Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5678–5682, August 1989
Biochemistry

The mouse homolog of the *hst/k-FGF* gene is adjacent to *int-2* and is activated by proviral insertion in some virally induced mammary tumors

(oncogenes/fibroblast growth factors/mouse mammary tumor virus/proviral activation/breast cancer)

GORDON PETERS*, SHARON BROOKES*, ROSALIND SMITH†, MARYSIA PLACZEK*‡, AND CLIVE DICKSON†

*Imperial Cancer Research Fund Laboratories, St Bartholomews Hospital, Dominion House, Bartholomew Close, London EC1A 7BE, United Kingdom; and †Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom

Communicated by George R. Stark, April 7, 1989

ABSTRACT **The fibroblast growth factor-related protooncogenes, *int-2* and *hst/k-FGF*, are within 17 kilobase pairs of one another on mouse chromosome 7 and are in the same transcriptional orientation. Approximately 70% of tumors induced in BR6 mice by mouse mammary tumor virus have proviral insertions adjacent to the *int-2* gene. We find that the murine homolog of the *hst/k-FGF* gene can also be transcriptionally activated by the insertion of mouse mammary tumor virus DNA either upstream or downstream of the gene. In most tumors, only one of these adjacent genes is activated, but in some cases both genes are expressed. One of the *hst*-expressing tumors also has a virally activated *int-3* gene. At least five distinct cellular genes (*int-1*, *-2*, *-3*, *-4*, and *hst/k-FGF*) can therefore contribute, either singly or in concert, to the development of histologically indistinguishable mammary tumors in mice infected by mouse mammary tumor virus.**

The *int-2* and *hst* (also known as *HSTF1*, *KS3*, and *k-FGF*) gene products have been shown by sequence similarity to belong to a family of related proteins of which the prototype is basic fibroblast growth factor (1–4). Both genes are also considered to be protooncogenes, but for different reasons. *int-2* was first identified within a common region for retroviral integration in tumors induced by mouse mammary tumor virus (MMTV), where it was subsequently shown that disruption of the locus by a provirus had activated the transcription of the gene (5, 6). In contrast, *hst* was identified as a dominant transforming gene when introduced into NIH 3T3 cells by DNA transfection (7, 8). In separate studies, high molecular weight DNAs extracted from a human stomach cancer (*hst/HSTF1*), a Kaposi sarcoma (*KS3/k-FGF*), and a melanoma, as well as from some normal tissues, were all shown to contain the same transforming gene, yet there was no evidence that it was expressed or played a direct role in the genesis of the original tumors (7–10). One possible explanation is that the expression of the gene is normally suppressed by a cis-acting element and that rearrangement or dissociation of these sequences during DNA preparation and transfection might permit the expression of the gene.

As well as being structurally related, the *hst* gene and the human homolog of *int-2* have been shown to map at approximately the same position on chromosome 11, at band q13 (9, 11–13). Indeed, it now appears that these genes are within 40 kilobases (kb) of one another and are consistently coamplified in about 10–20% of human breast cancers (13–18). This close linkage of the human genes prompted us to reexamine previously isolated clones of the genomic DNA flanking the mouse *int-2* locus. In particular, we had been investigating examples of MMTV-induced mammary tumors in which a provirus had integrated within the vicinity of the *int-2* gene but did not appear to activate *int-2* transcription. We now show that in two of these tumors the virus has activated a different cellular gene and that this gene is the murine homolog of *hst*. These data suggest that *hst* expression can directly influence the genesis of a naturally occurring tumor.

MATERIALS AND METHODS

Tumor DNA and RNA. The mouse mammary tumors discussed in this study were from the series analyzed in an earlier report (19). High molecular weight tumor DNA was prepared as described previously (5, 6). Total RNA was isolated by guanidinium thiocyanate extraction and centrifugation through cesium chloride, and the poly(A)$^+$ fraction was enriched by chromatography on poly(U)-Sepharose (6, 19).

The analysis of DNA by restriction enzyme digestion, agarose gel electrophoresis, and blot hybridization followed standard procedures that have been described in detail elsewhere (5, 6, 19). For RNA analyses, 5-μg aliquots of poly(A)$^+$ RNA (or 20 μg of total cellular RNA) were denatured in the presence of formaldehyde at 60°C and fractionated in agarose gels containing 2.2 M formaldehyde (19, 20). The separated RNAs were transferred to nylon membranes by blotting in 20× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate) and cross-linked by UV irradiation.

