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# Human galectin-2: nuclear presence *in vitro* and its modulation by quiescence/stress factors

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**Summary.** Galectins have the particular capacity to interact with distinct proteins, in addition to the typical reactivity of lectins with glycans. Therefore, they can be functionally active when residing at places other than the membrane or extracellular matrix. In fact, nuclear presence of galectins-1 and -3 is solidly documented but it is an open question whether these two cases are exceptional within this lectin family. Thus, galectin-2, which shares 43% sequence identity on the protein level with galectin-1, warrants study in this respect. Based on initial immunohistochemical evidence we herein address the issue as to whether this galectin can join the category of nuclear lectins. To do so we studied different types of cell *in vitro* using an antibody preparation free of cross-reactivity against other tested galectins. The immunocytochemical experiments revealed that galectin-2 was present in nuclei of murine 3T3 fibroblasts and also genetically engineered human colon carcinoma cells with stable ectopic expression. Transport of galectin-2 to the nucleus could be enhanced by physical (UV light), chemical (mitomycin C, serum withdrawal) or cell biological (coculture with stromal cells) treatment modalities. As a means of further characterizing the staining profile cytochemically, a series of markers with well-defined site of residency within the nuclear compartment was tested in parallel. Importantly, no colocalization with galectins-1 and -3 and the splicing factor SC35 was detectable, the former cases also serving as inherent specificity control. In contrast, a similarity was uncovered in the case of the promyelocytic leukemia (PML) protein as marker of PML nuclear bodies. In aggregate, nuclear localization is documented for galectin-2. This attribute should thus not be considered as an exceptional finding confined to galectins-1 and -3. That even closely related family members, here galectins-1 and -2, exhibit distinct intranuclear localization patterns gives ensuing research a clear direction.

**Key words:** Cell nucleus, Lectin, Nucleolus, Nucleostemin, Splicing factor

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## Introduction

Mammalian lectins are versatile regulators of cell adhesion and growth as well as glycoprotein maturation and routing (Gabius, 1997, 2001; Roth, 2002; Buzás et al., 2006; Villalobo et al., 2006). Among them, the family of galectins, whose carbohydrate specificity is directed to ß-galactosides of cellular glycoconjugates, is receiving increasing attention due to these proteins' capacity to be engaged in interactions, not only with carbohydrates, but also with peptide epitopes (Gabius, 1997, 2006; Kaltner and Stierstorfer, 1998; Liu et al., 2002; Gabius et al., 2004). Equally intriguing, evidence has emerged for galectins to be present at different cellular sites. These include the cell surface, the extracellular matrix and space, the cytoplasm and, last but not least, the nucleus (Wang et al., 2004; Smetana et al., 2006). Two cases are at present firmly documented, in which a galectin has nuclear localization. Following initial immunocyto- and histochemical as well as glycohistochemical detection of this feature, a correlation of nuclear presence to differentiation (galectin-1) or proliferative capacity (galectin-3) had then been delineated (Gabius et al., 1986, 1988; Moutsatsos et al., 1987; Vyakarnam et al., 1997, 1998; Choi et al., 1998; Openo et al., 2000). Nuclear entry appears to be by no means coincidental, because both lectins are known to target distinct ligands in this cellular compartment. In detail, they share reactivity with

spliceosomal proteins of Sm core components, such as Gemin4 (Park et al., 2001; Wang et al., 2006). Galectin-3, in addition, binds to RNA and ß-catenin, stabilizes protein-DNA complexes formed at the CRE site of the cyclin D1 promoter and interacts with the thyroid-specific transcription factor TTF-1, with Sufu (suppressor of fused), a binding partner of the Gli family of transcription factors, and importin-α (Laing and Wang, 1988; Wang et al., 1995; Lin et al., 2002; Paron et al., 2003; Paces-Fessy et al., 2004; Shimura et al., 2004; Nakahara et al., 2006). Its rather basic pI of 8.7 (approximately 9.3 for the truncated version) akin to histones might predestine this galectin to interact with nucleic acids. These data, while illustrating progress in the ongoing quest to elucidate nuclear functions of galectins-1 and -3, also prompt the question whether other members of the galectin family, especially proto-type proteins closely related to galectin-1, can exhibit nuclear localization. Indeed, the first respective histochemical evidence had recently been provided for galectin-2 when cataloguing its expression profile in human tissues and tumors (Saal et al., 2005).

The gene for this homodimeric proto-type galectin was first detected in a human HepG2 hepatoma cDNA library. The resulting protein shares 43 % sequence identity compared to galectin-1 (Gitt and Barondes, 1986, 1991; Gitt et al., 1992). Like galectin-1, galectin-2 is capable of exerting a growth-regulatory role on human neuroblastoma and activated T cells *in vitro* (Sturm et al., 2004; André et al., 2005a). Localization in the murine gut in zones with mitotic and undifferentiated epithelial cells intimated a relation to proliferation (Nio et al., 2005). Illuminating a further aspect of functionality, smooth muscle cells in the intima of atherosclerotic plaques, but not quiescent or normal medial cells, were found to express galectin-2 (Ozaki et al., 2004). Evidence for its binding to lymphotoxin-α and colocalization with tubulin in the cytoplasm, the lectin hereby apparently being involved in cytokine secretion, was provided too (Ozaki et al., 2004). That the single-nucleotide polymorphism C3279T in the first intron of the galectin-2 gene was found to be significantly correlated to incidence of myocardial infarction and affected gene transcription by a factor of two strengthens the case for galectin-2 as a risk factor for this disease (Ozaki et al., 2004; Ozaki and Tanaka, 2005).

Having previously analyzed nuclear presence of galectin-1 in squamous cell epithelia in detail (Purkrábková et al., 2003; Klíma et al., 2005), this background and cell system offered the attractive opportunity to address the issue as to whether galectin-2 (experimentally determined pI value = 5.97) can be found in the nucleus in cells *in vitro* when focusing on keratinocytes and fibroblasts. In the first step we detected nuclear presence in cultured cells, also studying epithelial colon cancer cells with ectopic expression in parallel to give results a wider scope. Next, we clarified the question as to whether this parameter can be affected by acquisition of quiescence and stress factors. Of note in this respect, murine 3T3 fibroblasts had been reported to lose nuclear staining for galectin-3 when subject to serum withdrawal to cause quiescence (Moutsatsos et al., 1987). We finally set galectin-2 localization in relation to staining profiles for galectins-1 and -3 and a set of distinct markers by using antibodies against Ki67, the promyelocytic leukemia (PML) protein characteristic of nuclear granules, the splicing factor SC35 and the nucleolar protein nucleostemin. These steps taken, the obtained results detail nuclear presence of galectin-2 and provide the first insights into dynamic regulation of this feature.

