The rat Calbindin-D9K (CaBP9K) gene is mainly expressed in intestine, uterus, and lung and is regulated in a complex tissue-specific manner. To analyze the role of potential regulatory elements, previously defined by DNase hypersensitivity, we made transgenic mice containing truncated rat CaBP9K fusion gene with simian virus 40 large T antigen and the chloramphenicol acetyltransferase as reporter genes. The transgenes contained CaBP9K promoter fragments with 5' end points at −4400, −1011, and −117 base pairs (bp), whereas the 3' end points was at +365 bp. Northern blot analysis of T antigen expression and chloramphenicol acetyltransferase enzyme-linked immunosorbent assay indicated that a positive element, probably the distal intestine-specific DNase HS, necessary to target the expression of the transgene in the intestine, is present between −4400 and −1011 bp. The cephalo-caudal gradient of expression of the transgene along the small intestine was similar to those of the endogenous gene, but an ectopic expression of the transgene was observed in the colon. The −1011 transgene was expressed in epithelial alveolar cells of the lung, in renal proximal tubule cells, and in uterine myometrium, as judged from immunocytochemical, histological, and Northern blot analyses. The shortest, −117 construct was only expressed in uterine myometrium, and it was under a strict estrogen dependence like the endogenous gene. Finally, responsiveness to vitamin D in the duodenum was observed with the largest, −4400 construct. Thus, different tissues utilize distinct cis-acting elements to direct and regulate the expression of the rat CaBP9K gene.

The expression of eukaryotic genes depends on the specific arrangement of unique DNA enhancer and promoter elements and DNA-protein and protein-protein interactions. The binding of transcriptional factors to DNA regulatory elements is an essential step in the activation or repression of a gene in a temporal or cell-specific pattern and in response to extracellular signals. The calbindin-D9K (CaBP9K) gene is a particularly valuable model for studying the hormonal, tissue-specific, and developmental control of genes expressed in several tissues. CaBP9K is an intracellular calcium-binding protein thought to be involved in intracellular calcium homeostasis (see Ref. 1 for a review). In adult rats, the CaBP9K gene is mainly expressed in intestine, uterus, and lung. The highest concentration of CaBP9K is in intestinal epithelial cells (2), with a concentration gradient along the gastrointestinal tract; the CaBP9K gene is actively expressed in the duodenum, but expression gradually decreases along the jejunum to the ileum, with no expression in the large intestine, except for the cecum (3, 4). In the villus itself, the concentration of CaBP9K increases from the crypt to the upper part of the villi. Intestinal CaBP9K gene activity that is controlled by calcitriol, the active hormonal form of vitamin D (3–5), also varies during development. It is maximal at weaning and decreases with age (3, 6). In the uterus, the CaBP9K gene is expressed mainly in the myometrium and the endometrial stroma of nonpregnant rats (7–9). In pregnant rats, the CaBP9K gene is also expressed in the uterine epithelium (10). In contrast with the intestine, the CaBP9K gene is not under the control of vitamin D in the uterus, although there are vitamin D receptors in this tissue; instead it is under the control of the sex hormones (7, 11–13). An estrogen-responsive element (ERE) involved in its regulation by estradiol has been characterized (11, 12). Finally, CaBP9K gene expression is regulated by neither vitamin D nor estradiol in the alveolar epithelial cells of the lung (14). Lastly, the CaBP9K gene is expressed in the mouse kidney but not in the rat kidney (15, 16).

