Transcription of the Chicken Histone H5 Gene Is Mediated by Distinct Tissue-Specific Elements within the Promoter and the 3' Enhancer

CECELIA D. TRAINOR AND JAMES DOUGLAS ENGEL*

Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208

Received 20 July 1988/Accepted 7 February 1989

Molecular genetic analysis of a number of vertebrate erythroid cell-specific genes has identified at least two types of *cis*-acting regulatory sequences which control the complex developmental pattern of gene expression during erythroid cell maturation. Tissue-specific cellular enhancers have been identified 3' to three erythroid cell-specific genes, and additional regulatory elements have been identified in the promoters of many erythroid genes. We show that the histone H5 enhancer, like the adult β -globin enhancer, is involved in mediating the developmental induction of histone H5 mRNA as erythroid cells mature. We also describe the preliminary characterization of a tissue-specific regulatory element within the 5' region of the H5 locus and describe investigations of the interaction between this element and the histone H5 enhancer in mediating histone H5 regulation.

Chicken erythrocyte histone H5 is a replacement variant linker histone which associates with chromatin in increasing quantity during erythroid cell maturation; its biological activity has not been definitively demonstrated, but H5 is thought to play a causal role in inactivation of the erythrocyte nucleus (4, 9, 32). Studies analyzing the histone H5 gene have revealed that it is single copy and is unlinked to other histone gene clusters in the chicken genome (20, 27).

Histone H5 synthesis is not cell cycle regulated, in contrast to that of most histone genes (8). In one respect, the expression pattern of histone H5 is similar to that of the adult β -globin gene in that both are expressed at higher levels in reticulocytes than in early erythroblasts (2, 24, 28). However, histone H5 is transcribed in avian erythroblastosis virus-transformed CFU-E stage erythroid progenitor cells, whereas the β -globin gene is inactive in these cells (3, 34). Quantitative analysis has shown that steady-state levels of histone H5 mRNA are lower (approximately 50-fold) than is β -globin mRNA at all maturation stages beyond CFU-E (23). It has previously been shown that this similarity between the expression patterns of the two genes is due, at least in part, to the presence of erythroid cell-specific enhancer elements localized 3' to both genes (6, 19, 29). The functional significance of this unusual location for these erythroid cell-specific enhancers remains unclear, although a model which suggests that collaborative activity between the promoter and enhancer may be required for β -globin transcription has been proposed (7).

The activity of the 3' erythroid cell-specific enhancer element was initially detected in the histone H5 gene through analysis of a series of deletion mutations of a marked H5 gene (H5*) with a transient transfection assay in HD6 cells, an erythroid cell line established by transformation of chicken bone marrow cells which is unable to differentiate (1, 3, 29). The histone H5 gene enhancer (H5E) element was definitively identified by using the simian virus 40 (SV40) enhancer trap vector pETlin (29, 31); the results obtained in those experiments indicated that the H5E is capable of inducing differential accumulation of T antigen, a reporter

gene product, in immature (35°C) versus differentiation-induced (42°C) HD3 cells in an orientation-independent manner. The 3' H5E was shown to cause fivefold stimulation of transcription of a reporter gene (*cat*) from either the H5 promoter or the SV40 early promoter in differentiation-induced HD3 cells, a cell line established by transformation of bone marrow cells with ts34 avian erythroblastosis virus which is conditionally inducible for erythroid cell differentiation (2, 16, 29). The effect of the H5E in immature erythroid cells (as represented by line HD6 or undifferentiated HD3) was much more subtle.

The studies presented here were conducted to establish whether or not the H5 promoter, in the absence of the enhancer, is capable of tissue-specific transcriptional activity and how the enhancer contributes to the transcriptional activation of this gene during the terminal stages of erythropoiesis. However, since the immunofluorescence assay does not directly measure transcription of the T-antigen gene, we were unable to accurately quantitate the effect of the enhancer by using this assay. To circumvent the problems inherent in quantitative analysis of immunofluorescence experiments, we turned to the study of reporter gene transfection assays and analysis using S1 nuclease protection of accumulated RNA.

