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Research Article

Alternative splicing facilitates internal ribosome entry on the ornithine decarboxylase mRNA

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Abstract. Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the biosynthesis of polyamines, which are required for optimal cell growth and proliferation. ODC is overexpressed in many tumors and, conversely, its overexpression induces transformation. We have previously reported that ODC mRNA alternative splicing relieves the translation repression normally imposed by a long and structured 5′ untranslated region (UTR), and that the ODC 5′ UTR contains an internal ribosome entry site (IRES). Here we show that ODC IRES activity is enhanced following inclusion of alternative sequences generated by splicing at cryptic acceptor sites. Furthermore, the alternative ODC IRES is more sensitive to cell-cycle-dependent changes in the rate of translation. These findings uncover a new biological property of differentially spliced transcripts. This is the first example of alternative splicing that modulates mRNA translation through the cell cycle in a cap-independent manner.

Key words. Ornithine decarboxylase; translation; cap; internal ribosome entry site; alternative splicing; cell cycle.

Introduction

All nuclear-encoded eukaryotic mRNAs are modified at their 5′ end with a cap structure, which is recognized by the eukaryotic initiation factor (eIF) 4F [reviewed in refs 1, 2]. eIF4F is composed of eIF4E (the cap-binding protein), eIF4A (an RNA helicase) and eIF4G (which bridges eIF4E and eIF4A). Two functional homologues of eIF4G, termed eIF4GI and eIF4GII, have been cloned [3]. eIF4G also interacts with the 40S ribosomal subunit via eIF3, another initiation factor. Thus, eIF4F brings both the eIF4A RNA helicase and the ribosome to the mRNA 5′ cap [1, 2]. eIF4A is thought to unwind the secondary structure in the 5′ untranslated region (UTR), thereby facilitating ribosome binding to the mRNA 5′ end [1, 2]. The 40S ribosomal subunit then scans from the 5′ cap downstream to the initiation codon. Translation of some cellular mRNAs is very inefficient because they possess a long and structured 5′ UTR that inhibits both ribosome binding to the mRNA 5′ end and scanning downstream to the initiation codon. However, a subset of mRNAs possessing a long and structured 5′ UTR, including ODC mRNA [4, 5], is translated independently of the cap, by recruitment of the 40S ribosomal subunit to an internal ribosome entry site (IRES). IRESs were first identified and characterized in picornavirus RNAs, which do not possess a 5′ cap structure [6, 7]. During poliovirus infection, eIF4G is proteolytically cleaved, rendering the eIF4F complex inactive for cap-dependent translation, yet functional for IRES-dependent ribosome binding [8, 9].

Ornithine decarboxylase (ODC) catalyzes the transformation of ornithine into putrescine, a rate-limiting step in the biosynthesis of polyamines (putrescine, spermidine and spermine). Polyamines are small polycations that

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exert multiple cellular functions [reviewed in ref. 10], such as DNA replication in S phase, chromosome condensation and mitotic spindle organization in mitosis. Accordingly, inappropriate accumulation of polyamines following ODC overexpression leads to transformation and, conversely, inhibition of ODC provokes cytostasis. Among the elaborated mechanisms that cells have evolved to maintain an optimal polyamine content, the control of ODC protein synthesis is a striking feature. It enables cells to manipulate polyamine production rapidly and transiently without de novo ODC mRNA synthesis, processing, or nucleocytoplasmic export. For example, ODC expression is transiently regulated through the cell cycle at the level of translation initiation. Indeed, ODC mRNA possesses a long and structured 5′ UTR, which can be divided into two segments (fig. 1A) that function autonomously to control translation [4]. The cap-proximal segment, composed of a stable hairpin structure followed by a short upstream open reading frame (uORF), functions as a repressor of cap-dependent translation. The downstream segment, located just upstream from the initiation codon, contains an IRES and functions as a translation enhancer in a cell-cycle-dependent manner.

We have previously reported that the ODC 5′ UTR is alternatively spliced in pancreatic tumor cells [11]. Inclusion of alternative sequences was shown to relieve the translation repression normally imposed in non transformed cells by the long and structured 5′ UTR. However, the molecular mechanism responsible for such a derepression of translation was not elucidated. Here we show that inclusion of alternative sequences in the ODC mRNA 5′ UTR facilitates cap-independent, IRES-mediated translation initiation.

