DNA target sites for a "multivalent" 11-zinc-finger CTCF-binding factor (CTCF) are unusually long (~50 base pairs) and remarkably different. In conjunction with the thyroid receptor (TR), CTCF binding to the lysozyme gene transcriptional silencer mediates the thyroid hormone response element (TRE)-dependent transcriptional repression. We tested whether other TREs, which in addition to the presence of a TR binding site require neighboring sequences for transcriptional function, might also contain a previously unrecognized binding site(s) for CTCF. One such candidate DNA region, previously isolated by Bigler and Eisenman (Bigler, J., and Eisenman, R. N. (1995) EMBO J. 14, 5710–5723), is the TRE-containing genomic element 144. We have identified a new CTCF target sequence that is adjacent to the TR binding site within the 144 fragment. Comparison of CTCF recognition nucleotides in the lysozyme silencer and in the 144 sequences revealed both similarities and differences. Several C-terminal CTCF zinc fingers contribute differently to binding each of these sequences. Mutations that eliminate CTCF binding impair 144-mediated negative transcriptional regulation. Thus, the 144 element provides an additional example of a functionally significant composite "TRE plus CTCF binding site" regulatory element suggesting an important role for CTCF in cooperation with the steroid/thyroid superfamily of nuclear receptors to mediate TRE-dependent transcriptional repression.

The CTCF1 transcription factor harbors an evolutionarily conserved 11-zinc-finger (ZF) DNA binding domain and recognizes unusually long (~50 bp) and remarkably different DNA target sequences in avian and mammalian c-myc promoters (1–3). This multiple sequence specificity of CTCF is achieved through its ability to employ different groups of individual ZFs to recognize highly divergent sequences, and we have described CTCF as a multivalent factor (3, 4).

This divergence of DNA target sequences recognized by CTCF makes it difficult to predict CTCF binding sites by sequence homology. For instance the AT-rich CTCF binding site within the F1 sequence of the S-2.4 lysozyme transcriptional silencer, which is required for optimal regulation by the thyroid hormone and/or retinoic acid receptors (5, 6), has little similarity to the GC-rich CTCF binding sequences in the promoters of avian and mammalian c-myc genes. Indeed CTCF utilizes different combinations of ZFs to bind the lysozyme F1 target sequences compared with the c-myc promoter targets (4). Moreover, CTCF also binds to a functionally important region of the amyloid β-protein precursor (APP) gene promoter (7, 8), and this site harbors no CTCF repeats and demonstrates overall homology to the CTCF target sequences in either the c-myc promoter or the F1 lysozyme silencer element (3, 4, 9).

CTCF binding to the F1 sequence of the S-2.4 lysozyme transcriptional silencer is of particular interest because in conjunction with the thyroid hormone receptor (TR) or v-ERBA it leads to a strong synergistic repression in transient transfection assays (4, 6). Other transcription factors that synergize with nuclear receptors including Octa- or CACCC-binding proteins could not replace CTCF in the S-2.4 lysozyme silencer-mediated transcriptional repression induced by v-ERBA (5). This composite TRE plus CTCF binding site regulatory element suggests an important role for CTCF in cooperation with the steroid/thyroid superfamily of nuclear receptors to mediate TRE-dependent transcriptional repression.

Here we identify another genetic element harboring a functionally significant composite TRE plus CTCF binding site. The TRE-containing genomic DNA segment 144 was originally isolated by Bigler and Eisenman (10) utilizing immunoprecipitation of nuclear TR-DNA complexes. In transient transfection assays this element is shown to function as a transcriptional repressor (11). We have identified a new CTCF binding site within the 144 DNA fragment some 160 bp away from the TRE that exhibits different sequence specificity compared with previously characterized CTCF sites. We show that the triiodothyronine (T3)-mediated transcriptional repression displayed by the 144 element is critically dependent on CTCF binding to this site. These observations indicate that the TRE-containing element 144 provides a second example of the synergistic, func-
nctional connection between a subset of certain CTCF target sites and T₃-responsive negative regulatory elements.

