Expression of axin2 Is Regulated by the Alternative 5'-Untranslated Regions of Its mRNA*

Thomas A. Hughes‡ and Hugh J. M. Brady
From the Molecular Haematology and Cancer Biology Unit, Camelia Botnar Laboratories, Institute of Child Health,
30 Guilford St., London WC1N 1EH, United Kingdom

Axin2 is a negative regulator of Wnt/β-catenin signaling with roles in early development and tumor suppression. We find that axin2 expression is regulated at both transcriptional and translational levels. The gene allows transcription of mRNAs with three alternative 5'-untranslated regions, and these are differentially expressed in various human cell types. These untranslated regions can differentially determine protein expression from messages by influencing mRNA stability and translational efficiency. We identify short upstream reading frames and structural motifs that are responsible for modulation of mRNA translational efficiencies. We show that the proportions of axin2 message expressing each 5'-untranslated region influence the amount of Axin2 protein expressed within cells. We discuss this complex regulation in the context of the function of Axin2 as a tumor suppressor.

Control of gene expression by elements within untranslated regions (UTRs) of mRNAs is a relatively little-studied regulatory mechanism despite recent evidence of its importance in both development (1, 2) and disease (3, 4). UTRs can influence the amount of protein produced from messages by altering message stability, localization, or translational efficiency (5). Within 5'-UTRs, the presence of stable secondary structures, binding sites for trans-acting factors or short open reading frames (ORFs) upstream of the main coding sequence can have a strong influence on cap-dependent translation (6). Some of these elements are fairly common, for example over 10% of human 5'-UTRs contain upstream ORFs (5, 6). Despite this, published studies that reveal regulation of human gene expression by these elements, as opposed to continuous activation or repression, remain rare.

Here, we have studied the regulation of human axin2. Axin2 is a negative regulator of Wnt/β-catenin signaling (7–9) and acts as a tumor suppressor by limiting the deregulation of Wnt signaling that is common in some cancers (8). Axin2 can be up-regulated by E2F1 allowing cross-talk between the pRb/E2F and Wnt/β-catenin pathways (9); two pathways that have critical roles in the development of many cancers (10, 11). Axin2 also has a crucial role in segmentation of the vertebrate embryo (12) and may regulate neuronal cell differentiation (13).

We have identified three previously unknown exons at the start of the human axin2 gene and show that these code for alternative 5'-UTRs for the axin2 message. These UTRs are expressed differentially in various cell types and, crucially, each can modulate gene expression to a different degree by influencing both message stability and translational efficiency. Therefore, expression of Axin2 protein depends not only on the total amount of axin2 mRNA but also on what proportions of the three different 5'-UTRs are present. Moreover, the effects of these UTRs on protein expression can be adapted by other cellular factors and consequently expression of axin2 is further modulated by the cellular context. This intricate regulation of expression may represent a level of control that is applicable to many other genes.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—MCF7 and HEK-293T cells were obtained from ATCC (LG C Promochem, UK) and were cultured in Dulbecco’s modified Eagles medium, 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1 mM glutamine at 37 °C in 5% CO2. Cells were seeded at 6 × 104 to 2 × 105 cells/cm2 and transfected using Lipofectamine (Invitrogen) (293T cells, 0.5 μg/ml) or Lipofectamine (Invitrogen) (293T cells, 0.5 μg/ml). Plasmid DNA (up to 1 μg/ml) and transfection reagent were added to equal volumes of Opti-MEM I (Invitrogen) separately (5 min), before being combined (20 min, forming 1/5 of the total medium volume). The mix was added in single drops to cells in either normal medium (MCF7) or Opti-MEM I (293T). Medium was replaced after 5 h with either fresh normal medium (MCF7) or medium containing 20% serum (293T). Transfection efficiencies were between 30 and 60% as determined by expression of green fluorescent protein (GFP) from pTH-GFPa.

Flow Cytometry—Cells were removed from culture plastic with trypsin/EDTA (Sigma) and re-suspended in medium containing phosphate-buffered saline. GFP expression was analyzed (104 events) at 525 nm on an Epics XL flow cytometer (Beckman-Coulter). Dead cells and debris were excluded (on the basis of forward activated light scatter versus side scatter), and gates were set so that the proportion of untransfected cells was less than 1%.

