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Ribosomal Protein Genes Are Overexpressed in Colorectal Cancer: Isolation of a cDNA Clone Encoding the Human S3 Ribosomal Protein

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We have isolated a cDNA clone encoding the human S3 ribosomal protein from a normal human colon cDNA library. The clone was identified as one of many that detected genes whose level of expression was increased in adenocarcinoma of the colon relative to normal colonic mucosa. Increased levels of the S3 transcript were present in the tumors of all eight patients examined. Moreover, the S3 mRNA was also more abundant in 7 of 10 adenomatous polyps, the presumed precursor of carcinoma. Additional studies demonstrated that increased levels of mRNAs encoding several other ribosomal proteins, including S6, S8, S12, L5, and P0, were present in colorectal tumors and polyps. These results suggest that there is increased synthesis of ribosomes in colorectal tumors and that this increase is an early event in colon neoplasia.

Tumorigenesis of the colon is thought to be a complex multistep process involving multiple molecular events that are seen as changes in gene structure, expression, and activity (24, 25). Some of these changes are important to the development and progression of the disease in that they directly contribute to the neoplastic or invasive behavior of the tumor. On the other hand, many of these changes may be the secondary result of tumorigenesis. Both types of events are of interest in that the former provide information concerning the molecular mechanism(s) leading to cancer, while the latter may serve as markers for specific steps occurring in this process.

Aside from its clinical importance, colorectal cancer offers a unique opportunity to study molecular events associated with the onset and progression of neoplasia. The disease occurs as several distinct stages that can be defined histopathologically, including benign tubular, tubulovillous, and villous adenomatous polyps, and different stages and pathologies of invasive adenocarcinomas. Most often the initial treatment is surgical removal of the tumor and neighboring tissue. This procedure provides from the same individual normal and tumor tissue that has not been exposed to the effects of ionizing radiation or chemotherapeutic drugs.

A number of laboratories have looked for changes in gene structure, expression, or activity that are important to the development of neoplasia in the colon. These studies have led to the observations that (i) the K-*ras* gene is frequently mutated at position 12 or 13 in adenocarcinomas and in large adenomatous polyps (8, 27, 35, 60); (ii) the p53 gene, located on chromosome 17, frequently undergoes a reduction to hemizygosity with mutation of the remaining allele in carcinomas (45, 60); (iii) a gene termed DCC shows an allelic loss in 71% of carcinomas (23), and other loci are also frequently altered (25, 44); (iv) allelic losses on chromosome 5 are associated with familial polyposis coli, a genetic syndrome that predisposes affected individuals to the development of colorectal cancer (5, 39, 55); (v) members of the *myc* gene family are overexpressed in a majority of adenocarcinomas and adenomatous polyps (22, 26, 54); (vi) the c-*erbB-2* gene product is overexpressed in carcinomas (19); and (vii) adenocarcinomas, large adenomatous polyps, and colon tumor cell lines have increased c-*src* kinase activity (6, 7, 9, 10, 51).

We have screened cDNA libraries constructed by using mRNA isolated from adenocarcinomas of the colon and from normal colonic mucosa to search for genes whose level of expression changes during tumorigenesis. In this report, we describe the isolation of a complete cDNA clone for the human S3 ribosomal protein from such a screen. The expression of this gene, as well as those encoding other ribosomal proteins, is increased in colorectal tumors and polyps.

MATERIALS AND METHODS

Plasmids. The β-actin probe (pBSactin) contains the full-length human cDNA clone of β-actin described by Ponte et al. (49) subcloned into pBluescript (Stratagene). Construction of plasmids pS6- 4, pS8-14, pS12-6, pL5-6-4, pL26-19, pL35-a, and pP0-3 have been described (12–14, 40, 47, 50, 57, 58). pGPDH contains the full-length cDNA clone of the rat glyceraldehyde-3-phosphate dehydrogenase gene (28).

Sequencing and DNA sequence analysis. Restriction endonuclease fragments were subcloned into M1310 and -11 (42) and sequenced by the dideoxy sequencing method, using a United States Biochemical Corp. Sequenase kit (52). DNA sequences were compared with those in the GenBank data base, and amino acid sequences generated by open reading frames (ORFs) were compared with sequences in the NBRF/PIR and Swiss-Prot data bases, using the FastA-Mail program (48) on Bionet.

