[Cell Cycle 3:2, 217-222; February 2004]; ©2004 Landes Bioscience

Report

Cell Cycle Regulation of NF-YC Nuclear Localization

Mattia Frontini[1,†]
Carol Imbriano[1]
Isabella Manni[2]
Roberto Mantovani[1,3,*]

[1]Dipartimento di Biologia Animale; Università di Modena e Reggio Emilia; Modena, Italy

[2]Istituto Regina Elena; Dipartimento di Oncologia Sperimentale; Roma, Italy

[3]Dipartimento di Scienze Biomolecolari e Biotecnologie; Università degli Studi di Milano; Milano, Italy

[†]Current adress: IGBMC 1; rue L. Fries; BP 10142; 67404 Illkirch CEDEX, France

[*]Correspondence to: Roberto Mantovani; Dipartimento di Scienze Biomolecolari e Biotecnologie; Università degli Studi di Milano; Milano, Italy; Tel.: 39.02.503.15005; Fax: 39.02.503.15044; Email: mantor@unimi.it

Received 10/16/03; Accepted 11/18/03

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=654

KEY WORDS

CCAAT, NF-Y, histone fold

ACKNOWLEDGEMENTS

We wish to thank Y. Chen for gift of NF-YCb vector, E. Lalli for gift of Leptomycin B and C. Vitale for his help with confocal images. R.M. is supported by grants from MIUR-COFIN and AIRC.

ABSTRACT

NF-Y is a trimeric activator with histone fold—HFM—subunits that binds to the CCAAT-box and is required for a majority of cell cycle promoters, often in conjuction with E2Fs. In vivo binding of NF-Y is dynamic during the cell cycle and correlates with gene activation. We performed immunofluorescence studies on endogenous, GFP- and Flag-tagged overexpressed NF-Y subunits. NF-YA, NF-YB are nuclear proteins. Unexpectedly, NF-YC localizes both in cytoplamatic and nuclear compartments and its nuclear localization is determined by the interaction with its heterodimerization partner NF-YB. Most importantly, compartmentalization is regulated during the cell cycle of serum restimulated NIH3T3 cells, accumulating in the nucleus at the onset of S phase. These data point to the control of HFM heterodimerization as an important layer of NF-Y regulation during cell cycle progression.

INTRODUCTION

Promoters and enhancers are a combinatorial puzzle of DNA elements recognized by sequence-specific regulators that act in a complex chromatin context. The CCAAT box is a common promoter element, usually positioned in either orientation between -60 and -100 bp with respect to the transcription start site. Over the last years, it has become evident that NF-Y is the principal, if not the only, CCAAT-binding activator.[1,2] Many genes regulated during the cell cycle are controlled by NF-Y (3 and references therein); in particular, key regulators such as CDC25A/B/C, Cdc6, E2F1, Cyclin A2, Cyclin B1/B2, Cdc2, Topoisomerase IIα and PLK all contain one, or often, multiple CCAAT boxes, shown to be crucial for the proper regulation of these genes. NF-Y is composed of three subunits: NF-YA, NF-YB and NF-YC, all necessary for DNA-binding. NF-YB and NF-YC dimerization is required for NF-YA association and subsequent DNA-binding. NF-YB and NF-YC contain histone fold motifs -HFM- which mediate dimerization and DNA-binding;[4] the HFM was first detailed as a common structure among the four core histones and is composed of a minimum of three helices—α1, α2, α3—separated by two loops, L1 and L2 (reviewed in ref. 5). This structure is shared by other proteins involved in transcriptional regulation and chromatin remodelling, such as most of the TBP-associated factors TAFs,[6] NC2/Dr1-DRAP1[7] and ChrAC15-17/YBL1-YCL1.[8,9]

NF-Y is considered as an ubiquitous transcription factor, mainly because no cell line without it has ever been described: in fact, the mRNA levels of NF-Y subunits have been found to be constant in all conditions tested so far; however, the three proteins are regulated at different levels. NF-YA and NF-YC have two major isoforms generated by alternative splicing, variously expressed in different cell types. NF-YA short form lacks 28 aminoacids in the Q-rich activation domain, with additional microheterogeneity at the border between the Q- and S/T-rich domains.[10,11] The recently described NF-YC short form lacks residues 36-97, coding for the α1-α3 helices of the HFM.[12] Another level of regulation involves acetylation of NF-YB[13] and acetylation and phosphorylation of NF-YA (14; Caretti G, Mantovani R; submitted). Reduction of NF-YA expression has been reported in IMR-90 fibroblasts after serum-deprivation[15] and in terminally differentiated C2C12 muscle cells,[16,17] whereas an increase is observed in human monocytes and in intestinal epithelial cells.[18,19] These studies show that the levels of NF-YA expression dictate the function of the NF-Y CCAAT-binding complex.