## Material and methods

### *Cell culture*

Cells of the murine 3T3 and human LEP19 fibroblast lines were cultured in H-MEM medium (commercial source for cells and medium: SevaPharma, Prague, Czech Republic) containing 10% fetal bovine serum (Biochrom, Berlin, Germany) at 37°C in an atmosphere containing 3.3% $CO_2$. Primary cultures of human dermal fibroblasts and keratinocytes were prepared from five skin specimens obtained during plastic surgery with the informed consent of the patients. D-MEM medium containing 10% fetal bovine serum (Biochrom, Berlin, Germany) was used in the case of dermal fibroblasts (37°C, 5% $CO_2$), H-MEM medium supplemented with 10 % fetal bovine serum, epidermal growth factor (10 ng/ml), hydrocortisone (0.5 μg/ml) and insulin (5 μg/ml) (all received from Sigma-Aldrich, Prague, Czech Republic) in the case of keratinocytes (Chovanec et al., 2004). Fibroblasts from the stroma of a case of basal cell carcinoma (BCC) were also prepared and kept in culture under conditions used for cells from normal skin samples, as described in detail in Lacina et al. (2007). Experiments were performed with BCC-stroma fibroblasts obtained at the 7th subculture (60 days) and the 15th passage (128 days), in the latter case indications for reduced contact inhibition consistently being observed. Routinely, the different fibroblast cultures were kept for one and five days, respectively, before immunocytochemical processing was carried out. Serum depletion to induce cell quiescence was maintained for three or six days. As chemical or physical stress factors, the different types of fibroblasts were treated with mitomycin C (24 μg/ml for three hours) (Sigma-Aldrich, Prague, Czech Republic) without or in coculture with human interfollicular keratinocytes, or were irradiated with UV light using an UV 1000 KL apparatus (Waldmann Lichttechnik GmbH & Co., Villingen-Schwenningen, Germany) emitting a wavelength of 311 nm (narrow band UV light) at a dose of 1.5 J/m$^2$. Cells of the FaDu line established from a squamous cell carcinoma of the hypopharynx were kept in culture using E-MEM medium (Biochrom, Berlin, Germany) with 10% fetal bovine serum. Transfection of

human HCT-15 colon carcinoma cells, proven to lack expression of galectins-1 and -2 by RT-PCR analysis (Lahm et al., 2001), with galectin-specific cDNA inserted into the pcDNA3.1 vector, selection of overexpressing clones using Western blotting and cell staining and maintenance of stable transfectants in culture were performed as described in detail previously (André et al., 2004, 2006).

### *Immunocytochemistry*

Cells were permeabilized by Triton-X100 (0.1% v/v for 10 minutes at room temperature) (Sigma-Aldrich, Prague, Czech Republic) and fixed with 2% paraformaldehyde in phosphate-buffered saline (pH 7.2). Galectins-1, -2 and -3 were localized by means of non-cross-reactive rabbit polyclonal antibodies after incubation for one hour at room temperature at a concentration of 20 $\mu$g/ml (Kaltner et al., 2002; Purkrábková et al., 2003; Saal et al., 2005). To exclude erroneous interpretation of staining profiles the different antibody preparations were checked for cross-reactivity against human galectins-1, -2, -3, -4, -7, -8 and -9, and any contaminating activity against another family member was removed by chromatographic depletion using the respective galectin as affinity ligand. In parallel, presence of galectins-1 and -3 was also assessed by applying commercial monoclonal antibodies (Vector Laboratories, Burlingame, CA, USA), that of Ki67 and PML protein was monitored by a monoclonal antibody from Dako (Brno, Czech Republic) and that of the splicing factor SC35 by a monoclonal antibody from Sigma-Aldrich (Prague, Czech Republic). A commercial polyclonal antibody preparation from goat against nucleostemin (Neuromics, Bloomington, MN, USA) was also added to the panel and applied as described (Lacina et al., 2006). Each antibody of the set of commercial markers was diluted as recommended by the supplier. FITC (fluorescein isothiocyanate)-labeled swine anti-rabbit antibody (SwAR-FITC; AlSeVa, Prague, Czech Republic), TRITC (tetramethylrhodamine isothiocyanate)-labeled donkey anti-goat antibody (Jackson Laboratories, West Grove, PA, USA) and TRITC-labeled goat anti-mouse antibody (Sigma-Aldrich, Prague, Czech Republic), also diluted as recommended by the supplier, were used as second-step reagents. The polyclonal antibodies against galectins were tested together with monoclonal antibodies against the mentioned markers in double-labeling experiments at the single-cell level, as described previously (Fronková et al., 1999). Nuclei were routinely counterstained with DAPI (4',6'-diamidino-2-phenylindole dilactate) (Sigma-Aldrich, Prague, Czech Republic). Control experiments were performed by omitting the incubation with the first-step probe and by introducing antibodies (in the case of monoclonal antibodies taking care of matching isotypes) against determinants (ßIII tubulin) not present in these cell types to pick up antigen-independent signals. Following careful washing to remove unbound second-step reagents the specimens were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA) and examined by fluorescence microscopy using a Nikon Eclipse 90i apparatus (Nikon, Prague, Czech Republic) equipped with filterblocks specific for FITC, TRITC and DAPI, respectively, a high resolution CCD camera and a LUCIA 5.2 computer-assisted image analysis system (Laboratory Imaging, Prague, Czech Republic).

## Results

### *Nuclear presence of galectin-2 in vitro*

Using the non-cross-reactive polyclonal antibody preparation against galectin-2, an antigen-dependent signal was detected in a small number of 3T3 cells (Fig. 1). The signal generally appeared in a punctate pattern in the nucleoplasm, clearly delimited from the nucleolus as shown in Fig. 2A-C. The other tested fibroblast preparations, i.e. LEP cells, as well as dermal or stromal fibroblasts, the latter originating from a basal cell carcinoma, were invariably negative, as were confluent cells of the FaDu hypopharyngeal squamous cell carcinoma line. To flank these experimental data on nuclear presence of galectin-2 *in vitro* after genuine gene expression by further independent evidence we also studied localization after ectopic expression. Following generation of stable clones by transfection with galectin-1/-2-specific cDNA preparations we detected presence of the produced lectin diffusely in nuclei and the cytoplasm of the engineered human HCT-15 colon carcinoma cells (Fig. 3B,C). Control cells, as exemplarily shown for wild-type cells (Fig. 3A), were negative. Within the preparation of galectin-2-producing clones, positivity was pronounced among cells of a diameter of less than 25 $\mu$m. Having thus shown evidence for nuclear presence *in vitro* after regular and ectopic expression, we next addressed the issue as to whether this factor can be controlled by acquisition of quiescence or stress factors.