Our previous studies focused on the cis- and trans-acting phenomena controlling the expression of the rat CaBP9K gene. We have cloned and characterized the rat CaBP9K gene and its 5' and 3'-flanking sequences (17) and mapped the DNase I-hypersensitive sites in several rat tissues to locate potential regulatory elements (18). A cluster of DNase I-hypersensitive sites (HS2 to HS5) was found in the promoter proximal region of the active gene (in the intestine and uterus). Two intestinal-specific DNase I-hypersensitive sites were also identified, HS4 close to the promoter region and HS1 located ~3.5 kbp upstream of the start site (18). An in vitro DNase I footprinting assay was used to show that a combination of intestinal-specific transcription factors (Cdx-2), liver-specific transcription factors (HNF-1, C/EBP, and HNF-4), and ubiquitously factors bind to the proximal promoter region and may be important for the control of the rat CaBP9K gene transcription in the intestine (19).
To get further insight in the understanding of the cis-regulatory regions involved in the multiple control of the rat CaBP9K gene in vivo, we choose to create lines of transgenic mice because ex vivo studies are hampered by the fact that no established cell line expressing the CaBP9K gene is available. We have also attempted to use targeted oncogenesis to isolate new cell lines, which may be suitable models for studying the molecular events implicated in the regulation of the CaBP9K gene. Simian virus 40 (SV40) large T antigen (Tag) was then selected as reporter gene, but some of these transgenic mice died immediately after birth. Consequently, the chloramphenicol acetyltransferase gene (CAT) was also used as a reporter gene. The main results from the present study show that distinct sequences are required for the expression of the rat CaBP9K gene in the intestine, uterus, and lung.

**MATERIALS AND METHODS**

**Construction of Hybrid Genes—Transgenes were constructed using standard recombinant technology (20). The 9K/4400-Tag and 9K/4400-CAT constructs were prepared as follows. A fragment (–22 to +365 bp) containing the promoter region (beginning at the SacI site), the first intron, and the beginning of the second exon (before the ATG initiation codon) was cloned by PCR. The resulting Sac-EcoRV fragment was then cloned, together with a EcoRI-SacI fragment containing 5′ regulatory sequences of the rat CaBP9K gene (–2232 to –22 bp) in the Bluescript KS vector. A BamHI-EcoRI fragment containing upstream 5′ regulatory sequences (–4400 to –2232 bp) was then inserted to yield the 9K/4400 construct, which contained 4.4 kbp of 5′ regulatory sequences, the promoter, the first exon, the first intron, and the beginning of the second exon of the rat CaBP9K gene. The 9K/–4400-Tag construct was prepared by inserting the klenow-blunted 2.4-kbp Stul-BamHI fragment of the SV40 early gene containing only the large T coding sequence (the small t sequence from –4636 to –4904 bp was deleted) into the 9K/–4400 construct at the EcoRV site (SacI-EcoRI fragment isolated from PBLCAT2 containing the coding region of CAT was similarly inserting to obtain 9K/–4400-CAT. 9K/–1011-Tag was prepared by cloning the SacI-EcoRV fragment obtained by PCR cloning together with a PstI-SacI fragment containing 5′ regulatory sequences of the rat CaBP9K gene (–1011 to –22 bp) in the Bluescript KS vector. The coding region for the SV40 large T antigen was then inserted in the same way as the 9K/–4400-Tag. The 9K/–117-Tag construct was prepared by cloning the SacI-EcoRV fragment obtained by PCR cloning containing and the beginning of the first intron, and the beginning of the second exon of the rat CaBP9K gene (–117 to –22 bp) in the Bluescript KS vector. The coding region for SV40 large T antigen was then inserted as for 9K/–4400-Tag.**

Transgenes were constructed using standard recombinant technology (20). The 9K/4400-Tag and 9K/4400-CAT constructs were prepared as follows. A fragment (–22 to +365 bp) containing the promoter region (beginning at the SacI site), the first intron, and the beginning of the second exon (before the ATG initiation codon) was cloned by PCR. The resulting Sac-EcoRV fragment was then cloned, together with a EcoRI-SacI fragment containing 5′ regulatory sequences of the rat CaBP9K gene (–2232 to –22 bp) in the Bluescript KS vector. A BamHI-EcoRI fragment containing upstream 5′ regulatory sequences (–4400 to –2232 bp) was then inserted to yield the 9K/4400 construct, which contained 4.4 kbp of 5′ regulatory sequences, the promoter, the first exon, the first intron, and the beginning of the second exon of the rat CaBP9K gene. The 9K/–4400-Tag construct was prepared by inserting the klenow-blunted 2.4-kbp Stul-BamHI fragment of the SV40 early gene containing only the large T coding sequence (the small t sequence from –4636 to –4904 bp was deleted) into the 9K/–4400 construct at the EcoRV site (SacI-EcoRI fragment isolated from PBLCAT2 containing the coding region of CAT was similarly inserting to obtain 9K/–4400-CAT. 9K/–1011-Tag was prepared by cloning the SacI-EcoRV fragment obtained by PCR cloning together with a PstI-SacI fragment containing 5′ regulatory sequences of the rat CaBP9K gene (–1011 to –22 bp) in the Bluescript KS vector. The coding region for the SV40 large T antigen was then inserted as for 9K/–4400-Tag.

