MOLECULAR AND CELLULAR BIOLOGY, May 2000, p. 3308–3315
0270-7306/00/$04.00+0
Vol. 20, No. 9

Zac1 (*Lot1*), a Potential Tumor Suppressor Gene, and the Gene for ε-Sarcoglycan Are Maternally Imprinted Genes: Identification by a Subtractive Screen of Novel Uniparental Fibroblast Lines

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Received 14 December 1999/Returned for modification 25 January 2000/Accepted 2 February 2000

Imprinted genes are expressed from one allele according to their parent of origin, and many are essential to mammalian embryogenesis. Here we show that the ε-sarcoglycan gene (*Sgce*) and *Zac1* (*Lot1*) are both paternally expressed imprinted genes. They were identified in a subtractive screen for imprinted genes using a cDNA library made from novel parthenogenetic and wild-type fibroblast lines. Sgce is a component of the dystrophin-sarcoglycan complex, Zac1 is a nuclear protein inducing growth arrest and/or apoptosis, and *Zac1* is a potential tumor suppressor gene. *Sgce* and *Zac1* are expressed predominantly from their paternal alleles in all adult mouse tissues, except that *Zac1* is biallelic in the liver and *Sgce* is weakly expressed from the maternal allele in the brain. *Sgce* and *Zac1* are broadly expressed in embryos, with *Zac1* being highly expressed in the liver primordium, the umbilical region, and the neural tube. *Sgce*, however, is strongly expressed in the allantoic region on day 9.5 but becomes more widely expressed throughout the embryo by day 11.5. *Sgce* is located at the proximal end of mouse chromosome 6 and is a candidate gene for embryonic lethality associated with uniparental maternal inheritance of this region. *Zac1* maps to the proximal region of chromosome 10, identifying a new imprinted locus in the mouse, homologous with human chromosome 6q24-q25. In humans, unipaternal disomy for this region is associated with fetal growth retardation and transient neonatal diabetes mellitus. In addition, loss of expression of *ZAC* has been described for a number of breast and ovarian carcinomas, suggesting that *ZAC* is a potential tumor suppressor gene.

The normal development of the mammalian embryo requires both the maternal and the paternal genomes (4, 27). Mouse embryos in which the entire genome is either of maternal origin (parthenotes) or of paternal origin (androgenotes) usually die at or shortly after implantation. Rarely do these embryos develop to E10, and none have developed to term. The basis for this failure in development is that some genes are imprinted and are expressed from only one parental allele. Thus, loss of the expressed allele can render the embryo null for the gene's function. For some imprinted genes, such as the maternally expressed Igf2/mannose-6-phosphate receptor allele (*Igf2r*) (3), this results in embryonic lethality, whereas mutation of the paternally inherited allele, which is not expressed, has no effect on viability (23).

In the mouse, most imprinted genes are found in clusters distributed among 10 regions over six chromosomes (C. V. Beechey and B. M. Cattanach, 1998, mouse imprinting data and references [http://www.mgu.har.mrc.ac.uk/imprinting/implink.html]). These regions were first defined by the elegant use of chromosomal translocations to derive embryos uniparental for specific chromosomal regions, with their inheritance having overt phenotypic effects on embryogenesis, postnatal growth, and behavior (6). Some of the imprinted genes responsible for these phenotypes have been identified (3, 8, 15, 24, 47, 48). Though these translocation studies may have been exhaustive in defining regions which when inherited uniparentally result in a severe phenotype, not all imprinted genes in the mouse map to these regions as defined above. Five imprinted genes have been localized outside imprinted loci (*Ins1* on chromosome 19 [14], *Grf1* on chromosome 9 [32], *Peg1* [*Mest*] on chromosome 6 [24], *Nnat* on chromosome 2 [46], and *Impact* on chromosome 18 [16]). Two of these genes, *Grf1* and *Peg1*, when mutated result in the newborns exhibiting subtle postnatal growth and/or behavioral defects (J. M. Itier, G. L. Tremp, J. F. Leonard, M. C. Multon, G. Ret, F. Schweighoffer, B. Tocque, M. T. Bluet-Pajot, V. Cormier, and F. Dautry, Letter, Nature **393:**125–126, 1998). These results reveal that imprinted genes are more widely distributed in the mouse genome than previously anticipated and that their mutation can result in subtle phenotypes. However, genes mapping to other imprinted regions, such as the proximal region of chromosome 6 and for the middle and distal regions of chromosome 12, both of which are associated with embryonic lethality, remain to be identified. Consequently, the identification of other imprinted genes in chromosomes 6 and 12 or other unknown imprinted regions is of major importance, as this may provide further insights into the role(s) of imprinting in mammalian development, its contribution to various disease processes in humans, and, ultimately, why and how this form of gene regulation evolved in mammals.

