BMC Molecular Biology

Research article

The major transcription initiation site of the *p27*^{Kip1} gene is conserved in human and mouse and produces a long 5'-*UTR*

Jennifer Coleman, Michelle Hawkinson, Robin Miskimins and W Keith Miskimins*

Address: Division of Basic Biomedical Sciences, University of South Dakota School of Medicine, Vermillion, South Dakota, USA

E-mail: Jennifer Coleman - jcoleman@usd.edu; Michelle Hawkinson - mhawkins@usd.edu; Robin Miskimins - rmiskim@usd.edu; W Keith Miskimins* - kmiskimi@usd.edu

*Corresponding author

Published: 11 October 2001

BMC Molecular Biology 2001, **2**:12

Received: 10 August 2001
Accepted: 11 October 2001

This article is available from: http://www.biomedcentral.com/1471-2199/2/12

Abstract

Background: The cyclin-dependent kinase inhibitor p27^{Kip1} is essential for proper control of cell cycle progression. The levels of p27^{Kip1} are regulated by several mechanisms including transcriptional and translational controls. In order to delineate the molecular details of these regulatory mechanisms it is important to identify the transcription initiation site within the *p27*^{Kip1} gene, thereby defining the promoter region of the gene and the 5'-untranslated region of the *p27*^{Kip1} mRNA. Although several previous studies have attempted to map *p27*^{Kip1} transcription start sites, the results vary widely for both the mouse and human genes. In addition, even though the mouse and human *p27*^{Kip1} gene sequences are very highly conserved, the reported start sites are notably different.

Results: In this report, using a method that identifies capped ends of mRNA molecules together with RNase protection assays, we demonstrate that *p27*^{Kip1} transcription is initiated predominantly from a single site which is conserved in the human and mouse genes. Initiation at this site produces a 5'-untranslated region of 472 nucleotides in the human *p27*^{Kip1} mRNA and 502 nucleotides in the mouse *p27*^{Kip1} mRNA. In addition, several minor transcription start sites were identified for both the mouse and human genes.

Conclusions: These results demonstrate that the major transcription initiation sites in the mouse and human *p27*^{Kip1} genes are conserved and that the 5'-UTR of the *p27*^{Kip1} mRNA is much longer than generally believed. It will be important to consider these findings when designing experiments to identify elements that are involved in regulating the cellular levels of p27^{Kip1}.

Background

The cyclin-dependent kinase inhibitor p27^{Kip1} plays an important role in regulating cell cycle progression by inhibiting the cyclin-Cdk complexes responsible for promoting the transition from G1-phase to S-phase [1,2]. In normal cells, p27^{Kip1} levels are high in GO and decrease rapidly in late G1 allowing entry into S-phase [3,4]. p27^{Kip1} has also been implicated as playing a role in tumor formation and progression. *p27*^{Kip1} knockout mice display internal organ hyperplasia and are highly prone to pituitary adenomas [5–7]. In human cancers, such as breast [8], prostate [9], colorectal [10], and others, low

levels of $p27^{Kip1}$ expression correlate with decreased survival rates. Since mutations of the *$p27^{Kip1}$* gene are rare in cancer cells [11], loss of $p27^{Kip1}$ must involve changes in the mechanisms that control its expression.

Changes in the levels of cellular $p27^{Kip1}$ are regulated by multiple mechanisms. In general, transcriptional control of the *$p27^{Kip1}$* gene is believed to play a minor role in regulating $p27^{Kip1}$ expression. However, recent reports indicate that transcription of the *$p27^{Kip1}$* gene can be activated by neuronal differentiation [12], treatment with vitamin D3 [13,14], interferon and cytokines [15–17], and exposure to hypoxic conditions [18] or xenobiotics [19]. Transcription of the *$p27^{Kip1}$* gene can be negatively regulated by growth factors [20] and by c-Myc [21]. Some of the growth factor and cytokine effects on *$p27^{Kip1}$* transcription may be mediated by the forkhead family of transcription factors that appear to be regulated downstream of the phosphatidylinositol 3-kinase signaling pathway [22–25]. The use of alternative promoters leading to multiple transcription start sites, as reported here, suggests an additional type of transcriptional control.

Post-transcriptional controls appear to be the major regulatory mechanisms that determine the levels of $p27^{Kip1}$ in the cell [26]. These include ubiquitination and proteasomal degradation [27] and sequestration and mislocalization [28] of the $p27^{Kip1}$ protein. In addition, changes in the rate of *$p27^{Kip1}$* mRNA translation have been shown to be important in regulating cellular $p27^{Kip1}$ levels. The rate of *$p27^{Kip1}$* mRNA translation is higher in quiescent cells than in growth factor stimulated cells [3]. $p27^{Kip1}$ translation is also enhanced during differentiation of HL60 cells [29].

