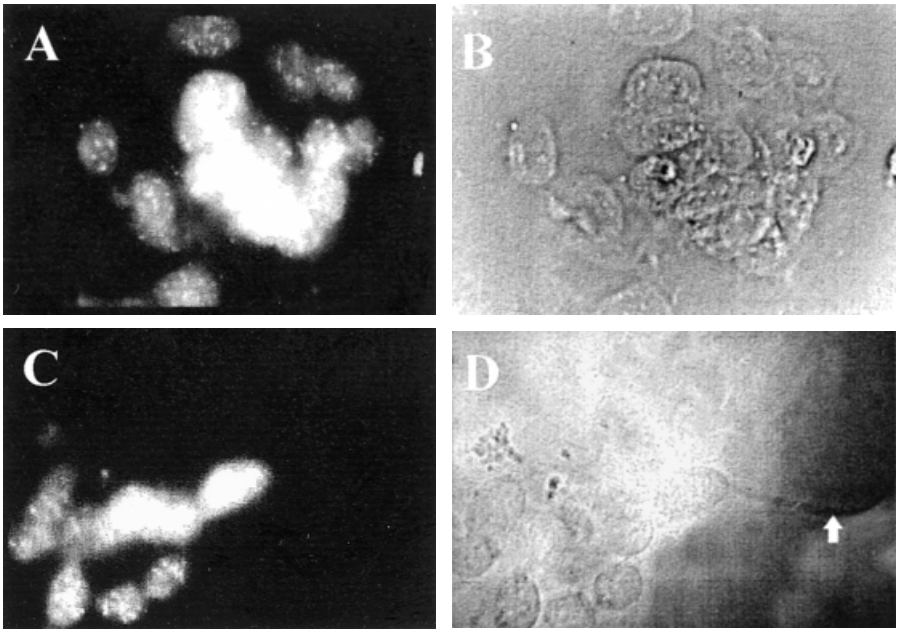

Fig. 4. Cellular localization of E2F4 protein in NB cells. Indirect immunofluorescence staining was carried out in uninduced (**A,B**) and RA-treated (10 days) LAN-5 cells (**C,D**). E2F4-specific immunofluorescence is shown in A and C. The corresponding fields are shown in phase contrast (B,D). The arrow in D indicates a neurite.

add support to the hypothesis that E2F factors participate in maintaining a proliferative or a quiescent/differentiated state by shifting their expression [Beijersbergen and Bernards, 1996; Sidle et al., 1996]. Recently, it has been demonstrated that, when E2F4 levels are markedly enhanced, its localization is invariably nuclear; on the other hand, when its levels are reduced, the protein is less compartmentalized [Lindeman et al., 1997]. In addition, the cellular localization of E2F4 seems related to the cell cycle: G_0/G_1 cells display a nuclear distribution, while S/G_2 cells display a cytoplasmic one. The strong upregulation of E2F4 and the accumulation of NB cells in G_0/G_1 phase after differentiation [Negroni et al., 1991] prompted us to evaluate its cellular localization in basal and differentiative conditions. Our finding that E2F4 was mostly nuclear in differentiated NB cells suggests that not only the levels of E2F4 but also its cellular localization can contribute to the transcriptional regulation of *B-myb* during differentiation.

In conclusion, our data confirm the relevance of the RB family proteins in NB differentiation [Kranenburg et al., 1995; Raschellà et al., submitted]. In addition, they also suggest that, where differentiation occurs necessarily through the downregulation of proliferation-related genes [Negroni et al., 1991; Thiele et al., 1985], the negative control of B-myb in differentiated cells could be fully achieved and maintained by the upregulation of pRb2/p130 and of nuclear E2F4 and by the formation of an inhibitory complex E2F-pRb2/p130 on the *B-myb* promoter. The recent report that a domain of the pRb2/p130 molecule acts as an inhibitor of cdk2 kinase activity [De Luca et al., in press] is also consistent with the role we attribute to pRb2/p130 in the negative control of cell proliferation.

