Zis: a developmentally regulated gene expressed in juxtaglomerular cells

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Karginova, Elena A., Ellen Steward Pentz, Irina G. Kazakova, Victoria F. Norwood, Robert M. Carey, and R. Ariel Gomez. Zis: a developmentally regulated gene expressed in juxtaglomerular cells. Am. J. Physiol. 273 (Renal Physiol. 42): F731–F738, 1997.—Renal juxtaglomerular (JG) cells are specialized myoepithelial cells located in the afferent arteriole at the entrance to the glomerulus. Their main function and distinctive feature is the synthesis and release of renin, the key hormone-enzyme of the renin-angiotensin system that regulates arterial blood pressure. Despite their relevance to health and disease, not much is known about factors that confer and/or maintain JG cell identity. To identify genes uniquely expressed in JG cells, we used a cDNA culture model and RNA differential display. JG cells cultured for 2 days express renin and renin mRNA, but after 10 days in culture they no longer contain or release renin and renin mRNA is reduced 700-fold. We report one cDNA differentially expressed in the 2-day JG cell culture that detects a 2.6-kb mRNA expressed at higher levels in newborn than adult kidney. Screening a 2-day culture JG cell cDNA library yielded clones representing differentially spliced transcripts. These cDNAs encode one unique protein (Zis) containing zinc fingers and domains characteristic of splicing factors and RNA binding proteins. Northern blot analysis confirmed Zis mRNA expression in differentiated JG cells, and identified an additional unique 1.5-kb transcript. The Zis transcripts are developmentally regulated in kidney and a number of other organs. The features of the Zis protein and its organ distribution suggest a possible role in regulation of transcription and/or splicing, both important steps for controlling developmentally expressed genes.

METHODS

Isolation and Culture of JG Cells

An enriched population of JG cells was isolated from kidneys of male Sprague-Dawley rats (100 g) by centrifugation in a Percoll density gradient as previously described (6). The enriched JG cell fraction was placed into cell culture using conditions described by Kurtz et al. (24) and maintained with daily changes of medium.

Immunostaining

Immunostaining for renin in cultured JG cells using anti-rat renin antibody was done as previously described (17).

Assay for Renin Activity

Renin enzymatic activity is reported as nanograms angiotensin I (ANG I) generated per milligram protein per hour measured by radioimmunoassay for ANG I as previously described (20, 34).

RNA Isolation

Total RNA was isolated from JG cells cultured for 2 and 10 days and from various organs of newborn and adult rats by the single-step RNA isolation method of Chomczynski (7) using Tri-Reagent (Molecular Research Center, Cincinnati, OH). Poly(A)+ mRNA was prepared according to the standard protocol of Okayama et al. (30) using oligo(dT) cellulose spin columns (5 Prime — 3 Prime, Boulder, CO).

Northern Hybridization

For Northern blot analysis, 15 µg total RNA or 2.5 µg of poly(A)+ mRNA was electrophoresed in a 1.2% agarose-
formaldehyde gel and transferred to positively charged nylon membranes (Zeta- Probe; Bio-Rad Laboratories, Hercules, CA) as previously described (16, 17). Probes were labeled with \([\alpha^{-32P}]dCTP\) by the random primer method (13) using a commercially available kit (DECAprime II; Ambion, Austin, TX). Probe specific activity ranged from 0.4 to 1.6 million counts min\(^{-1}\)µg\(^{-1}\). Northern blots were hybridized by the method of Church and Gilbert (8) as previously described (15, 16).

### Cloning and Analysis of cDNAs

Cloning and propagation of cDNAs in plasmid or phage vectors and restriction enzyme digestion and analysis followed standard molecular biology techniques (32) and manufacturers' protocols supplied with the various kits used.

### Renin mRNA Quantitative Reverse Transcription-Polymerase Chain Reaction

Construction of competitive template. The 1,433-bp renin cDNA (4) was digested with EcoRI to remove a 246-bp fragment. The EcoRI ends were blunted by filling in using Klenow DNA polymerase (32). A 322-bp RsaI-PvuII fragment isolated from pBluescript (Stratagene, La Jolla, CA) was ligated into the deleted renin cDNA to create a 322-bp insertion of heterologous DNA. cRNA was quantitated by measuring its absorbance at 260 nm. Primers for polymerase chain reaction (PCR) were designed to flank the insertion (5' upstream primer, 5'-ATGC-CTCTCTGGGCACTCTT; 3' downstream primer, 5'-GTGACTCCAC; AP-5, 5'- GTTGCGATCC). PCR amplification with these primers yields a 551-bp product from native renin and a 627-bp product from the competitive template (CT).