A possible further level of regulation of protein complexes involves the nuclear localization of single subunits. Indeed, a number of transcription factors are known to be regulated in their nuclear localization and nuclear localization signals—NLS—have been identified and

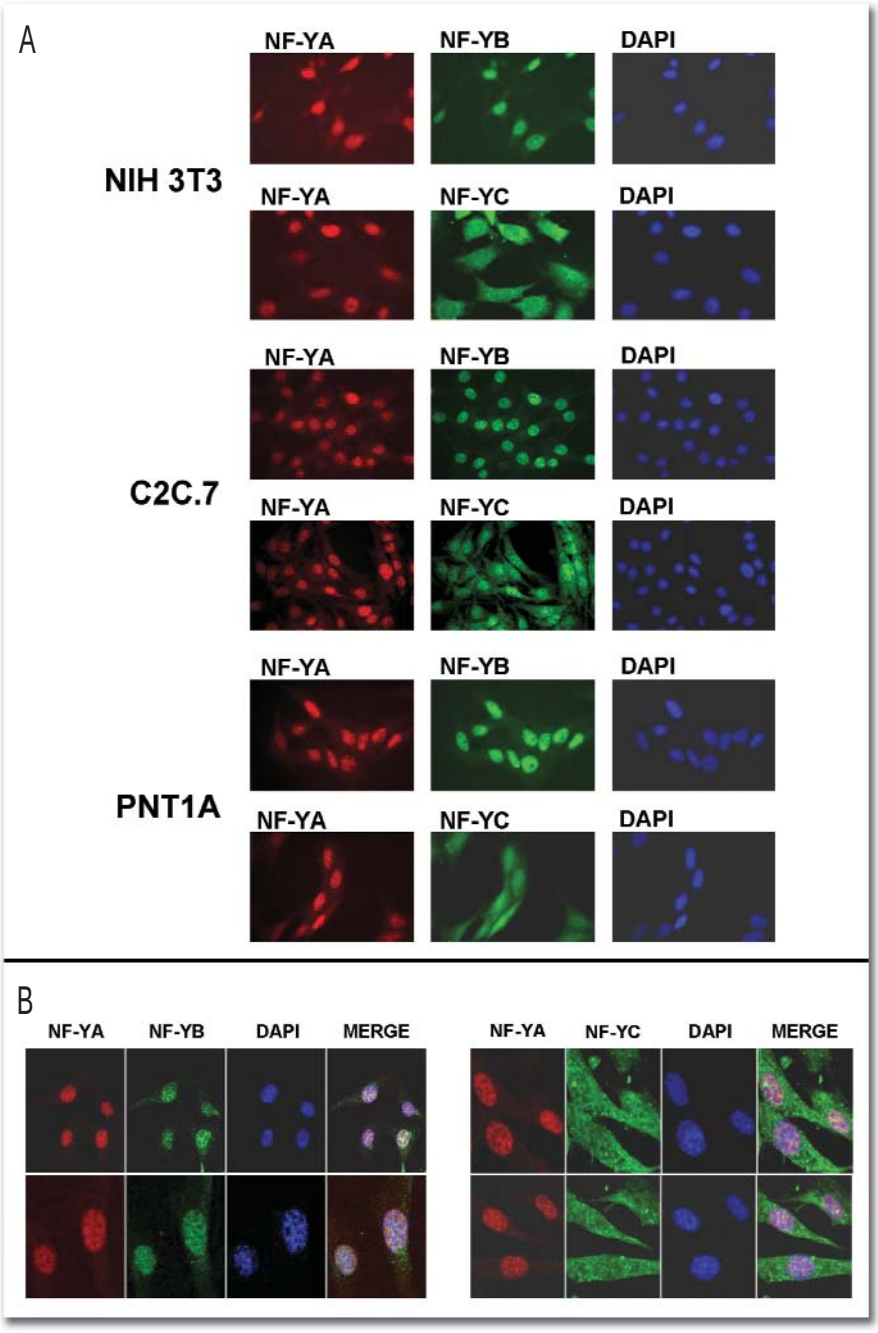

Figure 1. Immunostaining of endogenous NF-Y subunits. (A) Fluorescence microscopy images of exponentially growing NIH 3T3, C2C.7 and PNT1A stained with anti-NF-YA (mAb1a monoclonal; Mantovani, et al., 1992) and anti-NF-YB (Rabbit polyclonal; Mantovani et al., 1992) antibodies in the upper Panels and with anti-YA and anti-YC (Rabbit polyclonal; Bolognese et al., 1999) antibodies in the lower Panels. (B) Confocal microscopy images of exponentially growing NIH 3T3. Cells were stained with anti-YA mAb1a and anti-YB and with anti-YA and anti-YC antibodies.

catalogued.[20] In particular, the four core histones—H2A, H2B, H3 and H4—were analyzed by biochemical means and by direct fluorescence as fusion proteins:[21-23] the results revealed that they contain two portions—the basic amino acids in the N-terminal tails and the histone fold parts—mediating nuclear localization through importins-lead pathways.

As there are no indications about the behaviour of NF-Y in terms of nuclear targeting, we decided to study the localization of the three NF-Y subunits using direct and indirect fluorescence techniques.

MATERIALS AND METHODS

Plasmids Construction. GFP-YA13 was generated by cutting the long form of NF-YA from G4-YA13[24] with SmaI/BglII and inserting into SmaI/BamHI sites of pEGFP-C3 (Clontech). GFP-YB was generated using the BglII/SalI sites of G4-YB.[24] GFP-YC was similarly generated subcloning NF-YC from pET32bNF-YC[25] using BglII/SalI sites.

