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The Gene PC3$^{TIS21/BTG2}$, Prototype Member of the PC3/BTG/TOB Family: Regulator in Control of Cell Growth, Differentiation, and DNA Repair?

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PC3$^{TIS21/BTG2}$ is the founding member of a family of genes endowed with antiproliferative properties, namely BTG1, ANA/BTG3, PC3B, TOB, and TOB2. PC3 was originally isolated as a gene induced by nerve growth factor during neuronal differentiation of rat PC12 cells, or by TPA in NIH3T3 cells (named TIS21), and is a marker for neuronal birth in vivo. This and other findings suggested its implication in the process of neurogenesis as mediator of the growth arrest before differentiation. Remarkably, its human homolog, named BTG2, was shown to be p53-inducible, in conditions of genotoxic damage. PC3$^{TIS21/BTG2}$ impairs G_1–S progression, either by a Rb-dependent pathway through inhibition of cyclin D1 transcription, or in a Rb-independent fashion by cyclin E downregulation. PC3$^{TIS21/BTG2}$ might also control the G_2 checkpoint. Furthermore, PC3$^{TIS21/BTG2}$ interacts with carbon catabolite repressor protein-associated factor 1 (CAF-1), a molecule that associates to the yeast transcriptional complex CCR4 and might influence cell cycle, with the transcription factor Hoxb9, and with the protein-arginine methyltransferase 1, that might control transcription through histone methylation. Current evidence suggests a physiological role of PC3$^{TIS21/BTG2}$ in the control of cell cycle arrest following DNA damage and other types of cellular stress, or before differentiation of the neuron and other cell types. The molecular function of PC3$^{TIS21/BTG2}$ is still unknown, but its ability to modulate cyclin D1 transcription, or to synergize with the transcription factor Hoxb9, suggests that it behaves as a transcriptional co-regulator. J. Cell. Physiol. 187:155–165, 2001.
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PC3 (pheocromocytoma cell-3) is the prototype member of a novel family of antiproliferative genes, originally isolated by us as an immediate early gene activated by nerve growth factor (NGF) at the onset of neuronal differentiation in a cell line derived from a tumor of the adernal medulla, the PC12 cells (Bradbury et al., 1991). Concomitantly, the same gene was identified by the Herschman group as a tetradecanoyl phorbol acetate-induced sequence in mouse NIH3T3 cells, and named TIS21 (TPA-induced sequence 21, Flecher et al., 1991). Soon after, the antiproliferative gene BTG1 was cloned, that shared about 65% homology with PC3/TIS21 (Rouault et al., 1992), initially identified as a sequence associated to a chromosome translocation involving the MYC locus in a B-cell lymphocytic leukemia (Rimokh et al., 1991). The identification of several other related genes followed, thus creating a wide gene family (see the phylogenetic relationships among the different family members in Fig. 1). Namely, TOB (Matsuda et al., 1996; Yoshida et al., 1997), murine BTG3 (Guehenneux et al., 1997) and its human homolog ANA (Yoshida et al., 1998), TOB2 (Ikematsu et al., 1999; Ajima et al., 2000), and PC3B (Buanne et al., 2000), as well as the TOB-related homologs AmphiTOB in Amphioxus (Holland et al., 1997) and the more evolutionary distant FOG-3 in C. elegans (Chen et al., 2000), for a total number of at least six independent genes. The initial studies in my laboratory involved PC3 in the process of neuronal differentiation, but it is now clear that the whole family might have a role in cellular differentiation, not restricted to the neuron. Furthermore, the human homolog of the gene PC3/TIS21, named BTG2, was demonstrated to be induced by p53 and by p73, and to be involved in the response to DNA damage (Rouault et al., 1996; Zhu et al., 1998), a property that does not seem to be common to the other members of the gene family. The aim of this review is to summarize the current knowledge about PC3$^{TIS21/BTG2}$ (see also a previous review by Puiseux and Magaud, 1999), debating the main unsolved questions, and seeking where possible a comparison with the other genes of the family.

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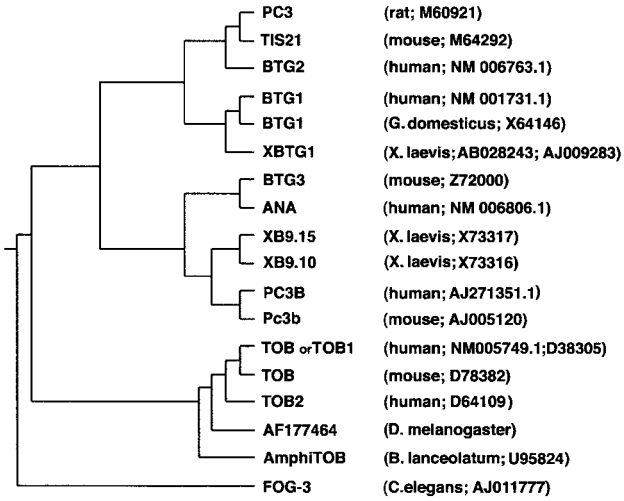

Fig. 1. Phylogenetic relationship in the PC3/BTG/TOB family. An evolutionary tree was calculated analyzing the sequences by the nearest neighbour algorithm with the software Align by Feng and Doolittle (1996). The evolutionary distance is shown by the total branch lengths (horizontal lines). The tree represents the relationship existing between the six independent genes composing the PC3/BTG/TOB family (October 2000): PC3$^{TIS21/BTG2}$, BTG1, ANA/BTG3, PC3B, TOB, TOB2. FOG-3, AmphiTOB and the mRNA with acc.n. AF177464, appear to be TOB homologs in Caenorhabditis elegans, Branchiostoma lanceolatum and Drosophila melanogaster, respectively. Species and accession numbers are indicated.

The predicted protein products of the different genes of the family are devoid of known functional motifs. However, a comparison between the protein sequences of the whole family, using an algorithm that pairs conserved aminoacids according to the criterion of frequency in substitution during evolution (Align; Feng and Doolittle, 1996), reveals that the first 100–120 aminoacids represent a homogeneous variant of a unique protein sequence. Conserved sequences are still detectable within the remaning carboxy-terminal half, common to all the family members, but in this case the assemblage is discontinuous, i.e., conserved blocks are interspersed by intervening stretches that form the "backbone" of the TOB proteins, especially of the D. melanogaster TOB homolog (acc. n. AF177464; see Fig. 2). Given that a pattern of homology between the amino- and the carboxy-terminal half can be found (applying, for instance, the algorithm FASTA to the FOG-3 or TOB sequences), a possible hypothesis is that following duplication of an ancestral gene, the carboxy-terminal region mutated in vertebrates more pronouncedly than in inverterbrates (and an example could be the C. elegans FOG-3 protein). The natural corollary of this hypothesis is that the amino-terminal and the carboxy-terminal regions could play different functional roles. Furthermore, within the conserved amino-terminal, two regions of higher homology are evident, named A or GR (for growth regulatory) and B box (Guehenneux et al., 1997; Guardavaccaro et al., 2000). The GR and B boxes, corresponding in PC3 to residues 50–68 and 96–115, respectively, appear to play a key role in the antiproliferative function (see below) and in the binding to a number of molecular targets.

CONTROL OF CELL DIFFERENTIATION
Evidence for a role of PC3 in neuronal differentiation

A number of evidence points to an involvement of PC3$^{TIS21/BTG2}$ in neuronal differentiation. Besides the initial observation that PC3 is rapidly induced following the NGF-dependent differentiation of a neural crest derivative, the chromaffin cells, we found that PC3 is transiently expressed in the neural tube (Bradbury et al., 1991; Iacopetti et al., 1994), at the moment and in the region—the ventricular zone—where the neuroblast undergoes the last proliferative cycle that precedes its differentiation into a mature neuron (Iacopetti et al., 1994, 1999). The spatio-temporal expression of PC3 qualified it as a marker for neuronal birth, and led to the hypothesis of a role of PC3 in neuronal differentiation, as inducer of the growth arrest required for differentiation (Iacopetti et al., 1994). This idea was also supported

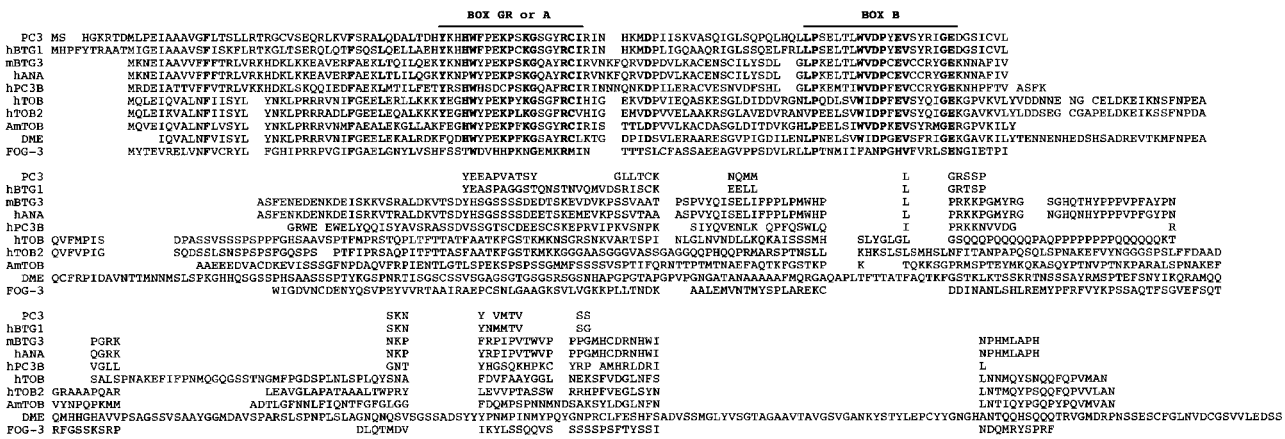

Fig. 2. Multiple alignment between protein sequences of the PC3/BTG/TOB family. The algorithm Align was used, as in Fig. 1. The two regions of higher homology, termed GR (or A) and B boxes are indicated. Aminoacids conserved throughout all the different protein sequences (or in the majority of them) are in bold. Nomenclature is the same as in Fig. 1, with the addition of a prefix to protein names where necessary: h, human; m, mouse; moreover: AmTOB, AmphiTOB; DME, acc. number AF177464.

