FEBS Letters 447 (1999) 160–166 FEBS 21741

Regulation of p53 mediated transactivation by the β-subunit of protein kinase CK2

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Received 7 December 1998; received in revised form 14 February 1999

Abstract The growth suppressor protein p53 plays a main part in cellular growth control. Two of its key functions are sequence specific DNA binding and transactivation. Functions of p53 in growth control are regulated at least in part by its interaction with protein kinases. p53 binds to protein kinase CK2, formerly known as casein kinase 2, and it is phosphorylated by this enzyme. CK2 is composed of two regulating β-subunits and two catalytic α- or α′-subunits and the interaction with p53 is mediated by the regulatory β-subunit of CK2. Recently we showed that the β-subunit could inhibit the sequence specific DNA binding activity of p53 in vitro. Based on this finding, we asked if a coexpression of the β-subunit of CK2 with p53 in mammalian cells could inhibit the DNA binding activity of p53 in a physiological context. We found that the coexpression of the β-subunit showed the same inhibitory effect as in the previous assays with purified proteins. Then, we investigated the effects of the coexpression of the β-subunit of CK2 on the transactivation and transrepression activity of p53. We found that transactivation of the mdm2, p21$^{WAF1/CIP1}$ and cyclin G promoter was inhibited in three different cell lines whereas transactivation of the bax promoter was not affected in COS1 cells but down-regulated in MCO1 and SaosS138V21 cells. p53 mediated transrepression of the fos promoter was not influenced by coexpression of the CK2 β-subunit. Taken together we propose a cell type dependent fine regulation of the p53 transactivation function by the CK2 β-subunit in vivo, which does not affect p53 mediated transrepression.

Key words: p53; Growth suppressor; DNA binding; Transcriptional activator; Protein kinase

1. Introduction

The growth suppressor protein p53 is an important cellular protein which governs the integrity of the human genome. It regulates cell growth, DNA repair and apoptosis (for review see [1,2]) at least in part because p53 is a potent transcriptional regulator. p53 can transactivate the expression of genes whose products are actively implicated in growth arrest, DNA repair or programmed cell death and it can repress the promoters of many cellular genes which are involved in stimulating growth or blocking apoptosis [3–5] or which are implicated in a self-regulating loop such as the mdm2 gene product [6]. p53 regulates transcription by binding to specific DNA sequences [7–9] or by interacting with transcription factors [10–13].

A number of different p53 responsive promoters were described which include the promoters of p21$^{WAF1/CIP1}$ [14], mdm2 [15], cyclin G [16], EGR-1 [17] and bax [18]. On the other hand the basal c-fos promoter and the bcl-2 promoter are down-regulated by p53 [19,20]. So far it is not clear how these different specificities of p53 are regulated.

The polypeptide chain of p53 consists of different functional domains [21] where an internal domain is required for contact with specific DNA sequences. A carboxy-terminal region is able to bind non-specifically to DNA [22] and to regulate the specific DNA binding activity. Binding of the p53 specific monoclonal antibody PAb421 to a C-terminal region of p53, binding of small peptides [23], deletion of a 30 amino acid long sequence of the C-terminus of p53 as well as phosphorylation of p53 by protein kinase CK2 at residue 392 activates p53 for a specific DNA binding.

Protein kinase CK2, formerly known as casein kinase 2, not only phosphorylates p53 at the C-terminus but also binds to p53 [24]. The protein kinase CK2 consists of two catalytic α- or α′-subunits and two regulatory β-subunits [25]. Mapping of the interaction between p53 and CK2 revealed that p53 binds to the regulatory β-subunit and not to the catalytic α-subunit [26]. The β-subunit of CK2 binds to a C-terminal region of p53 where a variety of other proteins such as p34^{cdc2}, the E4orf6 and tms1 bind [27–29]. Binding of the C-terminus of p53 to the CK2 holoenzyme stimulates the kinase activity of CK2 with respect to the phosphorylation of mdm2 [30]. On the other hand binding of the β-subunit of CK2 to p53 reduced the DNA binding activity of p53 at least in vitro in a dose dependent manner [31]. We now analysed whether the regulatory β-subunit of CK2 might also influence the transcriptional activity of p53.

