A growth-related mRNA in cultured mouse cells encodes a placental calcium binding protein

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ABSTRACT

We have characterized an mRNA that increases in abundance after serum stimulation of quiescent mouse fibroblasts. This mRNA, designated 18A2, encodes a predicted polypeptide of 101 amino acids with homology to known calcium binding proteins. A variety of mouse tissues express the 18A2 mRNA, with the highest levels detected in the non-pregnant uterus and in the placenta. The concentration of 18A2 mRNA in total placental RNA decreases from day 8 to day 10 of pregnancy, and is below detection throughout the latter half of gestation. In serum-stimulated fibroblasts, the increase in 18A2 mRNA is dependent on protein synthesis. The 18A2 mRNA is similar in size, serum-inducibility, and sequence to the 2A9 mRNA (1), but these mRNAs are derived from distinct genes. This suggests that the mouse genome harbors a family of serum-inducible genes encoding proteins predicted to bind calcium.

INTRODUCTION

As part of the transition from the resting to growing state, cultured mouse fibroblasts display altered patterns of gene expression. This is evidenced by the rapid accumulation of *c-fos*, *c-myc*, and other "early" mRNAs (2-6), and by increases later in the G1 period of proliferin (which is also known as mitogen-regulated protein) and p53 mRNAs (7-9), for example. The expression of these mRNAs as cells reenter the cell cycle suggests that the proteins they encode participate in the process, and perhaps the regulation, of cell growth, especially since *fos*, *myc*, and p53 are oncogenes, and proliferin is a member of a growth regulating hormone family (10). Consistent with this model are the observations that microinjection of a monoclonal antibody to p53 inhibits DNA synthesis in serum-stimulated fibroblasts (11) and that expression of anti-sense *fos* RNA interferes with cell proliferation (12,13).

Several laboratories have isolated cDNA clones corresponding to serum or growth factor induced mRNAs in cultured fibroblasts (2,6,7,14-18). The functions of the proteins encoded by these growth-related mRNAs remain largely unknown. We have previously isolated a cDNA clone for a serum-

inducible BALB/c 3T3 cell mRNA, designated 18A2 (7). The 18A2 cDNA clone was found to hybridize to a 0.6 kb mRNA that increased in amount following serum stimulation of quiescent BALB/c 3T3 cells, but with delayed kinetics. Only a slight increase in 18A2 mRNA levels was detected during the first 12 hr of serum stimulation, corresponding to the G₁ period in these cells; significantly higher levels of this mRNA accumulated by 18 hr after stimulation (i.e., in S phase). We have now characterized the 18A2 mRNA in terms of its structure, homology of its predicted translation product to known proteins, tissue distribution in the mouse, and dependence on protein synthesis for expression in cultured cells.

MATERIALS AND METHODS

Cell Culture

C3H 10T1/2 cells (19) were grown in Basal Medium Eagle (BME) supplemented with glutamine (2 mM), penicillin (10 units/ml), streptomycin (10 units/ml), and fetal calf serum (10%). Confluent cultures were maintained in BME with 0.5% serum for 48-72 hr to render them quiescent, and were stimulated with BME containing 20% serum. To some cultures, cycloheximide (Sigma) was added to a final concentration of 10 µg/ml. Primary fibroblasts were generated from late gestation mouse embryos by dissociation with dispase/collagenase (Boehringer-Mannheim), grown in Dulbecco's Modified Eagle's Medium (DME) until confluence, and then serum starved (DME with 0.5% serum) and stimulated (DME with 20% serum).

