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Sequence analysis of the rat *Brca1* homolog and its promoter region

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Abstract. Since the identification of the human breast and ovarian cancer gene, BRCA1, a large spectrum of germline mutations has been characterized that predispose women to developing these diseases. We have determined the complete coding sequence for the rat BRCA1 homolog and compared it with those of the mouse, dog, and human to help identify the important functional domains of the BRCA1 protein. The overall rat *Brcal* amino acid identity compared with the predicted mouse, dog, and human gene products is 81%, 69%, and 58%, respectively. In spite of this low overall homology, the amino terminal RING finger domain and one of two nuclear localization signals are highly conserved among these species. In addition, two BRCT domains at the carboxy terminus and a highly acidic region are relatively well conserved. We have also identified several putative regulatory elements through comparison of the bidirectional BRCA1 promoter regions among the rat, mouse, and human genes. These include motifs for CCAAT and G/C boxes, as well as potential SP1, CREB, and NFkB transcription factor binding sites. Finally, analysis of splice variants from rat mammary gland, ovary, testis, spleen, and liver tissues revealed that, while alternative transcripts are detectable, full-length transcripts are the predominant steady-state form.

Introduction

The early-onset breast and ovarian cancer gene, BRCA1, was identified in 1994 by positional cloning (Miki et al. 1994). The inactivation of both BRCA1 alleles appears to be necessary for neoplastic progression in the breast and ovary, suggesting BRCA1 acts as a tumor suppressor gene (Stratton 1996). Likewise, the introduction of wild-type BRCA1 into breast or ovarian cancer cell lines has been reported to result in growth inhibition, whereas its transfer into lung or colon cancer cell lines has no effect on growth rate (Holt et al. 1996). Although the primary function of BRCA1 remains to be determined, there is good evidence that this protein is involved in multiple cellular processes. The carboxy terminal region of the human BRCA1 protein can function as a transactivator of transcription (Chapman and Verma 1996; Monteiro et al. 1996, 1997; Zhang et al. 1998; Ouchi et al. 1998) and contains two BRCT (BRCA1 C terminus) regions (Koonin et al. 1996). The highly conserved zinc binding RING finger domain at the amino terminus of BRCA1 has been shown to interact with at least two proteins. The first of these interacting proteins, BARD1, also contains RING finger and BRCT domains (Wu et al. 1996). The BRCA1 RING domain also interacts directly with a newly identified ubiquitin hydrolase, BAP1, which can suppress cancer cell growth when overexpressed (Jensen et al. 1998). In addition, BRCA1 has been shown to complex with RAD51, suggesting that it may be involved in meiotic and mitotic recombination as well as one or more DNA damage response pathways (Scully et al. 1997a, 1997b). Two recent studies have demonstrated an interaction between BRCA1 and p53 that stimulates transcriptional activity (Zhang et al. 1998; Ouchi et al. 1998). These functions are consistent with the observation that BRCA1 localizes to discrete nuclear dots in a cell cycle-dependent manner, and these protein complexes are sensitive to various forms of DNA damage (Scully et al. 1996, 1997b).

We and others previously characterized the mouse homolog of the BRCA1 gene and mapped it to the distal region of mouse Chromosome (Chr) 11 (Able et al. 1995; Bennett et al. 1995). Murine *Brca1* transcripts are highly expressed in the mouse testis and moderately expressed in several lymphoid organs, consistent with observations in humans (Miki et al. 1994). *Brca1* is also highly expressed in various cell types of the mammary gland and ovary during normal growth and differentiation (Blackshear et al. 1998; Lane et al. 1995; Marquis et al. 1995; Phillips et al. 1997).

The human BRCA1 gene was originally reported to share a 295-nucleotide bidirectional promoter with the upstream NBR1 gene previously designated 1A1.3B (Brown et al. 1994). Surprisingly, there is a genomic duplication of this human region (Barker et al. 1996; Brown et al. 1996; Smith et al. 1996) which is not conserved in the mouse (Chambers and Solomon 1996). Two primary transcriptional start sites have been identified in the human BRCA1 sequence that give rise to transcripts containing exons 1a or 1b, yet there have been no reports of multiple mRNA species detectable in mouse tissues (Xu et al. 1995).

