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The genomic organization of LOT1, a gene implicated in the development of ovarian cancer

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The Genomic Organization of *LOT1*, a Gene Implicated In The Development

Of Ovarian Cancer.

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$\dfrac{12\ /\ 3\ /\ 98}{\text{Date}}$

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Abstract

We have recently identified the human homologue (*LOT1*, Lost On Transformation 1, GenBank accession #U72621) of a rat gene (*Lot1*, GenBank accession #U72620) whose expression is frequently lost, or aberrant decrease, when rat ovarian surface epithelial cells undergo spontaneous malignant transformation. The deduced proteins encoded by the human and rat genes contain seven zinc finger domains plus additional regions, suggesting that the proteins are transcription factors. Using Fluorescence *In Situ* Hybridization (FISH) analysis, *LOT1* has been localized to chromosome 6q25-6q24. Since loss of heterozygosity in this chromosomal region is a frequent occurrence in ovarian cancer, it is possible that *LOT1* is the targeted gene. To facilitate investigation of this possibility, we have determined the genomic organization of this gene. This was accomplished by a combination of PCR and genomic cloning techniques. PCR fragments generated from human genomic DNA with primers designed using *LOT1* cDNA sequence suggest that introns are not present in most of the open reading frame of *LOT1*. However, the 5'-portion of the gene could not thoroughly be studied using this strategy, which suggested the possibility that intron(s) are present in this area. Direct sequencing of genomic clones isolated from a human DNA libraries confirmed that introns were not present within the majority of the open reading frame and its 3' end. As anticipated by the PCR data, further analysis by this strategy revealed one intron residing within the nucleotides defining zinc-finger two and four introns within the 5'-untranslated region of the gene. Knowledge of the genomic organization of *LOT1* will allow human tumor DNA to be examined for mutations and clarify whether this gene is involved in the etiology and/or progression of ovarian cancer or other solid

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tumors where loss of DNA from the 6q25-6q24 chromosomal region is frequent.

Introduction

Every year, approximately 26,000 women are diagnosed with ovarian cancer and 15,000 women die from complications associated with the disease (1) . The cause of this disease, which arises as a result of the malignant transformation of the ovarian surface epithelium, is largely unknown. It is known that ovulatory frequency is a physiological factor contributing to the disease, however, only recently have some genes and chromosomal regions of possible relevance to the disease process been identified (1, 2) .

The aspect of ovulation believed to be critical to the disease process is the requirement for growth of surface epithelial cells to repair the wound at the site of ovulation (3). A strategy to examine the role of this repetitious requirement for growth in malignant transformation of ovarian surface epithelial cells can be carried out by isolating rat ovarian surface epithelial cells and subjecting them to growth stress *in vitro* by repeated subculture (4) . This experimental procedure frequently results in malignant transformation (5) and allows the genetic events that lead to multiple independent incidents of transformation to be examined in the context of cells which initially had the identical genetic background (6, 7) . We have used this model to identify a rat gene, *Lot1*, which frequently displays decreased, or disregulated, expression in transformed rat ovarian surface epithelial cells (6) . We have recently cloned the cDNA for human *LOT1* and have demonstrated that the gene is localized to chromosome 6q25-6q24. This chromosomal region is a frequent site for loss of heterozygosity in many solid tumor types (8-11) including ovarian cancer (6, 12) . The location of *LOT1*, the pattern of its expression in malignant cells, and the characteristics of the predicted protein which contains zinc-finger motifs and activator/repressor domains make it an

interesting candidate to consider for the probable tumor suppressor gene targeted for inactivation by deletion of genetic material at chromosome 6q25. In order to identify mutations and/or inactivating methylation differences, the genomic sequence of the tumor suppressor gene must be ascertained. Here we report the genomic structure of *LOT1*.