by the finding that the related gene BTG1 displayed antiproliferative properties (Rouault et al., 1992). Subsequently, the observation that PC3 was able to inhibit proliferation by arresting G_1 to S progression, in chromaffin PC12 cells and also in non-neuronal cells (Montagnoli et al., 1996; Rouault et al., 1996; see also below), gave an experimental ground to the above hypothesis for a role of PC3 in neurogenesis through cell cycle control. A more detailed study demonstrated that: (i) PC3/TIS21 mRNA was expressed, only during the G_1 phase, in a subset of neuroepithelial (NE) cells expressing the differentiation marker βIII-tubulin within the apical vetricular zone of the neural tube, i.e., in the subset of neuroblasts differentiating into post-mitotic neuron, and that (ii) the expression of the PC3/TIS21 protein (but not of the mRNA) persisted during the mitosis of the neuron-generating NE cells, and also for a short period (2–3 days) in the post-mitotic neuronal daughter cell (Iacopetti et al., 1999). This led the authors to conclude that PC3/TIS21 identifies single neuroepithelial cells that switch from proliferative to the neuron-generating division. Given that the change in the division mode of NE cells, from symmetric (that gives two proliferating daughter NE cells) to asymmetric (that generates one post-mitotic neuron and one NE cell), determines the onset of neurogenesis; this again points to PC3/TIS21 as an inducing signal for neurogenesis. This idea is further supported by a report which demonstrates that PC3 increases the rate of asymmetric division in primary cultures of cortical precursor cells (Malatesta et al., 2000). Nonetheless, PC3 does not appear to be endowed with neurogenic properties, given that no influence was seen on the ratio of glial cell birth from precursor, despite an increase in asymmetric division of presursors (Malatesta et al., 2000; F. Cremisi, personal communication).

Thus, the most comprehensive hypothesis appears that the expression of PC3 induces the asymmetric mode of division in neuronal precursor cells (NE neuroblast), and thus neurogenesis, by slowing down their cycling rate (G_1 to S transition) below a certain threshold, forcing the cell in a condition not permissive to maintain the proliferative symmetric mode (with an effect similar to the stimulation of neuronal differentiation by serum-free conditions). This mechanism would not be restricted to neurons. Also, preliminary data from our laboratory support the idea of an effect not restricted to neurons, as the phenotype of a transgenic mouse conditionally overexpressing PC3 preferentially in non-neuronal tissues, presents with a certain frequency a reduced body size.

Furthermore, also the other genes of the family might be involved in neuronal differentiation, judging by the developmental expression of ANA/BTG3, PC3B, and AmphiTOB, as detected by in situ analysis. In fact, ANA/BTG3 is expressed in the NE cells of the ventricular region of the neural tube at the stage E12–E14, similarly to $PC3^{TIS21/BTG2}$, as well as in mesenchymal cells deriving from cranial neural crest cells (see below; Yoshida et al., 1998). Conversely, PC3B appears to be selectively expressed, within the nervous system, in the intermediate and luminal region of the olfactory epithelium, at E16.5, where the olfactory neuron progenitors undergo the last divisions and begin the differentiation in post-mitotic neurons (Buanne et al., 2000). This localization is coincident with that of NeuroD, and it is worth noting that the olfactory epithelium has the unique peculiarity, within the nervous tissue, of being continuously regenerated throughout life by the olfactory neuron progenitors. Furthermore, AmphiTOB, the TOB homolog present in the invertebrate chordate Amphioxus (Branchiostoma lanceolatum), is highly expressed in the nerve cord and in the cerebral vescicle from the stage of neurula to that of larva, also suggesting that the TOB genes are involved in neurogenesis (Holland et al., 1997).

Evidence for a role of the PC3/BTG/TOB family in control of germ and muscle cell differentiation

The patterns of tissue expression within the PC3/BTG/TOB gene family are not identical. The most evident difference being that $PC3^{TIS21/BTG2}$, BTG1 and BTG3/ANA are—unlike the TOB genes—almost not expressed in the adult brain (Bradbury et al., 1991; Rimokh et al., 1991; Matsuda et al., 1996; Yoshida et al., 1988; Ikematsu et al., 1999), a fact that might indicate different functional roles between the two sub-categories of genes (PC3/BTGs and TOBs), which is in agreement with their different molecular structures.

Conversely, all the members of the PC3/BTG/TOB family of genes are expressed in the testis (Tippetts et al., 1998; Raburn et al., 1995; Matsuda et al., 1996; Yoshida et al., 1998; Ikematsu et al., 1999). An analysis of the expression of BTG1 during the maturation stages of the seminiferous epithelium indicates that BTG1 is highly expressed only in round spermatids, which have completed meiosis and are undergoing the differentiative process of spermiogenesis (Raburn et al., 1995). This is suggestive of a role of BTG1 in the irreversible exit from the cell cycle that precedes differentiation (Raburn et al., 1995), similarly to what is hypothesized for PC3 about the differentiation of the neuroblasts in post-mitotic neurons (Iacopetti et al., 1994, 1999). Interestingly, FOG-3, C. elegans ancestor gene of the family (certainly more related to the TOBs than to the BTG/PG3 genes) has been found to be necessary for the specification of the male sex in germ cell precursors. Mutational analysis reveals that in the absence of FOG-3, germ cells develop as oocytes and that the presence of FOG-3 is continuously required for spermatogenesis to occur (Chen and Ellis, 2000; Chen et al., 2000). This genetic analysis clearly indicates that FOG-3 can specify the commitment of cells already determined to the germ lineage, toward the fate of sperm. Furthermore, the family member PC3B recently isolated by us, shows in the adult animal a pattern of quite exclusive expression in the testis and oocyte, suggesting a role of PC3B as well in gematogenesis (Buanne et al., 2000). High expression in oocyte and ovary is seen also for TOB2 and ANA/BTG3 (Yoshida et al., 1998; Ikematsu et al., 1999).

Therefore, this evidence suggests a role for the PC3/BTG/TOB gene family in the control of germ cell differentiation. A further role for this gene family, in muscle cell development, is suggested by several observations.

In fact, it has been shown that overexpression of Xbtg1, a putative homologue of BTG1 in *Xenopus*, perturbed gastrulation and caused defects in posterior

tissues, in notochord and in muscle formation (Saka and Smith, 2000; see also Gawantka et al., 1998). It is worth mentioning that Xbtg1 expression is controlled by Brachyury and Pintavallavis (Saka and Smith, 2000), members of the T-box and of the forkhead/HFN3β-related families of transcription factors, respectively, which are essential for the formation of mesoderm and notochord in vertebrate development (O'Reilly et al., 1995). Thus, Xbtg 1 appears to be a transducer in these two crucial development pathways. Furthermore, it has been shown that overexpression of BTG1 in quail myoblasts stimulates terminal differentiation and inhibits myoblast proliferation (Marchal et al., 1995; Rodier et al., 1999). Remarkably, AmphiTOB also presents very high expression in the somitic musculature that will differentiate in axial trunk muscles, expression that persists during the larval stage until completion of the differentiative process (Holland et al., 1997).

An additional process in which TOB and ANA/BTG3 are involved is bone formation. This is indicated by evidence obtained in TOB−/− mice (Ikematsu et al., 1999; T. Yamamoto, personal communication), and from the observation that ANA/BTG3 is expressed in several tissues of mesenchymal origin (Yoshida et al.,1998).

As a whole, comparing the temporal windows of activity of these different genes, a general paradigm emerges. In fact, FOG-3 is necessary in sperm cells at the end of the process of sex determination, BTG1 is induced in post-meiotic sperms, and $PC3^{TIS21/BTG2}$ is induced in the neuroblast committing into adult neuron and in the chromaffin PC12 cell terminally differentiating upon NGF exposure. Thus, these genes appear to be active or induced at the transition between cell determination and commitment to terminal differentiation.

