MOLECULAR AND CELLULAR BIOLOGY, June 1984, p. 1057–1062
0270-7306/84/061057-06$02.00/0

Vol. 4, No. 6

Dexamethasone Increases the Number of RNA Polymerase II Molecules Transcribing Integrated Mouse Mammary Tumor Virus DNA and Flanking Mouse Sequences

JULIANE M. FIRZLAFF* AND HEIDI DIGGELMANN

Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland

Received 28 November 1983/Accepted 24 February 1984

In mouse Ltk$^-$ cells that were transfected with recombinant bacteriophage DNA containing a complete proviral copy of an integrated endogenous mouse mammary tumor virus (MMTV) with its flanking cellular sequences, the newly acquired MMTV proviruses were transcribed in a glucocorticoid-responsive fashion. After hormone treatment of selected cell clones in culture we isolated the nuclei, elongated the nascent RNA chains in vitro, and determined the number of RNA polymerase II molecules on the transcribed MMTV DNA as well as on the flanking mouse DNA sequences. We found that the specific increase in the polymerase loading after hormone treatment is proportional to the increase in the amount of stable MMTV mRNA. When the DNA sequences which are responsible for hormone-receptor binding and for the increased MMTV mRNA levels were deleted, no increase in RNA polymerase II loading on MMTV DNA was observed. Nuclear RNA chains which were transcribed in response to hormone treatment were detected not only from the transfected MMTV DNA but also from the mouse DNA sequences adjacent to the 3′ end of the provirus.

The expression of mouse mammary tumor virus (MMTV) in mice as well as in tissue culture cells is regulated by steroid hormones (18, 23, 25, 28, 38). Studies utilizing homologous and heterologous cell lines revealed that the strong stimulation of viral RNA synthesis could be attributed to the action of glucocorticoids alone. The regulation is mediated by a hormone-receptor complex; it is very rapid and independent of simultaneous protein synthesis (30, 34). The hormone-receptor complex binds specifically to sequences in the long terminal repeat (LTR) of MMTV DNA (9, 11, 26, 27, 32). In transfection experiments it was shown that the same DNA region is involved in glucocorticoid-regulated transcription (7, 13, 16, 17, 19). Upon deletion of some of these sequences, the hormonal effect on MMTV transcription is lost (2, 7, 21). It was demonstrated that the sequences required for the hormone response reside between ~100 and ~200 nucleotides upstream from the initiation site for viral RNA synthesis (2, 21). Larger DNA fragments containing this sequence but lacking the MMTV promoter were shown to confer hormone inducibility to other, normally non-hormone-responsive promoters (3, 21). As a result of hormonal treatment, the rate of viral RNA synthesis increases, as measured by pulse labeling (12, 29, 36, 37, 40), and higher levels of stable mRNA accumulate. From these studies it was deduced that the rate of initiation of viral RNA synthesis is increased, but they could not exclude the possibility of rapid metabolic effects of glucocorticoids, which may alter for example the stability of the MMTV mRNA. To further study the molecular events which are caused by hormone-receptor binding to DNA, it was therefore necessary to apply an approach which looks more directly at initiation of transcription. In the experiments presented here we measured the number of RNA polymerase II molecules transcribing MMTV DNA with and without hormone treatment. With the same experimental design, we also looked at transcription from adjacent cellular sequences to explore the possibility of promoter or enhancer insertion models for transformation by MMTV.

MATERIALS AND METHODS

Cell lines. Ltk$^-$ aprt$^-$ cells (provided by M. Perucho and M. Wigler via N. Hynes) were transfected with different recombinant pBR322 plasmids (2) and with a recombinant Charon 4A phage (6). The 15-1 cell line (6) had acquired a functional thymidine kinase gene (*tk*) of herpes simplex virus by transfection with a recombinant plasmid containing this gene (39). Cell line 16-3 (6) was co-transfected with the *tk* plasmid and a recombinant Charon 4A phage (AJ-1) harboring a complete endogenous MMTV provirus from the A/Jax mouse with its flanking mouse DNA sequences. It carries six copies of foreign DNA. The cell lines −600, −204, −149, and −105 were derived by transfection of a plasmid containing the MMTV LTR deleted to different extents fused to the *tk* coding sequence (2). The numbers indicate the remaining LTR sequences upstream from the cap site for the MMTV RNAs. These cell lines were generously provided by E. Buetti.

Preparation of the nuclei. The *tk*$^+$ cell lines were grown in HAT selective medium (20) without added hormone to 90% confluency. A small amount of medium with or without the synthetic glucocorticoid dexamethasone (final concentration, 10^{-6} M) was added, and after 2 h the cells were harvested in ice-cold phosphate-buffered saline. The nuclei were then purified according to a modification (33) of the method described by Hewish and Burgoyne (15) with the following adaptation for cells in tissue culture. The cells were resuspended in 0.5% Nonidet P-40 and 0.3 M sucrose in buffer A (60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 14 mM beta-mercaptoethanol, 0.5 mM EGTA [ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid], 2 mM EDTA, 15 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.5), homogenized in a Dounce homogenizer with pestle B and centrifuged (2,500 rpm, 4°C, 10 min, HB4 rotor of a Sorvall centrifuge) through a cushion of 30% sucrose in buffer A.