The rat has been used extensively as a model to study normal mammary gland development and carcinogenesis. Chen and associates have reported a partial sequence of the rat *Brca1* gene and mapped it to Chr 10, using a $(WKY \times WF)F_1 \times WF$ backcross (Chen et al. 1996). This observation is consistent with the remarkably high degreee of synteny observed among rat Chr 10, mouse Chr 11, and human Chr 17 (Remmers et al. 1992; Yamada et al. 1994). Interestingly, rat mammary tumors induced with 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine were shown to have specific allelic losses in the region of rat Chr 10 that is syntenic with the BRCA1 region on human Chr 17q (Toyota et al. 1996).

Interspecies protein comparisons of the BRCA1 gene product may identify functionally significant protein domains that should be highly conserved among species. In this report we determined the sequence of the complete coding region for the rat BRCA1 homolog. In addition, approximately 1.4 kb of upstream promoter regions from the rat and mouse were sequenced and compared with the homologous human BRCA1 region. Interspecies comparisons of these homologs have enabled us to identify several conserved regulatory elements in the promoter region that may control BRCA1 expression at the transcriptional level. Finally, in contrast to the two transcriptional start sites in the human BRCA1 gene, the rat homolog appears to use the single first untranslated exon to produce the full-length transcript that predominates in all tissues examined.

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Materials and methods

Characterization of the rat Brca1 homolog. The initial rat *Brca1* sequence was amplified from Sprague-Dawley genomic DNA with oligonucleotides on the basis of mouse exon 11 sequences (5′-GCACATTTATTACAGGACCACA and 5′-ACTTCCACCT-CAGCCTATTTTT). The resulting fragment was isolated, sequenced, and determined to be rat *Brca1* from nucleic acid homology with mouse and human. cDNA was generated from rat testis mRNA with the Reverse Transcription System (Promega). A series of rat-specific primers were used in combination with various mouse oligonucleotides to isolate the entire rat *Brca1* coding sequence and the bidirectional promoter region shared by *Brca1* and the upstream gene *Nbr1* (primer sequences available on request from L.M. Bennett). The PCR conditions were 94°C for 3 min followed by 35 cycles of: 94°C for 1 min 55°C for 1 min 72°C for 1 min. The coding information was generated by the direct sequencing of cDNA PCR products. Isolated PCR products were sequenced with a PRISM dye terminator kit on an ABI 373 automated fluorescent sequencer (Applied Biosystems). Sequence comparisons of the rat, mouse, and human BRCA1 genes were performed with GAP, BESTFIT, PILEUP, and MOTIFS software from the GCG package (Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group).

Northern analysis. *Brca1* expression was examined in multiple rat tissues by Northern analysis (Clontech) with a rat exon 2 through 7 cDNA probe generated with the primers 5′-AAATGGATTTATCTGCTGTTCG and 5′-ATCTGTTGAAGTTTTTTGACGC. The filter was hybridized in Hybrisol I (Oncor) at 42°C overnight and washed twice in 2×SSC (1×SSC: 1.5 M NaCl/0.15 M sodium citrate, pH 7.0) for 5 min at room temperature, twice in 2×SSC/1% SDS for 30 min at 65°C, and twice in 0.1× SSC/0.1% SDS at room temperature for 30 min. Autoradiography was performed at −70°C with X-Omat AR film (Kodak) for 7 days.

Cloning of the rodent promoter regions. The mouse *Brca1* promoter region was isolated from a previously described P1 genomic clone (Bennett et al. 1995) with a mouse primer specific for exon one 5′-AGAAAAAAGATCCGTACTTCCA and an SP6 vector primer. A PCR product was generated from rat genomic DNA with a murine primer from intron 1 of Nbr1 (5′-AATCGTCGCGGCCAGCT) and a rat-specific primer for exon 1 of *Brca1* (5′-CTTTCTTCCGAGAAAAACCT). These PCR products were cloned into pSKII (Stratagene) and sequenced as described above. The cloning of these promoter fragments by PCR may have resulted in *Taq* polymerase errors.