EXPERIMENTAL PROCEDURES

EMSA, Methylation Interference, and Missing Contact Analyses—Two consecutive DNA fragments, 144#1 and 144#2, covering DNA sequences upstream and downstream of the 144 TRE as shown in Fig. 1, were PCR amplified from the p144BS template and simultaneously 5'-end labeled at either strand by using two pairs of primers (one of which was 5'-end labeled with [γ-³²P]dATP and T4 polynucleotide kinase: 5'-TCGTTGCCACCTAGTACAGCACATGCCTTG-3' with the M13 reverse primer at the pBluescript polylinker (for the 144#1 fragment) and 5'-TAGATGTCATATCTATATAATCATT-3' with 5'-GGAAATGTGTATGTACATGTGGTTGAGCGGTGCCTG-3' (for the 144#2 fragment). A positive control DNA fragment bearing the CTCF binding sequence F1 of the S-2.4 transcriptional silencer from the chicken lysozyme gene (4, 6) was amplified from the genomic DNA. The 5'-end-labeled fragments were gel-purified and utilized for EMSA, methylation interference, and missing contact analyses as described previously (3). Binding reactions for EMSA were carried out in the buffer containing standard phosphate-buffered saline with 5 mM MgCl₂, 0.1 mM ZnSO₄, 1 mM dithiothreitol, 0.1% Nonidet P-40, and 10% glyceral in the presence of cold double-stranded competitors, namely poly(dI-dC) plus poly(dG) plus the 44-mer double-stranded oligonucleotide 5'-AGGAATGTGTATGTACATGTGGTTGAGCGGTGCCTG-3' (related to the classic AGGTCA half-sites that have been revealed earlier by methylation interference assays with TR elements (8, 10) and T3-responsive negative regulatory elements.

Therefore for human cell nuclear extract Ab2 antibodies serve as a negative control for the Ab1 anti-CTCF antibodies. Methylation interference (for guanines) and missing contact (for C and T nucleotides) analyses were carried out as described previously (3).

Generating a Set of CTCF Proteins with Serially Truncated ZFs—For generating in vitro translated proteins we utilized the TnT® Reticulocyte Lysate coupled in vitro Transcription-Translation System (Promega Co., Madison, WI). Human CTCF cDNA fragments with a BamHI site at the 5'-end and a SacI site at the 3'-end were PCR generated with the template being the p7.1 CTCF cDNA (3) and primers designed to cover selected regions coding for different segments of the human CTCF 11-ZF domain. These PCR fragments were then in-frame ligated to the BamHI-SacI ends of the parental vector pcITE4a(4)+ (Novagen, Madison, WI) under the EMC viral cap Independent Translational Enhancer, the CITE (12). Together 12 plasmids for expression of the serially truncated zinc finger forms of the CTCF DNA binding domain were generated. The pcITE4a-ZF(1–11) vector contains the reading frame of the full-length 11-zinc-finger domain from amino acid (aa) position 236 to 622 of the human CTCF protein (3). A number of N-terminal truncated ZF forms are encoded in the pcITE4a-ZF(2–11), beginning at the middle of the ZF1 at position 275 and ending at aa 236 in the pcITE4a-ZF(2–11), as 307–622; in the pcITE4a-ZF(3–11), as 307–622; in the pcITE4a-ZF(5–11), as 367–622; and in the pcITE4a-ZF(6–11), as 388–622. The vectors encoding a number of the C-terminal truncated forms are as follows: pcITE4a-ZF(1–10), aa 236–549; pcITE4a-ZF(1–9), aa 236–520; pcITE4a-ZF(1–8), aa 236–492; pcITE4a-ZF(1–7), aa 236–463; pcITE4a-ZF(1–6), aa 236–433; and pcITE4a-ZF(1–5), aa 236–404. Complete maps and sequences of these constructs are available upon request. Translation products, synthesized in the presence of [³⁵S] E. coli hydrolysate labeling reagent containing ~70% l-methionine (Trans³⁵S-Label, ICN Pharmaceuticals, Irvine, CA), were analyzed by denaturing electrophoresis and visualized and quantitated by fluorography of a scintillant impregnated SDS-polyacrylamide gel electrophoresis with x-ray film (Bio-Max MR, Eastman Kodak) essentially as described previously (15).

