Question 1
a. The data in Table show that insertions in four different regions of the receptor coding sequence cause decreased induction. This finding suggests that the glucocorticoid receptor has four separate functional domains. These four domains correspond to the following insertions: domain 1 = insertion D, E, and F; domain 2 = insertion I; domain 3 = insertion K, L, M, and N; and domain 4 = insertion Q, R, and S.

b. Only insertions Q, R, and S produce receptor proteins with decreased steroid-binding ability. Therefore, the region of the protein corresponding to these insertions is the steroid-binding domain.

c. 1) The data indicate that insertions in three receptor domains block induction without affecting steroid binding, but provide no evidence on which of the three domains is DNA binding region. (One could do is to compare the sequence of three domains with that of known DNA-binding domains in other steroid hormone receptors.) 2) same is true for transcription activation domain.

Question 2
A. Does the lymphocyte cell line contain the splicing and polyadenylation factors necessary to produce both calcitonin and CGRP mRNA? Why?

Because calcitonin mRNA is produced when the cells are transfected with the wild-type gene, and CGRP mRNA is produced when they are transfected with the exon-4 splice-site mutant, the lymphocytes must contain all the processing factors necessary to generate both mRNAs.

B. If differential processing results from polyadenylation-site selection, which mutant would you expect to produce CGRP mRNA when transfected into the lymphocyte cell line? Why?

If selection of a polyadenylation site was the critical choice in the expression of calcitonin mRNA in the lymphocyte cell line, then the mutant that was missing the exon-4 polyadenylation site would be expected to produce CGRP mRNA. If the splicing of exon 3 to exon 5 (to produce CGRP mRNA) is precluded by use of the polyadenylation site in exon 4, then removal of the site should permit CGRP mRNA production.

By contrast, the mutant lacking the exon-4 splice site might still be expected to be preferentially polyadenylated at exon 4, which would prevent production of CGRP mRNA. (As explained in part D, although CGRP mRNA is not made in the exon-4 splice-site mutant, the aberrant RNA that is generated does not match this simple expectation).

C. If differential processing results from splice-site selection, which mutant would you expect to produce CGRP mRNA when transfected into the lymphocyte cell line? Why?

If selection of the exon-4 splice site was the critical choice in the expression of calcitonin mRNA in the lymphocyte cell line, then the mutant that was missing the exon 4 splice site would be expected to produce CGRP mRNA. If the splicing of exon 3 to exon 4 is favored in lymphocytes,
then removal of the exon-4 splice site should permit the exon-5 splice site to be used, thus generating CGRPmRNA.

By contrast the mutant lacking the exon-4 polyadenylation site might still be expected to splice exon 3 to exon 4 preferentially, which might be expected to lead to an aberrant RNA containing the fourth intron along with exons 5 and 6. (As explained in part D, although an aberrant RNA is made, it is not the one expected by this simple reasoning.)

D. Which model for differential processing best explains the ability of the lymphocyte cell line to produce calcitonin mRNA but not CGRP mRNA. Why?

The predictions of the splice-site selection model best match the results from the two mutants. As explained in parts B and C, the splice-site-selection model correctly predicts that CGRP mRNA will be made by the mutant lacking the exon-4 splice site. The polyadenylation-site-selection model, by contrast, predicts incorrectly that CGRP mRNA will be make by the mutant that is missing the exon-4 polyadenylation site. Thus, these results favor splice-site selection as the critical choice that explains the ability of the lymphocyte cell line to produce calcitonin mRNA instead of CGRP mRNA.