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TRANSCRIPTION FACTORS AND THE DOWN-REGULATION OF G1/S BOUNDARY GENES IN HUMAN DIPLOID FIBROBLASTS DURING SENESCENCE

Kuang Yu Chen

Department of Chemistry and The Cancer Institute of New Jersey, Rutgers - The State University of New Jersey, Piscataway, NJ. 08855-0939

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1. ABSTRACT

The hallmark of cellular aging is the failure of senescent cells to initiate the DNA synthesis during the progression of cell cycle. Since most, if not all, of the G1/S genes exhibit a significant down-regulation during aging, an alteration of gene regulation at late G1/S boundary could be a major contributing factor for the loss of dividing potential during cell senescence. The underlying cause for the apparent global attenuation of gene expression at late G1/S boundary is not clear. Since we have shown that thymidine kinase (TK) and dihydrofolate reductase (DHFR) are transcriptionally regulated during aging, we suspect that a similar mechanism may be operative in the age-dependent down-regulation of other G1/S genes. DNA binding activities using Y-box containing sequence in TK promoter or E2F containing sequence in DHFR promoter show prominent serum-responsiveness in low passage cells and dramatic attenuation in senescent cells. Promoter analysis using GCG program reveals striking similarities in promoter organization of twelve age-dependent G1/S genes. Specifically, these genes can be divided into two groups, one group contains tandem multiple CCAAT element, similar to that in TK promoter and the other contains E2F site, similar to that in DHFR promoter. Further analysis shows that the promoter of transcription factor, NF-Y, which recognizes CBP/tk site contains a tandem, two Y-box motif, similar to that in TK promoter and that the promoter of E2F1 contains four E2F motifs and two tandem CCAAT elements. Thus, these two important transcription factors could undergo autoregulatory control themselves. It is possible that regulation of only a few of transcription factors such as CBP/tk (NF-Y) and E2F1 may be sufficient to cause a global attenuation of most of G1/S genes in human diploid fibroblasts during senescence.

2. INTRODUCTION

Normal diploid fibroblasts have a limited doubling potential in tissue culture (1). The remarkable consistency of the life span of these cells in culture, which is inversely related to the age of the donor, and the species specificity of the life span (2) has made them a useful model to study the biochemistry of cellular aging. Since the hallmark of cellular aging is the failure of old cells to initiate DNA synthesis (3), possible alterations of gene regulation at late G1/S boundary during senescence are of interest (4, 5). Many of early and mid-G1 genes are fully induced by serum in senescent cells, indicating that senescent cells still retain the ability to receive growth stimulatory signals, (6, 7). However, it has been reported that many genes at the G1/S boundary are suppressed

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Send correspondence to: Dr. Kuang Yu Chen, Department of Chemistry, Wright Chemistry Lab , Rutgers University Piscataway, NJ. 08855-0939 Tel: (732) 445-3739, Fax: (732) 445-5312, E.mail: kychen@rutchem.rutgers.edu

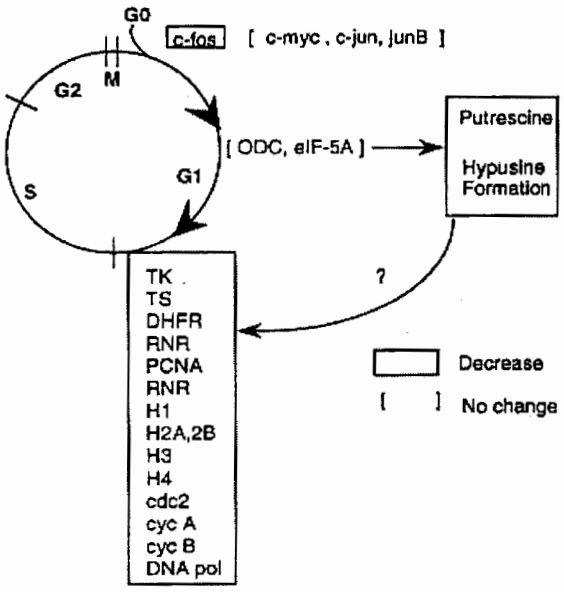


Figure 1. Schematic diagram of the expression of various cell cycle-dependent genes in serum-stimulated senescent human diploid fibroblasts. Among the early- and mid-G1 genes examined, only c-fos appears to be suppressed in senescent cells. In contrast, all the late G1/S genes examined appear to be suppressed in serum-stimulated senescent IMR-90 cells. Genes whose expressions show age-dependent attenuation are indicated in the box.

following serum stimulation in senescent cells (6-9). The attenuation of genes such as TK, DHFR, thymidylate synthase (TS), proliferating cell nuclear antigen (PCNA) and histones could lead to a defect in DNA synthetic machinery and thus renders cells incapable to enter S phase of the cell cycle.