Plasmids—Plasmids pTH-GFPa and pGL3-Reporter have been described previously (9). Vectors to express the UTRs were created by cloning UTRs upstream of the coding sequence for GFP in pTH-GFPa. UTRs were amplified from MCF7 or 293T cell cDNA by PCR adding 5′SacI and transcriptional start site and 3′ HindIII primer ends. UTRs were cloned into pTH-GFPa from plasmids pGL3-Basic, pGL3-Control, pGL3-β-galactosidase, and pGL3-TATA (Promega). UTRs were ligated directly into pTH-GFPa from MCF7 or 293T cell cDNA. pTH-GFPa was digested with SacI and HindIII to release the coding sequence for GFP fused to a constitutively active form of GL3-β-galactosidase. Vectors were engineered to direct expression of the UTRs in either direction, under the control of the CMV promoter and the LUC reporter enzyme sites.

This paper is available on line at http://www.jbc.org
cDNA Synthesis, Semi-quantitative PCR, and Real-time PCR—RNA was purified from cells using RNaseasy columns (Qiagen). Contaminating DNA was removed by digestion with DNase I (Roche Applied Science) if analyses of messages expressed from plasmids were to be carried out and RNA was further purified by phenol/chloroform extraction (∼2 ×) and ethanol precipitation using glycogen as a carrier. First strand cDNA was synthesized using SuperScript II (Invitrogen) and oligo(dT) primers. A control lacking reverse transcriptase was also performed. cDNA from human tissues and cell lines were purchased from Clontech. RNA and protein that had been purified simultaneously from single tissue samples was purchased from BioChain (AMS Biotechnology). cDNA samples were subjected to semi-quantitative PCR using RedTaq (Sigma) and the following primer pairs: primers for UTRa (CCCTCA-GAGCCGTAGGATTCGG), UTRb (GGGTGCCCAGCCGGCGGCGGC-GC), and UTRc (TGCCTGTGTTCTTCTCGG) were paired with UTR (GGAGGCAGATCGCAACATAGC); axin2 reading frame, GCC- TGGCCAAGGAGATGACC or AATTCCGGGGAGGGGC, and CTA-TTGCGTCTGTGGTGCAC; β-actin, AACCGACTGCTGTCACCT-TCAC and GGATCCAGGAAACTACTCCCTACA; GFP, CACAGGTTC-AGCGTGTCCG and CCCTCGAACTTCACCT; glyceraldehyde-3-phosphate dehydrogenase, TGAAGGTCGGTACGGATTTGGT and CATTGGGCCCAGGGATCTCACC; and CAGTGGGGCAGATGGTCC ACC. Products were analyzed on 2.5% agarose with 0.5 μg/ml ethidium bromide using 1× TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM disodium EDTA) and visualized on a UV trans-illuminator. We established that the products shown were taken from reactions within the linear range of amplification by examining reactions after at least three different numbers of cycles. PCR reactions were performed at least twice. Duplicate PCR reactions were performed using templates lacking reverse transcriptase (except with Clontech panels), and products were not seen with these templates. Products were used to ensure their specificity. Representative data are shown. Real-time PCR analysis was performed in triplicate using “as say on demand” reagents (Applied Biosystems: axin2, Hs00160344_m1; β-actin, Hs99999903_m1) on an ABI Prism 7000 machine.

Luciferase Assays—Cells were transfected with expression vectors and pGL3-Reporter in triplicate wells and were assayed after 20 h using “stop and glow” reagent from the dual luciferase kit (Promega). Data were analyzed using Srama tool Steve’s Graph Plot Analysis—Cells were washed in phosphate-buffered saline and removed from the substrate in 2 × Laemmli buffer. DNA was sheared by sonication and samples were boiled (5 min). Proteins were separated on 10% polyacrylamide gels and transferred to Hybond-C membrane (Amersham Biosciences). Membranes were blocked and stained with antibodies in phosphate-buffered saline, 5% milk, 0.2% Tween 20, and washed in phosphate-buffered saline, 0.2% Tween 20. Antibodies: rat anti-tubulin (Serotec) 1:2000; rat anti-HA (Roche Applied Science, clone 3F10) 1:1500; mouse anti-Axin2 (Santa Cruz Biotechnology, sc-25302) 1:250; goat anti-actin (Santa Cruz Biotechnology sc-1615) 1:500; Santa Cruz Biotechnology, secondary horseradish peroxidase-conjugated antibodies 1:1000. Horseradish peroxidase was visualized using ECL on ECL Hyperfilm (Amersham Biosciences). Density measurement was performed using a Bio-Rad GS-800 densitometer and Quantity One software.