We found that p53 expressed in mammalian cells binds to a consensus DNA sequence and the β-subunit of CK2 had an inhibitory effect on this DNA binding activity of p53. In COS1 cells we could show that coexpression of CK2 with p53 reduced the transactivation activity to at least 50% with mdm2, p21 and cyclin G promoters, while the bax promoter remained unaffected. The coexpression of the CK2 β-subunit had no effect on p53 mediated transrepression of the fos promoter. Using SaosS138V21 and MCO1 cells we showed that transactivation from all promoters was repressed by coexpression of the CK2 β-subunit.

2. Materials and methods

2.1. Cell culture

MCO1 cells (kindly provided by Moshe Oren) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS) [32]. Hep3B cells [33], a mouse cell line which

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PII: S0014-5793(99)00273-2

lacks endogenous mouse p53, COS1 cells, an SV40 transformed monkey cell line expressing wild-type p53 [34,35] and SaosS138V21 [36] were cultured in DMEM supplemented with 5% FCS. SaosS138V21 is a human osteosarcoma cell line lacking endogenous p53 [37] which was stably transfected with temperature sensitive human p53, Val-138 [36]. MCO and SaosS138V21 cells were grown to subconfluency and then shifted to 32°C for the indicated time period, control cells were cultured at 37°C.

2.2. Extraction of cells

Cells were harvested, washed three times with PBS (phosphate buffered saline, pH 7.3) and resuspended in lysis buffer (100 mM Tris-HCl, pH 9.0, 100 mM NaCl, 0.5% (v/v) NP40, 1% Trasylol). After three freeze thaw cycles and sonication for 30 s, proteins were extracted 30 min on ice. Cell debris was eliminated by centrifugation (4°C, 30 min, 13 000×*g*).

2.3. Western blot analysis

Proteins were separated on a 12.5% SDS polyacrylamide gel as previously described [38], blotted to a PVDF membrane (Boehringer Mannheim, Germany) and assayed by the appropriate antibody using the ECL system (Amersham, Braunschweig, Germany) according to the manufacturer's instructions.

2.4. Antibodies

For the immunodetection of tagged CK2 β-subunit we used monoclonal antibody 10C4 which is directed against a tag derived from the yeast tms1 protein [39,40].

2.5. Plasmids

A wild-type p53 cDNA was cloned into pRcCMV (pRcCMV p53) and the sequence was confirmed by DNA sequencing. The CK2 β-cDNA [41] was amplified by PCR and cloned into pCMVES, a vector with a 10C4 epitope tag (Schneider et al., submitted) and named pCMVES-β. The luciferase constructs mdm2-luc, WAF-luc (p21), cyclin G-luc, and bax-luc were described earlier [42]. The hfos-luc reporter was a kind gift from Klaus Roemer, Homburg, Germany. pCMV30 without insert was used as a control vector.

2.6. Transfection and reporter gene expression analysis

Transfection of cells (MCO1 and SaosS138V21) with the respective plasmids was performed by the DEAE-Dextran method (COS1) [43] or Hep38 with superfect transfection reagent (Qiagen, Düsseldorf, Germany) 1 day after seeding. Usually 1 μg of p53, 1 μg pCMVES or pCMV30 and 2 μg reporter plasmid were transfected per 6 cm dish of Hep3B cells as indicated in the figure legends. Cells were harvested 24 h post-transfection. For luciferase reporter assays cells were washed three times with PBS and lysed on the dish with 200 μl lysis buffer (Promega, Heidelberg, Germany) for 15 min at room temperature. The cell lysate was cleared by centrifugation (14 000×*g* for 5 min). Twenty μl of the lysate were mixed with 100 μl luciferase assay reagent and assayed in a scintillation counter according to the Promega instruction manual. Unless indicated each assay was performed in triplicate and repeated three to four times. Data are presented as mean values with the respective standard deviation.

2.7. Electrophoretic mobility shift analysis (EMSA)

Hep3B cells were transfected with 2 μg p53 and 2 μg pCMV30, or 2 μg p53 and pCMVES-β, harvested after 24 h with MF buffer (400 mM NaCl, 20% glycerol, 1 mM Na_3 EDTA, 10 mM HEPES, pH 7.9) and used for shift analysis. The assay was performed with ^{32}P-labeled consensus DNA as described earlier [31].

3. Results

In a previous study we have shown that the specific DNA binding activity of p53 was inhibited by the regulatory β-subunit of CK2 in a dose dependent manner [31].