Quantitative reverse transcription-PCR. Total RNA (200, 400, and 2,000 ng) from JG cells after 2, 6, and 10 days in culture was reverse transcribed with a known amount of mutant CT. Reaction conditions were as follows: 50 mM KCl, 10 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride, pH 9.0, 0.1% Triton X-100, 5 mM MgCl\(_2\), 1.25 mM each dNTP, 5 mM diithiothreitol (DTT), 15 U RNasin (Boehringer Mannheim, Indianapolis, IN). cDNA was quantitated by measuring its absorbance at 260 nm. Primers for polymerase chain reaction (PCR) were designed to flank the insertion (5' upstream primer, 5'-ATGC-CTCTCTGGGCACTCTT; 3' downstream primer, 5'-GTGACTCCAC; AP-5, 5'- GTTGCGATCC). PCR amplification with these primers yields a 551-bp product from native renin and a 627-bp product from the competitive template (CT).

mRNA Differential Display

Total RNA (50 µg) extracted from JG cells after 2 and 10 days in culture was treated with 10 U of deoxribonuclease I according to the manufacturer's protocol (MessageClean kit; GenHunter, Brookline, MA) to remove contaminating genomic DNA. The RNA was reverse transcribed with an oligo(dT) primer anchored to the beginning of the poly(A)- tail of the mRNAs [oligo(dT) primer T\(_2\)M, where M represents a degenerate base (see Ref. 27)] using the RNAMap Kit (Gen-Hunter). The RT reaction contained 20 µM dNTP, 0.01 µg/µl total RNA, 1 µM T\(_2\)M primer, 100 U MMLV reverse transcriptase in RT buffer (RT buffer contained 25 mM Tris-Cl, pH 8.3, 37.6 mM KCl, 1.5 mM MgCl\(_2\), and 5 mM DTT) in a total volume of 20 µl and was performed as follows: 65°C, 5 min; 37°C, 60 min; 95°C, 5 min. PCR amplification used the same oligo(dT) primer downstream and one of five arbitrary primers upstream (AP-1, 5'- AGCCAGCAGAA; AP-2, 5'- GACC-GCTTTG; AP-3, 5'- AGGTGACGGT; AP-4, 5'- GTTACCTCCAC; AP-5, 5'- GTTGCGATCC). The PCR reactions contained 2 µM dNTP, 0.2 µM AP-primer, 1 µM T\(_2\)M primer, 4 µl of RT mixture, 0.5 µM \([\alpha^{-35S}]dATP\) (1,200 Ci/mmol; New England Nuclear, Boston, MA), and 1 U of AmpliTag DNA polymerase (Perkin-Elmer, Branchburg, NJ) in PCR buffer (PCR buffer contained 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.5 mM MgCl\(_2\), and 0.001% gelatin) in a total volume of 20 µl. Amplification was as follows: 94°C, 30 s; 40°C, 2 min; 72°C, 30 s for 40 cycles; 72°C, 5 min. The amplified cDNAs were separated in a 5% polyacrylamide-urea sequencing gel. The gel was placed on blotting paper, dried under vacuum and exposed to BioMax film (Kodak, Rochester, NY) to visualize the labeled cDNAs. To minimize errors in the PCR procedure, several replicates of amplified cDNA (from the same RT reaction or a totally independent RT reaction) were separated in adjacent lanes of the same gel and only bands differentially expressed in several reactions were selected. Bands of interest (located by aligning the autoradiograph with the gel) were excised with a razor blade, and cDNA was extracted by boiling gel slices in 100 µl H\(_2\)O for 15 min. Eluted cDNAs were reamplified with the corresponding primer set using the same conditions and cycle times as the original PCR with the exception that the nucleotide concentration was reduced from 20 µM to 2 µM and no radioactive nucleotides were included. The reamplified cDNA fragments were electrophoresed in 1.2% agarose gels, the cDNA-containing bands were excised from the gel, and cDNA recovered using the Qiaex kit (Qiagen, Chatsworth, CA). Purified cDNA fragments were cloned into pNO TA, a vector designed for cloning of PCR products (31), following protocols supplied with the cloning kit (5 Prime → 3 Prime).