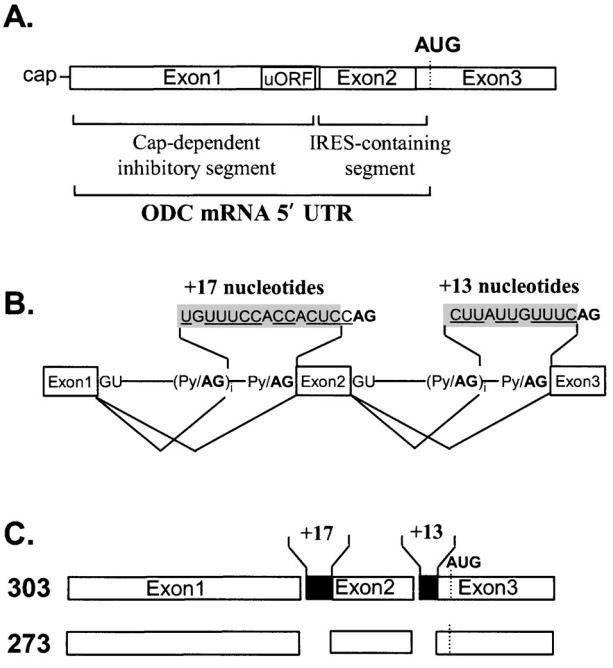

Figure 1. The ODC mRNA 5′ UTR and its spliced variants. (*A*) Translation regulatory elements. The cap proximal half of the ODC 5′ UTR is composed of a stable stem-loop structure followed by a short upstream open reading frame, which inhibit cap-dependent translation. The downstream less structured segment contains an IRES. (*B*) Intron 1 and intron 2 in the immature ODC mRNA both possess internal splicing acceptor sites (Py/AG_i), located 17 and 13 nucleotides upstream from the bona fide splicing acceptor site (Py/AG), respectively. (*C*) In pancreatic tumoral cells, the internal splicing acceptor sites are used to generate a 5′ UTR extended by 17 and 13 nucleotides at the exon 2 and exon 3 5′ borders, respectively. The resulting alternatively spliced 5′ UTR is 30 nucleotides longer (variant 303) than the wild-type 5′ UTR, which encompasses 273 nucleotides in rat.

Materials and methods

Plasmid construction and in vitro transcription and translation

$pcDNA_3$ plasmids containing the rat ODC 5′ UTRs (all spliced forms) upstream from CAT- or LUC-coding ORFs to generate monocistronic mRNAs, or inserted between CAT and LUC ORFs to generate bicistronic mRNAs, were described previously [4, 11]. The $pcDNA_3$-CAT-EMCV-LUC vector was also described previously [4].

To obtain cap-methylated mRNAs, linearized plasmids were transcribed with T7 RNA polymerase (Promega) for 2 h at 37 °C in the presence (tenfold molar excess relative to GTP) of m^7GpppG. mRNAs were then phenol-chloroform extracted, purified by gel filtration on NICK columns (Pharmacia) and ethanol precipitated. mRNA integrity was examined by formaldehyde-agarose gel electrophoresis and ethidium bromide staining. For in vitro translation, 12.5 µl of rabbit reticulocyte lysate (RRL; Promega) was programmed with 75 ng of mRNA at 30 °C for 90 min in the presence of [^{35}S]-methionine (20 µCi). Where indicated, RRL was pretreated with rhinovirus 2A protease ($2A^{PRO}$; a kind gift of Dr. T. Skern) for 5 min at 30 °C and then incubated for 10 min on ice in the presence of 0.7 mM elastatinal (Sigma), to inhibit the protease. Translation products were separated by SDS-12% PAGE and the gels were processed for fluorography using En3Hance (Dupont) followed by autoradiography and quantification of the products using a BAS-2000 PhosphorImager (Fuji).

Antibodies, Western blotting and far-Western analysis

The following antibodies were used: mouse anti-eIF4E [12], rabbit anti-eIF4GII [3] and rabbit anti-CAT [4]. Rabbit anti-eIF4GI antibodies were a kind gift of Dr L. Carrasco. To analyze proteins in RRL, 25 µg of total protein diluted in Laemmli sample buffer was separated by SDS-12% (eIF4E) or SDS-8% (eIF4Gs and far-Western) PAGE. Western blotting was then performed as described elsewhere [13].

For far-Western (filter-overlay assay) analysis, a triple fusion protein containing the heart muscle kinase (HMK) phosphorylation site and Flag-tagged eIF4E (HMK-Flag-eIF4E) was expressed in *Escherichia coli* BL 21 cells and purified using a Flag column (Sigma). HMK-Flag-eIF4E was phosphorylated using [γ-^{32}P]-ATP (Pharmacia) and bovine HMK (Sigma). Following SDS-10% PAGE and transfer, membranes were incubated with [^{32}P]-labeled HMK-Flag-eIF4E, as described before [14]. Interacting proteins were visualized by autoradiography.