Materials and Methods

PCR analysis of Human Genomic DNA to Determine LOT1 Structure/Sequence. The following primers were synthesized based on the DNA sequence of *LOT1* cDNA: p47(5'-TCA CCT GTG GGG TCT GTG CCC T--3'), p48(5'-TTG GGC GCA AGG ATC ACC TCA C-3'), p49(5'-CAC CCT CAG TCC CCC AGA ACA AG-3'), p51(5'-GCT CCT GTG AGT GGG TCT TCT TG-3'), p52(5'-CTG CAT AGG CTG GGC GGC TTG T-3'), p53(5'-CCC TTA GCC AGA TCA AAA CCT GG-3'), p54(5'-AAC CTG TGG GGC AAA GAT TCC CC-3'), p63(5'-TGG GTG TGA GGA GTG TGG GAA GA-3'), p69(5'-GCA GTT TGA TTA CAG AAC ACG CG-3'), p75(5'-CTT TCA CAG TGG TCG CAC TGG-3'), p95(5'-GCA CCA TCG TTC TGT TTG TGC-3'), pZ2(5'-GGC ACA GCA TTT GGT CAG CAC-3'), p898(5'-GCT TCT TTT CCT TGG TTC CGC-3'), p95r(5'-GCA CAA ACA GAA CGA TGG TGC--3'), pE1(5'--CTA CCT TCA GGG TTG GAT GTA G-3'), pE2(5'-CAG GGT TGG ATG TAG TTT AGT TAC-3'), pE3(5'-CGC GTG TTC TGT AAT CAA ACT GC -3), pA (5'-AGT GCT CAC TGC TCT ACT CCC C -3'), and pC(5'-GCT GTC ACT AGC TTT GCT GTG T-3') TH1341 (5'-AGA CAA ACT TCT GGG AGG ACT C-3') and TH1206 (5'-TGA ACA CTC GTC CGA GAA TAA CGA GTG GAT CTC GGT -3'). Various combinations of these primers were examined for their capacity to amplify 100 ng of human genomic DNA by PCR while others were used for DNA sequencing. A similar strategy was recently used to aid in defining the genomic structure of *MUTS* *(13)* . The conditions for the PCR were as follows: nineteen cycles of 94°C-15 seconds, 65°C-1 minute (-.5°C/cycle), and 72°C-1 minute. After these initial nineteen cycles, twenty-five cycles of 94°C-15 sec, 55°C-1 min and 72 °C-1 min were used, after which the reaction was extended for 5 minutes at 72°C. Aliquots of the reactions were visualized by

agarose gel electrophoresis (2%,w/v). PCR products were purified (Wizard Plus DNA purification system, Promega) according to the manufacturers instructions and subjected to automated fluorescent-based DNA sequencing (model 373A Automated Sequencer, Applied Biosystems) utilizing nested primers (see above).

In instances where long range PCR was performed (>1.2 kb), PCR conditions were the same as the "touch-down" program described, but an extension time of 3 minutes at 72 °C was used.

Isolation and Characterization of Genomic Clones Containing Portions of the LOT1 Gene. A human placental genomic DNA library [EMBL-3(SP6/T7), Clontech] was screened by standard methods (7) with F1 and RU5 PCR fragments which contained the majority of the open reading frame (bases -271 to +1623) of *LOT1* in fragment F1 and the 5' untranslated region (bases -271 to +1) in RU5. The genomic library was also screened using the ~1.2 kbp PCR fragment FB2-5' (discussed below). Bacteriaphage clones were grown and purified by standard methods (14). Phage inserts were sequenced with the aid of polylinker specific primers (EMBL-3, SP6/T7) and primers based on the cDNA sequence of *LOT1*. Additional insight into the genomic structure of *LOT1* was gained by analysis of the fragment sizes generated from PCR when phage were used as template in combination with *LOT1* cDNA based primers and/or phage specific primers.

A human P1 library (Genome Systems, St. Louis, MO) was also screened using the 1.2 kb FB2-5' fragment. This fragment was generated from

a new human partial LOT1 cDNA clone and contained bases -1547 to -567 with respect to LOT1's open reading frame. The resulting P1 clone (clone 17513) was purified by standard means (QIAGEN Plasmid Purification Kit, QIAGEN Inc.) and used in subsequent analysis.

Restriction Digests and Southern Blotting. A total of 1ug of purified P1 DNA was digested overnight with 20 Units of various restriction endonucleases (SalI, SacI, PstI, XbaI, NotI, BamHI) before being electrophoresed on 0.7% agarose gels. Gels were stained and DNA fragment sizes recorded before being transfered overnight onto nylon membranes (Genescreen Plus, NEN Life Sciences) under denaturing conditions (0.4 M NaOH). Blots were fixed for 45 seconds in the microwave before being prehybridized (0.2ml/cm^2) at 65 °C overnight in Church Buffer (0.5 M NaPO4, pH 7.1, 2 mM EDTA, 7% SDS, 0.1 % Sodium Pyrophosphate). Southern blots were hybridized with end labeled probes (see below) in fresh Church Buffer (0.2ml/cm^2) at 37 °C for 5 hours before being washed at 37 °C two times for 10 minutes in 5 x SSC / .1 %SDS. Fragments of interest were eventually cloned into pBluescript (Stratagene, La Jolla, CA) and sequenced.

Preparation of Probes. F1 and RU5 fragments were generated from clone FB4 (a plasmid containing the complete open reading frame for *LOT1*, (6)) by PCR using primers p57 (5'-GGT CTC CCT CCC CAG AAG CCC T-3')/p69 (5'-GCA GTT TGA TTA CAG AAC ACG CG-3') and primers p57/p60 (5'-TGG GCT TTG CTT CTC ACA CCT TC-3'). Fragment FB2-5' was generated from clone FB2 by PCR using primers TH1336 (5'- CCT CGT GTC ACA TCT GAA ACT - 3')/TH1340 (5'- CTG GAG TCA TGA GAT CCA GGA CAC -3'). The PCR

amplification of template (10ng) was under the following conditions: initial denaturation at 94°C for 2 minutes followed by 30 cycles of 94°C-25 seconds, 55°C-40 seconds, 72°C-2 minutes. The reaction product was purified (Wizard Plus DNA purification system, Promega) and labeled with α–^{32}P by random priming (Prime-IT II, Stratagene). For applications involving end labeling (γ–^{32}P), oligonucleotides specific for the extreme 5′ region of *LOT1's* cDNA and adjacent to intron 4 (TH1408/TH1339) were end labeled using the T4 Kinase Kit (Gibco BRL).