### DNA Sequencing

Cloned cDNA fragments were sequenced by the dideoxy chain-termination method (33) using the Sequenase version 2.0 kit (Amersham Life Science, Arlington Heights, IL) and \([\alpha^{-35S}]dATP\) (1,000–1,500 Ci/mmol; DuPont-NEN). Nested 5' and 3' deletions were generated (35) from cDNAs obtained from screening our JG cell cDNA library (see below) using exonuclease III (Life Technologies, Grand Island, NY). Both strands of each reported cDNA were completely sequenced.

### Construction of a JG Cell cDNA Library

Poly(A)+ mRNA was isolated from JG cells after 2 days in culture, and the integrity and size range (0.5–10 kb with majority at 2.5–3.5 kb) was determined by electrophoresis in a 1.2% agarose-formaldehyde gel. To synthesize first and second cDNA strands and to ligate adapters, the ZAP-cDNA Synthesis kit from Stratagene was used. The cDNA was
fractionated, sized on a nondenaturing polyacrylamide gel, and ligated into the Uni-ZAP XR vector (Stratagene). The library was packaged in Gigapack II packaging extract and plated on Escherichia coli XL1-Blue-MRF8 (Stratagene). The primary library was subjected to one round of amplification (final titer, $10^{10}$ plaque-forming units/ml).

cDNA Library Screening

The amplified library ($10^6$ plaques) was screened with $[\alpha-32P]dCTP$-labeled differential display-originated probes using protocols recommended by the library kit manufacturer (Stratagene). Positive plaques were excised in vivo to yield cDNAs in the more manageable pBluescript vector (Stratagene). Restriction enzyme digests were electrophoresed and blotted onto Zetaprobe membrane (Bio-Rad Laboratories), and the blots were hybridized with the probe used to screen the library to confirm that the clones contained cDNA homologous to the original probe. Sequences derived from selected clones were analyzed using the Wisconsin GCG sequence analysis software package available through the University of Virginia (14a).

RESULTS

Expression of Renin in Cultured JG Cells

Renin immunostaining of JG cells in culture is shown in Fig. 1. Virtually all JG cells that have been cultured for 2 days are positive for renin (Fig. 1A). However, after 10 days in culture, none of the cells contained immunoreactive renin (Fig. 1B). Renin enzymatic activity parallels the immunostaining, decreasing from 1,500 ng ANG I·mg$^{-1}$·h$^{-1}$ at 2 days in culture to 24 ng ANG I·mg$^{-1}$·h$^{-1}$ after 10 days in culture. The quantitative RT-PCR assay for renin mRNA showed that the decrease in renin protein during culture is paralleled by a marked decrease in renin mRNA (Table 1). The amount of renin mRNA in JG cells decreases 100-fold during the first 5 days of culture. By the 10th day of culture, the reduction is more than 700-fold. The protein and mRNA data show that the JG cells dedifferentiate in culture and lose their characteristic function of expressing renin.

mRNA Differential Display

To identify mRNAs that are expressed uniquely or in different abundance in differentiated versus undifferentiated JG cells, we analyzed RNA from JG cells cultured 2 days (differentiated) or 10 days (undifferentiated) by the method of mRNA differential display (26, 27). RNA was reverse transcribed to cDNA and then subjected to amplification using PCR and five different primer sets (see METHODS). Figure 2 shows a portion of a differential display gel autoradiogram. The cDNA fragments expressed in 2-day cultures but not in 10-day cultures and vice versa are numbered. In all, we found 27 cDNAs expressed only in 2-day cultures and 10 cDNAs only in 10-day cultures (only a fraction is shown). There were also a number of cDNAs whose abundance changed with culture duration but were not unique to the differentiated or undifferentiated state. Differentially expressed cDNA fragments were reamplified, and the purified fragments were used to probe Northern blots to confirm differential expression. For the initial screening, Northern blots of total RNA from newborn and

Table 1. Renin mRNA in juxtaglomerular cells after various times of culture

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>Renin mRNA, pg/µg total RNA</th>
<th>Relative Activity, %</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>16.75 ± 0.65</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>0.136 ± 0.06</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>0.023 ± 0.01</td>
<td>0.1</td>
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Values are means ± SE. Renin mRNA was quantitated using competitive reverse transcription-polymerase chain reaction as described in METHODS.
adult rat kidneys were used. There are more renin-expressing cells in the newborn kidney than in the adult, thus providing a greater proportion of mRNA from renin-expressing cells in newborn RNA samples (39). This screening paradigm was used, since RNA from staged cultured JG cells is scarce in comparison to that from whole kidneys. Six of seven cDNAs expressed in 2-day culture were differentially expressed in newborn kidneys and one of three cDNAs from 10-day culture was differentially expressed in adult kidney RNA. cDNA fragment 4 is therefore developmentally regulated and expressed more abundantly at the stage with a greater abundance of renin-expressing cells.

