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Ha-*ras* oncogene expression directed by a milk protein gene promoter: Tissue specificity, hormonal regulation, and tumor induction in transgenic mice

(whey acidic protein gene/whey acidic protein–*ras* transgene/Y chromosome integration/mammary gland tumors/salivary gland tumors)

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ABSTRACT **The activated human Ha-*ras* oncogene was subjected to the control of the promoter region of the murine whey acidic protein (*Wap*) gene, which is expressed in mammary epithelial cells in response to lactogenic hormones. The *Wap–ras* gene was stably introduced into the mouse germ line of five transgenic mice (one male and four females). *Wap–ras* expression was observed in the mammary glands of lactating females in two lines derived from female founders. The tissue-directed and hormone-dependent *Wap* expression was conferred on the Ha-*ras* oncogene. The signals governing *Wap* expression are located within 2.5 kilobases of 5′ flanking sequence. The other two lines derived from female founders did not express the chimeric gene. In the line derived from the male founder, the *Wap–ras* gene is integrated into the Y chromosome. Expression was found in the salivary gland of male animals only. After a long latency, *Wap–ras*-expressing mice developed tumors. The tumors arose in tissues expressing *Wap–ras*—i.e., mammary or salivary glands. Compared to the corresponding nonmalignant tissues, *Wap–ras* expression was enhanced in the tumors.**

The identification of oncogenes and the molecular mechanisms by which protooncogenes can be activated has provided a basis for the description of cancer as a genetic disease. A relatively small number of genes is able to subvert the growth program of cells and cause malignant transformation (1). *In vivo* and *in vitro* transformation studies indicate that tumorigenesis is a multistep process. The establishment of cell lines and their malignant transformation are two processes that can be distinguished in *in vitro* studies. The activated form of the Ha-*ras* oncogene has been shown to confer a stable tumorigenic phenotype to most established cell lines. Low levels of Ha-ras protein are not sufficient to transform primary rat fibroblasts. A second oncogene, which provides an establishment function (e.g., *myc*-, the *p53*-, or the *E1a* gene), is required (2–4). Additional genes and mutations may be crucial for tumorigenesis *in vivo* (5, 6).

The recent progress in obtaining transgenic mice that faithfully express foreign genes encourages the adaptation of this system for studies of oncogene effects *in vivo*. The information responsible for cell-type-specific gene expression is often located in the surroundings of the gene promoter. Recombination of these regulatory sequences has generally been sufficient to target chimeric gene expression in transgenic mice (7). This raises the possibility of analyzing the effect of an experimentally introduced oncogene on a particular cell type *in vivo* by coupling the oncogene to a tissue-specific promoter. The rat insulin II gene promoter directs the expression of the large tumor antigen (large T antigen) of simian virus 40 to the β cells of the endocrine pancreas in transgenic mice (8). The metallothionein gene promoter allows large T-antigen expression in liver cells (9). In both cases, tumors were observed in the T-antigen-expressing cells. The mouse mammary tumor virus-controlled expression of the *myc* oncogene in the mammary gland subsequently leads to mammary tumor formation (10). *myc* expression directed by the heavy- and light-chain immunoglobulin enhancers leads to the transformation of B lymphocytes in transgenic mice (11).

Our interest in transformation of mammary epithelial cells led us to study the effect of a specific oncogene on this particular cell type *in vivo*. Therefore, we introduced the activated human Ha-*ras*¶ oncogene under the putative control region of the murine whey acidic protein (*Wap*) gene into the genome of mice. The Wap is the major whey protein in the milk of rodents (13). The expression of the gene is regulated by the lactogenic hormone prolactin and is modulated by insulin and hydrocortisone. With the onset of lactation, Wap is synthesized in the differentiated mammary epithelial cells (14, 15). Here we describe that the *Wap* gene promoter confers tissue-directed and hormone-dependent expression onto the chimeric *Wap–ras* gene in transgenic animals. Mammary gland tumors were induced in a female mouse after several pregnancies and a high expression of the *Wap–ras* transgene was observed. A transgenic line carrying the *Wap–ras* gene on the Y chromosome expressed the transgene in salivary gland tissue. After a long latency, animals of this line developed salivary gland tumors expressing high levels of Wap–ras RNA.