Recent studies have shown that elements within the 5' untranslated region (5'-*UTR*) of the *$p27^{Kip1}$* mRNA are able to enhance translational efficiency [30,31] and several proteins that bind to the 5'-*UTR* have been identified [31]. The mouse *$p27^{Kip1}$* 5'-*UTR* has recently been shown to be able to mediate cap-independent initiation of translation [30]. In addition, a U-rich element within the *$p27^{Kip1}$* 5'-*UTR* may be involved in regulating *$p27^{Kip1}$* mRNA stability [32]. Together, these findings clearly demonstrate the importance of the 5'-*UTR* in controlling cellular $p27^{Kip1}$ levels. However, from the work published to date it is not clear what sequences constitute the full *$p27^{Kip1}$* 5'-*UTR* in either human or mouse.

The 5' end of the *UTR* is determined by the site of transcription initiation and several papers have reported putative *$p27^{Kip1}$* transcription initiation sites. In mouse, Kwon et al. [33] reported a major transcription start site 200 nucleotides upstream of the AUG start codon. They also reported a second transcription initiation site 253

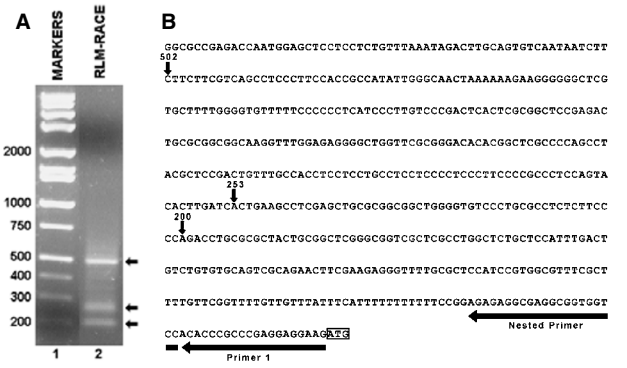

Figure 1
Mapping of mouse transcription initiation sites by RLM-RACE. (A) Agarose gel electrophoresis of nested PCR reaction products from the RLM-RACE procedure using mouse NIH 3T3 RNA. Molecular size markers (base pairs) are indicated on the left. Arrows on the right mark the major PCR products. (B) Sequence of the mouse $p27^{Kip1}$ gene upstream of the translation initiation codon. Vertical arrows above the sequence indicate transcription start sites identified by cloning and sequencing the RLM-RACE products shown in A. Horizontal arrows below the sequence indicate the gene specific primers used for the RLM-RACE procedure. The start codon is boxed. The sequence shown is derived from Genbank accession number U77915.

nucleotides upstream of the start codon. In contrast, Zhang and Lin [34] reported that the mouse *$p27^{Kip1}$* transcription start site is ~500 nucleotides upstream of the start codon. They found no evidence for the downstream initiation sites reported by Kwon et al. [33]. The gene sequences upstream of the *$p27^{Kip1}$* start codon, including the putative promoter regions, are highly conserved between mouse and human. However, it has been reported that the *$p27^{Kip1}$* transcription start site in human cells is only 153 nucleotides upstream of the AUG start codon [31,35]. In contrast to these findings, Ito et al. [36] reported multiple start sites in the region from 403 to 479 nucleotides upstream of the AUG as well as minor sites 280 and 273 nucleotides upstream of the AUG. Thus, for both mouse and human *$p27^{Kip1}$* genes there are discrepancies between the reported transcription start sites. In addition, despite the high level of conservation in *$p27^{Kip1}$* gene sequences, the reports in the literature show no correlation between mouse and human initiation sites, which would result in very different 5'-*UTRs* in the final *$p27^{Kip1}$* mRNAs.

Given the importance of the 5'-*UTR* in governing $p27^{Kip1}$ levels and the disparity among the reports described

above, we felt it necessary to further investigate the *p27*^{Kip1} gene transcription initiation sites. We have used a method that accurately identifies the capped 5'-end of mRNAs together with RNase protection assays. Our data indicate that the major transcription start site is at exactly the same sequence in mouse and human cells and that transcription initiation from this site produces a *p27*^{Kip1} 5'-*UTR* of 502 nucleotides in mouse and 472 nucleotides in human. These findings represent important sequence information relevant to studies being done on both transcriptional and post-transcriptional control of p27^{Kip1} levels.

