Expression of Human Sequences Related to Those of Mouse Mammary Tumor Virus

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Sequences related to those of the mouse mammary tumor virus (MuMTV) genome have been cloned from human DNA by screening a library prepared from the DNA of a human breast cancer cell line with MuMTV *gag-pol* DNA. Nine distinct groups of (MuMTV-related) sequences were identified among 100 lambda recombinants by cross-hybridization experiments with subcloned fragments containing *gag-pol*-related DNA. The largest group, of 64 recombinants, contains the MuMTV-related sequences cloned by others. The other eight groups contain MuMTV-related sequences that have not been described previously. The *gag-pol* regions of one recombinant from each of the nine groups were hybridized to RNA prepared from five human breast cancer cell lines, from placenta, and from two cell lines derived from other malignancies. RNAs were detected by probes for seven of the groups. The RNAs ranged in size from 1.2 to 12 kilobases. Probes for six of the groups detected large RNAs that could represent transcripts of full-length proviral DNA. Two of the probes detected RNA in one breast cancer cell line only. Most of the RNAs were detected in more than one cell line.

Retroviruses are involved in the etiology of a wide range of animal neoplasias (33). Recently a number of human retroviruses have been identified, one of which causes leukemia in humans (36). The full extent to which retroviruses are involved in human carcinogenesis, however, is unknown.

Mouse mammary tumor virus (MuMTV) is a type B retrovirus which causes mammary tumors in certain inbred strains of mice. Tumors can arise as a result of integration of proviral sequences, derived from exogenous or endogenous viruses, close to one of the *int* family of putative cellular oncogenes (9, 21, 25, 32).

Evidence has been presented for a human retrovirus that is related to MuMTV. Retroviruslike particles are elaborated by breast cancer cells in culture (15) and contain antigens recognized by antibodies against the MuMTV *env* protein (28).

Human endogenous retroviral sequences have been identified that show homology to type A (24), B (4, 6, 20, 24), and C (26, 27) retroviral genomes. Although many of the sequences examined to date are defective and do not contain significant open reading frames (4, 6, 27), some represent full-length proviruses (24, 27), and one contains open reading frames for *gag*, *pol*, and *env* proteins (24) and is transcribed into RNA (23, 24).

We (20) and others (4, 6) have demonstrated that human DNA contains a large number of endogenous sequences that are related to the MuMTV genome. We have recently cloned a large number of these human MuMTV-related sequences (18, 34) to facilitate analysis of their structure and function.

In this report we first show that although all of the MuMTV-related sequences hybridize to MuMTV *gag-pol* DNA, they are not all closely related to each other. We identify nine distinct groups of MuMTV-related sequences. A hybridization probe from each group was then used to

analyze the expression of MuMTV-related sequences in five human breast cancer cell lines, in two cell lines derived from other malignancies, and in human placenta.

MATERIALS AND METHODS

Cell culture. The MCF-7 (30), T47D (14), ZR-75 (7), EFM-19 (29), and MDA-MB 231 (3) human breast cancer cell lines and the HeLa (derived from a cervical carcinoma; 11) and A431 (derived from a vulval epidermoid carcinoma; 8) human cancer cell lines were maintained as described previously (19).

Preparation and analysis of DNA. DNA prepared from the MCF-7 cell line was digested to completion with *EcoRI* and transferred to nitrocellulose after electrophoresis on a 0.8% agarose gel. The DNA of the recombinants containing MuMTV-related sequences was immobilized in a matrix of dots as described previously (34).

RNA preparation. Total cellular RNA was prepared from the cell lines listed above by using the lithium chloride-urea precipitation method described previously (35). Poly(A)⁺ RNA was prepared by oligo(dT)-cellulose column chromatography (1). The same methods were also used to prepare total and poly(A)⁺ RNA from fresh human term placental tissue obtained by cesarian section (Princess Mary Maternity Hospital, Newcastle upon Tyne). Poly(A)⁺ RNA samples were denatured, electrophoresed on denaturing agarose gels, and transferred to nitrocellulose filters as described previously (19).

Hybridization. DNA was labeled with ³²P by nick translation to specific activities of 5 × 10⁸ cpm/μg. DNA filters were prehybridized for 24 h at room temperature and then hybridized for 3 days with 1 × 10⁶ to 5 × 10⁶ cpm/ml at 42°C. The prehybridization and hybridization solutions contained 35% formamide.