MATERIALS AND METHODS

Construction of the *Wap–ras* Gene and Production of Transgenic Mice. A 2.5-kilobase (kb) *Eco*RI/*Kpn* I fragment (Fig. 1) corresponding to the promoter and 5′ flanking sequences of the murine *Wap* gene was cloned into the polylinker of the pUC18 vector (pUC18/5′Wap). The coding part of the Ha-*ras* gene was isolated as a 4.9-kb *Sma* I/*Bam*HI fragment from pEJ plasmid (16). Subsequently, the *Sma* I site was converted into a *Bam*HI site by addition of *Bam*HI linkers, leading to a *Bam*HI fragment of 4.9 kb (R. Jaggi and B.G., unpublished results). We cloned the 4.9-kb *Bam*HI fragment into the *Bam*HI site of the pUC18/5′Wap plasmid.

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Abbreviation: Wap, whey acidic protein.
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¶The recommended designation for this gene is *HRAS* (12).

The details of the *Wap–ras* construct are given in Fig. 1. For cloning procedures, standard protocols were followed (17). The *Wap–ras* gene was purified from vector sequences and was injected as linear 7.4-kb *Eco*RI/*Hin*dIII fragment into fertilized eggs (≈200 copies per egg) of C57BL6 × SJL hybrid crosses. Microinjections were done as described (18) and the eggs were reimplanted on the same day into pseudopregnant recipients.

DNA Analysis. DNA was prepared from tails of 3- to 4-week-old mice. Wap–ras sequences were routinely detected by the dot blot hybridization technique using 5 μg of tail DNA (18). For restriction digestions, the DNA was further treated with RNase and proteinase K. Digested DNAs were separated on 0.8% agarose gels and analyzed according to Southern (19).

RNA Analysis. Cytoplasmic RNA from various tissues was prepared essentially as described by Brawermann *et al.* (20). After separation from the nuclei, the cytoplasmic supernatant was extracted twice with phenol using an extraction buffer containing 0.15 M Tris·HCl, 5 mM EDTA, and 1% sodium dodecyl sulfate (pH 9.0). The RNA was analyzed by RNase protection assays as described (21). As a probe to detect Wap–ras RNA, we used a fragment derived from the hybrid gene extending from the *Pst* I site in intron 1 (position 1860 in the ras sequence; ref. 18) to an *Xba* I site in the linker sequence that joins the *Wap* and the *ras* fragments (see Fig. 3). After RNase treatment, the protected fragment will comprise 143 nucleotides containing 133 nucleotides from the *ras* sequence (exon sequences from position 1780 to the *Sma* I site used for cloning on position 1647; ref. 16) and the additional linker sequences. To detect Wap RNA, we used a *Sac* I/*Pst* I fragment of the *Wap* gene (15) containing exon 1 and exon 2 (see Fig. 3). After RNase treatment, we expect two protected fragments of 110 (exon 1) and 135 (exon 2) nucleotides. The RNase-resistant fragments were separated on 6% polyacrylamide gels containing 8 M urea.

RESULTS

Acquisition of the *Wap–ras* Gene and Establishment of Transgenic Lines. Tissue specificity of gene expression can be conferred to chimeric genes by the 5′ flanking regions of several differentiated cell-specific protein genes (7). To study the possible involvement of Ha-*ras* expression in the transformation process of mammary epithelial cells, the coding part of the activated human Ha-*ras* gene was subjected to the control region of the mammary gland-specific murine *Wap* gene. The *Wap* gene is expressed in mammary epithelial cells during lactation (14). We linked the putative control region of the murine *Wap* gene contained within a 2.5-kb *Eco*RI/*Kpn* I fragment encoding the sequences up to one nucleotide 5′ of the translation start codon AUG to the Ha-*ras* oncogene. The restriction maps of the two genes and the resulting *Wap–ras* construct are shown in Fig. 1. The *Wap–ras* hybrid gene was purified from most vector sequences by *Eco*RI and *Hin*dIII digestion before injection. Then 560 fertilized eggs were injected and 71 mice were born. A dot blot analysis of tail DNA was carried out and five animals (female nos. 3, 11, 40, and 58; male no. 69) were found to contain the injected sequences.

