ELSEVIER

Ageing Research Reviews
1 (2002) 639–651

ageing
research
reviews

www.elsevier.com/locate/arr

Viewpoint

Transcriptional regulation of cellular ageing by the CCAAT box-binding factor CBF/NF-Y

Koozi Matuoka*, Kuang Yu Chen

Department of Chemistry and Chemical Biology, Rutgers—State University of New Jersey, 610 Taylor Road, Piscataway, NJ 08854, USA

Received 23 April 2002; accepted 24 April 2002

Abstract

Cellular ageing is a systematic process affecting the entirety of cell structure and function. Since changes in gene expression are extensive and global during ageing, involvement of general transcription regulators in the phenomenon is likely. Here, we focus on NF-Y, the major CCAAT box-binding factor, which exerts differential regulation on a wide variety of genes through its interaction with the CCAAT box present in as many as 25% of the eukaryotic genes. When a cell ages, senescing signals arise, typically through DNA damage due to oxidative stress or telomere shortening, and are transduced to proteins such as p53, retinoblastoma protein, and phosphatidylinositol 3-kinase. Among them, activated p53 family proteins suppress the function of NF-Y and thereby downregulate a set of cell cycle-related genes, including *E2F1*, which further leads to downregulation of E2F-regulated genes and cell cycle arrest. The p53 family also induces other ageing phenotypes such as morphological alterations and senescence-associated β-galactosidase (SA-gal) presumably by upregulation of some genes through NF-Y suppression. In fact, the activities of NF-Y and E2F decrease during ageing and a dominant negative NF-YA induces SA-gal. Based on these observations, NF-Y appears to play an important role in the process of cellular ageing.

Keywords: Cellular ageing; CCAAT box; Transcription factor; NF-Y; p53

1. Introduction

1.1. The CCAAT box and CCAAT box-binding proteins

The pentanucleotide “CCAAT” and its reverse “ATTGG” have been found to occur at a remarkably high frequency in the promoters of mRNA-encoding genes, the proximal regions upstream of the transcription initiation sites (Bucher, 1990). This motif is termed

* Corresponding author. Tel.: +1-732-445-5154; fax: +1-732-445-5312.
E-mail address: kmatuoka@mailhost.net (K. Matuoka).

1568-1637/02/$ – see front matter
PII: S1568-1637(02)00026-0

the CCAAT box. There are also other common sequences, including the cap signal, TATA box, and GC box. These elements have been analyzed in detail in terms of their roles in transcription. The cap signal is the sequence with which RNA polymerase II (Pol II) or TFIID interacts for initiation of transcription; the TATA box is the sequence that TATA box-binding protein (TBP) recognizes and binds to; and the GC box is typically that for the general transcription factor Sp1. As to the CCAAT box, however, there has been confusion.

A number of proteins were reported as factors interacting with the sequences containing the CCAAT box. These molecules were accordingly given names bearing the term "CCAAT". Unfortunately, subsequent analyses quite often led to a conclusion that they recognize a sequence different from "CCAAT" or that they are the same molecules as the factor NF-Y (or a complex containing or binding to NF-Y):

- α-CP1: originally described as a factor binding the CCAAT region of α-globin promoter. Identical with NF-Y.
- *Aspergillus nidulans* CCAAT-binding factor (AnCF): designated as a factor binding to the CCAAT sequence of acetamidase promoter. HAP/NF-Y in the fungus.
- CCAAT binding factor (CBF): purified as a DNA-binding factor from rat liver and found to bind to the CCAAT motifs of collagen gene promoters, Rous sarcoma virus long terminal repeat, etc. Identical with NF-Y.
- CCAAT-binding protein (CBP): purified as a binding protein for the CCAAT motif of herpes virus thymidine kinase promoter. Identical with C/EBPα.
- CCAAT displacement protein (CDP): identified as a factor binding to a sequence in the histone H2B promoter, thereby interfering sterically with binding of NF-Y to the CCAAT box therein. The binding sequence for CDP covers a CCAAT box; nonetheless, an oligonucleotide bearing CTTAT in place of CCAAT was equally effective, indicating dispensability of the CCAAT motif itself.
- CCAAT/enhancer binding protein (C/EBP): identified as a component of rat liver nuclear extracts that recognized the CCAAT-containing sequences. Since related proteins were cloned, the original molecule has been renamed as C/EBPα. Now these molecules form a family of transcription regulators, comprising several members, with the consensus binding motif RTTGCGYAAY.
- CCAAT-binding protein 1 (CP1 or Cp1): isolated as a factor binding to the CCAAT sequence of α-globin promoter. Identical with NF-Y.
- CCAAT-binding protein 2 (CP2): first described as a factor binding to the CCAAT sequence of γ-fibrinogen promoter; however, it appears to recognize a sequence other than the CCAAT motif.
- CCAAT transcription factor (CTF): a HeLa cell factor binding to the CCAAT motif of herpes virus promoter. The consensus sequence is TGGNNNNNNGCCAA.
- Enhancer binding protein p20 (EBP20): a rat liver factor interacting with DNA sequences common to several viral enhancers. Identical with C/EBFα.
- Heme activator protein (HAP): the CCAAT box-binding factor in the yeasts. Corresponding to (and functionally exchangeable with) the mammalian NF-Y.
- Heat shock protein–CCAAT box-binding factor (HSP–CBF): cloned as a binding protein to an HSP70 CCAAT sequence. Recently, this protein has been found to interact with NF-YB, thereby binding indirectly to the CCAAT box.

