MOLECULAR AND CELLULAR BIOLOGY, June 1982, p. 617–624
0270-7306/82/060617-08$02.00/0
Vol. 2, No. 6

Transcripts from the Cellular Homologs of Retroviral Oncogenes: Distribution Among Chicken Tissues

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Received 5 November 1981/Accepted 27 January 1982

The oncogenes (v-*onc* genes) of rapidly transforming retroviruses have homologs (c-*onc* genes) in the genomes of normal cells. In this study, we characterized and quantitated transcription from four c-*onc* genes, c-*myb*, c-*myc*, c-*erb*, and c-*src*, in a variety of chicken cells and tissues. Electrophoretic analysis of polyadenylated RNA, followed by transfer to nitrocellulose and hybridization to cloned *onc* probes showed that c-*myb*, c-*myc*, and c-*src* each give rise to a single mature transcript, whereas c-*erb* gives rise to multiple transcripts (B. Vennstrom and J. M. Bishop, Cell, in press) which vary in abundance among different cells and tissues. Transcription from c-*myb*, c-*myc*, c-*erb*, and c-*src* was quantitated by a "dot-blot" hybridization assay. We found that c-*myc*, c-*erb*, and c-*src* transcription could be detected in nearly all cells and tissues examined, whereas c-*myb* transcription was detected only in some hemopoietic cells; these cells, however, belong to several different lineages. Thus, in no case was expression of a c-*onc* gene restricted to a single cell lineage. There appeared to be a correlation between levels of c-*myb* expression and hemopoietic activity of the tissues and cells examined, which suggests that c-*myb* may be expressed primarily in immature hemopoietic cells. An examination of c-*onc* RNA levels in target cells and tissues for viruses carrying the corresponding v-*onc* genes revealed no obvious correlation, direct or inverse, between susceptibility to transformation by a given v-*onc* gene and expression of the homologous c-*onc* gene.

In the past few years, it has become apparent that most rapidly oncogenic retroviruses cause cell transformation in vitro and tumors in vivo by the expression of specific transforming genes or oncogenes (J. M. Bishop, Adv. Cancer Res., in press). These genes have homologs in normal cells; indeed, it is believed that rapidly oncogenic retroviruses have arisen by the incorporation of these cellular genes into the genomes of preexisting, replication-competent retroviruses which lack oncogenes.

The first such oncogene to be identified and characterized was the *src* gene of Rous sarcoma virus (RSV); convincing evidence has accumulated which indicates that *src* is responsible for the oncogenicity of RSV (38). It has more recently become clear that the avian acute or defective leukemia viruses (DLVs) also possess oncogenes (25, 28, 29). The avian myeloblastosis (AMV), erythroblastosis (AEV), and myelocytomatosis (MCV) viruses each cause distinct types of leukemias; in addition, both MCV and AEV cause other types of tumors. Table 1 summarizes in vitro transformation by and in vivo tumorigenicity of RSV, AMV, AEV, and MCV.

Several studies have shown that the various DLVs have different target cells (10, 15), although the range of target cell types for AMV and MCV overlap (9, 10). This is reflected in the oncogenic properties of the DLVs (Table 1) and in the observation that the transformed cells exhibit properties of the hemopoietic lineage to which the target cells belong. Thus it has been shown that the AEV target cell is an early erythroid cell (11), whereas AMV and MCV transform cells of the myelomonocytic lineage (9, 10, 15). Since the viral oncogenes (v-*onc* genes) dictate the target cell specificity of each virus (25, 29) and since they are derived from normal cellular genes, it has seemed possible that the function of the cellular homologs of the viral oncogenes (abbreviated here to c-*onc*) may be restricted to particular cell types or lineages. (The use of the abbreviation c-*onc* is a convenience and is not meant to imply that these genes are oncogenes; this nomenclature is described by Coffin et al. [3a].) In fact, it has been suggested that the target cells for the various DLVs are cells in which the corresponding c-*onc* genes are expressed (13, 14).

In this study, we quantitated the RNA transcribed from each of four c-*onc* genes, c-*myb*, c-*myc*, c-*erb*, and c-*src*, in a variety of chicken tissues and cells. Specifically, we wished to determine whether there are significant varia-

TABLE 1. Tumorigenesis and transformation by AMV, MCV, AEV, and RSV

Virus	Oncogene	Tumor in vivo	Transformation in vitro
AMV	*myb*	Myeloblastosis	Macrophages
MCV	*myc*	Carcinoma	Macrophages
		Myelocytomatosis	Fibroblasts
		Sarcoma	
AEV	*erb*	Erythroblastosis	Erythroid progenitors
		Sarcoma	Fibroblasts
RSV	*src*	Sarcoma	Fibroblasts

tions in the level of expression of these genes in different cells and tissues, whether expression of these genes is limited to any particular cell types or lineages, and whether any clear relationship exists between c-*onc* expression in a given cell type and its susceptibility to transformation by viruses carrying the corresponding v-*onc* genes.