Purification of RNA and DNA

RNA was isolated from cell cultures by centrifugation of a guanidinium thiocyanate lysate (20) through a CsCl cushion (21). RNA was isolated from mouse tissues using the same procedure, except that the tissues were first homogenized in the guanidinium thiocyanate solution with a Brinkmann polytron. Pelleted RNA was solubilized, extracted with phenol-chloroform, precipitated with ethanol, and solubilized in water. Mouse embryo DNA was purified as described (22). Bacterial plasmid DNA was purified from cell lysates by two rounds of CsCl/ethidium bromide centrifugation (22). The pSVc-myc-1 plasmid (23) was obtained from the American Type Culture Collection. Bacteriophage DNA was purified from phage particles isolated either on glycerol step gradients (22) or by immunoprecipitation with LambdaSorb (Promega Biotec).

Isolation and Sequencing of Placental 18A2 cDNA Clones

A λgt11 cDNA library was generated from day 10 (of pregnancy) placental RNA exactly as described (24); a more complete description of this library

will be presented elsewhere (P. Colosi and D. Linzer, manuscript in preparation). The library was screened with the nick-translated (25) insert from the original 18A2 cDNA clone (7), and the cDNA inserts from plaque-purified phage DNAs were subcloned into pGEM vectors (Promega Biotec). The sequence of these cDNAs was determined on both strands of the double stranded template by the dideoxy chain termination procedure (26). Nucleotide and protein homology searches were conducted with the FASTP computer programs (27).

RNA and DNA Hybridizations

Total cellular RNA was fractionated on formaldehyde agarose gels (28), transferred to nitrocellulose (29), and hybridized for 12-24 hr in 50% formamide at 42°C to nick-translated 18A2 cDNA. The exact conditions for hybridization and washing have been described previously (7). Restriction endonuclease digested mouse genomic DNA was fractionated on agarose gels, and transferred to nitrocellulose (30). Filters were then hybridized for 24-48 hr at 65°C to isolated, nick-translated cDNA inserts in 50 mM Tris-HCl, pH 7.4, 1 M NaCl, 5 mM EDTA, 0.5% sodium dodecyl sulfate, 10 µg/ml denatured salmon sperm DNA, 10⁶ dpm/ml probe, and 0.2% each bovine serum albumin, ficoll, and polyvinylpyrrolidone. Washes were carried out at 65°C in 0.1 X SSC (15 mM NaCl, 1.5 mM sodium citrate), 0.1% sodium dodecyl sulfate.

RESULTS

Serum-Stimulated Expression of 18A2 mRNA in Mouse Cells

We have previously reported an increase in amount of the 0.6 kb 18A2 following serum stimulation of quiescent BALB/c 3T3 cells (7). To determine if the serum-stimulated expression of 18A2 mRNA can be generalized beyond BALB/c 3T3 cells, 18A2 mRNA levels in another established embryo-derived cell line, C3H 10T1/2, and in primary mouse embryo fibroblasts were analyzed. RNA was isolated from subconfluent, actively growing C3H 10T1/2 cell cultures, and from quiescent and serum-stimulated cultures. Hybridization of the 18A2 cDNA to these RNA samples reveals that 18A2 mRNA is detectable in the resting cell population, but at a level significantly below that in growing and serum-stimulated cultures (Fig. 1A). No 18A2 mRNA had been detected originally in resting BALB/c 3T3 cell cultures (7), however that analysis used only a partial cDNA sequence as a probe, while the probe for Figure 1 is the full length cDNA (see below); the full length probe is also able to detect low levels of 18A2 mRNA in resting BALB/c 3T3 cells (data not shown).

RNA was also purified from BALB/c primary embryo fibroblasts that were grown to confluence and maintained in low serum for two days and then stimulated with fresh serum for up to 24 hr. 18A2 mRNA is present in the

Figure 1. 18A2 mRNA Levels in Mouse Fibroblasts. (A) Total cellular RNA was isolated from C3H 10T1/2 cells that were subconfluent and growing; confluent and serum-starved; and confluent, serum-starved, and then serum-stimulated for 12 hr. These RNAs (10 μ g per lane) were hybridized to the 18A2 cDNA clone. The positions of the 18S and 28S ribosomal RNAs are indicated. (B) Primary mouse embryo fibroblasts were grown to confluence, serum-starved, and then serum-stimulated for 0, 4, 8, 12, 16, or 24 hr. Total cellular RNA was prepared and 10 μ g of each preparation was hybridized to the 18A2 cDNA clone. Markers (in kb) are the RNA ladder from Bethesda Research Laboratories.