Results.

Genomic DNA Analysis by PCR. Initial Southern blot analysis suggested that the majority of the ORF of *LOT1* contained no introns or introns of small size (data not shown). To examine this possibility further, primers designed from the cDNA sequence were used to amplify portions of the *LOT1* gene from genomic DNA. This strategy allowed us not only to determine the fragment sizes, but also allowed us to subject fragments to sequencing in order to determine if small introns might exist within the sequences of DNA analyzed. Six primer pairs (p63/p51, p49/p54, p53/48, p52/p47, p54/p47, and p63/p69; Figure 1) gave unambiguous results with regard to fragment sizes when the PCR products were examined by electrophoresis (Figure 1). The PCR product generated by the p63/p69 primers encompassed the majority of the open reading frame and, thus, was purified and sequenced. Sequence data did not reveal any evidence of introns from the region of the gene encoding zinc-finger four to the end (3' end) of the open reading frame (data not shown). Efforts to use this approach to clarify the genomic organization of the 5' portion of the *LOT1* gene, i.e. upstream from zinc-finger four, yielded in no PCR product being formed.

Genomic Cloning and Characterization. Probe F1, which spanned the open reading frame of *LOT1* , was used to isolate a member (L-1a) of a human genomic placental DNA library. The phage clone contained an insert ~14 kb, which was partially sequenced using primers specific for the open reading frame of *LOT1*. Figure 2 displays the extent of the overlap between clone L-1a and the genomic organization of *LOT1* which was eventually elucidated.

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Sequence data based on phage clone L-1a indicated that the sequence between +153 (based on cDNA translation start site) and the last polyadenylation signal was devoid of introns. This finding was consistent with the previous PCR based strategy characterizing the genomic structure within the majority of the open reading frame. The area upstream of zinc finger #2 (+153 within cDNA) in clone L-1a was found to diverge from the known cDNA sequence and displayed characteristics of a 3' intron splice site.

Characterization of the 5' protein of the *LOT1* gene was aided by analysis of an additional *LOT1* cDNA clone (FB2) . This clone contain variations in the 5' UTR from that reported in GenBank (accession #U72621). As described below, use of FB2 to define the remaining exonic sequence of the *LOT1* gene yielded unambiguous results, i.e. all exonic sequences could be accounted for in genomic DNA, in contrast to the use of data in GenBank. W e speculate that the 5' UTR region of the GenBank entry, which diverges with cDNA clone FB2, is either a chimeric cDNA clone or an alternative transcript. To substantiate the validity of this new cDNA clone, FISH analysis of a ~1.2 kb fragment (FB2-5') derived from this clone was localized to chromosome 6q24 (data not shown).

We next isolated clone L-8a from the human placental genomic library using probe RU5, generated from bases -271 to +1 of the *LOT1* cDNA (Figure 3). This phage clone was purified, partially sequenced, and used to confirm the existence of an intron within zinc finger #2. PCR analysis on clone L-8a using primers flanking this intron have shown this intron to be ~5.5kbp in size (data not shown). Subsequent sequencing reactions upstream of this intron identified sequence consistent with the cDNA as well as divergent sequence. At this point of divergence, sequence was characteristic of a 3'

intron splice site. PCR analysis of this clone using *LOT1* specific primers downstream of this proposed 3' intron boundary and EMBL 3 SP6 primers produced a product ~4.5 kb in length (fragment f58La, Figure 2). Its sequence defined the 5' end of clone L-8a and was considered intronic based on divergence from the cDNA.

We next used the FB2-5' fragment described above to screen the human placental genomic library. One positive phage clone (L-2a, Figure 2) was isolated and a portion of it amplified using TH1341 and EMBL3 sp6 primers. The resulting ~3 kb fragment was cloned into pGEM-T vector (Promega), and sequenced, enabling the 5' end of this intron to be defined. However, this subclone (pL2-13) and phage L-8a did not span the length of the intron. To obtain the approximate size of this intronic region, long range PCR was performed with genomic DNA from normal individuals and primers designed using sequence from the 5' region of clone L-8a and the 3' region of clone pL2-13. A 6 kb product (fragment fTH1143) was consistently generated, whose sequence overlapped with the 5' and 3' region of clone L-8a and clone pL2-13. Through addition of these regions, the size of this intron (intron 4) has been estimated to be 12 kb.