Radiolabeled RNA probes were synthesized by transcription of the MuMTV-related sequences cloned into the plasmid pGEM1, as described previously (35). Filters were

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FIG. 1. Hybridization of probes for the nine groups of MuMTV-related sequences to the 100 lambda recombinants. MuMTV DNA (0.1 ng per dot; A1), EMBL 3A DNA (50 ng per dot; A2), or DNA prepared from the 100 recombinants (50 ng per dot) was immobilized on nitrocellulose filters, using a hybridot manifold. Nitrocellulose sheets containing the DNA matrix were hybridized with 10^6 cpm of the respective ^{32}P -labeled probes per ml as described in Materials and Methods. The prehybridization, hybridization, and wash solutions contained 35% formamide.

prehybridized for 4 h at 55°C and then hybridized for 24 h with 8×10^6 cpm of ^{32}P -labeled probe per ml at 55°C . The prehybridization and hybridization solutions were as described previously (35). Filters were washed five times for 15 min each time at 55°C in $0.1\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 15 mM sodium citrate) and exposed against preflashed X-ray film at -70°C . After satisfactory autoradiographs were obtained, filters were rewashed at 65, 75, and 80°C .

Identification of groups of MuMTV-related sequences. Groups of MuMTV-related sequences were defined as being sufficiently closely related to hybridize in the presence of 35% formamide at 42°C . MuMTV *gag-pol*-related DNA and repetitive DNA elements were mapped within the lambda recombinants by hybridization with the 4.0-kilobase (kb) *Pst*I fragment of MuMTV DNA that contains *gag* and *pol* sequences (12) and with unfractionated MCF-7 DNA, respectively. The restriction maps for the representative clones for the nine groups that were identified are shown in Fig. 2. A restriction fragment containing MuMTV *gag-pol*-related DNA but no repetitive DNA was subcloned from NMWV 4 into the plasmid pUC-9. Plasmid DNA was purified, labeled by nick translation, and hybridized to the collection of 100 lambda recombinants containing MuMTV-related sequences. A second recombinant was then chosen from among those that had not hybridized to the first probe, and the procedure was repeated to identify a second group. The process was repeated until all recombinants hybridized to the *gag-pol* probe from at least one of the representative recombinants. This procedure resulted in the identification of nine groups of MuMTV-related sequences.

RESULTS

Identification of groups of MuMTV-related sequences. The isolation of lambda recombinants that contain sequences related to the *gag-pol* region of MuMTV from a human DNA library has been reported previously (34). Previous experiments had shown that there are a large number of MuMTV-related sequences in the human genome (20, 34), and it seemed likely that, as they all hybridize to MuMTV *gag-pol* DNA, they would constitute a family of closely related sequences.

The *gag-pol*-related sequence contained within NMWV 4 (18) was subcloned and hybridized to the 100 lambda recombinants. Surprisingly, only four other recombinants cross-hybridized even under conditions of reduced stringency. This implied that the human MuMTV-related sequences do not constitute one closely related family of sequences.

To explore the relationships between the MuMTV-related sequences and to facilitate analysis of their expression, they were divided into groups whose *gag-pol* sequences were sufficiently homologous to cross-hybridize. The procedure used to identify these groups is described in Materials and Methods. Figure 1 shows the hybridization with subcloned *gag-pol* DNA of the representative recombinants for each group to the 100 lambda recombinants. The number of recombinants in each of the groups is given in Table 1. Nine groups were defined, containing from 1 to 64 recombinants each. The majority of the recombinants could be assigned to a single group; only six recombinants hybridized to probes for two different groups (Fig. 1, recombinants E2, E10, F8,

TABLE 1. Number of recombinants in each group

Group	Position in matrix (Fig. 1)	No. of recombinants
1	E11	64
2	A5	17
3	C10	8
4	E1	5
5	F6	4
6	H5	3
7	D9	3
8	A3	1
9	B1	1

H1, H6, and I2). Two of these six recombinants have been mapped and have two separated regions showing homology to MuMTV *gag-pol* DNA.

The *gag-pol* regions of a number of recombinants apart from the representative recombinants of each group were also subcloned into pUC-9 or pUC-19 and hybridized to the matrix of MuMTV-related sequences. Two examples (A6 belonging to group 1 and A4 belonging to group 3) are shown in Fig. 2 alongside the representative recombinant of each group. In both cases, the probes hybridized to the same recombinants. This was true for all the nonrepresentative recombinants tested in this way.