Here we describe a procedure by which androgenetic (AG) and parthenogenetic (PG) mouse embryonic fibroblast (MEF) lines that stably retain the parent-of-origin pattern of imprinted gene expression were established in culture. We used them as a source of mRNA for a suppressive subtractive screen for paternally expressed genes. We identified two novel im-

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printed genes, one of which, the gene for ε-sarcoglycan (*Sgce*), maps to proximal chromosome 6, while the other, *Zac1*, maps to chromosome 10 in a region previously not known to be imprinted in the mouse. The importance of *Zac1* and *Sgce* imprinting is discussed in the context of mouse development and human disease. Furthermore, these cell lines will be an important source of material for searching for other imprinted genes and for studying various questions related to imprinting.

MATERIALS AND METHODS

Embryos and the derivation of fibroblasts. MEFs were derived by explanting and culturing day 13 (d13) (day of plug ≡ d1) embryos after removing the head and internal organs. PG MEFs were generated from 13-day PG↔chimeric embryos made by aggregating PG embryos, constitutively expressing the Neo^r gene (36), with wild-type (WT) embryos. The WT MEFs from the chimeras were selected against by culturing the primary explants in medium supplemented with G418 for the first three passages, allowing only the PG cells carrying the neomycin gene to survive (37). AG MEFs were generated from chimeras made by injecting AG embryonic stem (ES) cells (25), transfected with the *Pgk*Neo cassette so that they constitutively expressed a Neo^r gene, into blastocysts (35) with their subsequent isolation by the same procedure used to derive the PG MEFs.

cDNA subtraction and differential screening. Total RNA from MEFs, PG MEFs, and AG MEFs was isolated using the RNeasy procedure (Qiagen), and poly(A)$^+$ RNA was purified with the poly(A) Track mRNA isolation system (Promega). Suppressive subtractive hybridization was carried out using the PCR-Select cDNA subtraction kit (Clontech) according to the manufacturer's protocol. MEF cDNA was used as the tester and PG MEF cDNA was used as the driver in the forward reaction which was designed to enrich the tester, MEF cDNA, for paternally expressed genes not present in the PG MEF cDNA. The subtraction was also performed in reverse by using PG MEF RNA as tester and MEF RNA as driver, to generate a probe lacking paternally expressed genes. Differential screening was performed by high-throughput cDNA array analysis using the PCR-Select differential screening kit (Clontech). Clones were blotted simultaneously on two membranes, hybridized with the forward probe and the reverse probes, and quantified by phosphorimager analysis.

Reverse transcriptase (RT) PCR. Total RNA was extracted with the RNeasy columns (Qiagen) and treated with RNase-free DNase I (Promega) to eliminate residual genomic DNA. Amplification consisted of 30 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 60 s followed by one cycle at 72°C for 7 min. Primers for *Igf2*, *Igf2r*, *H19*, and *Snrpn* were as described previously (41). Other primers were as follows: *Ndn*, 5′ CAGCCGAGGTCCCCGACTGTGAG and 3′ GCAG CCCGAACACTCTGGCGAGG; *p57^{kip2}*, 5′ CCGCGCAAACGTCTGAGAT GAG and 3′ CACCTTGGGACCAGCGTACTCC; *Grb10*, 5′ CAACGATATT AACTCGTCCGTGG and 3′ CCACTTCTCACATCTGCCACAATG; *Peg1* (*Mest*), 5′ AGCTCAGTGGTAGTGTGCCTGCC and 3′ TCCACGTCAGCCC TGGAGGAGCT; *Sgce*, 5′ GGGGTGGCAGAGTGCCGCTTCC and 3′ GGC AGCACATGATATAAGCGAG; *Zac1*, 5′ ATCCTGTTCCTACCTCATATGC and 3′ CTGGATCTGCAACTGAAACTGTGG; *Gas2*, 5′ CACAGAGAAGCT GTGTTTAGGATGATC and 3′ GATATGTCCTGGGTATACAGTCTGT; *Igfbp5*, 5′ GCAAGGGCTAAGGAGACACTCCCC and 3′ GGCTAGAGCTG AAAGCAAAAGGGC; and *Rpl19*, 5′ CTGAAGGTCAAGGGGAATG and 3′ GGACAGAGTTTTGATGATCTC.

Virtual Northern blotting. For virtual Northern blot analysis, double-stranded cDNA was synthesized by using the SMART cDNA synthesis kit (Clontech). Double-stranded cDNA (0.5 μg) was electrophoresed on a 1.2% agarose gel, transferred to a nylon membrane, and hybridized with the indicated labeled probe.