Nucleic Acid Hybridization. ^{32}P-labeled DNA probes were prepared from isolated restriction fragments by either nick-translation or priming with random oligonucleotides (Amersham). Hybridizations were performed in the presence of 50% (vol/vol) formamide at 42°C for DNA blots and at 50°C for RNA blots (6, 19). Filters were normally washed at high stringency at 65°C in 0.1× NaCl/Cit and 0.1% NaDodSO$_4$, but, for cross-species hybridization, the stringency was reduced to 2× NaCl/Cit at 50°C. In some experiments, RNA blots were hybridized with an antisense RNA probe for *int-2* prepared by transcribing cloned third exon sequences (*Hin*dIII–*Bam*HI fragment) with phage SP6 polymerase (21). Other probes used in these studies were the 0.79-kb *Eco*RI fragment from human *hst*, designated probe c in ref. 10, and a 1.5-kb *Eco*RI–*Bam*HI fragment from probe b for the *int-3* locus (22).

EC Cells. Mouse F9 embryonal carcinoma (EC) cells were maintained as previously described and treated with retinoic acid, N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP), and isobutylmethylxanthine to induce differentiated characteristics (21).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MMTV, mouse mammary tumor virus; LTR, long terminal repeat; Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate.

‡Present address: College of Physicians and Surgeons, Columbia University, New York, NY 10032.

Recombinant DNA Methods. Recombinant DNA clones were isolated from a λ library as described (5, 6). The library, originally constructed in Leroy Hood's laboratory (California Institute of Technology, Pasadena), contained a partial *Hae* III/*Alu* I digest of BALB/c mouse DNA inserted into the Charon 4A vector. A cosmid library (generously provided by Dimitris Kioussis, National Institute for Medical Research, London) was also screened for *hst* and *int-2* genomic sequences. This library consisted of partial *Sau*3A-digested C57BL/6 T-cell DNA in the COS202 vector (23). Subclones of selected DNA restriction fragments were prepared in appropriate plasmid vectors by standard procedures (20). DNA sequence analyses on specific segments of DNA are described in detail elsewhere (24).

RESULTS

Location of Proviruses in the *int-2* Locus. In a previous study, we surveyed a series of 30 spontaneous mammary tumors, arising in the BR6 strain of mouse, for proviral disruption and transcriptional activation of the *int-1* and *int-2* genes (19). As the limits of proviral influence have not been clearly defined, multiple combinations of restriction enzymes and genomic DNA probes were used to maximize the range of the analyses. In most cases, this established the location and orientation of the relevant MMTV proviruses, typically either 5′ or 3′ to the coding domains of the affected gene and at distances of up to 15 kb (refs. 19 and 25; Fig. 1). For *int-2*, the various enzyme/probe combinations spanned over 50 kb of mouse DNA, and this entire region is now represented in an overlapping set of λ DNA clones (ref. 25; Fig. 1).

Out of the 30 tumors analyzed in this way, 27 had sustained transcriptional activation of *int-1* or *int-2* and frequently both (19). Only three tumors, designated S40, E127A, and T103, scored negative for both genes. However, with one particular enzyme/probe combination (*Kpn* I-digested DNA with probe j; see Fig. 1), tumor S40 yielded both the expected >25-kb restriction fragment and a new fragment of ≈17.5 kb, that was consistent with insertion of MMTV proviral DNA downstream of the *int-2* gene.

To confirm the location and orientation of this provirus, a more detailed restriction map was constructed for the genomic DNA contained in the corresponding λ clone, λ1, and additional unique-sequence DNA probes were subcloned into plasmid vectors. The analysis of tumor S40 DNA with the probe designated EE1 in Fig. 1 produced novel virus–host junction fragments with the enzymes *Bam*HI, *Sac* I, and *Xba* I, as well as reproducing the result obtained with *Kpn* I and probe j (Fig. 2*a*). From the sizes of the novel fragments, the known restriction map of the BR6 strain of MMTV (26), and analogous data obtained using probe HH1 (data not shown), it was possible to deduce that the MMTV provirus in tumor S40 was apparently complete and in the position and transcriptional orientation depicted in Fig. 1. Moreover, the intensities of the novel bands on the Southern blot, relative to those from the normal allele, suggested that the tumor was essentially clonal for integration at this locus (Fig. 2*a*).