* Corresponding author.

The nuclei were then treated for 30 min on ice with 5 μg of RNase A per ml in 0.3 M sucrose in buffer A and centrifuged again twice through 30% sucrose in buffer A. In the first experiments described, an additional centrifugation step was performed. The nuclei were taken up in 2 M sucrose in buffer B (the same buffer as buffer A, but with 0.1 mM EGTA and 0.1 mM EDTA) and centrifuged through a cushion of the same composition in an SW50.1 rotor in a Beckman centrifuge at 3×10^4 rpm at 40°C for 1 h. This step proved to be unnecessary for nuclei isolated from cells in culture. The nuclei were resuspended in nuclei storage buffer (20 mM Tris-hydrochloride, pH 7.9, 75 mM NaCl, 0.5 mM EDTA, 0.85 mM dithiothreitol, 0.125 mM phenylmethylsulfonyl fluoride, 50% glycerol) and used directly or stored at −70°C. The DNA content of each preparation was measured by the method of Giles and Myers (10).

In vitro elongation and hybridization. The elongation of nascent RNA chains was carried out at 26°C following the protocol of Schibler et al. (33) according to the method of Gariglio et al. (8), omitting the RNase inhibitor. The RNA was extracted and hybridized to filter-bound DNA as described by Groudine et al. (14), except that 0.5% sodium dodecyl sulfate was added and no dextran sulfate was used in the hybridization mixture.

MMTV and flanking mouse DNA subclones used as hybridization probes. The pBR322 recombinant plasmid p2.0 (7) harbors the 2.0-kilobase (kb) *Pst*I fragment of an exogenous GR-MMTV. It was purified by banding in a cesium chloride-ethidium bromide gradient. The single-stranded probes C and D (a gift of N. Fasel) were derived by subcloning the *Pst*I-*Bgl*II fragment from the *gag-pol* region of exogenous GR-MMTV (1) into the single-stranded phages M13mp8 and M13mp9 and preparing the phage single-stranded DNA by the method of Sanger et al. (31). For the subclones A, B, E, F, and p1.1fl, the corresponding fragments were cut out from the lambda phage AJ-1 (6) and subcloned in M13mp8-11 and pBR322, respectively. The recombinant single-stranded or double-stranded DNA was purified as mentioned above. The single-stranded phage DNA was directly spotted onto nitrocellulose filters. The inserts of the different plasmids were cut out with the appropriate restriction enzyme, and the fragments were separated on 1% agarose gels and transferred to nitrocellulose filters.

RESULTS

In vitro elongation in isolated nuclei. We used the method of in vitro elongation of nascent RNA chains initiated in vivo (14) to show that the effect of glucocorticoid stimulation of transcription takes place at the level of initiation. An increase in the frequency of initiation results in a higher number of RNA polymerase II molecules bound to the DNA in the presence of hormone and therefore in a larger number of nascent RNA chains.

To perform this study, a tissue culture cell line (16-3) stably transfected with a cloned integrated endogenous MMTV provirus (6) was used. The newly acquired MMTV proviruses are transcriptionally active and regulated by glucocorticoid hormones (6). The nuclei of this cell line were purified as described above, and the preexisting RNA was removed by exposing the nuclei to RNase A. RNA fragments of 60 nucleotides in length are protected from the RNase A digestion by RNA polymerase II molecules bound to their DNA template (33). These RNA chains were then elongated in vitro in the presence of heparin (1 mg/ml), an RNase inhibitor, and ammonium sulfate (350 mM), which prevents reassociation of unbound RNA polymerase II molecules with DNA (4). A time course of such an elongation reaction with total cellular RNA is shown in Fig. 1. The elongation products after 2, 5, and 10 min of incubation were analyzed by agarose gel electrophoresis. The average length of the nascent RNA chains after 10 min of incubation (lanes 3 and 6) reached ca. 260 nucleotides, indicating an elongation rate of about 20 nucleotides per min at 26°C. The preceding RNase treatment had no effect on the reaction, as we found no difference in trichloroacetic acid-precipitable radioactivity after elongation in RNase-treated and untreated nuclei (data not shown). The addition of the synthetic glucocorticoid dexamethasone to the cells in culture for 2 h before preparation of the nuclei had no apparent effect on the overall incorporation, as measured by trichloroacetic acid-precipitable counts (data not shown), or on the elongation rate (Fig. 1). The UTP pool in the cells (determined by the method described by Cox [5]) was not changed by the hormone treatment (data not shown).

