Transcription termination occurs within a 1000 base pair region downstream from the poly(A) site of the mouse β -globin (major) gene

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ABSTRACT

Transcription of RNA from the β -globin (major) gene in nuclei from induced mouse erythroleukemia cells terminates within the region between 700 to 2000 bases downstream from the poly(A) addition site, but not at particularly favored sites. In addition we present the first analysis of *in vivo* labeled RNA from a cell transcription unit that shows RNA termination in the same region as diagnosed by analysis of *in vitro* labeled RNA. The region in which termination occurs is contained in 1414 bp that were sequenced beginning 600 bases downstream from the poly(A) site. There is an increased frequency of the sequence AATAAA at the beginning and a stem and loop structure followed by a string of Ts near the end of this region.

INTRODUCTION

Control of transcriptional termination at the level of attenuation, between genes of an operon, and between operons is an important element of gene regulation in bacteria (1-4). Despite the discovery of factor-assisted (*rho* dependent) termination in bacteria over fifteen years ago (5), the precise mechanisms of RNA termination are still not understood. In animal cells mRNA is produced from RNA polymerase II transcription units and the mechanism of transcriptional termination is not known. The analysis first of *in vivo* ^3H uridine labeled adenovirus-specific RNA and also ^{32}P labeled nuclear RNA elongated *in vitro* by isolated nuclei both showed that termination was not at the poly(A) site but downstream from the poly(A) site (reviewed in 6). These measurements in whole cells and in whole nuclei were the necessary first step to determine transcriptional termination sites because rapid RNA processing does not allow any conclusions about termination based on steady-state accumulation of nuclear RNA. Additional experiments with recombinant adenovirus DNA make clear that the 3' end of adenovirus mRNA is defined not by termination but by endonucleolytic cleavage at a poly(A) site within the primary transcription unit (7,8).

We have also investigated transcription downstream from the single poly(A) site of a single copy cell gene namely the β -globin (major) gene of mice (9-12), first to establish whether transcription passed the poly(A) site, which it did (9,10), and then to search for a specific termination site or sites (10-13). We reported last year that the labeled nascent RNA produced in isolated nuclei terminated at a specific site about 750 bases beyond the poly(A) site (12). That conclusion was, unfortunately, erroneous (as we have noted, 13) because the single-stranded phage DNA used to reach the conclusion did not contain the sequences it originally was thought to.

We have however now repeated the hybridization analysis of *in vitro* labeled nascent RNA downstream from the mouse β -globin transcription unit with newly prepared and characterized single-stranded phages. Again clear evidence was found for cessation of RNA synthesis between approximately 750 to 1500 nucleotides past the poly(A) site but no favored termination sites were found. To ascertain that isolated nuclei reveal the true picture of transcription within the cell, we examined the hybridization of *in vivo* labeled RNA to DNA within and beyond the putative termination region detected by labeled RNA from isolated nuclei. This is the first reported analysis of *in vivo* labeled RNA transcribed from a termination region of a cellular gene. The hybridization pattern of *in vivo* labeled RNA supports the conclusion that RNA synthesis ceases by the time RNA polymerase II has traversed ~2000 nucleotides past the poly(A) site.

The region within which termination occurs was sequenced and has been compared to prokaryotic and eukaryotic 3' flanking regions. Several conserved long oligonucleotides in these downstream regions were observed in globin genes but not other genes. The nucleotide sequence of β -globin region near the 3' most portion of the termination region contains a sequence reminiscent of strong termination sites (1-3) in eukaryotes.

MATERIALS AND METHODS

Cells and RNA-DNA Hybridization

The growth of Friend cells (DS 19 clone) and the erythroid induction by DMSO or HMBA treatment was carried out as previously described (10-12) as were the preparation and hybridization of ^{32}P -UTP labeled RNA from isolated nuclei (10-12). RNA from nuclei of cells labeled with ^3H -uridine was extracted as described (14) and prepared for hybridization by precipitation with 2 M LiCl followed by digestion by RNase-free DNase. Filters were prehybridized for 6 hrs. with Denhardt's solution (15) and were washed at 50° in 0.1 x SSC before RNase digestion to lower non-specific hybridization signals.