Provirus Adjacent to the Mouse *hst* Gene. It was initially assumed that the MMTV provirus in tumor S40 must represent an extreme example of activation of the *int-2* gene, particularly as it has been shown that a provirus at an equivalent distance upstream of the gene is capable of activating transcription (25). However, as judged by blot hybridization of poly(A)$^+$ RNA (Fig. 3*a*), as well as more sensitive RNase protection techniques (ref. 21; data not shown), there was no detectable expression of *int-2* in tumor S40. A number of possibilities were therefore considered: (*i*) that the location of the MMTV provirus close to *int-2* was coincidental and had no bearing on tumorigenesis, (*ii*) that transcription of the *int-2* gene had indeed been activated but was subsequently down-regulated during tumor progression (27), and (*iii*) that the provirus was affecting a different cellular gene.

The latter situation was the most readily testable, for example, by hybridizing tumor RNA with probes from around the S40 integration site (see below). However, we were also intrigued by the possibility that the gene in question might be the mouse homolog of *hst*, given the close linkage of *int-2* and *hst* on human chromosome 11 (9, 11–13, 17, 18). A 0.79-kb genomic DNA probe encompassing the second exon of human *hst* (10) was found to hybridize to the λ1 clone but not to any of the other λ clones from the mouse *int-2* locus. More specifically, this cross-reactivity was localized to a segment of λ1 DNA contained within the 1.65-kb *Hin*dIII

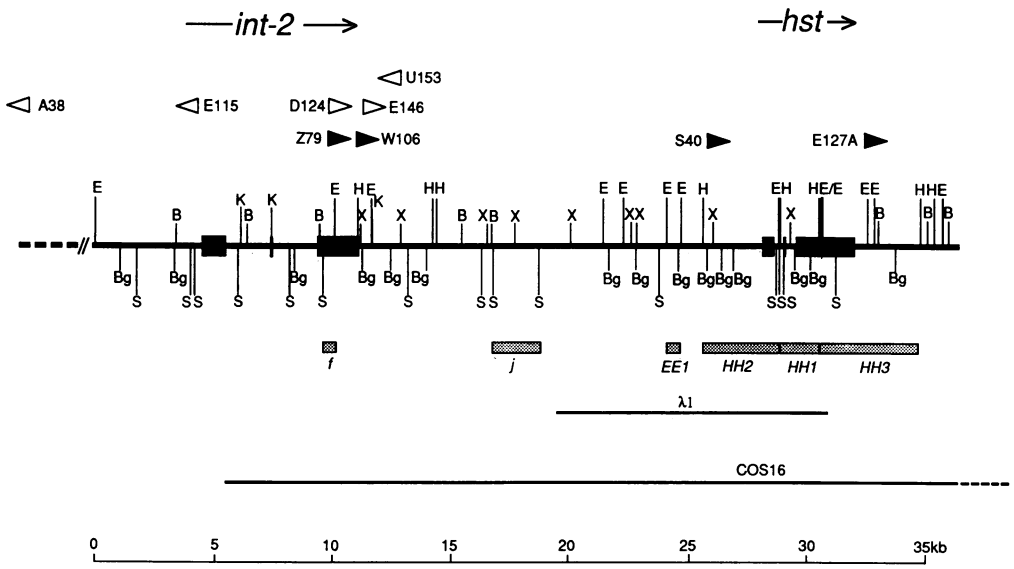

FIG. 1. Physical linkage of mouse *int-2* and *hst* genes. The linear map of mouse genomic DNA shows the sites of cleavage for the restriction enzymes *Bam*HI (B), *Bgl* II (Bg), *Eco*RI (E), *Hin*dIII (H), *Kpn* I (K), *Sac* I (S), and *Xba* I (X). The solid boxes represent coding exons for the respective genes, and the shaded boxes beneath the map depict genomic DNA restriction fragments used as hybridization probes. Each arrowhead above the map shows the position and transcriptional orientation of an integrated MMTV provirus in the numbered mammary tumor, with the filled symbols signifying the tumors in which the *hst* gene is expressed. The approximate extents of two of the recombinant DNA clones, λ1 and COS 16, that were used in the construction of the map are indicated.

