Communication

The Human Growth Hormone Gene Contains Both Positive and Negative Control Elements*

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A subset of DNA sequences in the 5'-flanking DNA of the human growth hormone (hGH) gene was examined by protein-DNA binding and gene transfer-expression experiments. Two adjacent cis-acting elements (I and II) were identified between nucleotides -308/-235 of the hGH gene that modulated the expression of a linked reporter gene in transfected HeLa cells. Elements I and II repressed gene expression whereas element II alone activated it. HeLa whole cell extracts contain two factors that bind hGH DNA carrying elements I and II. Factor I binds to single-stranded DNA, and its binding is correlated with repression of gene expression. Factor II binds between nucleotides -275/-257 of the hGH gene. This region is homologous to the binding site for the adenovirus major late transcription factor, and factor II binding to hGH DNA is competed by adenovirus major late promoter DNA, indicating that the hGH and major late adenovirus promoters share a transcription regulatory element.

Transcription is regulated by positive and negative control mechanisms including enhancer elements that activate expression (1) and silencers that inhibit transcription and share the orientation- and position-independence properties of enhancers (2). Several genes in yeast and higher eukaryotes are regulated by positive and negative control elements (3-6).

Our laboratory has been studying transcriptional control elements that regulate hormonal (7, 8) and tissue-specific (9) expression of the hGH1-related genes. In this communication, we describe two proteins, present in HeLa and rat anterior pituitary (GC) cell extracts, that bind to the hGH DNA at -5'flanking DNA. These protein-binding sequences and the proteins that bind to them regulate expression of a linked reporter gene in transfected HeLa cells. One element activates transcription and the other represses it.

MATERIALS AND METHODS

Plasmid Construction and DNA Transfer Experiments—Fragment hGH(-278/-250) with terminal BamHI linkers was synthesized by California Biotechnology Inc. Fragment hGH(-308/-235) was obtained from a Bal31 3' deletion fragment (nucleotide -235 converted to a BamHI site) of the hGH 5'-flanking DNA by digestion with Thal (nucleotide -308) and BamHI (nucleotide -235). The fragment was subcloned between the Smal and BamHI sites of pUC18 to yield plasmid pUCH GH(-308/-235). The EcoRI site in the pUC polylinker was converted to a BglII site, and hGH DNA was excised by BamHI/BglII digestion.

Elements I and I1 repressed gene expression whereas element II alone activated it. HeLa whole cell extracts contain two factors that bind hGH DNA carrying elements I and II. Factor I binds to single-stranded DNA, and its binding is correlated with repression of gene expression. Factor II binds between nucleotides -275/-257 of the hGH gene. This region is homologous to the binding site for the adenovirus major late transcription factor, and factor II binding to hGH DNA is competed by adenovirus major late promoter DNA, indicating that the hGH and major late adenovirus promoters share a transcription regulatory element.

The abbreviations used are: hGH, human growth hormone; MLTF, adenovirus major late transcription factor; ssDNA, single-stranded DNA; Hapes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

RESULTS

HeLa cell proteins in step-eluted phosphocellulose column fractions were tested for binding to a 32P-labeled hGH(-308/-235) DNA fragment using the gel electrophoresis binding assay (11). Two proteins bind the hGH(-308/-235) fragment; these proteins designated factors I and II are present in the 0.1 and 0.3 M KCl eluates, respectively (Fig. 1A). We determined that factor I binds optimally to ssDNA, since it bound to boilded hGH(-308/-235) DNA but not native DNA (Fig. 1B, lanes a-c) and binds to strand-separated DNA (Fig. 1B, lanes d and e). Factor I also binds both strands of the hGH(-308/-235) ssDNA (Fig. 1B, lanes a and c). As discussed below, factor II binds native DNA.