Results

Determination of transcription initiation sites using RNA ligase-mediated rapid amplification of 5' cDNA ends (5' RLM-RACE)

To identify transcription initiation sites, RLM-RACE was performed. This method has a major advantage over other methods of mapping transcription start sites, such as primer extension, nuclease protection assays, or traditional 5' RACE, in that only authentic capped 5' ends of mRNAs are detected. The first step in RLM-RACE is treatment of the RNA sample with phosphatase. This removes the 5' phosphate from incomplete mRNA fragments and from non-mRNAs eliminating their ability to participate in subsequent ligation reactions. In the second step of the procedure the 7-methylguanosine cap of mRNAs is removed using a pyrophosphatase that leaves a 5' phosphate group. These full-length decapped mRNAs are thus the only RNA molecules that can be ligated to an RNA oligonucleotide adapter in the next step of the procedure. Finally, the RNAs are reverse transcribed followed by PCR amplification using a gene-specific primer and a primer homologous to the RNA oligonucleotide adapter.

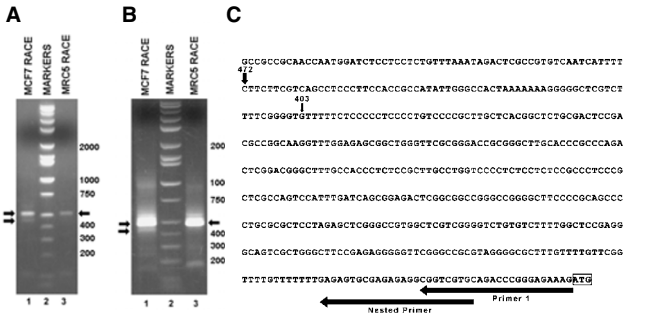

Figure 2
Mapping of human transcription initiation sites by RLM-RACE. (A) Agarose gel electrophoresis of the initial PCR reaction products obtained from the RLM-RACE procedure. Molecular size markers are in the middle well and sizes (base pairs) are indicated on the right. Arrows on the left mark the major PCR products obtained using RNA isolated from MCF7 cells and the arrow on the right marks the major product obtained using RNA from MRC5 cells. (B) Agarose gel electrophoresis of the nested PCR reaction products obtained from the RLM-RACE procedure. (C) Sequence of the human *p27*^{Kip1} gene upstream of the translation initiation codon. The large vertical arrow above the sequence indicates the major transcription start site identified by cloning and sequencing the RLM-RACE products shown in B. The small arrows indicate minor transcription start sites identified by RLM-RACE. Horizontal arrows below the sequence indicate the gene specific primers used for the RLM-RACE procedure. The start codon is boxed. The sequence shown is derived from Genbank accession number AB003688.

This procedure was carried out using total RNA from the mouse NIH 3T3 cell line, the non-transformed human MRC5 cell line, and the human breast cancer line MCF7. The initial PCR was performed using a primer complimentary to the *p27*^{Kip1} mRNA sequence just upstream of the AUG start codon (see Figs. 1B and 2C) together with the adapter primer. Nested PCR was then performed using the first PCR product as a template and the gene specific primers indicated in Figs. 1B and 2C. The nested PCR products were then cloned and sequenced.

In mouse, three bands were obtained after the 5' RACE nested PCR reaction (Fig. 1A) with estimated sizes of 200, 250, and 500 base pairs, with the 500 base pair band being the most intense. Cloning and sequencing of these PCR products revealed three different DNA sequences, each preceded by a sequence corresponding to the RNA oligonucleotide adapter and thus representing authentic cap sites. These sites map to 200, 253, and 502 nucleotides upstream of the ATG translation start codon in the mouse *p27*^{Kip1} gene (Fig. 1B).

When the RLM-RACE procedure was performed using RNA from the non-transformed human MRC5 cell line a single major band of approximately 480 base pairs was observed after the initial PCR reaction (Fig. 2A). With the human MCF7 breast cancer cell line two bands of approximately 480 and 400 base pairs were observed (Fig. 2A). Nested PCR confirmed these bands and revealed several faint bands of various sizes (Fig. 2B). Direct cloning of the RLM-RACE products followed by sequencing demonstrated that the major mRNA cap site in MRC5 cells is 472 nucleotides upstream of the AUG (Fig. 2C). For MCF7, cap sites were observed at 472 and 403 nucleotides upstream of the AUG start codon.