Preparation of RNA. RNA used for the preparation of the cDNA library was prepared by the method of Chirgwin et al. (16) and was selected twice on oligo(dT)-cellulose (21). The

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RNA that appears on Northern (RNA) blots was prepared either as described above or by the procedure of Chomczynski and Sacchi (17), with additional modifications by Cinna/Biotech. Briefly, tissue samples were homogenized in 20 ml of RNAzol (Cinna/Biotech) per g of tissue with a Polytron. Next, 1/10 volume chloroform was added, and the mixture was vortexed and left on ice for at least 15 min. After centrifugation for 15 min at 12,000 rpm in a Sorvall SS34 rotor, two more phenol-chloroform extractions and one chloroform extraction were done. The poly(A)$^+$ RNA that appears on Northern blots was selected once on oligo(dT)-cellulose.

cDNA library construction. The plasmid library was prepared essentially by the method of Gubler and Hoffman (30). Four micrograms of poly(A)$^+$ RNA was primed with oligo (dT)-cellulose and reverse transcribed with Moloney reverse transcriptase obtained from Bethesda Research Laboratories (BRL) as described by the manufacturer, using the BRL cDNA synthesis kit (catalog no. 8267SA). The double-stranded cDNA was tailed with oligo(dC) by using terminal deoxynucleotidyltransferase (38) and cloned into oligo(dG)-tailed pUC9 (Pharmacia).

Screening of cDNA libraries. Competent *Escherichia coli* DH5α cells, library efficiency from BRL, were transformed with the pUC9 clones. Colonies were picked and put onto fresh plates, which were incubated overnight. A nitrocellulose filter was applied to the plates, and the colonies were transferred to an ampicillin plate and incubated for several more hours. Colonies were then transferred to a chloramphenicol plate and incubated overnight. These filters were probed with a single-stranded cDNA derived from normal colonic mucosal RNA, using 200 to 500 ng of poly(A)$^+$ RNA for the first-strand reaction. The probe was treated with 50 mM sodium hydroxide at 65°C for 1 h to digest the RNA and then added to the hybridization buffer (0.725 M sodium chloride, 0.15 M sodium phosphate, pH 7.2, 1 mM EDTA, 1% sodium dodecyl sulfate [SDS], 100 μg of single-stranded DNA per ml) and hybridized for 16 to 35 h at 65°C. Clones that did not hybridize to the mucosa probe were rescreened by preparing DNA and spotting it onto duplicate nitrocellulose filters, which were hybridized to either a mucosa or a carcinoma-derived cDNA probe. Interesting clones were labeled by using the BRL nick translation kit and hybridized to Northern blots containing RNA from colon carcinoma and mucosa from the same individual.

To obtain a full-length clone of p1A-4, a human colon λgt11 cDNA library (HL1034b) from Clontech Laboratories was screened. Filter preparation, hybridization, and washes were performed as described by Maniatis et al. (41). Approximately 100 ng of p1A-4 insert was labeled by random priming, using the oligolabeling kit from Boehringer Mannheim.

RESULTS

Isolation clone p19E. To detect genes that were overexpressed in colorectal tumors relative to normal colonic mucosa, we prepared a cDNA library from a moderately well differentiated adenocarcinoma. A library composed of 470,000 transformants per μg of cDNA was screened with cDNA probes derived from mRNA isolated from the adenocarcinoma from which the library was prepared and from histologically normal colonic mucosa directly adjacent to the tumor. Approximately 600 transformants were screened, of which 11 showed differential hybridization by dot blot analysis. These were used as probes in subsequent Northern blot analyses. One of these clones, p1A-4, was characterized further.