- Histone H1 transcription factor 2 (H1TF2): a CCAAT-binding factor for the histone H1 promoter. A heterodimer, whose H1TF2A subunit exhibits homology to the NF-YA glutamine-rich domain.
- Nuclear factor erythroid-cell-specific (NF-E): described as a factor interacting with a region spanning the distal CCAAT box of γ-globin promoter. It actually recognizes GACAAGG and a separate G in the region rather than the CCAAT motif.
- Nuclear factor I (NF-I or NF I): identified as an essential cellular factor for adenovirus replication. Identical with CTF.
- Nuclear factor Y (NF-Y): originally described as a DNA-binding factor recognizing the Y box of MHC class II genes. Exhibiting a high affinity (Kd $= 10^{-10}$ to 10^{-11}) to the sequence RRCCAATSRGMR or the reverse YKCYWATTGGYY (R for A/G, Y for C/T, S for C/G, W for A/T, M for A/C, and K for G/T).
- Penicillin regulator 1 (PENR1): designated as a factor binding to the CCAAT sequence. Identical with AnCF, the fungal HAP/NF-Y.
- Y box-binding protein 1 (YB-1): one of the Y box factors.
- Y box factors: found as a fraction binding to MHC class II Y box. Initially its binding motif was postulated to be CTGATTGGCCAA; however, it seems that the molecules and their target sequences are substantially divergent.

Given this situation, one can state that the major factors recognizing the CCAAT box are molecules of the CBF/NF-Y/HAP family (for simplicity, referred to as NF-Y hereinafter). In this regard, there have been >5000 publications related to the CCAAT box thus far, among which approximately 400 concern NF-Y. Although we cite only recent references due to space limitations, we acknowledge the earlier studies by pioneering and active researchers, including B. de Crombrugghe, R.A. Currie, and R. Mantovani.

2. The genes regulated by NF-Y

The CCAAT box, which is the target motif of NF-Y, is present in approximately 25–30% of eukaryotic promoters (Bucher, 1990; Mantovani, 1998). However, it should be noted that the CCAAT box does not confine to the five nucleotides, but extends to the flanking sequences, giving the consensus motif "RRCCAATSR" (Bucher, 1990). This matches well the consensus for NF-Y-binding DNAs "RRCCAATSRGMR" (Mantovani, 1998). With vertebrate genes the CCAAT box preferably localizes to the region from −212 to −57 nucleotides (nt.) relative to the transcription initiation site (Bucher, 1990), suggesting this area for its efficient functioning.

Any CCAAT box satisfying those requirements (and the other CCAAT sequences at times) could be a potential target of NF-Y. Although NF-Y has been reported not to bind to some of them (Mantovani, 1998), that could be due to their position in the promoter, distance from other regulatory elements, or interference by another factor (as discussed later). Thus, as many as 25% of the eukaryotic genes are considered to be under regulation of NF-Y. The following are representative genes that have been shown to be regulated by NF-Y.

In relation to ageing (Meyyappan et al., 1996; Chen, 1997), genes upregulated in aged cells include fibronectin, α_1(I)-procollagen, α_2(I)-procollagen, and ferritin heavy chain. Genes

downregulated therein are cell cycle-related genes (cyclin A, cyclin B, *cdk-2*, ribonucleotide reductase R2, thymidine kinase (*TK*), histone H2B, and histone H3), transcription factors (*E2F-1* and A-*myb*), and a stress-response gene (*HSP70*). Examples of ageing-unaffected genes are β-actin and c-*jun*.

Besides *HSP70*, there are a number of genes related to cellular response to damages and stresses, indicating that NF-Y functions in removal of damaging agents and damaged cells. They include: phospholipid hydroperoxide glutathione peroxidase (Huang et al., 1999), which suppresses 12- and 15-lipoxygenases and cyclooxygenase-2, thereby reducing the amount of reactive oxygen species; mismatch repair gene *hMLH1* (Deng et al., 2001), whose dysfunction leads to microsatellite instability and cancer development; GADD45 (Jin et al., 2001), which is induced by DNA damaging agents such as ionizing or UV radiation and methanesulfonate; molecular chaperones and folding enzymes in response to accumulation of unfolded proteins in the endoplasmic reticulum (Yoshida et al., 2000); von Willebrand factor in vascular endothelial cells (Bertagna and Jahroudi, 2001); hyperexpression of catalase in H_2O_2-resistant cells (Nenoi et al., 2001).

In addition, although NF-Y functions basically as a transactivator of gene expression, it is also involved, directly or indirectly, in downregulation of transcription. For example, NF-Y mediates DNA damage-induced suppression of cyclin B1, cyclin B2, and *cdc25C* at G2 phase (Manni et al., 2001) and polyunsaturated fat-promoted inhibition of fatty acid synthase expression (Teran-Garcia et al., 2002). NF-Y binds to the CCAAT box in mouse renin enhancer, thereby blocking the positive regulatory element (Shi et al., 2001). In these cases NF-Y dysfunction would lead to failure to stop cycling of damaged cells, abnormalities of lipogenic metabolism and blood pressure control, respectively.

Since the above list covers either inducible or constitutive genes, tissue-specific or ubiquitous genes, and house keeping genes, there must be mechanisms for differential control of the NF-Y function on individual genes and in individual situations.

3. The structure of NF-Y

NF-Y has been found in a variety of eukaryotes, including yeasts, fungi, higher plants, and vertebrates, and is highly conserved throughout the evolution (Li et al., 1992b). In mammals, NF-Y consists of three subunits, termed NF-YA, YB, and YC. As regards another nomenclature CBF and yeast counterparts HAP proteins, NF-YA corresponds to CBF-B and HAP2, NF-YB to CBF-A and HAP3, and NF-YC to CBF-C and HAP5. The architecture of NF-Y has been described in detail (Mantovani, 1999; Matuoka and Chen, 1999): NF-YA contains a glutamine (Q)-rich region, a serine/threonine (S/T)-rich region, subunit interaction domain, and DNA-binding domain. NF-YB contains a histone-fold motif and a TBP-binding domain. NF-YC is similar to NF-YB, but has an additional C-terminal Q-rich region. In yeasts, HAP2 and HAP5 lack the sequence corresponding to the Q-rich regions; instead, there is a regulatory protein HAP4 (Mantovani, 1999). Despite the existence of the CCAAT box in some *Drosophila* genes (e.g. Hanai et al., 1998) the fruit fly may be devoid of NF-Y (Mantovani, 1999).