The activity of the 3' H5E was first detected in analysis of a deletion mutation (pH5* mutation 4) which is expressed in erythroid cells at an approximately fivefold reduced level relative to the complete H5 gene locus; pH5* mutation 4 is not expressed when cotransfected into chicken embryo fibroblasts (CEF) with pRSVCat (29). Two possible conclusions can be drawn from the fact that H5* mutation 4 is still expressed exclusively in erythroid cells. Either (i) there is another tissue-specific element (in addition to the enhancer element at the 3' end) in the H5 locus or (ii) the enhancer element has been damaged but not completely inactivated by deletion of enhancer sequences within the 3' enhancer-regulatory region defined by histone H5* gene mutation 4. No residual enhancer activity could be detected in the 3' one-third of the pH5* mutation 4 gene in the enhancer trap assay (data not shown), indicating that an additional tissue-

* Corresponding author.

FIG. 1. The histone H5 gene promoter is tissue specific. (A) A 580-bp *RsaI* fragment which extends from positions -312 to +268 of the histone H5 gene (20, 27) was inserted into pSV0Cat (a vector which was constructed from pSV2Cat by digestion with *AccI* and *HindIII* to remove the SV40 promoter and enhancer; 14) in the sense orientation 5' to the *cat* gene to generate H5Cat. H5Cat was further modified by insertion of the RSV LTR at a *BamHI* site 3' to the SV40 termination cassette in the antisense orientation relative to the inserted H5 gene fragment and the *cat* structural gene to form H5Cat/RSV. Histone H5 structural gene sequences are indicated by bold lines, *cat* structural sequences are represented by a stippled box, SV40 termination-polyadenylation sequences are represented by a cross-hatched box, and RSV LTR sequences are represented by a box with leftward-pointing arrowheads. pRSVCat (13) was used as a positive transfection efficiency control in all experiments. The S1 nuclease probe used to quantitate all plasmid-derived transcripts is a 336-bp *EcoRI* fragment from pRSVCat. This probe is predicted to protect 287 bases at the 5' end of mRNA transcribed from pRSVCat and 254 bases transcribed from either SVCat or H5Cat (see Fig. 2). (B) S1 nuclease analysis of histone H5 gene promoter test mutants in HD6 and CEF cells. Erythroid (HD6; 1) and fibroblast (CEF; 30) cells were cotransfected with either test gene (A) plus equimolar pRSVCat with DEAE-dextran (29, 31) or CaPO_4 (15, 25), respectively. An equal amount of recovered RNA (5, 12) was analyzed in each lane. Lanes: 1, RNA recovered from HD6 cells transfected with H5Cat and pRSVCat; 2, RNA recovered from HD6 cells transfected with H5Cat/RSV and pRSVCat; 3, RNA recovered from primary CEF transfected with H5Cat and pRSVCat; 4, RNA recovered from primary CEF transfected with H5Cat/RSV and pRSVCat. (C) Primer extension analysis of histone H5 promoter test mutants. RNA samples identical to those tested for panel B were analyzed by reverse transcription with a 24-nucleotide *cat* antisense primer (see text). A 118-nucleotide extension product would be expected for transcripts from pRSVCat, while a 353-nucleotide product would be expected from H5Cat if transcripts initiate correctly from the H5 cap site (20, 27).

specific element is likely to be present in the 5' region of the H5 locus.

In an attempt to localize the (presumptive) second erythroid cell-specific genetic element in the H5 locus, a 580-base-pair (bp) *RsaI* fragment (which extends from positions -312 to +268 of the H5 gene; 20, 27) was inserted into pSV0Cat (14) in the correct transcriptional orientation at the *HindIII* site 5' to the *cat* structural gene. This plasmid (termed H5Cat; Fig. 1A) was cotransfected with pRSVCat into erythroid (HD6) and nonerythroid (CEF) cells; the recovered cellular RNA was tested by S1 nuclease with an RSVCat probe (Fig. 1A). H5Cat is expressed at low levels in HD6 cells (Fig. 1B, lane 1) and is not expressed in CEF (Fig.

1B, lane 3). Thus, the 580-bp histone H5 gene *RsaI* 5' fragment contains erythroid cell-specific regulatory sequences distinct from those already identified in the 3' H5E.