For these studies we used extracts from insect cells infected with recombinant baculoviruses expressing p53 and purified regulatory β-subunit of CK2 together with the DNA consensus sequence [44]. We now wanted to analyse the influence of the β-subunit of CK2 on the DNA binding activity of p53

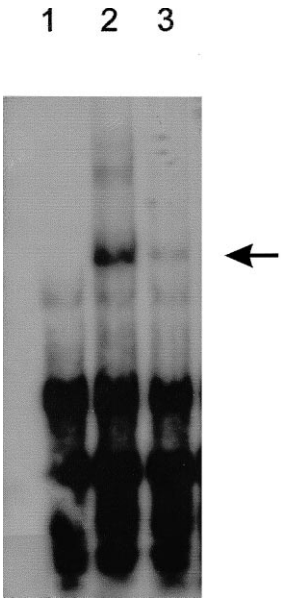

Fig. 1. DNA bandshift analysis with Hep3B cells transfected either with control vector (lane 1), p53 with control vector (lane 2), or p53 with CK2 β-subunit (lane 3). This figure represents one of three independent experiments. The arrow indicates supershifted ^{32}P-labeled DNA of the consensus sequence 5′-(CCGGGCATGT)$_3$-3′.

when p53 and CK2 β were expressed together in mammalian cells. Therefore, we transfected Hep3B cells with p53 (pRcCMVp53) together with a CK2 β-plasmid (pCMVES-β) or with control plasmid (pCMV30). After 24 h cells were harvested and the cell extracts were incubated with the DNA consensus sequence 5′-(CCGGGCATGT)$_3$-3′. DNA-protein complexes were analysed on a polyacrylamide gel. Fig. 1 shows that in cells transfected only with control plasmid no shift is detectable (lane 1). In cells transfected with the p53 expression plasmid and control vector, DNA is shifted to a higher molecular weight (lane 2). However, this shift is not detectable in cells, which were cotransfected with the p53 expression plasmid and the CK2 β-expression plasmid (lane 3). In agreement with our previously published data with p53 expressed in insect cells these data suggest an inhibitory effect of the CK2 β-subunit on the p53 DNA binding activity also in mammalian cells.

Sequence specific DNA binding is prerequisitory for p53 mediated transactivation. Therefore, we wanted to investigate the influence of the CK2 β-subunit on p53 mediated transactivation. For this type of analysis we used mdm2-luc, cyclin G-luc, WAF1-luc and bax1-luc reporter constructs which were already previously used for the analysis of p53 transactivation function [42].

We cotransfected COS1 cells with the p53 expression plasmid and the pCMV30 control vector or the p53 expression plasmid and the CK2 β-expression plasmid together with one of the luciferase reporter plasmids as indicated in Fig. 2A. Luciferase activity was measured in three different experiments where the activity of a promoter in the absence of CK2 β-subunit was regarded as 100%. As shown in Fig. 2A the reporter activity from the mdm2, cyclin G and $p21^{WAF1/CIP1}$ promoter was reduced to about 50% when CK2 β-subunit was expressed together with p53, whereas the transcription of the bax promoter was not affected (Fig.