MATERIALS AND METHODS

Animals and cell lines. White leghorn chickens and embryos were used for all uninfected tissue and cell preparations. Yolk sac cells and adherent macrophage cultures were prepared as described (20); the nonadherent cells were removed 4 days after isolation of the yolk sac cells. Fibroblasts were prepared as described (37). Bone marrow cells were obtained by flushing the femurs of 2- to 4-week-old chickens with Tris-glucose-saline (140 mM NaCl, 5 mM KCl, 6 mM glucose, 25 mM Tris, pH 7.4). Cell suspensions from thymus, spleen, and bursa were prepared by teasing the organs apart with dissecting needles and then squeezing the resultant slurry through a stainless steel mesh to isolate single cells. Preparations further enriched for lymphocytes were obtained as follows. The cell suspensions were centrifuged onto Ficoll-Hypaque (2,000 rpm for 30 min) to pellet dead cells and erythrocytes (22); the cells from the interface were washed, then incubated in RPMI 1640 containing 10% fetal calf serum in T-flasks for 16 h at room temperature followed by 1 h at 37°C, and the nonadherent cells were collected. The peripheral leukocytes were obtained from chicken blood as follows. After the addition of heparin, the blood was centrifuged at 2,000 rpm for 5 min, and the buffy coat was removed from the erythrocyte-plasma interface. These cells were then centrifuged onto Ficoll-Hypaque as described above, and the cells from this interface were collected and washed. The following transformed cell lines were used: erythroblasts transformed by AEV and by the temperature-sensitive mutant of AEV (*ts*34; kindly provided by T. Graf); the avian leukosis virus (ALV)-induced lymphoma cell line, 1104B-1 (17); the Marek's disease virus-induced T-cell leukemia line, MSB-1 (1); and the AMV-transformed nonproducer myeloblast line BM-2, obtained from C. Moscovici. A mass infection of fibroblasts with AEV was used to obtain AEV-transformed fibroblasts.

RNA preparation. Cells were washed by centrifugation with Tris-glucose-saline and resuspended in 0.1 M NaCl–10 mM Tris (pH 7.4)–1 mM EDTA at 10^7/ml. Whole organs were rinsed with Tris-glucose-saline and cut into small pieces. Proteinase K was added to 200 μg/ml, followed by sodium dodecyl sulfate (SDS) to 1%. Whole organ preparations were disrupted in a Virtis tissue homogenizer until a homogeneous suspension was obtained. The cells or organ digests were incubated at 37°C for 1 h, then homogenized in the Virtis for 1 min at medium speed. The homogenates were stirred with 2 to 3 ml packed volume of oligodeoxythymidylic acid-cellulose after adjusting the NaCl concentration to 0.4 M. After 1 to 2 h the oligodeoxythymidylic acid-cellulose was collected by centrifugation and poured into a column. The column was washed with 6 volumes of 0.4 M NaCl–10 mM Tris (pH 7.4)–1 mM EDTA–0.1% SDS and then with 2 volumes of 0.1 M NaCl–10 mM Tris (pH 7.4)–1 mM EDTA–0.1% SDS. Polyadenylated RNA was eluted with 4 volumes of 10 mM Tris (pH 7.4)–0.1 mM EDTA–0.1% SDS and concentrated by ethanol precipitation after adding sodium acetate to 0.2 M. Initially, we found that in RNA preparations from some whole organs, a small amount of high-molecular-weight DNA remained. Also, preparations from some normal cells and some whole organs gave absorbance at 260 nm/absorbance at 280 nm ratios that were unsatisfactory. To overcome these problems, all RNA preparations from whole organs and primary cell preparations were treated with 10 μg of RNase-free DNase I per ml for 20 min at room temperature in 10 mM Tris (pH 7.4)–7 mM $MgCl_2$. After adding SDS to 0.5% and EDTA to 10 mM, the RNA preparations were extracted with phenol-chloroform and ethanol-precipitated. This was not necessary for RNA from transformed cells or from fibroblasts. All RNAs used had absorbance at 260 nm/absorbance at 280 nm ratios greater than or equal to 1.9:1.

Analysis of polyadenylated RNA by formaldehyde-agarose gel electrophoresis. Samples of 4 μg of RNA were analyzed by formaldehyde-agarose gel electrophoresis as described (19), except that the buffer used was 20 mM morpholinepropanesulfonic acid (pH 7.0)–5 mM sodium acetate–1 mM EDTA and the gels were cast as slabs. RNA was transferred to nitrocellulose (34) and hybridized to radiolabeled DNA probes. Hybridization was carried out as previously described (12) either for 12 to 16 h in the presence of 10% dextran sulfate (39) or for 3 days without dextran sulfate. Washing and autoradiography have been described (12). Size standards were chicken rRNAs, which were visualized by staining with ethidium bromide.