ACKNOWLEDGMENTS

The authors thank Dr. A. Sala (Thomas Jefferson University, Philadelphia, PA), Dr. M. Levrero (A. Cesalpino Foundation, Rome, Italy), Dr. A. Felsani (CNR, Rome, Italy), and Dr. A. Baldi (Libero Istituto Universitario C.B.M., Rome, Italy) for useful suggestions and criticism and Dr. R.I. Lewis (University of Nebraska, Omaha, NE) for the anti-B-myb serum. The authors also thank V. Cesi and A. Di Stefano for their helpful technical assistance and M. Di Giovanni for the photographic work. F.B. is supported by an AIRC postdoctoral fellowship. A.D.L. is recipient of a NATO-CNR advanced fellowship.

REFERENCES

Baldi A, De Luca A, Claudio PP, Baldi F, Giordano GG, Tommasino M, Paggi MG, Giordano A (1995): The Rb2/p130 gene product is a nuclear protein whose phosphorylation is cell cycle regulated. J Cell Biochem 59:402–408.

Beijersbergen RL, Bernards R (1996): Cell cycle regulation by the retinoblastoma family of growth inhibitory proteins. Biochim Biophys Acta Rev Cancer 1287:103–120.

Bennett JD, Farlie PG, Watson RJ (1996): E2F binding is required but not sufficient for repression of B-*myb* transcription in quiescent fibroblasts. Oncogene 13:1073–1082.

Bies J, Hoffman B, Amanullah A, Giese T, Wolff L (1996): B-Myb prevents growth arrest associated with terminal differentiation of monocytic cells. Oncogene 12:355–363.

Cobrinik D, Lee MH, Hannon G, Mulligan G, Bronson RT, Dyson N, Harlow E, Beach D, Weinberg RA, Jacks T (1996): Shared role of the pRB-related p130 and p107 proteins in limb development. Genes Dev 10:1633–1644.

De Luca A, MacLachlan TK, Bagella L, Dean C, Howard CM, Claudio PP, Baldi A, Khalili K, Giordano A (1997): A unique domain of pRb2/p130 acts as an inhibitor of cdk2 kinase activity. J Biol Chem 292:20971–20974.

Helman LJ, Thiele CJ, Linehan WM, Nelkin BD, Baylin SB, Israel MA (1987): Molecular markers of neuroendocrine development and evidence of environmental regulation. Proc Natl Acad Sci USA 84:2336–2339.

Herwig S, Strauss M (1997): The retinoblastoma protein: A master regulator of cell cycle, differentiation and apoptosis. Eur J Biochem 246:581–601.

Hurford RK Jr, Cobrinik D, Lee MH, Dyson N (1997): pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. Genes Dev 11:1447–1463.

Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA (1992): Effects of an Rb mutation in the mouse. Nature 359:295–300.

Kranenburg O, Scharnhorst V, van der Eb AJ, Zantema A (1995): Inhibition of cyclin-dependent kinase activity triggers neuronal differentiation of mouse neuroblastoma cells. J Cell Biol 131:227–234.

Lam EW, Watson RJ (1993): An E2F-binding site mediates cell-cycle regulated repression of mouse B-myb transcription. EMBO J 12:2705–2713.

Lam EW, Bennett JD, Watson RJ (1995): Cell-cycle regulation of human B-myb transcription. Gene 160:277–281.

Lee EY, Chang CY, Hu N, Wang YC, Lai CC, Herrup K, Lee WH, Bradley A (1992): Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis [see comments]. Nature 359:288–294.

Lindeman GJ, Gaubatz S, Livingston DM, Ginsberg D (1997): The subcellular localization of E2F-4 is cell-cycle dependent. Proc Natl Acad Sci USA 94:5095–5100.

Lotan R, Francis GE, Freeman CS, Waxman S (1990): Differentiation therapy. Cancer Res 50:3453–3464.

MacLachlan TK, Sang N, Giordano A (1995): Cyclins, cyclin-dependent kinases and Cdk inhibitors: Implications in cell cycle control and cancer. Eukariotic Gene Expr 5:127–156.