Whole-mount in situ hybridization. Dissected embryos were processed for in situ hybridization as described previously (18). *Zac1* (1 to 1425) and *Sgce* (872 to 1422) sense and antisense riboprobes were synthesized from the appropriate mouse cDNA clones.

Chromosomal mapping. Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as described previously (7). A total of 205 N_2 mice were used to map the *Sgce* and *Zac1* loci. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described previously (19). By use of the *Sgce* probe, an ~500-bp fragment of mouse cDNA, fragments of 8.6, 3.4, 2.2, and 1.6 kb were detected in *Sca*I-digested C57BL/6J DNA and fragments of 8.6, 3.7, 2.8, and 1.6 kb were detected in *Sca*I-digested *M. spretus* DNA. The *Zac1* probe, an ~1.2-kb fragment of mouse cDNA, detected an ~20.0-kb *Sac*I fragment in C57BL/6J DNA and a 14.0-kb *Sac*I fragment in *M. spretus* DNA. The presence or absence of the *M. spretus*-specific fragments was monitored in backcross mice. A description of the probes and restriction fragment length polymorphisms (RFLPs) for the loci linked to *Sgce* including *Calcr*, *Met*, and *Cpa* has been reported previously (17, 44); those linked to *Zac1* include *Estra*, *Myb*, and *Lama2* (20, 29). Recombination distances were calculated using Map Manager, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

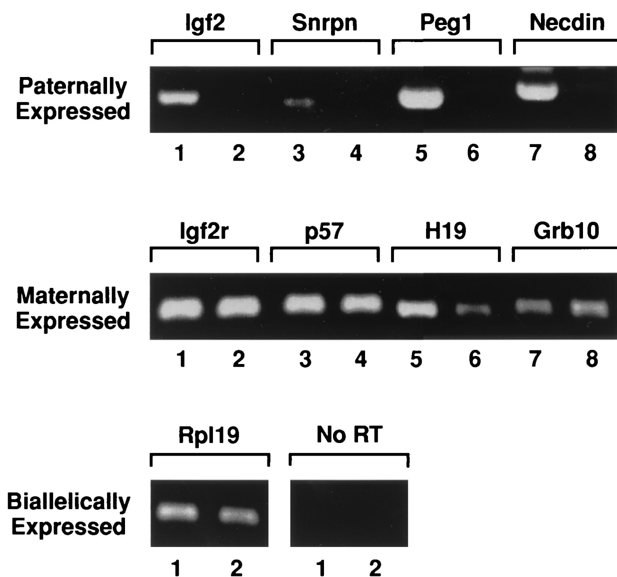

FIG. 1. Appropriate expression of known imprinted genes in PG MEFs. Total RNA isolated from PG and WT MEFs was analyzed by RT-PCR (lanes 1, 3, 5, and 7 are WT MEFs, and lanes 2, 4, 6, and 8 are PG MEFs).

RESULTS

Establishment of uniparental primary MEFs. To circumvent the difficulties of producing uniparental embryos in sufficient numbers, AG and PG primary MEFs were established from chimeras made between WT embryos and AG ES cells or PG embryos, respectively (25). The AG ES cells and the PG embryos used to make the chimeras both constitutively expressed the Neo^r gene and so were resistant to neomycin. AG and PG fibroblasts were isolated from the WT cells by culturing the explanted chimeras in high concentrations of neomycin for the first three passages. To ensure that these lines had stably retained their imprinted status, the expression of eight known imprinted genes, together with biallelically expressed genes, was analyzed by RT-PCR in WT MEFs, isolated from fertilized embryos, and PG MEFs. Paternally expressed genes such as *Igf2*, *Snrpn*, *Peg1* (*Mest*) (referred to as *Peg1*), and *Ndn* were detected in WT MEFs (Fig. 1, lanes 1, 3, 5, and 7) but not in PG MEFs (lanes 2, 4, 6, and 8). In contrast, maternally expressed genes such as *p57^{kip2}*, *Igf2r*, *H19*, and *Grb10* were expressed in both PG MEFs and WT MEFs (Fig. 1, middle panel). The biallelic genes *Rpl19* and *G3pdh* (data not shown) were expressed in both lines at similar levels (Fig. 1, bottom panel). These results clearly demonstrate that PG MEFs retained the appropriate expression pattern of several known imprinted genes. These lines were then used as a source of mRNA for cDNA screening of paternally expressed genes using a suppressive subtractive hybridization procedure.

Identification of novel imprinted genes. To identify genes exclusively expressed from their paternal allele, cDNA libraries made from WT and PG MEF mRNAs were used in a suppressive subtractive hybridization (9) as tester and driver, respectively. Following subtractive hybridization, a cDNA library was prepared and screened using probes enriched in paternally expressed genes (forward subtracted) versus those lacking paternally expressed genes (reverse subtracted).