DNA samples:

The λ gtWES clone containing the mouse β -globin DNA (16) and subclones of the region have been described (9-11). Single-stranded clones in bacteriophage M13 (17) were prepared for regions B1, B2, C, D, F, G and H (see Fig. 1) and some of these were used for Sanger sequencing (18). A clone of the entire 7 KB *Eco*RI β -globin (major) region into a pUC18 vector was generated and used for Maxam-Gilbert sequencing (19). Nine overlapping strands of DNA were sequenced to provide continuous sequence for the entire 1414 bp region of the F and G fragments (see Fig. 1).

RESULTS AND DISCUSSION

The restriction site map of the DNA and the position of the β -globin transcription unit in the 7 KB λ gtWES $\bullet$ M β G2 DNA (9,10,16,20) is shown in Fig. 1. The transcription termination region identified by previous *in vitro* experiments (9-12) occurs before the H region. RNA labeled *in vitro* by chain elongation in isolated nuclei hybridizes in an equimolar fashion to fragments B2, C, D and E.

Figure 1: Map of mouse β -globin (major) region. The 7KB *EcoRI* fragment was originally isolated by Tilghman *et al* (16) and fragment A through I were subcloned (9,10). The cap site and poly(A) site as well as the two introns and three exons are shown. The sizes of the various subcloned regions are taken from sequence data of Konkel *et al* (20) and this paper (Fig. 2). The arrows in the lower portion of the figure indicate the extent and 5' to 3' orientations of the strands sequenced. Dashed arrows denote dideoxy sequencing (18) and solid arrows indicate sequencing according to Maxam and Gilbert (19).

Definite submolar amounts of RNA complementary to F and G are also transcribed by RNA polymerase II in isolated nuclei (11,12, Fig. 2a). No labeled RNA hybridized to the H fragment. To be certain that these results with isolated nuclei truly reflected termination that occurred in whole cells, we returned to an examination of *in vivo* labeled RNA. The study of *in vivo* labeled RNA from single-copy eukaryotic genes is very difficult because even in a case such as induced mouse erythroleukemia cells, RNA from a specific gene makes up only about 2×10^{-5} of the total RNA output (9-11). In addition the time required to label the UTP pool is about 10-20 minutes and RNA processing proceeds on labeled molecules synthesized during that time (22-25). Nevertheless analysis of *in vivo* labeled RNA constitutes the only totally reliable method for determining initially where termination occurs. We therefore exposed 10^9 induced Friend cells to 30 mCi of ^3H -uridine for 20 min., extracted and hybridized the labeled RNA to excess DNA from various fragments within and downstream from the β -globin transcription unit. As shown in Table 1, the B2 and D fragments hybridized the most RNA, each to a normalized level of approximately 10 CPM/base. These fragments are 510 and 385 nucleotides long respectively and contain 222 and 116 nucleotides that are in exons (26). Fragment C which is 391 nucleotides long is entirely within the second intron and therefore will be present in the 15S nuclear RNA precursor to β -globin mRNA (20). However this fragment hybridized only about 2.7 CPM/base indicating that even within a 20 min. label time most labeled globin specific RNA has been processed. This result suggests that the half-lives of the unused sequences from the β -globin transcription unit must be considerably less than 10 minutes. By gel analysis fragment E is 450 to 500 nucleotides long and hybridized 1.5 CPM/base (comparable to the C fragment) and fragment F which is 809 nucleotides long hybridized only 0.43 CPM/base. The *in vivo* results

Figure 2: Hybridization of Nascent RNA from isolated nuclei.