Clone p1A-4 contains a 360-bp insert that detects a 1-kb mRNA on Northern blots. This transcript was about 10-fold more abundant in total RNA derived from the tumor than in RNA from the normal mucosa. To obtain a full-length clone of the message that p1A-4 detects, a commercially prepared λgt11 cDNA library from Clontech Laboratories was probed with the p1A-4 insert. Positive hybridizing plaques were picked, amplified, and analyzed by restriction enzyme digestion and hybridization. A clone with an 852-bp insert was obtained and subcloned into pUC19, and the resulting plasmid was termed p19E. When p19E was used to probe a Northern blot of normal mucosa and tumor RNA, it detected a transcript of the same size and abundance as detected by p1A-4.

p19E encodes ribosomal protein S3. Analysis of the sequence of nucleotides in p1A-4 revealed a 362-bp insert containing a continuous ORF of 120 codons. There was no poly(A) stretch or obvious polyadenylation signal. The DNA sequence of nucleotides in clone p19E revealed a 826-bp insert (Fig. 1). Nucleotides 6 to 334 of the p19E insert were identical to those of the insert present in p1A-4. The sequences differed at two positions near the 5′ ends and at three positions near the 3′ end of p1A-4. The sequence of a polymerase chain reaction-generated fragment corresponding to the 3′ end of p1A-4 derived from a primary colon carcinoma matched the sequence of p19E. Therefore, we assume the differences present in p1A-4 were due to cloning artifacts or sequencing errors. From the size of the mRNA detected by p19E in Northern blots, the insert present in this plasmid must be close to a full-length cDNA.

The p19E insert contained a continuous ORF of 243 codons terminated by a TAA stop codon. There were eight nucleotides at the 5′ end of the clone before the apparent initiation codon of the ORF and 88 additional nucleotides following the termination codon. A potential poly(A) addition signal (AATAAA) is located at nucleotides 798 to 803, 58 nucleotides beyond the stop codon. The methionine codon at nucleotide 9 is in a strong initiation context (37). The predicted protein of 243 amino acids has no obvious structural motifs, and secondary structure predictions using Chou and Fasman (18) analysis predict a protein with approximately 40% α-helical and 40% β-sheet content. The carboxy-terminal 40 residues tend to be hydrophobic, and this region of the molecule is predicted to exist largely as random coil.

Comparison of the putative 19E-encoded protein sequence with sequences in the NBRF/PIR and Swiss-Prot data bases on Bionet revealed a match of 61 of 63 consecutive amino acids with a *Xenopus laevis* ribosomal protein termed S1 by Amaldi et al. (1). The homology between the *Xenopus* S1 ribosomal protein and the translated ORF of p19E is between amino acids 19 and 79 in the *Xenopus* S1 sequence and between amino acids 146 and 208 in the p19E insert. The remainder of these two proteins show little or no sequence similarity. However, comparison of the translated p19E ORF with the rat ribosomal protein S3 (11) revealed that they were identical in 242 of 243 positions. On the basis of this close identity, we conclude that the p19E insert encodes the human S3 ribosomal protein.

Colorectal tumors and polyps contain increased levels of S3 mRNA. To determine whether the increased level of S3 mRNA observed in the differential screen of the cDNA library is common in colorectal cancer, we performed a Northern blot analysis on poly(A)$^+$ RNA derived from the

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          I
CGGGAAAG

ATG GCA GTG CAA ATA TCC AAG AGG AGG AAG TTT GTC GCT GAT GGC ATC TTC AAA GCT GAA 68
MET ALA VAL GLN ILE SER LYS LYS ARG LYS PHE VAL ALA ASP GLY ILE PHE LYS ALA GLU 20

CTG AAT GAG TTT CTT ACT CGG GAG CTG GCT GAA GAT GGC TAC TCT GGA GTT GAG GTG CGA 128
LEU ASN GLU PHE LEU THR ARG GLU LEU ALA GLU ASP GLY TYR SER GLY VAL GLU VAL ARG 40

GTT ACA CCA ACC AGG ACA GAA ATC ATT ATC TTA GCC ACC AGA ACA CAG AAT GTT CTT GGT 188
VAL THR PRO THR ARG THR GLU ILE ILE ILE LEU ALA THR ARG THR GLN ASN VAL LEU GLY 60

GAG AAG GGC CGG CGG ATT CGG GAA CTG ACT GCT GTA GTT CAG AAG AGG TTT GGC TTT CCA 248
GLU LYS GLY ARG ARG ILE ARG GLU LEU THR ALA VAL VAL GLN LYS ARG PHE GLY PHE PRO 80