Identification of 5′ ends of the rat Brca1 transcripts. 5′ ends of the rat *Brca1* gene were identified with Marathon-ready rat testis cDNA (Clontech) to generate 5′ cDNA fragments. A standard 50-μl PCR reaction was set up with 0.5 μl of rat cDNA, 1.5 mM $MgCl_2$, 200 nM forward primer (AP1, 5′ RACE System, Gibco/BRL) and a rat exon three reverse primer (5′-TTCTGGTTAAGGAGTTTCAGCA), 1× thermophilic DNA polymerase buffer (Promega), 100 nM dNTPs, and 0.13 units *Taq* polymerase. Nested PCR was performed with 5 μl of primary PCR product with the AP2 primer (5′ RACE System) and rat exon 2 reverse primer 5′-GGCCACGCGTCGACTAGTACACCGGTTCTTTGATCAGTTCC (modified to incorporate restriction enzyme sites for cloning manipulations). The products obtained from the nested PCR reaction were gel purified and cloned into pSKII. Twenty-two clones containing inserts of *Brca1* sequence were sequenced as described above.

Identification of alternatively spliced mRNA species. Total RNA isolated from rat tissues was used to prepare cDNA by reverse transcription (Promega). cDNA PCR was performed as described above with various combinations of rat-specific forward primers in exons 2, 9, 10, and 11 and reverse primers in exons 12, 13, and 16 (sequences available upon request). Discrete PCR products were isolated by agarose gel electrophoresis, cloned into pSKII, and sequenced as described above. The sequences were analyzed with the software contained in the Wisconsin GCG package.

Results

Interspecies comparison of the Brca1 coding region. A cDNA contig was assembled that spanned the entire rat *Brca1* coding region by use of a PCR-based approach with both rat genomic DNA and testis cDNA. The rat *Brca1* coding region of 5451 nucleotides encodes a predicted protein of 1817 amino acids (Fig. 1). This deduced amino acid sequence shares 81%, 69%, and 58% identity to the predicted mouse, dog, and human BRCA1 proteins, respectively. This was not unexpected, since the rat is evolutionarily more closely related to the mouse than to either the dog or human.

While overall sequence identity is low among these species, several regions are highly conserved (Fig. 1). The amino terminal end of the human BRCA1 gene product shares strong homology with a number of zinc-binding RING finger proteins. As previously reported, the amino terminal 100-amino acid region is the most homologous domain among the rat, mouse, dog, and human BRCA1 proteins (Bennett et al. 1995; Chen et al. 1995; Szabo et al. 1995). There is a 58-amino acid stretch between codons 17 and 74 that contains only two conservative substitutions among the four species. Rats and mice carry a D67E substitution, while the dog sequence has K56R alteration (Fig. 1). A bipartite nuclear localization signal (NLS) has been described in the human protein sequence (Chen et al. 1995; Thakur et al. 1997; Wilson et al. 1997), and the analogous regions in the rat (NLS1: 496–502 and NLS2: 599–610) are identical to human and mouse. Surprisingly, the dog sequence for NLS1 has diverged dramatically compared with the other mammalian species (Szabo et al. 1995). Consistent with other mammalian species, the rat *Brca1* protein contains a highly acidic domain (codons 1112–1447), including a region where only one basic amino acid is present among 16 acidic residues (codons 1301–1347). A distal carboxyl terminal domain (rat codons 1589–1740), which overlaps with the BRCT domains described previously (Koonin et al. 1996; Bork et al. 1997), shares 70% identity to mouse, dog, and human sequences.

Jensen and coworkers have proposed that the BRCA1 protein contains a granin motif that directs its secretion from the cell in a regulated manner (Jensen et al. 1996). Six of the seven amino acids that constitute the putative granin motif (rat codons 1178–1187) are conserved in rats; however, the leucine at position three, which is thought to be required for function, has diverged to aspartic acid and methionine residues in the rat, mouse, and dog proteins, respectively (Fig. 1). Likewise, 16 potential N-linked glycosylation sites are present in the human BRCA1 protein, but only one of these is preserved in the rat (codons 1509–1517), mouse, and dog gene products (Fig. 1).

The missense mutations, polymorphisms, and many of the unclassified human sequence variants that have been reported to the Breast Cancer Information Core database are highlighted in Fig. 1 (BIC database, June 1998). Missense mutations at 18 locations have been reported in the human. Twelve of the 18 amino acids at these positions are identical in the rat, mouse, and dog (Fig. 1), while four amino acid positions have highly conservative amino acid substitutions and two (Y105F in rodents and M1627S in the dog) represent nonconservative changes. In contrast, of the 25 residues where clear human polymorphisms have been identified, only 12 positions are identical among human, rodent, and dog sequences; four substitutions are conservative, and nine are nonconservative. Finally, 114 residues are designated as unclassified variants that alter an amino acid but have not been clearly shown to segregate with the disease in large cancer families, and their frequency in appropriate human control populations is unknown. Comparison of rat, mouse, and dog sequences reveals 50 residues that are identical among the four species, 23 at sites with conservative substitutions, and 41 with nonconservative changes.

