Legends for supplemental figures

Supplementary figure 1. Effect of exogenous TR on the action of T3 toward the TRH gene.

GH4C1 cells were transiently transfected with the TRH-Luc reporter gene and empty vector or wild-type TR, and luciferase assays were performed. Significant repression of the TRH promoter activity was induced by incubation with 100 nM T3. Addition of exogenous TR (indicated by +) did not affect the repression, suggesting that endogenous TR is sufficient for the full expression of the TRH gene in GH4C1 cells. The data are presented as the mean ± SE for three independent experiments.

Supplementary figure 2. The promoter activity of the thymidine kinase (TK) was not affected by exogenous TR and T3.

GH4C1 cells were transiently transfected with the TK-Luc reporter gene and wild-type TR or empty vector, and luciferase assays were performed. No significant effect on the promoter activity by exogenous TR or T3 was observed in GH4C1 cells. The data are presented as the mean ± SE for three independent experiments.

Supplementary figure 3. Effect of T3 on the amount of Histone H3 on the TRH promoter.

GH4C1 cells stably expressing the TRH promoter reporter gene and the wild-type TR were treated with 100 nM T3 for the period indicated. ChIP assays were performed using antibodies against histone 3 (H3). T3 caused no significant changes in the amount of H3 on the TRH promoter. Each level was compared to the basal level (0 min). The data are presented as the mean ± SE for three independent experiments.

Supplementary figure 4. Histone modifications in the coding region of the luciferase gene.

Histone modifications on the coding region of the luciferase reporter gene were assessed by ChIP assays in cells expressing the TRH promoter and the wild-type TR. No acetylation of H3K9 and H3K14, or methylation of H3K4, H3K9 and H3K27, was observed (indicated as coding). The promoter region of the TRH (indicated as promoter) was significantly acetylated at H3K9 and K14 and methylated at H3K4. Values were expressed as relative occupancy against the background with IgG. The data are presented as the mean ± SE for three independent experiments

Supplementary figure 5. The amount of TR was not affected by incubation with T3 in GH4C1 cells.

GH4C1 cells stably expressing the TRH promoter reporter gene and the wild-type TR were treated with 100 nM T3 for the period indicated. Western blot analysis indicated no significant change in the amount of TR during 24 h of incubation with T3.

Supplementary figure 6. Effect of T3 on the stability of luciferase mRNA.

GH4C1 cells stably expressing the TRH promoter reporter gene and the wild-type TR were treated with 100 nM T3 for 15 mins and with 1μg/ml of actinomycin D. After 15 mins, total RNA was isolated. The amount of Luc mRNA was measured by reverse transcription of the total RNA and real-time PCR. There was no significant change in the rate of degradation of Luc mRNA in 15 mins between T3-treated cells and vehicles. Values are presented as the ratio of the amount of Luc mRNA at time 0 of actinomycin D treatment. Values are presented as the means ± S.E. for three experiments.