In attempts to localize the regulatory element(s) within the 580-bp H5 5' *RsaI* DNA fragment, the Rous sarcoma virus (RSV) long terminal repeat (LTR) was inserted into the *BamHI* site lying 3' to the SV40 early region termination signal of H5Cat in the reverse transcriptional orientation relative to the H5 promoter (H5Cat/RSV; Fig. 1A). The RSV LTR was added to determine the effects of an enhancer having limited cell type specificity on the expression characteristics of H5Cat. We reasoned that analysis of the H5 promoter would be greatly facilitated if this promoter would respond to the RSV enhancer (RSVE), since this might allow an increase in absolute transcript abundance and this amplification might aid in the interpretation of subsequent promoter deletion analysis.

H5Cat and H5Cat/RSV were cotransfected into HD6 cells with pRSVCat and then assayed by S1 nuclease protection. RSV LTR-stimulated transcription from H5Cat was approximately fivefold in HD6 cells (Fig. 1B, lanes 1 and 2). To verify that the H5 promoter was indeed present in the test plasmid, primer extension analysis of these RNAs was performed. The 5' nucleotide of the *cat*-specific primer is 54 bases from the *HindIII* site (the insertion site of the 580-bp H5 *RsaI* 5' fragment) in pSV0Cat (14), and a 353-nucleotide extension product is expected from mRNA which correctly initiates transcription from the H5 promoter. Both H5Cat and H5Cat/RSV generated mRNAs which initiated transcription at the H5 cap site in HD6 cells (Fig. 1C, lanes 1 and 2), demonstrating both that the H5 promoter was intact in the H5Cat plasmid and that the H5 promoter could respond to a nonspecific enhancer element in erythroid cells.

Having established that the H5 promoter could respond to the RSVE element in erythroid cells, we also wished to determine whether or not the H5 promoter would also be active in nonerythroid cells under the same conditions and therefore transfected the identical DNA samples into CEF. Although H5Cat was not expressed in CEF when cotransfected with pRSVCat (Fig. 1B, lane 3), inclusion of the RSVE element in this plasmid induced transcription from the H5 promoter in nonerythroid cells (Fig. 1B and C, lanes 3 and 4). (We are uncertain as to the origin of the primer extension product that was ca. 50 nucleotides longer than the correctly initiated H5 transcript in CEF cells [Fig. 1C, lane 3]. Since S1 analysis of the identical RNA sample showed that H5Cat mRNA did not accumulate [Fig. 1B, lane 3] we feel that it is unlikely that the primer extension product was derived from the H5Cat promoter.)

The results of the previous experiment indicate either that the H5 promoter is intrinsically tissue specific or that an additional tissue-specific enhancer exists within the 580-bp H5 *RsaI* 5' fragment. We had previously tested the 5' segment of this *RsaI* fragment (nucleotides -385 to -47; 20) in both orientations in the SV40 enhancer trap and had been unable to detect enhancer activity (29). We subsequently tested the 3' segment of this fragment (-47 to +268) in the enhancer trap and could not detect enhancer activity in erythroid cells (data not shown).

To address the possibility that our inability to detect enhancer activity was due to destruction of a presumptive 5' enhancer sequence (by cleavage of the 580-bp H5 fragment precisely within the enhancer), we inserted the entire 580-bp *RsaI* H5 fragment into SVCat at the *BamHI* site 3' to the *cat* gene in both orientations (Fig. 2A). These plasmids were cotransfected into HD6 cells with pRSVCat, and the *cat*

FIG. 2. The histone H5 5' *RsaI* fragment does not contain an enhancer. (A) SVCat was constructed by removing the SV40 enhancer element from pSV2Cat by digestion with *AccI* and *SphI* (14). SVCat was further modified by insertion of the 580-bp histone H5 *RsaI* fragment into the *BamHI* site 3' to the SV40 termination cassette in both orientations [SVCat/Rsa(+)] and [SVCat/Rsa(-)]. The diagrams have the same designations as those in Fig. 1. (B) S1 nuclease analysis of RNA recovered from HD6 cells cotransfected with test genes (10 μ g) plus pRSVCat (2 μ g). Lanes: 1, SVCat plus pRSVCat; 2, SVCat/RSA(+) plus pRSVCat; 3, SVCat/RSA(-) plus pRSVCat.

transcript levels from the plasmids containing or lacking the histone H5 5' *RsaI* fragment were examined (Fig. 2B, lanes 2 and 3 versus lane 1). We were once again unable to detect enhancer activity in the H5 *RsaI* 5' fragment. We therefore conclude that the additional tissue-specific element resident in the 5' *RsaI* fragment of the H5 locus (Fig. 1B) cannot function as an enhancer, although this conclusion must be tempered with the caveat that it is based on the results of two independent negative experimental results.