Cell Cultures and Transfections. NIH3T3 fibroblasts and C2C.7 myoblasts (provided by Susanna Molinari, U. Modena) were maintained in Dulbecco's Modified Eagle Medium, 10% FCS the former 20% FCS the latter, and 2 mM L-Glutamine. PNT1A human epithelial prostate cells were maintained in RPMI, 10% FCS and 2 mM L-Glutamine. Tipically, 2.10^5 cells were seeded 8 hours before transfection in 35mm dishes and transfected with 2 μg of total DNA, 0.5 μg of each construct, using Lipofectamine Plus Reagent (Gibco Life Technologies) according to manufacturer's instructions. Cells were fixed 16–20 hours after transfection. Leptomycin B 10 ng/ml (Sigma) was added 16 hours after transfection for 6 hours.

Antibodies and Immunofluorescence. Cells were washed twice in PBS at room temperature, fixed with 2% paraformaldehyde in PBS for 10' at room temperature and permeabilized with 0,05% TritonX 100 in PBS. Samples were preincubated with 1% BSA in PBS for 20' and then incubated overnight at 4°C with the following primary antibodies diluted in PBS+1%BSA: αNF-YA Mab1a hybridoma supernatant 1:5, α-NF-YB 1:50,[26] α-NF-YC 1:10.[27] Cells were then washed twice with PBS and incubated 20' at room temperature in PBS+1%BSA before a further incubation, 60' at room temperature in the dark, with secondary antibodies: Orange Green or Alexa488 anti-rabbit and Alexa Red anti-mouse (Molecular Probes). After three washes in PBS and DNA counterstaining with DAPI monolayers were mounted with Vectashield and examined with a Zeiss Axioplan fluorescence microscope, images collected with a CCD Camera (Digital Instruments, Madison USA) and SPOT32 version 3.0 software package (Diagnostic Instruments). Confocal images were obtained using a Laica TCS4D instrument.

RESULTS

Localization of the Endogenous NF-Y Subunits. To investigate the distribution of the three NF-Y subunits, we used purified antibodies raised against NF-YA, NF-YB and NF-YC. These antibodies were previously used in EMSAs, Western blots, immunoprecipitations, in vitro transcription, and, more recently, in chromatin immunoprecipitation—ChIP—assays.[3,26,27] We employed them in immunofluorescence experiments on three cell lines: mouse NIH3T3 fibroblasts, C2C.7 myocytes and human prostate epithelial PNT1A cells. The results are shown in Figure 1A: in the upper Panels, colocalizations of NF-YA and NF-YB, in the lower, NF-YA and NF-YC. Patterns of nuclear localization are evident for NF-YA and NF-YB, whereas NF-YC staining is present in the cytoplasm and in the nucleus (Compare with NF-YA, NF-YB and DAPI staining in the upper Panels and NF-YA, NF-YC and DAPI in the lower). To further substantiate this latter point, confocal microscopy was used under similar conditions in colocalization experiments. Images are shown in Figure 1B: patterns of nuclear colocalization are evident for NF-YA and NF-YB, whereas NF-YC is

present in both the cytoplasm and nuclei. Two points need to be underligned: (i) the colocalizations of NF-YA and NF-YB (Fig. 1B, upper panel), or NF-YA and NF-YC could only be possible assuming that the third HFM partner is present (NF-YC in the upper and NF-YB in the lower panels), since innumerable biochemical experiments established that NF-YA can only interact with the NF-YB/NF-YC heterodimer.[1,4] (ii) None of these colocalizations fall in the well known heterochromatic dots of NIH3T3.

Regulation of NF-YC Localization During the Cell Cycle. The binding of NF-Y to cell cycle regulated promoters is dynamic during the different phases of the cycle in vivo, as assayed in ChIP analysis with anti-NF-YB antibodies.[3] We checked the localization of the different subunits in NIH3T3 cells arrested in G_0 after serum withdrawal, and restimulated at different time points after serum addition. Immunofluorescence analysis revealed that NF-YA and NF-YB are always localized in the nucleus, while NF-YC is found mainly in the cytoplasm during G_0 and the first 12 hours after serum addition; at 18 and 24 hours, only the nuclei showed positive staining for NF-YC (Fig. 2A). The relocalization of NF-YC coincides with the onset of the S phase, as checked by FACS and ChIP analysis.[3] We verified the increase of NF-YC and the short NF-YCb isoform in Western blots of cytoplasmic and nuclear extracts from G_0 and 18 hours post-stimulation cells (Fig. 2B). A decrease in the amount of NF-YC in the cytoplasm (upper panel) matched a corresponding increase in the nuclei, mostly due to the long isoform; the control nuclear NF-YB did not show a variation in its nuclear staining (lower panel). We therefore conclude that the nuclear levels of NF-YC are regulated during the progression of the cell cycle from G_0 to S phase.