Modeling of RNA Secondary Structure—Modeling was performed using mfold version 3.1 (available at bioinfo.rpi.edu/applications/) un- der the following conditions: 37 °C, 1M NaCl, no divalent ions, maximum interior bulge size of 30 nucleotides, and maximum asymmetry of bulge of 30 nucleotides.

RESULTS

mRNAs for axin2 with Three Alternative 5’-UTRs Are Differentially Expressed in Human Cells—We have identified expressed sequence tags (ESTs) from GenBankTM that contain sequences from the 5’-end of the axin2 gene. Some of these contain the sequence from exon 1, whereas their 5’-ends contain one of the alternative sequences of exon 2 upstream of the known gene. Fig. 1A shows an alignment of the 5’-end of the human axin2 gene (located on chromosome 17q24) with these ESTs. The ESTs contain sequence from additional exons, which we have termed E0a, E0b, and E0c. These non-coding exons were not previously identified when the structure of the axin2 gene was determined (14). The ESTs contain alternative 5’-ends of the message, upstream of the reading frame, that have we termed UTRa, UTRb, and UTRc.

We have investigated in which cell types these UTRs are expressed. Semi-quantitative PCR analysis was performed on cDNAs from human tissues and cell lines using forward primers specific for each UTR and a reverse primer for exon 1 (arrows, Fig. 1A). Products are only produced from templates that lack the intervening intron. Reactions were also performed using primers specific for sequence from the axin2 reading frame that is present in all axin2 messages, and for glyceraldehyde-3-phosphate dehydrogenase messages (HT, heart; Br, brain; Pl, placenta; Lu, lung; Li, liver; SM, smooth muscle; Ki, kidney; Pa, pancreas; also: 293, SKO-V3, Saos-2, A431, Du145, H1299, and HeLa cell lines). *, a product that was never visualized.

We also performed “rapid amplification of cDNA end” reactions from human placenta cDNA to identify the 5’-ends of the axin2 message (data not shown). We cloned species that represent all three of these alternative UTRs. The positions of the 5’-ends of some products were spread throughout exon 1 and the alternative E0s and we presume these to be from truncated messages. There was not a significant group of products with 5’-ends at or close to the start of exon 1, therefore we have no
Alternative UTRs Determine axin2 Expression

First, we examined whether the different 5' UTRs afford axin2 messages different stabilities. Cells were treated with an inhibitor of RNA polymerase II to halt synthesis of mRNA. Under these conditions, mRNAs continue to be turned over and their relative stabilities can be assessed by measurement of rates at which they are degraded. Cells were treated with actinomycin D for up to 300 min, and cDNA was prepared at various times.

Messages—Different 5’-UTRs Define axin2 Message Stability—It is clear that there is differential expression of the three UTRs in human cells. We wished to establish whether UTR expression has consequences for the production of protein from mRNAs. For these studies we used MCF7 cells (a breast epithelial adenocarcinoma line), because axin2 regulation has previously been studied in this cell type (15) and 293T cells, because these express axin2 mRNAs in a similar assay (data not shown).

In MCF7 cells, axin2 mRNAs containing UTRb were undetectable after only 60-min treatment with the inhibitor, indicating that they are relatively unstable, whereas those with UTRa remained after 300 min. In 293T cells, the quantification allows striking comparisons between stabilities. Messages for axin2 containing UTRc were dramatically less stable (47 min for 50% degradation) than those with either UTRa (102 min) or UTRb (155 min). These stabilities are reflected in the stability of total axin2 mRNA (89 min), which lies between the stability of those containing UTRc and those containing either UTRa or UTRb. We conclude that axin2 messages containing different 5'-UTRs have very different relative stabilities. We also conclude that these relative stabilities vary dramatically between cell types, because messages with UTRb are the most stable in 293T cells, whereas in MCF7 cells those containing UTRa are the most stable.

We also determined the relative stabilities of luciferase messages with each of the three axin2 5'-UTRs in a similar assay (data not shown). We found that luciferase messages containing these UTRs were all much more stable than axin2 messages and differences in stability were negligible. We conclude that axin2 UTRs are not sufficient to specify stability of heterologous messages.

