V-MYB ONCOGENE AND *C-MYB* PROTO-ONCOGENE EXPRESSION IN AVIAN CELLS: MORPHOLOGICAL CHANGES OF THE CELLS AND TOPOGRAPHIC LOCALIZATION OF MYB PROTEINS

J. KORB, J. ŠTOKROVÁ, V. KARAFIÁT, M. DVOŘÁKOVÁ, V. ČERMÁKOVÁ

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 37 Praha 6, Czech Republic

Received February 9, 1996; revised April 10, 1996

Summary. – Morphological changes of avian cells expressing the *v-myb* oncogene or *c-myb* proto-oncogene were studied by means of electron microscopy. Expression of both genes lead to distinct morphological changes of these cells. The nucleus of LSCC-BM2 cells espressing *v-myb* gene was of normal size but usually of irregular shape. It contained large unravelled nucleoli with typical interstices in some cells. Small nucleolar structures were also localized in the periphery of nuclear membrane. Nuclear envelope revealed reduced perinuclear space between two membranes. LSCC-BK3 cells expressing the *c-myb* gene were characterized by distinctly enlarged nucleus, in most cases of irregular shape. It contained only one nucleolus markedly enlarged, often unravelled, with apparent interstitial area. Nucleoli with nucleolonemas were observed in some cells. Nuclear envelope formed by two obscure membranes showed reduced perinuclear space. Topographic localization of v-Myb and c-Myb protein products was not basically different, both being detected in the nucleus of avian cells. v-Myb and c-Myb markers were distributed mostly in clusters, usually associated with interchromatin granules, but some marker was associated also with the nuclear membrane. Both Myb products were never detected in nucleolar structures of avian cells. Morphological changes of avian cells expressing *myb* genes and topographic localization of Myb proteins in these cells were different from those found in the insect cells expressing *myb* genes. The observed differences are discussed.

Key words: avian cells; v-Myb protein; c-Myb protein; topographic localization; morphology

Introduction

c-myb gene belongs to the members of proto-oncogene family many of which play a key role in the regulation of normall cellular growth and differentiation. Although the exact function of most proto-oncogenes is unknown, the protein product of *c-myb*, the $p75^{c\text{-}myb}$, is involved in regulation of differentiation and proliferation of haematopoietic cells (Gewirtz and Callabretta, 1988; Frampton *et al.*, 1995). *v-myb* oncogene represents a truncated and point-mutated version of *c-myb* proto-oncogene (Lipsick and Baluda, 1986) and its product $p48^{v\text{-}myb}$ possesses the characteristics of transcription factors. Both c-Myb and v-Myb are transactivating proteins containing three (c-Myb) and two (v-Myb) repeats of 52 amino acids, highly conserved, which are responsible for their DNA-binding activity (Klempnauer and Sippel, 1987; Biedenkapp *et al.*, 1988). They contain also several domains responsible for the regulation of their transactivating function (Klempnauer *et al.*, 1989; Garrido *et al.*, 1992).

The initial attempts to understand the mechanism of action of oncogenes and proto-oncogenes involved the subcellular localization of their gene products. It was found that *c-myb* proto-oncogene and *v-myb* oncogene belonged to the cellular and viral oncogenes, the products of which reside in the cell nucleus (Boyle *et al.*, 1984; Klempnauer *et al.*, 1984). A precise determination of subcellular distribution of oncogene products and study of their interactions

Abbreviations: DMEM = Dulbecco's modification of Eagle's medium

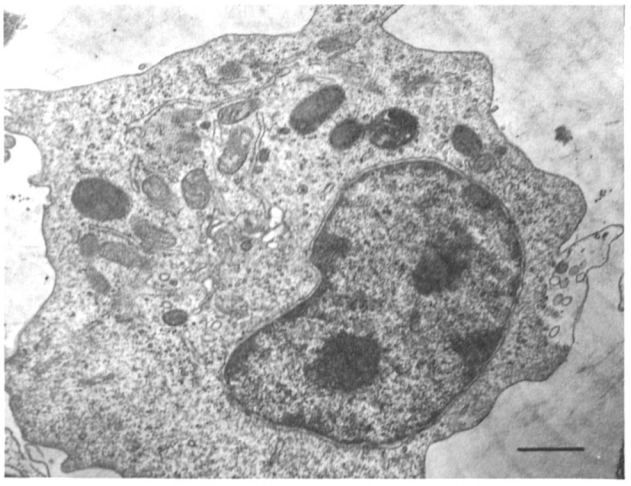

Fig. 1
Morphology of chicken thymocyte.
Bar = 1 μm.