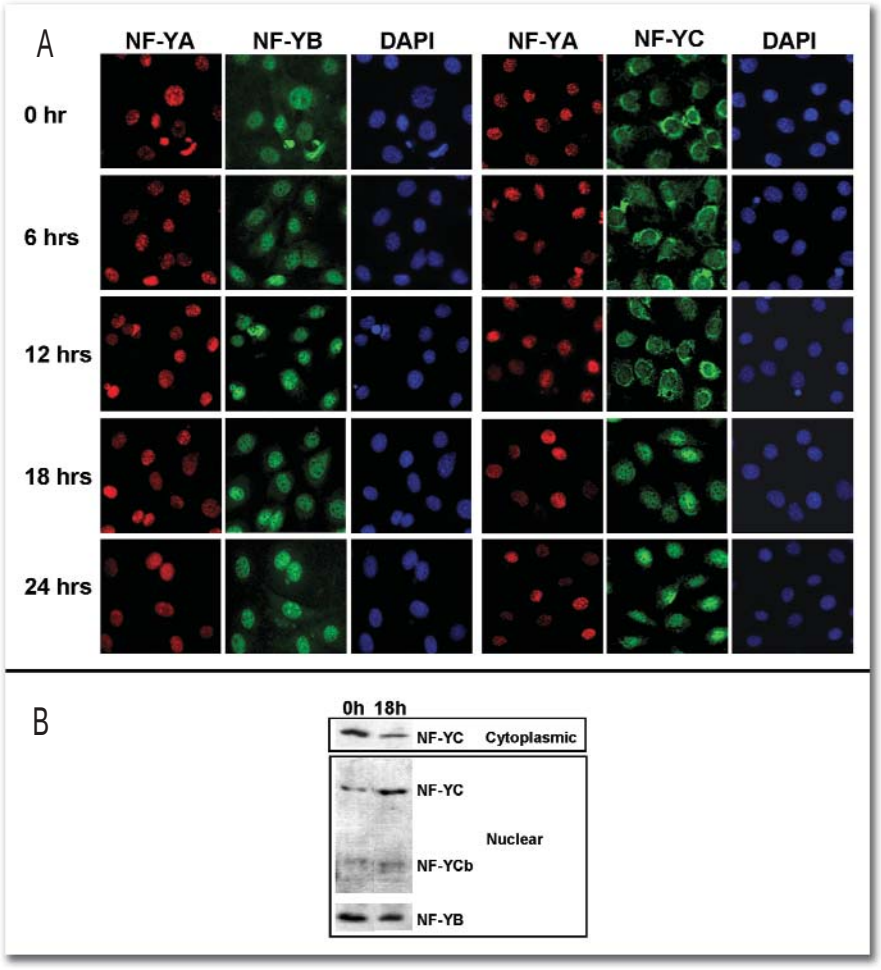

Figure 2. Cell cycle regulation of NF-YC localization in NIH 3T3 cells. (A) NIH 3T3 cells were fixed after serum starvation for 60 hours (0 hr), or after restimulation with 10% FCS for 6, 12, 18 and 24 hours. Cells were costained with anti-YA mab1a and anti-YB (Left Panels), or with anti-YA and anti-YC antibodies (Right Panels). (B) Western blot with anti-YC and anti-NF-YB antibodies on nuclear and cytoplasmic exctracts prepared from G_0 and 18 hours post-stimulation. The two NF-YC isoforms are indicated.

Nuclear Localization of NF-YC is Governed by NF-YB. To extend the results of Figures 1 and 2 and investigate the mechanism of NF-Y subunits localization, we transfected GFP fusions of the NF-Y subunits in the cell lines used in Figure 1: essentially the same patterns were revealed for NF-YA and NF-YB, that is, nuclear localization of fluorecence (Fig. 3A); on the other hand, NF-YC-GFP showed a largely cytoplasmatic localization (Fig. 3A), despite the nuclear/cytoplasmatic distribution of endogenous NF-YC. When NF-YC-GFP was overexpressed together with NF-YB, its localization changed, becoming mostly nuclear (Fig. 3B, left panels); note the colocalization of transfected NF-YB (Fig. 3B, central panels). A statistical analysis of these experiments is shown in Table 1.

Table 1 **Statistical Analysis of GFP Overexpressions**

	Number	Diffuse	Cytoplasmic	Nuclear
NF-YB-GFP	60	99%	--	
NF-YB-GFP / NF-YC	167	75%	-25%	
NF-YC-GFP	103	-99%	-	
NF-YC-GFP / NF-YB	164	31%	35%	34%
NF-YC-GFP / NF-YB / NF-YA	192	40%	22%	38%

As a further prove for the differential behaviour of NF-YC overexpression in the absence or presence of cotransfected NF-YB, we used Flag-tagged NF-YC: (Fig. 3C) shows that Flag-NF-YC is predominantly cytoplasmatic when expressed alone in NIH3T3 (upper panel, costaining with the anti-YC polyclonal and anti-Flag monoclonal antibodies), whereas it becomes nuclear, costaining with NF-YB, when the latter is coexpressed (lower panel). As a control, the Flag-NF-YA fusion maintained the nuclear localization pattern observed for the endogenous and GFP-fusions (Fig. 3C, right panel). Nuclear dots are visible depending on the amount of overexpressed NF-YA; they colocalize with PML bodies in PML overexpression experiments (Data not shown). As this behavior is apparently common to many transcription factors in overexpression assays (S. Minucci, personal communication), it was not further investigated. Taken together, these experiments indicate that NF-YB mediates NF-YC nuclear localization.