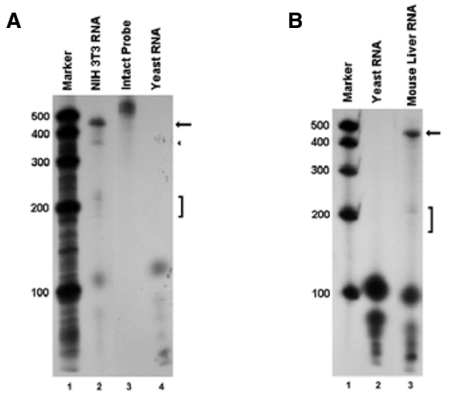

Figure 3
Transcription start site mapping by RNase protection analysis of mouse *p27*^{Kip1} mRNA. (A) RNase protection of *p27*^{Kip1} mRNA derived from NIH 3T3 cells. The intact probe (lane 3) was 565 bases in length including mouse *p27*^{Kip1} sequences from 17 to 502 nucleotides upstream of the AUG and 79 nucleotides derived from vector sequences. Molecular size markers are shown in lane one and the size of each marker in nucleotides is indicated on the left. Lane 2 shows the protected fragments obtained using RNA from NIH 3T3 cells. The arrow at the right indicates the major protected fragment. The arrowhead and bracket indicate minor protected fragments. A control reaction using RNA derived from yeast is shown in lane 4. (B) RNase protection of *p27*^{Kip1} mRNA derived from mouse liver. Details are as described in A.

By gel purifying DNA fragments from the faint bands obtained after the nested PCR reactions and then reamplifying, it was possible to obtain additional clones. These presumably correspond to minor start sites that map to 677, 316, and 289 nucleotides upstream of the *p27*^{Kip1} start codon in MCF7 cells. Using this procedure for MRC5 cells we were able to detect a minor cap site 403 nucleotides upstream of the AUG.

Determination of transcription initiation sites using ribonuclease protection

The RLM-RACE procedure identifies authentic transcription start sites but does not provide a quantitative measure of the efficiency at which the various sites are used. To verify the results of the RLM-RACE and to provide a measure of transcription start site usage an RNase protection assay was employed. Labeled antisense RNA probes were made by in vitro transcription using the cDNA sequence of the most abundant, longer transcripts identified by 5' RLM-RACE as a template. The human probe was 535 nucleotides in length, consisting of *p27*^{Kip1} sequences from 17 to 472 nucleotides upstream of the AUG with the remaining 79 nucleotides derived from non-*p27*^{Kip1} sequences. The mouse probe was 565 nucleotides in length and included *p27*^{Kip1} sequences from 17 to 502 nucleotides upstream of the AUG. The labeled probes were hybridized with total RNA from various sources followed by digestion with RNase and analysis of the protected RNAs on denaturing acrylamide gels.

Using either NIH 3T3 (Fig. 3A) or mouse liver (Fig. 3B) RNA for the RNase protection assay resulted in a single predominant band with an estimated size of 480 nucleotides (Figs. 3A and 3B, arrows). This corresponds to the distal cap site detected by RLM-RACE 502 nucleotides upstream of the AUG. Very faint bands of approximately 190 and 230 nucleotides (Figs. 3A and 3B, brackets) were also detected and these correspond to the other two cap sites mapped by RLM-RACE. Using NIH 3T3 RNA, an additional faint band of approximately 350 nucleotides (Fig. 3A, arrowhead) was detected. This band does not correspond to any known transcription start site. The various protected fragments were quantified by densitometry. After correction for length, the major band at ~480 nucleotides represents ~50.6 % of p27^{Kip1} mRNAs in NIH3T3 cells and 81.25 % in mouse liver. The other protected bands represent approximately equal proportions of the remaining p27^{Kip1} mRNA population.

RNase protection assays were performed using RNA from three separate human cell lines, MRC5, MCF7, and BT20. For all three lines a single band of approximately 450 nucleotides was observed (Fig. 4, arrow). This corresponds to the major transcription initiation site 472 nucleotides upstream of the ATG as identified by RLM-RACE.

Conclusions

In mouse, it was previously reported that the major transcription initiation site for *p27*^{Kip1} is 200 nucleotides upstream of the start codon with a minor start site 253 nucleotides upstream of the ATG [33]. We also detected transcription start sites 200 and 253 nucleotides upstream of the ATG but have shown that these are only rarely used. The RLM-RACE and RNase protection assays described here show that the overwhelming majority of mouse *p27*^{Kip1} transcripts are initiated at a position 502 nucleotides upstream of the ATG. This is true for both mouse liver and NIH 3T3 cells. Zhang and Lin [34], using nuclease protection assays, estimated the mouse *p27*^{Kip1} start site to be 495 nucleotides upstream of the ATG start codon. Our results agree with these investigators but precisely position the start site 502 nucleotides upstream of the AUG.