A total of 1,200 clones were screened for differential hybridization with the forward and reverse probes. Approximately 10% of these clones showed strong hybridization with the forward but not the reverse probe and were sequenced. Among

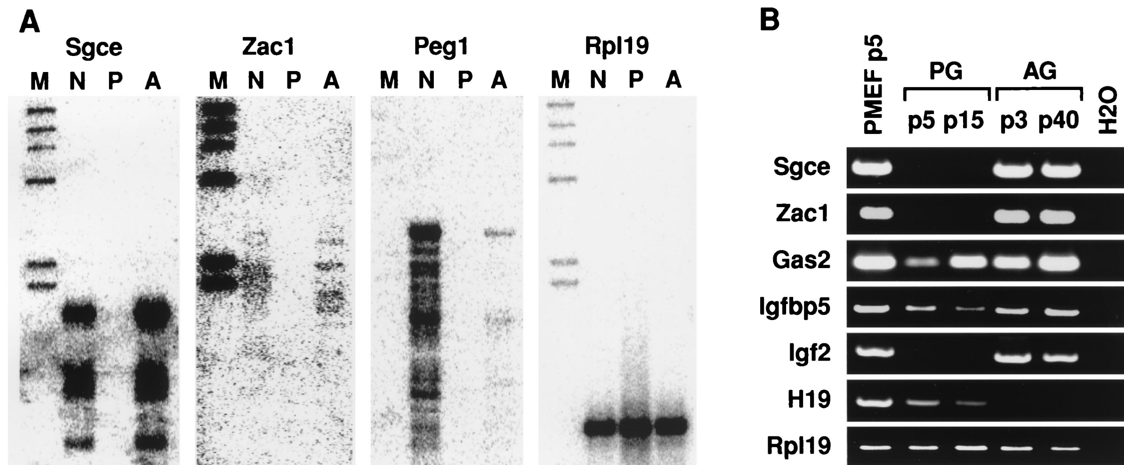

FIG. 2. Differential expression of clones isolated from the screen by virtual Northern blotting (A) and RT-PCR (B). *Rpl19* probe was used as a control for both the virtual Northern blotting and RT-PCR. Both *Sgce* and *Zac1* are expressed only in the AG and WT MEFs by both virtual Northern blotting and RT-PCR. Other genes such as *Gas* and *Igfbp5* are biallelically expressed. The maternally expressed *H19* was analyzed by RT-PCR as a control for AG MEFs not expressing maternally expressed imprinted genes (abbreviations: M, markers; N, WT MEFs; P, PG MEFs; A, AG MEF cDNA).

these differentially expressed clones, one was identified as *Igf2* and six were *Peg1* (*Mest*), these both being known as maternally imprinted genes, and as well there were two previously unidentified candidate imprinted genes, that for ε-sarcoglycan (*Sgce*) and *Zac1*. The presence of *Igf2* and *Peg1* (*Mest*) in the isolated clones provided a clear indication that the screened cDNA library was enriched in paternally expressed genes. The expression of candidate imprinted genes was further analyzed by virtual Northern blotting in which mRNA from the WT, AG, and PG MEFs was reverse transcribed into cDNA and probed with the candidate imprinted genes. AG MEF cDNA was used as a positive control to confirm the paternal expression of candidate genes which should be absent in PG MEFs but present in WT MEFs. As shown in Fig. 2A, *Sgce* (isolated in four independent clones) and *Zac1* (one clone) are expressed in AG and WT MEFs but not in PG MEFs. *Peg1*, a paternally expressed gene, and *Rpl19*, a biallelic gene, are shown as controls. The potential imprinted status of *Sgce* and *Zac1* was further analyzed by RT-PCR of the AG, PG, and WT cDNAs. We found high levels of expression of both *Sgce* and *Zac1* in AG MEFs and WT MEFs but no expression in PG MEFs (Fig. 2B). Other genes such as *Gas2*, *Igfbp5*, *Sarp*, and *Cnp6*, as well as two expressed sequence tags, were differentially expressed but not imprinted as shown by RT-PCR (Fig. 2B and data not shown). These results indicate that about 10% of the clones that were found to be differentially expressed in the enriched cDNA library were imprinted genes. The other nonimprinted genes showing differential expression were probably detected due to differences in the growth rates between our AG and PG fibroblast lines (unpublished observations).