$PC3^{TIS21/BTG2}$ as inhibitor of cell cycle

The antiproliferative activity of PC3, initially observed in my laboratory (Montagnoli et al., 1996; Guardavaccaro et al., 2000) and by Rouault et al. (1996) on the human homolog BTG2, is consistent with (i) the notion that NGF, which induces PC3, leads to an arrest of cell cycle followed by differentiation (Rudkin et al., 1989); (ii) the above evidence of a role of PC3 in neuroblast differentiation (Bradbury et al., 1991; Iacopetti et al., 1994); and with (iii) the sequence homology to the antiproliferative gene BTG1 (Rouault et al., 1992). However, we and others found that overexpression of exogenous PC3 in NIH3T3 fibroblasts induces an evident arrest in the G_1 phase of the cell cycle, without affecting the G_2 phase (Montagnoli et al., 1996; Lim et al., 1998a; Guardavaccaro et al., 2000), whereas the group of Rouault observed that genetic deprivation of TIS21 in mouse ES cells leads to disappearence of the arrest in the G_2 phase following DNA damage by adriamycin. Given the differences between the two experimental approaches (overexpression in somatic cells and loss of function in ES cells), it will be useful to analyze the G_2 checkpoint in $PC3^{TIS21/BTG2}$−/− somatic cells, since the Rb-dependent G_1 checkpoint is apparently dispensable during embryonic cell cycles, and possibly in ES cells as well (Jacks et al., 1992; Lee et al., 1992); and also after the induction of the expression of exogenous $PC3^{TIS21/BTG2}$ at selected time points of the cycle past G_1 phase (using a cell clone carrying an inducible PC3 vector), with or without genotoxic damage. Considering that a physiological induction of PC3/TIS21 in the G_1 phase has been seen in vivo in the neuroblast (Iacopetti et al., 1999), presently it can be hypothesized that $PC3^{TIS21/BTG2}$ plays a role in the cell cycle arrest of neuronal precursors and following genotoxic damage, possibly acting at different checkpoints.

Furthermore, the inhibition of G_1 to S transition following overexpression is an effect common also to BTG1 (Rouault et al., 1992), ANA/BTG3 (Yoshida et al., 1998), PC3B (Buanne et al., 2000), TOB (Matsuda et al., 1996), and TOB2 (Ikematsu et al., 1999).

As for the mechanism by which $PC3^{TIS21/BTG2}$ induces G_1 arrest, we demonstrated that this relies on the inhibition of cyclin D1 transcription, with consequent reduction of its protein levels. This impairs the ability of cyclin D1/CDK4 to inactivate pRb through phosphorylation, the preliminary step that triggers the cell cycle entry in G_1 (Guardavaccaro et al., 2000). However, it has also been reported that in 293 cells (which are devoid of functional pRb, p53 and cyclin D1), $PC3^{TIS21/BTG2}$ still inhibits the G_1 to S phase progression, by reducing cyclin E levels (Lim et al., 1998a). This finding is compatiable with our observation that the inhibition of cyclin D1 levels is the preferential but not the exclusive mechanism for the PC3-mediated G_1 arrest, and would imply that $PC3^{TIS32/BTG2}$ in some conditions (likely, the inactivation of both p53 and pRb) can act through Rb-independent pathways of inhibition of cell cycle progression, whose existence has recently been proposed (see for review Mittnacht, 1998).

Altogether, $PC3^{TIS21/BTG2}$ might exert cell cycle arrest through different pathways, depending on the cellular context, and it might behave as tumor suppressor, being able to inhibit proliferation even after pRb inactivation, a condition frequently seen in tumorigenesis.

A possible molecular mechanism for the PC3-$^{TIS21/BTG2}$ mediated growth arrest comes from the recent finding that $PC3^{TIS21/BTG2}$ binds mCAF-1 (carbon catabolite repressor protein CCR4-associated factor 1; Rouault et al., 1998). This is the mouse homolog of a yeast protein (yCAF-1) that participates in the multisubunit transcriptional complex CCR4, which is required in yeast for the transcriptional regulation of several genes, and comprises the transcriptional factor NOT and proteins also involved in cell cycle progression, such as the cell cycle-regulated serine/threonine kinase Dbf2 (Liu et al., 1997, 1998). yCAF-1 (and its mouse homolog mCAF-1) also binds BTG1 (Bogdan et al., 1998), TOB, and TOB2 (Ikematsu et al., 1999), and it has been shown that overexpression of rat CAF-1 inhibits colony formation in NIH3T3 and U2OS cells (Bodgan et al., 1998). Furthermore, a CDK2-mediated phosphorylation on Ser^{159} of BTG1 is required for the binding of BTG1 to CAF-1 and also for the antiproliferative activity of BTG1 (Bogdan et al., 1998). This would argue in favor of CAF-1 as an active mediator of the antiproliferative effect of BTG1. A further observation, that CAF-1 binds CDK4 and Cdc2, led the Yamamoto group to propose that the members of the PC3/BTG/TOB family might regulate the cell cycle by modulating CDK activities through their interaction with CAF-1 (Ikematsu et al., 1999). Yet for this, differences between the PC3/BTG/TOB proteins are evident: (i) PC3, unlike TOB2, can directly bind

CDK4 and Cdc2, at least in vitro (Guardavaccaro et al., 2000); (ii) differently from BTG1, the mutation of the serine of PC3 phosphorylated by CDK2 (aa 147) does not affect its antiproliferative activity (Guardavaccaro et al., 2000). Furthermore, the mechanism of cell cycle inhibition by negative modulation of cyclin D1 transcription through the GR box, shown for PC3 (Guardavaccaro et al., 2000), would likely differ from the postulated mechanism of CAF-1 binding, since this is mediated by the B box, as demonstrated for BTG1 (Rouault et al., 1998).

It is also worth a mention that the ability of $PC3^{TIS21/BTG2}$ to bind and stimulate the activity of protein-arginine methyltransferase 1 (PRMT1; Lin et al., 1996), together with the more recent observation that PRMT-1 binds the interferon-α receptor and positively modulates its growth arrest properties (Abramovich et al., 1997), can be seen as another possible mechanism for the growth arrest exerted by $PC3^{TIS21/BTG2}$, though limited to cells carrying interferon receptors. Furthermore, the binding of PRMT-1 to PP2A (protein phosphatase 2A), which has been implicated in several processes including DNA replication, extends to unexplored possibilities the likelihood of a control of cell cycle by $PC3^{TIS21/BTG2}$ through modulation of protein methylation. Just to mention one, the recent finding that the replication initiator protein PR59 might target PP2A on p107 (of the Rb family), leading to its dephosphorylation and consequent arrest of cycle (Voorhoeve et al., 1999).

Cell cycle expression of the PC3/BTG/TOB genes

At any rate, it should be emphasized that the induction of $PC3^{TIS21/BTG2}$ is not cell cycle-dependent, not occuring by default at defined steps of the cycle, but only after stimulus, at least in cell lines (Montagnoli et al., 1996). This confers on $PC3^{TIS21/BTG2}$ the character of a negative regulator of the cell cycle not constitutively part of the division machinery, induced only when activated by specific cellular cues (such as differentiation or DNA damage). Furthermore, the activation of $PC3^{TIS21/BTG2}$ in vivo during development seems to be triggered by cell autonomous signals (Iacopetti et al., 1999).

A remarkable difference within the family genes is suggested by the fact that BTG1 expression appears to be cell cycle-dependent. BTG1 expression is high in G_0–G_1 and more generally in quiescent tissues, and is down-regulated as the cell enters the S phase, to return high in the following G_1 (Rouault et al., 1992). This might indicate that BTG1 acts as a growth arrest gene responsible for the maintenance of the quiescent state, unlike it appears for $PC3^{TIS21/BTG2}$. Nonetheless, BTG1 expression, similarly to that of $PC3^{TIS21/BTG2}$, is also induced by stimuli that lead to growth arrest and differentiation. For instance, triiodothyronine and cAMP stimulate BTG1 expression concomitantly with myoblast differentiation (Marchal et al., 1995; Rodier et al., 1999), or prostaglandin E2 through the cAMP pathway activates BTG1, whose expression is associated with macrophage growth arrest (Sud et al., 1997). Further studies are thus necessary to analyze whether these differences correspond to different behaviours in cell cycle control.

More recently it has been observed that TOB localizes either in the nucleus or in the cytoplasm in a manner dependent on cell progression, and that TOB and TOB2 contain a nuclear localization signal which, at least in TOB, is functional, since mutants defective for the signal did not localize in the nucleus and were poorly antiproliferative (Yoshida et al., 2000; T. Yamamoto, personal communication). This would also indicate that the TOB gene localization is constitutively hooked to cell cycle progression. Furthermore BTG1 as well is induced to translocate in the nucleus upon myogenic stimulation by triiodothyronine and cAMP (Rodier et al., 1999).