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**Transgenic Mice—**

**Lung, kidney, and uterus tissues were fixed in Bouin’s liquid and embedded in paraffin. Histological analysis was performed on sections stained with hematoxylin-eosin safran. The SV40 large T antigen was immunolocalized on frozen tissue sections using a specific rabbit polyclonal antibody (kindly provided by Dr. D. Hanahan, University of California, San Francisco, CA). Lung, kidney, and uterus from transgenic mice were cut into small pieces, and rapidly frozen on a plastic book precooled with liquid nitrogen. Tissues were kept at −80°C until used. Frozen sections (7 μm) were cut with a cryostat (Bright), placed on gelatin-coated slides, and processed for immunofluorescence. Sections were fixed with 2% (v/v) paraformaldehyde for 10 min at room temperature, incubated with 0.25% (v/v) Nonidet P-40 (Boehringer Mannheim), rinsed in phosphate-buffered saline, and incubated with the anti-large T antigen polyclonal antibody (dilution, 1:2000) for 18 h at room temperature. The sections were washed and incubated with biotinylated anti-rabbit IgG antibody (dilution, 1:200; Vector) and streptavidin-fluorescein (dilution, 1:200; Vector). Control preparations were obtained by omitting the anti-Tag antibody showed no labeling (data not shown). Preparations were examined under a Zeiss photomicroscope equipped with epifluorescence optics and photographed.

**Hormonal and Developmental Studies—**

**F₁ or F₂ transgenic mice (6–8 weeks old) were given two kinds of hormonal treatment. The first group, transgenic mice carrying the 9K/–4400-CAT construct (line 94) were placed in an ultraviolet light free environment and fed ad libitum with a vitamin D-deficient diet (0.36% phosphorous, from UAR, France) for 3 weeks. They were then fed a vitamin D-free, low calcium diet (0.01% Ca²⁺, 0.36% phosphorous) supplemented with 0.8% SrCl₂ (17). This lead to a serum calcium level of less than 7 mg/dl. Vitamin D-injected mice (D group) were given intraperitoneally 25 ng of 1,25(OH)₂D₃ in 0.1 ml of 10% ethanol, 90% sesame oil and sacrificed 24 h later. Control mice (D group) were given vehicle alone. Duodena and colons were removed, rinsed in phosphate-buffered saline, frozen in liquid nitrogen, and tested for CAT activity.**

**The second group of transgenic mice, carrying the 9K/–117-Tag construct (line 116) were anesthetized and ovarioctomized. 10 days later each mouse was fitted with an Alzet mini-pump that released 0.5 μl/h vehicle (control group, –E₂) or diethylstilbestrol (100 μg/kg) (injected group, +E₂) for 15 days. The mice were then sacrificed, and the uterus were removed and frozen in liquid nitrogen. Uterine Tag were tested by RT-PCR. The developmental study was carried out on transgenic mice carrying the 9K/–4400-CAT construct (line 85). The intestines were removed from young mice (2–4 months old) and old mice (6–8 months old) and tested for CAT activity.**

**RESULTS**

**Generation of CaBP9K Transgenic Mice—The role of the potential regulatory elements corresponding to the DNase I hypersensitive sites (18) were investigated in vivo using three types of constructions. The 9K/–4400 construct contained 4.4 kbp of 5′-flanking region, the promoter region, the first exon, the first intron, and the beginning of the second exon (before the ATG initiation codon) was linked either to the SV40 large T antigen (construct 9K/–4400-Tag) or to the CAT coding sequence (construct 9K/–4400-CAT). These constructs contained all the**
CaBP9K Transgene Expression