Imprinting of *Sgce* and *Zac1* in vivo. To confirm that *Sgce* and *Zac1* were imprinted, allele-specific expression of the genes was analyzed in interspecific hybrid embryos and adult tissues using restriction enzyme polymorphisms in their cDNA sequences. A polymorphic change from GTAC to ATAC between *Mus musculus* and *M. spretus* deletes an *Rsa*I site in the cDNA of *Sgce* from *M. spretus* (Fig. 3A). After PCR, a 404-bp fragment was isolated and digested with *Rsa*I. cDNA from *M. musculus*, following *Rsa*I digestion, yielded two fragments of 280 and 124 bp (Fig. 3A, lane 5). In contrast, only the undigested fragment of 404 bp was found in *M. spretus* cDNA (Fig. 3A, lanes 6 and 7). When the cDNA from C57BL/6J × *M. spretus* interspecific hybrid embryos and a variety of adult tissues was analyzed, only the undigested *M. spretus* paternal 404-bp fragment (Fig. 3A, lane 8) was detected in the majority of these samples. The one exception was the adult brain, where, in addition to the predominant 404-bp paternal band, a signal from the 280- and 124-bp bands indicative of the maternal allele is weakly expressed in this tissue (Fig. 3A, lane 9). *M. musculus* males mated with *M. spretus* females do not breed successfully, and reciprocal crosses could not be made. As a control, to ensure that both alleles could be amplified in the same reaction, total RNA from both *M. musculus* and *M. spretus* was mixed and subjected to RT-PCR, and equal amplification of both alleles is shown in Fig. 3A, lane 13. This demonstrated that *Sgce* was transcribed from the paternal allele in the embryo and the majority of adult tissues and that therefore *Sgce* is maternally imprinted in vivo.

A similar analysis was performed to assess the imprinted status of *Zac1*. A polymorphism was found between *M. musculus* and *Mus musculus castaneus*. In both, the primers amplified a 465-bp fragment after PCR. A change in the sequence from TCTGG to CCTGG created an additional *Bst*NI site in *M. m. castaneus* cDNA. Restriction digestion with *Bst*NI resulted in three fragments (311, 124, and 30 bp) in *M. m. castaneus* (Fig. 3B, lane 1) versus two fragments (311 and 154 bp) in *M. musculus* cDNA (Fig. 3B, lane 4). Analysis of cDNA from E13 embryos of both reciprocal crosses (C57BL/6J × *M. m. castaneus* and *M. m. castaneus* × C57BL/6J) revealed that the restriction pattern always corresponded to that of the father, demonstrating that *Zac1* was expressed from the paternal allele and therefore is maternally imprinted (Fig. 3B, lanes 2 and 3). Subsequent analysis of cDNA from adult C57BL/6J × *M. m. castaneus* tissues revealed that paternal expression was retained in the pituitary, ovary, lung, brain, and heart tissue (lanes 5, 6, and 10 to 12) but that equal expression of the maternal and paternal alleles occurred in the liver (lane 9).

Expression of *Sgce* and *Zac1* in embryos and adult tissues. Using RT-PCR (Fig. 3) and Northern blotting (data not shown), we observed that *Sgce* and *Zac1* are expressed in all adult tissues analyzed, including skeletal muscle, kidney, liver, lung, brain, and heart. During embryogenesis, in midgestation (d9.5) embryos, *Sgce* and *Zac1* are both detected by in situ hybridization. *Zac1* is strongly expressed in the liver primor-

FIG. 3. (A) Monoallelic expression of *Sgce* in interspecific embryos by RFLP analysis. A PCR fragment was amplified from both C57BL/6J and *M. spretus* and sequenced. In lanes 1 to 4, the 404-bp fragment is amplified in both species. In lanes 5 to 7, only the C57BL/6J product is cut with *Rsa*I. In embryonic cDNAs from the interspecific cross, the 404-bp fragment is undigested by *Rsa*I, showing exclusive paternal (*M. spretus*) expression (lane 8), as seen also for adult tissues (lanes 10 to 12). Only in the adult interspecific brain is weak expression of the maternal allele detected (lane 9). Lane 13 is a control showing coamplification of *M. musculus* and *M. spretus* alleles after they are mixed in the same reaction. (B) Paternal expression of *Zac1* in interspecific embryos by RFLP analysis. A 465-bp PCR fragment was amplified from both C57BL/6J and *M. m. castaneus* and digested with *Bst*NI. *M. m. castaneus* produces three fragments, of 311, 124, and 30 bp (lane 1), whereas the C57BL/6 allele produces two fragments, of 311 and 154 bp (lane 4). Embryo and MEF interspecific cDNA from reciprocal crosses revealed that only the paternal allele is expressed (lanes 2, 4, 13, and 14). In adult tissues, biparental expression is most apparent in the liver (lane 9) and to a lesser extent in kidney and skeletal muscle (lanes 7 and 8).