Different 5’-UTRs Define Translational Efficiency of Messages—Next, we investigated whether axin2 UTRs directly influence the efficiency of translation of the subsequent ORF. Each UTR was cloned upstream of the GFP-reading frame in an expression vector. MCF7 and 293T cells were transfected with equal numbers of copies of either empty expression vector, vectors to express UTRa GFP, UTRb GFP, or UTRc GFP, or vector to express GFP with the 5'-UTR that is normally encoded by the vector as a control, and GFP expression was analyzed.

First, we included an additional vector in these transfections to allow overexpression of an unrelated protein, Renilla luciferase. Cells were assayed for luciferase expression (Fig. 3A). The same amount of luciferase was expressed when included with each vector, and we conclude that these combinations of plasmids are transfected equally efficiently. Expression of GFP in transfected cells was analyzed by Western blotting (Fig. 3B). In both cell types, there are clear and reproducible differences in GFP expression from these vectors. Because these transfected cells retain the ability to overexpress the same amount of another protein from equal amounts of vector (Fig. 3A), these differences in GFP expression are not caused by variation in transfection efficiencies or by effects on general protein synthesis.

We examined GFP expression in more detail using flow cytometry. Fig. 4A shows representative histograms of intensity of GFP fluorescence for populations of transfected cells. To simplify the analysis we have quantified expression in two ways (Fig. 4B). First, we have set a threshold level of fluorescence that excludes >99% of cells transfected with only empty vector, and we measured proportions of the populations that exceed this level (upper panel). These gates and proportions of GFP positive cells are shown in Fig. 4A. Second, we measured mean fluorescence intensities (MFI) of populations (lower panel) of GFP positive cells, which we have been unable to detect messages containing UTRc reliably, indicating that this species is expressed at very low levels if at all (Fig. 2A, UTRc). Messages containing UTRa and UTRb were detected in MCF7 cells, and, as expected, all three species were detected in 293T cells. The assay was repeated using 293T cells, and products were quantified to allow calculation of average times for the amount of each species to decrease by 50%. Average decay times (±S.D.) for the three species are shown in Fig. 2B.
Alternative UTRs Determine axin2 Expression

FIG. 3. Different amounts of protein are synthesized from messages containing different UTRs. MCF7 and 293T cells were transfected for 20 h with either empty vector alone (vector), or empty vector and equal numbers of copies of various vectors to allow expression of GFP. These vectors encode different 5'-UTRs: either axin2 UTRa, UTRb, UTRc, or the 5'-UTR normally encoded by pcDNA3.1 (GFP). A, triplicate wells of cells were transfected as above and with a vector to express Renilla luciferase, pGL3ren. Luciferase assays were performed and averages (± S.D.) are shown. B, cells were transfected and proteins were analyzed by SDS-PAGE and Western blots using antibodies against HA (GFP has a HA tag) and tubulin.

Panel). Each axin2 UTR causes a reduction in GFP expression (compare MFI's of each with control GFP vector). UTRb causes a further substantial reduction in GFP expression, which is detectable when expression is analyzed both by proportion of GFP positive cells and by MFI. UTRc allows expression at a level between that observed with UTRa and that observed with UTRb in MCF7 cells, whereas in 293T cells expression from UTRa and UTRc are similar.

Semi-quantitative PCR analysis was performed on cDNA from cells that had been transfected with these vectors using primers specific for GFP, and for β-actin (Fig. 4C). In both cell types, the levels of GFP message are similar between populations transfected with each GFP vector. It is clear that differences in transcription from these vectors do not account for the differences in protein expression. We conclude that these UTRs specify the efficiencies with which the reading frame downstream is translated. Each UTR specifies a different efficiency and, because that determined by UTRc varies relative to the other UTRs in the two cell lines, it follows that the cellular context can modulate these effects.

Upstream ORFs Reduce the Translational Efficiency of Messages Containing UTRa and UTRc—Each UTR contains two short ORFs upstream of the axin2 start codon (Fig. 5A). One is common to all three UTRs (lowercase), because it is wholly within the sequence of exon 1 (shaded gray), whereas the others are unique to each UTR (uppercase). These uORFs (‘u’ for upstream) can reduce the translational efficiency of a subsequent reading frame by stopping a proportion of scanning ribosomes from reaching the true start codon (6). The start codons of uORFs are required for their inhibition of expression of downstream reading frames. To test whether uORFs have an effect in these UTRs, we mutated the start codons of each uORF in each UTR GFP expression vector and analyzed effects on GFP expression. Within each UTR, we mutated the start codon of the unique uORF within UTRa (wt), or both uORFs. These mutations are called “m1,” “m2,” and “both.” We compared expression from vectors containing these mutations with expression from the wild type UTR (wt) and from the GFP control vector as before (GFP). Cells were transfected with equal numbers of copies of the vectors and expression of GFP protein and mRNA was analyzed using flow cytometry (in this case only MFI’s are shown, Fig. 5B) and semi-quantitative RT-PCR, respectively (Fig. 5C).