Moberg K, Starz MA, Lees JA (1996): E2F-4 switches from p130 to p107 and pRB in response to cell cycle reentry. Mol Cell Biol 16:1436–1449.

Moberg KH, Logan TJ, Tyndall WA, Hall DJ (1992): Three distinct elements within the murine c-myc promoter are required for transcription. Oncogene 7:411–421.

Mudryj M, Hiebert SW, Nevins JR (1990): A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. EMBO J 9:2179–2184.

Negroni A, Scarpa S, Romeo A, Ferrari S, Modesti A, Raschellà G (1991): Decrease of proliferation rate and induction of differentiation by a MYCN antisense DNA oligomer in a human neuroblastoma cell line. Cell Growth Differ 2:511–518.

Paggi MG, Baldi A, Bonetto F, Giordano A (1996): Retinoblastoma protein family in cell cycle and cancer: A review. J Cell Biochem 62:418–430.

Pertile P, Baldi A, De Luca A, Bagella L, Virgilio L, Pisano MM, Giordano A (1995): Molecular cloning, expression, and developmental characterization of the murine retinoblastoma-related gene Rb2/p130. Cell Growth Differ 6:1659–1664.

Raschellà G, Negroni A, Skorski T, Pucci S, Nieborowska-Skorska M, Romeo A, Calabretta B (1992): Inhibition of proliferation by c-myb antisense RNA and oligodeoxynucleotides in transformed neuroectodermal cell lines. Cancer Res 52:4221–4226.

Raschellà G, Negroni A, Sala A, Pucci S, Romeo A, Calabretta B (1995): Requirement of B-*myb* function for survival and differentiative potential of human neuroblastoma cells. J Biol Chem 270:8540–8545.

Raschellà G, Negroni A, Pucci S, Amendola R, Valeri S, Calabretta B (1996): B-myb transcriptional regulation and mRNA stability during differentiation of neuroblastoma cells. Exp Cell Res 222:395–399.

Raschellà G, Tanno B, Bonetto F, Negroni A, Claudio PP, Baldi A, Amendola R, Calabretta B, Giordano A, Paggi MG (submitted): Retinoblastoma family genes induce differentiation in neuroblastoma cells and downregulate B-myb promoter.

Richon VM, Lyle RE, McGehee RE Jr. (1997): Regulation and expression of retinoblastoma proteins p107 and p130 during 3T3-L1 adipocyte differentiation. J Biol Chem 272:10117–10124.

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

Seeger RC, Danon YL, Rayner S, Hoover F (1982): Definition of a Thy-1 determinant on human neuroblastoma, glioma, sarcoma and teratoma cells with a monoclonal antibody. J Immunol 128:983–989.

Sidle A, Palaty C, Dirks P, Wiggan O, Kiess M, Gill RM, Wong AK, Hamel PA (1996): Activity of the retinoblastoma family proteins, pRB, p107, and p130, during cellular proliferation and differentiation. Crit Rev Biochem Mol Biol 31:237–271.

Thiele CJ, Reynolds CP, Israel MA (1985): Decreased expression of N-myc precedes retinoic acid–induced morphological differentiation of human neuroblastoma. Nature 313: 404–406.

Thiele CJ, Cohen PS, Israel MA (1988): Regulation of c-myb expression in human neuroblastoma cells during retinoic acid–induced differentiation. Mol Cell Biol 8:1677–1683.

Tsokos M, Scarpa S, Ross RA, Triche TJ (1996): Differentiation of human neuroblastoma recapitulates neural crest development—study of morphology, neurotransmitter enzymes, and extracellular-matrix proteins. Am J Pathol 128:484–496.

Weinberg RA (1995): The retinoblastoma protein and cell cycle control. Cell 81:323–330.

Zwicker J, Liu NS, Engeland K, Lucibello FC, Müller R (1996): Cell cycle regulation of E2F site occupation in vivo. Science 271:1595–1597.