mammary gland. The absence of GnRH binding sites in non-neoplastic tissues suggests that carcinogenic transformation of normal breast cells is in some instances accompanied by the appearance of membrane proteins not normally present, or present only in undetectable amounts, in the nonlactating gland.

Evidence that the GnRH binding sites in tumor tissue are GnRH receptors may be provided by demonstrating that GnRH can affect cellular function. GnRH agonists have been reported to inhibit the estrogen-dependent growth of cultured mouse mammary tumor cells in a dose-dependent fashion (*14*), and inhibition of the growth of a single human breast cancer cell line in culture by GnRH analogs has been described (*15*). GnRH analogs also inhibit the incorporation of [^{3}H]thymidine and [^{14}C]leucine into MCF-7, MDA-MB-231, and ZR-75-1 human breast tumor cells in culture (*16*). These ductal cell lines were all positive for GnRH binding sites.

Although a major mechanism of inhibition of breast carcinomas by GnRH analogs is likely to be the inhibition of gonadotropin secretion and decline in gonadal steroid hormone production, the efficacy of the hormone in the treatment of some postmenopausal women with breast cancer and the effects demonstrated in vitro argue in favor of a direct effect of GnRH analogs on breast tumor cells. Our demonstration of specific GnRH binding sites in certain breast carcinomas illustrates a potential mechanism for these effects. A precedent for GnRH binding sites in a steroid hormone–sensitive tissue has been shown in rat prostatic tumors (*17*). The significance of GnRH binding sites in human breast carcinomas and their relevance in clinical management of breast cancer remain to be determined. The possibility of a correlation between the direct effects of GnRH on breast carcinoma cell lines and the presence of GnRH binding sites in the cell lines merit further study.

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19. Tumors and normal breast tissue were excised, rapidly frozen in liquid nitrogen, and stored at −70°C. The frozen tissue was diced and then homogenized in buffer containing 20 m*M* tris-HCl, 150 m*M* NaCl, 1 m*M* $CaCl_2$, and 1 m*M* phenylmethylsulfonyl fluoride at *p*H 8.0 on ice. Subsequent work was carried out at 0° to 4°C. The homogenate was centrifuged at 800*g* for 10 minutes, and the supernatant was centrifuged again at 800*g* for 5 minutes. The resulting supernatant was centrifuged at 10,000*g* or at 100,000*g* for 60 minutes. Membrane pellets were weighed and suspended in 125 m*M* tris-maleate, 160 m*M* NaCl, and 40 m*M* β-D-octylglucopyranoside (*p*H 6.0) to a final concentration of 100 mg/ml; the mixture was incubated at 0°C for 10 minutes and centrifuged at 10,000*g* for 15 minutes to remove nonsolubilized material. Membranes from tumors 27 and 18, which were prepared by centrifugation at 100,000*g*, exhibited low molecular weight bands (presumably nonspecific) that were not present in tumor membranes prepared by centrifugation at 10,000*g*.
20. Ligand immunoblotting was performed essentially as described [K. A. Eidne, D. T. Hendricks, R. P. Millar, *Endocrinology* **116**, 1792 (1985); this technique was based on the principle described by T. O. Daniel *et al.*, *J. Biol. Chem.* **258**, 4606 (1983)]. Protein (120 μg per lane) was loaded onto a 6 to 12 percent linear gradient polyacrylamide gel containing 0.1 percent sodium dodecyl sulfate; the protein was subjected to electrophoresis at 4°C under nonreducing conditions at 25 mA for 45 minutes and then at 35 mA for 60 minutes. The protein was then electrophoretically transferred to nitrocellulose in 50 m*M* tris, 200 m*M* glycine, and 20 percent (by volume) ethanol at 195 mA for 1 hour at 23° to 25°C. Protein molecular weight markers were stained with amido black, and the nitrocellulose strips with separated membrane proteins were incubated at 4°C overnight in buffer A [50 m*M* tris-HCl, 90 m*M* NaCl, and 50 mg of bovine serum albumin per milliliter (*p*H 8.0)] containing 10 percent fetal bovine serum. The nitrocellulose was then incubated in 10^{-6} *M* GnRH in buffer A for 45 minutes and washed eight times in buffer B [50 m*M* tris-HCl, 90 m*M* NaCl, and 5 mg of bovine serum albumin per milliliter (*p*H 8.0)], with subsequent incubation (45 minutes) in 10 ml of buffer A containing 200 μl of either antiserum 1076 (directed toward the middle region of GnRH) or antiserum 432 (directed toward the COOH and NH_2 termini of GnRH) or nonimmune rabbit serum. After the nitrocellulose was washed as before, incubations were carried out in 10 ml of buffer A containing 25 μl of affinity-purified horseradish peroxidase–conjugated sheep antibody to rabbit immunoglobulin G (Bio-Rad). The GnRH binding proteins were visualized by incubating them for 5 minutes with 4-chloro-1-naphthol [Sigma; 0.5 mg/ml in 50 m*M* tris-HCl, 150 m*M* NaCl (*p*H 8.0), and 0.0003 percent H_2O_2]. The reaction was terminated by several washes in distilled water.
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Tissue Factor Gene Localized to Human Chromosome 1 (1pter → 1p21)