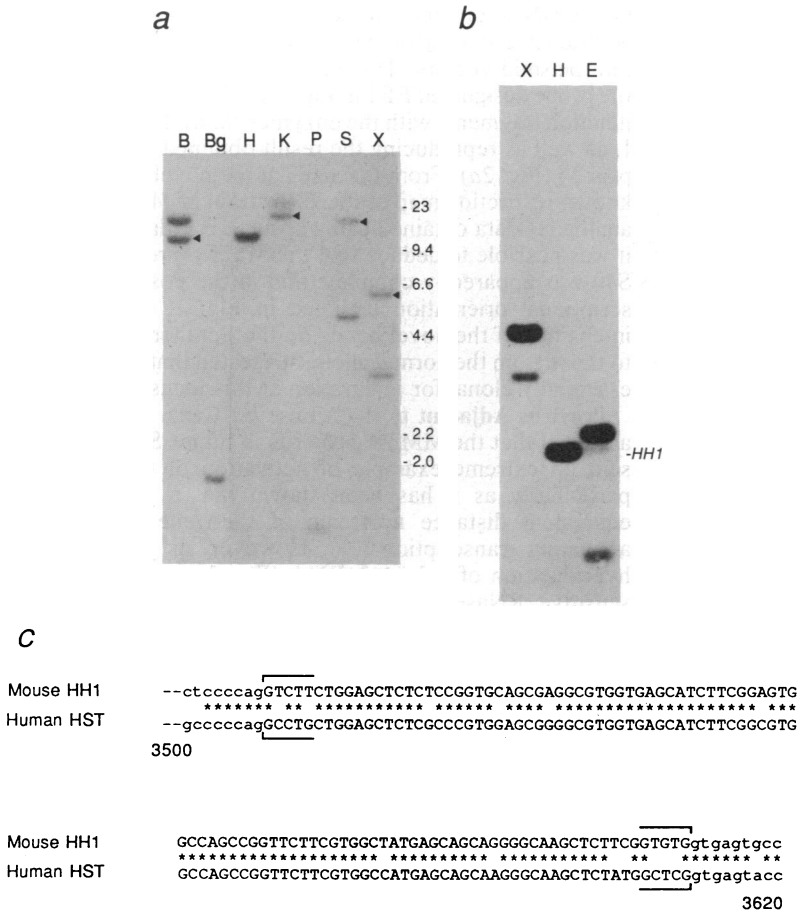

FIG. 2. An MMTV provirus adjacent to mouse *hst* sequences in tumor S40. (*a*) Southern blot of tumor S40 DNA cut with the restriction enzymes *Bam*HI (B), *Bgl* II (Bg), *Hin*dIII (H), *Kpn* I (K), *Pst* I (P), *Sac* I (S), and *Xba* I (X). The blot was hybridized with the EE1 probe depicted in Fig. 1. Novel junction fragments created by proviral insertion are indicated by arrowheads, and the numbers on the right indicate the positions and sizes of *Hin*dIII-digested λ DNA markers. (*b*) Southern blot in which DNA from the λ1 clone was digested with *Xba* I (X), *Hin*dIII (H), or *Eco*RI (E) and hybridized at reduced stringency to a 0.79-kb *Eco*RI fragment of genomic DNA from the human *hst* locus (10). The 1.65-kb *Hin*dIII fragment that we have designated HH1 is indicated. (*c*) The nucleotide sequence is shown for a segment of the HH1 fragment that has 88% sequence similarity with the second exon of human *hst* (from nucleotides 3500–3620 in the genomic sequence reported in ref. 10). Intron sequences are in lowercase and the boundaries of the exon are boxed.

fragment designated HH1 (Fig. 2*b*). DNA sequence analysis of HH1 identified a segment that is identical at 91 of the 104 residues that comprise the second exon of human *hst* (Fig. 2*c*). We have subsequently determined the exact location and organization of other exons of the mouse *hst* gene, as depicted in Fig. 1, by determining the complete genomic DNA sequence (24).