DNA blot analysis was used to investigate the presence, the integrity, and the approximate copy number of the *Wap–ras* gene in transgenic mice. DNA was digested with *Eco*RI and *Hin*dIII and blots were hybridized to a Ha-*ras*-specific probe (Fig. 2*A*). During the integration event, it is likely that the flanking *Eco*RI and *Hin*dIII restriction sites of the injected DNA are lost (22). For one integrated copy, we expect a fragment >7.4 kb hybridizing to the human Ha-*ras* probe. This can be seen in animal nos. 11, 40, and 58 (lanes 3–5). One band of 14.5, 10.5, and 9.5 kb, respectively, is found in the DNA of these animals. Animal no. 69 contains two copies of the transgene represented by bands of 18.5 and 8.8 kb (lane 1). Two to five copies were found in animal no. 3 (lane 2).

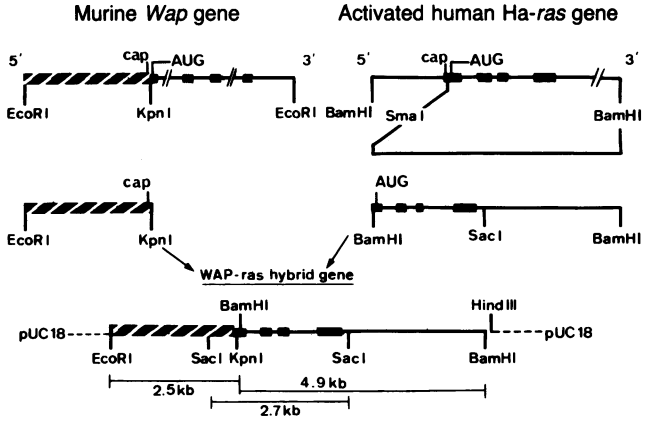

FIG. 1. Construction of the *Wap–ras* hybrid gene. Schematic drawing of the murine *Wap* gene, the human Ha-*ras* gene, and the fragments used to construct the hybrid gene, which is shown on the bottom line. Solid boxes, exon sequences; dashed box, the Wap promoter region; dotted lines, vector sequences. Cap indicates the transcriptional start and AUG indicates the translational start.

An internal 2.7-kb *Sac* I restriction fragment, which contains all four exons (Fig. 1) of the chimeric gene, was used to confirm the presence of nonrearranged Wap–ras DNA in the transgenic animals. This fragment can be visualized by DNA blot hybridization to a *ras*-specific probe in all five transgenic lines (Fig. 2*B*).

Breeding experiments have shown that in the four lines 3, 11, 40, and 58, 50% of the progeny, both male and female, contained the *Wap–ras* gene. This indicates autosomal transmission of the transgene. In line 69, 88 progeny of male no. 69 were analyzed. All 40 male offspring, but none of the 48 females, were found to be positive for the chimeric gene. The normal sex ratio in line 69 and the exclusive male transmission indicate integration of the *Wap–ras* gene into the Y chromosome.

Hormone-Dependent and Tissue-Specific Expression of the *Wap–ras* Gene in the Transgenic Lines. To test the potential of the 2.5-kb Wap regulatory region to direct the mammary

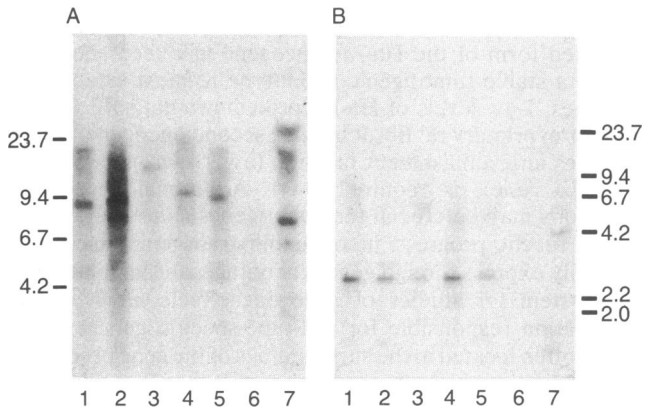

FIG. 2. DNA blot analysis of transgenic mouse tail DNA. (*A*) *Eco*RI/*Hin*dIII-digested DNA. (*B*) *Sac* I-digested DNA. Tail DNA (5 μg) from the transgenic founder animal nos. 69 (lane 1), 3 (lane 2), 11 (lane 3), 40 (lane 4), and 58 (lane 5). C57BL6 × SJL hybrid control mouse DNA (lane 6), and human placenta DNA (lane 7) were hybridized to a nick-translated ^{32}P-labeled 6.6-kb *Bam*HI fragment containing the human Ha-*ras* gene (16). *Hin*dIII-digested λ DNA was used as marker (indicated in kb).