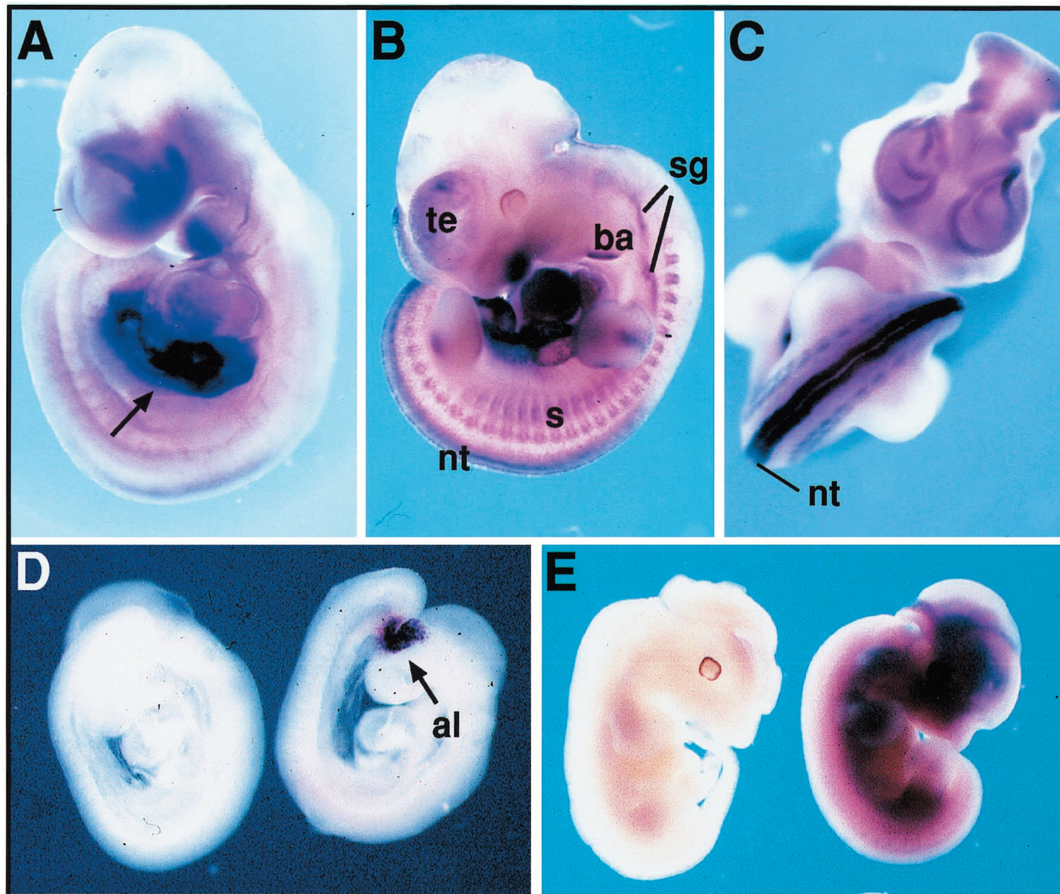

FIG. 4. Detection of *Zac1* and *Sgce* transcripts in mouse embryos by in situ hybridization. (A) *Zac1* is highly expressed in the liver primordium and body wall of the umbilical region (arrow) of a d9.5 embryo. (B) At d11, *Zac1* expression is observed in neural tube (nt), somites (s), sympathetic ganglia (sg), distal second branchial arch (ba), and telencephalic vesicles (te). (C) *Zac1* showed a strong expression in the neural tube (nt) at d10.5. (D and E) *Sgce* expression is restricted to the allantoic region (al) at d9.0 (D, right), whereas at d12 (E, right) this transcript is widely distributed (embryos hybridized with a sense probe are shown on the left of each panel).

dium as well as the umbilical region (Fig. 4A). Subsequently, in d11 to d12 embryos, *Zac1* showed high levels of localization to the neural tube, with weaker expression in the somites, sympathetic ganglia, distal second brachial arch, and telencephalic vesicles (Fig. 4B and C). *Sgce* is first detected in the allantoic region (Fig. 4D), and in later stages (d11 to d12), its expression becomes more widespread and diffuse among many tissues (Fig. 4E).

Mapping of *Sgce* and *Zac1*. The mouse chromosomal locations of *Sgce* and *Zac1* were determined by interspecific backcross analysis using progeny derived from matings of (C57BL/6J × *M. spretus*)F_1 × C57BL/6J mice (7). *Sgce* is located in the proximal region of mouse chromosome 6 linked to *Calcr*, *Met*, and *Cpa*. *Zac1* mapped to the proximal region of mouse chromosome 10 linked to *Estra*, *Myb*, and *Lama2* (Fig. 5). The proximal region of mouse chromosome 6 shares a region of homology with human chromosome 7q. Our placement of *Scge* in this interval is consistent with the assignment of *SGCE* to 7q21-q22 (28). The proximal region of mouse chromosome 10 shares a region of homology with human chromosome 6q, and *ZAC* has been mapped to human chromosome 6q24-q25 (43).