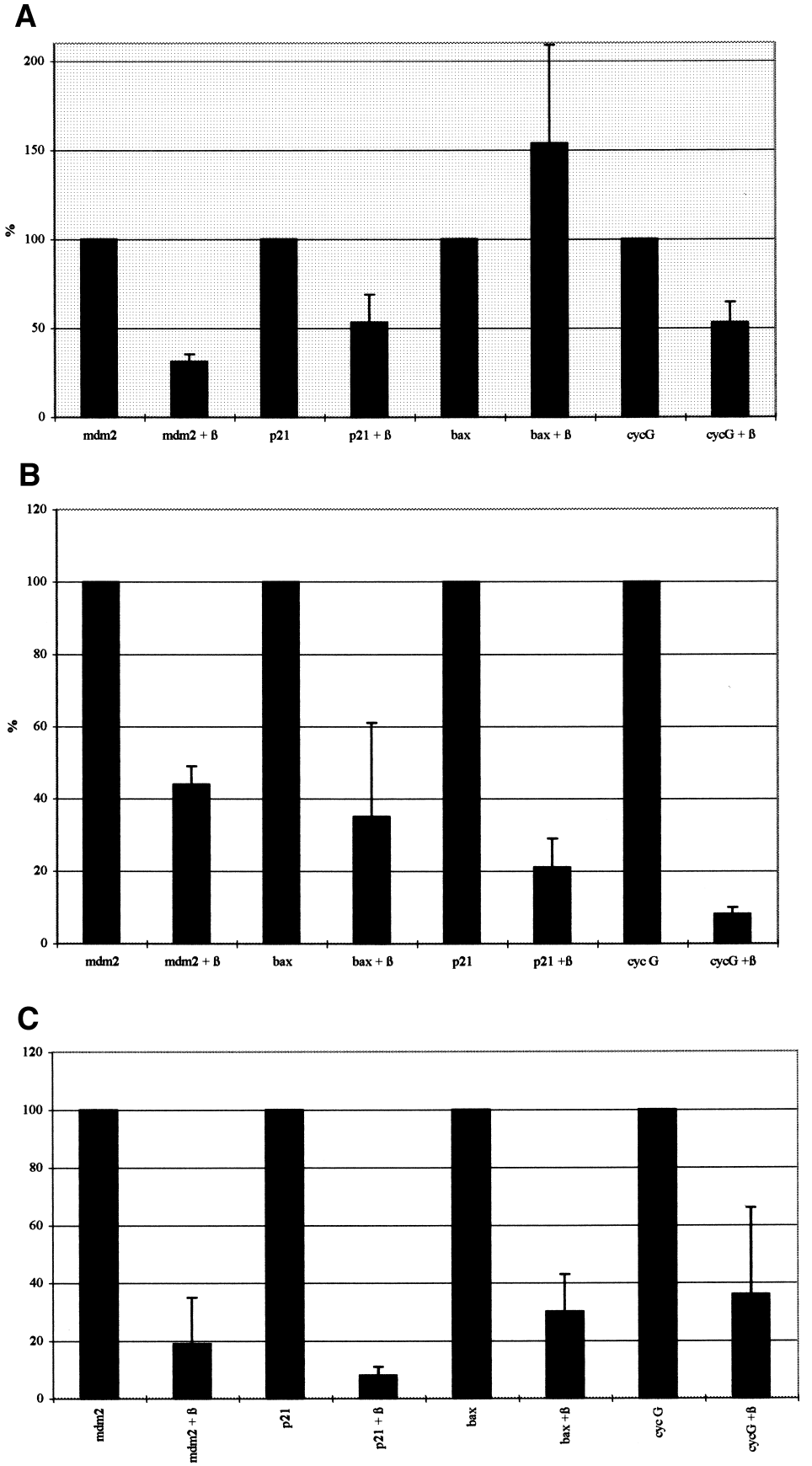

Fig. 2. Transactivation of the mdm2, $p21^{WAF1/CIP1}$, bax and cyclin G promoter in: A: COS1 cells; B: MCO1 cells; and C: SaosS138V21 cells. Cells were cotransfected with the reporter plasmid, p53 and control vector, or p53 and CK2 β-plasmid. COS1 cells were kept at 37°C whereas MCO1 and SaosS138V21 cells were shifted to 32°C. Cells were harvested after 24 h and analysed for luciferase activity as described in Section 2. Luciferase activity of cells transfected with p53 and control vector was set to 100%.

2A). Coexpression of the α- or α′-subunit had no effect (data not shown).

Since it was shown that the activity of p53 dependent promoters can be influenced differently in different cell lines [42], we tested if this would also be true for the inhibitory effect of CK2 β-subunit on the p53 mediated transactivation. For this type of analysis we used either a mouse cell line which lacks endogenous mouse p53 and which was stably transfected with temperature sensitive mouse p53 (tsp53 135 Ala-Val (MCO1)) [32] or a human osteosarcoma cell line which lacks also endogenous human p53 but expresses a temperature sensitive human p53 Val-138 (SaosS138V21) [36]. At 37°C both cell lines express the mutant form and at 32°C the wild-type form of p53. We transfected the two cell lines with either control vector (pCMV30) and reporter constructs, or the CK2 β-expression plasmid with reporter constructs as indicated. After transfection cells were shifted to 32°C to activate wild-type p53. Twenty-four h post-transfection cells were harvested and assayed for luciferase activity. For MCO1 cells results of three different experiments are shown in Fig. 2B. In the presence of the CK2 β-subunit the activity of all reporter constructs is repressed. The inhibition varies from about 58% for the mdm2 reporter construct to 85% for the cyclin G reporter construct. In contrast to our results obtained with COS1 cells the bax promoter is repressed in MCO1 cells.