gland-specific and lactogenic hormone-regulated *Wap–ras* expression, we analyzed the RNA of different tissues of transgenic animals for *Wap–ras* and for endogenous Wap sequences by RNase protection assays. To detect the Wap–ras RNA, we used a probe (*Xba* I/*Pst* I; Fig. 3) indicative for exon 1 of the hybrid gene that protects a fragment of 143 nucleotides. Endogenous Wap RNA can be detected by a probe that protects two fragments indicative for exon 1 (110 nucleotides) and exon 2 (135 nucleotides) of the murine *Wap* gene.

Fig. 3 shows the *Wap–ras* expression pattern in organs of a female representing line 3. The fourth mammary gland was surgically removed during the lactation period and the RNA was analyzed. Two weeks after the end of lactation, the animal was sacrificed and RNA was prepared from mammary glands, spleen, liver, lung, kidney, ovary, and brain. A protected fragment of 143 nucleotides is detected in the lactating mammary gland RNA (lane 1). After lactation, Wap–ras RNA is absent in mammary glands (lane 2) and in all other tissues analyzed (lanes 3–8). This indicates that stable Wap–ras RNA is only synthesized in the mammary gland during the stimulation by lactogenic hormones. The same pattern of expression was observed for the endogenous Wap RNA. It is present in large amounts in the lactating mammary glands (lane 11) and is not detectable in the mammary gland after lactation (lane 10) or in the tissues mentioned above (data not shown). The *Wap–ras* gene expression is subject to the same regulation by lactogenic hormones as the endogenous *Wap* gene. Although the transcriptional regulation of the *Wap* and the *Wap–ras* gene is very similar, the autoradiographic signals in Fig. 3 reflect

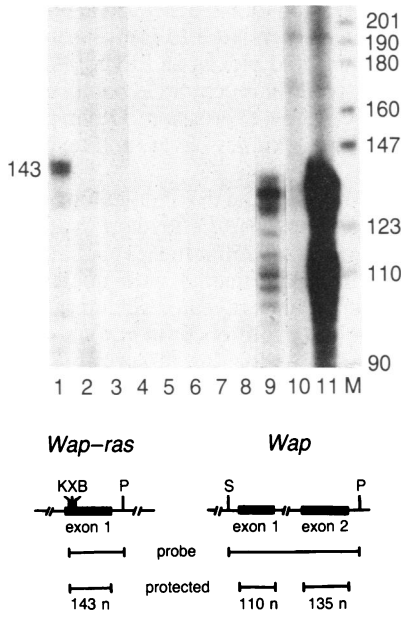

FIG. 3. Hormone-dependent expression of the *Wap–ras* gene. RNA (10 μg) was analyzed in an RNase protection assay as described. RNA was prepared from lactating mammary gland (7 days after parturition; lanes 1, 9, and 11), mammary gland (14 days after lactation; lanes 2 and 10), spleen (lane 3), liver (lane 4), lung (lane 5), kidney (lane 6), ovary (lane 7), and brain (lane 8) from a female of line 3. Lanes: 1–8, hybridized to the *Wap–ras*-specific probe; 9–11, with the *Wap*-specific probe. The exposure time was 18 hr, except line 9, which was exposed for 2 hr. Lane M contains *Hpa* II-digested ^{32}P-end-labeled pBR322 DNA (indicated in nucleotides). At the bottom, a scheme of the *Wap–ras* and the *Wap* probe used in the RNase protection assay is given. Wap–ras RNA protects a fragment of 143 nucleotides (n), Wap RNA protects two fragments of 110 nucleotides (exon 1) and 135 nucleotides (exon 2). For further details, see *Materials and Methods*. K, *Kpn* I; X, *Xba* I; B, *Bam*HI; P, *Pst* I; S, *Sal* I.

≈50-fold difference in the Wap and Wap–ras mRNA concentrations.