Gene expression is commonly regulated at the level of transcription initiation. This step, in turn, is controlled by general and gene-specific transcription factors which bind to cognate DNA sequences in gene promoter regions. The combined action of activating and repressing transcription factors determines the transcription of target genes, and ultimately specifies physiological phenotypes. We have investigated the mechanism of the age-dependent regulation of TK and DHFR. We have identified the CBP/tk (CCAAT Binding Protein for tk gene) and E2F1 as the key transcription factor, respectively, responsible for the down-regulation of TK and DHFR gene (10. 11). The TK-specific transcription factor, CBP/tk, recognizes either one of the two inverted CCAAT elements embedded in Y-box which has a consensus sequence CTGATTGGYYRR (Y=C or T, R=A or G) (10). DNA affinity purification and gel mobility supershift assay using antisera against NF-YA and NF-YB suggests that CBP/tk is either identical to NF-Y (12) or contains NF-Y like proteins (13). NF-Y is a heteromeric protein with three subunits, NF-YA, NF-YB, and NF-YC (14). The transcription factor, E2F, specific for DHFR regulation, was first identified as a DNA-binding protein for the sequence TTTCGCGC within the adenovirus E2 promoter (15). E2F is a heterodimer, consisting of one E2F family protein and one DP family protein (16, 17). Among the known E2F family and DP family members, the attenuation of E2F1 gene expression at both the mRNA and protein levels appears to be primarily responsible for the down-regulation of DHFR gene in senescent cells (11).

We found that the promoter organizations of most of G1/S genes share features resembling that of either TK or DHFR. Specifically, we found that five G1/S genes contain tandem CCAAT elements arranged in a manner similar to that in TK promoter whereas the other six G1/S genes, including DHFR, contain E2F sequence. Most of the CCAAT element in G1/S gene promoters is present as part of Y-box. The presence of these two prominent motif features in almost all age-dependent G1/S gene promoters suggests that the E2F and Y-box binding proteins such as E2F1 and CBP/tk may be important for the global attenuation of G1/S genes during cell senescence. Interestingly, the promoters of NF-Y subunits, A and B, also contain tandem multiple CCAAT elements, similar to that in TK promoter and the promoter of E2F1 contains E2F sites. Thus, it is likely that these two age-dependent transcription factors, CBP/tk and E2F1, may be subject to autoregulatory control by themselves. Further studies will determine whether the tandem, multiple CCAAT elements and the E2F site, individually or together, may represent unique cis-element motifs important for the age-dependent regulation of G1/S genes.

3 CHANGES OF GENE EXPRESSION DURING SENESCENCE

3.1 Global attenuation of G1/S genes

Normal human diploid fibroblasts at quiescent state can be stimulated by serum or appropriate growth factors to enter the cell cycle. We and others have compared the changes of gene expression during cell cycling between young (low PDL) and senescent (high PDL) human cells. The senescent cells can enter the cell cycle and are capable to fully express many cell cycle-dependent genes all the way to mid-G1 phase (6-9). However, the cell cycling journey of senescent cells appear to be blocked at the G1/S boundary. The results from these studies are summarized in Figure 1. All of the G1/S genes studied thus far show attenuation of gene expression during senescence (6-9). These include genes encoding TK, PCNA, TS, RNR, DHFR, histone proteins H1, H2A, H2B, H3, and H4, cdc2, cyclin A and cyclin B (18). In contrast to G1/S genes, all mid-G1 genes that we and others have examined, including ornithine decarboxylase (ODC) and eIF-5A, appear to be fully induced by serum in senescent cells. However, it should be noted that although expressions of mid-G1 genes such as eIF-5A and ODC do

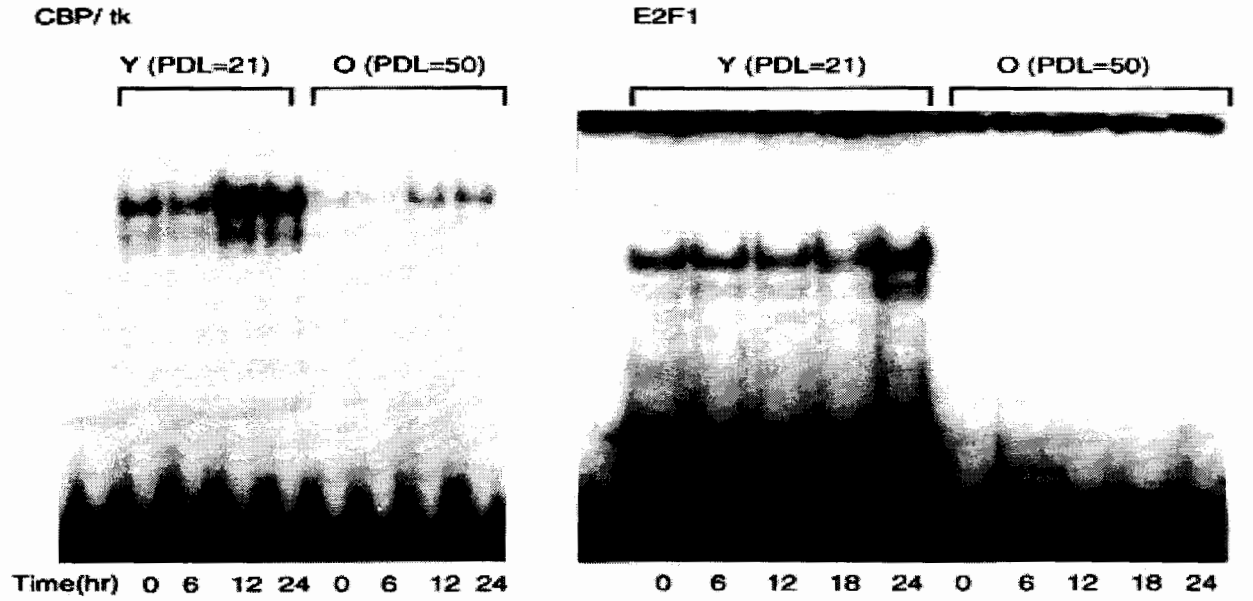


Figure 2. Gel mobility shift assay of the nuclear protein binding to the human TK and DHFR gene promoter in young (PDL=21) and old (PDL=50) IMR-90 cells following serum stimulation. The CBP/tk sequence used to perform the binding studies was the 28-bp fragment (-155/-128). The E2F sequence used was a 33-bp fragment spanning -77/ -45 bp relative to ATG codon