Role in apoptosis of the PC3/BTG/TOB gene family

$PC3^{TIS21/BTG2}$ is the primary response gene most rapidly and persistently induced, together with c-jun, at the onset of apoptosis induced by NGF deprivation in terminally differentiated neuronal PC12 cells (Mesner et al., 1995; Wang et al., 1997). Furthermore, $PC3^{TIS21/BTG2}$ is highly expressed in the process of glandular atrophy of the prostate, and it has been suggested that its expression, as antiproliferative regulator, might play a role in controlling atrophy (Walden et al., 1998). Functionally, we have recently observed in the PC12 model of NGF deprivation that overexpression of PC3 prevents apoptosis (as judged by nuclear morphology, D. Guardavaccaro and F. Tirone, unpublished data), which, together with the observation that genetic ablation of PC3 leads to increased cell death after DNA damage (Rouault et al., 1996), would argue for a cell survival effect by $PC3^{TIS21/BTG2}$. When terminal neuronal differentiation is attained, withdrawal of NGF from PC12 cells causes their death (Greene et al., 1986), in consequence of the attempt of terminally differentiated neurons to re-enter into the cycle. Such a process closely resembles programmed cell death in neurons (Mesner et al., 1992). Two pathways are essentially activated in neurons by NGF withdrawal, one being elicited by the c-Jun N-terminal kinase (JNK), and the other by CDK4 and CDK6 which are activated as a result of cyclin D1 increase (Kranenburg et al., 1996), with consequent inactivation of pRb. The final target of both pathways might be p53 that would induce death genes as BAX that trigger apoptosis (see for review Kaplan and Miller, 2000). As a whole, this leads to the idea that $PC3^{TIS21/BTG2}$ could exert an anti-apoptotic action in consequence of its ability to reduce cyclin D1 levels, thus preventing pRb inactivation and re-entry into the cycle.

A picture opposite to that seen for $PC3^{TIS21/BTG2}$ is given by a report on BTG1, which shows its expression in the regions of atherosclerotic lesions (e.g., aorta) occurring in an animal model of the Watanabe hyperlipidemia, and also its ability to induce apoptosis in NIH3T3 cells following its overexpression (Corjay et al., 1998). Considering that overexpression of $PC3^{TIS21/BTG2}$ does not cause apoptosis in NIH3T3 cells (Montagnoli et al., 1996), we cannot provide a rationale for this finding but a different action of the two genes.

Signal transduction pathways activating $PC3^{TIS21/BTG2}$

It is known that $PC3^{TIS21/BTG2}$ is induced by NGF, FGF, IL-6, TPA, serum, EGF, and cAMP (Bradbury et al., 1991; Fletcher et al., 1991; Montagnoli et al.,

1996), indicating that a number of stimuli triggering different transduction pathways can activate this gene. In fact, in PC12 cells NGF, FGF, and IL-6 induce neuronal differentiation, whereas EGF, TPA, and serum stimulate proliferation (Rydel and Green, 1987; Hall et al., 1988; Satoh et al., 1988). This appears contradictory, in particular knowing that PC3 plays a part in these processes, as a negative regulator of proliferation (Montagnoli et al., 1996). However, we should consider that NGF, EGF, and FGF act through tyrosine kinase receptors, which stimulate the Ras/MEK/mitogen-activated protein kinase (MAPK) pathway (see for reviews Campbell et al., 1998; Chao et al., 1998). In PC12 cells it has been shown that the duration of the activation of extracellular signal-regulated kinase (ERK)/MAPK pathway is crucial in determining the biological outcome, either differentiative or proliferative. A stimulus by NGF exerts longer activation, that leads to differentiation, whereas EGF exerts a shorter stimulation, that leads to proliferation (Cowley et al., 1994). Consistently, it is known that EGF also, when overexpressed, can induce neurite outgrowth in PC12 cells (Traverse et al., 1994). Given that in PC12 cells NGF and FGF induce PC3 to a greater extent than EGF, this suggests that PC3 regulation is encased within a system that integrates different stimuli by producing fine quantitative differences in gene expression. A cell having growth inhibitory and stimulatory cues both activating the same growth inhibitory gene would have the advantage to readily counteract an excess of proliferation. Examples of a negative feed-back to a proliferative stimulus are not uncommon, such as activation, following cyclin D1 induction by serum stimulation, of the proliferative molecule E2F-1, that in certain cell systems can inhibit cyclin D1 transcription (Watanabe et al., 1998). Interestingly, cyclin D1 is the main cell cycle target of the Ras pathway (Baldin et al., 1993; Quelle et al., 1993, Serrano et al., 1995), and in PC12 cells exposed to NGF the cyclin D1 associated kinase activity decreases (Yan and Ziff, 1995, van Grunsven et al., 1996). Furthermore, the activation in PC12 cells of endogenous PC3 by the NGF pathway occurs through high affinity NGF receptors TrkA and TrkB (M. Canossa and F. Tirone, unpublished results), and its physiological role seems to be that of a transient signal for cell cycle exit (Montagnoli et al., 1996).

In other cell systems employing selective kinase inhibitors, such as the adipocite during adipogenic stimulation, it has been shown that induction of TIS21 occurs mainly by protein kinase C and MEK pathways (Inuzuka et al., 1999).

$PC3^{TIS21/BTG2}$ is also induced by cellular depolarization that might indicate a role in the activity-dependent survival of neurons (Bradbury et al., 1991; Jung et al., 1996). The promoter elements activated by cellular depolarization in other NGF-inducible immediate early genes appear to differ, those being for instance, cAMP-dependent response elements (CREs) in the case of c-fos (Fisch et al., 1989; Sheng et al., 1990), or AP-1-like elements in the case of NGFI-B (Yoon and Lau, 1994). In the TIS21 promoter have been found CREs elements (Fletcher et al., 1991), that might account for the induction by depolarization and cAMP.

Induction of $PC3^{TIS21/BTG2}$ by DNA damage and by p53

$PC3^{TIS21/BTG2}$ is induced also in conditions of DNA damage (Rouault et al., 1996) and cellular stress (Fiedler et al., 1998). It has been shown that a wide variety of DNA damaging agents, such as ionizing radiations (IR), UV, and adriamycin, is able to induce $PC3^{TIS21/BTG2}$ (Rouault et al., 1996; Cortes et al., 2000), and that these agents are also able to induce BTG1 and TOB (Cortes et al., 2000). Such induction of $PC3^{TIS21/BTG2}$ has been demonstrated to be p53-dependent, using either cell lines carrying a temperature-sensitive p53 mutant (p53Val135), or cell lines stably transfected with a dominant negative p53. Conversely, the induction of BTG1 and TOB by DNA-damaging agents follows a delayed time course and is not p53-dependent (TOB appears to be rather down-regulated by p53; Cortes et al., 2000).

p53 plays a key role as tumor suppressor, being activated in conditions of DNA damage, hypoxia or nucleotide depletion by preventing the progression of the cell cycle and thus keeping the integrity of the genome (reviewed by Levine, 1997). This control is exerted by p53 at the G_1/S checkpoint through induction of p21, which results in the inhibition of CDK4 and CDK2 activity, p53 also controls the G_2/M checkpoint, albeit redundantly, through the induction of GADD45 that can destabilize Cdc2/cyclin B complexes, or, alternatively, by direct transcriptional repression of Cdc2 transcription (see for review Dasika et al., 1999). As mentioned above, genetic ablation of TIS21 in ES cells has been shown to prevent G_2 arrest that occurs after DNA damage, which suggests that $PC3^{TIS21/BTG2}$ might play a role in the p53-mediated G_2 arrest (Rouault et al., 1996). BTG2 in fact is directly induced by p53 through a p53 consensus element in the BTG2 promoter (Rouault et al., 1996), which appears to be quite conserved in TIS21 promoter. This induction of $PC3^{TIS21/BTG2}$ by p53 required the proline-rich PXXP motif within the p53 protein, which appears to be necessary to the apoptosis, but not to the growth arrest, induced by p53 (Zhu et al., 1998). The same proline-rich motif can be implicated in chromatin remodeling, given the differences observed between the induction of endogenous and exogenous p53-inducible genes by p53 mutants defective for this motif (Zhu et al., 1998).

However, the observations about the induction of $PC3^{TIS21/BTG2}$ by p53 so far concern the transcript, and do not regard the regulation of the protein. In our hands, the direct induction of PC3 mRNA by p53 is not accompanied by a detectable induction of the protein, whereas protein induction is seen following DNA damage (G. Corrente and F. Tirone, unpublished results). This suggests that p53 mediates the transcriptional induction of the $PC3^{TIS21/BTG2}$ gene, but that more complex post-translational regulations, involved in DNA damage and possibly p53-independent, are necessary to prolong the very short half-life of the PC3 protein (Varnum et al., 1994). This possibility would reconcile the observation that p53 overexpression induces cyclin D1 (Del Sal et al., 1994; Chen et al., 1995), whereas DNA damage or overexpression of PC3 reduces cyclin D1 levels (Shapiro et al., 1998; Guarda-

vaccaro et al., 2000). Indeed, we suggested that PC3 might be a mediator of the cyclin D1 inhibition occurring after DNA damage (Guardavaccaro et al., 2000). This hypothesis was also based on the previous demonstration by Pagano et al. (1994) that cyclin D1 binds and inhibits the proliferating cell nuclear antigen (PCNA), the auxiliary factor of DNA polymerases δ and ε required for DNA replication and repair (Barvo et al., 1987; Shivji et al., 1992; Bignami and Aquilina, 2001), and that the DNA damage-induced down-regulation of cyclin D1 is necessary for PCNA nuclear relocation and DNA repair synthesis. PC3 could thus act as a link between cell cycle and the process of DNA repair (see in this issue Bellacosa, 2001; Aquilina and Bignami, 2001).