We investigated whether the hormonally controlled expression of the *Wap–ras* gene is confined to the mammary gland. RNA from different tissues of a lactating female of line 3 was isolated and analyzed for *Wap–ras* expression. The results of RNase protection assays are shown in Fig. 4*A*. Wap–ras RNA was detected in the mammary gland (lane 1) and in brain tissue of lactating females (lane 3). The brain is known to express a high proportion of the genetic information (23) and might exert less restrictions on transcription than other tissues. It is interesting to note that the expression of the *Wap–ras* gene is still hormonally regulated in the brain (Fig. 3, lane 8; Fig. 4, lane 3). No Wap–ras RNA was detected in the muscle (lane 2), kidney, liver, spleen, lung, and ovaries (lanes 4–8) of a lactating female. The endogenous Wap RNA was only found in the lactating mammary gland. In other organs, we found no *Wap* expression. In the males of these lines, neither Wap–ras nor Wap RNA was detected in salivary gland, testis, brain, kidney, liver, lung, spleen, and muscle. *Wap–ras* expression was only found in mammary gland and brain of lactating animals of lines 3 and 58. We conclude that in these two transgenic lines the *Wap* fragment restricts ras expression to these two tissues and confers hormonal regulation. In females and males of lines 11 and 40, we failed to detect *Wap–ras* expression.

We analyzed the *Wap–ras* expression in males of line 69. In this line, the chimeric gene is integrated into the Y chromosome. The 143-nucleotide fragment indicative of the Wap–ras RNA was detected in the salivary gland RNA by RNase protection assays (Fig. 4*B*). All other male organs tested (liver, kidney, testes, brain, spleen, muscle, and lung) do not show *Wap–ras* expression. Expression of the transgene in salivary glands was not found in males of the other transgenic lines. It is peculiar to line 69 and may be based on the integration of the transgene into the Y chromosome.

The probe we used to detect Wap–ras RNA does not contain the transcriptional initiation site. RNase protection assays were done on RNA prepared from lactating mammary glands, salivary glands, and tumor tissue (see below) using a probe indicative only for the *Wap* and the linker sequences of the hybrid gene (i.e., a 0.4-kb *Sac* I/*Bam*HI fragment; see

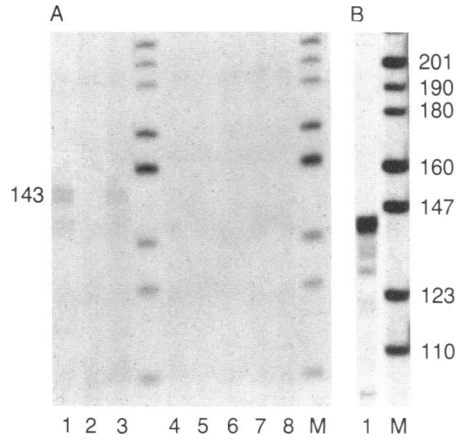

FIG. 4. Tissue-specific expression of the *Wap–ras* gene. RNA (10 μg) was analyzed in an RNase protection assay. (*A*) RNA was prepared from mammary gland (4 days after parturition; lane 1), muscle (lane 2), brain (lane 3), kidney (lane 4), lung (lane 5), liver (lane 6), spleen (lane 7), and ovary (lane 8) from a lactating female of line 3. (*B*) RNA was prepared from salivary gland (lane 1) from a male of line 69. The RNA was hybridized to the *Wap–ras* probe (see Fig. 3) resulting in a protected fragment of 143 nucleotides indicative for Wap–ras RNA. Lanes M, *Hpa* II-digested ^{32}P-end-labeled pBR322 DNA (indicated in nucleotides).

Fig. 1). For a correctly initiated Wap–ras RNA, we would expect a protected fragment of 35 nucleotides containing Wap sequences from position 1 (15) and the linker sequence. This fragment was not found; the Wap–ras RNA hybridized only to the linker sequences of the probe (data not shown). Using the same probe, we found a 19-nucleotide protected fragment after hybridization to endogenous Wap RNA. This is consistent with the previously mapped RNA initiation site (15). The Wap–ras RNA starts within the linker sequence between the *Wap* and the *ras* fragment. We assume that the construction of the hybrid gene created new sequences that can be used as the RNA start site in the transgenic animals.