Analysis and Cloning of cDNA Fragment 4

We report here cDNA fragment 4 (Fig. 2), which was differentially expressed in 2-day JG cell cultures. In the initial screening, cDNA fragment 4 detected a 2.6-kb transcript present in newborn kidney total RNA. A transcript of the same size was barely detectable in adult kidney RNA. cDNA fragment 4 is therefore developmentally regulated and expressed more abundantly at the stage with a greater abundance of renin-expressing cells.

cDNA fragment 4, which represents the 3′ end of the corresponding mRNA, was used to screen the cDNA library we prepared from JG cells cultured for 2 days. Three of the 21 positive plaques identified on the primary screen were purified and subjected to sequence analysis. The organization of these three clones is shown in Fig. 3. They share a common sequence which yields an uninterrupted 996-bp open-reading frame (ORF, open box) but differ in the lengths of their 5′- and 3′-untranslated regions. Clone 1511 has a 265-bp 5′-untranslated region, whereas clone 152 has 47 bp and clone 182 only 17 bp. The 3′-untranslated regions of 1511 and 152 are of similar length (152 and 149 bp to the poly-A sequence, respectively), whereas clone 182 has 1,633 bp at the 3′ end. The common ORF in clone 152 is interrupted by 1,247 bp of DNA. This clone may represent a cDNA from an incompletely processed mRNA, since the interrupting DNA has characteristics of an intron as revealed by exon-intron border analysis (37). A composite of the three sequences, shown in the bottom of Fig. 3, would produce a 4-kb mRNA.

Since each of the cDNAs analyzed had such different organization, we examined some of the other positive primary plaques to determine whether there were other forms and whether the original forms were repeated. Five additional cDNAs were purified and analyzed by restriction enzyme digestion and hybridization with probe a (Fig. 3). Three of the clones were the same as clone 182, and two were the same as clone 1511. The recovery of replicates of two of the original cDNAs indicates that they indeed represent alternate forms of the mRNA and are not cloning artifacts. No additional forms were found, suggesting that we had identified all the types present in the library.

The combined sequence of these three clones with the amino acid sequence of the coding regions in single letter code is shown in Fig. 4. The first methionine codon to all sequences was chosen as the translation start codon (22). The ORF yields a 332-amino acid protein. Analysis of this amino acid sequence (Fig. 5) revealed several features. There are two putative zinc finger motifs (characteristic of transcription factors and RNA/DNA binding proteins), a short stretch of basic residues (RKKKKYRGK) that corresponds to the consensus sequence for a nuclear localization signal (NLS) (38), and a 70-residue serine-arginine (SR)-rich region (characteristic of splicing factors). Because of the presence of these elements, we named this gene Zis (from “zinc finger, splicing”).

Comparison of the Zis nucleotide sequence with sequences in the GenBank and EMBL databases (14a) did not reveal significant overall homology to any
known sequence. Part of the sequence (as underscored on Fig. 4) has 83% nucleotide identity to an internal sequence of a X. laevis mRNA containing a zinc finger (GenBank accession no. X70647; data not published). The deduced amino acid sequence was also compared with the protein databases. The 70-residue SR-rich region shared 20–50% identity with various splicing factors (41) and other proteins with SR domains such as vitellogenin (40), natural killer (NK)-tumor recognition protein (1), and E2 human papilloma virus protein (12). The two zinc finger motifs in the NH2 terminus of the protein share 60% amino acid identity to a region of the human Ewing's sarcoma (EWS) RNA-binding protein (11) and human RNA-binding protein TLS (from "translocated in liposarcoma") (10). This is the same region which shares high nucleotide identity with the X. laevis zinc finger mRNA noted above. There is a low degree of homology with other RNA-binding proteins such as human RNA-binding protein (2) and Drosophila RNA-binding protein (19). Outside the zinc finger domain and the SR-rich region, no similarity to any known proteins was found.