The maps of the representative recombinants for the nine groups are shown in Fig. 3. The region hybridizing to the

FIG. 2. Hybridization of nonrepresentative recombinants of groups 1 and 3 to the 100 lambda recombinants. DNA from MuMTV, EMBL 3A, and the 100 recombinants was immobilized as a matrix on nitrocellulose filters as described for Fig. 1. The filters were hybridized with 10^6 cpm of the 32 P-labeled probes per ml as described in Materials and Methods. (A) Hybridization to the representative group 1 probe is shown alongside the hybridization to recombinant A6. (B) Hybridization to the representative group 3 probe is shown alongside the hybridization to recombinant A4. The prehybridization, hybridization, and wash solutions contained 35% formamide.

FIG. 3. Restriction maps of the representative recombinants of the nine groups of MuMTV-related sequences. The restriction sites in the human DNA contained within the nine lambda recombinants were positioned by analysis of single and double digests: S, *Sall*; H, *HindIII*; E, *EcoRI*; B, *BamHI*; and X, *XhoI*. The *Sall* sites and occasional *BamHI* sites at each end of the map are in the vector and delineate the junctions between the vector and insert DNA. The regions of the recombinants that hybridize with the MuMTV *gag-pol* DNA (solid bars) and contain repetitive DNA (open bars) are shown below the map. Strong (thick bars) and weak (thin bars) hybridization signals are indicated. The fragments subcloned for use as hybridization probes are marked above the maps by a line. NMWV 3 contains a large number of *HindIII* sites to the right-hand side of the insert DNA. The positions of these sites have not been mapped accurately, and there are additional sites within the sequences marked with a dashed line.

MuMTV *gag-pol* probe and the regions containing highly repetitive DNA elements are marked underneath the maps, and the restriction fragment containing the *gag-pol*-related sequence that was cloned into the plasmid vector pUC-9 or pUC-19 is indicated by a bar above the map. There were no obvious similarities between the restriction maps of the representative recombinants for each group, and this emphasizes the differences between the MuMTV-related sequences. Restriction mapping of recombinants within each group showed that few contained the same or overlapping fragments of DNA. For instance, only 6 of 29 group 1 recombinants that have been mapped contain overlapping DNA fragments. For the 23 nonoverlapping recombinants, similarities were often noted in the pattern of restriction sites around the region hybridizing to the MuMTV *gag-pol* probe.

Comparison of the restriction maps of the representative recombinants and their lack of cross-hybridization suggested

FIG. 4. Hybridization of the group 5 and 7 probes to the 100 lambda recombinants at reduced stringency. MuMTV DNA (0.1 ng per dot; A1), EMBL 3A DNA (50 ng per dot; A2), or DNA prepared from the 100 recombinants (50 ng per dot) was immobilized on nitrocellulose filters. Replica filters were hybridized to the group 5 probe (A) or the group 7 probe (B), as described in Materials and Methods, in the presence of 35% or 20% formamide.

that the different groups were only distantly related. To examine the relationships between the groups in more detail, the representative probes were hybridized at reduced stringency to all the recombinants. Two examples of the results obtained are shown in Fig. 4. At reduced stringency, the representative probes of group 5 and 7 hybridized to a large number of other recombinants. This hybridization is specific, as no hybridization was obtained to the vector EMBL 3A DNA. The pattern of hybridization with the representative probes, however, was complex. Many recombinants hybridized with various combinations of probes from groups 1, 3, 4, 5, 6, and 7, and half of the recombinants of group 2 hybridized with various recombinants of groups 1, 3, 5, 6, and 7. The recombinants of groups 8 and 9 did not hybridize to any other group. This analysis therefore showed that the majority of the groups of sequences are related, albeit distantly.

We have previously shown that MuMTV-related sequences have different degrees of mismatch to MuMTV *gag-pol* DNA (34). To ascertain whether any one group is particularly homologous to MuMTV, MuMTV *gag-pol* DNA was hybridized to the 100 recombinants at several different stringencies. An autoradiogram of the filter that had been hybridized in the presence of 30% formamide is shown in Fig. 5. These conditions discriminate between recombinants of various homology. Of the 10 recombinants giving the strongest hybridization signal, 5 belong to group 1 and 5 belong to group 2. As group 2 contains only 17 recombinants, this group contains a high proportion of the most related sequences.