***Wap–ras* Expression in Tumors of Transgenic Animals.** The tissue-specific expression of the *ras* oncogene in mammary glands of lines 3 and 58 and salivary glands of line 69 allowed us to investigate the phenotypic consequences of the presence of an activated oncogene product in these animals. Initially, the animals were morphologically normal and healthy. Only after a long latency did some of the animals develop tumors. We found two mammary tumors in the founder female no. 58 at the age of 325 days after having given birth to her fifth litter. Three weeks after parturition, RNA was prepared from both tumors and from the fourth histologically normal mammary gland and analyzed for the presence of *Wap–ras* sequences (Fig. 5*A*). Wap–ras RNA was detected in both tumors (lanes 2 and 3). The level of Wap–ras RNA is strongly enhanced in the malignant tissues compared to the normal mammary gland (lane 1). The endogeneous Wap RNA remains low in the tumor (lane 5) and in the normal mammary gland (lane 4). This low level of Wap RNA is consistent with the hormonal state of the animal near the end of the lactating period.

In line 69, the *Wap–ras* gene is constitutively expressed in the salivary gland. Five animals developed tumors in the neck region at the age of 9 months. The histological examination classified these as adenocarcinomas derived from the salivary gland. The Wap–ras RNA is present at high levels in all tumors compared to the levels found in the nonmalignant corresponding salivary gland of the same individual. The RNA analysis of two of these tumors and normal salivary tissue is shown in Fig. 5*B* (lanes 1–3). Hybridization experiments with an α-amylase-specific probe (24) were carried out to quantitate expression of this salivary gland-specific gene.

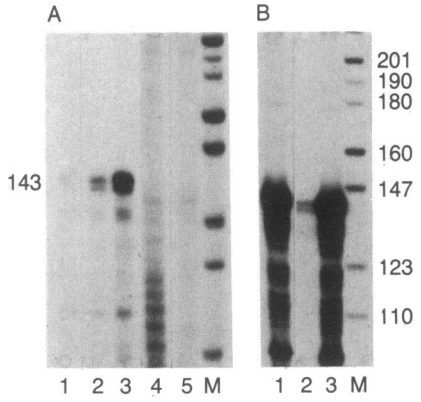

FIG. 5. Expression of the *Wap–ras* gene in tumors. RNA (10 μg) was analyzed in an RNase protection assay. (*A*) RNA was prepared from normal mammary gland (lanes 1 and 4), tumor 1 (lanes 2 and 5), and tumor 2 (lane 3) of female no. 58. Lanes 1–3 were hybridized to the *Wap–ras* probe; lanes 4 and 5 were hybridized to the *Wap* probe (see Fig. 3). (*B*) RNA was prepared from the salivary gland tumor of male no. 69 (lane 3), from the salivary gland tumor (lane 1), and the normal salivary gland (lane 2) of a male of the F_1 progeny of male no. 69 and hybridized to the *Wap–ras*-specific probe. The 143-nucleotide signal is indicative for Wap–ras RNA. Lanes M, *Hpa* II-digested ^{32}P-end-labeled pBR322 DNA (indicated in nucleotides).

The α-amylase mRNA accumulated to the same levels in these tumors as in normal salivary glands (data not shown). Mammary gland as well as salivary gland tumors show an enhanced expression of the *Wap–ras* transgene, indicating its involvement in the transformation process. No mammary or salivary gland tumors were observed in nontransgenic control animals. Comparison of DNA prepared from tumor tissue with DNA from normal tissue did not provide explanations for the overexpression. Amplifications, mutations, or rearrangements detectable at the level of restriction fragments of the hybrid gene were not observed.

DISCUSSION

The function of oncogenes is commonly associated with the regulation of differentiation and growth (1). To elucidate the effects of oncogenes on particular specialized cell types, it is desirable to restrict the expression of oncogenes to a limited subset of cells and possibly regulate their expression. Targeting of gene expression in transgenic animals by recombination of oncogene with differentiated cell-specific gene promoters provides an ideal system for analyzing the effect of oncogenes on a particular cell type.