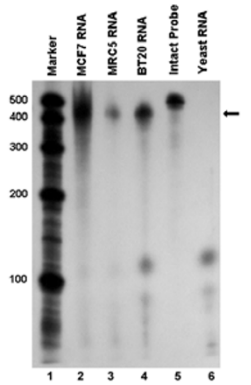

Figure 4
Transcription start site mapping by RNase protection analysis of human *p27*^{Kip1} mRNA. RNase protection assays were performed using *p27*^{Kip1} mRNA derived from MCF7 (lane 2), MRC5 (lane 3) or BT20 (lane 4). The intact probe (lane 5) was 535 bases in length including *p27*^{Kip1} sequences from 17 to 472 nucleotides upstream of the AUG and 79 nucleotides derived from vector sequences. Molecular size markers are shown in lane 1 and the size of each marker in nucleotides is indicated on the left. The arrow at the right indicates the major protected fragment observed in all three human cell lines. A control reaction using RNA derived from yeast is shown in lane 6.

In human cell lines, the major transcription initiation site is 472 nucleotides upstream of the ATG. Both RNase protection assays and RLM-RACE (Figs. 2 and 4) demonstrate this. This site exactly equates to the major transcription start site in mouse (Fig. 5). Using RLM-RACE we were also able to detect minor cap sites in human cells. An initiation site 403 nucleotides upstream of the ATG was easily identified in MCF7 human breast cancer cells (Fig. 2) and a significant portion of the initial clones were derived from RNAs capped at this site. In contrast, 100% of the initial clones from the RLM-RACE procedure using the non-transformed human MRC5 cell line were from products capped 472 nucleotides upstream of the AUG. By gel purification and reamplification we were able to detect additional cap sites 677, 316, and 289 nucleotides upstream of the AUG. However, these can only be very minor start sites since they were rarely observed by RLM-RACE and were not detected by RNase protection assays. Using an RLM-RACE procedure similar to that used for this study, Ito et al. [36] mapped a cluster of transcription start sites surrounding nucleotide 472 upstream of the ATG. However, their data did not demonstrate which site within this cluster served as the major initiation site.

In another study the transcription initiation site for the human *p27*^{Kip1} gene was reported to be 153 nucleotides upstream of the ATG [31,35]. The experiments reported here do not support these findings. Using RLM-RACE, a faint band of approximately 150 base pairs could be observed after nested PCR (Fig. 2A), but we were unable to clone an authentic RACE product equating to a start site 153 nucleotides upstream of the AUG. In addition, no bands corresponding to a start site at this position were detected by RNase protection assays indicating that, if there is a transcriptional start site at 153, it is extremely rarely used in any of the cell lines that we tested. The *p27*^{Kip1} 5'-*UTR* is predicted to have extensive secondary structure [31]. It is possible that this could interfere with primer extension analysis, which was used by Minami et al. [35] to map the putative transcription initiation site in human cells. It is also possible that differences exist between the cell types used here and those used in previous reports. However, this is unlikely since we observed exactly the same results with three separate human cell lines.

Both the mouse and human *p27*^{Kip1} promoter regions have previously been characterized using reporter gene constructs. All of the constructs that have significant levels of promoter activity contain the major transcriptional start sites identified in this study [33–36]. Mouse *p27*^{Kip1} reporter constructs containing ~500 base pairs upstream of the ATG start codon had virtually no activity [33,34]. Similarly a human *p27*^{Kip1} reporter construct containing 435 base pairs upstream of the ATG was inactive [35]. Our data indicate that this is because these constructs did not carry the major transcription initiation sites or the promoter sequences necessary to position the transcription complex. In this regard, it is noteworthy that the major transcription start site for both mouse and human is positioned ~30 base pairs downstream of an AT-rich element that may serve as a TATA box. This element in the *p27*^{Kip1} gene (TTTAAT) matches the consensus TATA box sequence (TATAA/TAA/T) at 6 out of 7 positions.

It has been shown that expression of *p27*^{Kip1} is controlled at the translational level [3,26,29] and that the *p27*^{Kip1} 5'-*UTR* is able to mediate enhanced translation rates [31]. In addition, it was recently reported that the *p27*^{Kip1} 5'-*UTR* has an internal ribosome entry site that allows cap-independent initiation of translation [30]. However, none of these reports have utilized the full 5'-*UTR* that results from transcription initiation at the major start site. The longest fragments of the *p27*^{Kip1} 5'-*UTR* that have been analyzed include only the first 153 nucleotides upstream of the AUG for human [31] and 217 nucleotides for mouse [30]. It is very likely that the additional ~300 nucleotides at the 5' end of the major *p27*^{Kip1} transcript,