DISCUSSION

Here we report the identification of two novel paternally expressed imprinted genes, *Sgce* and *Zac1*, by the subtractive hybridization screen of a cDNA library derived from the WT and PG MEF mRNAs. Previous attempts at deriving ES lines from AG and PG embryos to analyze imprinted gene expression in vitro were only partially successful since the cell lines showed extensive leakiness in retaining the appropriate expression of imprinted genes following their differentiation (2, 40). By making chimeras and then selecting for AG and PG fibroblasts from explanted midgestation embryos, we established lines that are uniform in their differentiation and stable in the expression of known imprinted genes. These lines provide distinct advantages in searching for novel imprinted genes in that they allow workers to avoid having to repeatedly produce AG and PG embryos, they can be expanded in vitro so that sufficient quantities of mRNA can be isolated, and they provide a quick and efficient means to screen for candidate imprinted genes.

We screened 1,200 clones, compared to some 50,000 analyzed in a previous report (21). Our screen resulted in the isolation of two known imprinted genes, *Peg1* and *Igf2*, and two genes that were not known to be imprinted, *Sgce* and *Zac1*. Using polymorphisms in the *Sgce* and *Zac1* cDNAs, we showed that both of these genes were imprinted in vivo, in embryonic fibroblasts and the majority of adult tissues.

Sgce is one of five members in the sarcoglycan family, transmembrane proteins which are components of the dystrophin-sarcoglycan complex (10, 28). This complex forms a structural

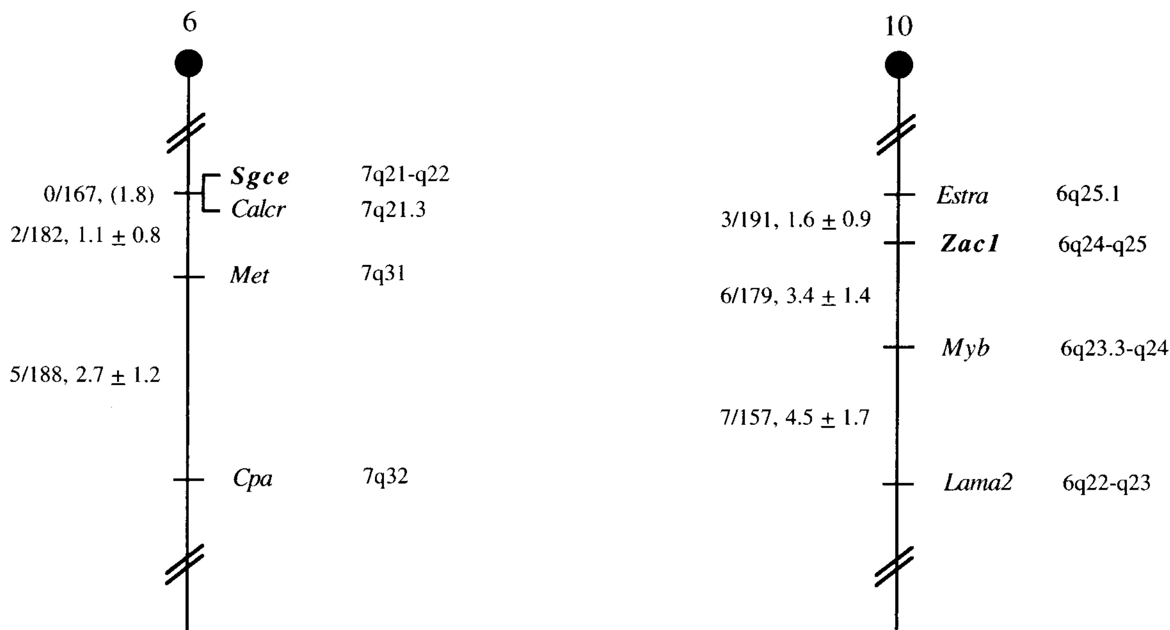

FIG. 5. Murine chromosomal location of *Sgce* and *Zac1*. Partial chromosome 6 and 10 linkage maps showing the location of *Sgce* and *Zac1* in relation to linked genes are shown. The number of recombinant N_2 animals over the total of N_2 animals typed together with the recombination frequency (genetic distance in centimorgans ± standard error) is shown for each pair of loci on the left of the map. Where no recombination was detected between loci, the upper 95% confidence limit of the recombination distance is shown in parentheses. The positions of homologous loci on human chromosomes are shown to the right.