GAG GGC AGT GTA GAG CTT TAT GCT GAA AAG GTG GCC ACT AGA GGT CTG TGT GCC ATT GCC 308
GLU GLY SER VAL GLU LEU TYR ALA GLU LYS VAL ALA THR ARG GLY LEU CYS ALA ILE ALA 100

                                  I
CAG GCA GAG TCT CTG CGT TAC AAA CTC CTA GGA GGG CTT GCT GTG CGG AGG GCC TGC TAT 368
GLN ALA GLU SER LEU ARG TYR LYS LEU LEU GLY GLY LEU ALA VAL ARG ARG ALA CYS TYR 120

GGT GTG CTG CGG TTC ATC ATG GAG AGT GGG GCC AAA GGC TGC GAG GTT GTG GTG TCT GGG 428
GLY VAL LEU ARG PHE ILE MET GLU SER GLY ALA LYS GLY CYS GLU VAL VAL VAL SER GLY 140

AAA CTC CGA GGA CAG AGG GCT AAA TCC ATG AAG TTT GTG GAT GGC CTG ATG ATC CAC AGC 488
LYS LEU ARG GLY GLN ARG ALA LYS SER MET LYS PHE VAL ASP GLY LEU MET ILE HIS SER 160

GGA GAC CCT GTT AAC TAC TAC GTT GAC ACT GCT GTG CGC CAC GTG TTG CTC AGA CAG GGT 548
GLY ASP PRO VAL ASN TYR TYR VAL ASP THR ALA VAL ARG HIS VAL LEU LEU ARG GLN GLY 180

GTG CTG GGC ATC AAG GTG AAG ATC ATG CTG CCC TGG GAC CCA ACT GGT AAG ATT GGC CCT 608
VAL LEU GLY ILE LYS VAL LYS ILE MET LEU PRO TRP ASP PRO THR GLY LYS ILE GLY PRO 200
                                                  SER

AAG AAG CCC CTG CCT GAC CAC GTG AGC ATT GTG GAA CCC AAA GAT GAG ATA CTG CCC ACC 668
LYS LYS PRO LEU PRO ASP HIS VAL SER ILE VAL GLU PRO LYS ASP GLU ILE LEU PRO THR 220

ACC CCC ATC TCA GAA CAG AAG GGT GGG AAG CCA GAG CCG CCT GCC ATG CCC CAG CCA GTC 728
THR PRO ILE SER GLU GLN LYS GLY GLY LYS PRO GLU PRO PRO ALA MET PRO GLN PRO VAL 240

CCC ACA GCA TAA CAGGGTCTCCTTGGCAGCTGTATTCTGGAGTCTGGATGTTGCTCTCTAAAGACCTTTAATAAA 803
PRO THR ALA                                                                    243

ATTTTGTACAAAGGCGGGAATTC                                                        826
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FIG. 1. Sequence of 19e. The sequence of nucleotides in 19e is shown with the longest ORF. The nucleotides that are identical in 1A-4 and 19e are underlined. The amino acid that differs in 19e and rat ribosomal protein S3 is shown below the amino acid sequence.

adenocarcinomas and normal mucosa of eight patients. Examples of these Northern blots are shown in Fig. 2. The Northern blots were also probed with the β-actin gene to correct for variability in RNA loading. The relative increase in RNA abundance for a typical tumor mucosa pair was determined by densitometry. All of these tumors except one contained 5- to 10-fold more S3 mRNA than was present in histologically normal mucosa directly adjacent to the tumor. The S3 mRNA was overexpressed about twofold in the other tumor. We conclude that increased levels of the S3 mRNA is a common occurrence in carcinoma of the colon. We also examined the levels of S3 mRNA in established cell lines derived from colorectal tumors. Figure 3 shows that the CaCo2 line expresses a greater amount of S3 mRNA than is found in a primary tumor, a level about 20-fold higher than that found in normal colonic mucosa. Similar results were obtained with the HT29 and LIM1863 cell lines (data not shown).