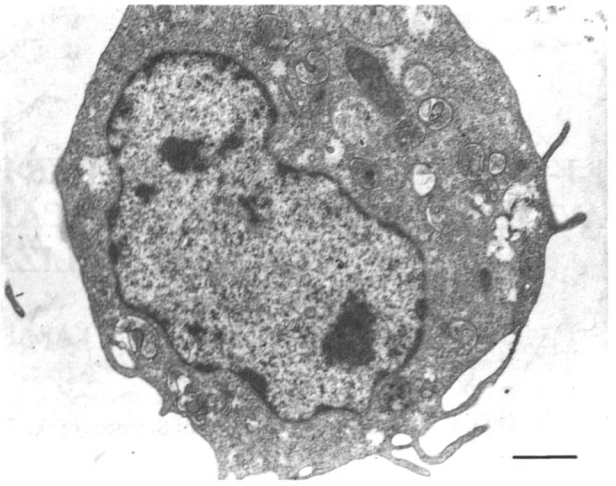

Fig. 2
Morphology of LSSC-BM2 cell expressing *v-myb* gene
Bar = 1 μm.

with cellular structures and their components represent one approach to the understanding of the oncogene functions.

Published data on the subcellular localization of *myb* gene products are rather controversial. Their association with chromatin but also with nuclear matrix was described depending on experimental conditions (Boyle *et al.*, 1985; Evan and Hancock, 1985; Klempnauer, 1988). In human cells c-Myb protein was found in the nucleoplasm, excluding nucleoli (Bading *et al.*, 1989). Since *v-myb* gene product was found also in the cytoplasm of the cells (Klempnauer *et al.*, 1984), its function in cooperation with cytoplasmic structures cannot be excluded.

Our previous studies were focused on the analyses of *v-myb* and *c-myb* gene product interactions with cellular components of insect cells in the baculovirus system expressing *v-myb* and *c-myb* inserts. It was found that expression of these genes was accompanied by extensive changes in the cell structure especially in the nucleus along with typical changes in the nucleolar structure (Štokrová *et al.*, 1995). *myb* gene protein products were localized predominantly in ring-shaped ribonucleoprotein structures in nuclei.

In this work we examined structural changes of avian cells expressing *v-myb* oncogene and *c-myb* proto-oncogene and topographic localization of their protein products in these cells.

Materials and Methods

Cells. Cell lines LSCC-BM2 expressing *v-myb* gene (Moscovici *et al.*, 1982) and LSCC-BK3 expressing *c-myb* (Hihara *et al.*, 1980) were grown in Dulbecco's modification of Eagle's medium (DMEM) containing 8% foetal calf serum, 2% chicken serum and antibiotics (penicillin and streptomycin). Chicken embryo fibroblasts were prepared from 10-day-old embryos and were grown in DMEM containing 4% calf serum, 1% chicken serum and antibiotics. Thymocytes were freshly isolated from the thymuses of the 2 to 3-week-old chicken.

Antibodies. Anti-Myb antibody (rabbit, polyclonal) recognizing v-Myb and c-Myb proteins, and antibody IL19 (rabbit, polyclonal) recognizing 19 amino acids of v-Myb and c-Myb proteins were obtained from Dr. M. Dvořák of this institute.

Electron and immunoelectron microscopy. Ultrastructural studies and immunoelectron microscopy were performed as described previously (Štokrová *et al.*, 1995).

Results and Discussion

Morphological features of the cells expressing myb genes

LSCC-BM2 and LSCC-BK3 cells represent avian haematopoietic cells transformed by avian retroviruses and expressing the *v-myb* and *c-myb* genes, respectively (Moscovici *et al.*, 1982; Hihara *et al.*, 1980). The morphological features of these cells were studied and compared with those of control cells where *myb* genes are not expressed (thymocytes, chicken embryo fibroblasts).

Expression of the *v-myb* oncogene and *c-myb* proto-oncogene in LSCC-BM2 and LSCC-BK3 cells, respectively, led to distinct morphological changes of the cells when compared with those observed in control avian cells.