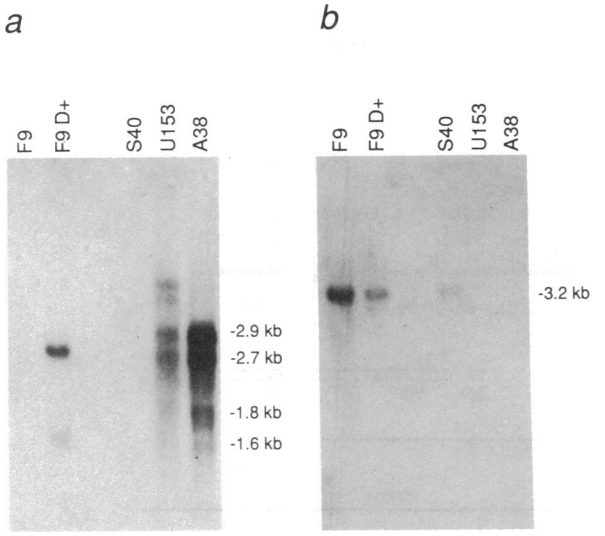

FIG. 3. Expression of *hst* RNA in tumor S40. Twenty micrograms of total RNA from F9 EC cells (F9) and F9 cells induced for 4 days with retinoic acid and Bt_2cAMP (F9 D+) and 5-μg samples of poly(A)$^+$ RNA from mammary tumors S40, U153, and A38 were fractionated on a formaldehyde/agarose gel and transferred to a nylon filter. The filter was hybridized sequentially with an antisense RNA probe for mouse *int-2* (*a*) and a randomly primed DNA probe corresponding to the HH1 fragment from mouse *hst* (*b*).

Expression of the *hst* Gene in Mouse Mammary Tumors. Having established that the HH1 fragment from λ1 contained part of the mouse *hst* gene, we tested whether it would hybridize to specific RNAs expressed in tumor S40. As shown in Fig. 3*b*, this probe detected a major transcript of ≈3.2 kb in S40 but not in two other tumors included in the analysis (Fig. 3*b*). As a further control for these experiments, we used RNA from the F9 EC cell line, since these cells are known to express *int-2*-encoded RNA when induced to differentiate with retinoic acid and Bt_2cAMP (ref. 21; and Fig. 3*a*). With *hst*, however, the 3.2-kb transcript was expressed in the undifferentiated F9 cells, and its level declined when the cells were treated with retinoic acid (Fig. 3*b*). A similar result has recently been reported by Yoshida *et al.* (28).

It was also important to establish whether the expression of *hst* in tumor S40 was an isolated case or whether other tumors in the original series expressed the gene. As exemplified in Fig. 4, we surveyed a total of 22 tumors, including many that had been shown to express both *int-1* and *int-2*, as well as the two examples (E127A and T103) that do not express either gene (19). Three additional *hst*-positive cases were identified, namely W106 and E127A (Fig. 4) and Z79 (data not shown), all of which contained substantially more *hst*-encoded RNA than was apparent in S40. This left only one tumor out of the original series (i.e., T103) that had not yielded evidence for gene activation by MMTV. However, when the RNA blot used in Fig. 4 was rehybridized with a riboprobe for *int-2*, a faint signal was detected in tumor T103 after prolonged exposure of the autoradiograph. The interpretation of this result remains unclear since the single band observed does not conform with the complex pattern of transcripts normally associated with *int-2*. It is nevertheless

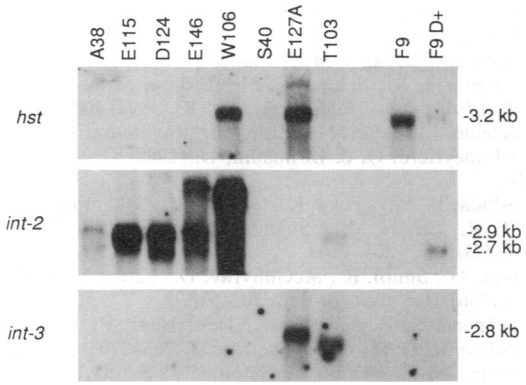

FIG. 4. Expression of *hst*, *int-2*, and *int-3* in a series of MMTV-induced mammary tumors. Poly(A)$^+$ RNAs (5 μg) from the indicated BR6 mouse mammary tumors were analyzed with probes for *hst*, *int-2*, and *int-3*. Samples (20 μg) of total RNA from F9 cells before (F9) and after induction with retinoic acid and Bt_2cAMP (F9 D+) were included as controls. The sizes of the major transcripts are indicated to the right. Note that the smaller 1.8- and 1.6-kb species of *int-2* RNA are not included.

possible that the improved sensitivity of the probe revealed a low level of *int-2* expression in a minor population of tumor cells that had not appeared positive for disruption of the *int-2* locus in the analyses of tumor DNA.