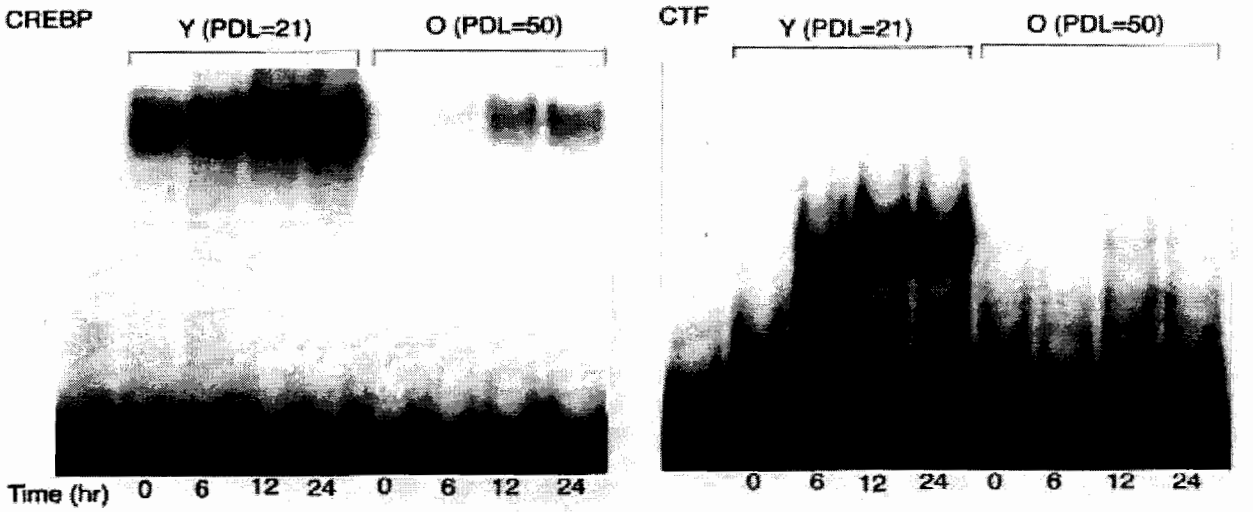


Figure 3. Gel mobility shift assay of the the CRE and CTF probes. The synthetic oligonucleotide probes with sequences CRE [TGACGTCA]5'-GATCTGACGTCATGACTGACGTCATGACTGACGTCATCA-3',CTF[GCCAAT]5'-GATCGCCAATGAT CGCCAAT GATC GCCAATGATC-3' were purchased from GIBCO-BRL. The nuclear extracts obtained from young (PDL=21) and old (PDL=50) IMR-90 cells at designated time points after serum stimulation were used for gel mobility shift assays with an oligonucleotide probe.

not show age-dependent attenuation at mRNA levels, a significant difference exists in the translation and/or post-translational modification of their gene products (6, 7, 19).

3.2 Molecular basis for the global attenuation of G1/S genes

The fact that G1/S genes that we and others have studied all exhibit age-dependent down-regulation in senescent cells raises a possibility that these genes may share a common or similar regulatory mechanism during cell senescence. To investigate this possibility, one needs to examine the regulatory mechanism of each G1/S gene in normal cells during senescence. The general strategy of this approach includes (i) performing nuclear run-on and promoter activity assay to determine whether the gene is transcriptionally regulated during the progression of cell