Adenocarcinomas of the colon are thought to arise from benign neoplastic growths called adenomatous polyps. Polyps are generally much smaller than tumors, and thus it is more difficult to obtain large quantities of RNA. For this reason, we isolated total RNA from 10 adenomatous polyps and from normal mucosa and examined the levels of S3 mRNA by Northern hybridization, again normalizing for the amount of β-actin mRNA present in each preparation. Examples of these results are shown in Fig. 4. Of the 10 polyps examined, 7 showed increased levels of the S3 transcript. In one patient from whom RNA from normal mucosa, adenomatous polyp, and carcinoma was available, the amount of S3 transcript in the polyp was approximately equivalent to the amount of S3 transcript in the tumor (data not shown). Thus, whatever the event(s) that leads to the increased abundance of the mRNA for this ribosomal protein, it precedes the onset of frank malignancy.

Overexpression of several ribosomal protein genes in colon tumors and polyps. To determine whether the genes for other ribosomal proteins were also overexpressed in tumors and polyps, we used rat probes to examine the mRNA levels for S6, S8, S12, L5, L26, L35, and P0. Examples of these results are shown in Fig. 5 and 6. Again the relative levels of

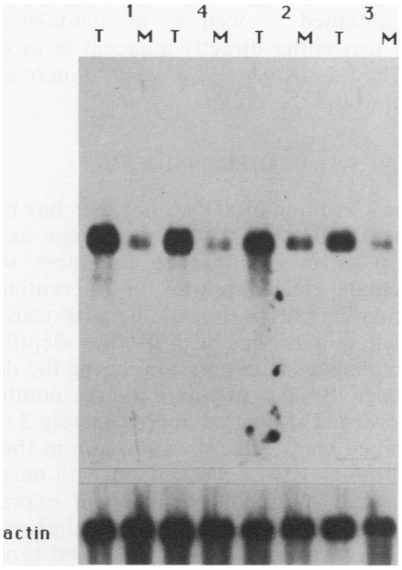

FIG. 2. Level of the transcript of 19e in colon tumors. Poly(A)$^+$ RNA (1 μg) from tumor (T) or mucosa (M) from patients 1 to 4 was separated by electrophoresis on a 1% agarose–3% formaldehyde gel. Gels were transferred to GeneScreen by capillary action in sodium phosphate buffer, pH 6.5. Plasmids p19e and pBSactin (200 ng) were nick translated by using a BRL kit. Hybridization was in a mixture containing 0.725 M sodium chloride, 0.15 M sodium phosphate, pH 7.2, 1 mM EDTA, 0.1% SDS, and 100 μg of single-stranded, salmon sperm DNA per ml for 12 to 16 h at 65°C. After being washed for 5 min at room temperature in 2× SSC–0.1% SDS, the blots were washed four times for 20 min at 65°C; the first two washes were in 25 mM sodium phosphate, pH 7.2–0.1% SDS, and the last two washes were in 25 mM sodium phosphate, pH 7.2–1 mM EDTA–1% SDS at 65°C. This blot was hybridized separately to p19e and to pBSactin; the filter was stripped between hybridizations by boiling the filter in 2 mM Tris, pH 8.0–2 mM EDTA–1% SDS twice for 20 min each time. Molecular weight standards (not shown) were purchased from BRL and were used to estimate the size of the message.

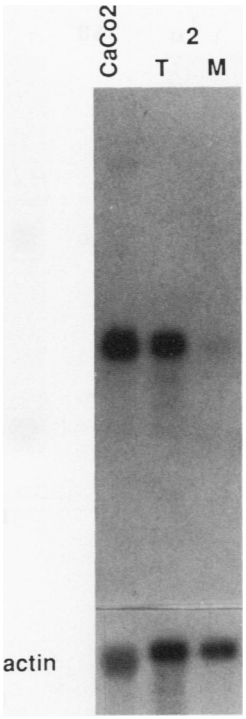

FIG. 3. Expression of 19e in a colorectal cell line. Total RNA from CaCo2 cells (4 μg) and tumor and mucosa poly(A)$^+$ RNA (100 ng) from patient 2 were analyzed as described in the legend to Fig. 2.

ribosomal protein mRNA and β-actin mRNA was determined by densitometry.