link between the extracellular matrix and cytoskeleton predominantly in the various types of muscle. The α-, β-, γ-, δ-, and ε-sarcoglycans are all found in skeletal and cardiac muscle (38). Unlike the other members, *Sgce* is more widely expressed among adult and embryonic tissues, as shown by our in situ analysis (10), which is consistent with the recent demonstration that it is predominantly localized to smooth muscle, particularly that of the blood vessels (39). Embryonic expression of the other sarcoglycans is first detected during later stages of myogenesis with expression being largely restricted to the various musculatures throughout adulthood. *Sgce* may therefore have a more widespread role in maintaining cell adhesion and tissue integrity than those of the other sarcoglycans. In the mouse, *Sgce* maps to the very proximal region of chromosome 6 close to the centromere. This is within the region where, according to the more recently refined imprinting map, maternal uniparental inheritance is associated with embryonic lethality, excluding *Peg1* (C. V. Beechey and B. M. Cattanach, 1998, mouse imprinting data and references [http://www.mgu.har.mrc.ak.uk/imprinting/implink.html]). Since *Sgce* is widely expressed in the embryo, it is a candidate gene for embryonic lethality associated with maternal uniparental isodisomy in this region of the chromosome. The proximal region of mouse chromosome 6 shares homology with human chromosome 7q21-q22 (Fig. 5), and maternal uniparental disomy for chromosome 7 has been sporadically associated with Silver-Russell syndrome (SRS) (45), a condition characterized by pre- and postnatal growth retardation. In mice, mutation of the paternal allele of *Peg1* (*Mest*) resulted in fetal growth retardation and was considered a candidate for SRS (24). However, a recent analysis has suggested that *PEG1* has no role in SRS (33). Therefore, *Sgce* is another candidate for this condition.

Zac1 has been independently identified in two previous screens, the first for genes regulated by neuropeptides (34) and the second for genes whose expression was lost on transformation in a rat ovarian carcinoma model (1). *Zac1* is a zinc finger DNA binding protein of the C_2H_2 family. Its biological functions remain unclear, although in mice (Fig. 4 and data not shown) and humans it is most strongly expressed in the pituitary gland and to a lesser extent in other tissues (43). *Zac1* maps to the proximal region of mouse chromosome 10, at a region homologous to 6q24-q25 in the human. Parent-of-origin defects have been reported for chromosome 6 with paternal duplication-isodisomy being associated with fetal growth retardation (often severe) and transient neonatal diabetes mellitus (12, 13, 42). However, maternal duplication or a deletion encompassing this region does not impair growth (22, 31). These observations suggest that *Zac1* (*ZAC*) or some closely linked gene(s) is a candidate gene which, when paternally duplicated, may be responsible for fetal growth retardation and transient neonatal diabetes mellitus.

The distal region of chromosome 6 also shows a high incidence of loss of heterozygosity in the development of a variety of tumors, particularly those of the breast, ovary, and cervix (11, 26, 30). Transfection of mouse and human cells with *Zac1* (*ZAC*) induces proliferative arrest at G_1 and apoptosis, suggesting that *Zac1* (*ZAC*) could function as a tumor suppressor gene (34, 43; our unpublished observations). Whether imprinting of *ZAC*, as a potential tumor suppressor, contributes to tumor formation is unclear. However, in an analysis of 42 primary breast carcinomas, none showed any detectable mutation in the coding sequence of *ZAC* but 8 failed to express *ZAC* or expressed it at very low levels (5). Expression could, however, be induced by treatment of the carcinomas with the demethylating reagent 5-azacytidine, suggesting that expression may be epigenetically regulated. It would be of interest to determine whether in these eight lines the paternal allele had been lost, leaving the intact but transcriptionally repressed maternal allele. Furthermore, *Zac1* can be induced in our PG fibroblasts following treatment with 5-azacytidine (unpublished observations). Gene targeting and transgenic approaches to manipulating the expression of *Zac1* and *Sgce* should aid in determining the role(s) of these imprinted genes

in the development and regulation of both embryonic and cellular growth.

ACKNOWLEDGMENTS

We thank Ruth Wolf, Terry Sulivan, Teresa Shatzer, Lori Sewell, Andreé Reuss, and Deborah B. Householder for excellent technical assistance; Anne Wang, Mark Lewandowski, and John Hagan for critical reading of the manuscript; and Rachel Wevrick for fruitful discussions and advice.

This research was supported, in part, by the National Cancer Institute, DHHS, under contract with ABL.

ADDENDUM IN PROOF

Kamiya et al. (Hum. Mol. Genet. **9**:453–460, 2000) reported that ZAC is imprinted in humans.

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