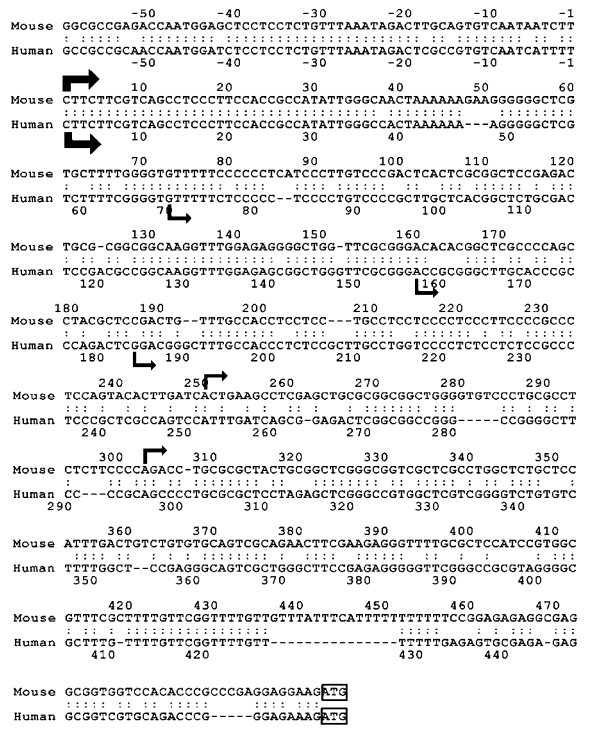

Figure 5

Alignment of the mouse and human *p27*^{Kip1} gene sequences. Bold arrows indicate the major transcription start sites. Minor start sites detected by RLM-RACE are indicated with small arrows. The genes are numbered with the major transcription start site as +1. A colon between the human and mouse sequences indicates identical nucleotides. Gaps in the alignment are indicated by dashes. ATG translation start codons are boxed. The sequence alignment was performed using the ALIGN program at the Biology WorkBench web site.

as reported here, will play an important role in regulating p27^{Kip1} expression. It will therefore be necessary to re-examine translational control of *p27*^{Kip1} in the context of its full 5'-*UTR*.

The data presented here show that, although there is a single major site, there are also alternative transcription initiation sites for both mouse and human *p27*^{Kip1}. This adds a new dimension to studies on *p27*^{Kip1} expression. One interesting possibility is that different start sites may be used at different points in the cell cycle or during cellular differentiation. It is known that *p27*^{Kip1} mRNA levels stay relatively steady throughout the cell cycle. However, usage of various transcription start sites could be regulated differently. This might affect the length of the 5'-*UTR* and thus translation of the *p27*^{Kip1} mRNA. Additional studies will need to be done to determine if and when different transcription start sites are used.

The region of the *p27*^{Kip1} gene upstream of the ATG start codon is highly conserved in mouse and human (Fig. 5). This includes the region encoding the 5'-*UTR* and the promoter region. The high conservation between the mouse and human genes extends at least an additional 300 nucleotides upstream of the sequences shown in Fig. 5 [35]. Our data indicate that the major transcription start site is at exactly the same sequence in both human and mouse (Fig. 5, large arrows). Based on these new results, we propose usage of the gene numbering system shown in Fig. 5. For mouse +1 is 502 nucleotides upstream of the ATG and for human +1 is 472 nucleotides upstream of the ATG. This information should be useful in designing experiments to analyze both transcriptional and translation control of p27^{Kip1} expression.

Materials and Methods

Cell culture and RNA extraction

Human breast cancer cell lines MCF7 and BT20, human fetal lung cell line MRC5, and the mouse NIH 3T3 cell line were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Total RNA was extracted using TRI Reagent (Molecular Research, Cincinnati, OH) and stored in ethanol at -70°C.

5' RLM-RACE

5' RLM-RACE [37–39] was performed using the GeneRacer kit (Invitrogen, Carlsbad, CA). In short, the total RNA was dephosphorylated using calf intestinal phosphatase then decapped using tobacco acid pyrophosphatase to target full-length messenger RNAs. An RNA oligonucleotide was then ligated to the full-length, decapped mRNAs and reverse transcription was performed using random primers. PCR was done to amplify the resultant cDNAs using the GeneRacer 5' primer and a primer consisting of bases immediately upstream of the translation start site of the *p27*^{Kip1} gene (CTTTCTCCCG-GGTCTGCACGACCG for human and CTTCCTCCTCG-GGCGGGTGT for mouse). Nested PCR was then done to eliminate the possibility of artifacts using the GeneRacer 5' nested primer and *p27*^{Kip1} primers CACGAC-CGCCTCTCTCGCACTCTC for human and GGACCAC-CGCCTCGCCTCTC for mouse (see Figs. 1 and 2).

Cloning and sequencing

The RACE nested PCR products were cloned into the pCR4Blunt-TOPO vector using a TOPO-cloning kit (Invitrogen). DNA obtained from the resultant colonies was sequenced with the USB Sequenase version 2.0 DNA Sequencing kit (Amersham, Cleveland, OH) using T3 and T7 primers.