Two explanations could be put forward for the NF-YC targeting: (i) it is devoid of a nuclear localization signal (NLS), so it needs its Histone Fold partner carrying one to be imported. (ii) NF-YC can enter the nucleus, but a nuclear export signal relocalizes it in the cytoplasm, when in excess with respect to NF-YB heterodimerization capacity (As it is the case in overexpressions).

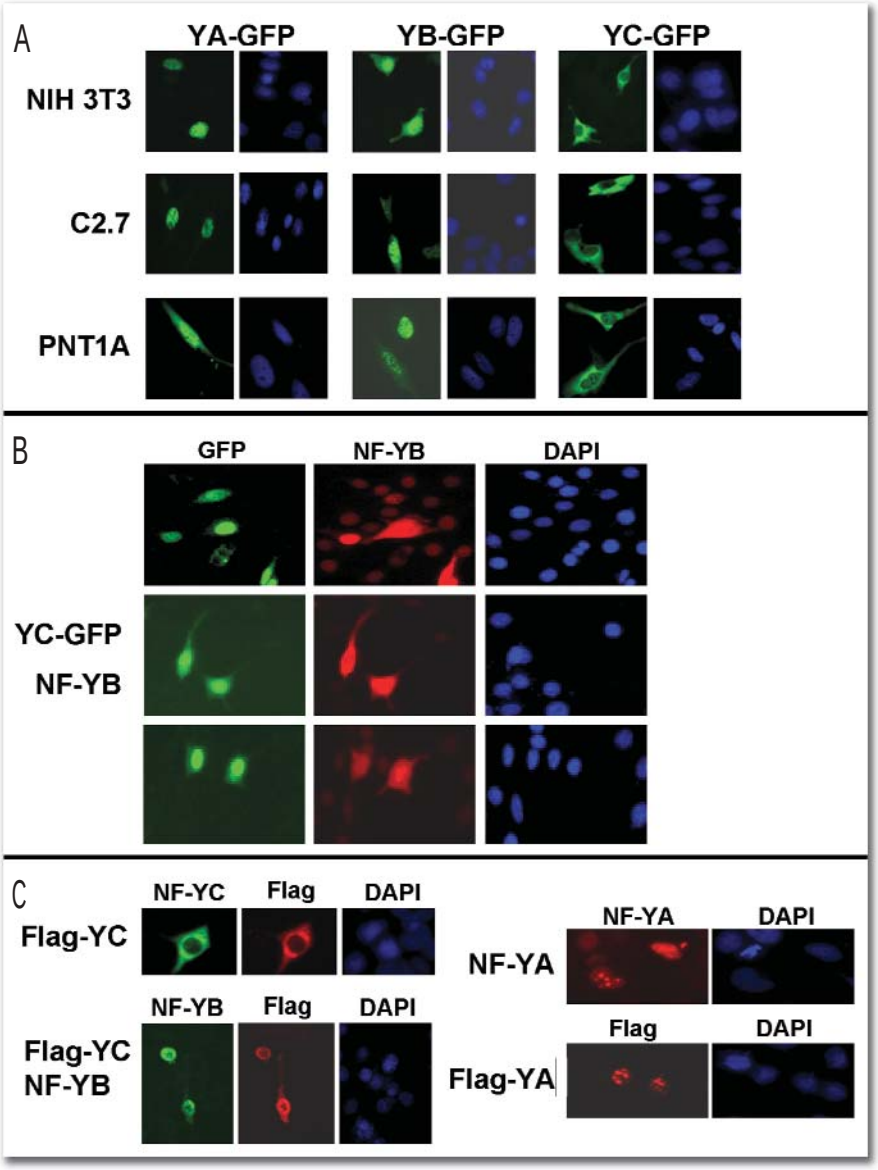

Figure 3. Localization of GFP- and flag-tagged NF-Y subunits. (A) Fluorescence of GFP-tagged NF-Y subunits overexpressed in NIH 3T3, C2C.7 and PNT1A. (B) Fluorescence of NF-YC-GFP transfected together with NF-YB in NIH 3T3 cells (left panels); costaining with anti-YB is shown in the central Panels. (C) Flag-NF-YC was transfected and revealed with anti-YC (upper left panel) and anti-Flag (upper central panel) antibodies. In the lower panel, Flag-NF-YC was cotransfected with NF-YB and costained with anti-YB (left panel) and anti-Flag (central panel) antibodies. Flag-tagged NF-YA was transfected was trasnfected and stained with anti-Flag (right panel), and compared with NF-YA stained with anti-YA Mab1a (upper right panel).

To discriminate between these possibilities, we performed two experiments. NIH3T3 cells were transfected with NF-YC-GFP and treated for 6 hours with Leptomycin B (LMB), a powerful inhibitor of CRM1 exportin: NF-YC clearly shows nuclear accumulation compared to untreated cells (Fig. 4A). This argues in favour of a mechanism in which overexpressed NF-YC does enter the nucleus and it is then exported to the cytoplasm via the CRM1-dependent pathway. In the second experiment, we used NF-YCb, a splicing isoform devoid of the HFM and hence of the NF-YB-interacting domain; we transfected the latter as a Flag-tagged protein (Fig. 4B): unlike NF-YC, NF-YCb is not largely confined to the cytoplasmic compartment, supporting the idea that residues from aminoacids 36-97, which are essential for NF-YB interactions, contain a NES.