DNase I-hypersensitive sites (Fig. 1). The second type of construct, 9K/–1011-Tag contained only the cluster of DNase I-hypersensitive sites in the promoter region of the rat CaBP9K gene (Fig. 1). This region, which contains 1011 bp of 5' regulatory sequences, was linked to SV40 Tag. The third type of construct was a minimal construct containing only 117 bp of 5' sequences upstream of the transcription start site linked to SV40 Tag (construct 9K/–117-Tag). All these regulatory sequences contained the ERE of the CaBP9K gene (12) (Fig. 1). These transgenes were microinjected into mouse eggs to produce founder animals. Three founders were obtained with the 9K/–4400-CAT construct; all died within 3 weeks of birth, so no transgenic lines were obtained. A nontoxic reporter gene (CAT) was used to analyze the role of the 4.4 kbp of regulatory sequences of the rat CaBP9K gene. This construct (9K/–4400-CAT) gave six founders, which were used to establish six transgenic mouse lines (Fig. 1). Finally, two lines with the 9K/–1011-Tag construct and four lines with the 9K/–117-Tag construct were obtained (Fig. 1). The number of transgenes integrated into the DNA of each founder mouse varied from 2 to 50 copies/cell. The transmission of the transgene in all transgenic mouse lines was Mendelian.

Tissue Specificity of Transgenes Directed by 4.4 kbp of 5' Regulatory Sequences—The expression of the construct bearing the longest CaBP9K 5'-flanking sequences was examined by CAT analysis of several tissues of the six mouse lines bearing the 9K/–4400-CAT construct (Fig. 1). A representative pattern of CAT distribution is shown in Fig. 2. It showed that 4.4 kbp of 5'-flanking sequences of the rat CaBP9K gene reproduced almost perfectly the pattern of expression of the endogenous CaBP9K gene. The CAT transgene was actively expressed in the duodenum, but no expression was detected in the jejunum or ileum. However, there was a strong ectopic expression of CAT in the colon, mainly in the distal part (Table I). The CAT transgene was also expressed in the kidney and uterus (Fig. 2). CAT activity was not closely dependent on the number of integrated copies (Table I), although the mice of line 84 with only two copies of the transgene had a very low CAT activity. Nevertheless, TLC chromatography allowed us to measure this low CAT activity, whose distribution was similar to that in the other lines (Table I).

Deletion Analysis of Transgene Expression—The roles of the distal and proximal potential regulatory elements defined by the DNase I hypersensitivity study were analyzed by generating 9K/–1011-Tag and 9K/–117-Tag constructs and examining the expression of these constructs in transgenic mice (Fig. 1). The 9K/–1011-Tag transgene lacking sequences –4400 to –1011 was not expressed at all in the intestine, indicating that the deleted fragment including the distal specific HS1 DNase I-hypersensitive site is essential for intestine specificity of the CaBP9K gene. As shown on Figs. 3A et 4A, Northern blot analysis showed Tag expression for line 46 in the lung and at a weakly level in kidney and only in kidney for line 39. RT-PCR detected very little Tag gene expression in the lung of line 39 mice carrying the 9K/–1011-Tag construct, indicating that ex-
CaBP9K Transgene Expression

The results were obtained by CAT ELISA assays and are expressed in pg/ml total protein. 10 μg of total proteins isolated from the various tissues were used for each assay. Values are the means ± S.D.

| Line | Copy no. a | Duodenum b | Distal colon c | Uterus | Kidney | Liver |
|------|------------|------------|---------------|--------|--------|-------|
| 38   | 35         | 3165 ± 1207 (8) d | 9800 ± 3656 (4) | 9052 ± 5863 (8) | 437 ± 193 (5) | <100 (3) |
| 94   | 30         | 8079 ± 3747 (8) | 3118 ± 1785 (10) | 3051 ± 4701 (9) | 599 ± 545 (5) | <100 (3) |
| 85   | 15         | 9375 (2)    | 3067 ± 284 (3)  | <100 (3) | 1490 (2) | <100 (3) |
| 105  | 50         | 5164 ± 2302 (5) | 12038 ± 1195 (4) | 6784 ± 4878 (3) | 1334 ± 1025 (5) | <100 (3) |
| 112  | 20         | 3548 ± 1594 (4) | 7360 ± 1126 (5) | 3164 ± 3461 (5) | 1220 ± 1198 (4) | <100 (3) |
| 84 d | 2          | <100 (3)    | <100 (3)       | <100 (3) | <100 (3) | <100 (3) |

a Number of transgene copies.
b Only results from the distal colon are shown; the proximal colon CAT ELISA values were at the limit of detection.
c n, number of mice analyzed.
d All mice from line 84 were retested by TLC assay, because values from CAT ELISA assays were at the limit of detection.