There are several variants of NF-YA due to alternative splicing at the Q-S/T domains (Li et al., 1992a). Although expression of these isoforms varies depending on tissues and cell

types, they all seem intact in terms of the transcriptional function. Nonetheless, for expression of metalloproteinase CD10, the long forms in epithelial cells appear to function but the short forms in lymphoid cells do not (Ishimaru et al., 1997). In addition, predominance of the short subtypes in SV40—transformed fibroblasts (Gu et al., 1999) and HeLa cells (Matuoka and Chen, unpublished) has been observed, while normal fibroblasts and skin epithelial cells mainly express the long subtypes.

Then, how does NF-Y interact with the chromatin to exert gene regulation? It is well documented (Struhl and Moqtaderi, 1998; Jenuwein and Allis, 2001), that chromatin consists of the nucleosomes of core histones (heterooctamers made of a pair of each of H2A, H2B, H3, and H4), the genomic DNA, and linker histones (normally H1, but H5 at times). In order to be functionally active, NF-Y needs to form a heterotrimer of the three subunits: NF-YB (homologous to histone H2B) and NF-YC (homologous to H2A) form a heterodimer via their histone fold motifs; then NF-YA binds to them via its interaction domain. Now the heterotrimer is capable of binding to the CCAAT box. NF-Y has the ability to interact with various TBP-associated factors (TAFs) (Frontini et al., 2002). Notably, NF-Y is capable of binding to a complex of $TAF_{II}31$ (H3-like TAF) and $TAF_{II}80$ (H4-like TAF). Since the NF-Y heterotrimer contains H2B-like (NF-YB) and H2A-like (NF-YC) molecules, the resultant complex possesses all the core histone components and, therefore, could mimic the interaction of the nucleosome core with genomic DNA (Struhl and Moqtaderi, 1998). Similarly, NF-YA/B/C trimer or YB/YC dimer has been found to bind to an H3/H4 tetramer (but not to an H2A/H2B complex) during nucleosome assembly (Caretti et al., 1999). Importantly, they also bind to the chromatin even after completion of nucleosome formation, indicating the ability of NF-Y to interact with the genomic DNA assembled in the nucleosome (Fig. 1B). Subsequently, NF-Y (or YB/YC having recruited YA) would “browse” the DNA strands in search of its target, the CCAAT sequence (Caretti et al., 1999). Upon binding to the double helix DNA, NF-Y recognizes the minor groove and bends the strands in a similar way to the DNA distortion by histone proteins for the nucleosome assembly. This interaction between DNA and NF-Y causes local disruption of the nucleosomal architecture (Coustry et al., 2001). If sufficient distortion extends to the transcription initiation site, the DNA strands undergo partial dissociation from the histone core, which, in turn, enables the general transcription machinery to “pinch” the freed portion of the genomic DNA, thereby permitting transcriptional initiation (Fig. 1B).

Another environmental factor also works therein; the highly basic N-termini of histone proteins surround negatively charged DNA strands to ensure tight binding of DNA to the nucleosome. However, when expression of a gene is required, the DNA for the gene needs to be relaxed or released from the histones. For the purpose the cell modifies the histone peptides by acetylation, phosphorylation, and methylation (Jenuwein and Allis, 2001). Particularly, acetylation of lysine residues of histone H3 and H4 neutralizes their positive charges, thereby weakening the interaction between DNA and histones. This process is performed by histone acetyltransferases, which are quite often integrated into the complexes of transcription regulators (Struhl and Moqtaderi, 1998). For example, TFIID is associated with p300/CBP; human PCAF complex has p/CAF; and yeast SAGA complex bears GCN5. Intriguingly, NF-Y subunits have been found to bind to these histone acetyltransferases: YB/YC to GCN5 (Currie, 1998); YA to p/CAF (Jin and Scotto, 1998); YB to p300

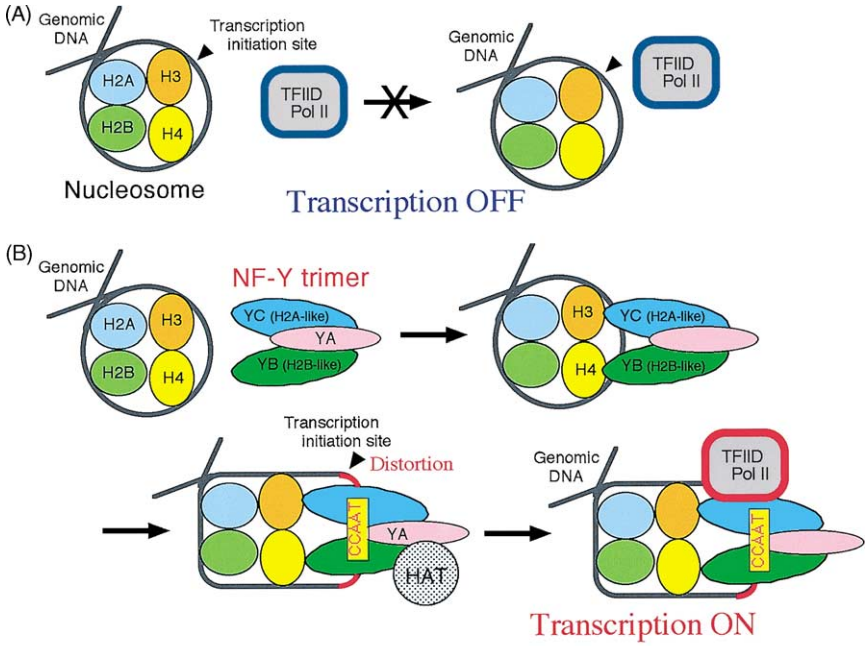

Fig. 1. NF-Y activation of transcription. (A) At the basal state, the genomic DNA is tightly assembled in the nucleosome. Thus, the TFIID-Pol II complex, the general transcription machinery, is unable to access to the transcription initiation site; hence, no transcription; (B) since the NF-Y heterotrimer bears histone H2A- and H2B-like components, the complex is able to interact with the H3–H4 complex. Then, NF-Y browses the DNA strands and "grabs" the CCAAT box, which causes distortion of the DNA. If sufficient distortion extends to the transcription initiation site, the DNA becomes loose in the region, enabling TFIID to access to the site; hence transcription activated. Histone acetyltransferases (HAT) located in the NF-Y complex further facilitate the process.