Differential Expression and Developmental Regulation of Zis

To confirm that the cDNA clones reflect the developmental regulation seen in the initial screening of cDNA fragment 4, we hybridized an XhoI-EcoRI fragment (2,116 bp) containing the common sequence of all the clones and the potential intron (probe a, Fig. 3) to mRNA from newborn and adult rat kidney (Fig. 6). The same 2.6-kb transcript found in the screening of total RNA was found in the poly(A)+ mRNA, as well as two other transcripts of 4 kb and 7 kb. All three transcripts were more abundant in newborn kidney mRNA than in the adult. The 2.6-kb transcript corresponds in size to the combined sequence of the three clones (Fig. 3). The 4-kb and 7-kb transcripts may represent partially spliced mRNAs. The membrane was also hybridized with a glyceraldehyde-3-phosphate dehydrogenase (housekeeping) probe which showed that equal amounts of RNA had been loaded in each lane (data not shown). Hybridization to mRNA from JG cells after 2 days in culture shows two transcripts, i.e.,

Fig. 4. Nucleotide and deduced amino acid sequence of the cDNA clones. Combined nucleotide sequence of the three different clones with the position of the coding region is shown. Double underline indicates ATG initiation codon, TAA termination codon, and AATAAA polyadenylation signal. Single underline indicates region similar to X. laevis zinc finger sequence. cDNA sequences have been submitted to GenBank: accession no. AF013965 for cDNA 1511, accession no. AF013967 for cDNA 182, and accession no. AF013966 for cDNA 152.
Similar to the kidney, the heart displays an increased expression of these mRNAs in the newborn. The brain also follows this pattern, but the difference is less marked. Both lung and liver show the opposite developmental regulation, having higher levels of the 2.6-kb transcript in the adult than in the newborn. The abundance of the two larger transcripts also appears to be developmentally regulated but not always in the same way with respect to the major transcript or themselves. For example, the 7-kb transcript in brain is more abundant in newborn, whereas the 4-kb transcript is more abundant in adult.

**DISCUSSION**

In this study we describe the isolation, structure, and expression of Zis, a novel gene expressed in JG cells. We isolated a pure preparation of JG cells that expresses a high level of renin protein and mRNA for up to 48 h in culture. However, after 10 days in culture, the JG cells dedifferentiate and lose their capacity to synthesize and release renin. We took advantage of this characteristic to identify genes potentially involved in JG cell differentiation.