The levels of GFP message are similar between populations transfected with each GFP vector, thus differences in transcription from these vectors do not account for differences in GFP protein expression (Fig. 5C). Mutation of the start codon of the unique uORF within UTRa causes a substantial increase in GFP expression (Fig. 5B, compare gray bars, wt and m1). In 293T cells, this mutation (UTRa m1) allows expression at the high level also seen in the control GFP vector (GFP). Mutation of the unique uORF within UTRc (black bars, m1) has different effects in the two cell lines. In MCF7 cells this mutation causes a small but significant increase in GFP expression (Student’s t test, p = 0.02), whereas in 293T cells there is no effect. Finally, mutation of the start codon of the unique uORF within UTRb (open bars, m1) has little effect in either line. Mutations in the
Alternative UTRs Determine axin2 Expression

Start codons of the uORF that is common to all three UTRs have only slight effects on expression of GFP whether this is alone (compare wt with m2) or in combination with mutations in the unique uORFs (compare m1 and both).

We conclude that uORFs repress translation of downstream reading frames in mRNAs containing some axin2 UTRs. The unique uORF within UTRa is responsible for the greater part, and in 293T cells perhaps all, of the repression of translation caused by UTRa. In UTRb, uORFs appear not to play a major role in repression of translation. Expression from UTRc is repressed by its unique uORF in MCF7 but not in 293T cells. These differences between the two cell lines underscore the fact that the cellular context can modulate the effects of the UTRs; thus these uORFs can be sites of regulation rather than continuous repression.

UTRs of axin2 Form Stable Secondary Structures That Reduce the Translational Efficiency of Messages—Regions of RNA secondary structure within 5'-UTRs can inhibit translation of subsequent reading frames (16). In some cases trans-acting factors bind these elements and regulate whether ribosomes continue to scan, whereas in others the RNA structure itself blocks ribosome passage (3). We have examined whether axin2 UTRs are capable of forming significant secondary structure using computer modeling. The degree and stability of these structures can be quantified using the theoretical change in free energy (ΔG); structures that are more stable release more energy as they form and have greater ΔG values. UTRa, UTRb, and UTRc can form structures with ΔG values of −74, −112, and −219 kcal/mol, respectively. For comparison, the ΔG values of the 5'-UTRs of GFP message from the mammalian expression vector (as used in Figs. 3–5) and human β-actin are only −24 and −13 kcal/mol. UTRs of axin2 contain significant secondary structure that must contribute to their ability to repress translation.

Modeling also revealed that the first 60 bases of UTRb can form an extremely stable stem-loop (ΔG = −40 kcal/mol) with 44 of its 60 nucleotides involved in base-pairing (Fig. 6A). The first 60 bases of UTRs and UTRc form structures with ΔG values of only −13 and −28 kcal/mol, respectively. Because uORFs appeared not to be responsible for inhibition of translation in UTRb (Fig. 5), we have tested whether these 60 bases, and thereby the structure they form, are responsible. MCF7 and 293T cells were transfected with equal numbers of copies of various vectors to express GFP. These vectors encode different 5'-UTRs: either UTRb as before (UTRb wt) or UTRb lacking the potentially inhibitory 60 bases (UTRb60), or the 5'-UTR that is normally present in this vector (GFP wt) or that UTR plus these 60 bases near its 5'-end (GFP +60). Expression of GFP protein and mRNA was analyzed as previously. The levels of GFP message are similar between populations transfected with each vector, and differences in transcription do not account for differences in GFP expression (Fig. 6C). Deletion of the 60 bases from UTRb caused a significant derepression of GFP expression (Fig. 6B, left panel), whereas addition of these 60 bases into the standard UTR of the expression vector caused a significant inhibition of expression (Fig. 6B, right panel). In both cases, the effects are considerably greater in 293T than in MCF7 cells. We conclude that the 60 base structural motif is responsible for a significant proportion of the translational repression of UTRb.

