from a common 5'-endpoint 58 nucleotides upstream from the fourth exon (Fig. 4a). Introduction of *KpnI* and *ClaI* restriction sites alone, allowing construction of these nested deletions, had little effect on cell-specific alternative splice-site selection (Fig. 4b, c). Deletion of a region between -58 and -38 (Δ -58/-38) had no apparent effect on alternative splicing. Further deletion from -58 to -27 (Δ -58/-27) resulted in continued use of the calcitonin acceptor in HeLa cells with increased levels of unprocessed RNA precursors. F9 cells transfected with this mutant transcription unit produced increased levels of unprocessed RNA species and an apparent increase in the ratio of calcitonin to CGRP mRNA. Additional deletion from -58 to -17 (Δ -58/-17) further increased the percentage of calcitonin-specific splice acceptor use in the CGRP-producing cell line. Sequence analysis of RNA from this transcription unit confirmed that accurate splicing of the exon 3 donor to the exon 4 acceptor had occurred in both cell types (data not shown). These results suggested that a *cis*-active element near the calcitonin acceptor inhibited the formation of calcitonin transcripts in F9 cells and that deletion of this element(s) permitted the increased production of calcitonin mRNA in the CGRP-producing cell line.

Further nested deletions near the calcitonin-specific acceptor were not feasible owing to the presence of the splice acceptor consensus sequence, (Py)₁₀GCAG, which has previously been shown to be critical for constitutive splicing¹⁸. In order to further define *cis*-active elements at the calcitonin acceptor itself, the entire splice acceptor region, corresponding to the coordinates -58 to +11 was removed and replaced with a heterologous splice acceptor that could be used by both cell types (Δ -58/+11 (Ex3); Fig. 4a). We chose a region of the exon 3 splice acceptor from -39 to +8 relative to the beginning of the third exon. When transfected into HeLa cells, this transcription unit resulted in the production of calcitonin transcripts only, whereas F9 cells now produced ~82% calcitonin mRNA (Fig. 4b, c). Sequence analysis of RNA from F9 cells transfected with this transcription unit confirmed the accuracy of splicing (data not shown). These results demonstrated that removal of sequence information from the calcitonin-specific 3'-splice junction and its replacement with an acceptor that could be used by F9 cells resulted in the efficient production of mature calcitonin mRNA in a CGRP-producing cell line.

We have demonstrated that tissue-specific processing of the calcitonin/CGRP primary transcript is the result of alternative splice-site selection primarily regulated by *cis*-active sequences at the calcitonin-specific 3'-splice junction. Deletion of sequence information at the calcitonin acceptor results in an alteration of splice-site selection in CGRP, rather than calcitonin-producing cells. These results are most consistent with a model in which *cis*-active sequences serve to inhibit the formation of calcitonin mRNA in the CGRP-producing cell line. Recent genetic analyses of developmental loci in *Drosophila melanogaster* have suggested that specific gene products, with putative RNA-binding domains, may regulate alternative splicing in the sex-determination pathway¹⁹⁻²¹. Such *trans*-acting factors may affect differential RNA processing by binding to specific *cis*-active pre-mRNA sequences, thereby promoting or repressing the selection of alternative splice sites²²⁻²⁴. Further definition of the *cis*-active element(s) involved in the production of calcitonin and CGRP messenger RNA may help to isolate and characterize the cell-specific *trans*-acting factor(s) involved in the tissue-specific RNA processing of this gene. □

Received 19 May; accepted 31 July 1989.

1. Breitbart, R. E., Andreadis, A. & Nadal-Ginard, B. *A. Rev. Biochem.* **56**, 467-495 (1987).
2. Leff, S. E., Rosenfeld, M. G. & Evans, R. M. *A. Rev. Biochem.* **55**, 1091-1117 (1986).
3. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Siller, S. & Sharp, P. A. *A. Rev. Biochem.* **55**, 1119-1150 (1986).
4. Guthrie, C. & Patterson, B. *A. Rev. Genet.* **22**, 387-419 (1988).
5. Cheng, S. C. & Abelson, J. *Genes Dev.* **1**, 1014-1027 (1987).