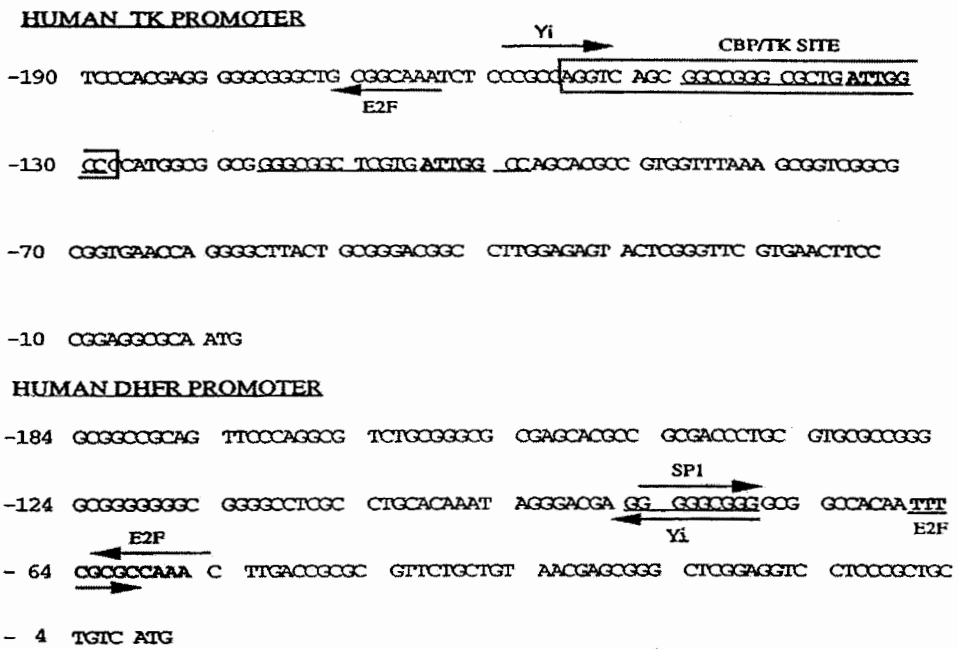


Figure 4. Organization of the promoter sequence in human thymidine kinase (TK) and dihydrofolate reductase (DHFR) gene. Human TK promoter: The distal CBP/tk site (28-bp) is indicated in the box. The 19-bp repeat within the CBP/tk site is underlined. The two inverted CCAAT elements (ATTGG) are shown in bold letters. The putative E2F site (7/8 match) does not show binding activity (23). Human DHFR promoter: The E2F sites in DHFR are arranged in two opposing directions as an imperfect palindrome. The arrows indicate the directions of E2F sites which are shown in bold letters. The SP1 (GGGGCGGG) and Yi site (CCCGCCCCCT) are also indicated with arrows.

cycle, (ii) performing nuclease protection assay to identify the promoter region that binds nuclear proteins, (iii) performing gel mobility shift assay to define the cis-elements which exhibit the cell cycle- and age-dependent binding activity, and finally (iv) using the cis-element as the probe or affinity media to isolate and characterize the trans-acting factor responsible for the age-dependent attenuation of the particular G1/S gene. Figure 2 shows that using this approach we have identified the promoter region and cis-elements responsible for the TK and DHFR gene during the progression of cell cycle and during senescence. The age-dependent CBP/tk binding activity is confined in the promoter region containing Y-box sequence. E2F1 is likely to be the key transcription factor for the age-dependent binding activity in DHFR promoter region containing E2F sequence (TTTCGCGC). Both CBP/tk and E2F binding activities are serum-responsive and age-dependent. Transcription factors such as CBP/tk and E2F1, may represent a new class of trans-acting factors which control gene expression not only during the cell growth but also during cell senescence.

3.3 Binding Activity as measured by gel mobility shift assay

Another approach to assess the role of transcription factors and their binding sites in cell aging is to use a synthetic oligonucleotide probe to examine whether a particular transcription factor may exhibit age-dependent binding activity. The probe contains the consensus binding sequence for that transcription factor. Figure 3 illustrates how this approach could lead to the identification of CREBP and CTF as putative age-dependent transcription factors. CREBP recognizes the sequence TGACGTCA whereas CTF recognizes GCCAAT. Using the synthetic probe containing tandem CRE or CTF, we show here that the CREBP and CTF binding activities in human IMR-90 cells are both serum-responsive and age-dependent. Similar results have been reported by Dimri and Campisi for WI38 cells (20). This approach, however, would not allow us to identify the target genes of these transcription factors. Thus, the physiological significance of this finding remains to be established.

3.4.The regulation of transcription factor activity during senescence

Once an age-dependent transcription factor is identified, it will be of interest to study as how this transcription factor is regulated during senescence. Studies have already shown that E2F1 is controlled at transcriptional level by E2F proteins during the progression of cell cycle (21). Since the E2F1 message is greatly reduced in senescent cells (11), it is likely that the E2F binding sites will be responsible for the down-regulation of E2F1 gene during senescence. With regards to the transcription factor specific for TK gene regulation,

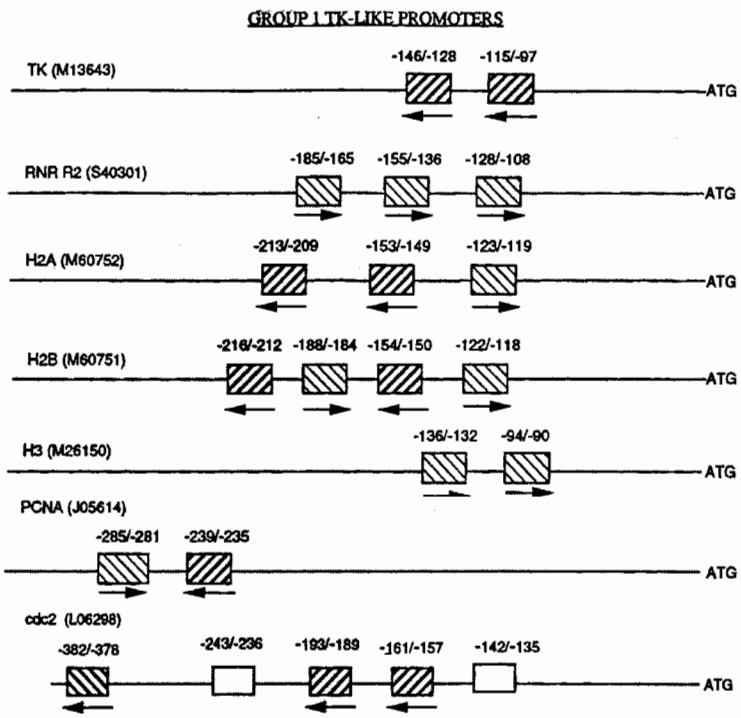


Figure 5. Promoter of the Group 1 genes that contain tandem CCAAT element in their promoters. The accession number of each gene is indicated in the parenthesis. The numbers above the shaded boxes indicate the position of cis-elements relative to the ATG codon since the transcription initial site for some of the genes has not been determined. The CCAAT element is indicated with a forward arrow and the inverted CCAAT element a backward arrow. The cdc2 gene also contains E2F site at positions -243/-236 and -142/-135.