Expression of Axin2 Protein Is Determined by the Relative Levels of axin2 UTRs—UTRs of axin2 had a profound differential effect on the translational efficiency of mRNAs (Fig. 3 and 4). We wished to examine whether this translational regulation modulates the expression of endogenous Axin2 protein. Human cell lines and tissues express different proportions of messages containing each axin2 UTR (Fig. 1B). Since these UTRs specify different translational efficiencies, we predicted that Axin2 protein expression would depend not only on the total amount of axin2 mRNA but also on the proportions of each 5'-UTR within these messages. For example, cells that express a large proportion of axin2 message containing UTRb would express relatively little Axin2 protein, because this UTR strongly represses translation.

Initially, we examined MCF7 and 293T cells. We determined relative expression of axin2 at three levels: total mRNA, using real-time PCR (normalized to β-actin mRNA); 5'-UTRs, using semi-quantitative RT-PCR (as Fig. 1B); and protein, using Western blots. 293T cells express in excess of 3-fold more total axin2 mRNA but also on the proportions of each 5'-UTR.
range. UTRc was not reliably detected in MCF7 cells, because it was expressed at very low levels if at all. Expression of Axin2 protein in 293T cells was 1.2-fold more than in MCF7 cells (average fold difference from densitometry on triplicate experiments, ±0.1 S.D., a representative blot is shown in Fig. 7C). 293T cells did not express as much Axin2 protein relative to MCF7 cells as would be expected from their levels of total axin2 mRNA alone. We explain this in terms of expression of different 5'-UTRs; 293T cells expressed high levels of axin2 mRNA containing UTRb that strongly inhibited translation (Fig. 4), thereby these messages did not contribute as much as others to synthesis of Axin2 protein.

We also examined axin2 mRNA, UTR, and protein expression in three human tissues that have strong differential expression of axin2 UTRs: placenta, lung, and pancreas (Fig. 1B), along with an additional sample, ovary. These tissues express similar amounts of total axin2 mRNA (Fig. 7D) but display differential UTR expression (Fig. 7E). UTR expression in placenta (Pl), lung (Lu), and ovary (Ov) was comparable, whereas in pancreas (Pa) UTRb was expressed at lower, and UTRa and UTRc at higher levels. The data for UTRa and UTRb mirror the relative expression patterns seen in Fig. 1B; however, UTRc was expressed in pancreas at a higher relative level than seen previously. The most striking difference in protein expression was in pancreas where more Axin2 protein was expressed relative to the other tissues (Fig. 7F). Again, translational differences specified by the UTRs appeared to be responsible. Although total axin2 mRNA levels in these tissues were similar, pancreas expressed relatively more UTRa and UTRc, UTRs that allow more translation (Fig. 4), and relatively little UTRb, which strongly inhibited translation (Fig. 4). This resulted in the synthesis of more Axin2 protein. There are other mechanisms by which total axin2 mRNA levels would not correlate with protein expression when compared across different cell types; for example, substantial differences in either the overall translational efficiency of axin2 messages, or in the stability of Axin2 protein. However, the data fully support our hypothesis that the relative proportions of different axin2 UTRs influence protein levels, and we conclude that differential UTR expression is reflected in the physiological levels of protein.

**DISCUSSION**

In this study, we have identified previously unknown exons within the human axin2 gene that allow expression of three alternative 5'-UTRs of the axin2 mRNA (Fig. 1A). The majority of vertebrate 5'-UTRs are <200 nucleotides and lack signifi-
cant secondary structure or reading frames (5). However, this is not the case for some proto-oncogenes, tumor suppressors, and other genes associated with proliferation (17), which may have long or structured non-coding RNA regions that influence the amount of protein translated from the message by altering message stability, localization or translational efficiency (5).

All three axin2 5′-UTRs are longer and structurally more complex than is typical. Expression of multiple UTRs has been demonstrated from a growing list of human genes (18–21), although in many cases their significance is not known. For multiple UTRs to provide regulation of gene expression they must be differentially expressed with respect to cell type or stimulus, and the UTRs must differentially modulate the amount of protein synthesized from their messages. The alternative axin2 5′-UTRs are differentially expressed in various human cell types (Fig. 1B), and therefore we have investigated whether this has implications for the expression of Axin2 protein.