Cell culture, transfection and synchronization

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum. Transient transfections were performed using lipofectamine (GIBCO-BRL), as previously described [13]. Cells were synchronized at the G1/S boundary by sequential thymidine (Sigma) and aphidicolin (Sigma) treatment as previously described [4]. Synchronization was monitored by flow cytometry analysis of cellular DNA stained with propidium iodide.

CAT and LUC assays

The CAT assay was performed as previously described [4]. Acetylated and non-acetylated forms of [^{14}C]-chloramphenicol (Amersham) were separated on thin-layer chromatography plates, which were autoradiographed. Results were quantified using a BAS-2000 PhosphorImager (Fuji). The LUC assay was performed with a luciferase assay system (Promega) and quantified using a luminometer (Bioorbit). CAT synthesis was determined by measuring the incorporation of [^{35}S]-methionine into the protein using a protein A-agarose-coupled anti-CAT antibody. Immunoprecipitated CAT was subjected to SDS-PAGE and transferred onto nitrocellulose membranes. Results were quantified using a BAS-2000 PhosphorImager (Fuji).

Results

Alternative splicing in the ODC mRNA 5′ UTR

In mammals, the ODC initiation codon lies in the third exon. Consequently, the 5′ UTR is composed of the first two exons and part of the third (fig. 1A). Intron 1 and intron 2 contain alternative internal splicing acceptor sites (fig. 1B; Py/ag$_i$). In pancreatic tumor cells, these alternative acceptor sites are used, and exon 2 and exon 3 in the mature mRNA are extended by 17 and 13 nucleotides, respectively [11]. The resulting alternative 5′ UTR is thus lengthened by 30 nucleotides (fig. 1C, variant 303), as compared to the wild-type 5′ UTR, which is composed of 273 nucleotides in rat. Surprisingly, translation driven by the alternative 5′ UTR was shown to be enhanced both in vitro and in transfected cells [11]. This result was counterintuitive since, according to the scanning of ribosome from the 5′ cap to the initiation codon, a longer 5′ UTR was expected to decrease the efficiency of translation initiation. However, following alternative splicing, the additional sequences are included in the IRES, downstream from the cap-proximal inhibitory segment, suggesting that they may modulate IRES-mediated, instead of cap-dependent, translation initiation.

Induction of IRES-mediated translation by alternative splicing

To examine the role of the alternative segments in IRES-mediated translation, monocistronic and bicistronic mRNAs containing the wild-type ODC 5′ UTR or its alternative form (fig. 2) were translated in RRL untreated or treated with 2A^{PRO}. Treatment with 2A^{PRO} results in the cleavage of eIF4G. The amino-terminal fragments of cleaved eIF4Gs associate with eIF4E but are inactive in cap-dependent translation initiation. The carboxy-terminal fragments, which contain the binding sites for eIF4A and eIF3 are, however, sufficient for IRES-mediated translation initiation [15, 16].

To verify the efficacy and specificity of 2A^{PRO} treatment, the integrity of eIF4E and eIF4Gs was analyzed prior and after treatment with the protease. While eIF4E remained unchanged (fig. 3A, compare lane 2 to 1), both eIF4GI (compare lane 4 to 3) and eIF4GII (compare lane 6 to 5) were cleaved following 2A^{PRO} treatment, as shown by Western blotting using antibodies specific to eIF4E and to the amino-terminal fragments (4GsN) of eIF4GI or eIF4GII. Furthermore, a far-Western analysis using ^{32}P-HMK-eIF4E as a probe showed that the amino-terminal fragments of eIF4Gs retain their capability to interact with eIF4E (compare lane 8 to 7).

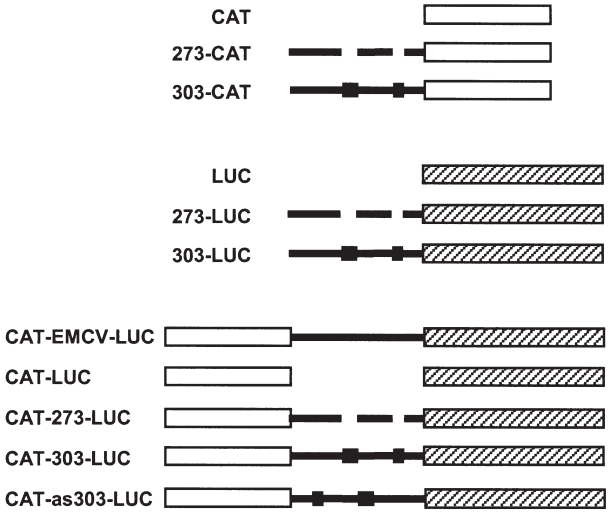

Figure 2. ODC 5′ UTR monocistronic (top) and bicistronic (bottom) constructs. The open and hatched boxes represent the CAT and LUC sequences, respectively. The thick lines represent different 5′ UTRs.