CBP/tk, it has been suggested that CBP/tk is identical to NF-Y, the binding protein for the Y box within E alpha gene promoter (12). However, we note that (i) the half-life of NF-Y is much longer than that of CBP/tk in IMR-90 cells; (ii) the effect of serum is more pronounced with CBP/tk (>10-fold induction) than with A subunit of NF-Y (~2-to 3-fold induction) in IMR-90 cells; (iii) B subunit of NF-Y is not serum responsive in IMR-90 cells; and (iv) the increase in NF-Y does not correlate with the increase in CBP/tk binding activity in HeLa cells (13). These discrepancies prompt us to speculate that either CBP/tk is similar but not identical to NF-Y or there exist other factors in CBP/tk binding complex. Recently, a third component of NF-Y, CBF-C (NF-YC), has been isolated and cloned (14). Thus, it remains to be seen whether NF-YC or other unidentified factor may be responsible for the unique age-dependent features of CBP/tk.

4. TRANSCRIPTIONAL BINDING SITES IN THE G1/S GENE PROMOTERS

4.1 Promoter analysis of G1/S genes

We have previously used the GCG program, FINDPATTERN, to scan the promoter region for trans-acting factor binding sites in nine G1/S genes in order to determine whether there is any common cis-element in the promoter regions of these genes (9). The most commonly present cis-element in these genes is the SP1 (promoter-specific transcription factor-1) binding site. However, SP1 in human normal diploid fibroblasts does not show any cell cycle- or age-dependent change in binding activity (10), suggesting that it could not be the candidate for the age-dependent "master switch". Although we could not find any common "master switch" motif, we noticed that the promoter regions of these G1/S genes share some common characteristics. We found that the age-dependent G1/S genes can be divided into two groups, termed Group 1 and Group 2, based on their promoter organizations. Genes in Group 1 contain multiple tandem CCAAT elements, similar to that in TK promoter whereas genes in Group 2 contain E2F site, similar to that in DHFR. Thus, TK and DHFR can be considered as the founding member for Group 1 and Group 2 genes, respectively.

4.2 Promoter organizations of TK and DHFR genes

Figure 4 schematically shows the features of the promoter organization of TK and DHFR genes. Two promoter regions in human TK gene, termed 28-bp (-155/-128) and 67-bp (-127/-61) fragment, give clear and prominent age-dependent binding activity as measured by gel mobility shift assay (10). The serum-induced CBP/tk binding activity is inversely proportional to the population doubling level (PDL) of IMR-90 cells and appears to be almost absent in cells derived from premature aging syndromes (22). Moreover, CBP/tk activity appears to be deregulated in transformed cells (22). CBP/tk recognizes both 28-bp and 67-bp fragments. Within each fragment there is a 19-bp repeat containing an inverted CCAAT element. The inverted CCAAT element and its flanking sequence match closely with the Y-box consensus sequence CTGATTGGYYRR (10/12 and 11/12 match). A 25-bp fragment (-172/-148) upstream to the distal CCAAT element in TK promoter contains an E2F-like (7/8 match)

FLANKING REGIONS OF CAAT ELEMENT IN G1/S GENES

Thymidine kinase

-83/-63 5' GGCCGGGCGCTGATTGGCCCC 3' (10/12)
-52/-32 5' GGCCGGCTCCTGATTGGCCAG 3' (11/12)

Ribonucleotide reductase R2

-185/-166 5' AGCCAATGGGAAGGGTCGGA 3' (9/12)
-155/-136 5' AGCCAATGGGAAGGGCCGGA 3' (9/12)
-128/-109 5' AGCCAATGGGAAGGGCCGGA 3' (8/12)

Histone H2A

-222/-204 5'CCAGCTCTGATTGGGCAAT 3' (11/12)
-162/-144 5'TTATTTCTAATTGGTTGGC 3' (11/12)
-132/-114 5'CGCAATTTGATTGGTTAAA 3' (rev) (11/12)

Histone H2B

-226/-205 5'GGGCTTTCTCATTGGGTACAAG 3' (9/12)
-198/-177 5'CTACCTTTTCATTGGCTATTTC 3' (rev) (9/12)
-164/-143 5'TGTCGTTTACATTGGCATTTGT 3'
-132/-111 5'TTCGTTTATGATTGGATTAATT 3' (rev) (9/12)

Histone H3

-139/-129 5'GGACCAATCCA 3' (9/12)
-97/-87 5'GGTCCAATGGT 3'

cdc2

-210/-178 5'AGCGTAGCTGGGCTCTGATTGGCTGCTTTGAAA 3' (11/12)
-178/-146 5'AGTCTACGGGCTACCTGATTGGTGAATCCGGGG 3' (10/12)

Figure 6. Sequence comparison of the flanking regions of inverted CCAAT elements. Sequence match with Y-box is indicated in the parenthesis. Rev in the parenthesis indicates that the antisense strand is used for comparison.

and Yi-like (9/10 match) element. However, this fragment does not show any binding activity in IMR-90 nuclear extracts (23). Although the significance of the dyad structure of the two 19-bp repeats is unclear, it can be certain that the CBP/tk binding sites represent the key cis-elements for cell cycle- and age-dependent regulation of human TK gene. In the case of DHFR, the functional promoter region contains two E2F sites, arranged in opposing directions as an imperfect palindrome. This region also contains an inverted Yi binding site and an SP1 site upstream to the E2F sites. The E2F site exhibits clear cell cycle- and age-dependent binding activities. In contrast, SP1 is constitutively active whereas Yi is absent in normal human cells (23). E2F complex is a heterodimer, consisting of one E2F family protein and one DP family protein (16, 17). Among the E2F and DP family proteins, E2F1appears to be the one responsible for the down-regulation of DHFR in senescent human IMR-90 cells (11).