A typical structure of a thymocyte cell is shown in Fig. 1. In ultrathin sections, the nucleus exhibited a moderately irregular shape and contained usually two or more nucleoli

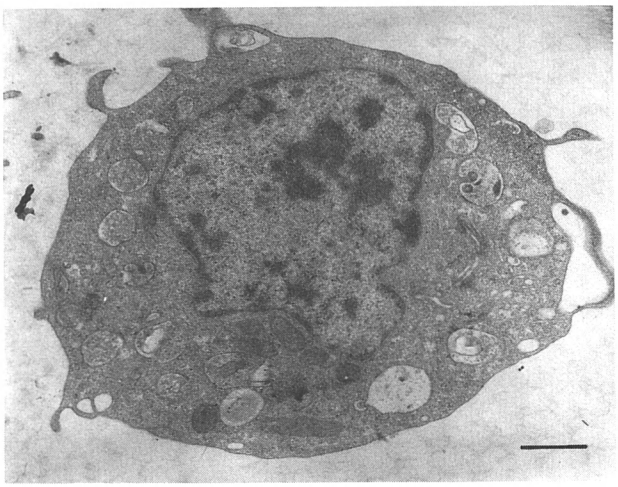

Fig. 3
The same as in Fig. 2 but with unravelled nucleolus
Bar = 1 μm.

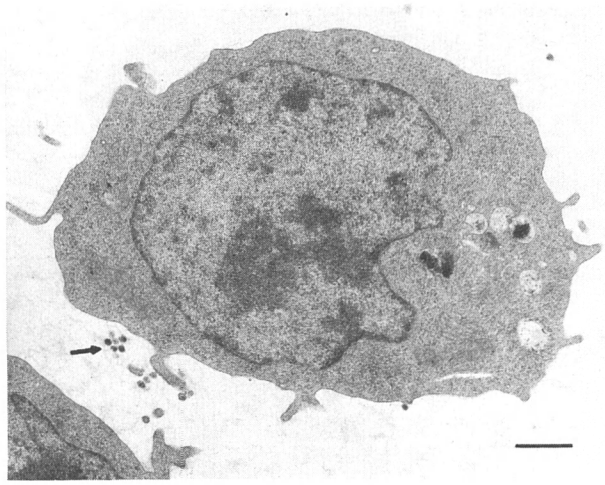

Fig. 4
Morphology of LSCC-BK3 expressing *c-myb* gene
Arrow indicates viral particles (bar = 1 μm).

of compact appearance. Chromatin was sometimes highly condensed with perinuclear localization. Nuclear envelope was made up of two membranes with a perinuclear space between them, whose width (about 0.03 μm) was somewhat irregular. No changes in cytoplasm area were observed in most of the cells.

The morphology of LSCC-BM2 cells expressing *v-myb* gene was different when compared with thymocytes and fibroblast cells. The nucleus of LSCC-BM2 cells was of normal size but usually of irregular shape. It contained one or two compact nucleoli in some cells (Fig. 2), but, in contrast to the control cells, large unravelled nucleoli with typical interstices were present in about 50% of LSCC-BM2 cells (Fig. 3). Small nucleolar structures were sometimes localized in the periphery of nuclear membrane. Perinuclear localization of condensed chromatin was relatively frequently observed, suggesting functional inactivation of some cells. Nuclear envelope formed by two membranes revealed significantly reduced perinuclear space between them (approximately 0.015 μm). In the cytoplasm of LSCC-BM2 cells, extensive vacuolization was observed in most of the cells. Structurally modified mitochondria were sometimes found.

On the other hand, the effect of *c-myb* proto-oncogene expression on cell structure of avian cells was different when compared with the *v-myb* gene expression. In contrast to LSCC-BM2 cells expressing the *v-myb* oncogene, LSCC-BK3 cells expressing the *c-myb* gene revealed distinctly enlarged nucleus, in most cases of irregular shape (Figs. 4, 5). Nuclear envelope was formed by two membranes which were less distinct in LSCC-BK3 than in LSCC-BM2 cells delimiting the perinuclear space to 0.01 — 0.015 μm. The nucleus usually contained only one markedly enlarged nucleolus (Fig. 4), often extensively unravelled (Fig. 5) with apparent interstitial area. Nucleoli with nucleolonemas were observed in some cells. Condensed chromatin was mostly found near the nuclear membrane, however, it was homogenously spread in the nuclear space in some cases. Vacuolization of LSCC-BK3 cells was much less when compared with LSCC-BM2 cells but higher than in control cells. Vacuoles were usually not round-shaped in this case, but rather of irregular shape. The viral particles were often either in vacuoles or in the periphery of cells (Fig. 4, arrow).