Plasmids, Stable and Transient Transfections, and CAT Assays—The p144BS plasmid contains the 144 genomic clone obtained by immunoprecipitation of solubilized chromatin with a TR-specific antisera, whereas the ptk144UTR-CAT reporter plasmid contains the 144 element cloned into the HpaI site of the pGLCAT4 reporter as described previously (10, 11).

Site-specific mutagenesis in the ptk144 UTR-CAT plasmid to alter
the CTCF binding site (Fig. 4) was carried out with the Quik Change site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. The presence of the correctly mutated site and orientation of the DNA sequence of the 144 element in the original construct (Fig. 1) were verified by DNA sequencing.

For both transient and stable transfections three CAT reporters driven by the TK promoter were utilized including the parental pGLCAT4 plasmid and the wild type and CTCF binding site-mutated ptk144UTR-CAT vectors (Fig. 1). The promoterless plasmid pBlCAT3 (14) was used to determine the nonspecific background CAT signal.

To obtain cell lines with different CAT plasmids stably incorporated into chromatin, DNA was transfected by CaPO₄ precipitation into C2C12 mouse myoblasts grown to 30–50% confluence on 10-cm dishes using 10 μg of each of the CAT reporter plasmids and 1 μg of the pSV2Neo. Selection of G418-resistant clones and the establishment of polyclonal mass cultures were performed as described previously (3).

Stably transfected C2C12 cells growing at approximately equal density in phenol red-free Dulbecco's modified Eagle's medium supplemented with 10% charcoal-stripped fetal calf serum (Sigma) were either induced for 24 h with 200 nM T₃ from a stock solution of T₃ dissolved in Me₂SO or mock-induced with an equal volume of Me₂SO (control), and whole cell extracts were prepared for CAT analysis. The relative number of stably integrated CAT reporter constructs/cell in C2C12 mass cultures was determined by Southern blot analysis utilizing as a probe the tk gene, both labeled with [³²P]dCTP as described (13). Direct phospho-image analysis of the Southern blots indicated that the relative amount of each integrated CAT reporter in one versus the other mass culture was essentially equal (data not shown).

Transient co-transfection experiments were carried out essentially as described (15). Expression vectors included the human CTCF pCIT3.1 (3) as well as RXR pZx1 and TRα pCE28 (10, 11). CV-1 cells and green monkey kidney fibroblasts were plated onto 6-well tissue culture plates at 2 × 10⁵ cells/well and cultured in phenol red-free Dulbecco's modified Eagle's medium supplemented with 10% charcoal-stripped fetal calf serum (Sigma). Transfection was performed the following day by standard CaPO₄ precipitation with 4 μg of an indicated CAT reporter and 2 μg of each effector vector in triplicate. The following day cells were transferred to fresh medium in the presence or absence of 200 nM T₃ for an additional 24 h before harvest.

In both stable and transient transfection experiments, CAT protein levels were measured in whole cell extracts and prepared from an equal number of transfected cells utilizing the sandwich enzyme-linked immunosorbent assay technique with anti-CAT antibodies prebound to microtiter plates according to the manufacturer (CAT-enzyme-linked immunosorbent assay kit 1263727, Roche Molecular Biochemicals). CAT values were normalized to total protein and in the case of stably transfected cells to the relative number of the integrated reporter plasmid.