4.3 Group 1 G1/S genes with CCAAT repeats within their promoters

TK promoter contains two Y-box arranged in tandem. This characteristic feature is shared by six other G1/S genes as shown in Figure 5. Thus, RNR R2 subunit, histones H2A, H2B, and H3, PCNA, and cdc2 all contain two to four CCAAT elements arranged close to each other in sense or in antisense direction. Figure 6 compares the flanking regions of each CCAAT or inverted CCAAT element in these genes. Almost all of the CCAAT elements in these genes are part of a sequence identical or similar to Y-box consensus sequence with a sequence match ranging from 9/12 to 11/12. Except for cdc2, which also contains E2F sites, the tandem CCAAT element (Y-box) represents the only prominent motif structure within the ~400 bp region upstream relative to the ATG codon in all these Group 1 genes. In some cases, the CCAAT elements and its flanking regions form long repeats, similar to that in TK promoter. The most striking example is the three almost identical 20-bp repeats in the RNR R2 subunit gene. Within each of the 20-bp repeat of the RNR R2 promoter, the sequence match with the Y-box consensus sequence is only 8/12 or 9/12. Nevertheless, the putative binding complex for R2 promoter has been suggested to be NF-Y proteins (24).

4.4 Group 2 G1/S genes with E2F site within their promoters

The most prominent feature of Group 2 gene promoters is that they all contain E2F site, similar to that in the DHFR promoter. These genes can be further divided into two subgroups, one contains only E2F site and the other contains a CCAAT element in addition to the E2F site. Figure 7 shows that DHFR and TS contain only E2F binding site, but no CCAAT element in their promoters. In contrast, the promoter regions of DNA