The remainder of the L-2a phage clone was characterized in a similar manner using PCR and a combination of *LOT1* cDNA specific primers and/or EMBL3 T7 specific primers. In this manner, amplification of portion of clone L-2a with primers TH1338 and TH1340 (Figure 3) consistently produced a ~1kb fragment devoid of introns as determined by sequence analysis. Similar amplifications using TH1405R/TH1407 produced a 2.8 kb fragment, which was cloned (subclone pTH1407). Sequence analysis of pTH1407 showed characteristics of exon/intron splice sites within the region encompassed by

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the TH1405R/TH1407 primers. PCR reactions using primers TH1405/EMBL T7 resulted in the 4.5 kb product which was cloned (pGEM-T). Sequence analysis of this clone (pT1, Figure 2) showed it to contain sequence homologous to FB2 (near the TH1405 primer site) and also contained sequence divergent with respect to a punitive 3' intron splice site (intron 1). PCR reactions using TH1405 in combination with further upstream *LOT1* specific cDNA primers failed to produce a product using either clone L-2a or normal human genomic DNA as templates, indicating the possible existence of an intron of substantial size upstream of the TH1405 primer site. Similar difficulties arose when attempting the amplification of the region between primer sites TH1408 and TH1339 using clone L2-a DNA. In both PCR reactions, products were not generated, regardless of altered extension times and enzyme type.

To resolve remaining issues with regard to LOT1 genomic organization, a human genomic P1 library was screened (Genome Systems) using the FB2-5' fragment, this resulted in the identification of P1 clone 17512. This P1 clone was digested with a number of restriction enzymes, southern blotted and probed with various *LOT1*-specific primers to validate the use of this clone for further analysis (data not shown). Restrictions fragments hybridizing to these primers specific for the regions upstream of primer TH1405 and between primers TH1408 and TH1339 were then eventually cloned into pBluescript. The sequence data produced on these clones hybridizing to primer TH1536 (pSAC9 and pNOT2) allowed us to characterize the 5' end of intron 1. Sequence data produced from genomic subclones pSAC3 and pXBA4.2, which hybridize to primers TH1339- and TH1408 respectively, verified the existence of intron 3.

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Discussion

The *LOT1* locus has been demonstrated to have a minimum size of 24 kb and composed of six exons and five introns. This was elucidated by screening a human genomic P1 (Genome Systems Inc.) and human genomic bacteriophage (Clontech) libraries with various *LOT1* cDNA fragments. All intron donor/acceptor sites follow the GT-AG rule (Table 1). The translation initiation codon (ATG) of *LOT1* is located in exon 5, and encodes zinc finger one and approximately half of zinc finger two which is interrupted by intron five (5.5 kb). The remainder of the open reading frame coding for zinc fingers 2-7 and the proline/glutamine regions, is located in exon 6. This last exon contains the 3' UTR of the gene which also processes five polyadenylation signals (AATTAAA and ATTAAA). A CpG island is present in the sequence we define as exon 1, and extends into the 5' flanking region of the gene. A classical TATA box element is not present. Therefore we cannot define with certainty the start site of transcription. We assume however that the sequence shown in Figure 4 contains this area. This notion is based on analysis of this sequence using the MatInspector (version 2.2) promoter database program, which has identified a number of transcription factor binding sites, including SP1, AP1, AP2, AP4, cAMP responsive binding protein, c-Myc and a CCAAT/enhancer binding protein element (Figure 4). These transcription factor binding sites are of course characteristic of the region 5'of the start site of transcription. RNAse protection assays and promoter reporter constructs will be used in the future to further elucidate the transcriptional start site and promoter activity of this region.

In summary, we have demonstrated that the genomic structure of the translated portion of *LOT1* and its 3' region is relatively simple. This portion of the gene contains only one intron, which is located within sequence defining zinc-finger #2. Ongoing work will clarify the genomic organization with respect to alternative transcripts and the gene's regulatory elements. Finally, the data presently available will facilitate analysis of tumor DNA for mutations in this gene.

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Chen, D.; Cerosaletti, K.; Fournier, R.; Todd, S.; Lewis, T.; Leach, R.; Naylor, S.; Weissenbach, J.; Mecklin, J.; Järvinen, H.; Petersen, G.; Hamilton, S.; Green, J.; Jass, J.; Watson, P.; Lynch, H.; Trent, J.; de la Chapelle, A.; Kinzler, K.; Vogelstein, B. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell **1993**, 75, 1215-1225.