6. Steitz, J. A. et al. *Structure and Function of Major and Minor SnRNPs* (ed. Birnstiel, M. L.) 115-154 (Springer, Heidelberg, 1988).
7. Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S. & Evans, R. M. *Nature* **298**, 240-244 (1982).
8. Sabate, M. I. et al. *J. Biol. Chem.* **260**, 2589-2592 (1985).
9. Amara, S. G., Evans, R. M. & Rosenfeld, M. G. *Mol. cell. Biol.* **4**, 2151-2160 (1984).
10. Crenshaw, E. B., Russo, A. F., Swanson, L. W. & Rosenfeld, M. G. *Cell* **49**, 389-398 (1987).
11. Leff, S. E., Evans, R. M. & Rosenfeld, M. G. *Cell* **48**, 517-524 (1987).
12. Bovenberg, R. A. L. et al. *Nucleic Acids Res.* **14**, 8785-8803 (1986).
13. Bovenberg, R. A. L., Adema, G. J., Jansz, H. S., Baas, P. D. *Nucleic Acids Res.* **16**, 7867-7883 (1988).
14. Reed, R. & Maniatis, T. *Genes and Dev.* **2**, 1268-1276 (1988).
15. Konarska, M. M., Grabowski, P. J., Padgett, R. A. & Sharp, P. A. *Nature* **313**, 552-557 (1985).
16. Weiringa, B., Meyer, F., Reiser, J. & Weissmann, C. *Nature* **301**, 38-43 (1983).
17. Smith, C. W. J. & Nadal-Ginard, B. *Cell* **56**, 749-758 (1989).
18. Mount, S. M. *Nucleic Acids Res.* **10**, 459-472 (1982).
19. Boggs, R. T., Gregor, P., Idriss, S., Belote, J. M. & McKeown, M. *Cell* **50**, 739-747 (1987).
20. Gorski, T. J., Edstrom, J. E. & Baker, B. S. *Cell* **56**, 1011-1018 (1989).
21. Bell, L. R., Maine, E. M., Schedl, P. & Cline, T. W. *Cell* **55**, 1037-1046 (1988).
22. Bandzulis, R. J., Swanson, M. S. & Dreyfuss, G. *Genes Dev.* **3**, 431-437 (1989).
23. Laski, F. A. & Rubin, G. H. *Genes Dev.* **3**, 720-728 (1989).
24. Streuli, M. & Saito, H. *EMBO J.* **8**, 787-796 (1989).
25. Chen, C. & Okayama, H. *Molec. cell. Biol.* **7**, 2745-2752 (1987).
26. MacDonald, R. J., Swift, G. H., Przybyla, A. E. & Chirgwin, J. M. *Meth. Enzym.* **152**, 219-227 (1987).
27. Ausubel, F. M. et al. (eds) *Current Protocols in Molecular Biology* (Wiley, New York, 1989).
28. Kunkel, T. A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 488-492 (1985).

ACKNOWLEDGEMENTS. We are grateful to Charles Nelson for maintenance of tissue culture cell lines and to Drs Alan Wells, Stuart Adler, Harry Mangalam, Xi He, Elliot Klein and Holly Ingraham for critical reading of the manuscript. This work was funded by grants from the National Institutes of Health and The American Cancer Society.

CORRECTION

Spatial and temporal expression of the retinoic acid receptor in the regenerating amphibian limb

Vincent Giguère, Estelita S. Ong, Ronald M. Evans & Clifford J. Tabin

Nature **337**, 566-569 (1989).

As part of the above report of the temporal and spatial expression of a retinoic acid receptor (RAR) during amphibian limb regeneration, we isolated cDNA sequences from a regenerating newt cDNA library using a human RAR α probe (in humans two distinct RARs were known, called RAR α and RAR β). As described, two non-overlapping cDNAs were interpreted as representing contiguous portions of a single newt gene product. A chimera constructed by fusing the two clones at a common *EcoRI* site was shown to function as a retinoic acid receptor in an *in vitro* assay.

Recent work by C. Ragsdale, P. B. Gates and J. P. Brockes has resulted in the isolation of a newt RAR cDNA referred to as RAR δ whose 3' sequence is identical to clone NB6 in our report but whose 5' sequence differs from one clone NB8. This suggests that the two cDNAs we isolated originated as transcripts from two distinct, although closely related genes. With 5' sequence information provided by J. Brockes and colleagues, we have used the polymerase chain reaction (PCR) to examine both genomic DNA and newt mRNA. The results verify the existence of two distinct genes and gene products. Moreover, RNase protection experiments using a probe spanning our chimaeric junction confirm that the two sequences we fused are not found on a contiguous RNA transcript. Accordingly, our published sequence should be interpreted as representing the partial sequence of two different genes: RAR β (the clone NB8 in our paper) and a novel β -like RAR (clone NB6).

Since the probe used for our *in situ* hybridizations was derived solely from the clone NB8 the revised sequence interpretation does not affect the data or the conclusions presented in our paper. In addition, our work together with the results from the Brockes laboratory leads to the intriguing conclusion that there are at least two β -like RARs expressed in the regenerating limb. □