RESULTS

The TRE-containing Genomic DNA Segment 144 Harbors a Novel CTCF Binding Site—To determine whether there was a new CTCF binding site within the 144 TRE, we performed EMSA utilizing two 5'-end-labeled PCR-amplified DNA fragments, 144#1 and 144#2 (Fig. 1B) incubated with the in vitro translated 11-ZF CTCF domain. Figure 2 demonstrates that one of these DNA fragments (144#1) turned out to be positive for binding to the 11-ZF CTCF domain, indicating the presence of a new CTCF binding site positioned within a 200-bp region upstream of the 144 TRE (Fig. 1). To determine which nucleotides within fragment 144#1 are required for specific recognition by the 11-ZF CTCF domain, we carried out methylation interference (for guanines) and missing contact (for pyrimidines) analyses (Fig. 3). The disposition map for these contact nucleotides in both DNA strands defines the 144 CTCF binding site as indicated in Fig. 1D. It contains an inverted repeat of the COCTC motif that has previously been found in some but not all CTCF binding sites (1, 3, 4, 9, 16, 17).

This novel CTCF binding site and the 144 TRE are positioned at a 160-bp distance from each other (Fig. 1). A gel shift analysis with the 144 fragment incubated with in vitro translated TR/RXR and CTCF indicated that both bind simultaneously to this fragment without any evidence of cooperativity or competition (data not shown).

CTCF Employs Different Groups of C-terminal ZFs for Bind-
Critical guanines, which interfere with CTCF binding when partially methylated with dimethyl sulfate, are indicated in T3-mediated transcription. transcriptional effects (11) suggesting that an additional factor flanking sequences resulted in a complete loss of the 144 transfected rat pituitary GH4 cells (10, 11). Deletion of the 144 TRE we constructed a number of plasmids for Ase described under “Experimental Procedures,” creates a new Ase1 restriction site (underlined).

Fig. 4. Optimal alignment of the S-2.4 silencer F1 and 144 CTCF binding sites reveals subregional differences and similarities. Critical guanines, which interfere with CTCF binding when partially methylated with dimethyl sulfate, are indicated in shaded boxes. Substitution of the three CTCF-contacting guanines for AAT by the site-directed mutagenesis as described under “Experimental Procedures,” creates a new Ase1 restriction site (underlined).

The 144 CTCF Binding Site Specifically Binds Endogenous CTCF.—We wished to determine whether the 144 site identified with the in vitro translated CTCF also bound endogenous CTCF. We performed the EMSA-utilizing nuclear extracts from HL-60 cells and the 144#1 DNA fragment. We observed a shifted band corresponding to the CTCF—DNA complex formed by the endogenous CTCF from the nuclear extract (Fig. 6, lane 2) with similar electrophoretic mobility as that generated by the in vitro translated CTCF (Fig. 6, lane 5). These bands disappeared with the addition of anti-CTCF antibodies (Ab1) but not control antibodies (Ab2) (Fig. 6, compare lanes 3 with 4 and lanes 6 with 7). Thus the 144 CTCF target sequence specifically interacts with the endogenous CTCF protein present in nuclear extracts. Moreover, under our EMSA conditions CTCF appears to represent the most abundant nuclear protein binding to the 144#1 sequence.

CTCF Binding to the 144 Element Positioned in the 3’-UTR Is Required for the TRE-mediated Transrepression.—As described previously the TRE-containing genomic DNA fragment 144 regulates transcription in a T3-dependent manner in transfected rat pituitary GH4 cells (10, 11). Deletion of the 144 TRE flanking sequences resulted in a complete loss of the 144 transcriptional effects (11) suggesting that an additional factor binding in the vicinity of the TRE is required to regulate the T3-mediated transrepression.

To test whether CTCF is the additional factor involved in the TRE-mediated 144 element function, we introduced a mutation within the 144#1 fragment that selectively eliminated CTCF binding. We substituted GAGGG for GAATT, which eliminated three CTCF-contacting guanines and conveniently created a unique Ase1 restriction site allowing us to verify accuracy of the site-directed mutagenesis (Fig. 4). This mutated fragment no longer binds to either endogenous CTCF from nuclear extracts or in vitro translated CTCF (Fig. 6, lanes 9–16). Moreover, the absence of any shifted bands in EMSA with this mutated fragment indicates that it does not harbor any fortuitously created binding sites for any other DNA-binding factors.