(Li et al., 1998; Faniello et al., 1999). These facts indicate that NF-Y is fully equipped to exert transactivation on the eukaryotic genes (Fig. 1B).

4. Regulation of NF-Y activity

The important question about this general transcription factor acting on the ubiquitous CCAAT sequences is, how NF-Y can discriminate individual genes and how differentially it can regulate each gene differentially.

It has been observed that, in the promoters with TATA box, the NF-Y-binding CCAAT boxes are generally located from −60 to −100 nt. from the transcription initiation sites but, in promoters without a TATA box, they are at −40 to −80 nt. (Mantovani, 1998). Since NF-Y having bound to the CCAAT sequence interacts with other juxtaposed factors, the above difference is likely to reflect the distance between the NF-Y-binding site and a putative neighboring factor (presumably, TBP, if there is a TATA box, or TFIID complex, if there is no TATA box) (Fig. 2A). As seen in this example, appropriate positioning of

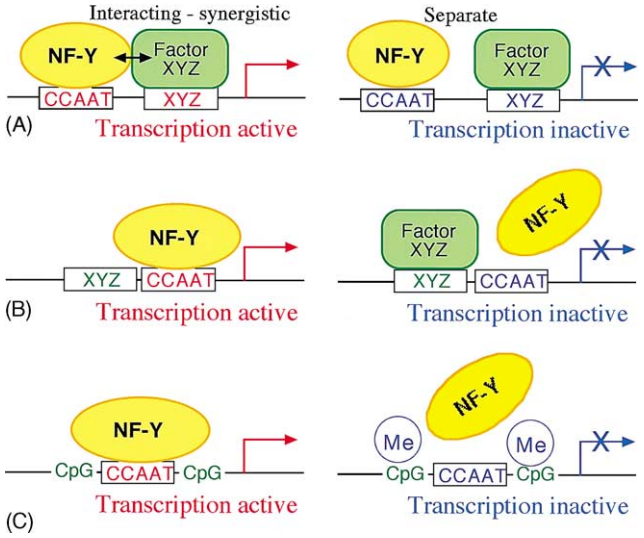

Fig. 2. Modification of NF-Y activity. (A) Quite often interaction with a neighboring factor is required for the NF-Y function or activates it in a synergistic fashion, as seen with TFIID in general, ATF6 for unfolded protein response, C/EBP for albumin promoter, and sterol regulatory element binding protein 1 for cholesterolgenic enzymes; (B) binding of a juxtaposed factor causes steric hindrance, thereby abolishing the function of NF-Y (or that factor). Examples are NF-E for Aγ-globin promoter, CDP for TK promoter, and ATPC-2 for photosynthesis gene *AtpC*; (C) CpG methylation around the CCAAT box impairs NF-Y binding to hMLH1 due to steric interference or structural modulation of the DNA strands. Besides, deviation of the CCAAT sequence from the consensus binding motif, quite naturally, results in a decrease in the NF-Y function. Intracellular redox state may also affect the NF-Y activity (see the text).

NF-Y relative to another factor is of importance, since that confers an efficient interaction between them. Molecular interaction of this type works for the promoters of albumin (with C/EBP; Milos and Zaret, 1992), HMG-CoA synthase and other cholesterolgenic enzymes (with sterol regulatory element binding protein 1 (Magana et al., 2000) and unfolded protein response chaperones (with ATF6a/b; Yoshida et al., 2000), etc.

On the contrary, in other cases, the positioning is important because it causes steric hindrance (Fig. 2B): NF-E binds to human Aγ-globin promoter (Superti-Furga et al., 1988), CDP binds to human TK promoter (Kim et al., 1997), and ATPC-2 binds to spinach photosynthesis gene *AtpC* (Bezhani et al., 2001), all interfering with NF-Y binding to the CCAAT box. With *hMLH1* gene, CpG methylation around the CCAAT box in cancer cells relates to NF-Y inability to bind to the sequence (Deng et al., 2001) (Fig. 2C).

The following observations exemplify possible differentiation of NF-Y function by subtle variation of the promoter sequences. As mentioned above, NF-Y is capable of bending DNA, but the angle of the bend varies from 62 to 82° depending on the sequences flanking the pentanucleotide CCAAT (Ronchi et al., 1995). The promoter of multidrug resistance protein 2 contains a CCAAT box; the rat promoter interacts with NF-Y, but the human promoter with another Y box factor YB-1 (Kauffmann et al., 2001). In addition, environmental factors, such as subcellular redox conditions (Nakshatri et al., 1996) and interaction with HMG-I

or -I(Y), abundant small nuclear non-histone proteins (Currie, 1997; Liberati et al., 1998), have also been suggested to affect the NF-Y function.

In short, one should consider that, under such multi-factor conditions, NF-Y functions on a case-by-case basis rather than on an all-or-none basis.

5. Ageing-related changes in NF-Y

With regard to changes in NF-Y during ageing, very few studies have been performed thus far, as seen by the fact that a database search returns <10 hits. In our hands, the basic observations with human fibroblasts are as follows (Good and Chen, 1996; Pang et al., 1996; Matuoka and Chen, 2000): expression of the NF-Y subunits at the mRNA level is affected little by ageing. At the protein level, however, NF-YA exhibits a marked decline with in vitro age. Consistently, the NF-Y function attenuates for the genes such as *TK*. Furthermore, treatment with H_2O_2, which induces premature senescence (Matuoka and Chen, 2002), causes a comparable change in the NF-Y activity (Pang et al., 1996). Interestingly, the binding activity of NF-Y for the oligonucleotide from the thymidine kinase promoter is greatly lowered in aged cells; however, that for the α_2(I)-procollagen promoter was unchanged or slightly higher therein (unpublished), confirming NF-Y discrimination of individual genes. In addition, a recent report has described NF-Y binding to TK promoter to be unaffected during aging (Chang and Huang, 2001). Although we have found our observations reproducible, this phenomenon should be analyzed in more detail.