Both factors I and II bind specifically to hGH DNA. DNA binding experiments with synthetic oligonucleotides indicate that there are two factor I binding sites located on the upper strand between nucleotides -308/-281 and the lower strand between nucleotides -279/-250 of the hGH gene, and factor
I binding to these sites is not competed by unrelated ssDNA. In addition, factor II binding to 32P-labeled hGH(-308/-235) native DNA was competed by a 500-fold molar excess of unlabeled hGH(-308/-235) DNA but not a 500-fold excess of a 75-base pair HinfI fragment of pBR322 (data not shown).

To map the factor I and II binding sites directly we employed DNase I footprinting assays. The 32P-labeled hGH(-308/-235) ssDNA and native DNA fragments were mixed with column fractions containing factors I and II, respectively, as described (11). Factor I binding to hGH(-308/-235) ssDNA yielded a prominent and reproducible hypersensitive site at nucleotide -280; however, conditions were not found which yielded a DNase I footprint (Fig. 2A). The inability to detect a footprint may be related to our finding of multiple factor I binding sites as discussed above. Factor II binding to hGH(-308/-235) native DNA produced a clear footprint at nucleotides -275/-257 (Fig. 2B). Factor II only binds native DNA and protects both strands of DNA between nucleotides -275/-257 (data not shown). This sequence contains a 10-base pair sequence (5'-GGTCACGTGG-3') present in the binding site for adenovirus major late transcription factor (MLTF), a protein found in uninfected HeLa cell extracts (11) that activates adenovirus transcription both in vivo and in vitro (11, 12).

To determine if factor II and MLTF may be identical, fragments of DNA derived from the adenovirus major late promoter and other viral and cellular promoters were used in competition experiments for factor II binding to hGH(-308/-235) DNA. The data in Fig. 3 demonstrate that the adenovirus DNA fragment containing the MLTF binding site competes with the hGH-derived probe for factor II binding, whereas the DNA fragments from the SV40, Rous sarcoma virus, and rat insulin promoters had little effect. Some competition was observed with a large amount of hepatitis B virus DNA, but the significance of this is unknown, since we could find no obvious sequence similarity to the factor II binding

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FIG. 1. DNA binding proteins in phosphocellulose-fractionated HeLa whole cell extracts. Unless specified otherwise, the hGH(-308/-235) DNA was 32P-end-labeled on the lower strand. A, binding to native DNA containing some denatured DNA. Lane a, crude extract (10 μg); lane b, column flowthrough (10 μg); lanes c–e, 0.1, 0.3, and 0.5 M KCl eluates (5 μg each). B, binding to native or denatured DNA. The probe was denatured by boiling for 5 min and cooling on ice or isolated from strand-separating gels. The 0.1 M KCl eluate was the source of protein (5 μg). Lane a, boiled probe; lane b, native probe; lane c, boiled probe labeled on upper strand; lane d, strand-separated probe; lane e, boiled strand-separated probe. I and II refer to nucleoprotein complexes formed by factors I and II, respectively. Bracket indicates free DNA; upper band is native DNA, lower band is denatured DNA.

FIG. 2. DNase I treatment of nucleoprotein complexes formed with hGH(-308/-235) DNA and factors I and II. A, comparison of DNase I-treated ssDNA–factor I complex (lane d) with similarly treated free DNA (lane c). The DNase I hypersensitive site at nucleotide -280 is indicated. B, comparison of DNase I-treated native DNA–factor II complex (lane d) with similarly treated free DNA (lane c). The boundaries (nucleotides -275/-257) of the footprint and the protected sequence are indicated. Lanes a and b (A and B) are hGH(-308/-235) DNA treated with Maxam and Gilbert G and G+A reactions, respectively.