Ribonuclease protection assay

For the ribonuclease protection assays, probes were made using the MAXIscript kit (Ambion, Austin, TX), ^{32}P-CTP, and T7 RNA polymerase. DNA from the RACE nested PCR clone containing the human $p27^{Kip1}$ sequence from -472 to -17 or the mouse $p27^{Kip1}$ sequence from -502 to -17 was linearized with Pme I and used as a template for probe synthesis. Numbering of these DNA sequences was assigned using +1 as the translation start site. After gel purification of the probe, the assay was carried out using the HybSpeed RPA kit (Ambion). Total RNA was co-precipitated with the labeled probe followed by denaturation and hybridization. Following digestion with RNase A/T1, the protected RNAs were precipitated and run on a 4% denaturing polyacrylamide gel and detected by autoradiography.

Acknowledgement

This work was supported by NIH grants NS36164 and CA84325.

References

1. Toyoshima H, Hunter T: **p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21.** *Cell* 1994, **78**:67-74
2. Kato JY, Matsuoka M, Polyak K, Massague J, Sherr CJ: **Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation.** *Cell* 1994, **79**:487-96
3. Agrawal D, Hauser P, McPherson F, Dong F, Garcia A, Pledger WJ: **Repression of p27kip1 synthesis by platelet-derived growth factor in BALB/c 3T3 cells.** *Mol Cell Biol* 1996, **16**:4327-36
4. Nourse J, Firpo E, Flanagan WM, Coats S, Polyak K, Lee MH, Massague J, Crabtree GR, Roberts JM: **Interleukin-2-mediated elimination of the p27Kip1 cyclin-dependent kinase inhibitor prevented by rapamycin.** *Nature* 1994, **372**:570-3
5. Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY: **Mice lacking p27Kip1 display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors.** *Cell* 1996, **85**:707-20
6. Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A: **Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27Kip1.** *Cell* 1996, **85**:721-32
7. Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K, Roberts JM: **A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27Kip1-deficient mice.** *Cell* 1996, **85**:733-44
8. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo EJ, Daling JR, Roberts JM: **Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients.** *Nat Med* 1997, **3**:222-5
9. Yang RM, Naitoh J, Murphy M, Wang HJ, Phillipson J, deKernion JB, Loda M, Reiter RE: **Low p27 expression predicts poor disease-free survival in patients with prostate cancer.** *J Urol* 1998, **159**:941-5
10. Thomas GV, Szigeti K, Murphy M, Draetta G, Pagano M, Loda M: **Down-regulation of p27 is associated with development of colorectal adenocarcinoma metastases.** *Am J Pathol* 1998, **153**:681-7
11. Ponce-Castaneda MV, Lee MH, Latres E, Polyak K, Lacombe L, Montgomery K, Mathew S, Krauter K, Sheinfeld J, Massague J: **p27Kip1: chromosomal mapping to 12p12-12p13.1 and absence of mutations in human tumors.** *Cancer Res* 1995, **55**:1211-4
12. Poluha W, Poluha DK, Chang B, Crosbie NE, Schonhoff CM, Kilpatrick DL, Ross AH: **The cyclin-dependent kinase inhibitor p21WAF1 is required for survival of differentiating neuroblastoma cells.** *Mol Cell Biol* 1996, **16**:1335-41
13. Inoue T, Kamiyama J, Sakai T: **Sp1 and NF-Y synergistically mediate the effect of vitamin D3 in the p27Kip1 gene promoter that lacks vitamin D response elements.** *J Biol* 1999, **274**:32309-17
14. Liu M, Lee MH, Cohen M, Bommakanti M, Freedman LP: **Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937.** *Genes Dev* 1996, **10**:142-53
15. Moro A, Santos A, Arana MJ, Perea SE: **Activation of the human p27Kip1 promoter by IFNalpha 2b.** *Biochem Biophys Res Commun* 2000, **269**:31-4
16. Kortylewski M, Heinrich PC, Mackiewicz A, Schniertshauer U, Klingmuller U, Nakajima K, Hirano T, Horn F, Behrmann I: **Interleukin-6 and oncostatin M-induced growth inhibition of human A375 melanoma cells is STAT-dependent and involves upregulation of the cyclin-dependent kinase inhibitor p27/Kip1.** *Oncogene* 1999, **18**:3742-53
17. de Koning JP, Soede-Bobok AA, Ward AC, Schelen AM, Antonissen C, van Leeuwen D, Lowenberg B, Touw IP: **STAT3-mediated differentiation and survival of myeloid cells in response to granulocyte colony-stimulating factor: role for the cyclin-dependent kinase inhibitor p27Kip1.** *Oncogene* 2000, **19**:3290-8
18. Gardner LB, Li Q, Park MS, Flanagan WM, Semenza GL, Dang CV: **Hypoxia inhibits G1/S transition through regulation of p27 expression.** *J Biol Chem* 2001, **276**:7919-26
19. Kolluri SK, Weiss C, Koff A, Gottlicher M: **p27Kip1 induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells.** *Genes Dev* 1999, **13**:1742-53
20. Servant MJ, Coulombe P, Turgeon B, Meloche S: **Differential regulation of p27Kip1 expression by mitogenic and hypertrophic factors: Involvement of transcriptional and posttranscriptional mechanisms.** *J Cell Biol* 2000, **148**:543-56
21. Yang W, Shen J, Wu M, Arsura M, FitzGerald M, Suldan Z, Kim DW, Hofmann CS, Pianetti S, Romieu-Mourez R, Freedman LP, Sonenshein GE: **Repression of transcription of the p27Kip1 cyclin-dependent kinase inhibitor gene by c-Myc.** *Oncogene* 2001, **20**:1688-702
22. Medema RH, Kops GJ, Bos JL, Burgering BM: **AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1.** *Nature* 2000, **404**:782-7
23. Collado M, Medema RH, Garcia-Cao I, Dubuisson ML, Barradas M, Glassford J, Rivas C, Burgering BM, Serrano M, Lam EW: **Inhibition of the phosphoinositide 3-kinase pathway induces a senescence-like arrest mediated by p27Kip1.** *J Biol Chem* 2000, **275**:21960-8
24. Nakamura N, Ramaswamy S, Vazquez F, Signoretti S, Loda M, Sellers WR: **Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN.** *Mol Cell Biol* 2000, **20**:8969-82
25. Dijkers PF, Medema RH, Pals C, Banerji L, Thomas NS, Lam EW, Burgering BM, Raaijmakers JA, Lammers JW, Koenderman L, Coffer PJ: **Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27Kip1.** *Mol Cell Biol* 2000, **20**:9138-48
26. Hengst L, Reed SI: **Translational control of p27Kip1 accumulation during the cell cycle.** *Science* 1996, **271**:1861-4
27. Pagano M, Tam SW, Theodoras AM, Beer-Romero P, Del Sal G, Chau V, Yew PR, Draetta GF, Rolfe M: **Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27.** *Science* 1995, **269**:682-5
28. Reynisdottir I, Massague J: **The subcellular locations of p15InK4b and p27Kip1 coordinate their inhibitory interactions with cdk4 and cdk2.** *Genes Dev* 1997, **11**:492-503
29. Millard SS, Yan JS, Nguyen H, Pagano M, Kiyokawa H, Koff A: **Enhanced ribosomal association of p27Kip1 mRNA is a mechanism contributing to accumulation during growth arrest.** *J Biol Chem* 1997, **272**:7093-8
30. Miskimins WK, Wang G, Hawkinson M, Miskimins R: **Control of cyclin-dependent kinase inhibitor p27 expression by cap-independent translation.** *Mol Cell Biol* 2001, **21**:4960-4967
31. Millard SS, Vidal A, Markus M, Koff A: **A U-rich element in the 5' untranslated region is necessary for the translation of p27 mRNA.** *Mol Cell Biol* 2000, **20**:5947-59
32. Zhao Z, Chang FC, Furneaux HM: **The identification of an endonuclease that cleaves within an HuR binding site in mRNA.** *Nucleic Acids Res* 2000, **28**:2695-701