As to the fluctuation of NF-YA, a possible reason is a post-transcriptional alteration of its metabolism such as translational inactivity or accelerated degradation. Intriguingly, parallel phenomena have been observed with cell cycling and differentiation. A decline in the amount of the NF-YA protein, without a change in its mRNA, has been correlated to an attenuated NF-Y function for expression of cyclin B2 during the cell cycle (Bolognese et al., 1999) (cyclin B1 in muscle cell differentiation (Farina et al., 1999), and MHC class II and other genes in monocyte-to-macrophage differentiation (Marziali et al., 1999). NF-YB and YC are unaffected therein. Thus, the same mechanism for NF-YA regulation as in the ageing seems to work in various situations. The protein level of NF-YA increases upon growth stimulation (Chang and Liu, 1994; Matuoka and Chen, 2000) and by DNA damaging agents (Jin et al., 2001). Thus, it is clear that NF-YA plays a major role in regulation of the NF-Y function.

In this regard, an additional possibility has been suggested for NF-Y regulation. Under an oxidative microenvironment, two cysteine residues of NF-YB are capable of forming a covalent loop between them (or with a cysteine residue of another molecule), which prevents NF-Y subunits from forming a heterodimer, hence no heterotrimer or interaction with DNA (Nakshatri et al., 1996) (Fig. 2). In fact, the intracellular environment becomes more oxidative when cells age (Hagen et al., 1997; Lee et al., 1999). With all the changes accompanying ageing, the function of NF-Y could become defective or attenuated.

6. Involvement of NF-Y in the cellular ageing

A number of reviews have addressed the ageing phenomena, counting >400 in the period of 2001–2002 alone. Although the definitive mechanism of cellular ageing has yet to be

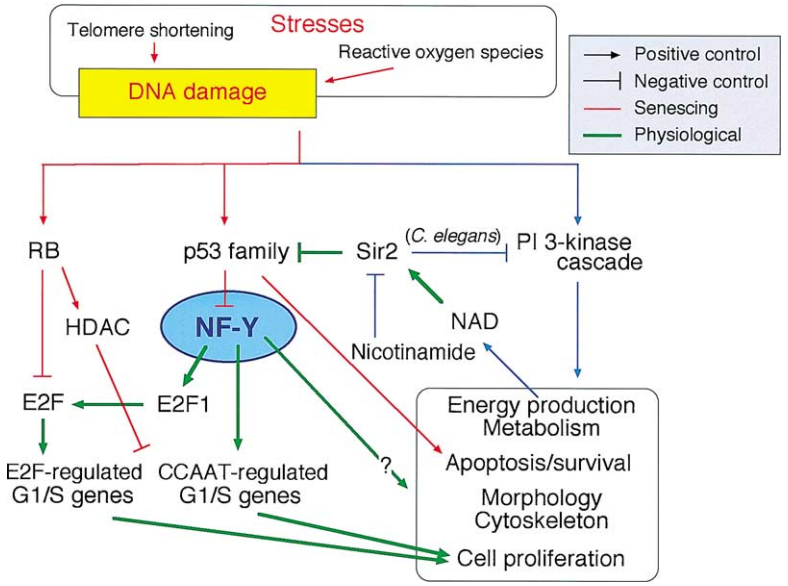

Fig. 3. Ageing-related signaling pathways and NF-Y function. The signals related to NF-Y are diagrammed. HDAC, histone deacetylase.

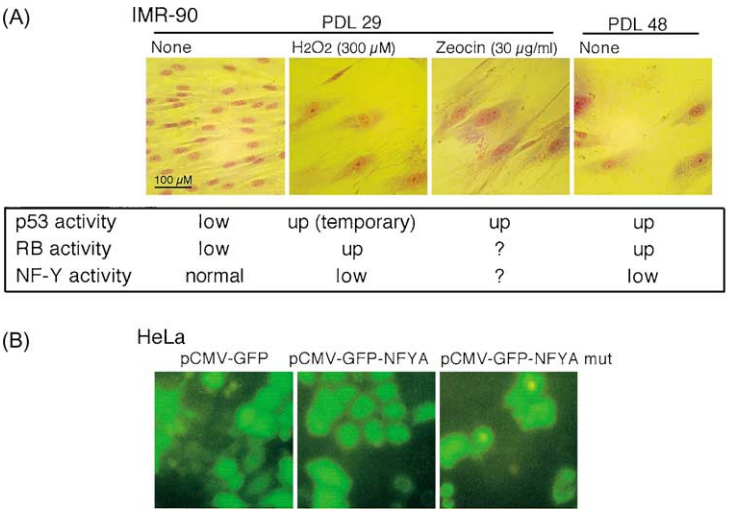

Fig. 4. Morphological changes due to ageing, chemical treatment, and mutant NF-YA. (A) Effects of H_2O_2 and Zeocin on IMR-90 human fibroblasts are shown in reference to the activities of p53, RB, and NF-Y (cf. Pang et al., 1996; Matuoka and Chen, 2002). Expression of senescent cell morphology may relate to a lowered NF-Y activity; (B) Dominant negative NF-YA mutant, but not wild-type NF-YA, induces aberrant cell shapes in HeLa cells.

established, recent progress has made the route to the senescence more visible (Campisi, 2001; Zhang and Herman, 2002). As major causes, stresses due to reactive oxygen species and/or telomere shortening may evoke aging responses signaling for ageing (Stewart and Weinberg, 2002; Matuoka and Chen, 2002). As diagrammed in Fig. 3, these events are most likely to involve p53, retinoblastoma protein (RB), and phosphatidylinositol 3-kinase (PI 3-kinase) as pivotal regulator molecules. Their downstream signaling pathways have been discussed in detail (e.g. Campisi, 2001; Zhang and Herman, 2002; Roymans and Slegers, 2001). Nevertheless, considering the global change in the gene expression during ageing (Meyyappan et al., 1996; Ly et al., 2000), we suggest that involvement of a general transcription regulator(s) should also be postulated in the ageing process.