Brca1 expression. BRCA1 expression has been shown previously to be high in the testis of both humans and rodents and moderately high in lymphoid tissues (Bennett et al. 1995; Chen et al. 1996; Miki et al. 1994). Northern analysis of mRNA from multiple rat

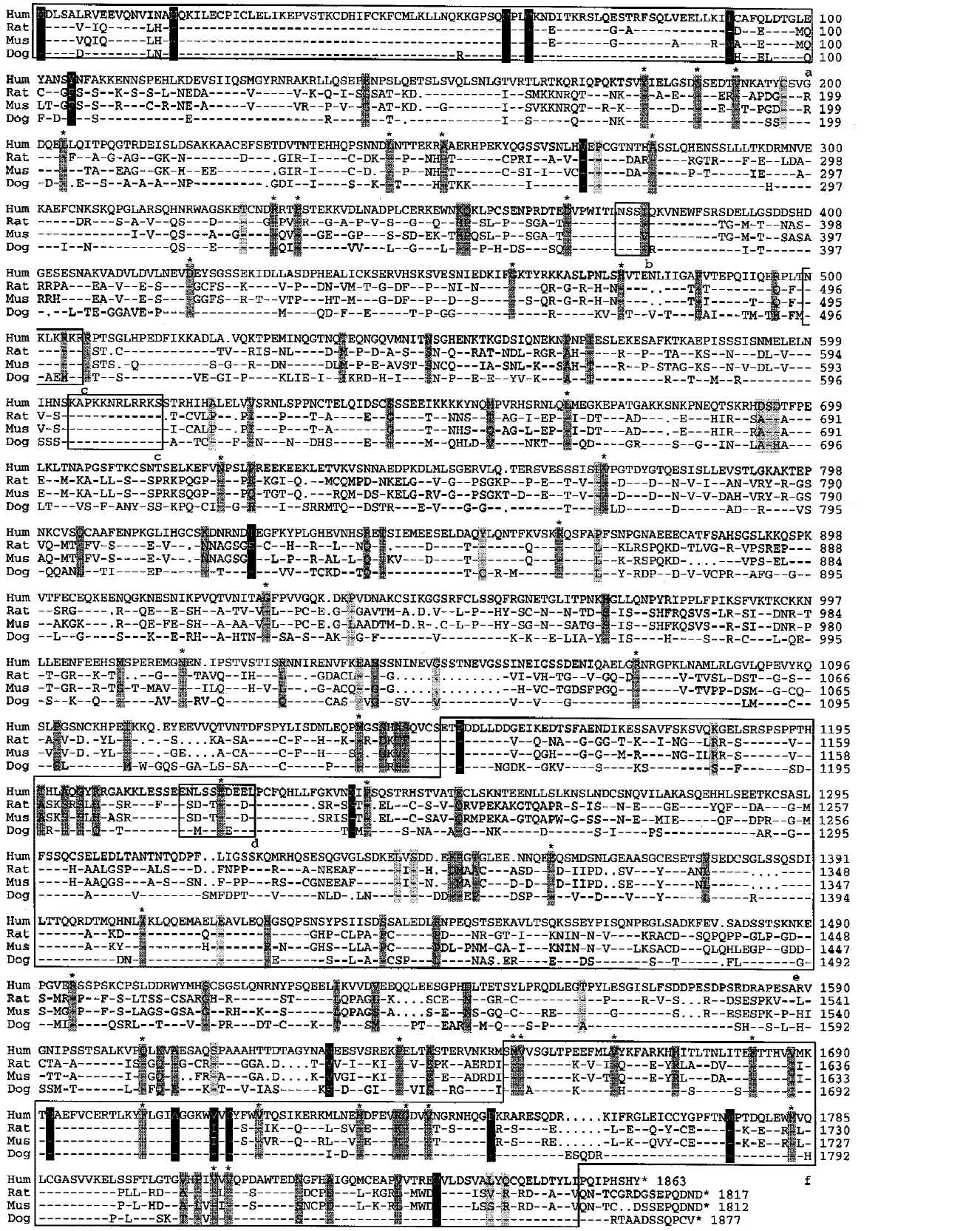

Fig. 1. Alignment of the predicted human (U14680), rat (AF036760), murine (U32446), and dog (U50709) *Brca1* amino acid sequences. Dashes represent amino acids of identity, and dots represent gaps in the sequence. Boxed regions include: (a) C3HC4 RING finger domain, (b) the only potential N-linked glycosylation site conserved among species, (c) bipartite nuclear localization signal, (d) putative granin motif, (e) acidic domain, (f) conserved BRCT repeats. Positions of missense mutations are indicated by black boxes, polymorphisms by light gray, and unclassified variants by dark gray. The asterisks designate missense changes at residues conserved in the four species.

