

to *tk* coding sequences at a convenient *Bgl*III site located within the untranslated leader region of both genes. As demonstrated previously³, this construct was highly inducible whereas a 5' deletion mutant extending to sequence position -42 (5' Δ -42) gave a low, uninducible level of TK activity (Fig. 3). The insertion of three copies of MRE-a' into the *Bam*HI site defining the end point of the deletion reconstituted a five-fold response to heavy metal. The addition of one MRE-d sequence immediately upstream of these three elements approximately doubled the expression of *tk* activity in both the presence and absence of Zn. Although MRE-d may have some capacity to respond to heavy metals, this relatively large increase in the induced level of expression could also result from the demonstrated ability of MRE-a' to interact with basal promoter elements³. The presence of an additional MRE-a' upstream of MRE-d in the latter construction caused only a slight increase in inducibility. However, the subsequent insertion of another copy of MRE-d increased both induced and uninduced expression, allowing an induced level of *tk* activity roughly equivalent to 70% of that obtained with a wild-type promoter. Although the induction observed with these model genes was slightly less than that observed with a wild-type promoter, it appears that both basal and metal-induced expression from a wild-type promoter may be accounted for by the action of two classes of promoter elements interacting with the *mMTI* 'TATA box' region. These results also demonstrate that promoter strength may be directly modulated by changing the copy number of individual promoter elements.

We have used synthetic oligonucleotides to test the function of specific MRE sequences found within the *mMTI* promoter. We have shown that four of the five MREs we recognized previously are individually able to confer metal responsiveness to the *tk* promoter when present as dimers. A comparison of the metal-responsive homologues suggests that the heptanucleotide TGCRCYC (R = purine, Y = pyrimidine) constitutes the core of a metal-dependent promoter element, which may be the recognition sequence for a metal-dependent transcription factor. This sequence closely resembles the nearly invariant consensus sequence TGCRCNC derived in Fig. 1a. It is noteworthy that this conserved minimal MRE sequence is small, and thus would be expected to occur frequently in the genome just by chance. The fact that relatively few genes are inducible by heavy metals can be attributed to the observed requirement for clustering of two or more such sequences within a promoter region in order to obtain a response to heavy metals⁵. The theme of multiple copies of relatively small functional sequences appears to be common among eukaryotic promoters and enhancers⁷⁻¹⁵. Although instances in which enhancer function is generated by duplication of a sequence present only once in the wild-type lead one to question the biological relevance of the single copy¹⁶, the fact that multiple related MRE sequences are found within *MT* promoters justifies our approach of examining the activity of duplicated elements.

We have also shown that the metal-independent promoter activity exhibited by one of our synthetic sequences is correlated with the presence of a strong Sp1 binding site homology within this sequence. Although the activity of this sequence apparently resulted from the chance juxtaposition of a partial Sp1 binding site homology found within the *mMTI* promoter and a synthetic *Bgl*III site, this finding may still be relevant to *mMTI* promoter function. One possibility is that metal-dependent regulatory factors enhance the binding or activity of other transcription factors such as Sp1, a suggestion strengthened by our previous observations of cooperative interactions between MREs and *tk* promoter elements³, and by the recent discovery of multiple Sp1 binding sites in the *hMTIA* gene promoter (W. Lee and M. Karin, personal communication). In addition to the observation that MREs and Sp1 binding sites coexist within *MT* gene promoters, we also note that many of the MRE homologues in Fig. 1a contain a 6 or 7 out of 8 bp match with the Sp1 binding site consensus sequence, (C/T)CCGCC(C/A) (K. Jones and R. Tjian, personal communication). This partial homology raises

the possibility that these two recognition sequences share a common evolutionary origin.

Our experiences have shown that the multiplicity of promoter elements and their potential to interact can hamper the interpretation of simple promoter mutant studies. The use of synthetic oligonucleotides to verify promoter sequence functions allows a complete dissection and definitive analysis of complex promoters. The identification of promoter elements with specific functions will eventually allow the production of synthetic promoters with designed responses to various environmental or developmental signals.