To localize the 5' tissue-specific genetic element within the histone H5 gene promoter, several promoter deletions of H5Cat were constructed and tested in the presence of the histone H5E placed 3' to the *cat* gene. These mutations contained H5 sequences from -312 to -2, -47 to +268, and -2 to +268 in the sense transcriptional orientation (Fig. 3A). The results of transfection of these plasmids into both cell types showed that none of the promoter deletions were expressed as well as the entire 5' 580-bp *RsaI* H5 fragment. The autoradiographs were quantitated by densitometric scanning, and the results were normalized to the RSVCat control cotransfection transcript levels (data not shown). Analysis of these data indicated that all of the promoter deletions were expressed at at least fivefold higher levels than pSV0Cat and between three- and fivefold more poorly than the whole 580-bp promoter. The -312 to -2 promoter (Fig. 3B, lane 3) accumulated the lowest relative level of the hybrid gene transcript and was the only H5 promoter construct which was not expressed more efficiently than the control transfection plasmid. Clearly, the absolute differences in signal intensities were small in comparisons between deletion mutations (Fig. 3B).

To investigate the role of the 5' flanking region in regulating H5 expression, the levels of transcription in two of the promoter test genes [H5Cat and H5Cat (Δ -312/-47); Fig. 3A] were compared after placement of either the RSVE or the H5E 3' to the *cat* gene. These four plasmids were individually cotransfected (with pRSVCat) into HD6 and into differentiation-induced HD3 cells, and equal weights of RNAs recovered from these transfections were analyzed by S1 nuclease protection. The results of these transfection experiments indicate that deletion of the -312 to -47 segment of the H5 locus reduced the transcript level more dramatically in the presence of the RSVE (3.5-fold) than in

FIG. 3. Localization of the histone H5 gene promoter. (A) Promoter deletion mutations of H5Cat. The mutations were prepared by using convenient restriction enzyme sites to create deletions from the promoter of nucleotides -312 to -47 (line 2), -2 to +268 (line 3), or -312 to -2 (bottom line). All of the mutations were tested in the presence of the histone H5E. The diagrams have the same designations as those in Fig. 1. (B) S1 nuclease analysis of promoter deletion mutations. HD3 cells were transfected with each of the test genes (10 μ g) plus pRSVCat (2 μ g) and induced for erythroid differentiation. A 50- μ g sample of recovered RNA was tested by S1 nuclease protection as described in the text. Lanes: 1, H5Cat/H5E; 2, H5Cat (Δ -312/-47); 3, H5Cat (Δ -2/+268); 4, H5Cat (Δ -312/-2); 5, pSV0Cat (Fig. 1A).

the presence of the H5E (1.7-fold) in immature (HD6) cells (Fig. 4A). The results obtained with the H5E and the -47 to +268 promoter construct are thus consistent with previous conclusions on analysis of H5* deletion mutations transfected into HD6 cells (29). In differentiation-induced erythroid cells (42°C HD3), the reduction seen upon deletion of -312 to -47 was about 2-fold in the presence of the RSVE and 3.5-fold in the presence of the H5E (Fig. 4B).

Under all of the conditions examined, the H5E-containing plasmids were expressed at a much higher level in differentiated cells (Fig. 4B, lane 3) than were the RSVE-containing plasmids (Fig. 4B, lane 1), while the reverse was true in transfected immature cells (Fig. 4A, lanes 1 and 3), confirming the conclusions concerning the role of the H5E in the temporal induction of histone H5. All of these plasmids contain the H5 promoter, but the plasmids which include the histone H5E are preferred transcription substrates in erythroid cells after HD3 cell differentiation.