### *Effect of quiescence/stress factors on nuclear presence*

To turn the cells quiescent we withdrew serum from the culture medium, leading to starvation. Cells responded to this treatment by an increase of nuclear positivity, as judged by the percentage of positive cells and intensity of staining (Fig. 1, Fig. 2J). This parameter change was not reversible by reestablishing nutrient supply through reconstitution of medium with serum (Fig. 1, Fig. 2I). Notably, the serum depletion was effective to induce expression and nuclear localization of galectin-2 in LEP cells and dermal but not stromal fibroblasts (Fig. 1, Fig. 2C). Thus, serum depletion led to quantitative and qualitative changes in nuclear galectin-2 presentation. The same held true for mitomycin treatment as chemical stress factor (Figs. 1, 2D, 4A-C). Interestingly, the presence of interfollicular keratinocytes

in LEP cultures reduced nuclear positivity of these fibroblasts when exposed to mitomycin (Fig. 1), whereas coculture conferred nuclear positivity to dermal fibroblasts, which were negative under routine culture conditions (Fig. 1). That stress could strongly affect nuclear content of galectin-2 was further substantiated by exposure to UV light, resulting in positivity for 3T3 and LEP cells, as well as dermal fibroblasts (Fig. 1, Fig. 2E-G). This effect was dependent on the cell type, because interfollicular keratinocytes failed to be responsive (Fig. 1). Besides these two chemical/physical stress factors, prolonged maintenance in culture leading to reduced contact inhibition was also able to engender nuclear localization in stromal fibroblasts obtained from a basal cell carcinoma (Fig. 2L, Fig. 5A). Albeit rapidly proliferating, the cells lacked reactivity indicative of Ki67 (Fig. 5). Having revealed acquisition of quiescence and stress factors as inducers of nuclear galectin-2 presence, this parameter was next set into relation to the reactivity profiles for distinct nuclear components.

### *Relation between galectin-2 staining and localization of marker proteins*

We first addressed the issue on comparison between the attributes of galectin-2 vs. galectins-1 and -3. At the

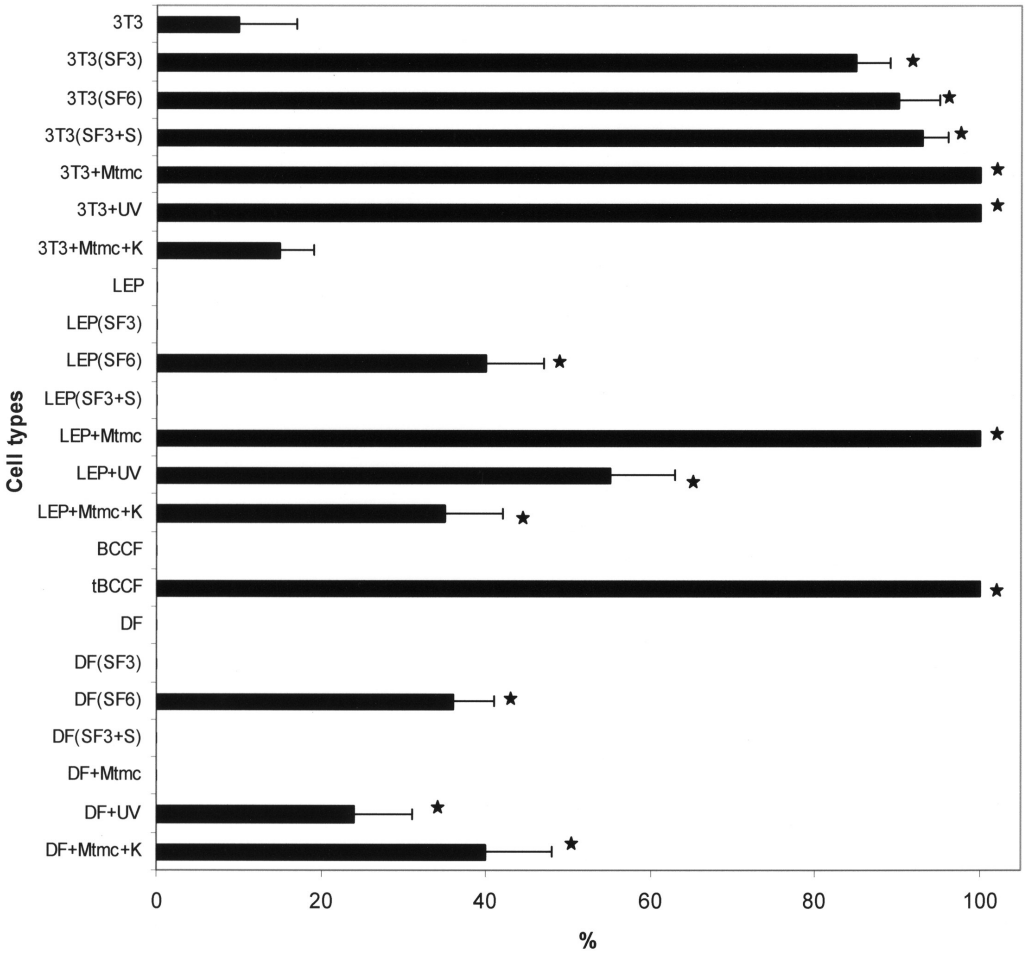
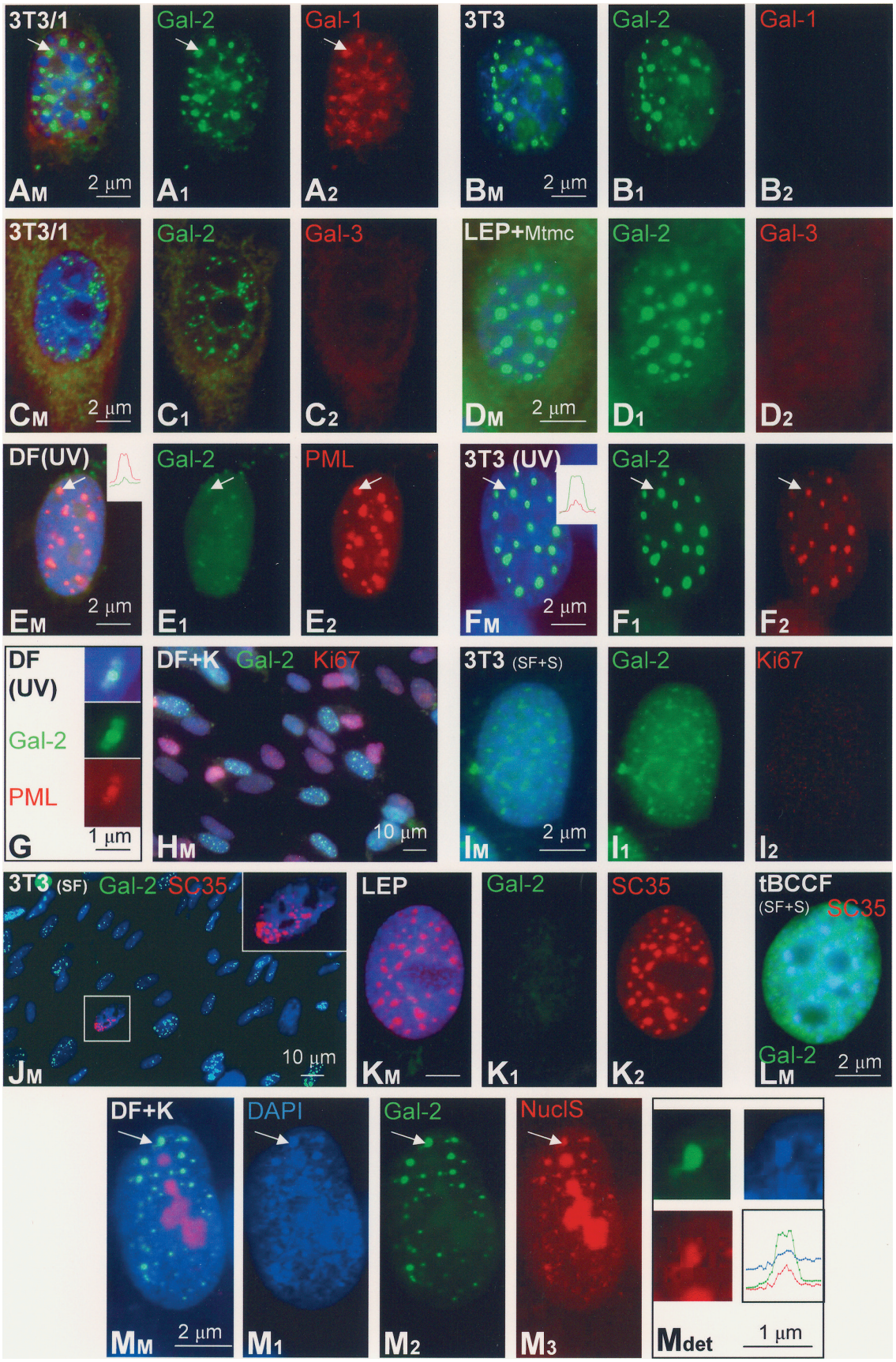