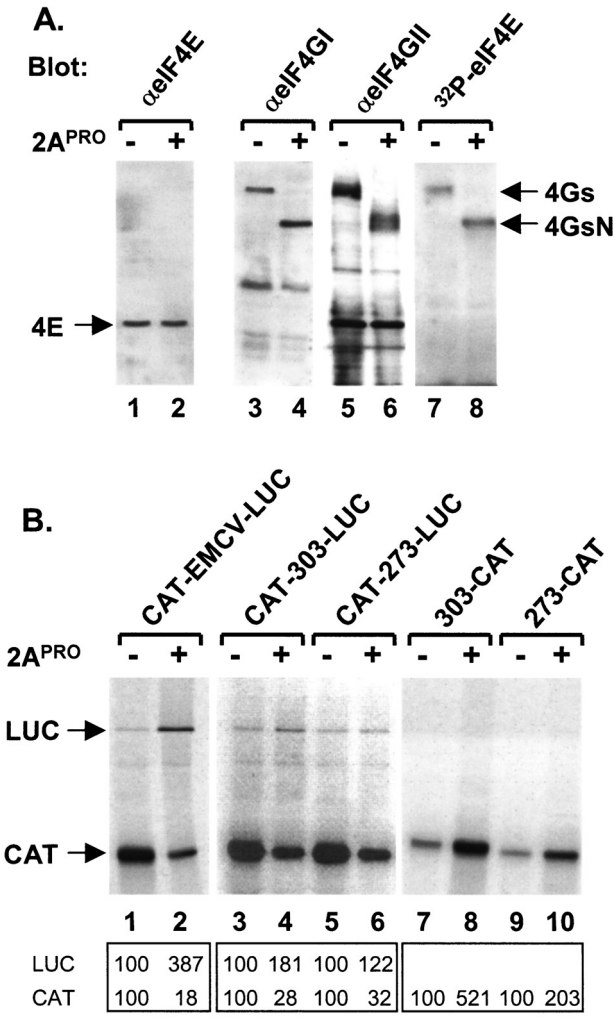

Figure 3. Induction of in vitro IRES-mediated translation by alternative splicing. (*A*) eIF4G cleavage in $2A^{PRO}$-treated RRL. Following $2A^{PRO}$ treatment, the RRL was analyzed by immunoblotting using specific antibodies indicated at the top of the figure (lanes 1–6), or by far-Western using ^{32}P-HMK-Flag-eIF4E as a probe (lanes 7 and 8), as described in Materials and methods. (*B*) Mono- or bicistronic capped mRNAs were translated in control or $2A^{PRO}$-treated RRL, as described in Materials and methods. Results are expressed as percentage of the value obtained for each mRNA in $2A^{PRO}$-untreated RRL.

To ensure that eIF4G cleavage did reduce cap-dependent translation, but enhanced IRES-mediated translation, a bicistronic mRNA possessing the encephalomyocarditis virus (EMCV) IRES inserted between CAT and LUC ORFs (CAT-EMCV-LUC) was tested. As predicted, $2A^{PRO}$ treatment inhibited cap-dependent translation of CAT (about sixfold), but in contrast induced EMCV IRES-mediated translation of LUC (about fourfold; fig. 3B, compare lane 2 to lane 1), thus validating the treatment of RRL with $2A^{PRO}$ to analyze cap- versus IRES-dependent translation. Translation of bicistronic reporter mRNAs harboring either the wild type (CAT-273-LUC) or the alternative ODC 5′ UTR (CAT-303-LUC) was then tested. IRES activity of the wild-type ODC 5′ UTR was slightly enhanced following $2A^{PRO}$ treatment, whereas cap-dependent translation of CAT on the same mRNA was inhibited (compare lane 6 to lane 5). Interestingly, enhancement of translation was more pronounced when the alternative 5′ UTR was tested (compare lane 4 to lane 3).