Five of the probes detected transcripts that were present at higher levels in adenocarcinomas than in matched normal colonic mucosa (Fig. 5). The pS6-4 and pS8-14 probes detected mRNAs of 820 and 980 bp respectively, that were overexpressed in all of three patients examined. The RNA transcripts detected by the S12 and P0 (data not shown) probes were 480 and 830 bp, respectively, and were more abundant in four of four tumors. The L5 gene probe hybridized to a 1,200-bp mRNA and was more abundant in two of two tumors. We did not detect a transcript in either tumors or normal tissue with use of the L26 or L35 probe. Within individual tumors, all of these ribosomal protein mRNAs were present at levels 4- to 10-fold higher than that found in adjacent normal colon, suggesting that the mechanism leading to the increased mRNA levels acts coordinately on these genes.

We next examined the mRNA levels for S6, S8, and L5 in adenomatous polyps. Figure 6 shows examples of Northern blots containing total RNA from polyps or normal mucosa hybridized to the S6, S8, or L5 probe. The S6 ribosomal protein gene was overexpressed in two of the four polyps examined. All three polyps examined showed increased levels of the S8 mRNA. The L5 gene was overexpressed in the single polyp examined. In this case we also examined the level of the L5 transcript in an adenocarcinoma that was simultaneously present in the same patient from which the

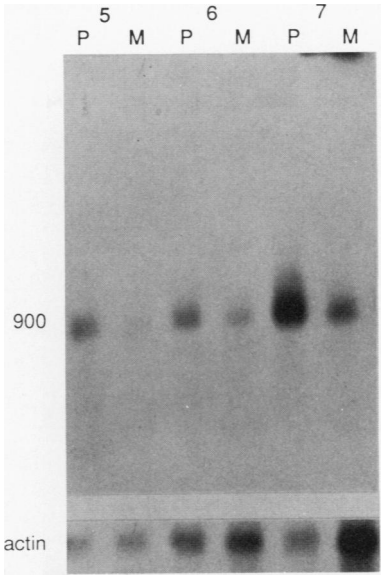

FIG. 4. Overexpression of 19e in colon polyps. Gels and blots were done as described in the legend to Fig. 2 except that 1 to 10 μg of total RNA from polyps (P) and mucosa (M) was loaded onto the gel.

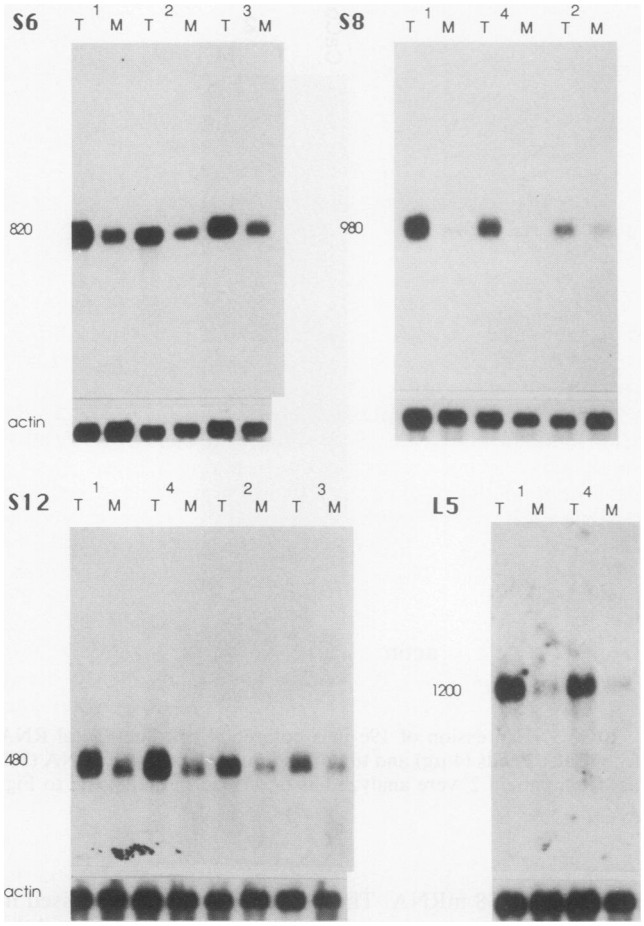

FIG. 5. Overexpression of S6, S8, S12, and L5 ribosomal proteins in colon tumors. Gels, transfer, and hybridizations were done as described in the legend to Fig. 2 except that the temperature of hybridization was 55°C. Estimated sizes of messages (in nucleotides) are shown at the left of each blot.

polyp was obtained as well as in histologically normal mucosa that was either directly adjacent to or distant from the tumor. The L5 mRNA was present at increased levels in both the tumor and the polyp.