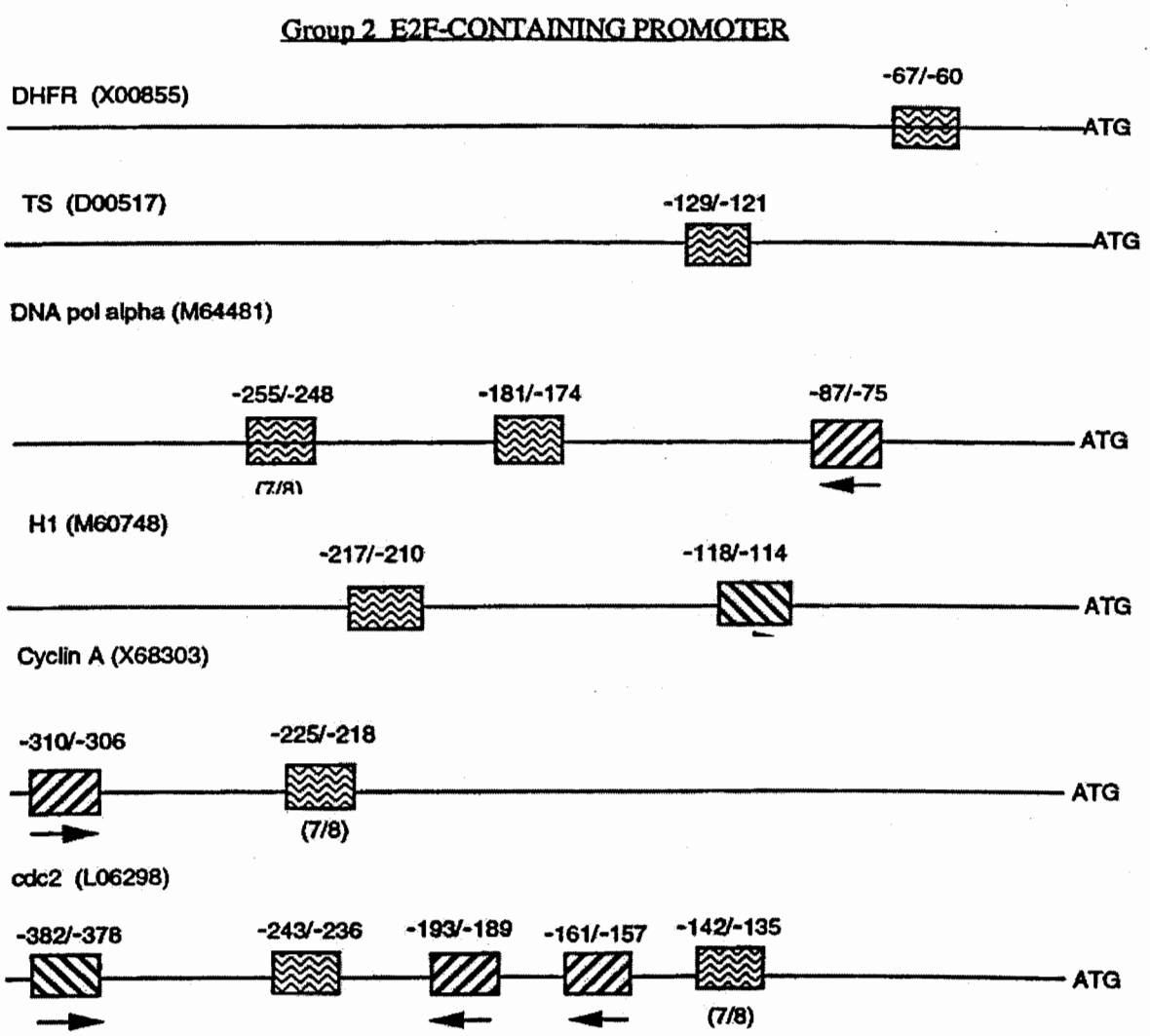


Figure 7. Promoter analysis of the Group 2 genes that contain E2F site in their promoters. The accession number of each gene is indicated in the parenthesis. The numbers above the boxes indicate the position of E2F site (8-bp) or CCAAT element (5-bp) relative to the ATG codon. The arrow on or below the shaded boxes indicates the direction of CCAAT element.

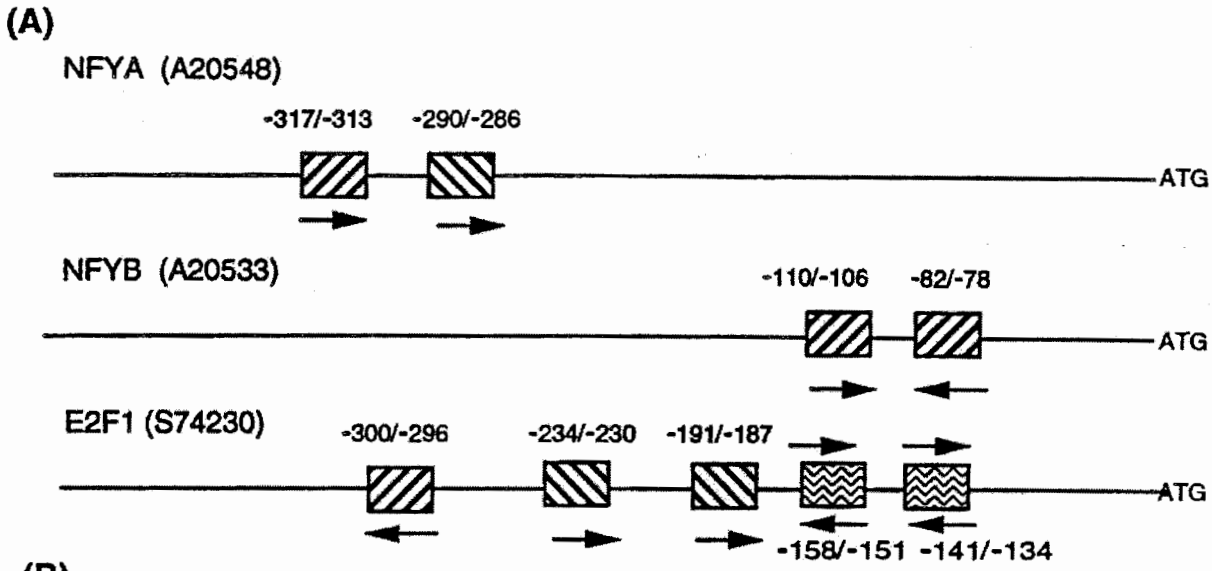
polymerase alpha, histone H1, cyclin A and cdc2 contain both E2F site and CCAAT element. The E2F site in TS, cdc2, DNA polymerase alpha and DHFR, match perfectly with the E2F consensus sequence. The putative E2F site in H1 and cyclin A has one mismatch. The CCAAT element in DNA polymerase alpha, H1, and cyclin A also appear to be part of Y-box with a sequence match of 9/12 to 10/12 (data not shown).

4.5 Late G1/S genes containing other cis-elements within their promoters

Among the G1/S genes that we have examined, histone H4 is the only one that contains neither CCAAT nor E2F element. Instead, it contains a CRE element with one mismatch at position -473/-466 relative to ATG codon. Although CREBP binding activity appears to be serum-responsive and age-dependent (9), it is not clear whether CREBP is responsible for the age-dependent attenuation of H4 gene expression in senescent cells.

4.6 Promoter analysis of NF-YA, NF-YB and E2F1

Promoter analysis as described above suggests that transcription factors such as Y-box binding protein CBP/tk (or NF-Y) and E2F1 may play a key role in controlling the expression of other G1/S genes, in addition to TK and DHFR, during cell senescence. Thus, the study of the regulation of these transcription factor genes during



(B)

E2F1 promoter

-312/-287	GCGGTACGG**CCAAT** GGCGGGGCGGGG	(rev)
-243/-218	GCGTTAAAG**CCAAT** AGGAACCGCCGC	
-200/-175	CCGGGGCAG**CCAAT** TGTGGCGGCGCT	

NF-YA promoter

-320/-309	CTG**ATTGG** GTTT	
-294/-283	CTG**ATTGG** CTCC	(rev)

NF-YB promoter

-90/-69	AGGGCTGC**ATTGG** AGGTTAAAA
-118/-97	AACAGCCG**ATTGG** AGACGGGAG

Figure 8. (A) Promoter analysis of transcription factors NF-Y and E2F1. The numbers indicate the positions of CCAAT and E2F element relative to ATG initiation codon. (B) Sequence comparison of the CCAAT elements and their flanking regions in NF-YA, NF-YB and E2F1 genes. Sequence match with Y-box is indicated in the parenthesis. Rev in the parenthesis indicates that the antisense strand is used for comparison.