Nascent nuclear RNA was prepared from isolated nuclei (9-11). In part (A), RNA was hybridized to excess filter bound phage DNAs deposited in dots on nitrocellulose. The phages used hybridized rightward reading RNA to C,D,F,G and H regions (21). In part (B) and (C) nascent nuclear RNA was hybridized to single stranded DNA as indicated; unpaired RNA was trimmed with RNase T1; RNA.DNA hybrids were collected by sucrose gradient sedimentation (12) and finally analyzed on denaturing acrylamide gels (19) along with indicated size markers of single-stranded DNA. The DNA used in protection experiments was B2 (rightward reading, see Fig. 1) F (both directions) and G (rightward reading) (G in leftward reading direction gave no protected bands). Four times as much RNA was used in the hybridization to G than to other DNA sequences.

obtained here, in conjunction with the transcription measured in pulse labeled isolated nuclei, provide a clear indication of declining hybridization probably beginning in the F fragment. G which is 600 nucleotides long and H which is 400 nucleotides long did not hybridize ^3H labeled RNA above background but a level of transcription of 1/10 as much as for G is not excluded and such low-level signals are seen by autoradiography with ^{32}P labeled nuclear RNA (Fig. 2a).

Since transcription termination clearly appears to occur in the F and G regions we had

Table 1

	B2	C	D	E	F	G	H
cpm in hybrid	5058	946	3647	673	345	25	19
Normalized CPM	10.1	2.7	9.3	1.5	0.43	--	--

The input was 88×10^6 CPM. The B2, C, D, F, G and H clones were in M13; the E clone was a plasmid. ^3H -uridine labeled RNA was prepared after a 20 min. label time (10^9 cells in 60 ml were labeled for 20 min. with 30 mCi ^3H -uridine. The extracted nuclear RNA was precipitated with LiCl (2 M) overnight at 4° and digested with RNAase free DNase. Filters containing either pBR or M13 DNA retained only 25 CPM.

attempted earlier to determine whether specific sites of termination exist. Nascent labeled nuclear RNA chains within the transcription unit (e.g. B2,C,D) could be protected against nuclease digestion by single-stranded probes that were equal in length to the B2, C, and D regions. We reported (12) that a single stranded F probe protected a nuclear RNA considerably shorter than its full length (156 instead of 809 nucleotides) and therefore concluded that a termination site existed in F. However, the single-stranded F clone was unfortunately deleted and experiments with nascent RNA had to be repeated.

We then prepared a fresh M13 clone (17) containing the entire F region and repeated the labeled nuclear RNA protection experiments. The labeled nuclear RNA protected by B2, F and G single-stranded phages is shown in Fig. 2, part b. The B2 cloned DNA protected a band 500 nucleotides long and the new F-specific clone protected a labeled RNA band that was 800 ± 10 nucleotides long; the F sequence itself is 809 nucleotides long (from *Xba*I to *Bgl*II site in Fig. 2). The F band is considerably less intense than the B2 band indicating frequent termination before F is completely traversed. The RNA that was protected was transcribed in the same direction as the β -globin mRNA; an F-specific clone of the opposite polarity did *not* protect specific sized RNA. The labeled nuclear RNA protected by the neighboring downstream fragments G was also examined (Fig. 3). A single-stranded phage containing the entire G sequence protected an RNA band approximately 600 nucleotides long (Fig. 2b). To observe the G specific band a four-fold higher input of labeled RNA was required compared to the F fragment so transcription concludes probably greater than 90% of the time before G is traversed. No protected RNA bands were seen with the H clone (data not shown).

There are numerous less than full-length bands within the autoradiogram of specific RNA protected by any of the single-stranded DNA clones. Since nascent RNA is under examination, we expect to observe transcriptional pause sites (12,27) and only if a termination site were highly effective would we be able to pick out a site beyond which no further transcription occurred. Therefore there may be either a fairly large number of true termination sites or termination may occur at any site beginning in the F region.