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Figure 1 :PCR Analysis of the *LOT1* Gene. 2% agarose gel depicting the PCR amplified regions of *LOT1*. 100bp marker (Lane 1) and 1 Kbp marker (Lane 8) were used as size standards. Lane 2 (p63/p51), lane 3 (p49/p54), lane 4 (p53/p48), lane 5 (p52/p47), lane 6 (p54/p47), and lane 7 (p63/p69). Fragment F63/69 (lane 7), spanning the majority of the open reading frame and parts of the 3' untranslated region, was later used for direct sequencing. The primer combinations used and the PCR product sizes are listed above. PCR reactions on genomic DNA utilizing primer combinations p95/p75 failed to consistently produce product (data not shown).
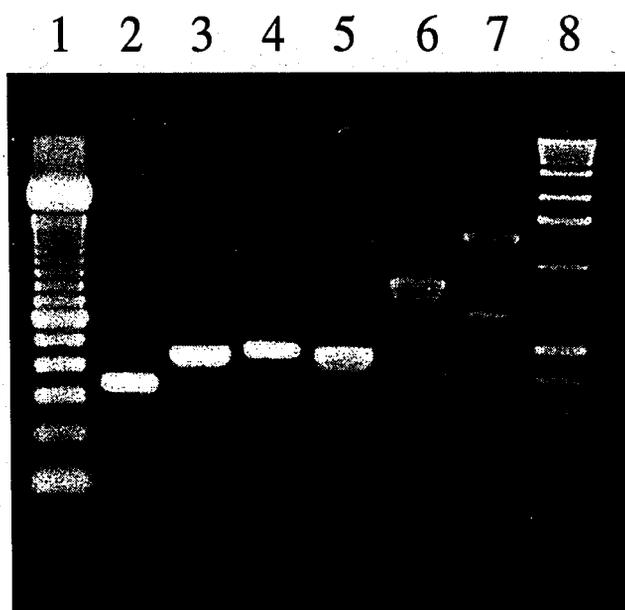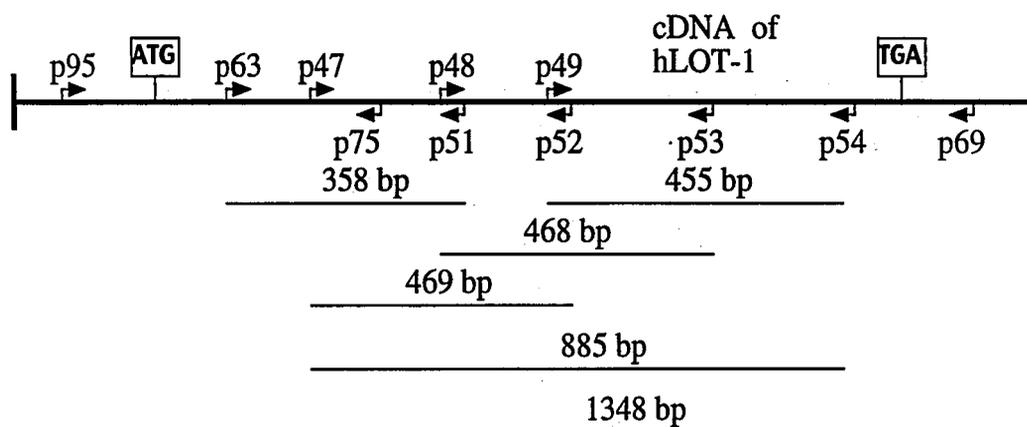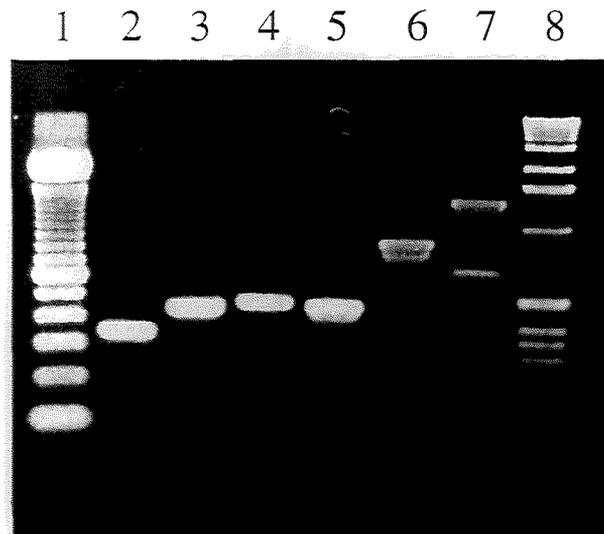