We thank Steve McKnight and Bob Kingsbury for providing *tk* linker-scanning mutants, and Gary Gryan and Joel Habener for providing synthetic oligonucleotides. This work was supported by NIH grant HD-09172.

Received 28 May; accepted 22 August 1985.

1. Stuart, G. W., Searle, P. F., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7318-7322 (1984).
2. Carter, A. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 7392-7396 (1984).
3. Searle, P. F., Stuart, G. W. & Palmiter, R. D. *Molec. cell. Biol.* **5**, 1480-1489 (1985).
4. Glanville, N., Durnam, D. M. & Palmiter, R. D. *Nature* **292**, 267-269 (1981).
5. Karin, M. & Richards, R. I. *Nature* **299**, 797-802 (1982).
6. Richards, R. I., Heguy, A. & Karin, M. *Cell* **37**, 263-272 (1984).
7. Searle, P. F. *et al. Molec. cell. Biol.* **4**, 1221-1230 (1984).
8. Karin, M. *et al. Nature* **308**, 513-519 (1984).
9. Palmiter, R. D., Stuart, G. W. & Searle, P. F. in *Eukaryotic Transcription: Role of cis- and trans-acting Elements in Initiation* (ed. Gluzman, Y.) 144-149 (Cold Spring Harbor Laboratory, New York, 1985).
10. McKnight, S. L. *Cell* **31**, 355-365 (1982).
11. Seguin, C., Felber, B. K., Carter, A. D. & Hamer, D. H. *Nature* **312**, 781-785 (1984).
12. Dynan, W. S. & Tjian, R. *Cell* **35**, 79-87 (1983).
13. Hinnebusch, A. G. & Fink, G. R. *J. biol. Chem.* **258**, 5238-5247 (1983).
14. Payvar, F. *et al. Cell* **35**, 381-392 (1983).
15. Travers, A. A. & Dudler, R. *Cell* **38**, 391-398 (1984).
16. Weber, F., de Villiers, J. & Schaffner, W. *Cell* **36**, 983-992 (1984).
17. Pustell, J. & Kafatos, F. C. *Nucleic Acids Res.* **10**, 4765-4783 (1983).
18. McKnight, S. L. & Kingsbury, R. *Science* **217**, 316-324 (1982).

Errata

Lymphokine dependence of *in vivo* expression of MHC class II antigens by endothelium

G. Groenewegen, W. A. Buurman & C. J. van der Linden
Nature **316**, 361-363 (1985)

IN the fifth line of the fourth paragraph, the drug concentration of cyclosporin A should be 0.2 and 0.4 mg l⁻¹.

Birth of the maser and laser

Nature **31b**, 307-309 (1985)

IN Professor Alfred Kastler's review of Bertolotti's book *Masers and Lasers*, two of the references are in error. The paper by Einstein, A. (1917) "Zur Quantentheorie der Strahlung" appears in *Physikalische Zeitschrift* **18**, 121-128 (1917) not *Z. Phys.*; and the paper by Boulouch, R. "Dédoublément des Franges D'Interférence en lumière naturelle" appears in *Journal de Physique Théorique et Appliquée* **2** (3rd Ser.) 316-320 (1893), not *J. Phys.*

Regulatory elements controlling chorion gene expression are conserved between flies and moths

S. A. Mitsialis & F. C. Kafatos
Nature **317**, 453-456 (1985)

THE words on the cover of the 3 October issue should have read "Moth genes into flies" and not vice versa.

Corrigendum

Amino-acid sequence of a Ca²⁺ + Mg²⁺-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence

D. H. MacLennan, C. J. Brandl, B. Korczak & N. M. Green
Nature **316**, 696-700 (1985)

ON page 697, the *M_r* of the ATPase should be corrected to 109,529; that of fragment A₂ to 22,089; fragment A₁ to 33,423; and fragment B to 54,053.