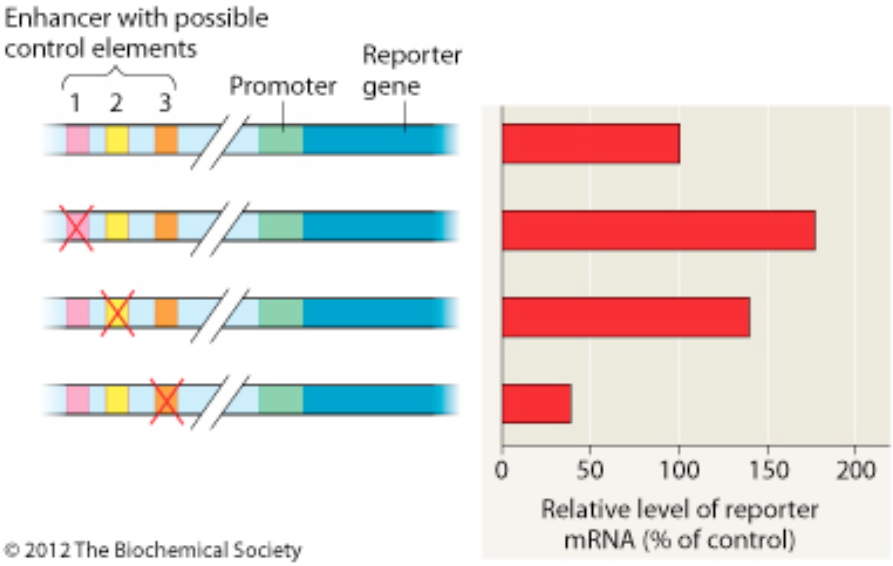
Analyzing DNA Deletion Experiments

The promoter of a gene includes the DNA immediately upstream of the transcription start site but the control elements that regulate the level of transcription of the gene (grouped in an enhancer) might be thousands of nucleotides upstream of the promoter. Because the distance and spacing of control elements make them difficult to identify, scientists began deleting possible control elements and measuring the effect on gene expression.

In this experiment, the researchers were looking at possible control elements for the human gene mPGES-1 which codes for an enzyme that synthesizes a type of prostaglandin, a hormone-like molecule made during tissue inflammation.

The researchers hypothesized that there were three possible control elements in an enhancer region located about 8 kilobases upstream of the mPGES-1 gene. Control elements regulate whatever gene is in the appropriate downstream location. Thus, to test the activity of the possible elements, researchers first synthesized molecules of DNA ("constructs") that had the intact enhancer region upstream of a "reporter gene," a gene whose mRNA could be easily measured experimentally. Next, they made three more DNA constructs, with one of the three proposed control elements deleted in each (see the left side of Figure 1.) The researchers then introduced each DNA construct into a separate human cell culture, where the cells took up the DNA constructs. After 48 hours, the amount of reporter gene mRNA made by the cells was measured. Comparing these amounts allowed researchers to determine if any of the deletions had an effect on expression of the reporter gene, mimicking the effect of deletions on mPGES-1 gene expression.



Data from J. N. Walters et al., Regulation of human microsomal prostaglandin E synthase-1 by IL-1b requires a distal enhancer element with a unique role for C/EBPb, *Biochemical Journal* (2012). doi:10.1042/BJ20111801

Figure 1 DNA Constructs and Reporter gene mRNA

Figure 1 show the intact DNA sequence (top) and the three experimental DNA sequences. A red X indicates the possible control element (1, 2, or 3) that was deleted in each experimental DNA construct. The area between the slashes represents the approximately 8 kilobases of DNA located between the promoter and the enhancer region. The horizontal bar graph shows the amount of reporter gene mRNA that was present in each cell culture after 48 hours relative to the amount that was in the culture containing the intact enhancer region (top bar = 100%).

1. a) What was the independent variable in this experiment?

b) What was the dependent variable in this experiment?

c) What was the control treatment in this experiment?

2. Do the data suggest that any of these possible control elements are actual control elements?

3. a) Did deletion of any of the possible control elements cause a reduction in reporter gene expression? How can you tell?

b) If deletion of a control element causes a reduction in gene expression, what must be the normal role of that control element?

c) Provide an explanation for how the loss of such a control element could lead to a reduction in gene expression.

4. a) Did deletion of any of the possible control elements cause an increase in reporter gene expression? How can you tell?

b) If deletion of a control element causes an increase in gene expression, what must be the normal role of that control element?

c) Provide an explanation for how the loss of such a control element could lead to an increase in gene expression.