Fig. 5. CTCF employs different groups of C-terminal ZFs in binding to the two TRE-containing elements, the 144 and the S-2.4 lysozyme silencer. Equal amounts of the CTCF proteins (C) containing different groups of ZFs were analyzed by EMSA with DNA fragments originating from the two TRE-containing regulatory elements. A, DNA probe, the 144#1 DNA fragment that does not include the TR/RXR site (Fig. 1). B, DNA probe, the F1 fragment of the S-2.4 silencer (4, 6). CTCF ZFs that can be deleted from the 11-ZF domain without significantly losing binding to the F1 or to the 144 sequence are schematically shown by filled boxes on the diagrams included at the bottom of each panel. Half-tone filled box in B indicates that deleting three C-terminal fingers, from 11 to 9 inclusive, partially reduces binding to the F1 DNA probe. Deleting of one more finger, as schematically depicted by open boxes, results in the complete loss of binding. The positions of the unbound (F) and protein-bound (B) DNA probes are indicated. Appearance of a second EMSA band after deleting N-terminal ZFs 1 and 2, an effect that was never observed with the entire 11-ZF domain, or with the other CTCF-target sequences (3, 4) may perhaps indicate the presence in the 144 element of another recognition subsite that, because of spatial constrains, can be occupied only by proteins with the N-terminally truncated ZF domain. C, the serially truncated from either terminus, 32P-labeled, forms of the 11-zinc-finger CTCF domain, marked for the C-terminal truncations as ZF(1–11) to ZF(1–5) and for N-terminal truncations as ZF(2–11) to ZF(6–11) were in vitro synthesized and quantitatively analyzed by SDS-polyacrylamide gel electrophoresis as described under the “Experimental Procedures” section.
In addition we found in gel shift analyses with in vitro translated TR/RXR and CTCF proteins that the “Ase1” mutation of the 144 CTCF binding site had no effect on TR/RXR binding to the 144 TRE (data not shown).

We then transiently co-transfected CV-1 cells with the parental ptk-CAT construct harboring no 144 element, with the ptk144UTR-CAT constructs with or without the Ase1 mutation of the CTCF binding site, together with expression vectors for all three proteins likely involved in mediating the 144 function including CTCF, TR, and RXR. We observed that the activity of the TK promoter-driven CAT construct is down-regulated by the presence of the 144 element, and this down-regulation is enhanced with the addition of T3 (Fig. 7A). This repression was considerably relieved with the reporter harboring the mutated 144 CTCF binding site, and this construct no longer responded to the addition of T3 (Fig. 7A).

We also performed stable transfection experiments in mouse myoblast C2C12 cells. These cells exhibit enhanced muscle differentiation with the addition of T3 (19) and harbor high endogenous levels of TR, RXR (18, 19), and CTCF.2 We stably transfected all three reporter constructs in these cells and normalized the relative copy number of each CAT reporter/cell as described under “Experimental Procedures.” Our observations in these stably transfected cells were similar to those with the transient transfections. Introducing the 144 element into these stably transfected cells were similar to those with the transient transfections. Introducing the 144 element into stably transfected C2C12 cells lines grown in the presence (+) or absence (−) of the hormone. See “Experimental Procedures” for more details.

The activity of this 144 CTCF binding site mutated reporter (Fig. 7B).

**DISCUSSION**

CTCF is a multivalent DNA-binding factor with multiple sequence specificity achieved through combinatorial usage of different groups of its individual fingers within the 11-zinc-finger DNA binding domain (3, 4, 9). CTCF is an abundant nuclear protein expressed at levels similar to that of the general transcription factor Sp1 (20). In many studies on nuclear DNA-binding factors, which commonly utilize 20–40-bp long oligonucleotides as DNA probes, CTCF has been “missed” because to bind DNA the 11-zinc-finger domain of CTCF requires additional flanking sequences outside of the ~50-bp long recognition motif(s) (1, 20).