DISCUSSION

Two notable results are reported here: (i) the differential behaviour of the two histone fold subunits of the NF-Y complex and (ii) complex regulation of nuclear localization of NF-YC during the cell cycle.

In recent years, cellular compartmentalization has emerged as an important level of regulation for DNA-binding proteins and cofactors involved in transcription activation and repression. The nuclear localization signals of many proteins have been studied in great details: at large, short stretches of basic aminoacids emerged as a common feature.[20] As for HFM proteins, only core histones have been studied so far: despite the small size, their nuclear import requires an active transport mechanism, via the binding to import receptors such as importins.[21-23] Histones contain several NLS that can be subdivided into two types of structures: the N-terminal tails, which are the focus of a wealth of post-translational modifications, and the HFMs.[21] The situation, as detailed in our study, is different in the case of NF-Y. Our results unambiguously prove that NF-YA, NF-YB and, to a certain extent, NF-YC are nuclear proteins. In particular, we find that only NF-YB behaves in a way similar to histones, in that it is intrinsically located into the nucleus. NF-YB is a highly acidic protein, with a pI of 4.1/4.5, as established by IEF (Mantovani R; unpublished). There are no basic residues outside of the HFM, thus it is likely that this structure, as in the case of the related H2B, mediates the nuclear localization. We hypothesize that L1 and L2 basic residues might be involved, particularly the KRK stetch at position 107-109 of L2.[4] A similar behaviour is observed for NF-YA, a highly basic protein with a pI of 10.5: indeed a small mutant harboring the 57 aminoacids homology domain—and all positively charged Lysines and Arginines—behaves much like the full length protein (Frontini M, Mantovani R; unpublished).

On the other hand, NF-YC undergoes a different regulation. It apparently enters the nucleus freely, since LMB experiments suggest that it is driven out by an active export mechanism. It is possible that an HFM NLS a la histone H2A is responsible for nuclear entry of the long NF-YC. In the NF-YCb short isoform, which lacks the HFM, yet it is found in the nucleus, it is not immediate to find a conventional NLS, as detailed in other proteins: it is possible that NF-YCb is associated to a shuttling factor, independently from the presence of the HFM. This hypothesis is particularly attractive, since NF-YCb originally emerged from yeast two hybrids screenings with SMAD factors,[12] which are transcriptional regulators that shuttle beetween the cytoplasm and the DNA upon TGF-β signaling.[28]

The nuclear localization of the long NF-YC is regulated via heterodimerization with NF-YB: what is most remarkable is that the two proteins can live separable lives, under specific cellular conditions. This could not be easily anticipated based on the structural features of the HFM couple, detailed by Romier et al.,[4] and on the available

data on core histones. Data supportive to the notion that apparently inseparable HFM proteins can be involved in different pathways came from biochemical and genetic analysis of the related NC2 in *S. cerevisiae*: the existence of multiple complexes involved in different functions in transcription emerged for both the alpha and beta subunits.[29] In general, a relevant open question concerns the amount of NF-YB within the nucleus which is stoichiometrically able to heterodimerize with NF-YC, and how this process is regulated. Acetylation of NF-YB by the HAT coactivator p300[13] does not seem to affect this function, nor acetylation appears to influence nuclear localization of NF-YB (Caretti G, Mantovani R; submitted).

Most importantly, NF-YC localization, but not its overall levels, dramatically changes during the cell cycle, being mostly cytoplasmatic during G_0 and the G_1 phase. It is clear that this type of regulation heads to a precise control of the levels of NF-YB/NF-YC dimer available for NF-YA association. Previous data obtained in several differentiation systems, human peripheral monocytes, C2C12, CaCo[16,18,19] and mouse tissues,[17] indicated that NF-YA is regulated in quantitative terms. Interestingly, NF-YC nuclear targeting coincides with the onset of the S-phase, between 12 and 18 hours after serum restimulation. This is the time where histones move into the nucleus to complex newly synthesized DNA into nucleosomes. In this respect, NF-YC behaves very much like core histones. A notable precedent for this behaviour is represented by E2F4, a transcriptional repressor of cell cycle genes:[30] in a cellular system similar to the one used here, E2F4 is escorted into the nucleus by p130 to represses transcription, as cells withdraw from the cell cycle.[31] The majority of E2F-regulated genes do have one or more CCAAT boxes (Salvatoni L, Mantovani R; in preparation). Interestingly, in vivo ChIP analysis indicated that the binding of E2F4 and NF-Y is never coincident on any coregulated promoters, E2F1, Cyclin A, Cdc2 and Cyclin B.[3] A reasonable working hypothesis is that a regulatory link exists between the exit of E2F4 from the nucleus and the entrance of NF-YC.

It is important to note that genes exist that are active in G_0 and require the trimer for their function:[3] in fact, a fraction of NF-YC is nuclear, and indeed CCAAT-bound, in serum-starved cells. Nevertheless, our data paint a scenario in which an additional effort must be made by the cell in terms of NF-Y CCAAT box binding capacity to meet a presumably larger set of promoters that need to be activated during the S phase: in accordance with this, a prevalence of NF-Y sites in promoters activated post-S has emerged from bioinformatics studies.[32] This is apparently accomplished through diverse mechanisms: increase in NF-YA stability[27] and relocalization of NF-YC, as shown here. Whether the latter mechanism involves pathways common to other histone-like proteins remains to be investigated.