In our attempts to account for tumorigenesis in all 30 of the BR6 mammary tumors, we also investigated the expression of *int-3*, a gene that can be provirally activated by MMTV in mammary tumors in Czech-II mice (22). Both T103 and E127A expressed an RNA from the *int-3* locus (Fig. 4), and both showed quasi-clonal discontinuities in the *int-3* genomic DNA (data not shown). Thus, it appears that the dominant cell population in tumor T103 expresses *int-3* rather than *int-2*. None of the other BR6 tumors in the survey expressed *int-3*, and no transcript was detected in F9 cells (Fig. 4).

Mode of Activation of *hst*. In tumor S40, it could be assumed that activation of *hst* expression resulted from the insertion of a complete MMTV provirus upstream of the gene (Fig. 1). However, in E127A, Z79, and W106, there had been no indication of a provirus in the vicinity of the *hst* gene. We therefore reexamined genomic DNA from each of these tumors for discontinuities on either side of *hst*. The λ1 clone did not encompass the complete *hst* gene, but a cosmid clone (COS 16) has since been isolated that extends downstream of the gene (Fig. 1; ref. 24). By using a genomic DNA probe derived from COS 16 (HH3 in Fig. 1) and appropriate restriction enzymes, it was possible to survey DNA at least 10 kb 3′ of *hst*. No discontinuities were observed in Z79 and W106, suggesting that activation of the *hst* gene may be mediated by the MMTV proviruses in *int-2*. In tumor E127A, however, we noted several additional restriction fragments that were consistent with the introduction of $\approx$1.3 kb of DNA in the position shown in Fig. 1 (Fig. 5). This is roughly the size of the MMTV long terminal repeat (LTR) and there are precedents for activation of *int-2* by a solo LTR (29). The presence of an LTR was confirmed by digestion with *Pst* I, which cleaves at a single site close to the 5′ end of the MMTV LTR, and with other combinations of enzymes, as well as by hybridizing selected digests with an MMTV LTR probe (data not shown). These data also established that the LTR was in the same transcriptional orientation as the *hst* gene (Fig. 1).

DISCUSSION

To date, four presumed protooncogenes (*int-1*, *-2*, *-3*, and *-4*) have been identified on the basis of their proximity to and activation by integrated MMTV DNA in mouse mammary

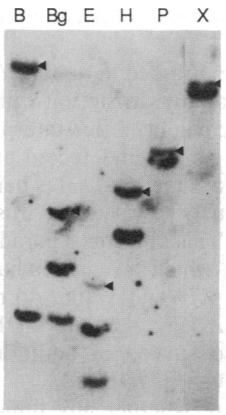

FIG. 5. Disruption of *hst* DNA in tumor E127A. A Southern blot is shown of tumor E127A DNA digested with the enzymes *Bam*HI, (B), *Bgl* II (Bg), *Eco*RI (E), *Hin*dIII (H), *Pst* I (P), or *Xba* I (X) and hybridized with probe HH3 (see Fig. 1). Additional bands created by insertion of MMTV LTR sequences are indicated by arrowheads.

tumors (30). Despite the similar strategies in their identification, these genes are quite distinct; they reside on different mouse chromosomes and encode unrelated proteins (30–32). We have now found a fifth gene that can be activated by MMTV; it is both related to and adjacent to *int-2* on mouse chromosome 7. Since there are at least two examples of proviral integration in the flanking chromosomal DNA, there is an argument for designating the locus as *int-5*. However, since the nomenclature is already confusing, we have elected to retain the acronym *hst* in this report, although it may be considered inappropriate for the mouse homolog.