Among the different regulatory DNA targets for CTCF, which others and we have identified, are promoters of vertebrate c-myc (1–3, 21) and POLO-like kinase dominant oncogenes3 as well as the critical region of the APP gene promoter.

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1. G. N. Filippova, Y. J. Hu, S. Collins, P. E. Neiman, and V. V. Lobanenkov, unpublished results.
2. G. N. Filippova, M. Macbeth, M. Lilley, Y. J. Hu, S. Collins, P. E. Neiman, and V. V. Lobanenkov, unpublished results.
mediator of repression by the TRE-containing 144 genomic fragment enhanced with the addition of T3 (Fig. 7). However, the same infection experiments the 144 DNA fragment repressed activity at the CTCF target site (Fig. 6). In both transient and stable transfection experiments the 144 DNA segment functioned as a transcriptional repressor when positioned within the 3′-UTR of a TK promoter-driven reporter construct and displayed a strict functional dependence on the presence of flanking DNA sequences (11). We identified a new CTCF binding site within this element some 160 bp away from the TRE and mapped the CTCF contact nucleotides (Figs. 2 and 3). We also showed that under our EMSA conditions CTCF appears to be a major protein in nuclear extracts that binds to the new 144 CTCF target site (Fig. 6). In both transient and stable transfection experiments the 144 DNA fragment repressed activity from a CAT reporter construct, and this repression was enhanced with the addition of T3 (Fig. 7). However, the same fragment harboring a mutated CTCF site, which was no longer capable of binding CTCF or any other nuclear proteins (Fig. 6), exhibited significantly less repression. Moreover, this fragment harboring the mutated CTCF binding site no longer mediated any effect of T3 on this repression. Thus CTCF is an important mediator of repression by the TRE-containing 144 genomic element, and the T3-induced transrepression via the 144 element requires binding of CTCF.

Thus in a manner similar to the composite “TR/RXR-CTCF binding element” of the S-2.4 lysozyme gene transcriptional silencer (4), specific binding of CTCF close to the 144 TRE appears to be an important determinant of the functional repressor activity of the 144 element providing another example of functional connection between certain CTCF target sites and TRE-containing elements. Previous reports have described functional interactions between nuclear hormone receptors and other “ancillary” transcription factors. For instance mutating the nuclear factor 1-like binding sites in the mouse MMTV-LTR or in the Xenopus laevis vitellogenin gene promoter abrogates the glucocorticoid response element-mediated glucocorticoid responsiveness (22) or the estrogen response element-mediated estrogen inducibility (23), respectively, of these promoters. In comparison with other transcriptional factors the unusual ability of CTCF to specifically recognize different DNA sequences might provide significant combinatorial flexibility in creating modular structures of composite hormone responsive elements. Therefore we would predict that there are numerous promoters and other regulatory regions that might harbor hormone response elements “linked” to CTCF activity.

Intriguingly, similar to the 144 CTCF and TR/RXR binding sites (Fig. 1), one of the recently described negative TR/RXR binding TREs is positioned in the promoter region of the APP gene (29) at the same distance (approximately 160 bp) from the CTCF binding site (9). Therefore it is likely that CTCF is also involved in negative regulation of APP gene transcription by thyroid hormone. Because APP plays a key role in the development of Alzheimer’s disease, testing this hypothesis with direct site-specific mutational analysis of the APP gene promoter would be important to evaluate a potential link between CTCF function, APP expression, and Alzheimer’s disease.

Comparison of CTCF contact nucleotides in the lysozyme gene transcriptional S-2.4 silencer F1 site with the 144 element revealed both subregional similarities and differences (Fig. 4). EMSA experiments with truncated proteins lacking individual ZFis consecutively deleted from either the N or C terminus of the 11-ZF domain clearly indicated that binding to the 144 TRE versus the F1 target sites requires different numbers of the C-terminal ZFis, whereas several N-terminal CTCF ZFis contribute similarly to both target sequences (Fig. 5). Thus for specific binding to the two different TRE-containing regulatory elements CTCF utilizes common N-terminal ZFis but different C-terminal ZFis. It is possible that despite clear differences in CTCF target sequences within these two TRE-containing elements, there may be a similarity in the resulting three-dimensional CTCF-DNA structure that could determine a common regulatory pathway(s) involving direct or indirect interaction between DNA-bound CTCF and adjacent TRE-bound TR/RXR.