Expression of the Tag gene led to the development of lung adenocarcinoma in all line 46 mice (Fig. 3, C and D). Histology of the lung of a 4-month-old transgenic mouse. Note the presence of tumor nodules (C) with intense nuclear Tag staining (D). Bars, 50 μm.

Deletion of sequences −117 bp (9K/−117-Tag construct) resulted in the loss of expression of the transgene in the tissues analyzed (intestine, kidney, and lung), as judged by Northern blot analysis (data not shown). However, all the females from the four transgenic mice lines later developed uterine leiomyomas (Fig. 5A). Immunocytochemical analysis always detected Tag expression only in the uterine myometrial tissue (Fig. 5B).

Altogether, these results indicate that the 9K/−4400 constructs containing 4.4 kb of 5′ regulatory elements possess the cis-acting elements needed to direct expression in the intestine, probably the distal intestine-specific DNase I-hypersensitive site HS1. A construct with 1011 bp of 5′-flanking sequences directed the expression in the lung, kidney, and uterus. As an endogenous gene, this transgene was expressed in epithelial alveolar cells and in uterine myometrium, whereas an unexpected expression was obtained in renal proximal tubule cells. A construct containing only −117 bp of 5′ regulatory sequences is sufficient to direct the low level expression of the transgene in the uterine myometrium.

**Tissue-specific Hormonal and Developmental Control of Transgene Expression**—The hormonal control of transgene exp-

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2 B. Romagnolo, T. Molina, G. LeRoy, C. Blin, A. Porbeaux, M. Thomasset, A. Vandewalle, A. Kahn, and C. Perret (1996) J. Clin. Invest., in press.
pression by vitamin D in the intestine and estrogen in the uterus was determined using vitamin D$_3$-deficient transgenic mice and ovariectomized transgenic mice. The mice fed the vitamin D$_3$-deficient diet had decreased CAT expression in the duodenum, whereas a single injection of 25 ng of 1,25(OH)$_2$D$_3$ restored CAT activity (Fig. 6A). Thus, both the 9K/–4400-CAT transgene and the endogenous CaBP9K are controlled by 1,25(OH)$_2$D$_3$ in the duodenum (Fig. 6C). This hormonal control was tissue-specific, because expression in the colon was independent of vitamin D$_3$ (Fig. 6B).

Because aging has been associated with alterations in calcium homeostasis (24) and decreased CaBP9K gene expression (6), we examined CAT expression in mice of different ages. CAT expression decreased with age (2–4 months old to 8 months old or more) (Fig. 7A). However, the age-dependent decrease in transgene expression was tissue-specific, because it occurred only in the duodenum and not in the colon (Fig. 7B). Because the serum 1,25(OH)$_2$D$_3$ and VDR contents decrease with age and can be partially reversed by exogenous 1,25(OH)$_2$D$_3$ (25), we examined the effect of 25 ng of 1,25(OH)$_2$D$_3$ on the decrease in CAT expression in old mice. Fig. 7 shows that the age-dependent decline in transgene expression in the duodenum was reversed by vitamin D$_3$.

Because the ERE of the rat CaBP9K gene was present in all constructs and the minimal promoter could direct the expression of the transgene in the myometrium, we investigated whether this ERE was functional in transgenic mice carrying the 9K/–117-Tag construct. RT-PCR detected neither Tag nor CaBP9K mRNA in uterus of ovariectomized line 116 transgenic mice but showed that they were strongly stimulated by treatment of these mice with 100 μg/kg diethylstilbestrol (Fig. 8). Thus, the 9K/–117-Tag transgene responded to estrogen in vivo, most likely via the previously characterized intragenic ERE.

**DISCUSSION**

The CaBP9K gene provides a useful model for dissecting the regulatory elements controlling its tissue expression and hormonal regulation (1). Previous studies indicated that a specific set of tissue-specific DNase I HSs are present in the chromatin of the rat CaBP9K gene and may be involved in the complex transcriptional control of that gene (18). Transgenic mice have now been used to assess the role of these potential cis-acting elements. The present results indicate that deletion of various upstream regions of the rat CaBP9K gene has tissue-specific effects on transgene expression in the three major CaBP9K-expressing tissues, intestine, uterus, and lung.