**Fig. 1.** Percentage of galectin-2-positive nuclei in cells of the 3T3 and LEP fibroblast lines and of primary cultures of dermal fibroblasts (DF) as well as in stromal cell populations of basal cell carcinoma cultured for either 60 days (BCCF) or 128 days, then showing phenotypical signs of transformation (tBCCF). In addition to routine culture, dermal fibroblasts exposed to serum-depleted medium for three (SF3) or six (SF6) days or three days with subsequent reconstitution of medium with serum (SF3+S) were studied. Stress factors included treatment with mitomycin C (Mtmc) and UV irradiation (UV). Nuclear positivity was also examined in mitomycin-treated fibroblasts cocultured with normal interfollicular keratinocytes (Mtmc+K). All measurements were performed in triplicates or centuplicates, respectively. Values statistically significantly different from the respective control cell population at $p<0.05$ evaluated by the Student unpaired t-test are marked by an asterisk.

**Fig. 2.** Detection of galectin-2 (green signal; A-M) in 3T3 and LEP cells, in dermal fibroblasts (DF) and fibroblasts from stroma of basal cell carcinoma after 128 days in culture exhibiting signs of transformation (tBCCF), with 3T3 cells being cultured for one day (3T3/1) and tBCCF for five days in the new passage. Parts of the cell populations were treated by mitomycin c (Mtmc) or irradiated with UV light (UV) as given in the respective panel. In addition to galectin-2, the closely related proto-type galectin-1 (red signal; AM, A2, BM, B2), the chimera-type galectin-3 (red signal; CM, C2, DM, D2), the PML protein (red signal; EM, E2, FM, F2, G), Ki67 (red signal; HM, IM, I2 ), the splicing factor SC35 (red signal; JM, KM, K2, LM) and nucleostemin (red signal; MM, M3) were also immunocytochemically detected. Profiles of fluorescence intensity for presence of galectin-2 and the PML protein and for galectin-2, nucleostemin and DAPI in marked granules were measured (EM, FM, MM). Figures marked by (M) document the result of merging the red, green and blue channels. A detailed illustration (Mdet) of a granule positive for galectin-2 and nucleostemin and reactive with DAPI is added for comparison. The blue signal reveals DAPI staining of DNA, visualized in combination with staining of distinct antigens or alone (M1).


3T3/1
Gal-2
Gal-1
AM
2 µm
A1
A2
3T3
Gal-2
Gal-1
BM
2 µm
B1
B2
3T3/1
Gal-2
Gal-3
CM
2 µm
C1
C2
LEP+Mtmc
Gal-2
Gal-3
DM
2 µm
D1
D2
DF(UV)
Gal-2
PML
EM
2 µm
E1
E2
3T3 (UV)
Gal-2
FM
2 µm
F1
F2
DF
(UV)
Gal-2
PML
G
1 µm
DF+K
Gal-2
Ki67
HM
10 µm
3T3 (SF+S)
Gal-2
Ki67
IM
2 µm
I1
I2
3T3 (SF)
Gal-2
SC35
JM
10 µm
LEP
Gal-2
SC35
KM
K1
K2
tBCCF
(SF+S)
SC35
Gal-2
LM
2 µm
DF+K
DAPI
Gal-2
NuclS
MM
2 µm
M1
M2
M3
Mdet
1 µm

initial stages of 3T3 culture with subconfluency the staining profiles of galectins-1 and -2 were rather similar, as though the noted sequence similarity might translate into cytochemically detectable overlap (Fig. 2A). However, the stage of confluent growth reached after five days was characterized by an absence of the galectin-1-dependent signal, only galectin-2 positivity still being present (Fig. 2B). No indication for co-expression with galectin-3 could be discerned, because no expression of Gal-3 was observed (Fig. 2C,D). In order to independently verify these results, we tested commercially available monoclonal antibodies against galectins-1 and -3 in the same experimental series. Their application yielded identical results, fully confirming the data given above. We next monitored nuclear markers not related to galectins.