The same experiment was performed with the SaosS138V21 cells and the results of three different experiments are shown in Fig. 2C. As with MCO1 cells in the presence of the CK2 β-subunit the reporter activity was repressed from all promoters including the bax promoter. The repression varied between about 60% with the cyclin G promoter and 90% with the $p21^{WAF1/CIP1}$ promoter. These results show that the extent of the inhibitory effect of the CK2 β-subunit on p53 mediated transactivation from various promoters is dependent on the cell line, but specific and detectable in all three investigated cell lines.

To show the specificity of the inhibition, we transfected COS1 cells with increasing amounts of the CK2 β-expression plasmid and constant amounts of the p53 expression plasmid, harvested the cells and assayed half of the cell extract for luciferase activity. The other half of the extract was used for a Western blot analysis. As shown in Fig. 3A luciferase activity from the mdm2 promoter after coexpression of increasing amounts of the CK2 β-subunit decreased in a dose dependent manner. Fig. 3B shows the corresponding Western blot analysis, which demonstrates the increase in the amount of CK2 β correlating with a decline in p53 transactivation activity. The amount of CK2 β was quantitated by densitometry from the corresponding Western blot showing that the amount of CK β increased by a factor of 3.5 (lane 3) and 10.8 (lane 4) compared to lane 2.

p53 not only transactivates a number of different promoters but also transrepresses several genes, i.e. the c-fos gene [19]. Now, we wanted to know if this inhibitory effect of the β-subunit is restricted to p53 mediated transactivation activity

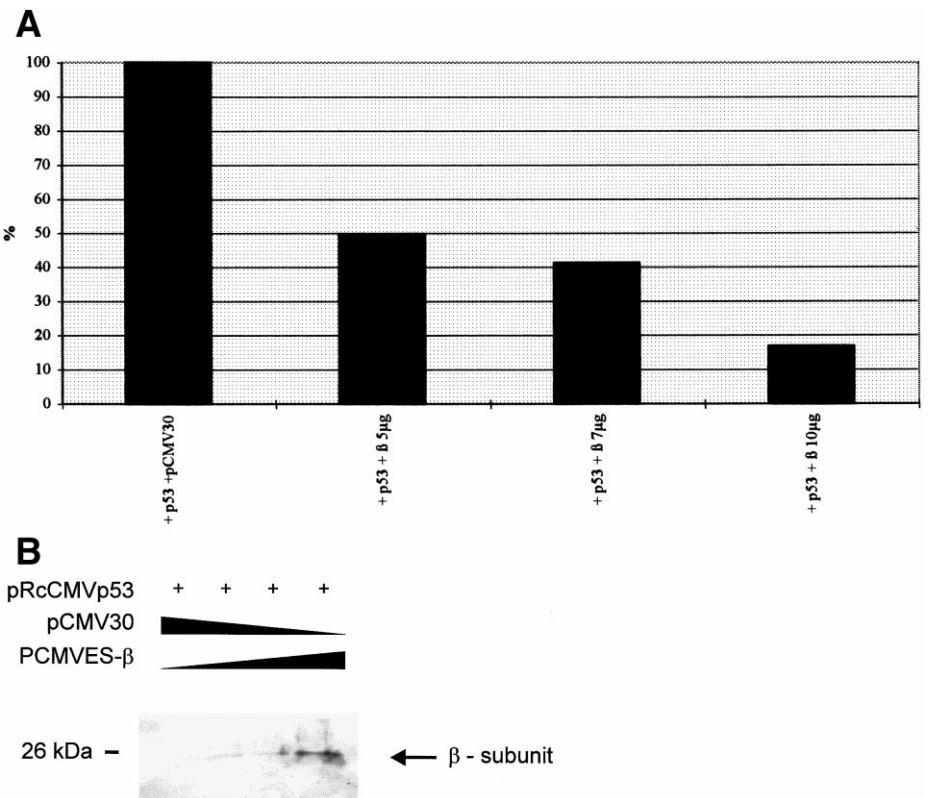

Fig. 3. Concentration dependent inhibition of the p53 transactivated mdm2 promoter by increasing amounts of CK2 β. COS1 cells were transfected with 10 μg p53 and either control vector (lane 1) or 5 μg (lane 2), 7 μg (lane 3) and 10 μg (lane 4) of the CK2 β-plasmid in 10 cm dishes. Cells were harvested for luciferase assay and Western blot analysis. One of five independent experiments is shown. A: Luciferase assay. B: Western blot analysis. 100 μg of cell extract were subjected to SDS polyacrylamide gel electrophoreses and blotted onto PVDF membrane. The epitope tagged β-subunit of CK2 was detected by 10C4 antibody.