Elongation of MMTV-specific transcripts. By using this assay, three different cell lines (16-3, 15-1, L-cells) were investigated for expression of MMTV-specific sequences. Cell clone 16-3 is derived from Ltk$^-$ cells which had been stably transfected with cloned endogenous integrated proviral DNA together with plasmid containing the herpes *tk* gene (6). This clone contains approximately six copies of foreign MMTV DNA in addition to the L-cell endogenous MMTV proviruses. The cell clone 15-1 was transfected with the *tk* plasmid alone, and the Ltk$^-$ cells served as a nontransfected control. These three cell lines were grown for 2 h in the presence of dexamethasone (10^{-6} M), and the nuclei were prepared and an in vitro elongation reaction was performed as described above. The labeled purified RNA chains were

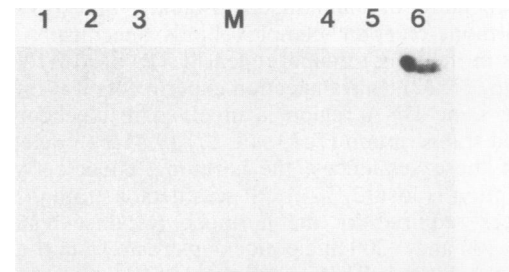

FIG. 1. RNA chain elongation in vitro. Nuclei of the cell line 16-3 were prepared as described in the text. The RNA chains were elongated for 2, 5, and 10 min and purified by extraction with hot phenol saturated with 20 mM sodium-acetate and 50 mM NaCl. After precipitation with 1 M ammonium-acetate in ethanol, the RNA was glyoxylated (22) and separated on a 2% agarose gel. Lanes 1 through 3: Nuclei from 16-3 cells treated for 2 h with 10^{-6} M dexamethasone. Lanes 4 through 6: Nuclei from untreated 16-3 cells. Lanes 1 and 4, 2-min incubation; lanes 2 and 5, 5-min incubation; lanes 3 and 6, 10-min incubation. Lane M: Denatured *Alu*I restriction fragments of pBR322 DNA.

then hybridized to DNA fragments, previously separated by agarose gel electrophoresis and transferred to nitrocellulose filters. The result of the analysis of nascent RNA molecules from these cell lines in presence of the hormone is shown in Fig. 2. In the nuclei of cell line 16-3, MMTV-specific transcripts were detected by their hybridization to the 2.0-kb *Pst*I fragment (p2.0 in Fig. 5) of exogenous MMTV (Fig. 2, lane B). This transcription was inhibited by actinomycin D (lane A). No MMTV-specific transcription was observed in nuclei of cell line 15-1 (lane C) or of L-cells (lane D), demonstrating that the endogenous MMTV proviruses present in L-cells are not expressed even in the presence of dexamethasone. This is in agreement with the results obtained by analyzing stable mRNA of these lines (6). With the RNA from the cell lines cotransfected with the unlinked ptk plasmid (39) (16-3 [lane B] and 15-1 [lane C]), weak hybridization also occurred to the linearized pBR322 DNA (4.3 kb) present on the filter. This suggests low levels of transcription of pBR322 sequences which could have been initiated either at the *tk* promoter or at TATA box-like sequences in the plasmid. These transcripts were not detected consistently and therefore not further analyzed.

Increased RNA polymerase II loading on the MMTV genome in response to hormone treatment. To investigate the effect of hormone treatment, cell line 16-3, kept in culture medium without added hormone, was grown for 2 h in the presence or absence of dexamethasone. After transcription-elongation in isolated nuclei, the labeled RNA was purified and hybridized to various DNAs bound to a filter. Hormone-responsive transcription was observed for the MMTV sequences, as determined by hybridization to the 2.0-kb *Pst*I fragment (Fig. 3, panels B and D, lanes 2 through 4). It is noteworthy that large amounts of unlabeled DNA must be present on the filter to produce conditions of excess DNA (compare lane 4 and lane 2 in panels B and D) in these hybridization reactions, an observation which has already been made (14). Only the amount of DNA in lane 4 was excessive, and higher amounts of DNA did not increase the signal (data not shown). Because most preexisting RNA molecules in the preparation of nuclei were digested with RNase A, there was little unlabeled RNA which might compete in the hybridization reaction with the labeled nascent RNA chains. The increased intensity of the hybridization signal (compare lane 4 of panels B and D) due to the hormone treatment was about 10-fold, as measured by densitometry of the autoradiographs. As the nuclei were treated with RNase A before the in vitro incubation and as de novo polymerase association with DNA was prevented, elongation only takes place on nascent RNA chains protected from nuclease digestion by RNA polymerase molecules. Therefore, the amount of labeled RNA specific for a certain DNA sequence is directly proportional to the number of RNA polymerase molecules bound to that sequence. The 10-fold increased hybridization signal therefore indicates that 10 times more polymerase molecules are active on the corresponding MMTV sequence in the presence of the hormone.