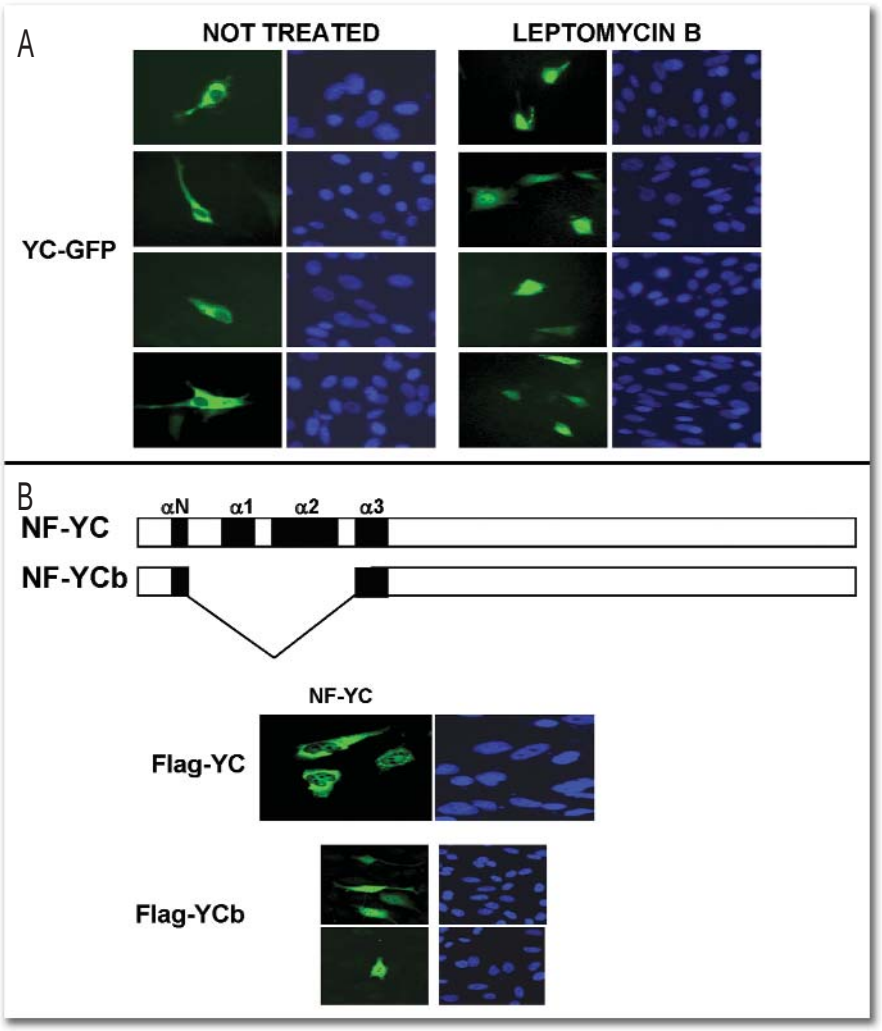

Figure 4. NF-YC contains a nuclear export signal located in the Histone Fold Domain. (A) NIH 3T3 cells transfected with NF-YC-GFP untreated (Left panels) or treated, 16 hours after transfection with 10 ng/ml of LeptomycinB for 6h (Right Panels). (B) Schematic representation of the two NF-YC isoforms. Flag-NF-YC (Upper Panel) and Flag-NF-YCb (Lower Panel) were transfected and NIH 3T3 cells stained with the anti-YC polyclonal antibody.

References

1. Maity SN, de Crombrugghe B. Role of the CCAAT-binding protein CBF/NF-Y in transcription. Trends Biochem Sci 1998; 23:174-8.
2. Mantovani R. The molecular biology of the CCAAT-binding factor NF-Y. Gene 1999; 239:15-27.
3. Caretti G, Salsi V, Vecchi C, Imbriano C, Mantovani R. Dynamic recruitment of NF-Y and histone acetyltransferases on cell cycle promoters. J Biol Chem 2003; 278:30435-40.
4. Romier C, Cocchiarella F, Mantovani R, Moras D. The NF-YB/NF-YC structure gives insight into DNA binding and transcription regulation by CCAAT factor NF-Y. J Biol Chem 2003; 278:1336-45.
5. Luger K, Richmond TJ. DNA binding within the nucleosome core. Curr Opin Struct Biol 1998; 8:33-40.
6. Gangloff YG, Romier C, Thuault S, Werten S, Davidson I. The histone fold is a key structural motif of transcription factor TFIID. Trends Biochem Sci 2001; 26:250-7.
7. Kamada K, Shu F, Chen H, Malik S, Stelzer G, Roeder RG, et al. Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex. Cell 2001; 106:71-81.
8. Bolognese F, Imbriano C, Caretti G, Mantovani R. Cloning and characterization of the histone-fold proteins YBL1 and YCL1. Nucleic Acids Res 2000; 28:3830-8.
9. Poot RA, Dellaire G, Hulsmann BB, Grimaldi MA, Corona DF, Becker PB, et al. HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. EMBO J 2000; 19:3377-87.
10. Li X-Y, van Huijsduijnen R, Mantovani R, Benoist C, Mathis D. Intron-exon organization of the NF-Y genes: tissue specific splicing modifies an activation domain. J Biol Chem 1992; 267:8984-90.