primary cultures, and a slight enhancement (approximately 2-fold by 24 hr, as estimated by densitometry) in the level of this mRNA is detected after treatment of the cultures with serum (Figure 1B). Thus, serum-stimulated expression of 18A2 mRNA, originally detected in BALB/c 3T3 cells, is also found in another established embryo fibroblast cell line, but only a slight increase in 18A2 mRNA levels is found in serum-stimulated primary embryo fibroblasts.

Tissue Distribution of 18A2 mRNA

If 18A2 gene expression is a general feature of mouse cell growth, then this mRNA might be detected in many mouse tissues. RNAs were purified from mouse liver, kidney, brain, thymus, pituitary, testes, uterus, placenta, and fetus and hybridized to 18A2 cDNA (Fig. 2). The highest concentration of 18A2 mRNA was detected in the non-pregnant uterus and placenta. Lower levels were found in the embryo and in adult kidney, while very low amounts were

Figure 2. Distribution of 18A2 mRNA in the Mouse. RNA was isolated from the indicated mouse tissues and 10 μ g of RNA per lane were hybridized to the 18A2 cDNA clone. The positions of the 18S and 28S ribosomal RNAs are shown.

Figure 3. Placental 18A2 mRNA Levels During Pregnancy. Placental tissue was isolated from days 8 through 18 of gestation, with day 0 defined by the appearance of a vaginal plug. Total cellular RNA was purified from each tissue sample, and equal amounts of RNA (10 μ g in each lane) were hybridized to the 18A2 cDNA clone. Markers are the 18S and 28S ribosomal RNAs.

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                                ACGGTTACC
                                10
10 ATG GCA AGA CCC TTG GAG GAG GCC CTG GAT GTA ATT GTG TCC
   MET Ala Arg Pro Leu Glu Glu Ala Leu Asp Val Ile Val Ser

                                20
52 ACC TTC CAC AAA TAC TCA GGC AAA GAG GGT GAC AAG TTC AAG
   Thr Phe His Lys Tyr Ser Gly Lys Glu Gly Asp Lys Phe Lys

                                30                                40
94 CTG AAC AAG ACA GAG CTC AAG GAG CTA CTG ACC AGG GAG CTG
   Leu Asn Lys Thr Glu Leu Lys Glu Leu Leu Thr Arg Glu Leu

                                50
136 CCT AGC TTC CTG GGG AAA AGG ACA GAT GAA GCT GCA TTC CAG
   Pro Ser Phe Leu Gly Lys Arg Thr Asp Glu Ala Ala Phe Gln

                                60                                70
178 AAG GTG ATG AGC AAC TTG GAC AGC AAC AGG GAC AAT GAA GTT
   Lys Val MET Ser Asn Leu Asp Ser Asn Arg Asp Asn Glu Val

                                80
220 GAC TTC CAG GAG TAC TGT GTC TTC CTG TCC TGC ATT GCC ATG
   Asp Phe Gln Glu Tyr Cys Val Phe Leu Ser Cys Ile Ala MET

                                90
262 ATG TGC AAT GAA TTC TTT GAG GGC TGC CCA GAT AAG GAG CCC
   MET Cys Asn Glu Phe Phe Glu Gly Cys Pro Asp Lys Glu Pro