FIG. 4. Enhancer response of the histone H5 gene promoter. Two of the promoter test mutants [H5Cat and H5Cat (Δ -312/-47)] were tested for transcriptional activity by S1 nuclease assay in the presence of both the H5E and the RSVE. Equal weights (50 μ g) of all of the recovered RNAs were tested. Panel A represents the results from transfection into HD6 cells, and panel B represents the results of transfection of the identical plasmids in the same order into HD3 cells, followed by differentiation induction. Lanes: 1, H5Cat/RSVE plus pRSVCat; 2, H5Cat (Δ -312/-47)/RSVE plus pRSVCat; 3, H5Cat/H5E plus pRSVCat; 4, H5Cat (Δ -312/-47)/H5E plus pRSVCat; 5, pRSVCat alone.

In conclusion, we have shown that two erythroid cell-specific *cis*-acting regulatory elements were identified in the histone H5 locus. One is an enhancer element which is found 3' to the gene (29) and is differentially active in a manner directly dependent on the erythroid cell maturation state, while the other(s) is contained within a 580-bp 5' fragment capable of generating a reduced level of the correctly initiated tissue-specific transcript from a reporter gene in immature erythroid cells. The 3' histone H5E was more active in differentiation-induced erythroid cells than in immature cells in two separate assays: increased T-antigen synthesis from the SV40 enhancer trap (data not shown) and increased steady-state levels of the SVCat/H5E transcript (relative to the SVCat transcript) in differentiated HD3 cells (29).

Although the H5 promoter is unable to function in non-erythroid cells, it does respond to a viral enhancer element which is not tissue restricted in both erythroid and nonerythroid cells, indicating that the tissue specificity of the promoter element can be overcome. Previous deletion mutagenesis failed to detect any tissue-specific negative regulatory elements in the H5 locus (29). These data are most consistent with the existence of a cell type-independent promoter-erythroid cell-specific upstream promoter element (21) combination within the 580-bp 5' H5 *Rsa*I fragment we studied in this analysis, since we have been unable to detect enhancer activity in this fragment by two separate assays in which other erythroid cell-specific enhancers have been shown to be active (6, 29).

An enhancerlike activity was reported to exist in the H5 gene between positions -90 and +313 (33). This activity was detected in a microinjection assay in *Xenopus laevis* oocytes when plasmids containing the histone H5 gene (and other histone genes) were coinjected with various nuclear extract protein fractions prepared from avian erythroblastosis virus-transformed erythroid progenitor cells. The enhancerlike activity could reside within the 45-bp segment of the H5 gene, which differs between the test genes used in the assays reported here and the previously published assays (33), or the discrepancy may be the result of the differences between the two assay systems used. We are unable to explain these differences but do not feel that the low-level expression of the H5 promoter deletion mutations could be due entirely to lack of an enhancer element, since the enhancers (both H5E and RSVE) artificially restructured into the reporter gene plasmids tested here should complement the removal of any other (putative 5') H5E element.

The data presented here show that the 580-bp H5 fragment contains a tissue-specific promoter which can function in enhancer-independent fashion in erythroid cells but is functional in fibroblasts only in the presence of a viral (RSV) enhancer element. This finding distinguishes the H5 promoter from most other tissue-specific promoters which do not respond to active enhancers in heterologous cell types (10, 11, 17, 22, 26). The rat albumin promoter may, therefore, be the closest model available for the histone H5 promoter, since a 150-bp region of the albumin promoter immediately 5' to the cap site is sufficient to induce tissue-specific expression of a reporter gene in a transient expression assay but expression in non-albumin-producing cells is restored upon addition of the S40 enhancer and no independent enhancer activity has been found in this region of the albumin gene (18).

The nature of the H5 tissue-specific promoter defined by the experiments reported here is not completely clear, since we were unable to precisely localize its activity in examination of a rather simple promoter deletion set. H5 sequences

both 5' and 3' to position -2 contributed to the accumulated reporter gene transcript level in erythroid cells; -312 to -2 and -47 to +268 histone H5 promoters were not expressed significantly more efficiently than a -2 to +268 promoter, and none of them were expressed at the level of a -312 to +268 promoter. Possible explanations for these results are that the -312 to -2 promoter does not contain an efficiently utilized transcription initiation site or that a regulatory element which affects H5 transcription is 3' to position -2 of the gene.