The deduced amino acid sequence of Zis yields a 332-amino-acid, ~40-kDa protein that contains several interesting motifs which implicate it in protein-nucleic acid interactions, e.g., two zinc fingers, an NLS, a glutamic acid-rich region and an SR-rich domain. The sequence of the first 137 amino acids (including the zinc finger region and NLS) is virtually identical (124/127 matches) to that of C4SR, an RNA binding protein from X. laevis containing zinc fingers (GenBank accession no. X70647; data not published). This protein also has a glutamic acid-rich region and an SR region, but in these the identity with Zis is less pronounced. The carboxyterminal parts of Zis and C4SR share no identity. The Xenopus sequence was identified by virtue of its RNA binding capacity; however, a specific function is unknown. The Zis zinc finger region matches a sequence in the carboxy-terminal portion of two other RNA binding proteins, EWS and TLS (10, 11), but distal to their RNA binding domains. The functions of EWS and TLS are also not known. An NLS sequence is just distal to the potential nucleic acid binding region. This organization is typical of many DNA or RNA binding proteins (25) most of which interact with their substrates in the nucleus. The glutamic acid-rich region is found particularly in hnRNP ("heterogeneous nuclear ribonucleoprotein particles") C-type proteins. These proteins have been shown to bind to sites on pre-mRNAs that are critical for the formation of mature and functional mRNA (3). The distinctive SR domain is a characteristic element of splicing factors (14). RNA binding proteins are modular proteins that are built of several elements that vary with the final function of the protein (3). For example, the domain organization of an RNA-binding element followed by an SR-rich region is commonly found in splicing factors (14, 41). In addition, it has been shown that the proximity of an RNA binding region to an NLS may influence its functional regulation (25) as found in the adenovirus E1a oncprotein.
common transcript cDNA clones lack this GC-rich structure is found in the cell-specific transcript. The translation (23). It is interesting to note that this regulatory genes and suggests that production of the presence of such sequences is common for critical that might create a barrier to effective translation. The rich and can assume an extensive secondary structure of the 5'-untranslated region of transcript in all organs is 2.6 kb, and the 1.5-kb transcript is expressed only in JG cells. The brain has the greatest abundance of the 2.6-kb Zis transcript, followed by kidney and heart. In these organs, the expression of Zis is higher in the newborn than in the adult. Lung and liver have lower levels of Zis, and the developmental regulation is different, i.e., more in the adult than in the newborn. Transcripts of 4 and 7 kb are also present in all organs surveyed. As suggested by our analysis of the cDNA clones, the 4-kb transcript may be an incompletely processed mRNA. In the same way, we think the 7-kb transcript represents an even less completely processed species for which we did not detect a cDNA in our library. It is interesting to note that in the brain the levels of these two transcripts are also developmentally regulated, i.e., the 4-kb transcript is more abundant in the adult, whereas the 7-kb transcript is more abundant in the newborn. The presence of multiple transcripts, some of which are incompletely processed mRNAs and therefore untranslatable, has been observed with other developmentally important genes such as some of the homeobox genes (9). It has been suggested that the generation of such regulated, untranslatable transcripts may be a way to modulate the amount of translatable mRNA available at any particular time in development. Analysis of the 5'-untranslated regions of Zis cDNAs also suggests that controlling the translatable of Zis mRNA is important. From our JG cell cDNA libary, two classes of translatable cDNAs were recovered representing the major 2.6-kb transcript (clone 182) and the 1.5-kb JG cell-specific transcript (clone 1511). All the clones have the same sequence around the ATG initiation codon. This sequence (TCC AAG ATG T) differs from the consensus (GCC A/GCC ATG G) by having a T in the +4 position instead of the usual G and differing in three other positions (20). This results in an unfavorable context for initiation and may result in inefficient translation. This situation is found in mRNAs for some potent regulatory proteins (such as growth factors and cytokines), suggesting that it might be necessary to control the yield of protein that could be harmful if overproduced (21, 23). The two classes of cDNAs differ in the lengths of their 5'-untranslated regions. Analysis of the 5' sequences suggests that the different transcripts could be translated with different efficiencies. The 5'-untranslated region of clone 1511 is very GC-rich and can assume an extensive secondary structure that might create a barrier to effective translation. The presence of such sequences is common for critical regulatory genes and suggests that production of the corresponding proteins is regulated at the level of translation (23). It is interesting to note that this structure is found in the cell-specific transcript. The common transcript cDNA clones lack this GC-rich region and thus should be more readily translated. Although the role of the cell-specific transcript is as yet unknown, it is present at levels similar to that of the major transcript in JG cells and thus may require special regulation.

The results from analysis of the DNA structure, predicted amino acid sequence, and transcript patterns of Zis all point to it being a regulatory protein that interacts with RNA, probably as a regulated splicing factor. The expression of untranslatable transcripts and the unfavorable context for translation of the translatable ones all point to the importance of controlling the level of Zis protein. A number of genes involved in the early determination as well as maintenance of the differentiated state (such as the sex determination loci in Drosophila) are splicing factors that autoregulate their own expression levels and influence splice site selection in downstream genes in their pathway (36). Zis has the features of being such a developmentally regulated, and self-regulating, splicing factor. The regulation of the levels of SR proteins themselves in different cells has been shown to have a role in cell-specific splice site selection (28, 36, 41). A number of mRNAs that can be alternatively spliced in a tissue-specific or developmentally controlled manner have been identified (36) and could be candidate targets for regulation by Zis.

JG cells are specialized myoendothelial cells whose major identifying characteristic is the synthesis, storage, and secretion of renin. During development of the fetal kidney, renin-expressing cells are present along much of the kidney vasculature, extending from afferent arterioles to the major branches of the renal artery. As development proceeds, the vascular location of renin-expressing cells is restricted, so that in the newborn there is expression along only part of the afferent arteriole, and in the adult, renin-expressing cells are found normally only at the entrance to the glomerulus (17). Various pharmacological and physiological manipulations that modulate renin levels in the whole animal result in expression of renin in cells along the afferent arteriole (15) in a pattern similar to that found in the fetus. It appears that there is plasticity in the cells of the vasculature such that adult cells that were not expressing renin, but probably did at an earlier stage in development, can regain their capacity to do so in response to certain stimuli. This plasticity in renin expression admits a wide range of potential regulatory levels. In this survey of mRNAs characteristic of renin-expressing cells, we identified Zis, a potential regulated splicing factor. Zis could affect renin expression directly by regulating the processing of renin pre-mRNA and thus influencing the amount of translatable mRNA or indirectly by regulating the expression of other proteins that modulate renin.

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REFERENCES