                                100
304 CGG AAG AAG TGA AGACTCCTCAGATGAAGTGTGGGGTGTAGTTTGCCAGT
   Arg Lys Lys TER

355 GGGGGATCTTCCCTGTTGGCTGTGAGCATAGTGCCTTACTCTGGCTTCTTCGCAC

410 ATGTGCACAGTGCTGAGCAAATTCAATAAAAGGTTTTGAAACTATT      455

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Figure 4. Nucleotide Sequence of 18A2 cDNA. The nucleotide sequence of 18A2 cDNA was determined for both strands by the dideoxy nucleotide chain termination method. Numbers at the side refer to nucleotide positions; numbers over each line refer to the amino acid residue positions. The translation initiating methionine codon is placed by homology to the other polypeptides shown in Fig. 5. The poly(A) addition hexanucleotide signal AATAAA occurs at residues 434-439, and an Eco RI site is located at nucleotide positions 271-276.

seen in testes and thymus. No 18A2 mRNA could be detected in liver, brain, or pituitary preparations. One mouse that we examined displayed high levels of 18A2 mRNA in the ovary, but ovaries from other mice (including those treated with estradiol) were negative for 18A2 mRNA (data not shown). These results indicate that 18A2 expression occurs in several tissues, including the rapidly growing embryo and placenta, and that 18A2 mRNA can also be detected in adult tissues that are not undergoing active proliferation.

To follow the temporal pattern of expression of 18A2 mRNA in the placenta during pregnancy, we analyzed RNA isolated from the placenta at various days of gestation. This mRNA is present at day eight of pregnancy, at a lower level at day 10, and is not detectable throughout the remainder of

Figure 5. The Family of 18A2-Related Proteins. The amino acid sequences of the rat calcium binding protein (rCaBP), the bovine S100a protein, the human 2A9 polypeptide, the human cystic fibrosis antigen (CFag), and the porcine p11 tyrosine kinase substrate are compared to the putative 18A2 polypeptide sequence. Identical amino acid residues are boxed; gaps have been added for alignment, and do not represent missing amino acid residues.

pregnancy (Fig. 3). Rehybridization of this filter with a cDNA probe for mouse placental lactogen II (24) indicated that all of these RNA samples were intact (data not shown). It is possible that this mRNA remains at a high level in expressing cells, but that the percentage of cells in the developing placenta that transcribe the 18A2 gene into a stable mRNA decreases during gestation. The presence of this mRNA in both the fetus and the uterus suggests that expression in the placenta may occur in cells derived from the mother as well as from the embryo.

Nucleotide Sequence Analysis of 18A2 cDNA

The initial 18A2 clone isolated from the serum-stimulated BALB/c 3T3 cell cDNA library was sequenced and found not to be full length. Additional

18A2		ACGG	4
2A9	CATCCCCGACCGCTCGCGTCGCATTGGCCGCCTCCCTACCGCTCCAAGCCCAGCCCT		60
18A2	TTACCATGGCTAGACCCCTGGAGGAGGCCCTGGATGTAATTGTGTCCACCTTCCACAAAT		64
2A9	CAGCCATGGCATGCCCTTGGATCAGGCCATTGGCCTCCTCGTGGCATCTTCCACAAGT		120
18A2	ACTCAGGCAAAGAGGGTGACAAGTTCAGCTGAACAAGACAGAGCTCAAGGAGCTACTGA		124
2A9	ACTCCGGCAGGGAGGGTGACAAGCACCCCTGAGCAAGAAGGAGCTGAAGGAGCTGATCC		180
18A2	CCAGGGAGCTGCCTAGCTTCCTGGGGAAAAGGACAGATGAAGCTGCATTCCAGAAGGTGA		184
2A9	AGAAGGAGCTCACCATTGGCTCGAAGCTGCAGGATGCTGAAATTGCAAGGC-----TGA		234
18A2	TGAGCAACTTGGACAGCAACAGGGACAATGAAGTTGACTTCCAGGAGTACTGTGTCTTC		244
2A9	TGGAAGACTTGGACCGGAACAAGGACCAGGAGGTGAAGTCCAGGAGTATGTACCTTTC		294
18A2	TGTCCTGCATTGCCATGATGTGCAATGAATTCTTTGAGGGTGCCCGATGAAGGAGCCCC		304
2A9	TGGGGGCCTTGGCTTTGATCTACAATGAAGCCCTCAAGGGTGAAAATAAATAGGGAAGA		354
18A2	GGAAGAAGTGAAGACTCC-----TCAGATGAAGTGTGGGGGTAGTTTGCCA		352
2A9	TGGAGACACCCCTCTGGGGGTCTCTCTGAGTCAAATCCAGTGGTGGGAATTGTACAATA		414
18A2	GTGGGGGATCTTCCCTGTGGCTGTGAGCATAGTGCCTTACTCTGGCTTCTTCGCACATG		412
2A9	AATTTTTTTTGGTCAAATTT		434
18A2	TGCACAGTGCTGAGCAAAATTCATAAAAGGTTTTGAAACTATT		455