No increase in transcription of the cotransfected *tk* gene and the lambda Charon 4A DNA was observed after hormone treatment (panels B and D, lane 1 and 6). Lane 5 of panels B and D shows hybridization of in vitro elongated transcripts to the recombinant plasmid p1.1fl (see Fig. 5) which contains a DNA sequence adjacent to the A/Jax endogenous MMTV provirus (6) but which does not contain MMTV sequences. Transcripts hybridizing to this plasmid seem to increase after hormone treatment. This effect was

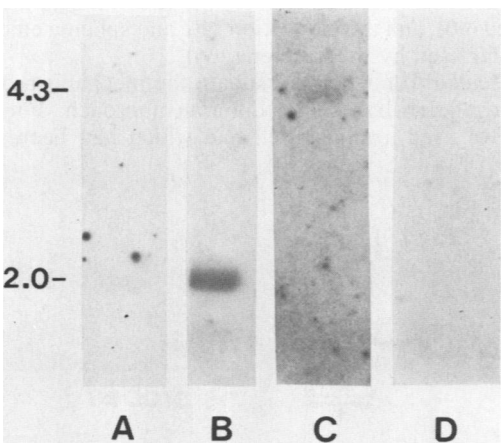

FIG. 2. MMTV-specific transcription in cell line 16-3. The transfected cell lines 16-3 and 15-1 and Ltk$^-$ cells were grown for 2 h in the presence of 10^{-6} M dexamethasone. Nuclei were prepared and the RNA was elongated as described. The ^{32}P-labeled RNA was hybridized to a filter containing 30 μg of DNA of the plasmid p2.0 cut with *Pst*I. Source of RNA: (lane A) 16-3, elongation reaction in the presence of actinomycin D (200 μg/ml), (lane B) 16-3, (lane C) 15-1, (lane D) L-cells.

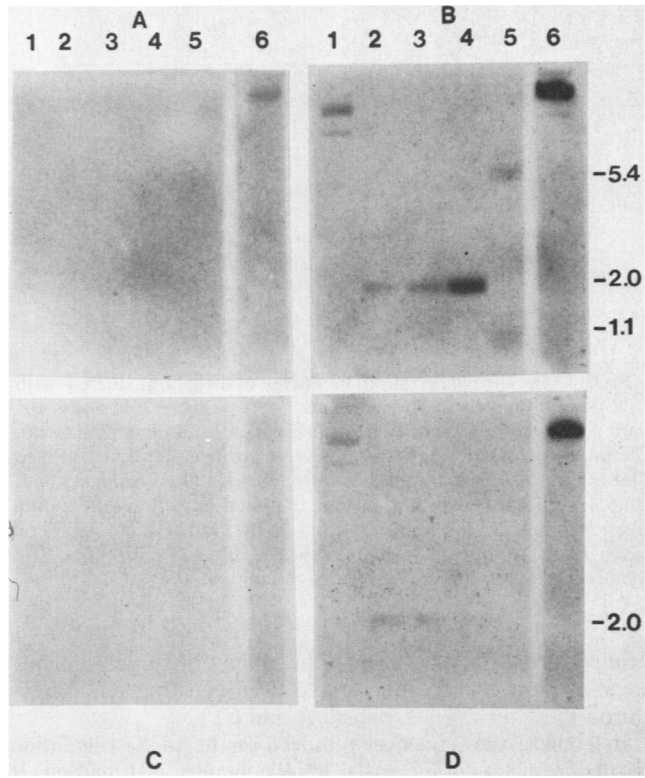

FIG. 3. Hormone-responsive transcription of MMTV DNA by RNA polymerase II. Cell line 16-3 was grown in the presence (A, B) or absence (C, D) of dexamethasone, and the nuclei were prepared as described. RNA chains were elongated in the absence (B, D) or presence (A, C) of alpha-amanitin (20 μg/ml), purified as described, and hybridized to filter-bound DNA. Lanes 1, ptk DNA; lanes 2 through 4, p2.0 DNA cut with *Pst*I, 4, 10, and 20 μg, respectively; lanes 5, p1.1fl DNA (1.4 μg) partially cut with *Hin*dIII, linearized plasmid (5.4 kb), and excised insert (1.1 kb); lanes 6, Charon 4A DNA.