Having experimentally demonstrated that transcriptional termination occurs in the mouse β -major globin gene between 700 and 1500 nucleotides past the poly(A) site we determined the entire sequence of the F and G regions (Fig. 3). A search of the Los Alamos compilation of known

*λ*bal 50 100
 TCTAGAGAGCA ATAGCAATAT CTAGTTTITA **ACGCTCATTA** CTGGGCTCTT ATGAAATTC CATAAATCC TGTAAATCGA AGCATTTATT TTTT**AAATAA**
 AGATCTCTCT TATTCTTATA GATCAAAAAA TCCGAGTAAT GACCCACAAA TACTTTAAAG GTATTAATCGA ACATTTACCT TCGTAAATAA AAAAGTTATT

150 200
 ΔTCTATCTTG AATATCCAGT GTCCGTTAGC ATTAATATCT TCCCTCATAC AGTTTCACTG C **TTTTATTTA** TATCGAGTTA CTAGACTTAA CACAATAAGT
 TAGATACAAK TTATAGGTC A CACCACAATCC TAATTTAGAG AGCAGATATG TCAACCTGAC GAAAAATAAT ATACCTCAAT GATCTCAAT GTGTATTCTA

250 300
 AATATACCCCT TGATTGTGTT TTCTTTTCCAT AACCAACAGC TTATCGCAAA TCCCGA**AAAT** **AAAT**CTGTGT TCCCAAGAGT TCTTTACGCT ACCTCTCTGCT
 TTATATCGGA ACTAAACAAA AAGCAACGTA TTCTGTGCTC AATACGCTTT TTTTACAC AAGCTTCTCA AGAAATCGGA TCAAGACACA

350 400
 ACAGTTTATG TCGATTTTGT AATGACTAC ATATAATAAG TGGCTTTTAA TTACAGAAAT GTTGTGTGAC GTACAG**ATAA** **AAAT**ACACCA AATATTATGA
 TGTCAAAATC ACTCTAAAC TTACTGATG TATATTATTC ACCCGAAAT TATGTCTTAC CAACACATC CATGTCTTAT TTTATGTGCT TTTAATTAAT

450 500
 GTTTCAGTCA TTGTCATGAG TCATAAAAAA GACAGTCCAA ACGAAGTAAA GAGTTAGAGT ATGGTCAAGAA ATTATAAAC ATCAAGAAAA **AAATACAGCA**
 CAAATCTAGT AACAGTACT GATATTTTAA CGTGAGGCTT TGTCTATT CTCAATCTCA TACCATCTT TAAATTTGG TAGTCTCTTT TTTATGCTCT

550 600
 CCCATAAAGG TAGTGTGCTG GCAAGCTATT TCGTGCATAT TTATAATCTT TATTTATTA TACTAAGAA CCAAGCAGTA TTTATAAAAT ATGGTCTCTCT
 GGTATTTTCC ATCAACACGC CAGTCCATAA AGCAGCTATA AATATTAGCA ATAAATAATA ATGATCTTTC GATCTGCTAT AATATTTTTA TACCAGCAKA

650 700
 CTGAATGCAA TGTCCAATCG TCTAAAAACC ATATCTTAGT GTTCTCAAGC CAGTATCTTC TGTTCGAAA TAGAACTGAA TTTTATATA CTGTCTCTA
 GACTTACCTT ACAGGTTACC AGATTTTGGG TATAGATCA CAGAGCTCTC GTCTAGAGAG ACAAGCGTTT ATCTTGACCTT AAAAAATATT GACAGAGTAT

750 800
 ATTTATGTAA GACTTTTGGC TAGCCATAAA GATAGCATGA GCAATCTTT TTGCACTAGC TAGAACCTT GCTGTCTTTT TCTTGACTTA ATGAAAGTCA
 TAAATACATT CTGAAACCG ATCGGTATTT CTATCTTACT CGTTAGAGAA AACGTCATCC ATCTTGGGAA CCGACAAAAA AGAAGCTGAA TACTTCTAGT

850 900
 BglI
 GTAATAGATC TTGGTTTATA GAGAGTTAGT TGGTAGTAGA CATCATTTCA CAGTTGCTT CCTCGACTGA ATCTAATTA AATGTATTA GTTTTCTTT
 CATTTATCTAG AACCAATAT CTCTCAATCA ACCATCATCT GTAGTAAAGT GTCAAGCTAA GAGAGTGAAT TAGCATTAAT CAAAGAAAA