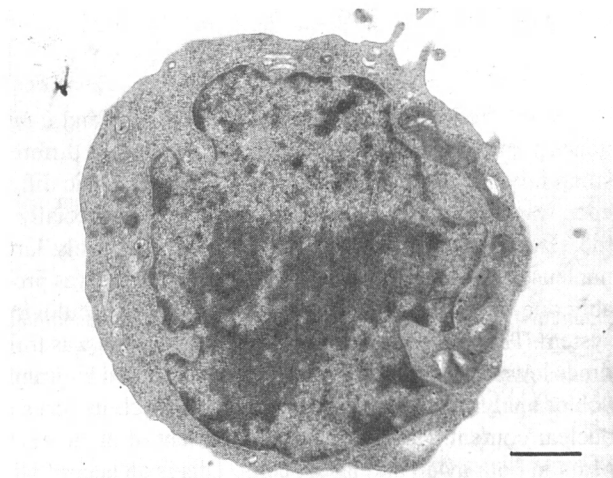

Fig. 5
The same as in Fig. 5 but with unravelled nucleolus
Bar = 1 μm.

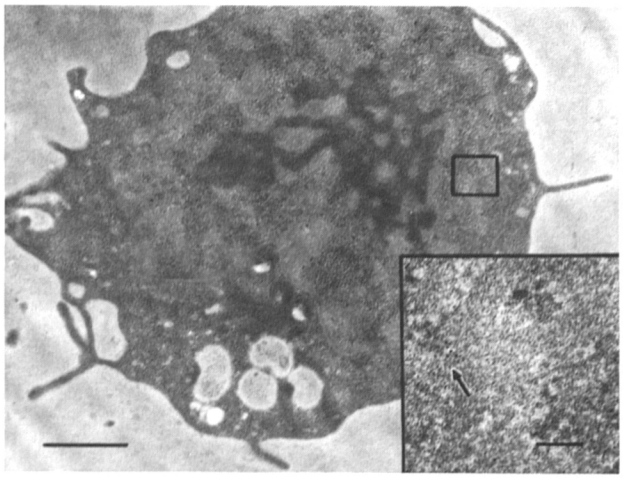

Fig. 6
Immunoelectron microscopic localization of v-Myb protein in LSCC-BM2 cells
Bar = μm. Insert: selected area visualized at high magnification. Arrow indicates cluster of Au-marker (bar = 100 nm).

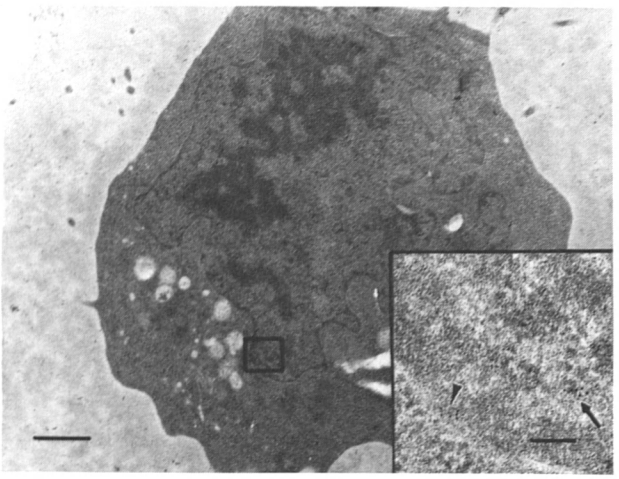

Fig. 7
Immunoelectron microscopic localization of c-Myb protein in LSCC-BK3 cells
Bar = 1 μm. Insert: selected area visualized at high magnification. Arrow indicates clusters of Au-marker, arrowhead indicates labelling of nuclear membrane (bar = 100 nm).

As reported earlier, the expression of *v-myb* and *c-myb* genes in the insect cells was accompanied by quite different stuctural changes (Štokrová *et al.*, 1995). The basic difference was apparent in the size of nucleus and especially in the size and structure of nucelolus. The extremely large nucleus of insect cells expressing the *v-myb* gene was probably due to overexpression of this gene in the baculovirus system. The expression of the *c-myb* gene which was three times lower than that of *v-myb* gene, as revealed by immunoblot analysis, was not accompanied by such increase of nuclear content. However, the enlargement of nucleoli appears in both avian and insect cells. This is an accord with published data that nuclear size is greater in progressing malignant cells of mammals (Wachler and Stahl, 1993) and that nucleolar morphology is often aberrant in cancer cells. Ring-shaped nucleoli typical for cancer cells (Busch and Smetana, 1970) were detected frequently only in the insect cells (Štokrová *et al.*, 1995).