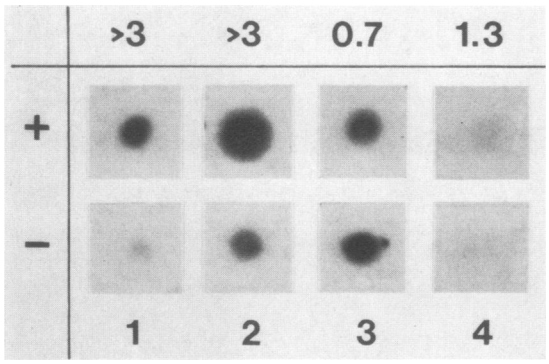

FIG. 4. In vitro elongation in nuclei of cells transfected with LTR deletion mutants. The cell lines −600, −204, −149, and −105 were grown in the absence (−) or presence (+) of dexamethasone. The in vitro elongated RNA chains were purified and hybridized to plasmid ptk (4 μg) and dotted to nitrocellulose filters as described. Lane 1: Cell line −600, 5×10^5 cpm applied in each hybridization reaction. Lane 2: Cell line −204, 0.9×10^6 cpm. Lane 3: Cell line −149, 1.2×10^6 cpm. Lane 4: Cell line −105, 1.6×10^5 cpm. The stimulation factor is indicated for each cell line.

studied further (see below). Alpha-amanitin strongly inhibited transcription corresponding to the DNA fragments present on the filter (Fig. 3, panels A and C).

Deletion of the sequences required for hormone regulation results in the loss of increased RNA polymerase II loading. It has recently been shown that removal of certain DNA sequences from the viral LTR leads to a loss of hormone responsiveness of transcription (2, 3, 17, 21) and to a loss of the hormone-receptor binding (32). DNA sequences between about −100 and −200 base pairs upstream from the initiation site of viral transcription are required for glucocorticoid stimulation (2, 21).

Cell lines stably transfected with a partially deleted LTR linked to the herpes *tk* coding region (2) were grown in the presence or absence of dexamethasone for 2 h. Nuclei were purified and the RNA was elongated in vitro as described. As hybridization probe to detect MMTV transcripts we used the DNA of the plasmid ptk, bound to nitrocellulose filters. Figure 4 shows that increased RNA polymerase II loading in the presence of the hormone can be detected until the deletion reaches −204 base pairs upstream from the initiation site of transcription (lanes 1 and 2). The removal of the sequences between −204 and −105 base pairs upstream of the cap site previously shown to be required for increased accumulation of stable mRNA in the same cell lines (2) leads to a loss of the hormone-induced increase in RNA polymerase II loading (lanes 3 and 4 with and without dexamethasone). The absolute level of transcription observed in the individual cell clones transfected with the deletion mutants cannot be directly compared, as the cell clones contain different copy numbers of the foreign DNA (e.g., lanes 1 through 4, without dexamethasone).

Transcription of sequences flanking MMTV DNA. As shown in Fig. 3 (panel B, lane 5), we detected hybridization of the nuclear RNA elongated in vitro to cellular sequences flanking an integrated MMTV provirus. To investigate further the nature of the transcripts in the vicinity of the integrated provirus, single-stranded probes of 5′ and 3′ MMTV flanking mouse sequences were prepared (Fig. 5) and used for the hybridization with in vitro elongated RNA extracted from cells transfected with the AJ-MMTV proviral DNA (16-3 cells). The result of this experiment is shown in Fig. 6. As demonstrated previously (Fig. 3), transcripts complementary to the coding strand of MMTV DNA (probe D) were synthesized in response to dexamethasone (compare in Fig. 6 panels A and B, dots D). Very little hybridization was observed to probe C, which represents the opposite strand. Hormone-dependent transcripts also hybridized with roughly the same intensity to the coding strand of the 3′ flanking mouse DNA (probe F), whereas no hybridization was observed to the 5′ flanking mouse DNA in presence or absence of the hormone (dots A and B). Thus, transcription initiated under the control of a hormone-regulated promoter can be detected in downstream mouse sequences flanking the integrated provirus.

DISCUSSION

Several attempts have already been made to define precisely the event which finally leads to the increased levels of stable MMTV-specific mRNA in hormone-treated cells. The assumption that the crucial step is the initiation of RNA synthesis is based on experiments where transcription was measured after pulse labeling with radioactive precursors (12, 29, 36, 37, 40). This conclusion was supported by the observation that the half-life of the viral mRNA was not increased (40), and the elongation rate and splicing efficiency were unaffected by the hormone (36).