In contrast to galectins-1 and -3, co-expression of galectin-2 with the nuclear body marker PML protein was picked up (Fig. 2E,F). It is noteworthy that UV irradiation consistently caused a spreading of the signals for these two proteins from the granule in the center of interchromatin space (Fig. 2G). Enzymatic treatment with DNase and RNase revealed conspicuous sensitivity of the galectin-2 signal to RNA digestion (Fig. 6). The granular or diffuse galectin-2-dependent staining was not correlated to Ki67 presence. As already noted above, the case of the stromal fibroblasts kept in long-term culture revealed that proliferation and galectin-2 presence could even be observed without Ki67 positivity (Fig. 2L, 5A). To underscore this lack of correlation to Ki67 expression, keratinocytes with Ki67-positive nucleoli were found to be positive for galectin-2 exclusively in the cytoplasm but not in the nucleus, even after UV irradiation (Fig. 5B). In view of the accumulated evidence on a role of galectins-1 and -3 in pre-mRNA processing, the lack of colocalization between the splicing factor SC35 and galectin-2 served as an internal control (Fig. 2J-L). The expectable colocalization between galectin-1 and this factor could indeed be verified (not shown).

As a marker of nucleoli in proliferating cells, we included nucleostemin. A part of the population of fibroblasts (up to 10%), usually with large nuclei containing galectin-2-positive granules, harbored nucleoli with strong signal for nucleostemin (Fig. 2M). This observation was characteristic only for cells in which granules at the same time positive for galectin-2 and nucleostemin and also reactive with DAPI were detectable. Fig. $2M_{det}$ documents an example. However, the majority of cells with nuclear positivity for galectin-2 exhibited granules lacking presence of nucleostemin and a DAPI-dependent signal. DAPI staining of nuclei was further exploited as approach to relate structural aspects of chromatin to galectin-2 presence because DNA containing chromatin is DAPI-positive. The results obtained after image analysis evaluating homogeneity/heterogeneity within the monitored cell batches revealed that galectin-2-positive nuclei frequently contained

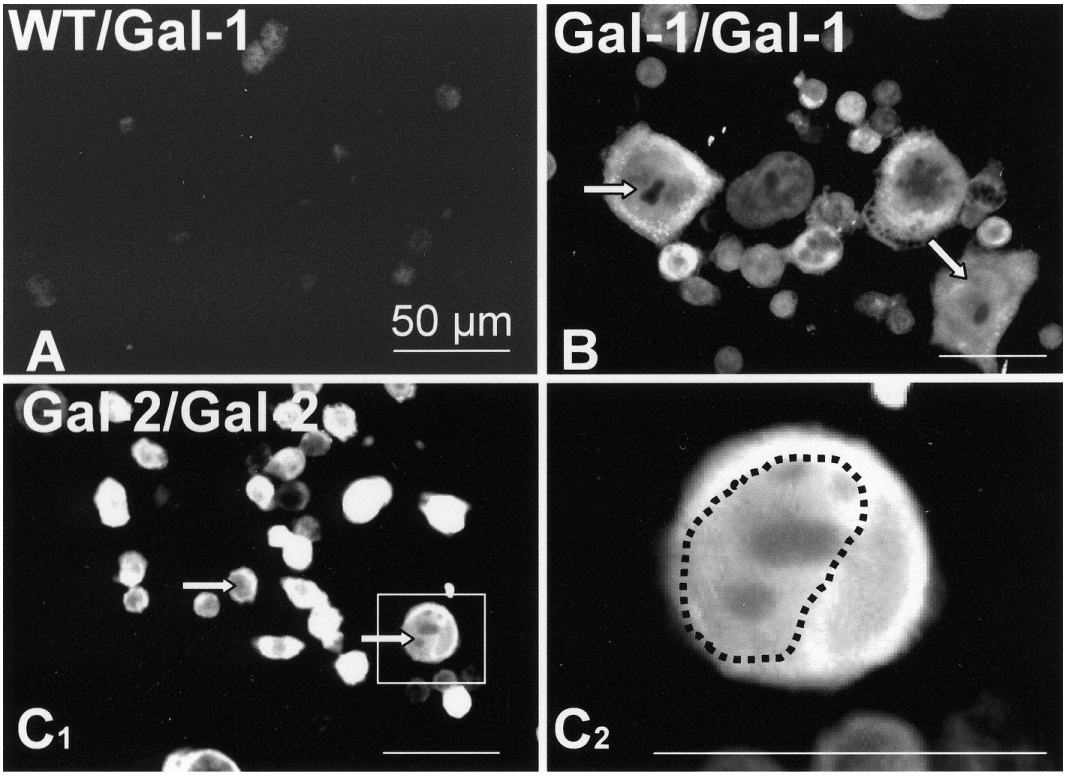



**Fig. 3**. Immunocytochemical detection of galectin-1 (**A, B**) and galectin-2 (**C**) in different cell populations of the human HCT-15 colon carcinoma line. Wild-type cells negative for galectin-1 (**A**) were used to generate the stable transfectants harboring the expression vector with either galectin-1- (**B**) or galectin-2-specific cDNA (**C1**), both exhibiting strong cytoplasmic and moderate nuclear positivity (arrows). Enlargement of a sector illustrates distribution of signal intensity in a galectin-2-positive nucleus (**C2**; encircled area). Arrows mark cells with nuclear positivity.