senescence would represent a further step in the understanding of the regulatory network controlling cell aging. We, therefore, have also examined the promoter organization of these transcription factor genes. Figure 8A shows the putative transcription factor binding sites in the promoter regions of NF-YA, NF-YB and E2F1. Both NF-YA and NF-YB contain two CCAAT elements in tandem, similar to that found in Group 1 gene promoters. The sequences of 12-bp containing CCAAT (-320/-309 and -292/-281) in NF-YA are almost identical to that in TK gene (Figure 8B). In the case of E2F1 gene, its promoter contains four E2F-binding sites arranged in two imperfect palindromes. In addition to E2F sites, E2F1 promoter also contains two CCAAT element and one inverted CCAAT element upstream to E2F sites. Studies are currently underway to determine (i) whether NF-Y and E2F1 are transcriptionally regulated and (ii) how the cis-elements such as E2F site and multiple CCAAT elements contribute to the down-regulation of these transcription factors.

5. PERSPECTIVE AND SUMMARY

Biochemical and molecular biological studies have shown that suppression of CBP/tk and E2F binding activities is responsible, respectively, for the attenuation of TK and DHFR gene expression in senescent cells (10, 11). A similar approach can be used to investigate the mechanism of regulation of other G1/S genes during cell

senescence. The regulation of some G1/S genes during the progression of cell cycle has already been studied. For example, Schultze *et al* (25) have shown that a variant E2F site within cyclin A is responsible for cell cycle regulation of cyclin A gene. On the other hand, a CCAAT binding protein, termed CBP/cycA, has been suggested to be responsible for the G1/S transition in normal, adhesion-dependent, mesenchymal cells (26). Cyclin A contains two E2F sites (7/8 match) and a CCAAT element (Figure 7). It is certainly of interest to examine which promoter region in cyclin A, E2F or CCAAT, or both, is responsible for age-dependent regulation.

The age-dependent G1/S genes can be divided into two groups based on their promoter organizations. The Group 1 genes contain the tandem CCAAT elements (Figure 5) whereas the Group 2 genes contain E2F consensus sequence (Figure 7). The CBP/tk is likely to be a Y-box family protein. Since the Y-box family proteins may represent a large number of loosely related CCAAT binding proteins (27), it remains to be investigated whether CBP/tk is equivalent to NF-Y (12, 13). NF-Y has also been suggested to be involved in the cell cycle regulation of RNR R2 subunit (24). Recently, a CCAAT binding protein, termed CBP/cdc2, was shown to be involved in the cell cycle-dependent regulation of cdc2 gene (28). It remains to be seen whether CCAAT binding proteins CBP/tk (10), CBP/R2(24), CBP/cycA (26), and CBP/cdc2 (28) are identical or related to NF-Y or other Y-box binding proteins. Based on the similarity of the promoter organization among the Group 1 genes, it is tempting to speculate that these multiple CCAAT elements-containing regions may be responsible for the age-dependent down-regulation of these genes. This possibility is now under investigation. If indeed, the CCAAT binding proteins are involved, it will be of interest to compare the biochemistry of these CCAAT binding proteins with that of CBP/tk. At the same time, it is also important to carry out further study on the role of NF-Y in controlling target genes during cellular aging. All the genes in Group 2, with exception of H11 and cyclin A, contain perfect E2F consensus sequence within their promoter. The variant E2F site (7/8 match) in H1 and cyclin A has been shown to be involved in cell cycle regulation (26). Four of the Group 2 genes also contain CCAAT element in their promoters (Figure 7). Of particular note is the high sequence homology (15/19 match) between the Y-box containing region of cdc2 (-205/-187) and the 19-bp repeat (-52/-34) within the TK promoter. Most of the CCAAT element in Group 2 genes also shares Y-box feature. It is likely that E2F site alone (e.g. DHFR and TS) or together with some Y-box protein (e.g. cyclin A) will be sufficient to control the regulation of the Group 2 genes during the progression of cell cycle and during cell senescence.

Once the key transcription factors involved in the age-dependent down-regulation of G1/S genes have been characterized, attention will certainly be directed toward the regulation of these transcription factor genes. It is interesting to note that the characteristic promoter organization associated with the age-dependent G1/S genes is also present in NF-Y and E2F1 genes. Thus, NF-YA, NF-YB and E2F1 all contain multiple CCAAT repeats in tandem within their promoters. In addition, E2F1 contains four E2F sites. This raises a possibility that these transcription factors are part of autoregulatory circuit which may be an integral component of the genetic program of aging. If indeed this is the case, we will have to look for the earlier events in the cell cycle that lead to the suppression of transcription factors such as E2F1 and CBP/tk. Although the mid-G1 genes such as ODC and eIF-5A are fully expressed in senescent cells (Figure 1), we note that their gene products, namely ODC enzyme and eIF-5A protein, are greatly reduced in senescent cells (6, 19). Both proteins have been implicated in growth control and are essential for proliferation (6, 19). We wonder whether they may have a role in the down-regulation of transcription factors such as CBP/tk and E2F1, and the global attenuation of G1/S genes during senescence.

6. ACKNOWLEDGMENTS

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