We directed the expression of the activated human Ha-*ras* gene to the lactating mammary epithelial cells with a fragment from the murine *Wap* gene, containing the promoter and ≈2.4 kb of additional 5′ flanking sequences. Lines 3 and 58 show a similar expression pattern of the *Wap* and the *Wap–ras* gene. Since they are only active during lactation, the *Wap* fragment conferred the hormone dependence to the ras expression. Under the hormonal stimulus, the expression of the chimeric gene is predominantly restricted to the mammary gland. Expression is also observed in brains of lactating females. This might be related to the presence of prolactin receptors in the choroid plexus and hypothalamus (25). But the presence of prolactin receptors is not a sufficient prerequisite for *Wap–ras* gene expression. Other prolactin receptors bearing tissues as kidney, liver, and testis (25) show no *Wap–ras* expression.

Although the *Wap–ras* and the endogenous *Wap* gene show similar expression patterns, the level of Wap–ras mRNA in the mammary gland was 1/50th the level of endogenous Wap mRNA. "Run-on" experiments with nuclei isolated from lactating mammary glands revealed similar rates of transcription (data not shown). Differences in mRNA stability (26) are likely to be responsible for the different mRNA levels that accumulate in this tissue.

The lack of *Wap–ras* expression in lines 11 and 40 may be due to the integration of the transgene into an inactive chromosomal domain. It is possible that dominantly acting integration sites exist that repress transcription and overrule the regulatory sequences present in the *Wap* promoter region. Numerous examples for silent transgenes have been described (e.g., see refs. 27 and 28). Tissue-specific expression of introduced genes can be influenced by the integration sites (27, 29). The chromosomal location may be of particular significance in line 69. Integration into the Y chromosome and the expression of the *Wap–ras* gene in salivary glands are correlated. It will be interesting to analyze the integration site and its potential influence on salivary gland-specific gene expression.

The *Wap–ras* construct is expressed in only a few tissues. How can this expression be correlated to tumor formation in transgenic animals? The occurrence of tumors in the tissues where the chimeric gene was expressed suggests its involvement in tumorigenesis. The long latency of tumor formation indicates that the observed expression of the activated Ha-*ras* gene is not sufficient to transform differentiated cells *in vivo*. In rodents, mammary tumors are efficiently induced by chemical carcinogens. These tumors often contain an

activated Ha-*ras* oncogene (30). However, analysis of dose–response curves suggests that at least two events are required for transformation (31). A similar situation was described for transgenic mice bearing the c-*myc* oncogene fused to the long terminal repeat of the mouse mammary tumor virus (10, 32). The possibility of secondary mutations might increase with age. This could result in expression of cooperating oncogenes.

In our transgenic animals, all tumors showed a remarkably enhanced *Wap–ras* expression compared to the nonaffected tissues of the same animal. This could reflect the fact that the tumor tissue represents a more homogenous population of *Wap–ras*-expressing cells. Consequently, the level of endogenous Wap RNA should also be enhanced in mammary tumors, provided that both genes are identically regulated. High expression of Wap RNA in the tumor tissue of female no. 58 was not found. Several possible explanations can be invoked for the enhanced and possibly hormone-independent *Wap–ras* expression in tumor tissue. Mechanisms could involve trans- or cis-acting events. Franza *et al.* (33) found that transfection of *ras*-containing cells with the adenovirus *E1a* gene led to a 10-fold enhancement of *ras* expression. The enhanced expression of the *Wap–ras* gene in tumor tissue could therefore be the consequence of a mutation in a second gene functionally analogous to the *E1a* gene. An alternative trans-acting mutation could occur in a gene regulating the Wap–ras mRNA half life and result in higher steady-state levels. Cis-acting secondary mutations that lead to a higher Wap–ras mRNA level could also occur directly in the mRNA sequence involved in RNA stability. Finally, a mutation affecting the Wap promoter sequence can be envisaged. The negative regulation of enhancers by cellular factors has recently been demonstrated (34). We identified sequences upstream of the promoter region that are specifically recognized by nuclear proteins from mammary epithelial cells (unpublished data). It is conceivable that those sites of protein–DNA interaction might play a role in the regulation of *Wap* gene expression. The derivation of cell lines from the mammary and salivary gland tumors and introduction of gene constructs into these cells, as well as the recloning and molecular analysis of the *Wap–ras* transgene from these tumor cells will be useful in the dissection of the possible explanations for enhanced *Wap–ras* expression.

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