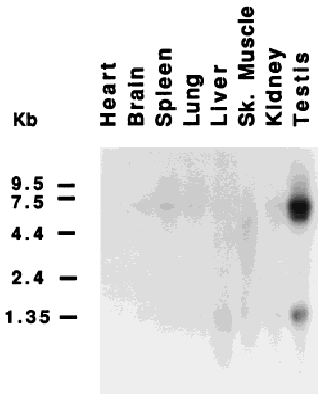

Fig. 2. Northern analysis of the expression of *Brca1* in mRNA isolated from various rat tissues. The filter was hybridized with an exon 2–7 probe.

tissues with a probe spanning rat exon 2 to exon 7 revealed a 7.5-kb transcript consistent with that predicted for the full-length *Brca1* gene (Fig. 2). As expected, the expression of *Brca1* is highest in testis, but present at lower levels in other tissues. In addition, transcripts are detectable in several tissues of approximately 1.4 kb, suggesting alternatively spliced forms of *Brca1*.

Splice variants that remove all or most of human exon 11 yield BRCA1 products that are localized to the cytoplasm instead of the nucleus where they may have distinct cellular functions relative to full length BRCA1 (Chen et al. 1995; Thakur et al. 1997; Wilson et al. 1997). RNA from five rat tissues was examined by cDNA PCR with a variety of primers, and five unique alternatively spliced Brca1 messages were identified (Table 1). Two of the mRNA species excise exons 3 or 5 through 15, resulting in severely truncated proteins, and are likely to represent the 1.4-kb species detected by Northern analysis (Fig. 2). Another spliced form removes only exon 5, which encodes 26 amino acids of the RING finger domain, and maintains the reading frame. This transcript is analogous to one identified previously in a normal human breast cDNA library (Miki et al. 1994). In addition, alternative transcripts were detected that excised exons 10 and 11, resulting in a severe frameshift, or deleted the 3′ end of exon 10 and all of exon 11, but maintained the open reading frame (Table 1). Although these rat Brca1 transcripts resemble the alternatively spliced forms observed in humans that lack exon 11, their steady state (ca. 4.5-kb transcripts) were undetectable by Northern analysis in multiple rat tissues (Fig. 2).

Interspecies comparison of the bidirectional Brca1/Nbr1 promoter region. The human and mouse BRCA1 and NBR1 genes lie in a head-to-head conformation, with transcription occurring in opposite directions (Brown et al. 1996; Chambers and Solomon 1996; Smith et al. 1996). The rat promoter sequence shares 90% and 68% identity to the mouse and human sequences, respectively. Comparison of the rat Brca1 promoter region to the human and mouse promoters indicates that the bidirectional nature of this region is conserved in rats and the rat Brca1/Nbr1 promoter lacks TATA boxes as previously reported for the human and mouse genes. Several putative transcriptional control elements are conserved among all three species in the 1.4 kb of upstream sequence that we have examined. Two CCAAT sites are conserved at positions 156 and 911 (nucleotide numbers given are for rat sequence; see Fig. 3 for alignment with mouse and human) and one G/C box at 274. In addition, there is a conserved SP1 site in exon 1 of the NBR1 gene at nucleotide 17, a potential NFkB site at 1131 in exon 1 of the *Brca1* gene, and a CREB site at 886. The rat promoter region contains CpG islands between nucleotides 18 and 401, including the first exon of Nbr1, and from nucleotides 991 to 1191, which encompasses the first exon of *Brca1* (GCG Wisconsin Package). Although wide variations exist among these species, sequence analysis indicates that the homologous regions in mouse and human are also significantly enriched for CpG sites.