33. Kwon TK, Nagel JE, Buchholz MA, Nordin AA: **Characterization of the murine cyclin-dependent kinase inhibitor gene p27Kip1.** *Gene* 1996, **180**:113-20
34. Zhang Y, Lin SC: **Molecular characterization of the cyclin-dependent kinase inhibitor p27 promoter.** *Biochim Biophys Acta* 1997, **1353**:307-17
35. Minami S, Ohtani-Fujita N, Igata E, Tamaki T, Sakai T: **Molecular cloning and characterization of the human p27Kip1 gene promoter.** *FEBS Lett* 1997, **411**:1-6
36. Ito E, Iwahashi Y, Yanagisawa Y, Suzuki Y, Sugano S, Yuasa Y, Maruyama K: **Two short sequences have positive effects on the human p27Kip1 gene transcription.** *Gene* 1999, **228**:93-100
37. Maruyama K, Sugano S: **Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides.** *Gene* 1994, **138**:171-4
38. Volloch V, Schweitzer B, Rits S: **Ligation-mediated amplification of RNA from murine erythroid cells reveals a novel class of beta globin mRNA with an extended 5'-untranslated region.** *Nucleic Acids Res* 1994, **22**:2507-11
39. Schaefer BC: **Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends** *Anal Biochem* 1995, **227**:255-73