The observation that groups 1 and 2 contained the sequences that were most homologous to MuMTV DNA suggests that groups 3 through 9 might contain fewer recombinants simply because they hybridized weakly when the

FIG. 5. Hybridization of the MuMTV *gag-pol* probe to the 100 cloned, MuMTV-related human sequences. A DNA matrix was prepared as described in the legend to Fig. 2. The filter was hybridized with 1.2×10^6 cpm of ^{32}P -labeled MuMTV *gag-pol* DNA per ml in the presence of 30% formamide at 42°C as described in Materials and Methods.

library was screened. To examine whether the sizes of the various groups reflect the number of sequences present in human DNA or their homology to MuMTV, the probes for the nine groups of sequences were hybridized to Southern transfers of *Eco*RI-digested DNA from MCF-7 cells (Fig. 6). The probes gave very different patterns of hybridization, and a large number of bands were detected by the probes for groups 1 through 7. In the case of the group 1 probe, very intense hybridization was observed to a 2.1-kb fragment, indicating that several MuMTV-related sequences have fragments of this size.

In general, the number of fragments hybridizing correlated with the size of the group. For example, the probe for group 1 hybridized to a large number of fragments, whereas those for groups 8 and 9 hybridized to few fragments. The most

FIG. 6. Hybridization of the probes for each of the nine groups of MuMTV-related sequences to MCF-7 cell DNA. *Eco*RI-digested DNA (10 μg) prepared from MCF-7 cells was electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose, and then hybridized with 4×10^6 cpm of the subcloned probes, used to define the nine groups, per ml. The number of the group is indicated below each track. Hybridization was in the presence of 50% formamide as described in Materials and Methods. The positions of the molecular-weight markers (in kilobases) are indicated on the left.

notable exception was the probe for group 5. This group contains only four recombinants, yet the probe for this group hybridized to a large number of fragments. The recombinants belonging to this group are among the least related to MuMTV (Fig. 6), and this could account for their being underrepresented in the collection of recombinants.

Expression of the human MuMTV-related sequences. The *gag-pol*-related sequences of the nine groups were subcloned into pGEM1. Strand-specific RNA probes were synthesized and hybridized to poly(A)⁺ RNA prepared from five breast cancer cell lines, two cell lines (HeLa and A431) derived from other human malignancies, and normal human placenta.

A number of RNAs were detected; in all cases, only one of the two strand-specific probes hybridized to the RNA (Fig. 7). The NMWV 1 and NMWV 3 probes hybridized to RNAs of 8 and 8.3 kb, respectively, in A431 poly(A)⁺ RNA. The 8-kb RNA was detected by RNA transcribed from the SP6 promoter but not the T7 promoter, whereas the 8.3-kb RNA was detected by RNA transcribed from the T7 promoter through an *EcoRI* fragment cloned in one but not the opposite orientation.

Seven of the nine probes detected one or more RNA species in at least one of the cell lines tested. There was a marked variation in the intensity of the hybridization signal and in the number of RNA species detected (Fig. 8).

The most intense hybridization signals were obtained using the NMWV 1, NMWV 3, NMWV 4, and NMWV 6 probes. The NMWV 1 probe detected RNAs of 12 and 8 kb. The 8-kb RNA hybridized most strongly and was most abundant in A431 cell and placental RNA, but was also present in ZR-75, T47D, and EFM-19 cells. The 12-kb RNA was detected in T47D, ZR-75, and EFM-19 cells.

Radiolabeled RNA probes synthesized from the subcloned *gag-pol*-related DNA of recombinant B4 (also belonging to group 1) detected RNAs of 8 kb in T47D, ZR-75B, EFM-19, A431, and placental RNA. The restriction maps of recombinants B4 and NMWV-1 show little similarity. This experiment therefore showed that two recombinants of one group detect the same pattern of expression and validates the use of representative recombinants of closely related groups of MuMTV-related sequences to detect expressed RNAs.

The NMWV 3 probe detected principally an RNA of 8.3 kb. This RNA was detected in all cells. It was most abundant in EFM-19, HeLa, A431, and placental cells and somewhat less abundant in T47D, ZR-75, MDA-MB 231, and MCF-7 cells. In contrast, the NMWV 4 and NMWV 6 probes hybridized principally to shorter RNAs. The most abundant RNA detected by the NMWV 4 probe was 1.2 kb long, but RNAs of 10, 4.5, 3.5, 2.8, and 2.2 kb were also visible. Hybridization to the 1.2-kb RNA was of similar intensity in all the cells tested. The NMWV 6 probe detected two RNAs of 2.9 and 2.2 kb. These RNAs were most abundant in the placenta and HeLa cells and were detected in A431 cells after a longer exposure. They were not detected in the breast cancer cell lines. Densitometric scanning of the autoradiographs showed that the 2.2-kb RNA was ninefold more abundant in placental RNA than it was in HeLa cell RNA.