It has been demonstrated that the activity of p53 increases during ageing (Atadja et al., 1995) and that it is required for induction of senescence (Bond et al., 1996). Recently p53 and its family members p63 and p73 have been shown to inhibit NF-Y function through direct binding to NF-Y (Jung et al., 2001). Activation of p53 due to DNA damage or another signal accompanying ageing downregulates the NF-Y function, which is critical for expression of CCAAT-regulated G1/S genes prerequisite for cell cycling (Chen, 1997), including another transcription activator E2F1 (Fig. 3). The decline in E2F1 further causes suppression of another set of genes, E2F element (TTTCGCGC)-regulated G1/S genes (Good et al., 1996; Matuoka and Chen, 1999). As a result, cell cycling is severely impaired. As to the morphology and other phenotypes prominent in aged cells (Fig. 4A), RB has been implicated in their premature induction by an oxidative stress with H_2O_2 (Chen et al., 2000) and, on the other hand, we have observed involvement of PI 3-kinase therein (Matuoka et al., submitted). Intriguingly, Zeocin, which upregulates p53 (Houser et al., 2001), induces the morphology typical of senescent cells (Fig. 4A), implicating p53 in the phenomenon. In this connection, nicotinamide, which is an inhibitor of Sir2, an NAD-dependent histone deacetylase suppressing the activity of p53 (Luo et al., 2001), causes a reversion of ageing cell phenotypes (Matuoka et al., 2001). These mechanisms do not necessarily exclude a possible role of NF-Y downstream of those molecules in expression of the ageing phenotypes. As already discussed, NF-Y inactivation can exert differential modulation of various genes, including even their upregulation, as the case may be.

Does attenuation of the NF-Y function lead to cellular ageing? That seems to be the case. Human bladder carcinoma cells become senescent upon suppression of NF-Y by expression of p53 family proteins, (Jung et al., 2001). Exposure to H_2O_2, an oxidative stress, induces parallel changes to the natural in vitro ageing in both NF-Y activity and senescence phenotypes (Pang et al., 1996; Matuoka and Chen, 2002). Human fibroblasts express SA-gal upon transfection of a dominant negative NF-YA mutant (Matuoka and Chen, 2000). In addition, the mutant NF-YA, but not the wild type NF-YA, brings about morphological aberrations in HeLa cells (Fig. 4B), indicating an important role of NF-Y in the cellular physiology.

7. Concluding remarks

Since a global change in the gene expression accompanies ageing, general transcription regulators have been implicated. The accumulated observations concerning the NF-Y

transcription factor are suggestive enough to prompt further investigations on the role of NF-Y in the cellular ageing. For the analysis, recently identified NF-Y inhibitors, including genistein (Zhou and Lee, 1998), HMN-154 (Tanaka et al., 1999), and ET-743 (Minuzzo et al., 2000), will be of great use. The question as to how the NF-YA protein decreases during ageing is also important for elucidation of the mechanism for post-transcriptional cell regulation.

References

Atadja, P., Wong, H., Garkavtsev, I., Veillette, C., Riabowol, K., 1995. Increased activity of p53 in senescing fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 92, 8348–8352.

Bertagna, A., Jahroudi, N., 2001. The NFY transcription factor mediates induction of the von Willebrand factor promoter by irradiation. Thromb. Haemost. 85, 837–844.

Bezhani, S., Sherameti, I., Pfannschmidt, T., Oelmuller, R., 2001. A repressor with similarities to prokaryotic and eukaryotic DNA helicases controls the assembly of the CAAT box binding complex at a photosynthesis gene promoter. J. Biol. Chem. 276, 23785–23789.

Bolognese, F., Wasner, M., Dohna, C.L., Gurtner, A., Ronchi, A., Muller, H., Manni, I., Mossner, J., Piaggio, G., Mantovani, R., Engeland, K., 1999. The cyclin B2 promoter depends on NF-Y a trimer whose CCAAT-binding activity is cell-cycle regulated. Oncogene 18, 1845–1853.

Bond, J., Haughton, M., Blaydes, J., Gire, V., Wynford-Thomas, D., Wyllie, F., 1996. Evidence that transcriptional activation by p53 plays a direct role in the induction of cellular senescence. Oncogene 13, 2097–2104.

Bucher, P., 1990. Weight matrix descriptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. J. Mol. Biol. 212, 563–578.

Campisi, J., 2001. Cellular senescence as a tumor-suppressor mechanism. Trends Cell Biol. 11, S27–S31.

Caretti, G., Motta, M.C., Mantovani, R., 1999. NF-Y associates with H3–H4 tetramers and octamers by multiple mechanisms. Mol. Cell. Biol. 19, 8591–8603.

Chang, Z.F., Liu, C.J., 1994. 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. 269, 17893–17898.

Chang, Z.F., Huang, D.Y., 2001. Regulation of thymidine kinase expression during cellular senescence. J. Biomed. Sci. 8, 176–183.

Chen, K.Y., 1997. Transcription factors and the down-regulation of G1/S boundary genes in human diploid fibroblasts during senescence Front. Bioscience 2, d417–d426.

Chen, Q.M., Tu, V.C., Catania, J., Burton, M., Toussaint, O., Dilley, T., 2000. Involvement of Rb family proteins, focal adhesion proteins and protein synthesis in senescent morphogenesis induced by hydrogen peroxide. J. Cell Sci. 113, 4087–4097.

Coustry, F., Hu, Q., de Crombrugghe, B., Maity, S.N., 2001. CBF/NF-Y functions both in nucleosomal disruption and transcription activation of the chromatin-assembled topoisomerase IIα promoter. Transcription activation by CBF/NF-Y in chromatin is dependent on the promoter structure. J. Biol. Chem. 276, 40621–40630.