The modest induction observed for ODC IRES-mediated translation following $2A^{PRO}$ treatment could result from the use of bicistronic constructs, a context that can alter the proper function of certain IRESs [17]. We therefore tested ODC IRES activity in a monocistronic context. Translation of a monocistronic mRNA containing the wild-type ODC 5′ UTR (273-CAT) was enhanced (about twofold) in $2A^{PRO}$-treated RRL (fig. 3B, compare lane 10 to lane 9), while the enhancement of translation driven by the alternative form (303-CAT) was significantly more pronounced (about fivefold; compare lane 8 to lane 7). Similar results were obtained with the LUC ORF, indicating that effects are independent of downstream sequences (data not shown).

Thus, inclusion of alternative sequences in the ODC 5′ UTR enhances IRES activity in vitro.

Induction of IRES-mediated translation by alternative splicing in transfected and synchronized cells

To examine whether the alternative ODC mRNA 5′ UTR also facilitates IRES-mediated translation in vivo, HeLa cells were transfected with bicistronic constructs, and CAT and LUC activities were assayed 36 h following transfection. While cap-dependent translation of CAT remained unchanged, IRES-mediated expression of LUC was stimulated to a higher extent (~3-fold) when the alternative 5′ UTR was inserted in the intercistronic space (fig. 4A, compare CAT-303-LUC to CAT-LUC), as compared to the wild-type 5′ UTR (~1.7-fold, compare CAT-273-LUC to CAT-LUC). This induction was sequence specific, as translation of LUC from a bicistronic mRNA containing the ODC 5′ UTR in the antisense orientation was not stimulated but rather inhibited (compare CAT-as303-LUC to CAT-LUC).

As observed in vitro, the modest induction of ODC IRES activity measured in transfected cells could result from the use of bicistronic constructs (see above). Also, we utilized an asynchronous population of cells, which is inappropriate since we and others have shown that the rate of ODC synthesis is cell cycle dependent see [e.g. ref. 4]. Important, therefore, was to test whether the extra fragments included in the ODC 5′ UTR were the target of a cell-cycle-dependent translational control in a monocistronic context. To this end, translation of mRNAs containing the ODC 5′ UTR fused to the CAT ORF was examined at different times following release from a sequential thymidine and aphidicolin block, which arrests cells at the G1/S boundary. Cell cycle progression was monitored by flow cytometry of propidium-iodide-stained

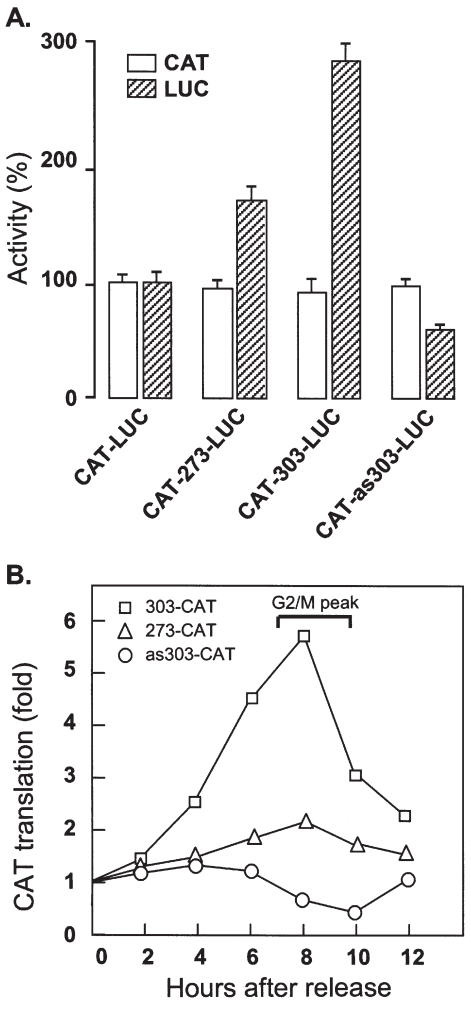

Figure 4. (*A*) Induction of IRES-mediated translation by alternative splicing in transfected HeLa cells. Bicistronic constructs were transfected in HeLa cells, and CAT and LUC activities were assayed as described in Materials and methods. Results are expressed as a ratio relative to the activity measured for the CAT-LUC construct containing no 5′ UTR, and are the mean + SE of two independent experiments performed in triplicate. (*B*) Cell-cycle-dependent IRES activity. HeLa cells were transfected with monocistronic CAT constructs and synchronized at the G1/S boundary. CAT synthesis was measured at various times after release from the aphidicolin block, as described in Materials and methods. CAT synthesis was quantified using a PhosphorImager and is expressed as a ratio relative to the value obtained at time 0. Results are representative of three different experiments.

cells (not shown). Following release, cells rapidly entered S phase, were predominantly in G2/M after 8–10 h and subsequently in G1. Translation of the 303-CAT mRNA, which possesses the alternative fragments, peaked in G2/M (fig. 4B), while translation of the 273-CAT mRNA (lacking the alternative fragments) was less markedly increased. Translation of mRNAs possessing the entire alternative ODC 5′ UTR but placed in the antisense orientation (as303-CAT) was in contrast inhibited during G2/M.

