similar to several binding elements of transcription factor YY1. Binding activity to NRE's was detected in both HeLa and GH_3 nuclear extracts. Using HeLa extracts, one major bound complex was detected when NRE1 was used as a probe. Complex with similar mobility and also several other bands were detected when NRE2 or 3 was used as a probe. In GH_3 nuclear extract, there was strong binding activity to NRE1 whose electrophoretic mobility was similar to the binding activity in HeLa cells. The binding pattern was quite different between HeLa and GH_3 extracts when NRE3 was used as a probe. From the competition analysis using HeLa nuclear extract, it was shown that the factor bound to NRE1 was specific and that it had affinity, although very weak, to NRE2 and NRE3. When GH_3 nuclear extract was used, the pattern of competition was similar to that of HeLa nuclear extract. The recombinant human YY1, which was expressed in *E.coli*, bound to NRE1 very strongly and to NRE2 and NRE3 weakly. The relative strength of binding to NRE1, 2, and 3 was 133, 1, and 7, respectively. Judged from these results, it is very likely that the factor bound to NRE1 in HeLa and GH_3 nuclear extracts is YY1, or a close homologue. Increasing amount of YY1 in HeLa cells caused decrease in the bGH promoter activity, suggesting that YY1 indeed might be involved in negative regulation of bGH gene via binding to NRE's, especially NRE1.

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Characterization of the Regulatory Elements and Their Binding Factor(s) in Bovine Growth Hormone Gene

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The upstream region of bovine growth hormone gene was analyzed. When the region between nucleotides -335 and -238 was deleted from bGH promoter linked to CAT gene in the replicating plasmid containing SV40 replication origin, gene expression from bGH increased by about five fold in HeLa cells. This region was named as negative regulatory site (NRS). When various viral enhancers replaced replication origin of SV40 to increase the basal activity of CAT, the CAT activity also increased when NRS was deleted from bGH promoter regardless of the type of viral enhancer used. The insertion of NRS decreased CAT expression from various combinations of SV40 promoter and viral enhancers. Deletion of NRS increased CAT expression from bGH promoter in rat pituitary GH_3 cells as well. However, the level of increase was lower than in HeLa cells. When NRS was inserted in front of heterologous rGH and TK promoters, the CAT expression from each promoter was reduced. The extent of reduction was larger in HeLa than in GH_3 cells. These results suggest that the negative regulatory effect be more pronounced in nonexpressing cells. By fine deletion analysis of bGH promoter region within NRS, three regions were identified to act negatively, and they were named NRE1, 2, and 3, respectively. From sequence comparison, three NRE's exhibited some sequence homology to one another and the sequence was