It is well known that the morhology of avian and insect cells differs substantially. Also the expression of *myb* genes, postranslational modifications and cellular transport of their protein products are different in both types of cells. However, the comparison of both types of cells expressing *myb* genes with corresponding control cells (not expressing *myb* genes) revealed that the expression of these genes was acompanied by significant changes in cell morphology. The observed differences in response of avian and insect cells suggest different interactions of the *myb* gene products with structural components of the two cell types.

Topographic localization of v-myb oncogene and c-myb proto-oncogene products

Localization of *myb* gene products in avian cells was studied by immunoelectron microscopy. v-Myb protein was detected in the nucleus of LSCC-BM2 cells similarly as described before in other cell types (Boyle *et al.*, 1984; Klempnauer *et al.*, 1984). This protein product was found predominantly in interchromatin regions of the cell nucleus (Fig. 6). The collodial gold marker was distributed mostly in clusters and it was usually associated with interchromatin granules (Fig. 6, arrow). It is well known that many biological macromolecules involved in transcription and splicing processes are found in these nuclear structures (Puvion *et al.*, 1984; Turner and Franchi, 1987; Gottlieb and Steitz, 1989; Spector *et al.*, 1991). Interchromatin granules are apparently involved in ribosomal biogenesis, however, their exact function still has to be elucidated. Although most of the label was detected in the interchromatin region some marker clusters were associated also with the nuclear membrane suggesting some interaction of v-Myb protein with nuclear matrix.

There was found no basic difference in the topographic localization of c-Myb and v-Myb protein products. The distribution of c-Myb protein in LSCC-BK3 cells is shown in Fig. 7. Also in this case most of the marker was detected in the interchromatin region (Fig. 7, arrow), but in contrast to the v-Myb protein nuclear membrane of LSCC-BK3 cells was more frequently labelled with clusters of gold particles

(Fig. 7, arrowhead). Both v-Myb and c-Myb products were never detected in nucleolar structures of avian cells.

Although Myb protein products have been descriebed to be associated with cell nuclei (Boyle *et al.*, 1984; Klempnauer *et al.*, 1984), published data on their interactions with chromatin and nuclear matrix differ substantially (Boyle *et al.*, 1985; Klempnauer, 1988; Shen-Ong, 1990). Their localization in avian cells may suggest that Myb proteins preferably interact directly with the transcription complex. This is in accord with data on another product of oncogene also residing in the cells nucleus, the Myc protein, which was co-localized with a small nuclear ribonucleoprotein, raising the possibility that these nuclear constituents may function in related processes (Spector *et al.*, 1987). However, some interaction of Myb proteins with nuclear membrane of avian cells was also detected, suggesting possible cooperation with nuclear matrix. Some nuclear matrix proteins are known to play a role of transcription factors (Guo *et al.*, 1995).

Topographic localization of Myb proteins in avian cells is substantially different from those found in insect cells infected with recombinant baculovirus containing *v-myb* or *c-myb* genes (Štokrová *et al.*, 1995). Whereas our data obtained on insect cells indicated preferential interaction of Myb proteins with nucleolar structures, their interactions with other components were much less intensive. Despite basic difference in structure of both cell types and *myb* gene expression, these findings revealed different interaction of Myb proteins with structural components of avian and insect cells, suggesting that they might participate in different processes.

Acknowledgements. We thank Prof. K. Smetana and Dr. I. Raška for valuable discussion. This work was supported by grant No. 304/93/0210 from Grant Agency of the Czech Republic.

References

Bading H, Rauterberg EW, Moelling K (1989): Distribution of *c-myc, c-myb* and *Ki-67* antigens in interphase and mitotic human cells evidenced by immunofluorescence staining technique. *Exp. Cell Res.* **185**, 50–59.

Biedenkapp H, Borgmeyer U, Sippel AE, Klempnauer K-H (1988): Viral *myb* oncogene encodes a sequence-specific DNA-binding activity. *Nature* **335**, 835–837.

Boyle WJ, Lampert MA, Lipsick JC, Baluda MA (1984): Avian myeloblastosis virus and E26 virus oncogene products are nuclear proteins. *Proc. Natl. Acad. Sci. USA* **81**, 4265–4269.