The data in Fig. 4 are most consistent with the interpretation that an element lies within the -312 to -47 histone H5 5' fragment that is crucial to the response of this promoter to enhancers. In immature erythroid cells (as represented by HD6), the RSVE stimulated transcription from the H5 promoter approximately fourfold (Fig. 1A, lanes 1 and 2); this is similar to the level of the reduction in the accumulated *cat* message that was seen when the -312 to -47 sequences were removed from the H5Cat gene in the presence of the RSVE (Fig. 4A, lanes 1 and 2). However, deletion of this region of the H5 locus had little, if any, effect on the accumulated H5Cat transcript levels in immature cells in the presence of the H5E (Fig. 4A, lanes 3 and 4), which is itself only a weak enhancer in immature cells (29). In contrast, in differentiation-induced erythroid cells (42°C HD3), removing the -312 to -47 region of the H5 gene had a 3.5-fold effect in the presence of the H5E (congruent with the previously established four- to fivefold transcriptional stimulation of H5 promoter-directed *cat* gene transcription upon addition of the H5E; 29), but a substantially smaller effect was detected with the RSVE in differentiated cells (Fig. 4B). The decrease in accumulated *cat* mRNA abundance caused by deletion of the -312 to -47 histone H5 gene sequence thus correlated, in three cases tested, with the independently assayed activity of the RSVE and 3' H5E elements in the erythroid cell types used in these studies. Although we did not conclusively demonstrate that sequences within the -312 to -47 5' histone H5 gene fragment are involved in enhancer response, we were unable to arrive at another interpretation which is consistent with all of the available data; deletion of this 5' element had a differential effect on accumulated transcript levels in erythroid cells when two different enhancers were examined. Although the accumulated *cat* mRNA abundance derived by transcription of the whole 580-bp (-312 to +268) H5 gene promoter was dependent on the enhancer used, the transcript generated in a -312 to -47 deletion mutation promoter did not vary in abundance with the enhancer type.

We thank S. J. Stamler for technical assistance in several of these experiments, D. Linzer for the gift of the antisense *cat* primer, and many colleagues in the laboratory for patient discussions and fruitful suggestions.

This work was partially supported by Molecular and Cellular Biology training grant GM 08061 to Northwestern University from the National Institute of General Medical Sciences and by Public Health Service grant GM 28896 to J.D.E. from the National Institutes of Health.

LITERATURE CITED

1. Beug, H., G. Doederlien, C. Freudenstein, and T. Graf. 1982. Erythroblast cell lines transformed by a temperature-sensitive mutant of avian erythroblastosis virus: a model system to study erythroid differentiation *in vitro*. *J. Cell. Physiol.* 1:195-207.
2. Beug, H., S. Palmieri, C. Freudenstein, H. Zentgraf, and T. Graf. 1982. Hormone-dependent terminal differentiation *in vitro* of chicken erythroleukemia cells transformed by ts mutants of avian erythroblastosis virus. *Cell* 28:907-919.