Currie, R.A., 1997. Functional interaction between the DNA binding subunit trimerization domain of NF-Y and the high mobility group protein HMG-I(Y). J. Biol. Chem. 272, 30880–30888.

Currie, R.A., 1998. NF-Y is associated with the histone acetyltransferases GCN5 and P/CAF. J. Biol. Chem. 273, 1430–1434.

Deng, G., Chen, A., Pong, E., Kim, Y.S., 2001. Methylation in hMLH1 promoter interferes with its binding to transcription factor CBF and inhibits gene expression. Oncogene 20, 7120–7127.

Faniello, M.C., Bevilacqua, M.A., Condorelli, G., de Crombrugghe, B., Maity, S.N., Avvedimento, V.E., Cimino, F., Costanzo, F., 1999. The B subunit of the CAAT-binding factor NFY binds the central segment of the Co-activator p300. J. Biol. Chem. 274, 7623–7626.

Farina, A., Manni, I., Fontemaggi, G., Tiainen, M., Cenciarelli, C., Bellorini, M., Mantovani, R., Sacchi, A., Piaggio, G., 1999. 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 18, 2818–2827.

Frontini, M., Imbriano, C., diSilvio, A., Bell, B., Bogni, A., Romier, C., Moras, D., Tora, L., Davidson, I., Mantovani, R., 2002. NF-Y recruitment of TFIID, multiple interactions with histone fold TAF(II)s. J. Biol. Chem. 277, 5841–5848.

Good, L.F., Chen, K.Y., 1996. Cell cycle- and age-dependent transcriptional regulation of human thymidine kinase gene: the role of NF-Y in the CBP/tk binding complex. Biol. Signals 5, 163–169.

Good, L., Dimri, G.P., Campisi, J., Chen, K.Y., 1996. Regulation of dihydrofolate reductase gene expression and E2F components in human diploid fibroblasts during growth and senescence. J. Cell. Physiol. 168, 580–588.

Gu, Z., Kuntz-Simon, G., Rommelaere, J., Cornelis, J., 1999. Oncogenic transformation-dependent expression of a transcription factor NF-Y subunit. Mol. Carcinog. 24, 294–299.

Hagen, T.M., Yowe, D.L., Bartholomew, J.C., Wehr, C.M., Do, K.L., Park, J.Y., Ames, B.N., 1997. Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. Proc. Natl. Acad. Sci. U.S.A. 94, 3064–3069.

Hanai, S., Uchida, M., Kobayashi, S., Miwa, M., Uchida, K., 1998. Genomic organization of *Drosophila* poly(ADP-ribose) polymerase and distribution of its mRNA during development. J. Biol. Chem. 273, 11881–11886.

Houser, S., Koshlatyi, S., Lu, T., Gopen, T., Bargonetti, J., 2001. Camptothecin and Zeocin can increase p53 levels during all cell cycle stages. Biochem. Biophys. Res. Commun. 289, 998–1009.

Huang, H.S., Chen, C.J., Chang, W.C., 1999. The CCAAT-box binding factor NF-Y is required for the expression of phospholipid hydroperoxide glutathione peroxidase in human epidermoid carcinoma A431 cells. FEBS Lett. 455, 111–116.

Ishimaru, F., Mari, B., Shipp, M.A., 1997. The type 2 CD10/neutral endopeptidase 24.11 promoter: functional characterization and tissue-specific regulation by CBF/NF-Y isoforms. Blood 89, 4136–4145.

Jenuwein, T., Allis, C.D., 2001. Translating the histone code. Science 293, 1074–1080.

Jin, S., Scotto, K.W., 1998. Transcriptional regulation of the *MDR1* gene by histone acetyltransferase and deacetylase is mediated by NF-Y. Mol. Cell. Biol. 18, 4377–4384.

Jin, S., Fan, F., Fan, W., Zhao, H., Tong, T., Blanck, P., Alomo, I., Rajasekaran, B., Zhan, Q., 2001. Transcription factors Oct-1 and NF-YA regulate the p53-independent induction of the GADD45 following DNA damage. Oncogene 20, 2683–2690.

Jung, M.S., Yun, J., Chae, H.D., Kim, J.M., Kim, S.C., Choi, T.S., Shin, D.Y., 2001. p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. Oncogene 20, 5818–5825.

Kauffmann, H.M., Vorderstemann, B., Schrenk, D., 2001. Basal expression of the rat, but not of the human, multidrug resistance protein 2 (*MRP2*) gene is mediated by CBF/NF-Y and Sp1 promoter-binding sites. Toxicology 167, 25–35.

Kim, E.C., Lau, J.S., Rawlings, S., Lee, A.S., 1997. Positive and negative regulation of the human thymidine kinase promoter mediated by CCAAT binding transcription factors NF-Y/CBF, dbpA, and CDP/cut. Cell Growth Differ. 8, 1329–1338.

Lee, A.C., Fenster, B.E., Ito, H., Takeda, K., Bae, N.S., Hirai, T., Yu, Z.X., Ferrans, V.J., Howard, B.H., Finkel, T., 1999. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. J. Biol. Chem. 274, 7936–7940.

Li, X.Y., van Huijsduijnen, R., Mantovani, R., Benoist, C., Mathis, D., 1992a. Intron-exon organization of the *NF-Y* genes. Tissue-specific splicing modifies an activation domain. J. Biol. Chem. 267, 8984–8990.

Li, X.Y., Mantovani, R., Hooft, van Huijsduijnen, R., Andre, I., Benoist, C., Mathis, D., 1992b. Evolutionary variation of the CCAAT-binding transcription factor NF-Y. Nucl. Acids Res. 20, 1087–1091.

Li, Q., Herrler, M., Landsberger, N., Kaludov, N., Ogryzko, V.V., Nakatani, Y., Wolffe, A.P., 1998. Xenopus NF-Y pre-sets chromatin to potentiate p300 and acetylation-responsive transcription from the Xenopus hsp70 promoter in vivo. EMBO J. 17, 6300–6315.