Thus, these data indicate that inclusion of extra sequences in the ODC mRNA 5′ UTR facilitates cell-cycle-dependent, IRES-mediated translation.

The 57-kDa polypyrimidine-tract-binding protein does not enhance ODC IRES activity in vitro

The induction of IRES activity measured following inclusion of the alternative pyrimidine-rich extra sequences could be mediated by trans-acting factors that specifically bind UC-rich stretches. In an attempt to characterize RNA-binding proteins that could be involved in the 5′ UTR-dependent regulation of ODC translation, Manzella and Blackshear [18] detected a 58-kDa protein [18], which bound preferentially to a UC-rich stretch in the ODC mRNA IRES. One proposed candidate was the 57-kDa polypyrimidine-tract-binding-protein (PTB), known to be involved in splice acceptor site recognition [19], and to modulate the activity of certain IRESs [20]. We tested this possibility using recombinant PTB in untreated or $2A^{PRO}$-treated RRL. Unfortunately, the presence of PTB did not enhance but instead inhibited 303-CAT mRNA translation in both untreated (fig. 5, compare lane 2 to lane 1) and $2A^{PRO}$-treated RRL (compare lane 4 to lane 3). Translation of the 273-CAT mRNA was also attenuated (lanes 5–8), while translation of a CAT mRNA with no

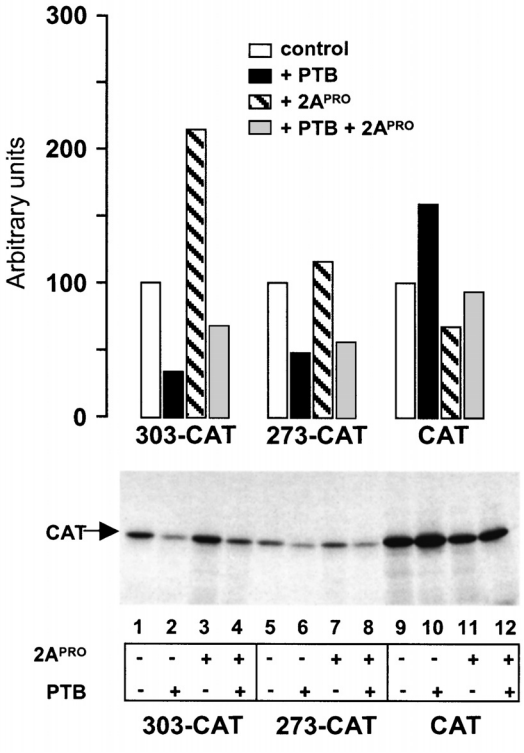

Figure 5. PTB does not enhance ODC IRES activity in vitro. Monocistronic capped mRNAs were translated in control or $2A^{PRO}$-treated RRL, in the presence or absence of recombinant PTB, as described in Materials and methods. Results are expressed as percentage of the value obtained for each mRNA in the absence of PTB and in $2A^{PRO}$-untreated RRL.

5′ UTR was in contrast slightly enhanced in the presence of PTB (lanes 9–12). Further analyses also revealed that, although PTB actually bound to the ODC IRES in vitro, the binding was not specific to alternative sequences (not shown). Thus, PTB is unlikely to be the trans-acting factor that facilitates IRES-driven translation of the ODC mRNA. Similar negative results were obtained using two other recombinant RNA-binding proteins, YB-1 and hnRNPA1 (not shown).

Discussion

In the ODC pre-mRNA, intron 1 and intron 2 contain cryptic splicing acceptor sites, which are used in pancreatic tumor cells. The use of these cryptic splicing acceptor sites is not restricted to pancreatic tumoral cells. For example, in a molecular-biological approach to investigate ODC gene regulation upon induction by tumor promoters, van Kranen et al. [21] isolated an ODC cDNA from a cDNA library of testosterone-induced rat kidney. Such a cDNA is spliced at both cryptic acceptor sites, further supporting the idea that alternative splicing in the ODC 5′ UTR may facilitate ODC expression.