Control of CaBP9K Gene Expression in the Intestine—A 4400 bp 5′-flanking sequence is necessary to direct the expression of the transgene in the intestine. Deletion to –1011 abolishes the expression in the intestine, indicating that there is a strong cis-acting element between –4400 and –1011 bp involved in intestinal-specific control of transcription of the rat CaBP9K gene. Because a major intestine-specific DNase I hypersensitive site (HS1) lies between –4400 and –1011 bp, it is tempting to propose that HS1 is the cis-acting element involved in the intestine specificity CaBP9K gene. It would thus form part of a group of cis-acting elements associated with DNasel
HS and found in tissue-specific genes, e.g., the genes for albumin (26), α-feto protein (27), tyrosinase (28), lysozyme (29), and Wap (30).

Another intestine-specific DNase I HS site (HS4) has been located close to the promoter of the rat CaBP9K gene. We have shown by in vitro DNase I footprinting assays that Cdx-2, an intestine-specific transcriptional factor, together with hepatic transcriptional factors (HNF1, HNF4, and C/EBP), bind to that region (19). Transfection experiments in CaCo2 cells suggested that proximal and distal elements cooperate to confer intestine specificity on the rat CaBP9K gene (19), which is consistent with the results obtained in transgenic mice. However, although deletion of the distal element suppresses expression of the transgene in the intestine of transgenic mice, it only decreases activity of the CaBP9K gene promoter tested by transient transfection in CaCo2 cells (19). This type of difference between the results obtained in transgenic mice and transiently transfected cells has been reported several times (31, 32) and could be ascribed to such phenomena as the chromatin influence in transgenic mice or differences between tissues in vivo to cultured cell lines. For instance, Cdx-2 and other factors could bind to the promoter in transiently transfected cells but not in the chromosomal context in which, in the absence of HS1, the chromatin around the promoter could be inaccessible to these factors. In other observations all the elements necessary for in vivo expression in the intestine are proximal, in the case of liver fatty acid binding protein (Fabp-L), intestinal fatty acid binding protein (Fabp-I), and intracellular lipid binding protein (Iibp) (33, 34).

Although the 9K/−4400-Tag transgene was intensively transcribed in the duodenum but not in the jejunum or ileum, mimicking the pattern of expression of the endogenous gene, it was also actively expressed in the distal colon and, to a lesser extent, in the proximal colon. This aberrant transgene expression in the colon suggests that regulatory elements involved in suppressing the activity of the rat CaBP9K gene promoter are absent from the −4400 bp of 5′-flanking sequences. These results extend those obtained by others, showing that the small and large parts of the intestine require somewhat different regulatory elements to recapitulate the correct patterns of fatty acid binding protein (Fapb) and sucrase isomaltase gene activities (34, 35). Additional deletion analyses, together with constructs including more 5′- and 3′-flanking sequences should help to define the cis-acting elements involved in the control of the rat CaBP9K gene expression along the length of the gastrointestinal tract.

Control of Transgene Expression in the Lung—Tag mRNA was detected in the lungs of 9K/−1011-Tag mice, once by Northern blot and once by RT-PCR. This difference in the level of expression between the lines reflects the influence of the integration site. In contrast, the transgene was not expressed at all in the lung of 9K/−117-Tag mice, which allows us to locate elements involved in the lung specificity of the CaBP9K gene between −1011 and −117 bp. This gene is known to be expressed in epithelial alveolar cells (14), which is consistent with immunodetection of the T antigen in lung of the 9K/1011-Tag mice and with the development of lung adenocarcinoma. The gene encoding surfactant apoprotein SpC has a similar specificity, but the regulatory region involved in epithelial alveolar cell gene expression in transgenic mice shows no similarity to the −1011 to −117 bp 5′-flanking sequence of the CaBP9K gene (36).