Two alternative pathways regulated by $PC3^{TIS21/BTG2}$ following DNA damage have also been suggested. The first is highlighted by the observation that disruption of yCAF-1 in yeast suppresses a mutation of RAD52 (Schild, 1995; Rouault et al., 1998), a protein involved in DNA repair, that binds DNA with double strand breakage and directs the end-to-end assembly (see for review Dasika et al., 1999). This indicates a functional interaction of yCAF-1 with RAD52 (Schild et al., 1995), in which $PC3^{TIS21/BTG2}$ might have a part.

A second pathway might rely on the ability of $PC3^{TIS21/BTG2}$ (and also BTG1) to bind and modulate the activity of protein-arginine methyltransferase 1 (PRMT1; Lin et al., 1996, see below), one of whose substrates is histones. In fact, the group of Puisieux proposed that in conditions of DNA damage p53 might control transcription by protein methylation through $PC3^{TIS21/BTG2}$. This hypothesis was based on the notion that histone methylation might regulate the assembly into chromatin and thus transcription, and on finding that methylation of histones is increased by DNA damage with a kinetic similar to the induction of $PC3^{TIS21/BTG2}$ and BTG1 transcripts (Cortes et al., 2000). Such a mechanism appears to be non-target-specific, but could certainly explain some transcriptional effects of $PC3^{TIS21/BTG2}$ so far described.

Another aspect to consider is that p53 is also involved in the control in vivo of neural development and neural apoptosis, exemplified by the observation that a subset of p53 null mice develops anencephaly as the consequence of failure in neural tube closure (Armstrong et al., 1995), and that two other molecules of the p53 family, p73 (which induces $PC3^{TIS21/BTG2}$ mRNA; Zhu et al., 1998) and particularly p63, are in fact involved in the process of neuronal differentiation more than in DNA damage (see for review Lohrum and Vousden, 2000). Thus, it cannot be excluded that $PC3^{TIS21/BTG2}$ could also play a role in pathways elicited by the p53 family different from those related to the DNA damage response.

Possible role of PC3/BTG/TOB genes as a tumor suppressors

The possibility that $PC3^{TIS21/BTG2}$ behaves as a tumor suppressor, though it presently remains speculative, is based on the fact that PC3 is involved in both the p53 and the Rb pathways, which are primary targets in cells escaping growth control. No informative studies have so far been produced defining whether the $PC3^{TIS21/BTG2}$ gene undergoes mutations in tumors, although it is known that the 1q23-32 region, where the gene maps (at 1 q32, Rouault et al., 1996), presents loss of heterozygosity in breast carcinomas with a 25% frequency (Chen et al., 1989). Nonetheless, transgenic mice studies have clearly evidenced that some genes never found to be mutated in tumors do possess tumor-suppressor activity (see for review Macleod, 2000).

Also, in the case of BTG1, the t(8:12) translocation in a B-cell lymphocytic leukemia of a genomic sequence containing the MYC locus and the BTG1 sequence, did not change the structure of BTG1, since the breakpoint was localized upstream to BTG1 itself (Rimokh et al., 1991).

There is, however, evidence indicating that TOB can inhibit proliferation of some human pancreatic cancer cell lines (viz., AsPC-1, BxPC-3, SOJ; Yanagie et al., 2000), and that TOB−/− mice develop spontaneous tumors in a variety of tissues, presenting a higher incidence of hepatic tumors following exposure to the carcinogen diethylnitrosamine (Yoshida et al., 2000). All this supports the idea that TOB might act as a tumor suppressor. Whether this possibility is related to the ability of the carboxy-terminal of TOB to bind the protein-tyrosine kinase receptor erbB-2, remains to be established. In fact TOB fails to inhibit the proliferative activity of the rat c-erbB-2 gene *neu* oncogenically activated by a point mutation (Matsuda et al., 1996). In this regard, it has been recently shown that growth stimulation by activated *neu* occurs through the Ras and p38 pathways, and depends on the induction of the cyclin D1 promoter with an E2F-1-dependent mechanism (Lee et al., 2000). This would suggest that the inhibitory action of TOB on the cell cycle is either upstream to cyclin D1, or that TOB targets cyclin D1 (or other regulators downstream) less effectively than the *neu*-activated stimulatory signal.

Molecular targets of $PC3^{TIS21/BTG2}$

Recently, by yeast two hybrid analysis some $PC3^{TIS21/BTG2}$ interacting proteins have been identified, that can also interact with other PC3/BTG/TOB genes.

PRMT-1—As mentioned above, $PC3^{TIS21/BTG2}$ and BTG1 bind and regulate the activity in vitro of PRMT1 (Lin et al., 1996). This is a ubiquitous enzyme able to methylate arginine residues of a variety of substrates, in some cases modulating their activity, such as heterogeneous ribonucleoproteins involved in RNA processing, PP2A, interferon and cytokine receptors, and histones (see for review Aletta et al., 1998). This interaction might be involved in transcriptional control following DNA damage and in cell cycle inhibition by $PC3^{TIS21/BTG2}$ (see also sections related to cell cycle and DNA damage). However, such experimental evidence is not yet available and it is not known which domain of the $PC3^{TIS21/BTG2}$ molecule interacts with PRMT-1. Interestingly, TIS21 itself is a substrate of PRMT1, suggesting the existence of a feed-back regulatory pathway (Lim et al., 1998b).

CAF-1—See section above, - $PC3^{TIS21/BTG2}$ as inhibitor of cell cycle.

Hoxb9—Recently it has been shown that $PC3^{TIS21/BTG2}$ (and BTG1) binds and positively modulates the transcriptional activity of the homeogene Hoxb9 (Prevot et al., 2000). The Hox genes have been demonstrated to

be responsible for the regionalization of the embryo along its major axis (see for review Deschamps et al., 1999; Burke, 2000) and, in the case of Hoxb-9, its targeted disruption leads to defects in the development of the first and second rib (Chen and Capecchi, 1997). More generally, the expression of Hoxb9 during development is detected in the mouse from E9 to about E12, in the posterior thoracic region, and, in the chicken, also in the corresponding dorsal root ganglia (Burke et al., 1995). Indeed, the activity of Hox genes in defining the identity of a certain body segment is the result of a complex and concerted interaction with other homeogenes as well as with genes of different categories. The transcriptional state of Hox genes can be, for instance, reprogrammed by more posterior locations, involving RA and FGF signaling. Given that the expression of PC3 in the neural tube is colinear with that of Hoxb9, and that one of the putative transcriptional targets of Hoxb9 is the neural cell adhesion molecule (N-CAM), the group of Corbo suggested that PC3 might control neuronal differerentiation by modulating the transcriptional activity of Hoxb9 (Prevot et al., 2000). The hypothesis that PC3, expressed continuously along the nervous system of the embryo, could contribute to specify a body segment by influencing the activity of a target homeogene is certainly plausible and interesting. However, this idea has to be reconciled with the fact that Hoxb9, in particular, does not seem to be involved in neurogenesis if not marginally, whereas PC3, in the region of colinearity with Hoxb9, is present only in the neuroblast of the ventricular region of the neural tube and in dorsal root ganglia (Iacopetti et al., 1994). An additional point is that ANA/BTG3 is expressed not only in the ventricular region of the neural tube at the stage E12—E14, as $PC3^{TIS21/BTG2}$, but also, at that period, in tissues of mesenchymal origin, such as the ribs (Yoshida et al., 1998). ANA/BTG3 has thus a colinearity of expression with Hoxb9 certainly higher than $PC3^{TIS21/BTG2}$, and it would be worth analyzing its binding with Hoxb9, and the chance of it being a potential in vivo regulator of Hoxb9 action in the development of the thoracic segment of the body.

From a molecular point, the binding of BTG1, and presumably of PC3, to Hoxb9 does not occur through the GR or B boxes (rather through the initial amino-terminal region), and no functional effect of Hoxb9 on proliferation has been observed. This fact, together with our observation that the transcriptional control of cyclin D1 and the consequent inhibition of proliferation is exerted by the GR domain (A box) of PC3, would clearly suggest that the control of proliferation is a function separate from the modulation of the transcriptional activity of Hoxb9, although the final outcome might be in both cases the control of differentiation (see above; Iacopetti et al., 1994, 1999; Malatesta et al., 2000).

PC3 as a putative transcriptional regulator

The observation that $PC3^{TIS21/BTG2}$ regulates the transcriptional activity of Hoxb9 by direct binding, favors the possibility that $PC3^{TIS21/BTG2}$ functions as a transcriptional co-regulator. $PC3^{TIS21/BTG2}$ might act as a modulator of a transcriptional complex, being by itself deviod of a transactivation domain, as judged by the lack of transcriptional activity of the GAL4-PC3 chimeric protein (Prevot et al., 2000; our unpublished data). The idea that $PC3^{TIS21/BTG2}$ could assemble with transcriptional complexes is also suggested by binding to the yCAF-1 (Rouault et al., 1998) component of the yeast CCR4 transcriptional complex. So far, the transcriptional complex putatively interacting with CAF-1 in mammal is still unknown, and no evidence so far has been produced that $PC3^{TIS21/BTG2}$ modulates the transcriptional activity of CCR4 or of its targets. Furthermore, the ability of $PC3^{TIS21/BTG2}$ to bind and directly modulate the enzymatic activity of pRMT-1 can be compatible with the above hypothesis, if we think the possibility of a transcriptional modulation by histone methylation mediated by PRMT-1. However, this latter point remains to be demonstrated, with the additional possibility that between the substrates of PRMT-1 are specific transcriptional regulatory factors.