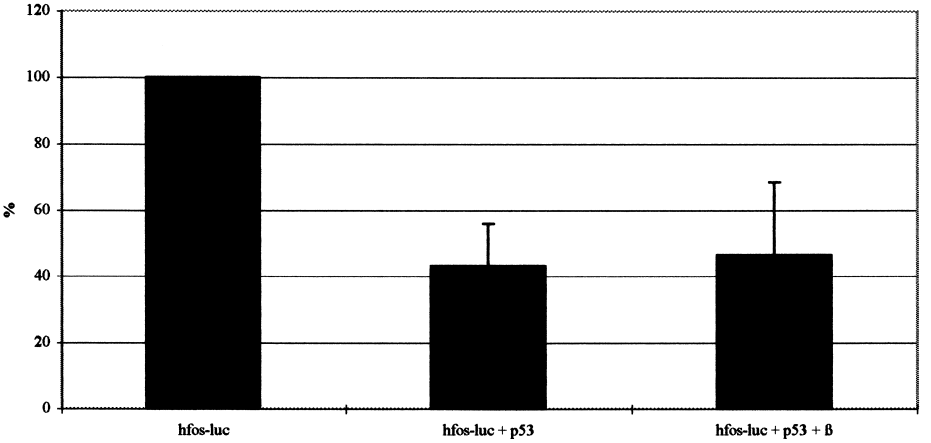

Fig. 4. Influence of CK2 β-subunit on the transrepression activity of p53. COS1 cells were transfected with hfos-luc, control vector, or p53 with CK2 β-subunit. Luciferase activity of cells transfected with hfos-luc and control vector was set to 100%.

or if the transrepression activity is also affected. We transfected COS1 cells only with control vector pCMV30, with p53 expression plasmid and pCMV30 control vector or the p53 expression plasmid and the CK2 β-expression plasmid together with a fos luciferase reporter (hfos-luc). Half of the cell extract was used for the measurement of the luciferase activity, the other half of the cell extract was analysed for the expression of p53 and CK2 β by Western blot analysis (data not shown). Although we found constant amounts of p53 and CK2 β the results of four different experiments revealed that p53 represses the reporter activity to about 50% and that this repression is not affected by coexpression of the CK2 β-subunit (Fig. 4). This finding strongly confirms the specificity of the inhibitory effect on the transactivation activity of p53, while the transrepression activity of p53 remains unaffected.

4. Discussion

The growth suppressor p53 functions as a transcriptional transactivator protein with a sequence specific DNA binding activity [8,9,45]. In addition p53 interacts with various members of the general transcription machinery including members of the TFIID complex such as the TATA-box binding protein (TBP) [46], TAFII40 and TAFII60 [47] and the TFIIH transcriptional complex [48]. The domain for specific DNA binding is located in the central part of the p53 polypeptide chain whereas the transcription factor activity was localised in the N-terminal acidic domain of the p53 molecule. The C-terminus of p53 confers a non-specific DNA binding activity and it seems to regulate the specific DNA binding activity [21]. p53 is phosphorylated by protein kinase CK2 at the penultimate residue [49] and this phosphorylation activates the specific DNA binding activity of p53 [50,51]. Protein kinase C phosphorylates p53 at several C-terminal residues at least in vitro and this phosphorylation again stimulates the sequence specific DNA binding activity of p53 [52–54]. Binding of the monoclonal antibody PAb421 to C-terminal sequences and a deletion of the last 30 amino acids of p53 also activates p53 for sequence specific DNA binding [50]. Some years ago, we found that protein kinase CK2 not only phosphorylates p53 but also binds via the regulatory β-subunit to p53 [24,26,55]. The binding region for CK2 on the polypeptide chain of p53 was recently mapped to a region between amino acids 330–339 [29], a region where several other proteins such as $p34^{cdc2}$, tms1, protein kinase C and the E4orf6 protein bind [27,28,56]. Binding of CK2 β to this region of the p53 molecule leads to a dose dependent reduction in the DNA binding activity of p53 which was expressed either in insect cells [31] or in mammalian cells as shown here (Fig. 1).