In the alternatively spliced ODC mRNA, exon 2 and exon 3 are extended at their 5′ end by 17 and 13 nucleotides, respectively. These two short sequences are composed of a pyrimidine-rich stretch followed by an AG dinucleotide, which is used as a bona fide splice acceptor site in non-transformed cells: 5′-UGUUUCCACCACTCCAG-3′ and 5′-CUUAUUGUUUCAG-3′. They both contain a UGUUUC motif. In a previous work, we showed that mutation of the UGUUUC stretch contained in the 13-base insert into UGAAAC (Py1 mutant of the 303 variant [4]) dramatically decreased ODC IRES activity. The effect of such a mutation has not yet been evaluated in the 17-base insert. However, when tested independently, the 13-base insert and the 17-base insert enhance translation to a similar extent, suggesting that the UGUUUC motifs conserved in each insert play a similar role. The molecular mechanism by which UC-rich sequences stimulate IRES activity remains to be elucidated. Trans-acting factors other than the three RNA-binding proteins we tested (PTB, YB-1 and hnRNPA1) are likely to be involved. That PTB, YB-1 and hnRNPA1 inhibited the translation of IRES-containing mRNAs but enhanced translation of mRNAs with no 5′ UTR (fig. 5) is consistent with the idea that general RNA-binding proteins render translation cap dependent. Indeed, unless a protein that specifically activates IRES activity is used, one function of general mRNA-binding proteins has been proposed to be to promote ribosome loading by a 5′ end, cap mediated mechanism [22]. In mRNAs containing the ODC 5′ UTR, however, rendering translation cap dependent by the addition of RNA-binding proteins does not promote ribosome loading but instead inhibits translation (fig. 5). This apparent discrepancy may be due to the fact that the ODC mRNA 5′ UTR possesses a strong cap-dependent translation inhibitory sequence next to the 5′ cap. Thus, masking the IRES with unspecific RNA-binding proteins precludes IRES-mediated translation, and renders cap-dependent translation more sensitive to the cap-proximal inhibitory sequence. The translation of CAT mRNAs containing no ODC 5′ UTR, and hence no inhibitory sequence, is in contrast enhanced.

Polyamines are critical for cell cycle functions, such as DNA replication in S phase, chromosome condensation and mitotic spindle organization in mitosis. Accordingly, ODC expression varies through the cell cycle. We [4] and more recently Tinton et al. [23] have shown that the ODC IRES is the target of a cell-cycle-dependent translational control. This control is negatively exerted at the G1/S transition but functions positively in G2/M. In this paper we show that the ODC IRES generated following alternative splicing is more sensitive to cell-cycle-dependent changes. Thus, alternative fragments in the ODC IRES are likely specifically targeted by cell-cycle-dependent trans-acting factors. Possible candidates are the proteins that form the exon-exon junction complex (EJC), a multiprotein complex recruited to the mRNA during splicing [24, 25]. The EJC is highly dynamic, and several of its proteins shuttle between the nucleus and the cytoplasm [26, 27]. Also, certain members of the EJC are found attached to polysomes together with mRNAs [28, 29]. Thus, following alternative splicing, insertion of alternative fragments between exon-exon junctions impinges upon EJC assembly on mRNA and, consequently, affects mRNA translation.

Recent data indicate that IRES-mediated initiation of translation does not necessarily need the presence of trans-acting factors. For example, ribosomes can be loaded on the cricket paralysis virus (an insect picornavirus) IRES through direct interaction with complementary sequences found in the ribosome [30, 31]. Another possibility then is that inclusion of alternative sequences favors direct base-pairing between the ODC IRES and the ribosome, or provokes conformational changes that render the IRES more accessible for ribosome loading. This may be supported by the fact that picornavirus and ODC 5′ UTRs share a certain degree of sequence similarity, particularly in elements important for IRES function [4]. However, computer modeling of several picornavirus IRES elements has shown that a Y-type structure followed by a short stem-loop is also an important feature for IRES activity [32]. Such a structure could be detected neither in the wild-type ODC 5′ UTR nor in the alternative form (fig. 6), suggesting that the ODC IRES may use another strategy to attract the ribosome.However, the nature of secondary structures present in the ODC IRES is likely to play an important role. This is supported by data showing that