On the other hand, $PC3^{TIS21/BTG2}$ appears to be localized mainly in the cytoplasm, which implies that the interaction with the component of a given transcriptional complex would not occur in the nucleus. Conversely, TOB and BTG1 have been found able to localize in the nucleus. In the case of TOB, this is cell cycle-dependent and is correlated to the antiproliferative effect, and for BTG1 depends on stimulation by T3 or cAMP (Rodier et al., 1999; Yoshida et al., 2000; T. Yamamoto, personal communication). Since no specific studies of endogenous $PC3^{TIS21/BTG2}$ localization have been so far carried out, it cannot be excluded that a similar translocation to the nucleus might occur for $PC3^{TIS21/BTG2}$, in some specific conditions.

As far as we are concerned the target genes whose transcription is regulated by $PC3^{TIS21/BTG2}$, it has been identified the endogenous cyclin D1 (Guardavaccaro et al., 2000), and shown that the activity of the N-CAM promoter can be modulated by $PC3^{TIS21/BTG2}$ (Prevot et al., 2000).

CONCLUSIONS

According to experimental data, the inhibition of G_1–S progression by $PC3^{TIS21/BTG2}$ can be either Rb-dependent, by inhibition of cyclin D1 transcription, or Rb-independent, by cyclin E down-regulation. An Rb-dependent mechanism of growth arrest might be common to all family proteins, since also TOB and TOB2 inhibit pRb phosphorylation (Ikematsu et al., 1999). Moreover, there is evidence also that the G_2 checkpoint could be affected by $PC3^{TIS21/BTG2}$ following DNA damage.

This ability of $PC3^{TIS21/BTG2}$ to inhibit the cell cycle might have a functional role in the growth arrest triggered by a variety of stimuli, such as DNA damage or other types of cellular stress (e.g., hypoxia, Gubits et al., 1993), cellular differentiation, and apoptosis. In fact $PC3^{TIS21/BTG2}$ can be induced following DNA damage in a p53-dependent manner, or during apoptosis, and by growth factors as well as by cell autonomous stimuli. Given that after DNA damage or apoptosis is observed a decrease of cyclin D1 levels, a role of $PC3^{TIS21/BTG2}$ in these processes is further suggested, chiefly through impairment of the Rb checkpoint.

The molecular function of $PC3^{TIS21/BTG2}$ is still undefined, although much evidence (the binding to CAF-1 which in yeast is part of the CCR4 transcriptional

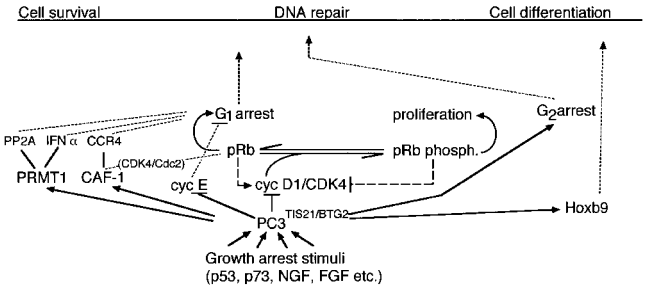

Fig. 3. A model for $PC3^{TIS21/BTG2}$ activity. Dotted lines indicate pathways either putative or requiring further confirmation. Following induction by DNA damage, cell autonomous stimuli for differentiation, or apoptosis, $PC3^{TIS21/BTG2}$ induces G_1 arrest through Rb-dependent (cyclin D1/CDK4) or Rb-independent pathways (cyclin E), and/or G_2 arrest. Cell cycle arrest might be attained through other targets of $PC3^{TIS21/BTG2}$ such as PRMT1 and CAF-1, impinging on different cell cycle pathways. Thus, modulation of G_1 and G_2 checkpoints by transcription-dependent and independent pathways would influence cell survival, DNA repair and cell differentiation. Furthermore, $PC3^{TIS21/BTG2}$ might regulate cell differentiation by targets such as Hoxb9, not directly involving cell cycle control.

complex, and the binding to PRMT1 that might regulate chromatin assembly by histone methylation) points to the possibility that $PC3^{TIS21/BTG2}$ might behave as a transcriptional co-regulator. So far, a domain of $PC3^{TIS21/BTG2}$, the GR or A box, has been directly involved in the inhibition of proliferation through the Rb pathway, whereas other domains, i.e., the B box and possibly the most amino-terminal region, have been involved in the binding to other target molecules, that might have a role in control of cell cycle perhaps by other pathways—CAF-1—or in differentiation—Hoxb9—(see Fig. 3). It is thus possible that different domains might exert different molecular functions, an occurrence not uncommon between cell cycle molecules. These functions might be partly transcription-dependent, partly -independent (as might be the modulation of PP2A by PRMT1). Furthermore, within all the genes of the family another domain might be represented by the carboxy-terminal region, though this appears to have undergone extensive deletion except in the TOB and FOG genes. In the case of TOB this domain has been shown to be responsible for the binding to ErbB2, suggesting that it is an additional region that has evolved and/or has been conserved only for some genes of the family, to identify and bind specific molecular targets.

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LITERATURE CITED

Abramovich C, Yakobson B, Chebath J, Revel M. 1997. A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I interferon receptor. EMBO J 16:260–266.

Ajima R, Ikematsu N, Ohsugi M, Yoshida Y, Yamamoto T. 2000. Cloning and characterization of the mouse tob2 Gene. 253:215–220.

Aletta JM, Cimato TR, Ettinger MJ. 1998. Protein methylation: a signal event in post-translational modification. Trends Biochem Sci 23:89–91.

Armstrong JF, Kaufman MH, Harrison DJ, Clarke AR. 1995. High-frequency developmental abnormalities in p53-deficient mice. Curr Biol 5:931–936.

Baldin V, Lukas J, Marcote MJ, Pagano M, Draetta G. 1993. Cyclin D1 is a nuclear protein required for cell cycle progression in G_1. Genes Dev 7:812–821.

Bellacosa A. The role of Med1 (MBD4) gene in DNA repair and human cancer. J Cell Physiol, in press.

Bignami M, Aquilina G. Mismatch repair in correction of replication errors and processing of DNA damage. J Cell Physiol, in press.

Bogdan JA, Adams-Burton C, Pedicord DL, Sukovich DA, Benfield PA, Corjay MH, Stoltenborg JK, Dicker IB. 1998. Human carbon catabolite repressor protein associative factor 1: cloning, expression and characterization of its interaction with the B-cell translocation protein BTG1. Biochem J 336:471–481.

Bradbury A, Possenti R, Shooter EM, Tirone F. 1991. Molecular cloning of PC3, a putatively secreted protein whose mRNA is induced by nerve growth factor and depolarization. Proc Natl Acad Sci USA 88:3353–3357.

Bravo R, Frank R, Blundell PA, Macdonald-Bravo H. 1987. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta Nature 326:515–517.

Buanne P, Corrente G, Micheli L, Palena A, Lavia P, Spadafora C, Lakshmana MK, Rinaldi A, Banfi S, Quarto M, Bulfone A, Tirone F. 2000. Cloning of PC3B, a novel member of the PC3/BTG/TOB family of growth inhibitory genes, highly expressed in the olfactory epithelium. Genomics 68:253–263.

Burke AC. 2000. Hox genes and the global patterning of the somitic mesoderm. Curr Top Dev Biol 7:155–181.

Burke AC, Nelson CE, Morgan BA. Tabin C. 1995. Hox genes and the evolution of vertebrate axial morphology. Development 121:333–346.

Campbell SL, Khosravi-Far R, Rossman KL, Clark GJ. 1998. Increasing complexity of Ras signaling. Oncogene 17:1395–1413.

Chao M, Casaccia-Bonnefil P, Carter B, Chittka A, Kong H, Yoon SO. 1998. Neurotrophin receptors: mediators of life and death. Brain Res Brain Res Rev 26:295–301.

Chen LC, Dollbaum C, Smith HS. 1989. Loss of heterozygosity on chromosome 1q in human breast cancer. Proc Natl Acad Sci USA 86:7204–7247.

Chen P, Ellis RE. 2000. TRA-1A regulates transcription of fog-3, which controls germ cell fate in C. elegans. Development 127:3119–3129.

Chen PJ, Singal A, Kimble J, Ellis RE. 2000. A novel member of the TOB family of proteins controls sexual fate in Caenorhabditis elegans germ cells. Dev Biol 217:77–90.

Chen F, Capecchi MR. 1997. Targeted mutations in Hoxa-9 reveal synergistic interactions. Dev Biol 181:186–196.

Chen X, Bargonetti J, Prives C. 1995. p53, through p21 (WAF/CIP1), induces cyclin D1 synthesis. Cancer Res 55:4257–4263.