Figure 6. Nucleotide Sequence Homology of 18A2 and 2A9 cDNAs. A comparison of the mouse 18A2 and human 2A9 cDNA sequences is shown. Asterisks over the Hae III and Sau 3A sites in 18A2 and the Nco I and Dra I sites in 2A9 mark the boundaries of the DNA fragments used as hybridization probes for Fig. 7.

clones were isolated from this library and from a placental cDNA library by hybridization to the cDNA insert from the original clone. The nucleotide sequence of these clones was determined (Fig. 4); the sequence of 455 nucleotides is consistent with an mRNA of approximately 600 nucleotides including the poly(A) tail. The hexanucleotide signal for poly(A) addition, AATAAA (31), occurs at positions 434-439. The sequences of the cDNAs derived from BALB/c 3T3 and BALB/c placental RNA were found to be identical, and to contain an open reading frame capable of encoding a polypeptide of 101 amino acids beginning with a methionine codon at residues 10-12. While it remains possible that translation initiates further upstream in this mRNA, homology of the predicted 18A2 protein to proteins of known sequence suggests that the assignment of this methionine as the first amino acid is correct.

Figure 7. Comparison of 18A2 and 2A9 Genomic DNA Fragments. Mouse genomic DNA was digested with Bam HI, Eco RI, or Hind III and hybridized to (A) a Hae III to Sau 3A fragment of the 18A2 cDNA insert or (B) an Nco I to Dra I fragment of the 2A9 cDNA insert (see Fig. 6). Markers (in kb) are bacteriophage λ DNA cut with Hind III.

Homology of 18A2 to Calcium Binding Proteins

The 18A2 sequence was compared to entries in the GenBank DNA and the NBRF protein sequence databases, and significant homology was detected with placental/intestinal calcium binding proteins (32) and with the related S-100 proteins (33) (Fig. 5). Homology to these calcium binding proteins has been found for the tyrosine kinase substrate pll (34,35), the cystic fibrosis antigen (36), and another growth-related mRNA, designated 2A9, that has been detected in hamster, mouse, and human cells (1). The reported sequence of 2A9 is from a human cDNA clone; it has 57% nucleotide (Fig. 6) and 62% amino acid homology with 18A2 (including conservative changes).

To determine if the 2A9 and 18A2 genes represent human and mouse homologues, or if they are members of a family of homologous genes that share growth-related regulation of expression, restriction endonuclease digested mouse genomic DNA was hybridized to the 18A2 or the 2A9 cDNA sequences under