We demonstrate that axin2 messages containing different 5′-UTRs have significantly different stabilities (Fig. 2). The UTRs are not sufficient to specify these differences in the context of heterologous messages (Fig. 4C). Other sequences from the axin2 message are likely to be required for this, because stability is determined by interactions between the 5′-cap, 3′-poly(A) tail, trans-acting factors, and, often, sequences within the message (22). A range of mRNA species with different stabilities is also expressed from the cystic fibrosis gene (23), and, although the significance of this is not clear for either of these genes, it must relate to modulation of the rapidity of gene expression changes.

We also demonstrate that these 5′-UTRs directly inhibit the translation of downstream reading frames in their messages (Figs. 3 and 4). Inhibition was caused by a combination of translational inhibition, with UTRb consistently causing the least efficient translation of downstream reading frames in their messages. The alternative axin2 5′-UTRs are differentially expressed in various human cell types (Fig. 1B), and therefore we have investigated whether this has implications for the expression of Axin2 protein.

Alternative UTRs, axin2, and Cancer—Axin2 is a tumor suppressor (8, 9), and it is probable that bypass of its effects as a negative regulator of Wnt/β-catenin contributes to tumor progression in many cases. This bypass is achieved by loss of heterozygosity or rearrangements in the locus containing the axin2 gene in some cancers (32–34), whereas the gene itself is mutated (and probably functionally inactivated) in some hepatocellular carcinomas (35) and colorectal cancers (8). Still other cancers accumulate mutations in Wnt/β-catenin signaling components, which act downstream of the inhibitory effect of axin2 (36). In this study we describe a further mechanism by which inhibitory effects of axin2 may be bypassed: down-regulation of axin2 by modulation of either the relative expression of axin2 UTRs, or of other factors that influence the degrees to which the UTRs repress axin2 expression. The expression of a number of genes is deregulated in this way during carcinogenesis (3). The oncogene mdm2 is up-regulated in some choroid-carcinomas by expression of an alternative 5′-UTR that allows more efficient translation than the normal UTR (19). In some breast cancers, the tumor suppressor Brca1 is down-regulated by expression of an alternative 5′-UTR that leads to inefficient translation of the protein (18).

Acknowledgments—We thank Jo Buddle for technical assistance, members of the Brady group for helpful discussions, and Jon Gilley and Dale Moulding for critical readings of the manuscript.

REFERENCES

1. de Moor, C. H., and Richter, J. D. (2001) Int. Rev. Cytol. 203, 567–608
2. Brents, S., and Goodwin, E. B. (2003) Nat. Rev. Genet. 4, 626–637
3. Stoneley, M., and Willis, A. E. (2003) Curr. Mol. Med. 3, 597–603
4. Ruggiero, D., and Pandolfi, P. P. (2003) Nat. Rev. Cancer 3, 179–192
5. Pesole, G., Mignone, F., Gissi, C., Grillo, G., Liuni, S., and Liuni, S. (2001) Gene 276, 73–81
6. Meijer, H. A., and Thomas, A. A. (2002) Biochem. J. 367, 1–11
7. Kikuchi, A. (1999) Cytokine Growth Factor Rev. 10, 255–265
8. Liu, W., Dong, X., Mai, M., Sestan, R. S., Taniguchi, K., Krishnadath, K. K., Halling, K. C., Cunningham, J. M., Boardman, L. A., Qian, C., Christensen, E., Schmidt, S. S., Roche, P. C., Smith, D. I., and Thiobudeau, S. N. (2000) Nat. Genet. 26, 146–147
9. Hughes, T. A., and Brady, H. J. M. (2005) Exp. Cell Res. 303, 32–46
10. Giles, R. H., van Es, J. H., and Clevers, H. (2005) Biochim. Biophys. Acta 1653, l–24
11. Classon, M., and Harlow, E. (2002) Nat. Rev. Cancer 2, 910–917
12. Aulehla, A., Wehrle, C., Brand-Saberi, B., Kemler, R., Gossler, A., Kanzler, B., and Herrmann, B. G. (2003) Dev. Cell 4, 395–406
13. Zeiher, D., Fujita, Y., Hulsen, J., Muller, T., Walther, I., Taketo, M. M., Crenshaw, E. B., 3rd, Birnmeier, W., and Birnmeier, C. (2003) Dev. Biol. 258, 406–418
14. Dong, X., Sestan, R. S., Qian, C., Mai, M., and Liu, W. (2001) Cytogenet. Cell Genet. 93, 26–28
15. Leung, Y. J., Killegy, P. T., Wu, R., Zhang, Y., Kucik, R., Hanash, S., Cho, K. R., and Fearon, E. R. (2002) J. Biol. Chem. 277, 21657–21665
16. Feldman, J., and Sonenberg, N. (1985) Cell 40, 515–526
17. Kozak, M. (1991) J. Cell Biol. 115, 887–903
18. Sobczak, K., and Kryzosiak, W. J. (2002) J. Biol. Chem. 277, 17349–17358
19. Landers, J. E., Cassel, S. L., and George, D. L. (1997) Cancer Res. 57, 3562–3568
20. Bar, I., Tissier, F., Lambert de Rouvray, C., De Backer, O., and Goffinet, A. M. (2003) J. Biol. Chem. 278, 5802–5812
21. Grobe, K., and Esko, J. D. (2002) J. Biol. Chem. 277, 30699–30706
22. Gubaniov, J., and Brewer, G. (2001) Gene (Amst.) 265, 11–23
23. Davies, W. L., Vandenbarg, J. L., Sayers, R. A., and Trezise, A. E. (2004) Biochem. Biophys. Res. Commun. 319, 410–418
24. Wilkie, G. S., Dickson, K. S., and Gray, N. K. (2003) Trends Biochem. Sci. 28, 182–188
25. Davies, W. L., Vandenbarg, J. L., Sayers, R. A., and Trezise, A. E. (2004) J. Biol. Chem. 279, 15877–15887
26. Newton, D. C., Bevan, S. C., Choi, S., Robb, G. B., Millar, A., Wang, Y., and Marsden, P. A. (2003) J. Biol. Chem. 278, 636–644
27. Wang, Y., Newton, D. C., Robb, G. B., Kau, U. C., Miller, T. L., Cheung, A. H., unpublished observations.