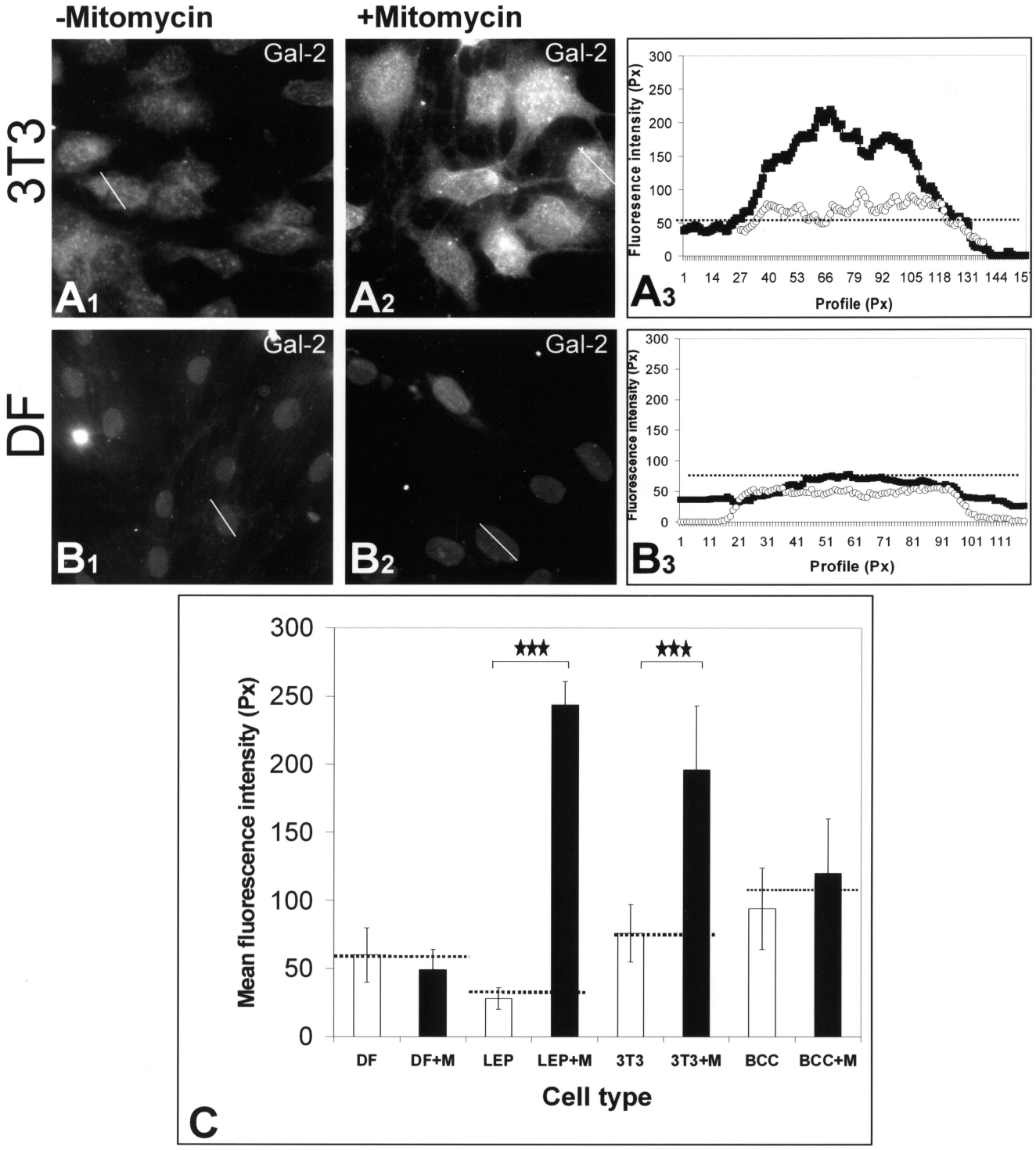



**Fig. 4.** Influence of mitomycin c on the extent of intranuclear presence of galectin-2. Microphotographs of 3T3 cells without **(A1)** and after mitomycin treatment **(A2)** including profiling of fluorescence intensity **(A3)** in the cases of the two cells marked by a white line, which in each case defines the course of profiling, are presented. The same type of monitoring is documented for dermal fibroblasts (DF) **(B)**. **C.** The mean fluorescence intensity for nuclear galectin-2 in at least 500 cells without (white column) and after mitomycin treatment (black column) is shown. Differences for LEP and 3T3 cells were statistically significant at the level of $p<0.001$ (three asterisks) using the non-paired Student t test. The dashed line indicates the level of the non-specific background signal.