stringent conditions (final wash in 0.1 x SSC at 65°C). Comparison of the hybridization patterns (Fig. 7) reveals that these two probes detect different sets of genomic DNA fragments. Two Bam HI hybridizing fragments are detected with the 2A9 probe, while a single Bam HI fragment of a different size is seen with the 18A2 probe; Eco RI cleaves within the 18A2 gene to generate two hybridizing fragments, as predicted by the presence of an Eco RI site at positions 271-276 of the 18A2 cDNA sequence, while the 2A9 probe hybridizes to one Eco RI fragment; and, each probe detects a unique Hind III fragment. The sizes of the Hind III fragments, as well as the 2A9 and large 18A2 Eco RI fragments, are similar but not identical. The 18A2 and 2A9 probes were generated from corresponding regions of the cDNA sequences, residues 32 - 362 from 18A2 and 65 - 431 from 2A9 (see Fig. 6), thereby minimizing the possibility that differences in the hybridization patterns could be attributed to terminal sequences present in one probe but not the other. These results imply that the mouse genome harbors two distinct serum-inducible genes corresponding to the 18A2 and 2A9 cDNA clones. Furthermore, the hybridization patterns suggest that each of these genes is present in a single copy (or a very low number of copies) in the haploid mouse genome.

Effect of Protein Synthesis Inhibition on 18A2 Expression

Maximal levels of 18A2 RNA are not detected in BALB/c 3T3 cells until approximately 18 hr after serum stimulation (7). This is similar to the time course of 2A9 RNA levels in a hamster kidney derived cell line (16). Considering the long delay in accumulation of these RNAs following serum stimulation, it seems likely that 18A2 expression is not directly induced by growth factors, rather that it represents a late response to a series of mitogen-induced molecular events. Some of these molecular events may include the synthesis of new polypeptides, but addition of cycloheximide along with serum to the hamster kidney cells did not block a small (2-fold) increase in 2A9 RNA levels seen after 4 hr (37).

In contrast to the reported effect of cycloheximide on 2A9 expression, serum-stimulated 18A2 RNA levels reveal a marked dependence on protein synthesis. Confluent, serum-starved C3H 10T1/2 cells were stimulated with serum or with serum plus cycloheximide for 2 to 18 hr. In the absence of cycloheximide, the time course of 18A2 RNA levels is equivalent to that found in BALB/c 3T3 cells: a high level of 18A2 RNA is present 18 hr after addition of serum, but only a slight increase in 18A2 RNA concentration is seen within the first 12 hr after serum addition (Fig. 8A). Cultures treated with both serum and cycloheximide display no increase in the amount of 18A2 RNA, and by 18 hr 18A2 RNA levels have decreased relative to resting cell levels. These

Figure 8. Effect of Protein Synthesis Inhibition on 18A2 mRNA Levels. Confluent, serum-starved C3H 10T1/2 cell cultures were stimulated with serum for 2, 4, 12, or 18 hr in the absence (- CHX) or presence (+ CHX) of cycloheximide. RNA was harvested from resting cells (-) and from the stimulated cultures and analyzed by hybridization to (A) the 18A2 cDNA clone or (B) the pSVc-myc-1 clone. Markers are the RNA ladder from Bethesda Research Laboratories.

RNA samples were also hybridized with a *c-myc* clone (22): as shown previously by others (3), *c-myc* mRNA levels increase by 2 hr after serum stimulation and then decrease, while these levels are superinduced and remain elevated through the 18 hr time point in the presence of cycloheximide (Fig. 8B). Thus, the increase in 18A2 RNA concentration in serum-stimulated cells appears to require protein synthesis, and cessation of protein synthesis results in a gradual decrease in accumulated 18A2 RNA.

DISCUSSION

18A2 mRNA is expressed at elevated levels during S phase of serum-stimulated BALB/c 3T3 and C3H 10T1/2 cells. The timing of the increase in 18A2 mRNA is therefore similar to that of various histone mRNAs in synchronized cell populations (38,39); it remains to be determined if 18A2 mRNA levels fluctuate during the cell cycle in actively growing cultures, or if changes in the level of this mRNA are only seen in cells released from quiescence. Serum-stimulated 18A2 mRNA accumulation is dependent on protein synthesis, since addition of cycloheximide along with serum to resting cells eliminates the increase in 18A2 mRNA levels. Furthermore, cycloheximide causes a decrease in the amount of 18A2 mRNA present 18 hr after stimulation