Figure 1 :PCR Analysis of the *LOT1* Gene. 2% agarose gel depicting the PCR amplified regions of *LOT1*. 100bp marker (Lane 1) and 1 Kbp marker (Lane 8) were used as size standards. Lane 2 (p63/p51), lane 3 (p49/p54), lane 4 (p53/p48), lane 5 (p52/p47), lane 6 (p54/p47), and lane 7 (p63/p69). Fragment F63/69 (lane 7), spanning the majority of the open reading frame and parts of the 3´ untranslated region, was later used for direct sequencing. The primer combinations used and the PCR product sizes are listed above. PCR reactions on genomic DNA utilizing primer combinations p95/p75 failed to consistently produce product (data not shown).
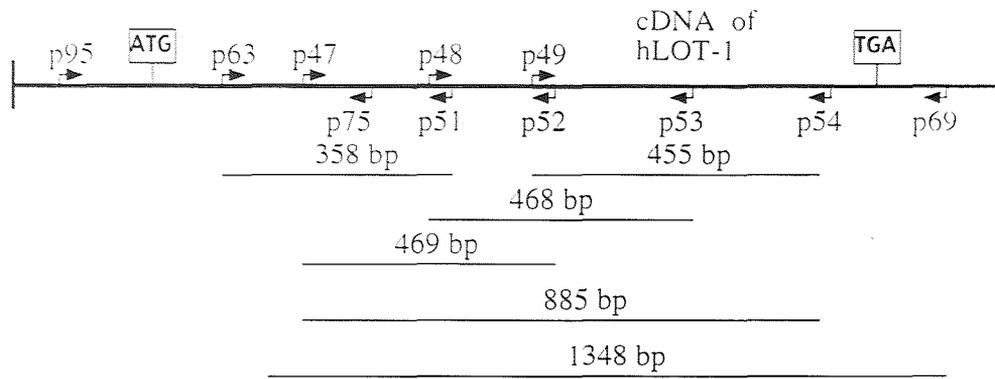
Genomic Organization and Contig Map of *LOT1*.
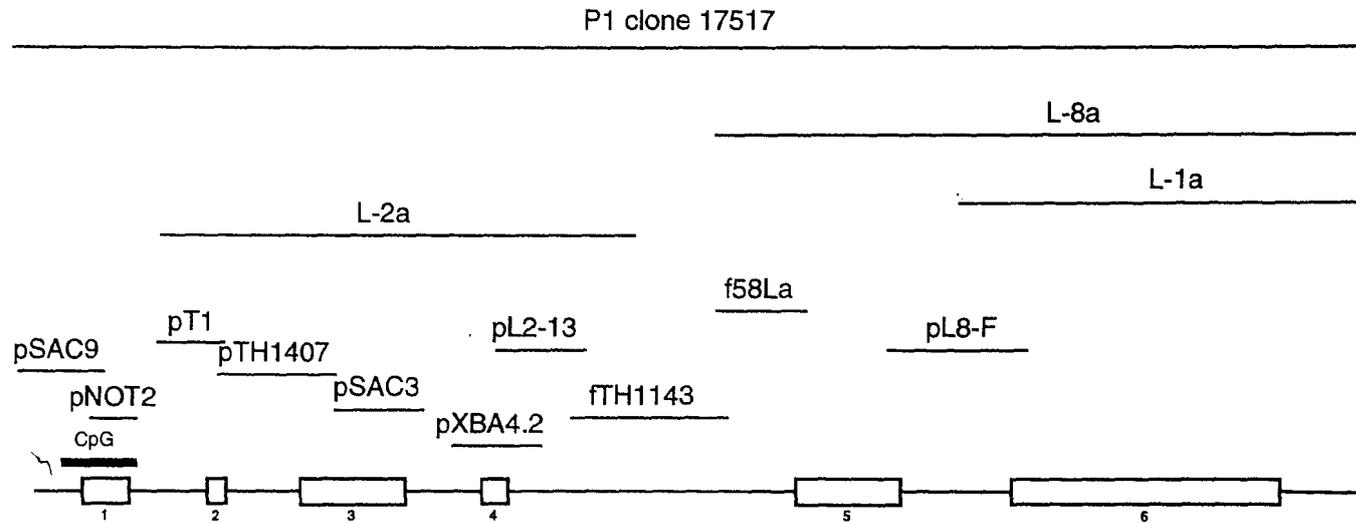