In contrast to a large number of relatively well characterized positive TREs that are derepressed in response to binding of ligand, the 144 TRE is not activated by T3 but rather like other previously described “negative TREs” (24–30) mediates enhanced repression with the addition of T3 (Fig. 7). Our data clearly indicate that CTCF is an important player in mediating this T3-induced repression. Because CTCF does not interact with TR or RXR in vitro pull down assays (data not shown) or in the yeast two hybrid system, additional proteins are likely to be involved in mediating cross-talk between juxtaposed DNA-bound CTCF and nuclear hormone receptors.

In addition to numerous previously characterized factors that interact with nuclear receptors in either a ligand-dependent or -independent manner (31) including SMRT (32), N-CoR (33), and TRIP15/Alien (34), an interesting candidate protein to serve as a bridge between CTCF, TR, and the general transcriptional machinery is the tumor suppressor p53. The human TRβ physically interacts with p53 via its DNA binding domain (35), and in co-transfection experiments the wild type p53 represses the hormone-dependent transcriptional activation by TRβ (36). Moreover, the C-terminal domain of p53, which is not involved in TRβ binding, has been reported to interact with CTCF (37). Therefore it is possible that p53 can interact with both CTCF and TRβ resulting in a functionally significant regulatory trimeric complex. This possibility is particularly intriguing because CTCF is itself a candidate tumor suppressor gene, mapped to the chromosome locus 16q22.1, which frequently displays loss of heterozygosity in prostate, breast, and a number of other cancers developing from hormone-dependent cell lineages (38).

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REFERENCES

1. Lobanekov, V. V., Nicoles, R. H., Adler, V. V., Paterson, H., Klenna, E. M., Polatskaia, A. V., and Goodwin, G. H. (1990) Oncogene 5, 1743–1753
2. Klenna, E. M., Nicoles, R. H., Paterson, H. F., Carne, A. F., Heath, C. M., Goodwin, G. H., Neiman, P. E., and Lobanekov, V. V. (1993) Mol. Cell. Biol. 13, 7612–7624
3. Filipovic, G. N., Fagerlie, S., Klenna, E. M., Myers, C., Dehner, Y., Goodwin, G., Neiman, P. E., Collins, S. J., and Lobanekov, V. V. (1996) Mol. Cell. Biol. 16, 2892–2813
4. Burcin, M., Arnold, R., Lutz, M., Kaiser, B., Runge, D., Lottspeich, F., Filipovic, G. N., Lobanekov, V. V., and Renkawitz, R. (1997) Mol. Cell. Biol. 17, 1281–1286
5. Kohn, A. C., Baniahmad, A., and Renkawitz, R. (1993) J. Mol. Biol. 232, 747–755
6. Baniahmad, A., Steiner, C., Kohn, A. C., and Renkawitz, R. (1999) Cell 98, 505–514
7. Quitschke, W. W., and Golgadger, D. (1992) J. Biol. Chem. 267, 17362–17368
8. Quitschke, W. W. (1994) J. Biol. Chem. 269, 21229–21233