We decided to further investigate the mechanism of hormonal regulation by using a different approach. For these studies we used a mouse cell line which had been stably

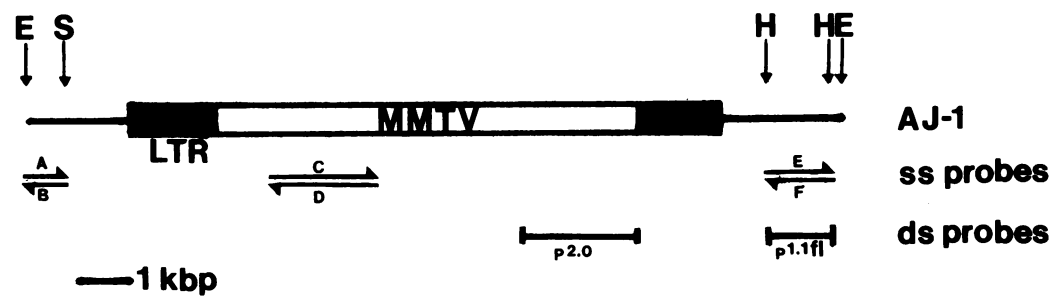

FIG. 5. Insert of the recombinant Charon 4A clone AJ-1 with description of the subcloned DNA fragments used as hybridization probes. The restriction sites utilized for the subcloning of the flanking mouse sequences are indicated: E, *Eco*RI; S, *Sst*I; H, *Hin*dIII. The single-stranded (ss) probes A through F were generated from recombinant M13mp8-11 phage DNA. The arrows are pointing from the 5′ to the 3′ direction. Probe D represents the coding strand for MMTV-initiated transcripts. The double-stranded (ds) probes p2.0 and p1.1fl were derived by cloning the corresponding restriction fragments into the plasmid pBR322 (for details see the text).

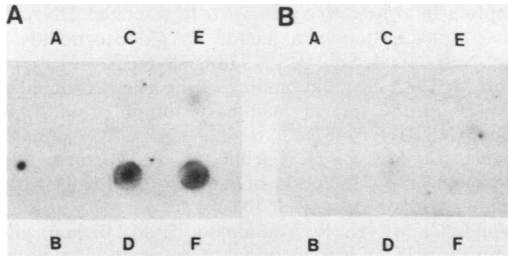

FIG. 6. Hormone-regulated transcription in mouse flanking sequences. Cell line 16-3 was grown in the presence (A) or absence (B) of dexamethasone, and the nuclei were prepared as described. The in vitro elongated RNA was hybridized to single-stranded recombinant phage M13 clones spotted onto nitrocellulose. Spots A and B: 5′ flanking mouse sequences. Spots C and D: Internal MMTV sequences. Spots E and F: 3′ flanking mouse sequences. Spots A, C, and E: Noncoding strand for MMTV transcripts. Spots B, D, and F: Coding strand for MMTV transcripts. See also Fig. 5.

transfected with the recombinant phage AJ-1 containing an endogenous MMTV provirus of A/Jax mouse and adjacent flanking mouse DNA (Fig. 5). It was previously demonstrated (6) that the transfected MMTV copies are expressed in a hormone-responsive fashion. Stable MMTV-specific mRNA accumulates to about 10-fold higher levels in the presence of glucocorticoids. We prepared nuclei from these cells and measured the number of RNA polymerase II molecules bound to the MMTV provirus by in vitro elongation of nascent RNA chains. The data presented here demonstrate that the number of active RNA polymerase II molecules on MMTV DNA increases to the same extent as the specific mRNA level and can therefore account entirely for the effect of glucocorticoid hormones on MMTV transcription. This increase in polymerase loading most likely reflects a higher frequency of initiation of viral RNA synthesis in the presence of the hormone. Moreover, we show that this hormone-mediated increase requires upstream DNA sequences extending to nucleotide −204 from the cap site. Further deletion of the DNA between positions −204 and −149 abolished the increase, whereas a constitutive level of transcription was still detectable. These results confirm and extend those obtained by analyzing stable mRNA transcribed from deletion mutants (2, 21) in that they establish the initiation of transcription as the primary target for the glucocorticoid stimulation. Several authors have reported binding in vitro of glucocorticoid hormone-receptor complexes to a DNA region including these sequences (9, 11, 26, 27, 32). Taken together, these findings are consistent with the model that the hormone-receptor complex binds to a specific DNA sequence which does not comprise an RNA start site and destabilizes the DNA in this region so that the initiation of transcription can take place more frequently by providing a polymerase entry site (35). As this sequence can be turned around (3) or moved away with respect to the promoter (3, 17, 21), it has the features of an enhancer element, analogous to those found in several different transcription units (24).

With the assay used in this study which measures the in vitro elongation of RNA chains initiated in vivo, we could address another important question, namely, whether transcription downstream from MMTV DNA in flanking cellular sequences does occur. In previous reports (36, 37) no stable transcripts corresponding to adjacent cellular DNA fragments were detected in the infected cell lines used. If such transcripts exist, they may not have been stable enough to be detected in those experiments. In our assay, where we used nascent RNA chains, we detected as much hybridization to downstream flanking mouse sequences as to internal MMTV DNA sequences. The origin of these transcripts has not been determined precisely. They may be initiated in the left LTR and run through the site of polyadenylation in the right LTR or they may be initiated in the right LTR. A third possibility is that they may start at a cellular promoter located very near the provirus and become hormonally regulated due to the presence of the adjacent MMTV right LTR. It has been shown (3, 17, 21) by using chimeric DNA molecules in transfection experiments that MMTV sequences responsible for glucocorticoid stimulation can confer hormonal control to a heterologous, nonregulated promoter. S1 nuclease mapping of nuclear RNA and primer extension experiments will be required to distinguish between the above possibilities.