950 1000
 hamalu49a
 CTTCTAGGCA GACAGTCTG GTCCGTTTT CATTGTGCT TCCAGCTACT GGTACCATAG GTTCTAGTCC AAGTTGCTC TCTGATCTG GAAGAAAGAG
 GAGATCTCGT CTGTACGAC ACCCGAAAG GGTAAACAGA AGCTCCATGA CCATCGTATC CAGATCAGC TTTCAACGAG AGACCTAGAC CTCTTCTCC

1050 1100
 musrsblb, b, c, afp
 CAGAGGCTGG TTGAATGTTA CCTCTCGAT GAACGTAGC TCGAGCAGTG TATCAAAAGT TCTTCCCACT TACTTTCTTA AGAAAAAT ACACCTTCA
 GTCTCCGACC AACTTACAA CCGAGACCTA CTGACATCG ACCTCCGATC ATACCTTCA AGAAGCGGTG ATGAAGAGAT TCTTTGTTTA TGTGAAACCT

1150 1200
 AclI
 TATGTGTTT AAAAGTTATA TGTGATGAC ACACCAATA GTTAGCTGA TCTGTACGA GCCAGATCAT CCAATGTGCT GATGTGTTGA TACAAAAA
 ATACACAAAA TTTTCAATAT ACCATTCATG TGTGGTTAT CAATCGACAT AGACAGTCTG CCGTCTAGTA CGTTACAGCA CTACACAACT ATGTTTTTGT

1250 1300
 musrsalu22
 TTACTGCTGA CTGTCTATC GTGATAGAAA TATTGTGTA GTTACTTAA GTTAGTACCA AAAATGAAA TANGTAGCA TTCAATATA TTTATCTCAA
 AATGACGACT GACAGAGTAG CACTATCTT ATAAACAACT CAATCAATG ATCTCATGCT TTTTACTTT ATTCGATCGT AAGTTAATAT AAGTAGAGTT

1350 1400
 humhbgga
 TACTCTATTA TTTAGTACC ACATATCTT **CTAGCTACAC** AAACAGCAAG CGACTTAA CA GTGTACTTA **CA** **TTTTTTTT** **TTTTTTTT** TACA AGCCCTCTCA
 ATGAGATAAT AAGTCAATG GTATAGAAC GATCGATGTG TTGTCTCTT CCTGAATTGT CACATCAAAAT CCAAAAAA AAAAAATGT TCCGAGACGT

1400
 BclI
 ATCGACACTG ATCA
 TACCTGTGAC TAGT

Figure 3: Sequence of F and G regions. The sequence of the F fragment which starts approximately 600 bp downstream from the β -globin poly(A) addition site (Fig. 1) begins at position 1 and ends at the *Bgl*I site at 806. The G fragment immediately follows ending at the *Bcl*I site at 1409. Dyad symmetries occur at positions 150 and 1333 and are indicated by arrows. T rich strings discussed in the text are shown in bold italics. AATAAA sub-sequences, underscored, are found at positions 96, 258, and 377. Regions homologous to other known sequences are printed in bold type and include several repetitive sequences from hamster and mouse DNA (GenBank loci hamalu49a, musrsblb, musrsblb, musrsblc, musrsblafp, and musrsalu22). Two regions in the human α_1 γ globin cluster (GenBank locus humhbgga) and a segment of the human δ - β -globin sequence (provided by S. Surrey) also share homology as indicated. No other significant homologies were observed between this downstream sequence and the few other sequences in GenBank 21.0 containing 31 flanking regions greater than 500 bases. The sequence homologies are summarized in Table 2.

This sequence has been made available to GenBank.