Previous studies have suggested that proviral activation of *int-2* leads to expression of the normal gene product, in that no examples have been observed in which the protein coding domain of the gene has been perturbed (ref. 19 and unpublished results). Moreover, of the proviruses mapped within *int-2* in the BR6 tumors, all but three conformed to the pattern associated with enhancement of gene expression by the viral LTRs. Thus, proviruses located 5′ to the gene are in the opposite transcriptional orientation to *int-2*, whereas proviruses located 3′ of the coding domain are transcribed in the same direction as the gene (6, 19). The exceptions include cases where the provirus is in a "promoter insertion" mode, driving *int-2* expression directly from the MMTV promoter (19, 29), and an unusual situation where a deleted provirus has been mapped downstream of the gene but in the opposite transcriptional orientation (D. Baines, R. Moore, G.P., S.B., R.S., and C.D., unpublished results). This tumor (U153) expresses additional *int-2*-encoded RNAs that terminate within the viral LTR but apparently initiate at the normal *int-2* promoters (Fig. 3*a*). Although it defies the accepted norms for activation of *int-2*, because the viral promoter in the 3′ LTR comes between the enhancer and the target gene, this deleted provirus would be in the appropriate orientation to enhance expression of *hst*, albeit at a distance of 12 kb. However, as indicated in Fig. 3*b*, there was no detectable expression of *hst* RNA in tumor U153. Similarly, the provirus in tumor S40, and indeed the solo LTR in E127A, would be in the appropriate orientation for enhancing *int-2* expression, but they do not appear to do so. Such observations, coupled with the noticeable lack of proviral insertions within the 10 kb separating the integration sites in U153 and S40, are intriguing. If the mouse *hst* gene is under the control of a cis-acting suppressor, as suggested for human *hst*, then it is conceivable that the lack of insertions in this region may reflect an unusual chromatin conformation associated with the negative element. The proviral activation in tumor S40 could therefore represent a dissociation of the gene from its silencer rather than classical promoter insertion, since the *hst* transcript observed in the tumor is superficially similar to the presumably normal RNA expressed in F9 cells. Such an interpretation might also explain the low levels of *hst* RNA detected in tumor S40 compared to those seen in E127A. In the latter

tumor, the expression of *hst* is presumably enhanced by the downstream LTR.

However, the fact that *hst* is also expressed in tumors Z79 and W106, in the apparent absence of any disruption within the present limits of the *hst* locus, challenges this interpretation. Unless these tumors contain as yet undetected proviruses downstream of *hst*, we must assume that *hst* is being activated at a distance by the previously mapped proviruses in the 3′ untranslated region of *int-2*. This presents a paradox since other *int-2*-positive tumors, in which proviruses have been located very close to those in Z79 and W106, do not express detectable levels of *hst* RNA (Fig. 4, tumors D124 and E146). A more comprehensive survey of additional mammary tumors may help to resolve this issue.

Further studies might also indicate whether any tumors have sustained proviral disruptions in both *int-2* and *hst*, analogous to the previously reported activation of both *int-1* and *int-2* in ostensibly clonal populations of cells (19), and the dual activation of *hst* and *int-3* seen here in tumor E127A. Disruption of two genes can be explained if each event provided a selective advantage to the developing tumor cell. Although tumors Z79 and W106 featured among the examples that expressed both *int-1* and *int-2* (19), it is not clear whether coexpression of *hst* in these tumors was coincidental or provided an additional stimulus to neoplastic growth. This becomes an important question given the structural and perhaps functional similarities between *hst* and *int-2* and the possibility that mammary epithelial cells might respond to both factors, through the same or separate receptors. Interactions between these presumed growth factors and cognate receptors could also prove significant for the growth and differentiation of EC cells and other embryonic lineages. Given the strict spatial and temporal regulation of *int-2* expression during mouse development and the suggestion of multiple inductive roles, it will be interesting to determine the patterns of *hst* expression (33, 34). Whatever the answers, the independent activation of *hst* in tumors S40 and E127A is one of the strongest pieces of evidence to implicate *hst* in a naturally occurring cancer and suggests that at least five distinct cellular genes (*int-1*, *-2*, *-3*, *-4*, and *hst*), either singly or in various combinations, can contribute to the genesis of pathologically indistinguishable mammary tumors.

We are grateful to M. Terada and P. Little for communicating results and providing the probe for human *hst*, to R. Callahan for the probe for *int-3*, and to D. Kioussis for supplying us with the cosmid library. We also thank R. Watson and G. Stark for comments on the manuscript and J. Thurlow and A. Holder for additional help.