Since it was described that the adenovirus E4orf6 protein not only interacts with C-terminal sequences of the p53 molecule but also blocks p53 mediated transcriptional activity [56] we asked in the present study whether the β-subunit of CK2 might also block transcription mediated by p53. For the transactivation assays we used luciferase reporter gene constructs driven by the mdm2, $p21^{WAF1/CIP1}$, bax or cyclin G promoter which were previously used to analyse the effects of phosphorylation of rat p53 on transactivation [42]. It is generally accepted that a major control by p53 on the cell cycle at G_1/S phase is through transcriptional control of the $p21^{WAF1/CIP1}$ gene [57]. $p21^{WAF1/CIP1}$ is an effective inhibitor of G_1/S cyclin dependent kinases (cdks) [58]. Cyclin G is another transcriptional target of p53 [16] which upon overexpression also enhances cell cycle progression. A p53 DNA binding sequence has also been found in the bax gene promoter [59] and after DNA damage and overexpression of p53 the expression of bax is known to increase. There is ample evidence that bax may be part of a p53 dependent apoptosis pathway [60]. Finally, the mdm2 gene is also regulated by p53. The mdm2 gene product is implicated in a regulatory network which controls the subcellular trafficking and the expression of p53 [61]. Transactivation of the mdm2, $p21^{WAF1/CIP1}$ and cyclin G promoter by p53 was down-regulated by coexpression of the β-subunit of CK2 regardless of which cell line was used. It is striking that only transactivation of the bax promoter by p53 is not affected in COS1 cells whereas a considerable decrease was observed in the two other cell lines. Cell type specific variations in transcriptional activities of p53 have been described earlier [42,62,63]. We now present evidence for a cell type specific repression of the p53 transactivation function by the β-subunit of CK2.

p53 not only transactivates gene expression but also can transrepress some genes such as the c-fos gene [64]. As shown in the present study the influence of the β-subunit of CK2

seems to be restricted to an influence on the transactivation and not on the transrepression function of p53.

CK2 isolated from a wide variety of organisms and tissues consists of a spontaneously active heterotetramer composed of two catalytic subunits (α- and/or α′-subunits) and two regulatory β-subunits. It is an intriguing question whether there is additional α- or β-subunit which is not in the heterotetramer of the holoenzyme. Since we found a distinct inhibitory function of the β-subunit for the DNA binding activity of p53 which is different from the activity of the holoenzyme, these experiments might argue for the presence of at least uncomplexed β-subunit. This hypothesis is strengthened by the fact that the free subunits of CK2 are transported separately to the nucleus where they are assembled [65]. Furthermore, binding partners for the CK2 α such as hsp 90, nucleolin and PP2A as well as for the CK2 β such as Nopp140, A-raf or p53 were described [66–71], indicating that both subunits have individual functions which are different from their function in the holoenzyme. Finally, in human kidney tumour cells as well as in lymphoid cell lines an asymmetric expression of protein kinase CK2 subunits were described [72,73]. Elevated levels of CK2 are found in highly proliferating cells in comparison to normal proliferating cells (for review see [25]). According to our present results elevated levels of CK2 β would mean that transactivation of p53 dependent genes such as $p21^{WAF1/CIP1}$ is reduced which would favour cell growth. Our results might further indicate a dual role of protein kinase CK2 in regulating p53 functions. Phosphorylation of p53 by CK2 stimulates DNA binding and transactivation functions of p53 whereas binding of the regulatory β-subunit of CK2 to p53 seems to have the opposite effect, i.e. the CK2 β-subunit reduces the DNA binding activity and the transactivation function of p53.

Acknowledgements: The authors thank Moshe Oren for MCO1 cells, N. Tsuchida for SaosS138V21 cells and Klaus Roemer for the hfos-luc reporter construct. This work was supported by grants B4 (SFB 399) and Mo309/11-1 from Deutsche Forschungsgemeinschaft, and a grant from Fonds der Chemischen Industrie to M.M.

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