Variability in 5′ ends of the rat Brca1 transcripts. 5′ RACE experiments were performed to determine whether the rat *Brca1* gene utilized an alternative first exon, as has been observed in the human BRCA1 promoter region (Xu et al. 1995). A rat Brca1 exon 5 primer was used to reverse transcribe 5′ ends from rat testis RNA followed by cDNA PCR. The resulting products were subjected to a second round of PCR with nested rat *Brca1* and anchor primers. This resulted in a diffuse product of approximately 250 bp that was purified by gel electrophoresis. Variation in the RACE product sizes obtained was explained by differences in the 5′ end start sites. Of the 25 clones examined there were 9 distinct transcriptional start sites. However, no alternative first exon was detected among the 25 RACE clones of various sizes that were isolated and sequenced.

Discussion

The BRCA1 gene product is likely to play multiple roles in normal growth control and/or differentiation. Mutations in the coding region that truncate the protein or result in missense mutations have been implicated in the development of a significant subset of hereditary breast and ovarian cancers (Couch and Weber 1996; Shattuck-Eidens et al. 1995). We have identified several important structural and regulatory domains of the rat *Brca1* gene and its protein by studying sequence conservation between humans and rodents, yet the precise functions of the BRCA1 gene product remain elusive. The overall sequence homology between the deduced human BRCA1 gene product and the rat homolog was low, but similar to that observed previously for the predicted mouse and dog proteins (Bennett et al. 1995; Szabo et al. 1995).

The conservation of the zinc-binding RING finger domain and the bipartite nuclear localization signal motifs among species are consistent with the reported localization of BRCA1 to the nucleus (Scully et al. 1996, 1997a, 1997b). The net negative charge of the deduced Brca1 amino acid sequences from rat and other mammalian species is suggestive of acidic transactivators of transcription that have distinct DNA-binding and activation domains (Hahn 1993; Tijan and Maniatis 1994). The BRCA1 protein was initially proposed to regulate gene expression on the basis of its zinc-binding RING finger motif and the highly acidic carboxy terminal domain (Miki et al. 1994). Consistent with these predictions, the highly conserved carboxyl terminal region of the BRCA1 protein transactivates transcription of reporter constructs in yeast and mammalian cells (Chapman and Verma 1996; Monteiro et al. 1996; Zhang et al. 1998). Several missense mutations in this car-

Table 1. Alternatively spliced forms of rat *Brca1*.

Splice	Tissue	mRNA (kb)	Junction	Predicted protein
Deletes exons 3–15	liver, testis, mammary gland, and spleen	~1.4	ATCTG/AAGGA	ΔAA 27–1817
Deletes exons 5–15	liver, spleen, and ovary	~1.4	TGCAA/AAGGA	ΔAA 45–1817
Deletes exon 5	liver	~7.5	TGCAA/GAGCC	ΔAA 45–71
Deletes exons 10–11	liver, mammary gland, and ovary	~4.5	TGCAG/TGGCG	ΔAA 215–1817
Deletes internal 3′ exons 10–11	liver	~4.5	TGAAG/TGGCG	ΔAA 198–1328