Table 2

Gene	Sequence	Matches/Length to FG Sequence	Distance to poly(A) Site
Mouse β -globin	AGAAAAAATA		1085
Human A_γ -globin	AGAAAAAATA	11/11	1262
Human G_γ -globin	AGAAAAAATA	11/11	1169
Mouse β -globin	GAAATAAAATGT		856
Human β -globin	GAAATAAAATAT	11/12	900
Human δ -globin	GAAAGAAATAT	10/12	720
Mouse β -globin	TTTTTTTTTTTTTTA		1973
Human A_γ -globin	TTTTTTGTTTTTTTA	15/16	1527
Human G_γ -globin	TTTTTTTTTTTA	12/12	1669

sequences (GenBank release 21.0) was made for sequences in common to the F and G region sequences. Homologies of 15 to 20 bases with *Alu* sequences were found in the G region (nucleotides 901-919, 986-1016, and 1132-1116). A known full length *Alu* sequence has been located in the I region (S. Haynes and W. Jelinek, pers. comm.). The short homologies in the G region would not be expected to contribute stable hybridized RNA under the conditions used in our experiments. To identify sequences that could possibly be associated with termination signals, we compared known genomic sequences located in other genes more than 500 base pairs downstream from poly(A) sites. These sequences included the human G_γ , A_γ , δ and β -globin genes (28,29). There are two small sequences that appear statistically significant by virtue of their occurrence in similar locations in three globin genes and these are shown in Table 2 and Fig. 3. The sequence GAAATAAAATGT at 856 bases downstream from the mouse β -globin poly(A) site is greater than 80% homologous to sequences 900 and 720 bp downstream from the poly(A) sites of the human δ - and β -globin sequences (pers. comm., S. Surrey). The T rich sequence 1973 bp from the mouse β -globin poly(A) site is greater than 90% with human A_γ - and G_γ -globin sequences 1527, and 1669 bp to their respective poly(A) sites. These homologies may of course indicate simply that these downstream regions are part of the duplicated domains in the β -globin region and may have nothing to do with termination. No sequence comparison with downstream regions in other genes were made because no termination regions have been mapped in regions that have been sequenced.

In the mouse β -globin gene, there is one striking structure that might be related to the termination. Near the downstream end of the G-fragment the primary sequence can be folded into a stem and loop structure that is followed within 5 nucleotides by a stretch of 15 T residues (Fig. 2). This structure closely resembles prokaryotic termination sites (1-3, 30) and is potentially related to the hybridization profile which shows that all transcription ceases before the H fragment is reached.

The presence in the F region of another sequence of interest, AAUAAA (the poly(A) signal) is worth noting as well. There are three of these hexanucleotides within 400 bases of the beginning of the F fragment at positions 96, 256 and 376. In a random sequence each hexanucleotide would occur

once in 4096 bases. It is possible that these sequences, possibly by causing the RNA polymerase to pause, contribute to the gradual loss of transcription begins in the F and is complete by the end of the G region. In fact a bimodal recognition mechanism might be responsible for transcriptional termination. First passage of the transcription complex past a designated site of polyadenylation into a region of pause sites (possibly the AAUAAA signal sequence) reducing transcription rates >90% followed by complete blockage at a sequence such as the T-rich sequence seen 1973 bases past the mouse β -major globin poly(A) site.

We have turned to the task of transplanting the β -globin region to other genomic locales to determine whether the termination region functions in other situations. Preliminary evidence is that the sequence of F alone, when inserted into the intervening sequences of adenovirus transcription units greatly diminishes but may not completely stop transcription (E. Falck-Pedersen, J. Logan, J. E. Darnell and T. Shenk; unpublished results). One consideration in such analyses is that any termination might only occur if the termination sequence lies downstream from a poly(A) site. A termination effect that only occurred after polyadenylation demands the ability to measure RNA transcription (not a stable cytoplasmic product) from such a recombinant genome. For this reason we have turned to reconstructed adenoviruses containing the β -globin gene (31,32) (E. Falck-Pedersen, J. Logan, J.E. Darnell and T. Shenk) so that large amounts of nuclear RNA can be produced in order to assay the potential effect of downstream globin sequences on termination.