Figure 2: Genomic organization and contig map of the *LOT1* gene. Exons are shown as boxes while introns are shown as solid lines. Genomic phage clones (L-8a, L-1a, and L-2a) and subclones derived from phage and P1 clone 17517 which overlap with the *LOT1* gene are represented as horizontal lines. PCR fragments (f58La and fTH1143) which were used for sequencing are also displayed.

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                                              TH1536
-2041  GCCGTGCTCA CAGCTCAGCA GCGCGGGGCC TTGGCGCGCG GGGCGCTTCC CCGGGTCGCC GTCATGGCCG CGGAGGTGGC

-1961  ACGCCCGAGC GGCCTCGCCT GAGCTCCGGG GGTCGTCGCC CCGCAGGGAT TGCTGTCACG ĮCTAATGTGG CTGCTGCCTC
            TH1405                                          ▼
-1881  GTGTCACATC TGAAACTCAT CTGTACCTCA CTTAGAAAGT GGTTCTGATT AGACAAGACT TTTCGTTGCA GTCGACAGAA
         TH1405R
-1801  ACCTAATGGG ACCATTGAAG AATTCCAAAC AGGCAAGTGA CAGGAACATA TTTGCATGTT AGAAGAATCA GCCTGGCAGC

-1721  AGCATTGTGA TTAGAATGAA GGGGAACCGT CCAAAAACAG ACTGGGGAAC CAATATGGAT CTGCCTCTAG CATGCGGAAA

-1641  TATCTCTCTA CACTGACCTA ACTGACTCAC TAAGCTTGTG GTTTGTTTAA AGGAAGCCAC ATGAAAATTG AGTTTGGGCC
                               TH1407
-1561  AATAGAGTGA ACTCGTTCTC CATTCTCTAG CATCCCTCCC CATGCAACCA CCCTATCCCT GCCAAGTTTC TGGGCACCAC
                                        TH1338
-1481  AGATTGGAAA GTAGCCTCCT GTAGGTATTT GCCCATGTTA AGTGGTGGGG TCTCTTTCTG TCTTCCCTTC TTGGTCTCAC

-1401  CTGTCTGGTG TGACAAGGGA AGCATGTGCC AACCAAGGCT AGACCCTTGT GAGACCAGAG CAGCCCCACT TTCGGTAAAG

-1321  CAAGCAACCT CTGCTTACTT GCCCAACACA CCCTAGCTTC CGTGTTCCTT GCCTTGTGAG TTATCTTCTT GGGTACAATT

-1241  TAACACAGCG TTCCCACCTC CTTATAACTA AAATAGCTAG AGGGGGCTTT GTGCCTCAAA CCCCAAGGAG ACCATCTTGG

-1161  CAATCTCTGC CTCTGCAACA GGGCACTTGC TCCCTGGGCA ACTCCTTTCC CAGGTCATCT TCTCCCTTGA CATGCCCATC

-1081  AGATTTAATA GTGTGCCTAT TTGGCAGAAA AATGGGGTCC CTTGGTTGCT GATTCTTAGG AATGTGAGGT TCTCCCAGCC

-1001  AGAGCTCTCA GCACTAAAAG TTCTAGTCTC TGCGTAATGG CCGGCATATC TTTGGCGTAT CTAAAATCCT CTTCAGTTTC

 -921  CAGCAGGGTC TTTATAAGCT TCTTCTGAAG GTCTCAGACA TTCAGCCACG GAACTCAAGT GGAATTTGTT TGGATAACGT

 -841  GACTGATTTC AAAGCCCGGA CCCTTAGACG TGCCCATTTG GTGCTGCAAG TACTGAACAT TACTACAACA TCTTACAGTC

 -761  AACAAATTTG ACACATTAAA GATTTATATC CTTTCTTTTG GGTTAGGATC TTCTCTCCCC TAAAGCATCT CAGTTTCCAG

 -681  CATGCAATCA TTTCCATCTT ATGGAAATCA GCCATCCCGC TCCGTGCCAG CATGCTACCC TGGGAGGCAC ATCCAGGCTT
                               TH1340
 -601  GGGAAACGGG GGTGTCCTGG ATCTCATGAC TCCAGCAGCA CCAGCTGCTC TCTTTCCTCT TCCAAGTAGA CTTCCGTTCC
                               TH1339
 -521  CCCCCCACTT GGGTGTTTTT GTTTGTTTTA GCAATTCAGA GCTCAAGATA AAGACCTTAA AGATAACTTT GTGTGTCTCT
                                                 ▼                             TH1408
 -441  CCCTTTCTAG GTATTTGCAT AGGAATCAGA GGAGTTAATC TTGTTTGAAT CTTCAGACAA ACTTCTGGGA GGACTCGGTC
                                                ▼
 -361  CCTGCCTCGC AGCAGATGTT TCCCTGTCAC TCAGTAGCCA ATCCGGGGGA CCCAGGACAT GCCCCAGCTA TAGTGATGCA