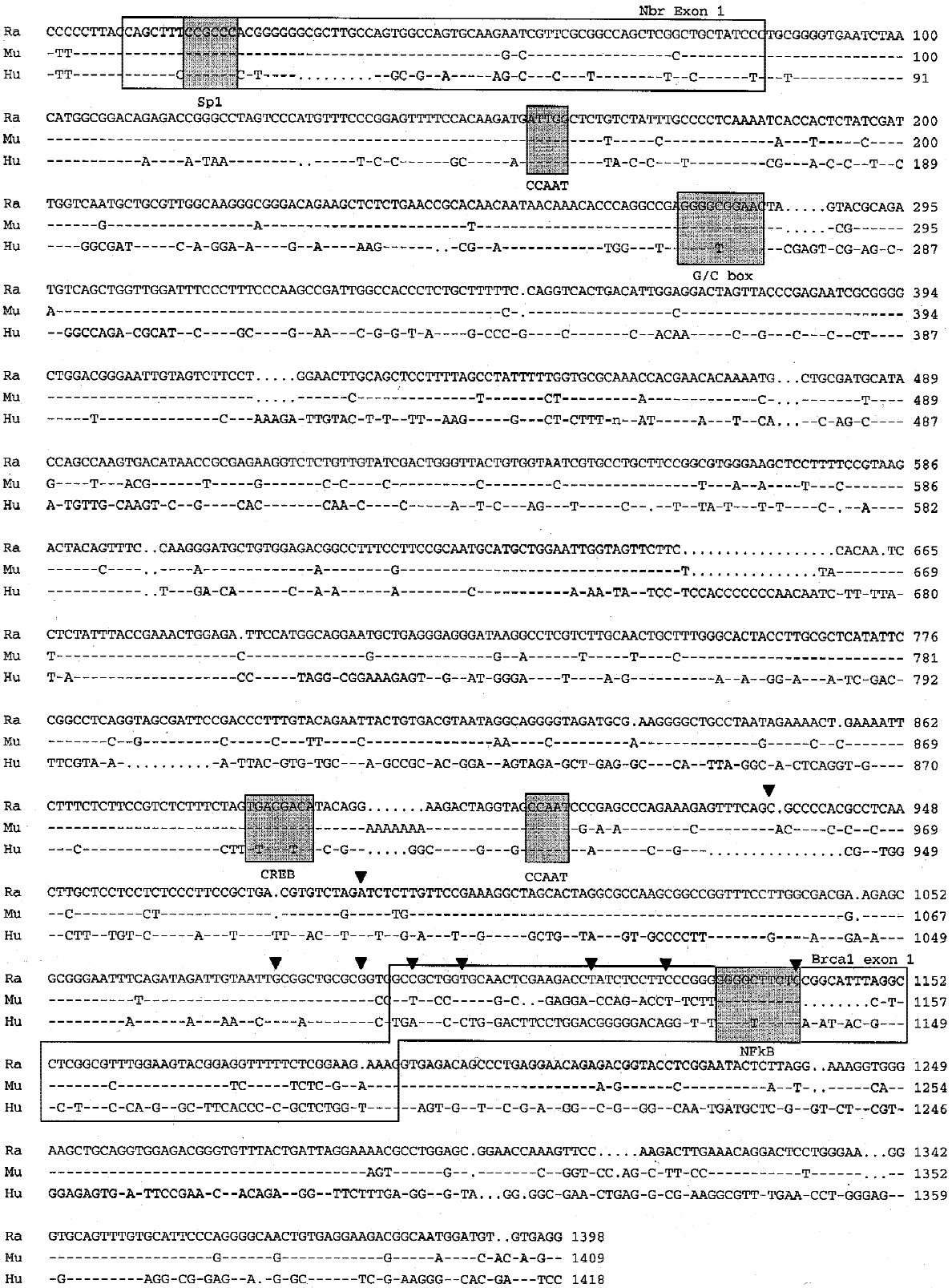

Fig. 3. Alignment of the bidirectional promoter regions of the rat, mouse, and human BRCA1/NBR genes with the identification of several putative transcriptional control elements conserved among all three species. Arrows indicate the alternative start sites identified by 5′ RACE analysis. [Genbank accession numbers: AF080589 (mouse) and AF080590 (rat)].

boxyl region, identical to those identified in breast cancer patients, abrogate the transactivating activity of fusion proteins (Chapman and Verma 1996; Monteiro et al. 1996), while some common BRCA1 polymorphisms retain the ability to activate transcription (Monteiro et al. 1997).

The majority of mutations identified in the human BRCA1 gene are frame shift, nonsense, and splice site mutations, which result in the premature truncation of the protein product (Couch and Weber 1996). In addition, approximately 160 amino acid residues have been identified in the human coding region that harbors putative missense mutations, polymorphisms, and unclassified variant sequences (Fig. 1, BIC database, June 1998). Comparisons of the codons at these positions with those of other mammalian species may help identify amino acid substitutions that merit detailed human case-control studies to distinguish between neutral polymorphisms and biologically significant missense mutations that may be of relatively low penetrance. The evolutionary conservation data presented in this and previous reports support the notion that mutation of amino acids that are conserved in rodents and the dog are more likely to be missense mutations in humans than are those that are not conserved (Able et al. 1995; Bennett et al. 1995; Szabo et al. 1995).

Alternative BRCA1 transcripts resulting from the use of two first exons were detected in all human tissues examined by RT PCR (Xu et al. 1995). Exon 1a was the primary transcript detected in breast tissue, while the exon 1b species was expressed more frequently in human placenta. Analysis of 5′ rat RACE products from testis failed to reveal an alternative exon 1b as described for the human BRCA 1 locus. This finding was not unexpected, since genomic sequence analysis of the complete human BRCA1 locus indicates that almost 42% of the 81 kb are comprised of ALU repeats (Smith et al. 1996), and the comparison of exon 1b to human repetitive elements revealed that this exon shares 83% identity to an ALU element (L.M. Bennett, unpublished observations). The observation that ALU elements can act as exons is not unprecedented. In fact, exon 4 of the human BRCA1 gene, which was detected in a single cDNA clone from a human placental library (Accession U15595), also is a reverse ALU sequence.