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REFERENCES

1. Yanofsky, C. (1981). *Nature* 289, 751-758.
2. Burton, Z. E., Gross, C. A., Watanabe, K. K. and Burgess, R. R. (1983). *Cell* 32, 335-349.
3. Adhya, S. and Gottesman, M. (1982). *Cell* 29, 939-974.
4. Roberts, J. W. (1975). *Proc. Natl. Acad. Sci. USA* 72, 3300-3304.
5. Roberts, J. W. (1969). *Nature* 224, 1168-1174.
6. Darnell, J. E. (1982). *Nature* 297, 365-371.
7. Montell, C., Fisher, E. F., Caruthers, M. H. and Berk, A. J. (1983). *Nature* 305, 600-605.
8. McDevitt, M., Ali, H. and Nevins, J. R. (1984). *Cell*, in press.
9. Hofer, E. and Darnell, J. E. (1981). *Cell* 23, 585-593.
10. Hofer, E., Hofer-Warbinek, R. and Darnell, J. E. (1982). *Cell* 29, 887-893.
11. Salditt-Georgieff, M., Sheffrey, M., Krauter, K., Darnell, J. E., Rifkind, R., and Marks, P. A. (1984). *J. Mol. Biol.* 172, 437-450.
12. Salditt-Georgieff, M., and Darnell, J. E. (1983). *Proc. Natl. Acad. Sci. USA* 80, 4694-4698.
13. Salditt-Georgieff, M., and Darnell, J. E. (1984). *Proc. Natl. Acad. Sci. USA* 81, 2274.
14. Salditt-Georgieff, M., Harpold, M., Sawicki, S., Nevins, J., Darnell, J. E., Jr. (1980). *J. Cell Biol.* 86, 844-848.
15. Denhardt, D. (1966). *Biochem. Biophys. Res. Comm.* 23, 641- 646.
16. Tilghman, S. M. Tiemeier, D. C., Polsky, F., Edgell, M. H., Seidman, J. G., Leder, A., Enquist, L. W., Norman, B., and Leder, P. (1977). *Proc. Natl. Acad. Sci. USA* 74, 4406-4410.
17. Messing, J., Crea, R. and Seeburg, P. H. (1981). *Nuc. Acids. Res.* 9, 309-321.
18. Sanger, F., Nicklen, S. and Coulson, A. R. (1977). *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
19. Maxam, H., and Gilbert, W. (1980). In *Methods in Enzymology* 65, 499-560; L. Gross and K. Moldave, Eds. Acad. Press, N. Y.

20. Konkel, D. A., Tilghman, S. M., and Leder, P. (1978). *Cell* 15, 1125-1132.
21. Kafatos, F., Jones, C. W., and Efstradiatis, A. (1979). *Nuc. Acids Res.* 7, 1541-1551.
22. Bogenhagen, D. F. and Brown, D. D. (1981). *Cell* 24, 261-270.
23. Harpold, M., Wilson, M., and Darnell, J. E. (1981). *Mol. and Cell. Biol.* 1, 188-198.
24. Salditt-Georgieff, M., and Darnell, J. E. (1982). *Mol. and (1977). J. Cell Phys.* 90, 521-534.
25. Puckett, L. and Darnell, J. E. (1977). *J. Cell Phys.* 90, 521-534.
26. Curtis, P. J., Mantei, N., and Weissman, C. (1977). *Cold Spring Harbor Symp. Quant. Biol.* 42, 971-984.
27. Mills, D. R., Dobkin, F., and Kramer, F. B. (1978). *Cell* 15, 541-550.
28. Shen, S., Slighton, J. L., and Smithies, D. (1981). *Cell* 26, 191-203.
29. Ponez, M., Schwartz, E., Ballantine, M., and Surrey, S. (1983). *J. Biol. Chem.* 258, 11599-11609.
30. Rosenberg, M., and Court, D. (1979). *Ann. Rev. Genet.* 13, 319-353.
31. Stow, N. D. (1981). *J. Virol.* 37, 171-180.
32. Hearing, P. D., and Shenk, T. (1983). *Cell* 33, 695-703.