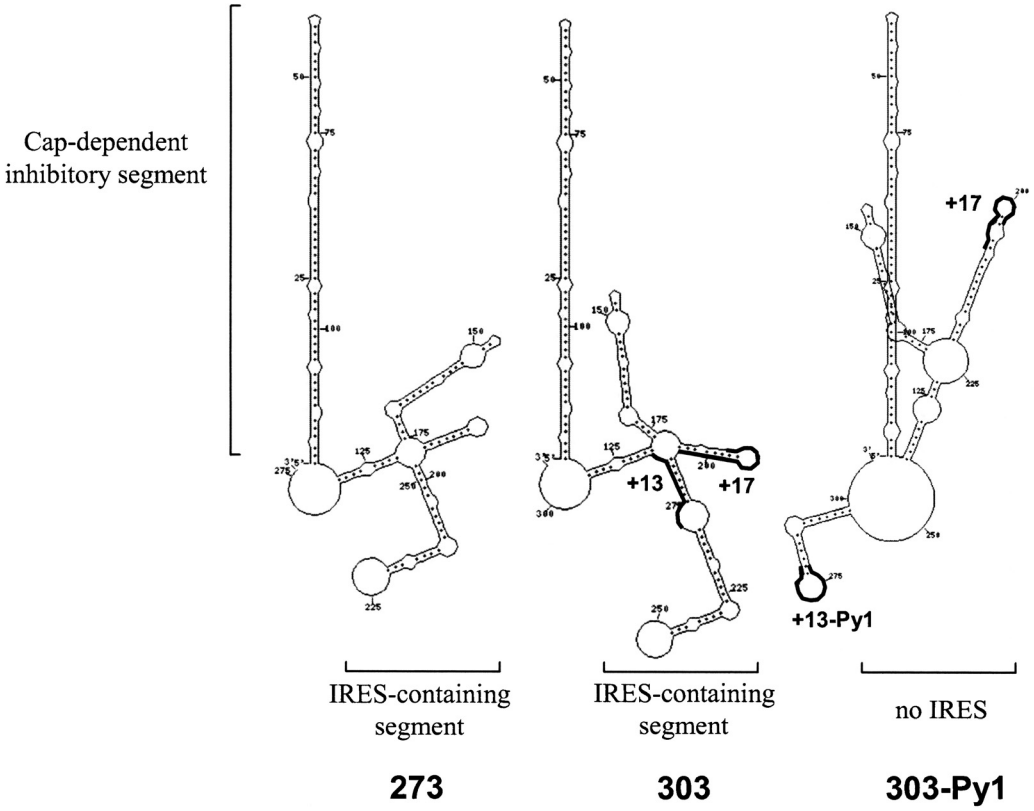

Figure 6. Secondary structure of ODC IRES variants (273 and 303) and that of the 303 variant harboring point mutations in the 13-base insert (303-Py1). RNA folding patterns of the ODC 5′ UTRs were predicted by the MFOLD algorithm of Zuker and Stiegler [35]. The two additional fragments (+17 and +13) are highlighted. In the three structures, the cap-proximal part folds into a unique very stable stem-loop structure known to block cap-dependent translation. The downstream segment forms three less stable stem-loops whose shape is globally conserved in the 273 and 303 variants, but destroyed in the 303 variant harboring a mutated 13-base insert.

point mutations in the 13-base insert reported previously [4] were more effective than deletion of the entire insert (this work) in preventing IRES activity. This apparent discrepancy can be actually explained by the observation that point mutations profoundly affect the IRES secondary structure, while deletion of the entire insert has moderate effects (fig. 6, compare the structures of the 273, 303 and 303-Py1 variants).

Alternative splicing in pancreatic carcinomas is not restricted to the ODC mRNA. For example, an abnormal splice form of the CCK-B receptor has also been detected [33, 34]. This tumor-specific splice variant is of particular interest because it emanates from the presence of a suboptimal acceptor site at the 3′ end of CCK-B intron 4 [35], as is the case for intron 1 and intron 2 in the ODC mRNA (our study). The CCK-B mRNA splice variant was shown to generate an aberrant protein, which exhibits constitutive activity and consequent mitogenic effects [33, 34]. The possibility that a unique alteration in the molecular mechanism of splicing targets both ODC and CCK-B mRNAs processing is intriguing and suggests that other mRNAs may be affected in pancreatic cancer.

Thus, in this study, we showed that the use of cryptic acceptor splice sites in the ODC mRNA generates a longer 5′ UTR which facilitates cell-cycle-dependent, IRES-mediated ODC production. The resulting accumulation of ODC protein may contribute to the transformation of pancreatic cells.

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