ACKNOWLEDGMENTS

We thank E. Buetti and N. Fasel for gifts of material, O. Hagenbüchle for advice, and P. Beard and R. Eisenman for critical reading of the manuscript.

This work was supported by the Swiss National Science Foundation and a grant from the Swiss Cancer League. J.M.F. was a recipient of a fellowship of the Deutsche Forschungsgemeinschaft.

LITERATURE CITED

1. **Buetti, E., and H. Diggelmann.** 1981. Cloned mouse mammary tumor virus DNA is biologically active in transfected mouse cells and its expression is stimulated by glucocorticoid hormones. Cell **23**:335–345.
2. **Buetti, E., and H. Diggelmann.** 1983. Glucocorticoid regulation of mouse mammary tumor virus: identification of a short essential DNA region. EMBO J. **2**:1423–1429.
3. **Chandler, V. L., B. A. Maler, and K. R. Yamamoto.** 1983. DNA sequences bound specifically by glucocorticoid receptor in vitro render a heterologous promoter hormone responsive in vivo. Cell **33**:489–499.
4. **Cox, R. F.** 1973. Transcription of high-molecular weight RNA from hen-oviduct chromatin by bacterial and endogenous form-B RNA polymerases. Eur. J. Biochem. **39**:49–61.
5. **Cox, R. F.** 1976. Quantitation of elongating form A and B RNA polymerases in chick oviduct nuclei and effects of estradiol. Cell **7**:455–465.
6. **Diggelmann, H., A. L. Vessaz, and E. Buetti.** 1981. Cloned endogenous mouse mammary tumor virus DNA is biologically active in transfected mouse cells and its expression is stimulated by glucocorticoid hormones. Virology **122**:332–341.
7. **Fasel, N., K. Pearson, E. Buetti, and H. Diggelmann.** 1982. The region of mouse mammary tumor virus DNA containing the long terminal repeat includes a long coding sequence and signals for hormonally regulated transcription. EMBO J. **1**:3–7.
8. **Gariglio, P., M. Bellard, and P. Chambon.** 1981. Clustering of RNA polymerase B molecules in the 5′ moiety of the adult beta-globin gene of hen erythrocytes. Nucleic Acids Res. **9**:2589–2598.
9. **Geisse, S., C. Scheidereit, H. M. Westphal, N. E. Hynes, B. Groner, and M. Beato.** 1982. Glucocorticoid receptors recognize DNA sequences in and around murine mammary tumor virus DNA. EMBO J. **1**:1613–1619.
10. **Giles, K. W., and A. Myers.** 1965. An improved diphenylamine (DPA) method for the estimation of DNA. Nature (London) **206**:93.
11. **Govindan, M. V., E. Spiess, and J. Majors.** 1982. Purified glucocorticoid receptor hormone complex from rat liver cytosol binds specifically to cloned mouse mammary tumor virus long terminal repeats *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **79**:5157–5161.
12. **Groner, B., N. E. Hynes, U. Rahmsdorf, and H. Ponta.** 1983. Transcription initiation of transfected mouse mammary tumor virus LTR DNA is regulated by glucocorticoid hormones.