3. Beug, H., A. von Kirschbach, G. Doederlein, J. Conscience, and T. Graf. 1979. Chicken hematopoietic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. *Cell* 18:375-390.
4. Brasch, K., G. H. M. Adams, and J. M. Neelin. 1974. Evidence for erythrocyte-specific histone modification and structural changes in chromatin during goose erythrocyte maturation. *J. Cell Sci.* 15:659-677.
5. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299.
6. Choi, O.-R., and J. D. Engel. 1986. A 3' enhancer is required for temporal and tissue-specific transcriptional activation of the chicken adult β -globin gene. *Nature (London)* 323:731-734.
7. Choi, O.-R. B., and J. D. Engel. 1988. Developmental regulation of β -globin gene switching. *Cell* 55:17-26.
8. Dalton, S., J. R. Coleman, and J. R. E. Wells. 1986. Transcription of the histone H5 gene is not S-phase regulated. *Mol. Cell. Biol.* 6:601-606.
9. Dardick, I., and G. Setterfield. 1976. Volume of condensed chromatin in developing primitive-line erythrocytes of chick. *Exp. Cell Res.* 100:159-171.
10. Edlund, T., M. D. Walker, P. J. Barr, and W. J. Rutter. 1985. Cell-specific expression of the rat insulin gene: evidence for role of two distinct 5' flanking elements. *Science* 230:912-916.
11. Foster, J., J. Stafford, and C. Queen. 1985. An immunoglobulin promoter displays cell-type specificity independently of the enhancer. *Nature (London)* 315:423-425.
12. Glisin, V., R. Crkvenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry* 13:2633-2637.
13. Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard. 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc. Natl. Acad. Sci. USA* 79:6777-6781.
14. Gorman, C. M., L. F. Moffat, and B. H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* 2:1044-1051.
15. Graham, F. L., and A. J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-467.
16. Graf, T., N. Ade, and H. Beug. 1978. Temperature sensitive mutant of avian erythroblastosis virus suggests a block in differentiation as a mechanism of leukaemogenesis. *Nature (London)* 275:496-501.
17. Grosschedl, R., and D. Baltimore. 1985. Cell type specificity of immunoglobulin gene expression is regulated by at least three DNA sequence elements. *Cell* 41:885-897.
18. Heard, J., P. Herbomel, M. Ott, A. Mottura-Rollier, M. Weiss, and M. Yaniv. 1987. Determinants of rat albumin promoter tissue specificity analyzed by an improved transient expression system. *Mol. Cell. Biol.* 7:2425-2434.
19. Hesse, J. E., J. M. Nikol, M. R. Lieber, and G. Felsenfeld. 1986. Regulated gene expression in transfected primary chicken erythrocytes. *Proc. Natl. Acad. Sci. USA* 83:4312-4316.
20. Krieg, P. A., A. J. Robins, R. D'Andrea, and J. R. E. Wells. 1983. The chicken H5 gene is unlinked to core and H1 histone genes. *Nucleic Acids Res.* 11:619-627.
21. Maniatis, T., S. Goodbourn, and J. A. Fischer. 1987. Regulation of inducible and tissue-specific gene expression. *Science* 236:1237-1244.
22. Mason, J. O., G. T. Williams, and M. S. Neuberger. 1985. Transcription cell type specificity is conferred by an immunoglobulin VH gene promoter that includes a functional consensus sequence. *Cell* 41:479-487.
23. Molgaard, H. V., M. Perucho, and A. Ruiz-Carrillo. 1980. Histone H5 messenger RNA is polyadenylated. *Nature (London)* 283:502-504.
24. Neelin, J. M. 1968. Common histones of avian erythropoiesis. *Can. J. Biochem.* 46:241-247.
25. Parker, B. A., and G. R. Stark. 1979. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus or viral DNA. *J. Virol.* 31:360-369.
26. Picard, D., and W. Schaffner. 1985. Cell type preference of immunoglobulin κ and λ gene promoters. *EMBO J.* 4:2831-2838.
27. Ruiz-Carrillo, A., M. Affolter, and J. Renaud. 1983. Genomic organization of the genes coding for the six main histones of the chicken: complete sequence of the H5 gene. *J. Mol. Biol.* 170:843-859.
28. Sotirov, N., and E. W. Johns. 1972. Quantitative difference in the content of the distal F2C between chicken erythrocytes and erythroblasts. *Exp. Cell Res.* 73:13-16.
29. Trainor, C. D., S. J. Stamler, and J. D. Engel. 1987. Erythroid-specific transcription of the chicken histone H5 gene is directed by a 3' enhancer. *Nature (London)* 328:827-830.
30. Vogt, P. K. 1969. Focus assay of Rous sarcoma virus, p. 198-211. In K. Habel and N. P. Salzman (ed.), *Fundamental techniques in virology*. Academic Press, Inc., New York.
31. Weber, F., J. deVilliers, and W. Schaffner. 1984. An SV40 "enhancer trap" incorporates exogenous enhancers or generates enhancers from its own sequences. *Cell* 36:983-992.
32. Weintraub, H. 1978. The nucleosome repeat length increases during erythropoiesis in the chick. *Nucleic Acids Res.* 5:1179-1188.
33. Wigley, P. L., and J. R. E. Wells. 1987. H5 gene-specific *trans*-activation by nuclear extracts from avian erythroid cells. *Mol. Cell. Biol.* 7:3853-3856.
34. Zenke, M., P. Kahn, C. Disela, B. Vennstrom, A. Leutz, K. Keegen, M. Hayman, H.-R. Choi, N. S. Yew, J. D. Engel, and H. Beug. 1988. *V-erbA* specifically suppresses transcription of the avian erythrocyte anion transporter (band 3) gene. *Cell* 52:107-119.