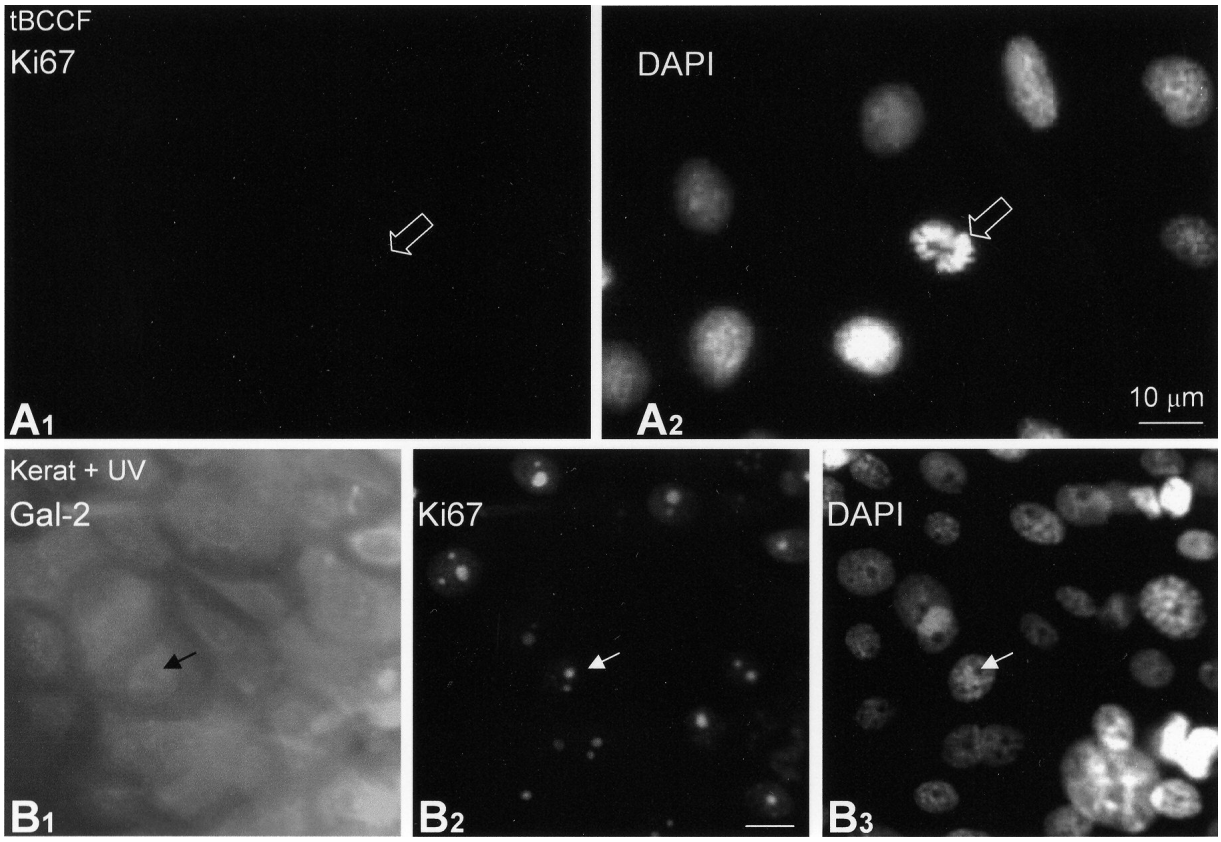



**Fig. 5.** Correlation between the presence of galectin-2 and the Ki67 antigen. "Transformed" BCCF (tBCCF) cells lacking the contact inhibition of growth were negative for Ki67 **(A1)**, although numerous mitotic cells were visible after DNA staining by DAPI **(A2)**. Keratinocytes, even after UV irradiation, were weakly positive for galectin-2 in the cytoplasm and harbored only background signal intensity in nuclei **(B1)**. Ki67-positive nucleoli **(B2)** were frequently seen in these cells. Nuclei were counterstained with DAPI **(B3)**, arrows indicating the position of the same structure in figures processed in distinct color channels.

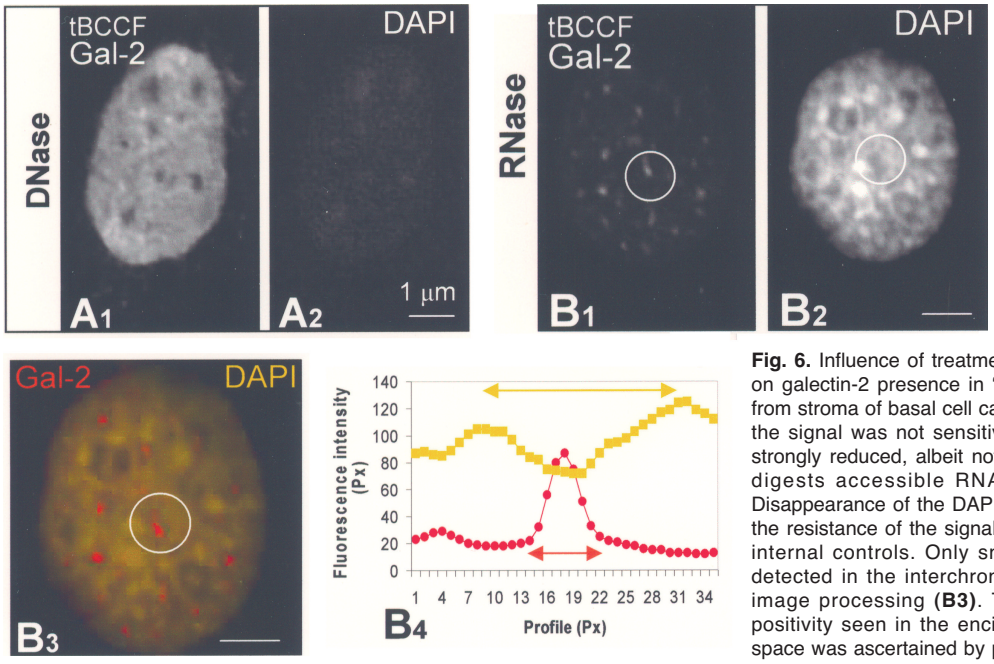



**Fig. 6.** Influence of treatment by DNase and RNase, respectively, on galectin-2 presence in "transformed" BCCF (tBCCF) prepared from stroma of basal cell carcinoma by long-term culture. Whereas the signal was not sensitive to presence of DNase **(A1)**, it was strongly reduced, albeit not fully abolished by RNase **(B1)**, which digests accessible RNA in ribonucleoprotein complexes. Disappearance of the DAPI signal after DNase digestion **(A2)** and the resistance of the signal to RNase treatment **(B2)** are given as internal controls. Only small dots containing galectin-2 were detected in the interchromatin compartment as is visible after image processing **(B3)**. The assignment of residual galectin positivity seen in the encircled area **(B3)** to the interchromatin space was ascertained by profiling the fluorescence intensity **(B4)**, where the red peak representing galectin-2 presence is located in the DNA-free space (yellow signal).

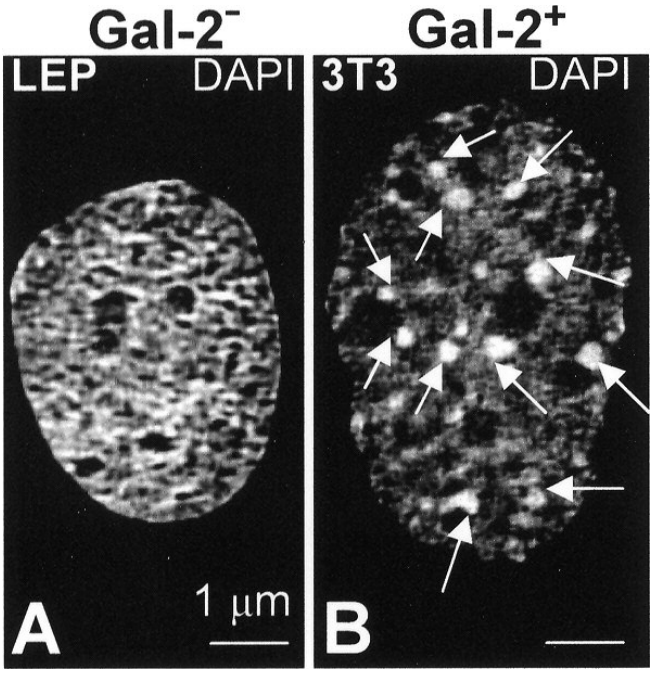



**Fig. 7.** Correlation between the presence of galectin-2 and the characteristics of the DAPI signal. Galectin-2-negative nuclei of LEP cells exhibited dispersed fine chromatin **(A)** as opposed to galectin-2-positive 3T3 cells with numerous DAPI-positive granules (B, arrows). The intensity of the rather diffuse DAPI signal was stronger in galectin-2-negative nuclei (**A**) than in galectin-2-positive nuclei (**B**). Of note, the DNA content of distinct granular structures was characteristic for galectin-2-positive nuclei (**B**).