11. Ge Y, Jensen TL, Matherly LH, Taub JW. Synergistic regulation of human cystathionine-beta-synthase-1b promoter by transcription factors NF-YA isoforms and Sp1. Biochim Biophys Acta 2002; 1579:73-80.
12. Chen F, Ogawa K, Liu X, Stringfield TM, Chen Y. Repression of Smad2 and Smad3 transactivating activity by association with a novel splice variant of CCAAT-binding factor C subunit. Biochem J 2002; 364:571-7.
13. Li Q, Herrler M, Landsberger N, Kaludov N, Ogryzko VV, Nakatani Y, et al. Xenopus NF-Y presets chromatin to potentiate p300 and acetylation-responsive transcription from the Xenopus hsp70 promoter in vivo. EMBO J 1998; 17:6300-15.
14. Yun J, Chae HD, Choi TS, Kim EH, Bang YJ, Chung JK, et al. Cdk2-dependent phosphorylation of the NF-Y transcription factor and its involvement in the p53-p21 signaling pathway. J Biol Chem 2003; 278:36966-72.
15. Chang ZF, Liu CJ. Human thymidine kinase CCAAT-binding protein is NF-Y, whose A subunit expression is serum-dependent in human IMR-90 diploid fibroblasts. J Biol Chem 1994; 269:17893-8.
16. Farina A, Manni I, Fontemaggi G, Tiainen M, Cenciarelli C, Bellorini M, et al. Down regulation of cyclin B1 gene transcription in terminally differentiated skeletal muscle cells is associated with loss of functional CCAAT-binding NF-Y complex. Oncogene 1999; 18:2818-27.
17. Gurtner A, Manni I, Fuschi P, Mantovani R, Guadagni F, Sacchi A, et al. Requirement for down-regulation of the CCAAT-binding activity of the NF-Y transcription factor during skeletal muscle differentiation. Mol Biol Cell 2003; 14:2706-15.
18. Bevilacqua MA, Fanello MC, Iovine B, Russo T, Cimino F, Costanzo F. Transcription factor NF-Y regulates differentiation of CaCo2 cells. Arch Biochem Biophys 2002; 407:39-44.
19. Marziali G, Perrotti E, Ilari R, Coccia M, Mantovani R, Testa U, et al. The activity of the CCAAT-binding factor NF-Y is modulated through the regulated expression of its A subunit during monocyte to macrophage differentiation. Blood 1999; 93:519-26.
20. Kaffman A, O'Shea EK. Regulation of nuclear localization: A key to a door. Annu Rev Cell Dev Biol 1999; 15:291-339.
21. Baake M, Bauerle M, Doenecke D, Albig W. Core histones and linker histones are imported into the nucleus by different pathways. Eur J Cell Biol 2001; 80:669-77.
22. Mosammaparast N, Guo Y, Shabanowitz J, Hunt DF, Pemberton LF. Pathways mediating the nuclear import of histones H3 and H4 in yeast. J Biol Chem 2002; 277; 862-8.
23. Muhlhausser P, Muller EC, Otto A, Kutay U. Multiple pathways contribute to nuclear import of core histones. EMBO Rep 2001; 2:690-6.
24. diSilvio A, Imbriano C, Mantovani R. Dissection of the NF-Y transcriptional activation potential. Nucleic Acids Res 1999; 27:2578-84.
25. Bellorini M, Zemzoumi K, Farina A, Berthelsen J, Piaggio G, Mantovani R. Cloning and expression of human NF-YC. Gene 1997; 193:119-25.
26. Mantovani R, Pessara U, Tronche F, Li X-Y, Knapp AM, Pasquali JL, et al. Monoclonal antibodies to NF-Y define its function in MHC Class II and albumin gene transcription. EMBO J 1992; 11:3315-22.
27. Bolognese F, Wasner M, Lange-zu Dohna C, Gurtner A, Ronchi A, Muller H, et al. The cyclin B2 promoter depends on NF-Y, a trimer whose CCAAT-binding activity is cell cycle regulated. Oncogene 1999; 18:1845-53.
28. Moustakas A, Souchelnytskyi S, Heldin, CH. Smad regulation in TGF-beta signal transduction. J Cell Sci 2001; 114:4359-69.
29. Creton S, Svejstrup JQ, Collart MA. The NC2 alpha and beta subunits play different roles in vivo. Genes Dev 2002; 16:3265-76.
30. Cam H, Dynlacht BD. Emerging roles for E2F: Beyond the G_1/S transition and DNA replication. Cancer Cell 2003; 3:311-6.
31. Rayman JB, Takahashi Y, Indjeian VB, Dannenberg JH, Catchpole S, Watson RJ, et al. E2F mediates cell cycle-dependent transcriptional repression in vivo by recruitment of an HDAC1/mSin3B corepressor complex. Genes Dev 2002; 16:933-47.
32. Elkon R, Linhart C, Sharan R, Shamir R, Shiloh Y. Genome-wide in silico identification of transcriptional regulators controlling the cell cycle in human cells. Genome Res 2003; 13:773-80.