Three probes (from NMWV 2, NMWV 7, and NMWV 9) gave weaker hybridization. The NMWV 2 probe hybridized to an 8-kb RNA present only in EFM-19 RNA. The NMWV 7 probe hybridized to a 7.0-kb RNA in all samples. The NMWV 9 probe hybridized to RNAs of approximately 8 and 7.2 kb that were only present in MDA-MB 231 cells.

Two probes (from NMWV 5 and NMWV 8) did not give clear hybridization to any of the RNA samples tested.

FIG. 7. Strand specificity of RNA probes used to detect MuMTV-related RNAs. Poly(A)⁺ RNA (10 µg) prepared from A431 cells was denatured, separated on a 1.2% agarose gel, and transferred to nitrocellulose as described in Materials and Methods. The filters were hybridized with ³²P-labeled RNA transcribed from NMWV 1 and NMWV 3. For NMWV 1, the 3.6-kb *Sall-BamHI* fragment was cloned in the pGEM1 vector and linearized with *HindIII* or *EcoRI*, and the linearized DNA was transcribed with SP6 or T7 polymerase, respectively. For NMWV 3, the 3.9-kb *EcoRI* fragment was cloned into pGEM1 in both orientations. Both constructs were digested with *HindIII* and transcribed with T7 polymerase.

NMWV 5 gave no detectable hybridization, and NMWV 8 hybridized to a smear of RNAs of different sizes (Table 2).

The variation in the intensity of the hybridization signal is presumed to reflect both the abundance of the RNA and the degree of homology between the probe and the RNA. To determine whether the RNAs detected were closely related to the probes, the filters were washed at increasing temperatures. The autoradiographs of filters hybridized to the NMWV 1, NMWV 3, NMWV 4, and NMWV 7 probes and then washed at 55, 65, and 75°C are shown in Fig. 9. For some RNAs (e.g., those detected by probes from NMWV 1 and NMWV 3), the probe remained hybridized after a 75°C wash, whereas for others (e.g., the RNA detected by NMWV 7), the probe was removed by a 65°C wash. In the case of NMWV 4, the hybridization to the 1.2-kb RNA was removed by a 65°C wash, whereas for the longer RNAs the hybridized probe remained on the filter even at 75°C. These two RNAs may represent transcripts from different related sequences, rather than different posttranscriptional products of the same sequence. The highest temperature at which the radiolabeled probe was retained on the filter is listed for each RNA in Table 2.

DISCUSSION

A large number of endogenous retroviral sequences that show homology to different classes of animal retroviruses have been identified in human DNA (2, 4, 20, 22, 27). We have been interested in the possibility that a type B-related retrovirus is elaborated in breast epithelial cells, following reports of MuMTV-related antigens and retroviruslike particles in the T47D human breast cancer cell line. We have cloned a large number of MuMTV-related sequences from human DNA to study their structure and to provide hybridization probes to identify proviral sequences that are expressed in breast cancer cells. The large number of MuMTV-related sequences, however, complicates their analysis. In

FIG. 8. Hybridization to RNA of RNA probes corresponding to the nine groups of MuMTV-related sequences. Poly(A)⁺ RNA was prepared from the MDA-MB 231 (MDA), MCF-7, T47D, ZR-75, and EFM-19 (EFM) human breast cancer cell lines, from HeLa and A431 cell lines, and also from placenta (Plac). Samples of 10 μ g were denatured, separated on 1.2% agarose gels, transferred to nitrocellulose, and hybridized with the ³²P-labeled RNA probes as described in Materials and Methods. Only the seven probes that gave hybridization signals are shown. The filters hybridized with NMWV 4 and NMWV 7 were washed at 55°C. The filters hybridized with the other probes were washed at 65°C. The names of the recombinants from which the probes were derived are shown above the filters, and the lengths of the RNAs detected are marked at the side in kilobases.

this study, groups of MuMTV-related sequences have been identified that are sufficiently closely related to cross-hybridize. Probes for each of the groups have then been used to identify MuMTV-related RNAs.

Group 1 is by far the largest of the groups, and it is noteworthy that it contains within it the MuMTV-related sequences cloned by others. Recombinant C5 (Fig. 1) contains the HLM-2 sequence described by Callahan et al. (4), recombinants A6 and E11 contain members of the HM family described by Deen and Sweet (6), and recombinants E2 and H6 contain HERV-K sequences (24). It is therefore likely that the 8-kb RNA detected by NMWV 1 is the same

as the 8.8-kb RNA detected in T47D cells by Ono et al. (23). The precise relationship of the sequences in the other groups to the group 1 sequences and MuMTV remains to be established. However, restriction mapping of a large number of the recombinants of groups 2 through 9 indicates that they do not correspond to human retroviral sequences with homology to Moloney murine leukemia virus (31), baboon endogenous virus (22), or other human retroviruslike sequences (16, 17).