FIG. 3. Competition of DNA-factor II complex formation with DNA from other promoters. As indicated, 5 or 20 ng of each competitor DNA was used in incubations with 32P-end-labeled hGH(-308/235) native DNA. Lane a, no competitor DNA control; lanes b and c, adenovirus (AdV) major late promoter, XhoI/HindIII fragment (nucleotides 5788/6241); lanes d and e, SV40, SphI fragments (nucleotides 200/270); lanes f and g, Rous sarcoma virus (RSV), NruI/HindIII fragment (nucleotides 6020/6185); lanes h and i, hepatitis B virus (HBV), SphI/Stul fragments (nucleotides 1235/1115), containing hepatitis B virus enhancer; lanes j and k, rat insulin I (INS) I gene, subcloned fragment (nucleotides -247/-104).
site on this fragment. The ability of adenovirus DNA to compete with hGH DNA for factor II binding suggests that these genes bind the same protein.

To determine whether the DNA containing elements I and II has transcription regulatory activity, two fragments, hGH(-279/-250) (containing element II) and hGH(-308/-235) (containing elements I and II), were independently fused upstream from the -100hGHp.cat gene (9). The hybrid genes were introduced into HeLa cells, and chloramphenicol acetyltransferase activity was measured after 48 h (9) (Fig. 4). The -279/-250hGH.-100hGHp.cat gene expressed 3-fold more activity than the -100hGHp.cat gene. The -308/-235hGH.-100hGHp.cat gene expressed only 50% of the -100hGHp.cat gene activity and 6-fold less activity than the -279/-250hGH.-100hGHp.cat gene. These results indicate that positive and negative control elements are located between nucleotides -308/-235 of the hGH gene and suggest that factor II activates gene expression and factor I represses it. Since the hGH(-308/-235) DNA fragment contains binding sites for both factors I and II, repression by factor I must be dominant to activation by factor II.

**DISCUSSION**

We have shown that the hGH 5′-flanking DNA contains two cis-acting elements (I and II) that mediate negative and positive control, respectively, of hGH promoter activity and have identified two trans-acting factors (I and II) that may mediate this activity. Based on chromatographic (Fig. 1A), DNA-binding (Figs. 1–3), and functional properties (Fig. 4), we conclude that factor II is related to MLTF (11, 12) or upstream stimulatory factor (13, 14) that activates adenovirus major late promoter transcription and that the adenovirus and hGH genes share a common transcription element. Interestingly, a yeast centromere DNA-binding protein that also binds to many yeast promoters binds to a sequence that is homologous to the MLTF binding site (15), suggesting that MLTF-related DNA binding motifs may represent conserved protein binding sites (15). Our studies indicating that MLTF-related proteins can affect hGH gene expression and recent evidence that MLTF activates the rat fibrinogen promoter (16) support this concept. Evidence has been presented that the interaction of upstream stimulatory factor with transcription factor IID (adenovirus major late promoter TATAAA element binding factor) is involved in promoter activation (13, 14), and similar mechanisms have been proposed for yeast gene activation by the MLTF-related protein (15). Thus, factor II interactions with transcription factor IID-related proteins may be important for hGH gene expression as well.

The physiological significance of factors I and II on hGH gene expression is unknown. Our data indicate that the repressor activity that is correlated with the binding of factor I to ssDNA (Fig. 1B) is dominant over activation by factor II (Fig. 4). It is possible that factor II activity may be exhibited when factor I levels are limiting, suggesting a potential switch mechanism. Both factors I and II are found in HeLa cells which lack hGH gene expression and rat somatomammotropin gene expression (GC) cells (data not shown) that express the rat GH gene and serve as an excellent model for studying hormonal (7, 8) and tissue-specific (9, 17) regulation of the hGH gene. Our earlier work demonstrates that deletion of elements I and II does not affect hGH gene expression in transfected GC cells (9). This suggests that elements downstream of elements I and II such as the tissue-specific control elements (9, 17) may act to overcome the effects of these elements. Accordingly, it is possible that the activity associated with factor I may be involved in repressing hGH gene expression in nonpituitary cells.

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**FIG. 4.** Effect of subcloned regulatory elements I and II on gene expression in transfected HeLa cells. Average values for chloramphenicol acetyltransferase (CAT) activity and standard deviations were obtained from three independent experiments.