*Arabidopsis thaliana* can normally grow quickly and complete its life cycle in less than two months. Plants homozygous for the *año* mutation, however, grow very slowly and take a whole year to mature. In order to learn more about the *AÑO* gene and its function, you have made a series of deletion and substitution mutations in the *AÑO* gene and have tested these altered versions for their ability to be transcribed. The constructs made are shown below. Each x indicates that a single nucleotide has been altered to another one at that location.

After placing each of these gene constructs into transgenic plants and measuring the rate of transcription (shown above) it is time to interpret what the results mean.

1. **What general type of regulatory element is likely to be present near –90? Explain.**

   **It must be a critically important promoter element.** A 1 nt change drastically reduced transcription and deletion of this element completely eliminates transcription.

2. **What type of regulatory element is likely to be present between –200 and –300? Explain.**

   **It must be an enhancer element.** Even if this element is very badly mutated or completely deleted, the gene can still be transcribed reasonably well.

3. **Why do you think that construct #1 had such a low level of transcription?**

   **The TATA box (which is essential for transcription) has been ruined in this construct.**

4. **You have observed that transgenic plants containing construct #4 have 6 times as much *AÑO* mRNA as do transgenic plants containing construct #5. What is the most reasonable explanation for this result?**

   **The mutations introduced into construct #5 have resulted in decreased mRNA stability.** (Perhaps an RNA stability determinant has been ruined by the mutations.)
The famously eccentric biologist Pool Greenwater has dedicated his life to the study of gene expression in mammalian cells, using mice as a convenient model system. In his favorite mouse strain, sqk49, Pool has found that the growth factor GRW causes proliferation of liver cells. Pool has recently developed some mutant versions of his mice with the following genetic changes:

- **sqk49-IRP** mice have a mutation in the gene encoding the iron response protein that completely blocks transcription of this gene.
- **sqk49-TfRIRE** mice have a deletion in the gene encoding the transferrin receptor that removes the entire region that forms IREs in the mRNA.
- **sqk49-GRW-AT** mice have a deletion that removes a ATTTAATTTAATTTAATTTA sequence from near the 3’ end of the GRW gene.

1. What effects (if any) would you expect the mutation in the **sqk49-IRP** mice to have on the production of transferrin receptor protein? Explain your answer, being as specific as possible.

   The IRP protein will be absent in cells of these mice. As a result, the IREs on the TfR mRNA will never be bound by IRP. Thus, the TfR mRNA will always be unstable (even at low iron concentration) because the cleavage recognition will never be blocked by IRP. The mouse’s cells will produce low levels of TfR protein, even when cellular [Fe] is low.

2. What effects (if any) would you expect the mutation in the **TfRIRE** mice to have on the production of transferrin receptor protein? Explain your answer, being as specific as possible.

   The TfR mRNA will lack the cleavage recognition site, so it will be always stable, even at high [Fe], when IRP protein is not available. The mouse’s cells will produce high levels of TfR protein, even when cellular [Fe] is high.

3. Pool has noticed that he has trouble keeping his **sqk49-GRW-AT** mice, as most of them die from liver cancer. Can you provide him a logical explanation for this observation? Please be as specific as possible.

   It would appear that a sequence encoding an AU-rich element has been deleted from the gene. The GRW mRNA is normally made unstable by this element. The mutant version of GRW mRNA is too stable, and results in the production of too much GRW protein. The causes excessive liver cell proliferation (cancer).