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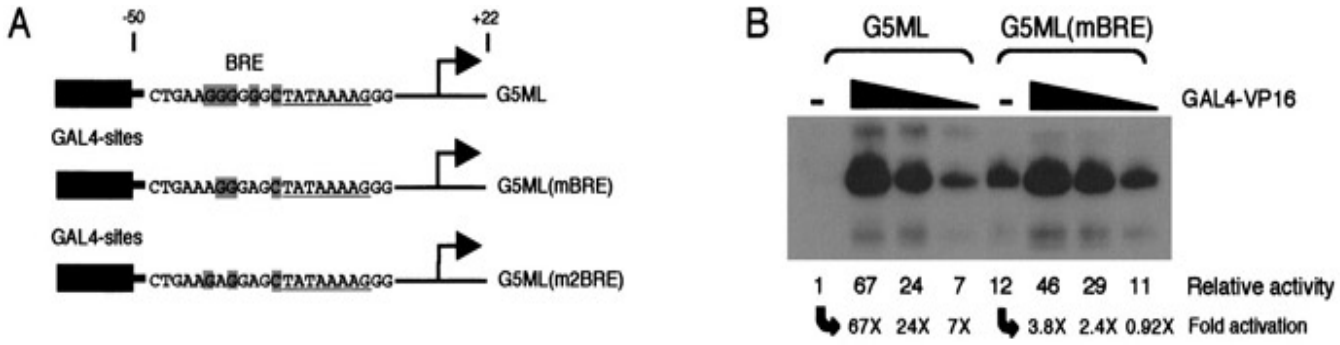
Biology 463: Gene Regulation in Development.

Part I. A recent paper by Evans et al. **Genes & Development** 15:2945-2949 (2001) reveals a different role for the general transcription factor, TFIIB. At some promoters, including the major late promoter for adenovirus, TFIIB will bind to a specific site called a BRE (TFIIB response element) located just 5' to the TATA box. The authors examined the effect of mutating the BRE in a hybrid promoter (called G5ML), in which GAL4 binding sites (or UAS; upstream activating sequence) are present upstream of the adenovirus late promoter. (See panel A in the figure below; the start site for transcription is marked by the arrow.) Constructs with a mutated BRE are called G5ML(mBRE) and G5ML (m2BRE). The amount of in vitro transcription from the G5ML and G5ML(mBRE) promoters was measured using a crude HeLa cell nuclear extract, which contains RNA polymerase and all the general transcription factors (but no GAL4). These results are shown in panel B in the lanes labeled -. The effect of adding increasing amounts of the artificial transcriptional activator GAL4-VP16, which binds to the GAL4 sites, was also examined, as shown in the lanes under the black triangle (the higher the triangle, the more GAL4-VP16). The results of the assay of transcripts by S1 nuclease protection are shown in the gel in panel B below. The promoter activity relative to that of the basal adenovirus major late promoter is reported as "relative activity" and the increase in the activity in the presence of GAL4-VP16 is reported as "fold activation". Similar results were obtained for G5ML(m2BRE) as for G5ML(mBRE).



1. In one sentence, what is the effect of TFIIB binding to the BRE with respect to activity in the absence of the GAL4-VP16 activator?

2. In one sentence, what is the effect of TFIIB binding to the BRE with respect to activity in the presence of the GAL4-VP16 activator?

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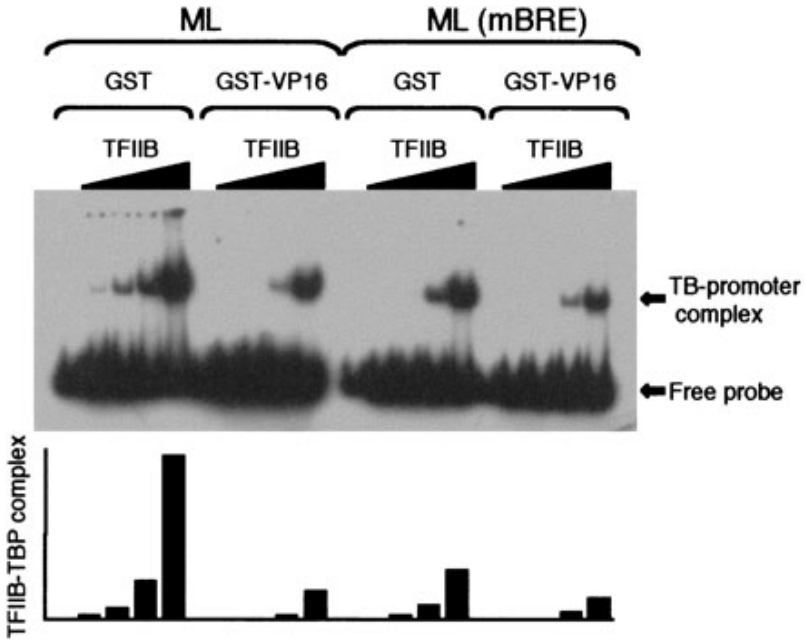
Part II. The ability of the VP16 activation domain to affect the TFIIB-BRE interaction was examined by EMSA assays of proteins bound to a radiolabelled major late promoter fragment containing a TATA box and a BRE. These are basically the DNA fragments shown in the figure for Part I but with no GAL4-sites; both wild-type ML and the major late promoter with mutated BRE, called ML(mBRE) were examined. The labeled DNA was incubated with TBP, with and without increasing amounts of TFIIB (shown as triangles of increasing thickness). VP16 was added as a hybrid between a convenient affinity tag (GST) and the VP16, called GST-VP16. GST alone was added for the lanes without VP16. The band representing a complex of the promoter, TBP, and TFIIB is labeled TB-promoter complex. The amount of this complex is plotted underneath the gel image.



1. In one sentence, why was GST added alone?

2. Which of the following can be concluded from these data (there may be more than one answer)?
a. On the major late promoter (ML), VP16 reduces the amount of the TFIIB-TBP-promoter complex.
b. On the major late promoter (ML), VP16 increases the amount of the TFIIB-TBP-promoter complex.
c. Mutation of the BRE in the major late promoter (ML(mBRE)) reduces the amount of TFIIB-TBP-promoter complex formed.
d. VP16 causes a small reduction in the amount of the TFIIB-TBP-promoter complex formed on the major late promoter mutated in the BRE (ML(mBRE)).

Which conclusion can be drawn from the data as a whole (there may be more than one answer)?
a. TFIIB bound to the BRE increases transcription, and synergistically activates transcription in the presence of VP16.
b. TFIIB bound to the BRE represses basal transcription and blocks activation by VP16.
c. TFIIB activates transcription by binding to the BRE.
d. TFIIB competes with TBP for binding to the TATA box.
e. TFIIB bound to the BRE represses basal transcription, and disruption of the TFIIB-BRE interaction by VP16 results in a strong activation of transcription.