2 T. A. Hughes and H. J. M. Brady, unpublished observations.

3 T. A. Hughes and H. J. M. Brady, submitted for publication.
Alternative UTRs Determine axin2 Expression

8588

Hall, A. V., VanDamme, S., Wilese, J. N., and Marsden, P. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12150–12155

28. Pontrelli, L., Sidiropoulos, K. G., and Adeli, K. (2004) Biochemistry 43, 6734–6744

29. Frankton, S., Harvey, C. B., Gleason, L. M., Fadel, A., and Williams, G. R. (2004) Mol. Endocrinol. 18, 1631–1642

30. Hernandez-Sanchez, C., Mansilla, A., de la Rosa, E. J., Pollerberg, G. E., Martinez-Salas, E., and de Pablo, F. (2003) EMBO J. 22, 5582–5592

31. Hernandez-Sanchez, C., Rubio, E., Serna, J., de la Rosa, E. J., and de Pablo, F. (2002) Diabetes 51, 770–777

32. Phelan, C. M., Borg, A., Cuny, M., Crichton, D. N., Baldersson, T., Andersen, T. I., Caligo, M. A., Lidereau, R., Lindblom, A., Seitz, S., Keisel, D., Hamann, U., Rio, P., Thorlacius, S., Papp, J., Ohah, E., Ponder, B., Bignon, Y. J., Scherneck, S., Barkardottir, R., Borresen-Dale, A. L., Eyfjord, J., Theillet, C., Thompson, A. M., and Larsson, C. (1998) Cancer Res. 58, 1004–1012

33. Plummer, S. J., Paris, M. J., Myles, J., Tubbs, R., Crowe, J., and Casey, G. (1997) Genes Chromosomes Cancer 20, 354–362

34. Barlund, M., Tirkkonen, M., Foreman, F., Tanner, M. M., Kallioniemi, O., and Kallioniemi, A. (1997) Genes Chromosomes Cancer 20, 372–376

35. Taniguchi, K., Roberts, L. R., Aderca, I. N., Dong, X., Qian, C., Murphy, L. M., Nagorney, D. M., Burgart, L. J., Roche, P. C., Smith, D. I., Ross, J. A., and Liu, W. (2002) Oncogene 21, 4863–4871

36. Wong, N. A., and Pignatelli, M. (2002) Am. J. Pathol. 160, 389–401
Expression of axin2 Is Regulated by the Alternative 5′-Untranslated Regions of Its mRNA
Thomas A. Hughes and Hugh J. M. Brady

J. Biol. Chem. 2005, 280:8581-8588. doi: 10.1074/jbc.M410806200 originally published online December 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M410806200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 11 of which can be accessed free at http://www.jbc.org/content/280/9/8581.full.html#ref-list-1