Boyle WJ, Lampert MA, Li AC, Baluda MA (1985): Nucleare compartmentalization of the *v-myb* oncogene product. *Med. Cell. Biol.* **5**, 3017–3023.

Busch H, Smetana K (1970): *The Nucleolus.* Academic Press, Press, New York and London, pp. 105-114.

Evan GI, Hancock DC (1985): Studies on the interaction of the human *c-myc* protein with cell nuclei: $p62^{c\text{-}myc}$ as a member of discrete subset of nuclear proteins. *Cell* **43**, 253–261.

Frampton J. McNagny K, Sieweke M, Philip A, Smith G, Graf T (1995): v-Myb DNA binding is required to block thrombocytic differentiation of Myb-Ets-transformed multipotent haematopoietic progenitors. *EMBO J.* **14**, 2866–2875.

Garrido C, Grasser F, Lipsick JS, Stéhelin D, Saule S (1992): Protein truncation is not required for *c-myb* proto-oncogene activity in neuroretina cells. *J. Virol.* **66**, 6773–6776.

Gewirtz AM, Calabretta B (1988): A *c-myb* antisense oligodeoxynucleotide inhibits normal human hematopoiesis *in vitro*. *Science* **242**, 1303–1306.

Gottlieb E, Steitz JA (1989): Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J.* **8**, 851–861.

Guo B, Odgren PR, van Winen AJ, Last TJ, Nickerson J, Penman S, Lian JB, Stein GS (1995): The nuclear matrix protein NMP-1 is the transcription factor YY1. *Proc. Natl Acad. Sci. USA* **92**, 10526–10530.

Hihara H, Yamamoto H, Arai K, Okazaki W, Shimizu T (1970): Conditions for successful cultivation of tumor cells from chickens with avian lymphoid leukosis. *Avian Dis.* **24**, 971–979.

Klempnauer K-H, Symonds G, Evan GI, Bishop JM (1984): Subcellular localization of proteins encoded by oncogenes of avian myeloblastosis virus and avian leukemia virus E26 and by the chicken *c-myb* gene. *Cell* **37**, 537–547.

Klempnauer K-H, Sippel AE (1987): The highly conserved amino-terminal region of the protein encoded by the *v-myb* oncogene functions as a DNA-binding domain. *EMBO J.* **6**, 2719–2725.

Klempnauer K-H (1988): Interaction of Myb proteins with nuclear matrix *in vitro*. *Oncogene* **2**, 545–551.

Klempnauer K-H, Arnold H, Biedenkapp H (1989): Activation of transcription by *v-myb*: evidence for two different mechanism. *Genes Develop.* **3**, 1582–1589.

Lipsick JS, Baluda MA (1986): The *myb* oncogene. In Papas TS, Vande Woude GF (Eds): *Gene Amplification Analysis.* Vol. 4, Oncogenes, Elsevier, Amsterdam, pp. 73–98.

Moscovici C, Zeller N, Moscovici MG (1982): Continuous lines of AMV-transformed nonproducer cells: growth and oncogenic potential in the chick embryo. In Revotella RF (Ed.): *Expression of Differential Functions of Cancer Cells.* Raven Press, New York, pp. 435–449.

Puvion E, Viron A, Xu FX (1984): High resolution autoradiographical detection of RNA in the interchromatin granules of DRB-treated cells. *Exp. Cell Res.* **152**, 357–367.

Shen-Ong GLC (1990): The *myb* oncogene. *Biochim. Biophys. Acta* **1032**, 39–52.

Spector DL, Watt RA, Sullivan NF (1987): The *v-* and *c-myc* oncogene proteins colocalize *in situ* with small nuclear ribonucleoprotein particles. *Oncogene* **1**, 5–12.

Štokrová J, Korb J, Dvořáková M, Čermáková V (1995): Morphological changes of the insect cells in the baculovirus system as a function of *v-myb* and *c-myb* inserts expression

and topographic localization of v-Myb and c-Myb proteins. *Acta Virol.* **39**, 99—108.

Turner BM, Franchi L (1987): Identification of protein antigens associated with the nucleare matrix and with clusters of interchromatin granules in both interphase and mitotic cells. *J. Cell Sci.* **87**, 269—282.

Wachtler F, Stahl A (1993): The nucleolus: A structural and functional interpretation. *Micron* **24**, 473—500.