Abstract. *Tissue factor (tissue thromboplastin, coagulation factor III), a protein component of cell membranes, is an essential cofactor for factor VII–dependent initiation of blood coagulation. Since no tissue factor–deficient condition has been described, it is one of only a few proteins of the coagulation system for which the pattern of inheritance has not been ascertained. Because of the species–specificity of tissue factor activity and the availability of a very sensitive chromogenic assay, it was possible in the present study to use somatic cell hybrids to assign the chromosomal location of the tissue factor structural gene* (F3) *to human chromosome 1 (1pter → 1p21).*

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Tissue factor is a glycoprotein present in the membranes of most cells and is traditionally assigned to the extrinsic pathway of blood coagulation (*1, 2*). Also known as tissue thromboplastin or coagulation factor III, tissue factor serves as a lipid-dependent cofactor for factor VII–mediated activation of factor X (*3–6*) and factor IX (*7*). That it may be the primary physiological initiator of blood coagulation (*1*) is supported by the observation that tissue factor is the only known protein in the pathway leading to blood coagulation for which a congenital deficiency has not been reported (*8, 9*). Tissue factor regulation, both in terms of expression of activity at the cell membrane and at the genetic level, is impor-

Table 1. Human chromosomes and tissue factor activity in human-mouse hybrid cells (*30*).

Cell hybrid	Tissue factor score	Assay value ($\times 10^6$ A_{405}/min^2)	Human chromosome																					Translocations
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
WIL1	−	0	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+	
WIL6	−	0	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+	
WIL7	−	0	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	−	−	−	+	−	+	
WIL14	+	10.3	+	−	+	−	−	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+	
SIR3	+	94.1	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	
SIR8	+	92.7	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
SIR11	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	
REW7	+	37.0	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
REW15	+	11.8	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	
DUA1A	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	t	−	−	−	−	−	−	−	t	X/15
DUA1CsAzF	−	0	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
DUA1CsAzH	−	0	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
TSL1	−	0	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	−	+	−	−	−	
TSL2	−	0	−	−	t	−	+	+	−	−	−	+	−	+	−	−	−	−	t	+	−	+	+	−	+	17/3
TSL2CsBF	−	0	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	−	−	
XTR1	+	162.2	+	−	t	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	X/3; 3/X
XTR2	−	0	−	−	t	−	+	−	−	+	−	+	−	+	+	−	−	−	−	+	−	+	+	−	t	3/X
XTR3BsAgE	+	6.9	+	−	t	−	−	+	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+	−	t	3/X
XTR22	−	0	−	+	t	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	+	+	+	t	X/3
XER9	−	0	−	+	−	+	−	+	−	+	−	t	t	+	−	+	+	−	−	+	+	+	+	−	t	11/X
XER11	+	7.4	+	−	+	+	−	−	+	+	−	+	t	+	+	−	+	−	+	+	+	−	+	+	t	X/11; 11/X
REX12	−	0.4	−	−	+	−	−	+	+	−	−	+	+	+	+	+	+	−	+	+	−	−	−	+	t	22/X
JSR29	+	16.9	+	+	+	+	+	+	t	+	t	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7/9
JVR22	+	93.0	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2/1; 1pter → 1p21
JWR22H	+	8.4	t	t	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+	−	+	+	+	−	−	2/1; 1pter → 1p21
ALR2	+	91.1	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	X/9
ICL15	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−	−	
ICL15CsBF	−	0	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	
MH21	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	
% Discord			0	32	17	24	31	21	21	31	21	24	30	21	21	28	14	24	21	28	17	34	41	21	27	

tant not only because of the role tissue factor plays in normal hemostasis but also because of its potential role in pathological conditions such as disseminated intravascular coagulation, atherosclerosis, and metastasis of malignant cells (*1, 2*).

In this report we describe our mapping of the tissue factor gene, designated *F3*. The study was made possible by the use of human-mouse somatic cell hybrids (*10, 11*), a sensitive chromogenic assay for factor X activation (*12, 13*), and the use of purified bovine factors VIIa and X (*14–16*). The material remaining insoluble after homogenization of the cells (*17, 18*) was used as the source of tissue factor. In the presence of bovine factor VIIa and factor X, calcium, and chromogenic substrate (*13, 19, 20*), the parent mouse cell lines showed no tissue factor activity (produced no detectable activation of factor X) whereas the parent human cell lines tested were rich in tissue factor activity. No significant activation of factor X occurred with material from any cell line in the absence of factor VIIa, confirming that the activity measured was that of tissue factor. Hybrid lines uniformly expressed less tissue factor activity than the human parent lines, but this had no effect on the analysis as indicated by the concordance of activity with the eventual chromosome assignment. To assure the reliability of the results, the samples were coded and the presence or absence of tissue factor was determined by one investigator (S.D.C.) who remained unaware of the identities or chromosome complements of the hybrids.