DISCUSSION

Plus/minus screening of cDNA libraries has been used to identify genes whose mRNA levels change as a result of differentiation or in response to treatment with growth factors. Screening cDNA libraries for differential expression of genes in familial polyposis, colonic adenocarcinoma, and normal colonic mucosa has been used to identify the genes that undergo changes in expression during the development of colon cancer. Studies in which a large number of genes have been screened show that approximately 2 to 7% of the clones examined show altered expression in the carcinoma or in familial polyposis in comparison with normal mucosa (2, 3). With this approach, changes in expression of a laminin-binding protein (62), the ribosomal protein L31 (15), cytochrome *c* oxidase (32), and type I and type II keratin genes (53) have been observed. In this study, we prepared a cDNA library by using mRNA isolated from an adenocarcinoma of the colon and used differential screening with cDNA probes prepared from the carcinoma and from adjacent normal mucosa to detect genes whose level of expression changes during tumorigenesis. We anticipated that some of the products of the genes detected by such a screen would be involved in determining the biological properties of the tumor, while many would be genes whose transcript levels change as a result of tumorigenesis.

We were able to isolate a cDNA clone (p19E) encoding the human S3 ribosomal protein because the mRNA is more abundant in adenocarcinomas than in normal colonic epithelium. We identified the protein encoded in the cDNA by its similarity to the rat S3 amino acid sequence (11). The deduced rat and human amino acid sequences differ at only 1 of 243 positions. The rat and human ORFs are 90% identical. Thus, the coding sequences for this protein have been highly conserved between rats and humans, as is

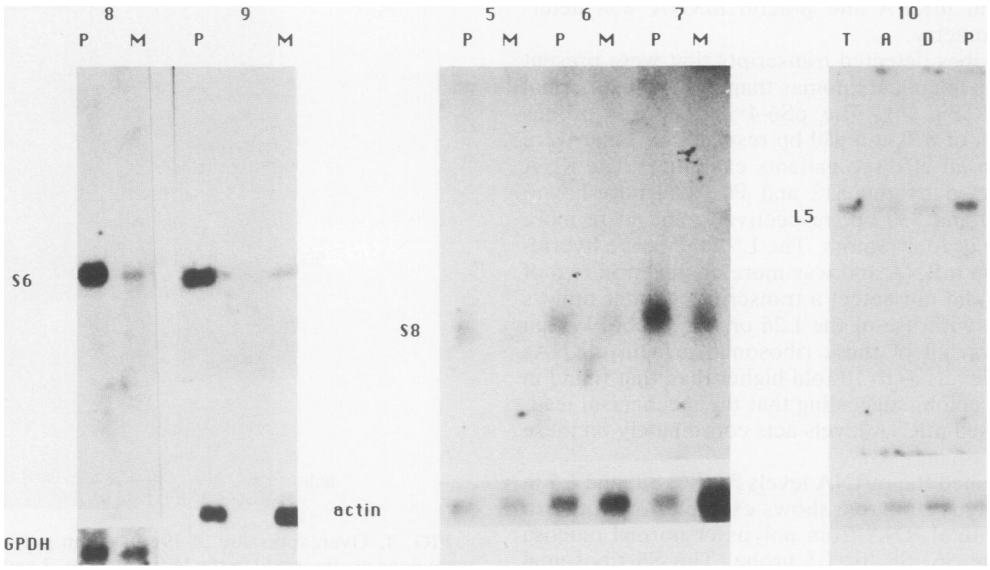

FIG. 6. Overexpression of S6, S8, and L5 in colon polyps. Gels, transfers, and hybridizations were done as described in the legend to Fig. 5 except that the amount of total RNA loaded varied between 1 and 10 μg. Both pGPDH and pBSactin were used as probes.

usually the case (61). An independent cDNA encoding human S3 has recently been isolated from WISH cells (63).

Both the rat and human S3 proteins possess a remarkable stretch of homology with the predicted amino acid sequence of a protein identified as the S1 ribosomal protein from *X. laevis* (1). The amino acid sequence of the human S3 protein as derived from the DNA sequence of p19E was identical to the *Xenopus* S1 protein sequence in 61 of 63 positions toward the center of the molecule. Little or no identity existed between these two molecules in either the amino-terminal or carboxy-terminal region.