1. Abraham, J. A., Whang, J. L., Tumulo, A., Mergia, A., Friedman, J., Gospodarowicz, D. & Fiddes, J. C. (1986) *EMBO J.* **5**, 2523–2528.
2. Delli Bovi, P., Curatola, A. M., Kern, F. G., Greco, A., Ittmann, M. & Basilico, C. (1987) *Cell* **50**, 729–737.
3. Dickson, C. & Peters, G. (1987) *Nature (London)* **326**, 833.
4. Zhan, X., Bates, B., Hu, X. & Goldfarb, M. (1988) *Mol. Cell. Biol.* **8**, 3487–3495.
5. Peters, G., Brookes, S., Smith, R. & Dickson, C. (1983) *Cell* **33**, 369–377.
6. Dickson, C., Smith, R., Brookes, S. & Peters, G. (1984) *Cell* **37**, 529–536.
7. Delli Bovi, P. & Basilico, C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5660–5664.
8. Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M. & Sugimura, T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3997–4001.
9. Adelaide, J., Mattei, M., Marics, I., Raybaud, F., Planche, J., De Lapeyriere, O. & Birnbaum, D. (1988) *Oncogene* **2**, 413–416.
10. Yoshida, T., Miyagawa, K., Odagiri, H., Sakamoto, H., Little, P. F. R., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7305–7309.
11. Casey, G., Smith, R., McGillivray, D., Peters, G. & Dickson, C. (1986) *Mol. Cell. Biol.* **6**, 502–510.
12. Huebner, K., Ferrari, A. C., Delli Bovi, P., Croce, C. & Basilico, C. (1988) *Oncogene Res.* **3**, 263–270.
13. Yoshida, M. C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T. & Terada, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4861–4864.
14. Fantl, V., Brookes, S., Smith, R., Casey, G., Barnes, D., Johnstone, G., Peters, G. & Dickson, C. (1989) *Cancer Cells* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 7, pp. 283–287.
15. Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2980–2984.
16. Lidereau, R., Callahan, R., Dickson, C., Peters, G., Escot, C. & Ali, I. (1988) *Oncogene Res.* **2**, 285–291.
17. Nguyen, C., Roux, D., Mattei, M., de Lapeyriere, O., Goldfarb, M., Birnbaum, D. & Jordan, B. R. (1988) *Oncogene* **3**, 703–708.
18. Wada, A., Sakamoto, H., Katoh, O., Yoshida, T., Yokota, J., Little, P. F. R., Sugimura, T. & Terada, M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 828–835.
19. Peters, G., Lee, A. E. & Dickson, C. (1986) *Nature (London)* **320**, 628–631.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Smith, R., Peters, G. & Dickson, C. (1988) *EMBO J.* **7**, 1013–1022.
22. Gallahan, D. & Callahan, R. (1987) *J. Virol.* **61**, 66–74.
23. Kioussis, D., Wilson, F., Daniels, C., Leveton, C., Taverne, J. & Playfair, J. H. L. (1987) *EMBO J.* **6**, 355–361.
24. Brookes, S., Smith, R., Thurlow, J., Dickson, C. & Peters, G. (1989) *Nucleic Acids Res.* **17**, 4037–4045.
25. Peters, G., Brookes, S., Placzek, M., Schuermann, M., Michalides, R. & Dickson, C. (1989) *J. Virol.* **63**, 1448–1450.
26. Moore, R., Dixon, M., Smith, R., Peters, G. & Dickson, C. (1987) *J. Virol.* **61**, 480–490.
27. Sonnenberg, A., van Balen, P., Hilgers, J., Schuuring, E. & Nusse, R. (1987) *EMBO J.* **6**, 121–125.
28. Yoshida, T., Muramatsu, H., Muramatsu, T., Sakamoto, H., Katoh, O., Sugimura, T. & Terada, M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 618–625.
29. Moore, R., Casey, G., Brookes, S., Dixon, M., Peters, G. & Dickson, C. (1986) *EMBO J.* **5**, 919–924.
30. Nusse, R. (1988) in *Breast Cancer: Cellular and Molecular Biology*, eds. Lippman, M. E. & Dickson, R. (Nijhoff, Boston), pp. 283–306.
31. Gallahan, D., Kozak, C. & Callahan, R. (1987) *J. Virol.* **61**, 218–220.
32. Peters, G., Kozak, C. & Dickson, C. (1984) *Mol. Cell. Biol.* **4**, 375–378.
33. Wilkinson, D. G., Peters, G., Dickson, C. & McMahon, A. P. (1988) *EMBO J.* **7**, 691–695.
34. Wilkinson, D. G., Bhatt, S. & McMahon, A. P. (1989) *Development* **105**, 131–136.