DAPI-reactive granules in the vicinity of the galectin-2-containing structures (Fig. 2L, 7). Apparently, galectin-2-positive granules were not a common part of heterochromatin in the majority of the studied cells.

## Discussion

Our study demonstrates that a) galectin-2 definitely belongs to the group of nuclear lectins, and b) its nuclear localization is subject to quantitative and qualitative regulation by the proliferative status and exposure to stress factors, intimating functional relevance. In this respect, the disparity of expression profiles for galectin-2 and the splicing factor SC35 and, even more important, the absence of signals for galectins-1 and -3 in fibroblasts at the stage of confluency, which are characterized by strong nuclear positivity for galectin-2, are arguments for non-overlapping roles of the three galectins in nuclei. Despite the high degree of sequence identity this situation also holds true for the mechanistic details during the induction of caspase-mediated apoptosis in activated T cells by the two proto-type galectins. In essence, galectin-1 uniquely recruits caspase-8 (Sturm et al., 2004). Whereas this process is initiated by protein-carbohydrate interaction, glycans of CD7 playing a notable role for galectin-1 also on the clinical level but not galectin-2 (Rappl et al., 2002; Sturm et al., 2004), crucial determinants of the nuclear targets will most likely be peptide motifs. Weak protein-protein interactions were concluded to be responsive to tether galectins-1 and -3 in a mutually exclusive manner to their binding partner(s) in spliceosomes (Wang et al., 2006), and dependence of nuclear transport of galectin-3 on the carbohydrate recognition domain in an ectopic model, as shown by Gaudin et al. (2000), should not be interpreted to infer carbohydrates as ligands. Peptide binding is relevant, too, to account for the reported interaction of galectin-2 with lymphotoxin-α and tubulin (Ozaki et al., 2004; Ozaki and Tanaka, 2005). This evidence combined with the respective information given in the introduction underscores the versatility of distinct galectins to select ligands of glycan and peptide nature. Galectin-1 even harbors potential to perturb membrane properties after binding (Gupta et al., 2006). As the ongoing structural elucidation of the extended binding site of galectin-1 for ganglioside GM1's pentasaccharide and for the oncogenic H-ras attests (Siebert et al., 2003; López-Lucendo et al., 2004; Rotblat et al., 2004), highly selective contacts can be established despite the nominal sugar specificity of galectins to the common ß-galactoside lactose. In other words, not every glycan with common branch-end ß-galactoside qualifies as potent ligand. In addition to ligand structure, the density of natural ligand presentation matters considerably (Wu et al., 2004, 2006, 2007), and functional grading of affinity constants in multivalent systems adds a new level of regulation (Dam et al., 2005). The precise definition of the peptide motifs, which in certain cases have glycomimetic properties when screening peptide libraries (André et al., 2005b, 2007), and their contact sites on the lectin is an arising challenge for structural work and targeting peptides is apparently rather common physiologically within this lectin family is also substantiated by the shared affinity of galectins-3 and -4 for the apoptosis-associated protein nucling (Liu et al., 2004) and will obviously be a fertile field for further research.

With respect to galectin-2's intranuclear localization, the parallel detection of selected markers was helpful to support assignment to intranuclear compartments. Additionally, it served a methodological purpose. Because the intranuclear distribution of galectin-3 in 3T3 fibroblasts was shown to differ when monitored by immunofluorescence and -gold labeling in light and electron microscopy, bringing nucleolar positivity in this cell type into dispute (Hubert et al., 1995), the internal validation by simultaneous marker analysis can help to address this issue properly. As a consequence, the presence of both galectin-2 and the PML protein in nuclear granules is considered to raise solid evidence for inclusion of this lectin into PML nuclear bodies. These special structures are found in the interchromatin compartment and represent a subnuclear entity that is connected to responses of cells to stress, such as damage of DNA or viral infection, eventually capable of causing apoptosis (Dellaire and Bazett-Jones 2004; Zimber et al., 2004). Certainly, swift changes of the actual protein

composition at a given cell status underlie the wide spectrum of activities of PML nuclear bodies. Our findings on inducibility of nuclear galectin-2 presence by stress factors pinpoint a so far not known aspect of remodeling of their protein composition. Notably, the association of DNA, an attribute of galectin-2-containing granules, has also been reported for PML nuclear bodies during heterochromatin reorganization or viral replication (Jul-Larsen et al., 2004; Luciani et al., 2006). Equally interesting, preliminary application of biotinylated galectin-2 has raised evidence for homogeneous to punctuate distribution of binding sites reactive with this enodogenous lectin (Smetana et al., 2006). The approach of using a labeled galectin as a marker definitely deserves to be pursued, combining immuno- with galectin histochemistry.

In aggregate, this study on galectin-2 describes in detail the lectin's nuclear localization and its dynamic modulation by stress factors. It herewith provides salient information to solidify the concept that members of the galectin family are endowed with the capacity to reside at different cellular sites. Homing in on glycan and diverse peptide epitopes with conspicuous selectivity most likely accounts for this particular localization profile. Thus, the term lectin should in such a case be used with the explicit understanding of operativity of versatility in intermolecular recognition, i.e. the ability to "moonlight", that is to have more than one function and/or binding partner in a cell. This is an attribute galectins apparently share with a steadily growing panel of other proteins from diverse classes (Gabius, 1994; Jeffery, 2003). That said, and in view of the galectin network constituted by proto-type, chimera-type and tandem-repeat-type family members (Lahm et al., 2004), it becomes immediately obvious that the current status of respective information on tandem-repeat-type galectins is rather low. On the grounds of recent immuno-histochemical reports for nuclear presence of the tandem-repeat-type galectins-8 and -9 with indications for association either biologically to malignancy or cytologically to nucleoli (Danguy et al., 2001; Lensch et al., 2006) the next stage of immunocytochemical analysis on this topic will consequently be to investigate the characteristics of staining profiles of these family members in the manner given herein. The availability of non-cross-reactive antibodies will be crucial to eventually reach the aim of accurately defining the nuclear presence and the pattern of intranuclear staining of proto-type, chimera-type and tandem-repeat-type galectins.

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