4 M. Lutz, R. Arnold, and R. Renkawitz, unpublished results.
New CTCF+TR/RXR Negative Element

9. Vostrov, A. A., and Quitschke, W. W. (1997) J. Biol. Chem. 272, 33553–33559
10. Bigler, J., and Eisenman, R. N. (1994) Mol. Cell. Biol. 14, 7621–7632
11. Bigler, J., and Eisenman, R. N. (1995) EMBO J. 14, 5710–5723
12. McCormick, M., and Mierendorf, R. (1994) In: Novations 1, 4–6
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Luckow, B., and Schutz, G. (1987) Nucleic Acids Res. 15, 5490
15. Klenova, E. M., Fagerlie, S., Filippova, G. N., Kretzner, L., Goodwin, G. H., Loring, G., Neiman, P. E., and Lobanenkov, V. V. (1998) J. Biol. Chem. 273, 26571–26579
16. Lobanenkov, V. V., and Goodwin, G. H. (1989) Proc. Acad. Sci. USSR (Moscow) 309, 741–745
17. Tevosian, S. G., Gromova, E. S., Adler, V. V., and Lobanenkov, V. V. (1991) Mol. Biol. (Moscow) 25, 1013–1023
18. Carnac, G., Albagli-Curiel, O., Vandromme, M., Pinset, C., Montarras, D., Laudet, V., and Bonneil, A. (1992) Mol. Endocrinol. 6, 1185–1194
19. Muscat, G. E., Mynett-Johnson, L., Dowhan, D., Downes, M., and Griggs, R. (1994) Nucleic Acids Res. 22, 583–591
20. Lobanenkov, V. V., Adler, V. V., Klenova, E. M., Nicolas, R. H., and Goodwin, G. H. (1989) In: Gene Regulation and AIDS: Transcriptional Activation, Retroviruses and Pathogens (Papas, T. S., ed) pp. 45–68, Portfolio Publishing Corp., Woodlands, Texas
21. Neiman, P. E., Churman, R. E., and Lobanenkov, V. V. (1997) Curr. Top. Microbiol. Immunol. 224, 231–238
22. Chavez, S., and Beato, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2885–2890
23. Chang, T. C., and Shapiro, D. J. (1990) J. Biol. Chem. 265, 8176–8182
24. Sanchez-Pacheco, A., Palomino, T., and Aranda, A. (1995) Mol. Cell. Biol. 15, 6322–6330
25. Madison, L. D., Ahlquist, J. A., Rogers, S. D., and Jameson, J. L. (1993) Mol. Cell. Endocrinol. 94, 129–136
26. Radoja, N., Diaz, D. V., Minars, T. J., Freedberg, I. M., Blumenberg, M., and Tumie-Canic, M. (1997) J. Invest. Dermatol. 109, 566–572
27. Hollemburg, A. N., Morden, T., Flynn, T. R., Boers, M. E., Cohen, O., and Wondisford, F. E. (1995) Mol. Endocrinol. 9, 549–550
28. Tagami, T., Madison, L. D., Nagaya, T., and Jameson, J. L. (1997) Mol. Cell. Biol. 17, 2642–2648
29. Belandia, B., Lataza, M. J., Villa, A., and Pascual, A. (1998) J. Biol. Chem. 273, 30366–30371
30. Carr, F. E., and Wong, N. C. (1994) J. Biol. Chem. 269, 4175–4179
31. Torchia, J., Glass, C., and Rosenfeld, M. G. (1998) Curr. Opin. Cell Biol. 10, 373–383
32. Chen, J. D., and Evans, R. M. (1995) Nature 377, 454–457
33. Horlein, A. J., Naar, A. M., Heinzl, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) Nature 377, 397–404
34. Dressel, U., Thornmeyer, D., Altincicek, B., Paululat, A., Eggert, M., Schneider, S., Tenbaum, S. P., Renkawitz, R., and Banasham, A. (1999) Mol. Cell. Biol. 19, 3383–3394
35. Yap, N., Yu, C. L., and Cheng, S. Y. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4273–4277
36. Bhat, M. K., Yu, C., Yap, N., Zhan, Q., Hayashi, Y., Seth, P., and Cheng, S. (1997) J. Biol. Chem. 272, 28989–28993
37. Wadaogkan, R., Spatis, R., and Abarzua, P. (1996) Proc. Annu. Meet. Am. Assoc. Cancer Res. 37, A4092
38. Filippova, G. N., Lindblom, A., Meineke, L. J., Klenova, E. M., Neiman, P. E., Collins, S. J., Doggett, N. A., and Lobanenkov, V. V. (1998) Genes Chromosomes Cancer 22, 26–36