We observed 5′ transcriptional start sites of the rat *Brca1* gene to be variable, as reported previously for the human 5′ start sites for exon 1a (Xu et al. 1995). The GCG Wisconsin package program FOLDRNA was used to predict the hairpin loop structures of the 5′ sequences of rat *Brca1*. Of the nine transcript species detected, three were interrupted in a putative loop structure and six stopped in the putative stem region similar to that observed for the human BRCA1 promoter region (Xu et al. 1995). This observation is consistent with the suggestion that a complex secondary structure in the 5′ untranslated region of the BRCA1 mRNA may block first-strand reverse transcription, resulting in 5′ RACE cDNA PCR products of variable lengths.

The large genomic duplication of the human BRCA1 and NBR1 bidirectional promoter region was conserved in neither the mouse (Chambers and Solomon 1996) or rat homologs. The NBR1 gene was initially isolated as a candidate for the CA125 antigen that is used for ovarian cancer screening and clinical management (Campbell et al. 1994). Several putative transcriptional control elements described for the exon 1a and 1b human and mouse BRCA1 transcripts (Brown et al. 1994; Chambers and Solomon 1996; Xu et al. 1995) are conserved in the bidirectional promoter region of the rat and may play a critical role in BRCA1 regulation. The CREB site (nucleotide 867, Fig. 3) is of particular interest since it is methylated in some sporadic human breast cancers (Mancini et al. 1998). Cyclic AMP mediates signaling by the pituitary hormones, follicle stimulating hormone and luteinizing hormone, which in turn regulate normal growth control and differentiation of developing ovarian follicles. Thus, the CREB protein is an excellent candidate for regulating normal *Brca1* expression in ovarian and breast tissue during specific stages of the cell cycle. Interestingly, BRCA1 has recently been shown to interact directly with RNA helicase A in the RNA polymerase II holoenzyme complex, which also includes the CREB binding protein transcriptional coactivator (Anderson et al. 1998). Interactions of these and other various transcription factors within the bidirectional promoter region are likely to regulate *Brca1* expression. Site-directed mutagenesis studies of this complex promoter region are needed to dissect critical regulatory elements governing *Brca1* transcription in target tissues.

The biological significance of any alternatively spliced Brca1 message identified in various rat tissues is unknown. If alternatively spliced forms of BRCA1 are biologically significant, they would be expected to be conserved in other mammalian species. While it is unlikely that the forms resulting in severely truncated Brca1 proteins have functional significance, two spliced forms that maintain large portions of the full-length protein merit further investigation. Possible roles for the spliced species lacking exon 5 (ΔAA 45–71) are intriguing, since the resulting protein has lost a significant portion of the RING finger domain. The RING finger domain has been shown to interact with at least two other proteins: BARD1 (Wu et al. 1996) and BAP1 (Jensen et al. 1998). Interestingly, a similar spliced transcript has been observed in normal human breast tissue (Miki et al. 1994). Missense mutations in exon 5 altering the cysteines at codons 61 and 64, which are critical for the RING structure, result in strong predisposition to tumor development in human BRCA1 mutation carriers. The rat Brca1-Δ10/11 is structurally most similar to the previously identified human BRCA1-Δ11b and Δ11 spliced forms which appear to be preferentially localized in the cytoplasm rather than the nucleus (Thakur et al. 1997; Wilson et al. 1997; Cui et al. 1998). It will be interesting to determine whether this is coincidence or whether this spliced form harbors an important and perhaps conserved biological function.

This report describes and compares a variety of features among the rat *Brca1* gene and previously reported mammalian homologs. These studies provide a foundation upon which more detailed examinations can be made with regard to (1) unclassified sequence variants that merit further investigation as disease-related missense mutations; (2) regulatory elements in the promoter region; (3) biological significance of alternatively spliced mRNA species; and (4) the transcriptional transactivation activity of BRCA1. Since rats are widely used in carcinogenesis experiments, this report will facilitate additional studies addressing the role of BRCA1 defects in hereditary breast and ovarian cancer.

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