Nucleic Acids Res. **11**:4713–4725.
13. **Groner, B., N. Kennedy, U. Rahmsdorf, P. Herrlich, A. van Ooyen, and N. E. Hynes.** 1982. Introduction of a proviral mouse mammary tumor virus gene and a chimaeric MMTV-thymidine kinase gene into L cells results in their glucocorticoid responsive expression, p. 217–228. *In* J. E. Dumont, J. Nunez, and G. Schultz (ed.), Hormones and cell regulation, vol. 6. Elsevier/North-Holland Biomedical Press, Amsterdam.
14. **Groudine, M., M. Peretz, and H. Weintraub.** 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. **1**:281–288.
15. **Hewish, D. R., and L. A. Burgoyne.** 1973. Chromatin substructure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. Biochem. Biophys. Res. Commun. **52**:504–510.
16. **Huang, A. L., M. C. Ostrowski, D. Berard, and G. L. Hager.** 1981. Glucocorticoid regulation of the Ha-MuSV p21 gene conferred by sequences from mouse mammary tumor virus. Cell **27**:245–255.
17. **Hynes, N., A. J. J. van Ooyen, N. Kennedy, P. Herrlich, H. Ponta, and B. Groner.** 1983. Subfragments of the large terminal repeat cause glucocoticoid-responsive expression of mouse mammary tumor virus and of an adjacent gene. Proc. Natl. Acad. Sci. U.S.A. **80**:3637–3641.
18. **Lasfargues, E. Y., A. B. Vaidya, J. C. Lasfargues, and D. H. Moore.** 1976. In vitro susceptability of mink lung cells to mouse mammary tumor virus. J. Natl. Cancer Inst. **57**:447–449.
19. **Lee, F., R. Mulligan, P. Berg, and G. Rinegold.** 1981. Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumour virus chimaeric plasmids. Nature (London) **294**:228–232.
20. **Littlefield, J. W.** 1964. Selection of hybrids from mating of fibroblasts *in vitro* and their presumed recombinants. Science **145**:709–710.
21. **Majors, J., and H. E. Varmus.** 1983. A small region of the mouse mammary tumor virus long terminal repeat confers glucocorticoid hormone regulation on a linked heterologous gene. Proc. Natl. Acad. Sci. U.S.A. **80**:5866–5870.
22. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. U.S.A. **74**:4835–4838.
23. **Nandi, S., and C. M. McGrath.** 1973. Mammary neoplasia in mice. Adv. Cancer Res. **17**:353–414.
24. **Parker, M.** 1983. Enhancer elements activated by steroid hormones? Nature (London) **304**:687–688.
25. **Parks, W. P., E. M. Scolnick, and E. H. Kozikowski.** 1974. Dexamethasone stimulation of murine mammary tumor virus expression: a tissue culture source of virus. Science **184**:158–160.
26. **Payvar, F., O. Wrange, J. Carlstedt-Duke, S. Okret, J.-A. Gustafsson, and K. R. Yamamoto.** 1981. Purified glucocorticoid receptors bind selectively *in vitro* to a cloned DNA fragment whose transcription is regulated by glucocorticoids *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **78**:6628–6632.
27. **Pfahl, M.** 1982. Specific binding of the glucocorticoid-receptor complex to the mouse mammary tumor proviral promoter region. Cell **31**:475–482.
28. **Ringold, G. M., R. D. Cardiff, H. E. Varmus, and K. R. Yamamoto.** 1977. Infection of rat hepatoma cells with mouse mammary tumor virus. Cell **10**:11–18.
29. **Ringold, G. M., K. R. Yamamoto, J. M. Bishop, and H. E. Varmus.** 1977. Glucocorticoid-stimulated accumulation of mouse mammary tumor virus RNA: increased rate of synthesis of viral RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:2879–2883.
30. **Ringold, G. M., K. R. Yamamoto, G. M. Tomkins, J. M. Bishop, and H. E. Varmus.** 1975. Dexamethasone-mediated induction of mouse mammary tumor virus RNA: a system for studying glucocorticoid action. Cell **6**:299–305.
31. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in a single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
32. **Scheidereit, C., S. Geisse, H. M. Westphal, and M. Beato.** 1983. The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumor virus. Nature (London) **304**:749–752.
33. **Schibler, U., O. Hagenbüchle, P. K. Wellauer, and A. C. Pittet.** 1983. Two promoters of different strength control the transcription of the mouse alpha-amylase gene *Amy*-1[a] in the parotid gland and the liver. Cell **33**:501–508.
34. **Scolnick, E. M., H. A. Young, and W. P. Parks.** 1976. Biochemical and physiological mechanisms in glycocorticoid hormone induction of mouse mammary tumor virus. Virology **69**:148–156.
35. **Sluyser, M.** 1983. Interaction of steroid hormone receptors with DNA. Trends Biochem. Sci. **8**:236–238.
36. **Ucker, D. S., G. L. Firestone, and K. R. Yamamoto.** 1983. Glucocorticoids and chromosomal position modulate murine mammary tumor virus transcription by affecting efficiency of promoter utilization. Mol. Cell. Biol. **3**:551–561.
37. **Ucker, D. S., S. R. Ross, and K. R. Yamamoto.** 1981. Mammary tumor virus DNA contains sequences required for its hormone-regulated transcription. Cell **27**:257–266.
38. **Vaidya, A. B., E. Y. Lasfargues, G. Heubel, J. C. Lasfargues, and D. H. Moore.** 1976. Murine mammary tumor virus: characterization of infection of nonmurine cells. J. Virol. **18**:911–917.
39. **Wilkie, N. M., J. B. Clements, W. Boll, N. Mantei, D. Lonsdale, and C. Weissmann.** 1979. Hybrid plasmids containing an active thymidine kinase gene of Herpes simplex virus 1. Nucleic Acids Res. **7**:859–877.
40. **Young, H. A., T. Y. Shih, E. M. Scolnick, and W. P. Parks.** 1977. Steroid induction of mouse mammary tumor virus: effect upon synthesis and degradation of viral RNA. J. Virol. **21**:139–146.