relative to the level detected in unstimulated cells. Presumably the synthesis or stabilization of 18A2 mRNA is dependent on a labile factor(s) that decreases in quantity or activity during this 18 hr period. This result differs from the reported lack of a cycloheximide effect on the serum-stimulated increase in concentration of the related 2A9 mRNA (37). The 2A9 increase in those experiments was monitored at 4 hr after treatment, well before the peak level of 2A9 (16,37) or 18A2 mRNA is attained. The early, 2-fold increase in 2A9 levels after serum addition may therefore be independent of protein synthesis; we do not detect a significant increase in 18A2 mRNA levels by this time in serum-stimulated cells either treated or not treated with a protein synthesis inhibitor.

Both the 18A2 and 2A9 serum-inducible mRNAs encode predicted polypeptides with homology to calcium binding proteins, to a substrate for viral tyrosine kinase activity, and to the cystic fibrosis antigen. While the sequences of these proteins are derived from several mammalian species, they appear to define a family of related, yet distinct, proteins. None of these protein sequences are sufficiently similar to suggest that they represent the same proteins in two different species. This was especially important to demonstrate for 18A2 and 2A9, since they are both encoded by 0.6 kb mRNAs with similar degrees and timing of serum inducibility. Comparison of the hybridization patterns of these cDNAs to mouse genomic DNA demonstrates that these mRNAs are derived from independent genes, each of which appears to be present in a single copy. Thus, at least two closely related mRNAs encoding putative proteins similar to known calcium binding proteins are expressed in response to serum in cultured mouse cells.

18A2 mRNA is detected in several tissues in the mouse and in primary mouse embryo fibroblast cell cultures. Primary cell cultures do not show a large serum effect on 18A2 expression, however. This may be due to a heterogeneous cell population compared to the cloned BALB/c 3T3 and C3H 10T1/2 cell lines, or to a difference between the responses to serum deprivation/serum stimulation by primary and immortalized cells. 18A2 mRNA is detected in the placenta at day 8 of pregnancy, and at lower levels at day 10. The inability to detect this mRNA in placental tissue from later stages of gestation suggests either that 18A2 expression is regulated temporally during pregnancy, or that a subpopulation of placental cells expresses this mRNA, and that this group of cells decreases as a percentage of total placental mass during pregnancy. Since the half-life of 18A2 mRNA may be short relative to the half-life of the 18A2 protein, it is possible that 18A2 protein remains and functions in the placental tissue beyond day 10 of pregnancy.

Placental and intestinal tissue are major sites of synthesis of the 9 kD calcium binding protein homologous to 18A2 (32,40). In the rat placenta, synthesis of this calcium binding protein occurs primarily in trophoblast cells, which are derived from the developing embryo (41). The localization of this calcium binding protein to the intestine and to placental trophoblasts is indicative of a role in calcium absorption and transport (41). By analogy, the 18A2 gene product may also participate in calcium uptake in the mid-pregnant placenta, in the developing embryo, and in other tissues.

Homology of the 18A2 protein to the placental/intestinal calcium binding protein, the cystic fibrosis antigen, the S100 proteins, the phosphotyrosine acceptor polypeptide, and the 2A9 gene product demonstrates that a family of structurally similar proteins that are predicted to share the property of calcium binding exists in mammalian cells. The association of the proteins in this family with cystic fibrosis, viral transformation, and cell growth suggests that their activities are crucial to normal cell function. Calcium plays a fundamental role in muscle contraction and nerve signal transmission, for example, and one of the early events following mitogenic stimulation of mammalian cells is the mobilization of calcium (42). The increase in 18A2 mRNA well after the initial mitogenic stimulation makes it unlikely that the 18A2 protein is an essential participant in this process of calcium mobilization. Instead, the timing of 18A2 mRNA accumulation is most consistent with a role for this protein during cellular DNA synthesis.

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