Liberati, C., Sgarra, R., Manfioletti, G., Mantovani, R., 1998. DNA binding of NF-Y: the effect of HMGI proteins depends upon the CCAAT box. FEBS Lett. 433, 174–178.

Luo, J., Nikolaev, A.Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L., Gu, W., 2001. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 107, 137–148.

Ly, D.H., Lockhart, D.J., Lerner, R.A., Schultz, P.G., 2000. Mitotic misregulation and human aging. Science 287, 2486–2492.

Magana, M.M., Koo, S.H., Towle, H.C., Osborne, T.F., 2000. Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. J. Biol. Chem. 275, 4726–4733.

Manni, I., Mazzaro, G., Gurtner, A., Mantovani, R., Haugwitz, U., Krause, K., Engeland, K., Sacchi, A., Soddu, S., Piaggio, G., 2001. NF-Y mediates the transcriptional inhibition of the cyclin B1, cyclin B2, and *cdc25C* promoters upon induced G2 arrest. J. Biol. Chem. 276, 5570–5576.

Mantovani, R., 1998. A survey of 178 NF-Y binding CCAAT boxes. Nucl. Acids Res. 26, 1135–1143.

Mantovani, R., 1999. The molecular biology of the CCAAT-binding factor NF-Y. Gene 239, 15–27.

Marziali, G., Perrotti, E., Ilari, R., Coccia, E.M., Mantovani, R., Testa, U., Battistini, A., 1999. The activity of the CCAAT-box binding factor NF-Y is modulated through the regulated expression of its A subunit during monocyte to macrophage differentiation: regulation of tissue-specific genes through a ubiquitous transcription factor. Blood 93, 519–526.

Matuoka, K., Chen, K.Y., 1999. Nuclear factor Y (NF-Y) and cellular senescence. Exp. Cell Res. 253, 365–371.

Matuoka, K., Chen, K.Y., 2000. Possible role of subunit A of nuclear factor Y (NF-YA) in normal human diploid fibroblasts during senescence. Biogerontology 1, 261–271.

Matuoka, K., Chen, K.Y., 2002. Telomerase positive human diploid fibroblasts are resistant to replicative senescence but not premature senescence induced by chemical reagents. Biogerontology 3, in press.

Matuoka, K., Chen, K.Y., Takenawa, T., 2001. Rapid reversion of aging phenotypes by nicotinamide through possible modulation of histone acetylation. Cell. Mol. Life Sci. 58, 2108–2116.

Meyyappan, M., Atadja, P.W., Riabowol, K.T., 1996. Regulation of gene expression and transcription factor binding activity during cellular aging. Biol. Signals 5, 130–138.

Milos, P.M., Zaret, K.S., 1992. A ubiquitous factor is required for C/EBP-related proteins to form stable transcription complexes on an albumin promoter segment in vitro. Genes Dev. 6, 991–1004.

Minuzzo, M., Marchini, S., Broggini, M., Faircloth, G., D'Incalci, M., Mantovani, R., 2000. Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. Proc. Natl. Acad. Sci. U.S.A. 97, 6780–6784.

Nakshatri, H., Bhat-Nakshatri, P., Currie, R.A., 1996. Subunit association and DNA binding activity of the heterotrimeric transcription factor NF-Y is regulated by cellular redox. J. Biol. Chem. 271, 28784–28791.

Nenoi, M., Ichimura, S., Mita, K., Yukawa, O., Cartwright, I.L., 2001. Regulation of the catalase gene promoter by Sp1, CCAAT-recognizing factors, and a WT1/Egr-related factor in hydrogen peroxide-resistant HP100 cells. Cancer Res. 61, 5885–5894.

Pang, J.H., Good, L.F., Chen, K.Y., 1996. The age-dependent binding of CBP/tk, a CCAAT binding protein, is deregulated in transformed and immortalized mammalian cells but absent in premature aging cells. Exp. Gerontol. 31, 97–109.

Ronchi, A., Bellorini, M., Mongelli, N., Mantovani, R., 1995. CCAAT-box binding protein NF-Y (CBF, CP1) recognizes the minor groove and distorts DNA. Nucl. Acids Res. 23, 4565–4572.

Roymans, D., Slegers, H., 2001. Phosphatidylinositol 3-kinases in tumor progression. Eur. J. Biochem. 268, 487–498.

Shi, Q., Gross, K.W., Sigmund, C.D., 2001. NF-Y antagonizes renin enhancer function by blocking stimulatory transcription factors. Hypertension 38, 332–336.

Stewart, S.A., Weinberg, R.A., 2002. Senescence: does it all happen at the ends? Oncogene 21, 627–630.

Struhl, K., Moqtaderi, Z., 1998. The TAFs in the HAT. Cell 94, 1–4.

Superti-Furga, G., Barberis, A., Schaffner, G., Busslinger, M., 1988. The -117 mutation in Greek HPFH affects the binding of three nuclear factors to the CCAAT region of the gamma-globin gene. EMBO J. 7, 3099–3107.

Tanaka, H., Ohshima, N., Hidaka, H., 1999. Isolation of cDNAs encoding cellular drug-binding proteins using a novel expression cloning procedure: drug-western. Mol. Pharmacol. 55, 356–363.

Teran-Garcia, M., Rufo, C., Nakamura, M.T., Osborne, T.F., Clarke, S.D., 2002. NF-Y involvement in the polyunsaturated fat inhibition of fatty acid synthase gene transcription. Biochem. Biophys. Res. Commun. 290, 1295–1299.

Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., Mori, K.A., 2000. TF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol. Cell. Biol. 20, 6755–6767.

Zhang, Y., Herman, B., 2002. Ageing and apoptosis. Mech. Age. Dev. 123, 245–260.

Zhou, Y., Lee, A.S., 1998. Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy. J. Natl. Cancer Inst. 90, 381–388.