Corjay MH, Kearney MA, Munzer DA, Diamond SM, Stoltenborg JK. 1998. Antiproliferative gene BTG1 is highly expressed in apoptotic cells in macrophage-rich areas of advanced lesions in Watanabe heritable hyperlipidemic rabbit and human. Lab Invest 78:847–858.

Cortes U, Moyret-Lalle C, Falette N, Duriez C, Ghissassi FE, Barnas C, Morel AP, Hainaut P, Magaud JP, Puisieux A. 2000. BTG gene expression in the p53-dependent and -independent cellular response to DNA damage. Mol Carcinog 27:57–64.

Cowley S, Paterson H, Kemp P, Marshall CJ. 1994. Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cell. Cell 77:841–852.

Dasika GK, Lin S-CJ, Zhao S, Sung P, Tomkinson A, Lee EY-HP. 1999. DNA damage-induced cell cycle checkpoints and DNA stand break repair in development and tumorigenesis. Oncogene 18:7883–7899.

Del Sal G, Loda M, Pagano M. 1996. Cell cycle and cancer: critical events at the G1 restriction point. Crit Rev Oncog 7:127–142.

Deschamps J, van den Akker E, Forlani S, De Graaff W, Oosterveen T, Roelen B, Roelfsema J. 1999. Initiation, establishment and maintenance of Hox gene expression patterns in the mouse. Int J Dev Biol 43:635–650.

Erickson KL, Hubbard NE, Chakrabarti R. 1995. Modulation of signal transduction in macrophages by dietary fatty acids. J Nutr 125 (6 Suppl):1683S–1686S.

Feng DF, Doolittle RF. 1996. Progressive alignment of amino acid sequences and the construction of phylogenetic trees from them. Methods Enzymol 266:368–382.
Fiedler F, Mallo GV, Bodeker H, Keim V, Dagorn JC, Iovanna JL. 1998. Overexpression of the PC3/TIS21/BTG2 mRNA is part of the stress response induced by acute pancreatitis in rats. Biochem Biophys Res Commun 249:562–565.
Fisch TM, Prywes R, Simon MC, Roeder RG. 1989. Multiple sequence elements in the c-fos promoter mediate induction by cAMP. Genes Dev 3:198–211.
Fletcher BS, Lim RW, Varnum BC, Kujubu DA, Koski RA, Herschman HR. 1991. Structure and expression of TIS21, a primary response gene induced by growth factors and tumor promoters. J Biol Chem 266:14511–14518.
Gawantka V, Pollet N, Delius H, Vingron M, Pfister R, Nitsch R, Blumenstock C, Niehrs C. 1998. Gene expression screening in *Xenopus* identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. Mech Dev 77:95–141.
Greene LA, Aletta JM, Rukenstein A, Green SH. 1986. PC12 pheochromocytoma cells: culture, nerve growth factor treatment, and experimental exploitation. Methods Enzymol 147:207–216.
Guardavaccaro D, Corrente G, Covone F, Micheli L, D'Agnano I, Starace G, Caruso M, Tirone F. 2000. Arrest of G(1)-S progression by the p53-inducible gene PC3 is Rb dependent and relies on the inhibition of cyclin D1 transcription. Mol Cell Biol 20:1797–1815.
Gubits RM, Burke RE, Casey-McIntosh G, Bandele A, Munell F. 1993. Immediate early gene induction after neonatal hypoxia-ischemia. Brain Res Mol Brain Res 18:228–238.
Guehenneux F, Duret L, Callanan MB. Bouhas R, Hayette S. Berthet C, Samarut C, Rimokh R, Birot AM, Wang Q, Magaud JP, Rouault JP. 1997. Cloning of the mouse BTG3 gene and definition of a new gene family (the BTG family) involved in the negative control of the cell cycle. Leukemia 11:370–375.
Hall FL, Fernyhough P, Ishii DN, Vulliet RP 1988. Suppression of nerve growth factor-directed neurite outgrowth in PC12 cells by sphingosine, an inhibitor of protein kinase C. J Biol Chem 263:4460–4466.
Holland ND, Zhang SC, Clark M, Panopoulou G, Lehrach H, Holland LZ. 1997. Sequence and developmental expression of AmphiTob, an amphioxus homolog of vertebrate Tob in the PC3/BTG1/Tob family of tumor suppressor genes. Dev Dyn 210:11–18.
Iacopetti P, Barsacchi G, Tirone F, Maffei L, Cremisi F. 1994. Developmental expression of PC3 gene is correlated with neuronal cell birthday. Mech Dev 47:127–137.
Iacopetti P, Michelini M, Stuckmann I, Oback B, Aaku-Saraste E, Huttner WB. 1999. Expression of the antiproliferative gene TIS21 at the onset of neurogenesis identifies single neuroepithelial cells that switch from proliferative to neuron-generating division. Proc Natl Acad Sci USA 96:4639–4644.
Ikematsu N, Yoshida Y, Kawamura-Tsuzuku J, Ohsugi M. Onda M, Hirai M, Fujimoto J, Yamamoto T. 1999. Tob2, a novel anti-proliferative Tob/BTB1 family member, associates with a component of the CCR4 transcriptional regulatory complex capable of binding cyclin-dependent kinases. Oncogene 18:7432–7441.
Inuzuka H, Nanbu-Wakao R, Masuho Y, Muramatsu M, Tojo H, Wakao H. 1999. Differential regulation of immediate early gene expression in preadipocyte cells through multiple signaling pathways. Biochem Biophys Res Commun 265:664–668.
Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA. Weinberg RA. 1992. Effects of an Rb mutation in the mouse. Nature 359:295–300.
Jung HY, Kang UG, Ahn YM, Joo YH, Park JB, Kim YS. 1996. Induction of tetradecanoyl phorbol acetate-inducible sequence (TIS) genes by electroconvulsive shock in rat brain. Biol Psychiatry 40:503–507.
Kaplan DR, Miller FD. 2000. Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol 10:381–391.
Kranenburg O, van der Eb A, Zantema A. 1996. Cyclin D1 is an essential mediator of apoptotic neuronal cell death. EMBO J 15:46–54.
Lee RJ, Albanese C, Fu M, D'Amico M, Lin B, Watanabe G, Haines GK IIIrd, Siegel PM, Hung MC, Yarden Y, Horowitz JM, Muller WJ, Pestell RG. 2000. Cyclin D1 is required for transformation by activated *Neu* and is induced through an E2F-dependent signaling pathway. Mol Cell Biol 20:672–683.
Lee EY, Chang CY, Hu N, Wang YC, Lai CC, Herrup K, Lee WH, Bradley A. 1992. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 359: 288–294.
Levine AJ. 1997. p53, the cellular gatekeeper for growth and division. Cell 88:323–331.
Lim IK, Lee MS, Ryu MS, Park TJ, Fujiki H, Eujiki H, Paik WK. 1998a. Induction of growth inhibition of 293 cells by downregulation of the cyclin E and cyclin-dependent kinase 4 proteins due to overexpression of TIS21. Mol Carcinog 23:25–35.
Lim IK, Park TJ, Kim S, Lee HW, Paik WK. 1998b. Enzymatic methylation of recombinant TIS21 protein-arginine residues. Biochem Mol Biol Int 45:871–878.
Lin WJ, Gary JD, Yang MC, Clarke S, Herschman HR, 1996. The mammalian immediatearly TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. J Biol Chem 271:15034–15044.
Liu HY, Badarinarayana V, Audino DC, Rappsilber J, Mann M, Denis CL. 1998. The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. EMBO J 17:1096–1106.
Liu HY, Toyn JH, Chiang YC, Draper MP, Johnston LH, Denis CL. 1997. DBF2 a cell cycle-regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. EMBO J 16:5289–5298.
Lohrum MAE, Vousden KH. 2000. Regulation and function of the p53-related proteins: same family, different rules. Trends in Cell Biology 10:197–202.
Macleod K. 2000. Tumor suppessor genes. Curr Opin Genet Dev 10:81–93.
Malatesta P, Gotz M, Barsacchi G, Price J, Zoncu R, Cremisi F. 2000. PC3 overexpression affects the pattern of cell division of rat cortical precursors. Mech Dev 90:17–28.
Marchal S, Cassar-Malek I, Magaud JP, Rouault JP, Wrutniak C, Cabello G. 1995. Stimulation of avian myoblast differentiation by triiodothyronine: possible involvement of the cAMP pathway. Exp Cell Res 220:1–10.
Matsuda S, Kawamura-Tsuzuku J, Ohsugi M. Yoshida M, Emi M, Nakamura Y, Onda M, Yoshida Y, Nishiyama A, Yamamoto T. 1996. Tob, a novel protein that interacts with p185erbB2, is associated with anti-proliferative activity. Oncogene 12:705–713.
Mesner PW, Winters TR, Green SH. 1992. NGF withdrawal-induced cell death in neuronal PC12 cells resembles that in sympathetic neurons. J Cell Biol 119: 1669–1680.
Mesner PW, Epting CL, Hegarty JL, Green SH. 1995. A timetable of events during programmed cell death induced by trophic factor withdrawal from neuronal PC12 cells. J Neurosci 15:7357–7366.
Mittnacht S. 1998. Control of pRB phosphorylation. Curr Opin Gen Dev 8:21–27.
Montagnoli A, Guardavaccaro D, Starace G, Tirone F. 1996. Overexpression of the nerve growth factor-inducible PC3 immediate early gene is associated with growth inhibition. Cell Growth Differ 7:1327–1336.
O'Reilly M-AJ, Smith JC, Cunliffe V. 1995. Patterning of the mesoderm in *Xenopus*: dosedependent and synergistic effects of Brachyury and Pintallavis. Development 121:1351–1359.
Pagano M, Theodoras AM, Tam SW, Draetta GF. 1994. Cyclin D1-mediated inhibition of repair and replicative DNA synthesis in human fibroblasts. Genes Dev 8:1627–1639.
Prevot D, Voeltzel T, Birot AM, Morel AP, Rostan MC, Magaud JP. Corbo L. 2000. The leukemia-associated protein Btg1 and the p53-regulated protein Btg2 interact with the homeoprotein Hoxb9 and enhance its transcriptional activation. J Biol Chem 275:147–153.
Puisieux A, Magaud JP. 1999. Mechanisms of BTG2 activity, a transcriptional target of p53: evidences and hypothesis. Bull Cancer 86:358–364.
Quelle DE, Ashmun RA, Shurtleff SA, Kato J-y, Bar-Sagi D, Roussel MF, Sherr CJ. 1993. Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. Genes Dev 7:1559–1571.
Raburn DJ, Hamil KG, Tsuruta JK, O'Brien DA, Hall SH. 1995. Stage-specific expression of B cell translocation gene 1 in rat testic. Endocrinology 136:5769–5777.
Rimokh R, Rouault JP. Wahbi K. Gadoux M. Lafage M, Archimbaud E, Charrin C, Gentillhomme O, Germain D, Samarut J, Magaud JP. 1991. A chromosome 12 coding region is juxtaposed to the MYC protooncogene locus in a t(8;12)(q24;q22) translocation in a case of B-cell chronic lymphocytic leukemia. Genes Chromosomes Cancer 3:24–36.
Rodier A, Marchal-Victorion S, Rochard P, Casas F, Cassar-Malek I, Rouault JP, Magaud JP, Mason DY, Wrutniak C, Cabello G. 1999. BTG1: a triiodothyronine target involved in the myogenic influence of the hormone. Exp Cell Res 249:337–348.