Control of Transgene Expression in the Uterus—The expression of all the transgenes in the uterus indicates that the sequence downstream of position −117 is sufficient for expression in the uterine myometrium. Although it is difficult to compare the expression of CAT transgenes measured by CAT ELISA to that of Tag transgenes measured by Northern blot analysis, the 9K/−117-Tag construct seems to be less actively expressed in the uterus than the 9K/−4400-CAT construct. This indicates that although strict cell and tissue specificities are conferred by a −117 to +365 bp fragment of the rat CaBP9K gene, other elements are needed to obtain full activity in the uterus. These elements are probably located upstream of position −1011 bp, because expression of the 9K/−1011-Tag transgene was similar to that of the 9K/−117-Tag transgene.

Control of CaBP9K Gene Expression in Other Tissues—The CaBP9K gene is expressed in the kidney of the mouse but not in the rat. However, transgenes directed by 4.4 kb or 1011 bp of 5′-flanking sequences of the transgene were expressed in the mouse kidney, indicating that the lack of CaBP9K gene expression in the rat is due to the absence of trans-acting factors from the rat kidney. However, cell specificity appears to be unexpected, because the 9K/−1011-Tag construct was expressed in the proximal tubule cells and not in the distal tubule, like the endogenous gene. This abnormal targeting of transgene expression in the kidney may result from the use of a rat transgene in mice or from the lack of important regulatory elements in the transgene.

Except for the abnormal targeting in the colon, there was no other ectopic expression of the transgenes. In particular neither the endogenous CaBP9K gene nor the 9K/−4400-CAT transgene were transcribed in the liver, although the intestine-specific DNase I HS site is also detected in this organ. Fine mapping revealed that the rat liver contains another specific DNase I HS site HS5, 100 bp 5′ to HS1 (18). HS1 could reflect the endodermal lineage of the origin of gut and its appendages, particularly the liver, whereas HS5 could be a liver-specific silencer complex blocking the influence of HS1 (18). HS1 could also require the presence of intestine-specific factors bound to the promoter (Cdx-2) in order to stimulate expression of the CaBP9K gene.

Tissue-specific Regulation of the Transgene by Vitamin D and Estradiol—Hormonal control of both the endogenous rat CaBP9K gene (1) and transgenes in mice is tissue-specific. The expression of the 9K/−117-Tag transgene, which contains the intragenic CaBP9K ERE (12) in the uterus, is under the strict control of estradiol. Therefore, the previously identified estrogen response element is fully functional in transgenic mice, even with only 117 bp of 5′-flanking sequence. In contrast, the elements required for control by 1,25(OH)2D3 in the intestine are contained in the 9K/−4400 construct. The 1,25(OH)2D3 control of transgene expression is also restricted to the duodenum and does not act in the colon, although the expression of
the transgene in these two intestinal tissues was similar, and the colon contains a high concentration of VDR (37). The vitamin D tissue-specific control can have several explanations: 1) transgene and VDR are not expressed in the same cells in the colon, 2) the VDR in the colon is not functional, and 3) the control of the CaBP9K gene expression by 1,25(OH)2D3 is tissue-specific and requires cis-acting factors that are present in the duodenum but missing from the colon or is under negative constraint in the colon. The first possibility is unlikely because the VDR concentration in the colon epithelium is high, and we have found that most of the CAT activity was detected in this epithelium (data not shown). The second possibility is also unlikely because the transcriptional regulation by vitamin D3 has been described in the colon (38), and epidemiological studies suggest that 1,25(OH)2D3 protects against colorectal carcinogenesis (39, 40). The third explanation is consistent with the notion that tissue-specific factors frequently cooperate with nuclear hormone receptors to confer tissue-specific hormone-dependent responsiveness on various genes (see Ref. 41 for a review). However, the cis-acting elements responsible for such a tissue-specific responsiveness of the CaBP9K gene to vitamin D3 have yet to be firmly identified. A putative VDRE has been ascribed to position −467 bp with respect to the cap site of the rat gene and hence lies in the 9K/−4400-CAT construct (42).

In conclusion, we have shown that the 4.4 kb of the 5′-flanking sequence of the rat CaBP9K gene contain all information required to direct expression of the transgene in a hormone-dependent and tissue-specific fashion. The data from deleted constructs indicate that distinct sets of regulatory sequences are responsible for expression in the three main CaBP9K-containing tissues, the intestine, uterus, and lung.

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