Each hybrid line was evaluated in at least four tissue factor assays, and the results were compared to negative controls (assays containing either material from the mouse parent line or dilution buffer). Twenty-nine hybrid lines gave results that were clearly either positive or negative when compared to the controls (*21*). A comparison of the result of the tissue factor evaluation and the complement of human chromosomes in the hybrids is presented in Table 1. Only human chromosome 1 was present in all lines with tissue factor activity and absent in all lines with no tissue factor activity (100 percent concordance). From these results we conclude that the structural gene for human tissue factor is located on chromosome 1.

The chromosome complement of hybrid JWR22H did not include chromosome 1, but a 2/1 translocation chromosome [t(1;2)(p21;q37)] was present (*22*). This cell line expressed tissue factor

activity ($P < 0.02$, Wilcoxon rank-sum test), so we were able to assign the tissue factor gene to the p arm of human chromosome 1 (1pter → 1p21).

While the mode of inheritance (recessive or dominant, autosomal or sex-linked) is known for most of the blood coagulation factors, only a few of them have been mapped to a human chromosome. The enzyme (factor VII) and one substrate (factor X) involved in tissue factor-initiated coagulation have only recently been mapped to chromosome 13 from studies of patients with chromosomal abnormalities (*23*). The assignment of factor IX (the other substrate) to the X chromosome was deduced from the sex-linked inheritance of hemophilia B (*8, 9*), and factor IX has been shown to be closely linked with the gene for factor VIII (*24*).

The evidence that tissue factor preparations from various tissues have similar functions, molecular weights, and immunological properties (*1, 2, 25–28*) is not sufficient to conclude that only one tissue factor protein is common to all cells that express tissue factor activity. Because of the limited variety of human parental cell types used to construct the somatic cell hybrids in this study (*17*) as well as potential perturbations in gene expression resulting from hybridization, we are not able to eliminate the possible existence of tissue factor loci on other chromosomes. Furthermore, multiple tissue factor genes may be present within the region 1pter → 1p21. Despite these possibilities, the similarities among tissue factor isolates and the gene mapping results are consistent with a single gene locus. The successful demonstration that human tissue factor can be expressed in human-mouse hybrid cells and the localization of the human tissue factor gene expressed in those hybrids should expedite efforts to isolate the gene and resolve these questions.

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17. Cell hybrids were constructed from mouse cells (RAG or LM/TK$^-$) and human cells (fibroblasts or leukocytes) and characterized as described (*10, 11, 18*). Cells were harvested at 80×10^6 to 100×10^6 cells per milliliter and disrupted with a homogenizer (Dounce) in 0.05*M* tris (*p*H 7.5). After centrifugation the supernatants were used for isoenzyme analyses to establish the hybrid genotype (*18*). The centrifugate was stored frozen until used in this study.
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30. A + sign in any column indicates that the column attribute (either tissue factor or a specific human chromosome) was present in the cell hybrid, a "t" that a translocation chromosome was present, and a − sign that the column attribute was absent. Discordancies were calculated with translocation chromosomes omitted. For example, TSL2 contained a 17/3 translocation chromosome [t(3;17)(p21;p13)] and was not used in calculating discordancies for either chromsome 17 or chromosome 3. Tissue factor activity, expressed as the rate of acceleration of substrate hydrolysis (*13*), is the median value of at least four analyses. Cellular material equivalent to 140 nmol of phosphate produced a turbid suspension that interfered with the spectrophotometric assay when the absorbance at 405 nm was negligible. Therefore, samples with high turbidity relative to the increase in the rate of change of absorbance often provided negative assay results and median activities less than zero. These are reported as having zero activity. Median values for the increase in the rate of change of absorbance for mouse cells and dilution buffer was 1.3×10^{-6} and 2.2×10^{-6} A_{405}/min^2, respectively, whereas assay values for the human parent fibroblasts were greater than 1×10^{-3} A_{405}/min^2. The dose-response curve plotted for a homogenized extract of human brain (*29*) as the source of tissue factor was smooth over a wide range of dilutions. For example, a 1 to 800 dilution of the brain homogenate resulted in an increase in the rate of change of absorbance of 5×10^{-3} A_{405}/min^2 whereas a 1 to 1.64×10^6 dilution resulted in an increase of 1×10^{-5} A_{405}/min^2.

31. We thank S. Ross, L. Haley, M. Byers, and R. Eddy for their technical assistance. Supported in part by NIH grants HL-31408, GM-20454, and HD-05196 and American Cancer Society grant CD-62. Facilities at the Department of Pathology, University of Colorado, are partially supported by a gift from R. J. Reynolds Industries and by grant CA-15823.

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