Rouault JP, Falette N, Guehennuux F, Guillot C, Rimokh R, Wang Q, Berthet C, Moyret-Lalle C, Savatier P, Pain B, Shaw P, Berger R, Samarut J, Magaud JP, Ozturk M, Samarut C, Puisieux A. 1996. Identification of BTG2, an antiproliferative p53-dependent component of the DNA damage cellular response pathway. Nat Genet 14:482–486.

Rouault JP, Prevot D, Berthet C, Birot AM, Billaud M, Magaud JP, Corbo L. 1998. Interaction of BTG1 and p53-regulated BTG2 gene products with mCaf1, the murine homolog of a component of the yeast CCR4 transcriptional regulatory complex. J Biol Chem 273:22563–22569.

Rouault JP, Rimokh R, Tessa C, Paranhos G, French M, Duret L, Garoccio M, Germain D, amarut J, Magaud JP. 1992. BTG1, a member of a new family of antiproliferative genes. EMBO J 11: 1663–1670.

Rudkin BB, Lazarovici P, Levi B-Z, Abe Y, Fujita K, Guroff G. 1989. Cell cycle-specific action of nerve growth factor in PC12 cells: differentiation without proliferation. EMBO J 8:3319–3325.

Rydel RE, Greene LA. 1987. Acidic and basic fibroblast growth factors promote stable neurite outgrowth and neuronal differentiation in cultures of PC12 cells. J Neurosci 7:3639–3653.

Saka Y, Tada M, Smith JC. 2000. A screen for targets of the xenopus T-box gene Xbra. Mech Dev 93:27–39.

Satoh T, Nakamura S, Taga T, Matsuda T, Hirano T, Kishimoto T, Kaziro Y. 1988. Induction of neuronal differentiation in PC12 cells by B-cell stimulatory factor 2/interleukin 6. Mol Cell Biol 8:3546–3549.

Schild D. Suppression of a new allele of the yeast RAD52 gene by overexpression of RAD51, mutations in srs2 and ccr4, or mating-type heterozygosity. 1995. Genetics 140:115–127.

Serrano M, Gomez-Lahoz E, DePinho RA, Beach D, Bar-Sagi D. 1995. Inhibition of ras-induced proliferation and cellular transformation by p16INK4. Science 267:249–252.

Shapiro GI, Edwards CD, Ewen ME, Rollins BJ. 1998. p16INK4A participates in a G1 arrest checkpoint in response to DNA damage. Mol Cell Biol 18:378–387.

Sheng M, McFadden G, Greenberg ME. 1990. Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. Neuron 4:571–582.

Shivji MK, Kenny MK, Wood RD. 1992. Proliferating cell nuclear antigen is required for DNA excision repair. Cell 69:367–374.

Suk K, Sipes DG, Erickson KL. 1997. Enhancement of B-cell translocation gene-1 expression by prostaglandin E2 in macrophages and the relationship to proliferation. Immunology 91:121–129.

Tippetts MT, Varnum BC, Lim RW, Herschman HR. 1998. Tumor promoter-inducible genes are differentially expressed in the developing mouse. Mol Cell Biol 8:4570–4572.

Traverse S, Seedorf K, Paterson H, Marshall CJ, Cohen P. Ullrich A. 1994. EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor. Curr Biol 4:694–701.

Van Grunsven LA, Billon N, Savatier P, Thomas A, Urdiales JL, Rudkin BB. 1996. Effect of nerve growth factor on the expression of cell cycle regulatory proteins in PC12 cells: dissection of the neurotrophic response from the anti-mitogenic response. Oncogene 12:1347–1356.

Varnum BC. Reddy ST, Koski RA, Herschman HR. 1994. Synthesis, degradation, and subcellular localization of proteins encoded by the primary response genes TIS7/PC4 and TIS21/PC3. J Cell Physiol 158:205–213.

Voorhoeve PM, Watson RJ, Farlie PG, Bernards R, Lam EW. 1999. Rapid dephosphorylation of p107 following UV irradiation. Oncogene 18:679–688.

Walden PD, Lefkowitz GK, Ficazzola M, Gitlin J, Lepor H. 1998. Identification of genes associated with stromal hyperplasia and glandular atrophy of the prostate by mRNA differential display. Exp Cell Res 245:19–26.

Wang S, Dibenedetto AJ, Pittman RN. 1997. Genes induced in programmed cell death of neuronal PC12 cells and developing sympathetic neurons in vivo. Dev Biol 188:322–336.

Watanabe G, Albanese C, Lee RJ, Reutens A, Vairo G, Henglein B, Pestell RG. 1998. Inhibition of cyclin D1 kinase activity is associated with E2F-mediated inhibition of cycling D1 promoter activity through E2F and Spl. Mol Cell Biol 18:3212–3222.

Yan G-Z, Ziff EB. 1995. NGF regulates the PC12 cell cycle machinery through specific inhibition of the Cdk kinases and induction of cyclin D1. J Neurosci 15:6200–6212.

Yanagie H, Summimoto H, Nonaka Y, Matsuda S, Hirose I, Hanada S, Sugiyama H, Mikamo S, Takeda Y, Yoshizaki I, Nakazawa K, Tani K, Yamamoto T, Asano S. Eriguchi M, Muto T. 1998. Inhibition of human pancreatic cancer growth by the adenovirus-mediated introduction of a novel growth suppressing gene, tob, in vitro. Adv Exp Med Biol 451:91–96.

Yoon JK, Lau LF. 1994. Involvement of JunD in transcriptional activation of the orphan receptor gene nur77 by nerve growth factor and membrane depolarization in PC12 cells. Mol Cell Biol 14:7731–7743.

Yoshida Y, Kawamura-Tsuzuku J, Nakamura T, Umemori H, Minowa O, Ikematsu N, Kuno J, Noda T, Yamamoto T. June 22–25, 2000. Disruption of TOB, a new tumor suppressor gene, leads to increased tumor susceptibility in mice. 16th Annual Meeting on oncogenes: the evolution of the cancer cell. The Salk Institute, La Jolla, CA. Abstract, p. 83.

Yoshida Y, Matsuda S. Ikematsu N. Kawamura-Tsuzuku J, Inazawa J, Umemori H. Yamamoto T. 1998. ANA, a novel member of Tob/BTG1 family, is expressed in the ventricular zone of the developing central nervous system. Oncogene 16:2687–2693.

Yoshida Y, Matsuda S, Yamamoto T. 1997. Cloning and characterization of the mouse tob gene. Gene 191:109–113.

Zhu J, Jiang J, Zhou W, Chen X. 1998. The potential tumor suppressor p73 differentially regulates cellular p53 target genes. Cancer Res 58:5061–5065.

Zhu J, Jiang J, Zhou W, Zhu K, Chen X. 1999. Differential regulation of cellular target genes by p53 devoid of the PXXP motifs with impaired apoptotic activity. Oncogene 18:2149–2155.