 -281  GATTACCTTT CTGCTCCTGA ATCGCACCTG TGCCTCAGAC TTTCTCCCCT CAGCTTGAGA CTGCATGTAA ACTGGGATGT

 -201  GTGAAAGCAG GAAGCAAAGC TAGTGACAGC TGAGAGGTCC ATGTCTGGGT AGAACCAGGC CCACGATGCT GCCTCTCCCG

 -121  TGTTCTGGAG TTCAGCTGCA GGGATTCTGC TGATGTGCCC AGCACCATCG TTCTGTTTGT GCTTAAATGG CACAGCATTT

  -41  GGTCAGCACA TCTGAAAAGG AAGGTGTGAG AAGCAAAGCC CATGGCCACG TTCCCCTGCC AGTTATGTGG CAAGACGTTC

   40  CTCACCCTGG AGAAGTTCAC GATTCACAAT TATTCCCACT CCAGGGAGCG GCCGTACAAG TGTGTGCAGC CTGACTGTGG

  120  CAAAGCCTTT GTTTCCAGAT ATAAATTGAT GAGGCATATG GCTACCCATT CTCCCCAGAA ATCTCACCAG TGTGCTCACT
                                        ▼
  200  GTGAGAAGAC
```

Figure 3. Partial cDNA Structure of *LOT1*. Shown are the first five exons and the 5' end of exon 6. The first codon of *LOT1* is located in exon 5 and is underlined. Also included are the areas where introns are present, as indicated by inverted arrows. Sense and antisense primers are also shown as solid lines below and above the primary sequence (respectively) which were used to characterize the gene.

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                                              CCAAT/enhancer
-2911 GAGCTCCTTA CACCTCTCTT AATCTTTTTA CGTAATGCTT ATAGGCCTTT
                                       cAMP Responsive Element
-2861 TAAAAAGCAC GTCTTTTAGG GAAGAAAGCA ATAGAGATTC CCTTCAGTTT

-2811 ATTTTTCTTA AATTCCAGTA ACATGAAAAA TTTGAAATTA TATGAAGCAG
                 OCT-1
-2761 ACGTAGTGTG ATACCGTAAT TAGAAAATTA CTTCTGTCTA CCCTTTCTGC
                 GATA-1       AP-4
-2711 GTCTTCGTTT CTTCAGCTGT GAGACGGGTA TTTCTCCCAG TGTTGCTGTG

-2661 AGGATTACAC GTATTTACCT GCAAAGTGCT TAGGACAGTG CCTGGCTCAC

-2611 GGTCAGTGCT CACGTTTGGG CAGCTGCACT TGGGCGCTGC TGGCACAGNA

-2561 GGTAAGTTAG TTTGGCCTAT TGCAGCGTCC CAGCATCTGT CGCGTTTCTC

-2511 ATGTGTGATT GGGCTCTGGC GGCCCATCCT GGCGGAGACT TCGGCTAGCA
                         AP-2
-2461 GGCCCCGCTG CAGACCCCAG GCCGGCTCGG GTCTACCTGC GCCAGCGCTG
                                          AP-1       AP-2
-2411 TACCTGGGCG ACCTTGGCTT TGCCCCCACC GGTGACCCGG CCCGCAGGAC
                 AP-4
-2361 GTGTGGGTGC CGTTCAGCTC CCCCCGCTTC GGCCGCCGAC CCCCAGCTCC
            SP1                  c-Myc
-2311 CCGGCGGGGC CTCCNTCTTG CCACGTGACG CCCCCGCCAG GGGCCCCAGC

-2261 GCCCTCCTCG CGGCCGCGCC GTTCCGGCTC CCAAGCCCCG CCTGCGCGCG

-2211 GCCTCCTCGG CGCAGCCATC CTCTTGGCTG CCGCGGGCGG CAAAGCCCAC
                         AP-1
-2161 GGCATCTGCC ATTTGTCATT CAGCCCGTCG GTACCGCCCC GAGCCTTGAT
                                              GC Box
-2111 TTAGACACGG CTGGGGCGTG CTCTGGCCTC ACTCTCCGGG CGGGTGCTGG

-2061 ACGGACGGAC GGACGGGGCA GCNGTGCTCA CAGCTCAGCA GCGCGGGGCC

-2011 TTGGCGCGCG GGGCGCTTCC CCGGGTCGCC GTCATGGCCG CGGAGGTGGC
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Figure 4: Nucleotide Sequence of the 5' UTR and 5' Flankng Region of *LOT1*. The numbering of nucleotides to the left of the sequence corresponds to the first ATG start site. A number of different transcription factor binding sites are also present, as indicated by the boxed regions.

Table 1: Exon/Intron Boundries and Exon Sizes of the *LOT1* Gene.

EXON	EXON SIZE	3' INTRON/EXON SEQUENCE	5' INTRON/EXON SEQUENCE
1	---	---	CGCAGGgtaggtgttt
2	72	tttcctttagGATTGC	AGAAAGgtaaatacgt
3	1443	caactttcagTGGTTC	AATCTgtgagtatac
4	75	cttctcacagGTTTGA	CAGTAGgtgcgcatgt
5	475	ctctccacagCCAATC	GATGAGgtgagaggct
6	2380	ctgctcctagGCATAT	TTGCAAtttggcttga

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Vita

David Roberts was born on the 30[th] day of September, 1973, in the small northern shore town of Port Jefferson, Long Island. The son of Dona A. Roberts of Blue Point, N.Y., David attended both primary school at Blue Point Elementary School and secondary school at Bayport-Blue Point High School before graduating in the spring, 1991. David continued his education in the fall of 1991 by pursuing a B.Sc. in Molecular Biology by attending Lehigh University. Following commencement in the spring of 1995, David was one of the first students awarded a Presidential Scholarship, enabling him to continue his studies for a full year, tuition free. Using this scholarship, he applied to the Chemistry Department at Lehigh University in pursuit of a Master's degree, and accepted a very rewarding position as a Teaching Assistant for freshman chemistry (Chem 21 and 22). In July 1996, David joined the Medical Oncology department of Fox Chase Cancer Center as a graduate student and completed his M.S. degree research requirements under the guidance of Dr. Thomas C. Hamilton. Besides characterizing the genomic organization of a potential transcription factor, David also oversaw the characterization of the LOT1 antibody and promoter region of the gene. His true interests lie in the utilization of suppression subtractive hybridization in identifying genes lost during the tumorgenic process in ovarian cancer.

END

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