The sequences in groups 2 through 9 and some of the sequences in group 1 therefore represent a large and novel collection of endogenous human retroviral sequences. The

TABLE 2. RNAs detected by probes for the nine groups of human MuMTV-related sequences

Probe	Fragment (kb)	RNAs (kb)	Expressed in:	Hybrid stable at temp ^a (°C):
NMWV 1	<i>Sall</i> - <i>Bam</i> HI (3.6)	12	T47D, ZR-75, EFM-19	75
		8	T47D, ZR-75, EFM-19, A431, placenta	75
NMWV 2	<i>Eco</i> RI (2.3)	8	EFM-19	75
NMWV 3	<i>Eco</i> RI (3.9)	8.3	All cells	75
NMWV 4	<i>Eco</i> RI (4.0)	10	All cells	75
		4.5	Placenta	65
		3.5	All cells	65
		2.8	MDA-MB 231, MCF-7, T47D, ZR-75, EFM-19, A431	55
		2.2	A431, placenta	55
		1.2	All cells	55
NMWV 5	<i>Bam</i> HI- <i>Eco</i> RI (1.4)	ND ^b		
NMWV 6	<i>Eco</i> RI- <i>Sall</i> (2.2)	2.9	HeLa, A431, placenta	75
		2.2	HeLa, A431, placenta	75
NMWV 7	<i>Eco</i> RI (4.2)	7	All cells	55
NMWV 8	<i>Bam</i> HI (0.7)	NDD ^c		
NMWV 9	<i>Hind</i> III (4.0)	8	MDA-MB 231	65
		7.2	MDA-MB 231	65

^a Wash temperature, the highest temperature at which the radiolabeled probe was retained on the filter.

^b ND, No RNAs detected.

^c NDD, No discrete RNAs detected.

nine human MuMTV-related probes detected a number of RNAs that show considerable variation in size, intensity of hybridization, and pattern of expression in the cell lines tested. All these RNAs gave strand-specific hybridization, and this therefore eliminates the possibility that the hybridization is to contaminating chromosomal DNA.

Early reports of human endogenous retroviral sequences showed that they were replication defective, containing only short open reading frames (27), and either were not expressed or were transcribed into relatively small RNAs (10). Two recent papers, however, have reported the detection of much larger RNAs expressed from retroviruslike human sequences (13, 23). We have detected a number of large RNAs in the range of 7 to 12 kb. These are of particular interest as they could represent full-length proviral transcripts, as has recently been reported by Ono et al. (23). Alternatively, they could represent composite RNAs containing both retroviral and cellular sequences, as reported for ERV3 mRNAs by Kato et al. (13). This second possibility would seem likely for the RNAs of 10 kb or more, as these are larger than would be expected for the expression of a full-length provirus.

A number of the detected RNAs showed marked differences in expression between the cell lines tested. The RNAs detected by the NMWV 2 and NMWV 9 probes were specifically expressed in EFM-19 and MDA-MB 231 cells, respectively. Most of the RNAs, however, were not specifically expressed in the breast cancer cell lines. Tissue-specific expression of human retroviral sequences has been reported by others (13), but the factors involved are not understood.

The expression of MuMTV is hormonally regulated by glucocorticoids, progestins, and androgens via an enhancer-like element in the long terminal repeat (5). Steroid hormones and their receptors may therefore contribute to the regulation of the expression of human endogenous MuMTV-related retroviral sequences. The RNAs we have detected were generally most abundant in placental tissue, and this has also been found for ERV3-related RNAs (13). Ono et al. have recently reported that the expression of the HERV-K human retroviral sequence is stimulated by estradiol and

FIG. 9. Elution of hybridized probes from immobilized RNA at increasing temperatures. Placental poly(A)⁺ RNA (10 µg) was electrophoresed, transferred to nitrocellulose, and hybridized with the NMWV 1, NMWV 3, NMWV 4, and NMWV 7 probes as described in the legend to Fig. 6. The filters were washed sequentially at 55, 65, and 75°C before autoradiography.

progesterone in T47D cells (23). We have found that the RNAs detected by NMWV 4 are regulated by progesterone (unpublished data).

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