Northern hybridization analysis showed that the S3 ribosomal protein genes had higher levels of expression in all eight adenocarcinomas of the colon examined than in matched normal colonic mucosa. To determine whether this change in mRNA levels was restricted to the S3 gene, we performed Northern analysis using probes for the genes encoding the S6, S8, S12, L26, L5, L35, and P0 ribosomal proteins. The L26 and L35 probes failed to detect any transcript in either normal or malignant colon and were not studied further. However, all of the remaining probes detected the expected transcript, and in each case overexpression was seen in tumors relative to normal mucosa. Single tumors showed increased levels of all of these ribosomal protein mRNAs, suggesting that these levels were coordinately controlled. Furthermore, the transcripts for these genes were also overexpressed in adenomatous polyps, the presumed precursor to adenocarcinomas. In an independent study, Ou et al. (46) found increased transcript levels detected by a cDNA clone that showed sequence similarity to the L3 *E. coli* ribosomal protein in hepatocellular carcinoma when compared with normal liver and in several transformed cell lines when compared with a "non-transformed" cell line. In addition, using slot blot hybridization, Chester et al. (15) have detected higher transcript levels for the human ribosomal protein L31 in colon carcinoma than in mucosa. These observed increases could be due to increased mRNA levels in tumor epithelial cells or in proliferating invading T cells and/or blood vessels and stromal tissue associated with the tumor. We favor the former hypothesis, since pure epithelial cell lines derived from colorectal tumors also have levels of the S3 transcript equivalent to those found in tumor, that is, much greater than are present in normal colonic mucosa.

It is not likely that increased transcription of ribosomal protein genes contributes directly to tumorigenesis. Rather, some earlier genetic event(s) signals affected cells to synthesize more ribosomes, hence the presence of more ribosomal protein transcripts. We have also observed that colorectal tumors and tumor-derived cell lines contain higher levels of rRNAs than does normal colonic mucosa (data not shown), consistent with increased numbers of ribosomes. The step-up of ribosomal protein synthesis occurs in most of the adenomatous polyps examined, implying that it occurs early in tumorigenesis, possibly concomitant with the onset of neoplasia.

It has been known for several years that many cell types undergoing active proliferation show increased levels of ribosomal proteins (36, 43, 56, 59). Indeed, an increase in ribosome biogenesis is an early event whenever growth increases, whether it be normal or malignant. However, the proliferative index of colorectal carcinomas is not significantly different from that of normal colonic mucosa (4, 33). Thus, it is unlikely that the increased levels of ribosomal protein mRNAs is simply due to the presence of a higher percentage of dividing cells in tumor. Furthermore, the regulation of ribosomal protein levels does not generally involve increased transcription or mRNA stability. In fact, several investigators examining a mechanism for the increase in ribosomal protein synthesis have concluded that growing cells more efficiently translate ribosomal protein mRNAs than do resting cells (20, 29, 31, 34). Furthermore, in chicken embryo fibroblasts and in mouse fibroblasts in which ribosomal protein synthesis is stimulated by insulin and serum, respectively, the levels of ribosomal protein mRNAs change little with the addition of the mitogens (20, 29). Our studies on colorectal cancer indicate that in this system the levels of mRNAs encoding ribosomal proteins are increased. It is not clear whether the higher mRNA levels are the result of increased transcription or decreased mRNA degradation.

While it would seem most likely that the increased level of ribosomal protein transcripts in tumor tissues is the result of neoplasia and not a causal event in carcinogenesis, there is one example of a ribosomal fusion protein that has been isolated from transformed cells that appears to have an active role in transformation. Ziemiecki et al. (64) have isolated the *trk*-2h oncogene from NIH 3T3 cells transformed with DNA from a human breast carcinoma cell line. The *trk*-2h gene codes for 41 amino acids of L7 a ribosomal protein fused to the receptor kinase domain of the *trk* proto-oncogene. This protein appears to have tyrosine kinase activity, and it has been shown to be closely associated with ribosomes. Thus, it remains possible that changes in ribosome activity could contribute to the neoplastic phenotype.

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