Dot-blot hybridizations. These were performed essentially as described by Thomas (34). Nitrocellulose sheets were wetted with water and then soaked in 3 M NaCl–0.3 M Na_3 citrate. The sheets were dried, and then RNA solutions (2 μl) in 10 mM Tris–1 mM EDTA were spotted onto the filters. RNA dilutions were approximately threefold, such that every second spot was a 10-fold dilution. The most concentrated sample was usually 1 mg/ml. The sheets were dried and baked at 80°C for 2 h under vacuum. Hybridization, washing, and autoradiography were carried out as described above for RNA transferred from gels, except that preflashed film was used (18).

Preparation of DNA probes. Radiolabeled probes were prepared by copying cloned DNA templates with AMV DNA polymerase, using randomly cleaved calf thymus DNA primers as described (12, 23). Templates

used were: for *src* probe, the 0.8-kilobase (kb) *Pvu*II fragment E of cloned RSV DNA (5); for *erb* probe, the 2.5-kb *Pvu*II fragment of cloned AEV DNA (35); for *myc*, the 1.5-kb *Pst*I fragment of cloned MCV DNA (36), or a 3.0-kb *Sac*I fragment of cloned c-*myc* DNA, which contains most of the sequences homologous to v-*myc* (B. Vennstrom, D.S., and J.M.B., manuscript in preparation; this probe was kindly provided by G. Payne); and for *myb*, a 1.3-kb *Hin*dIII fragment of cloned c-*myb* DNA, which contains about 25% of the sequences homologous to v-*myb* (T.J.G., unpublished data). The c-*myc* clones were isolated after screening a "library" of chicken genomic DNA fragments in the bacteriophage Charon 4A with AMV-specific cDNA (T.J.G., unpublished data); probe made from the 1.3-kb *Hin*dIII fragment reacts only with AMV-specific viral RNA species and the cellular RNA species described below (T.J.G., unpublished data). All of these templates were derived from plasmid subclones.

Quantitation of dot-blot data. The standards used for determining c-*src* and c-*myc* levels were either chicken embryo fibroblast RNA or whole chicken embryo RNA, which both give $C_rt_{1/2}$ values of 1.5×10^4 when hybridized with *src*-specific cDNA (32, 33, 35) and 5×10^3 when hybridized with *myc*-specific cDNA (25, 30). Fibroblast RNA was also used for the c-*erb* standard ($C_rt_{1/2} = 1.5 \times 10^4$) (25, 26). These values were used to calculate the proportion of polyadenylated RNA comprised by each c-*onc* RNA, on the basis of 2% of the total RNA being polyadenylated, and assuming that all of the c-*onc* RNA is polyadenylated (3, 31). The *myb* standard was a preparation of AMV 70S virion RNA which had previously been analyzed by solution hybridization with AMV-specific cDNA (12); the $C_rt_{1/2}$ value was 6×10^{-2}, which implies that one third of this preparation was AMV genomic RNA. Thus, after correction for the size of the c-*myb* transcript, the amount of c-*myb* RNA could be determined. (The highest concentration of virion RNA used was 10 μg/ml.)

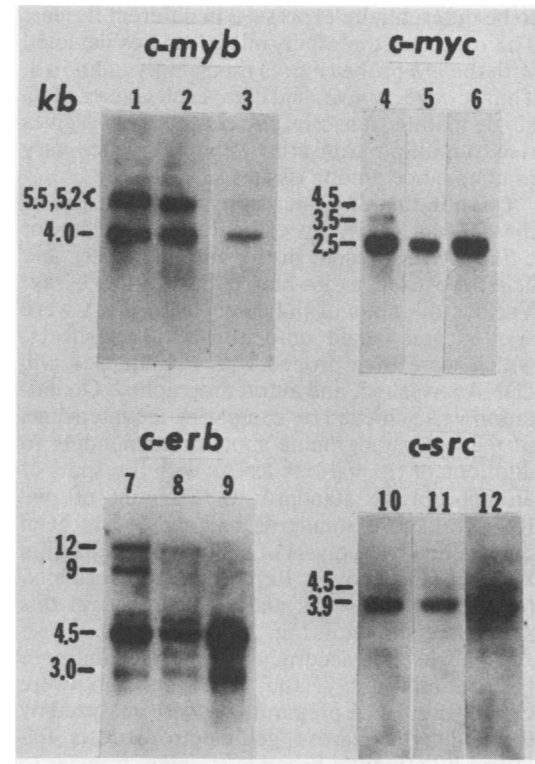

FIG. 1. Autoradiograms showing sizes of c-*onc* RNAs in several chicken cells and tissues. Polyadenylated RNA was fractionated on formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized to *onc*-specific DNA probes, as indicated at the top of each panel. RNA was from: lanes 1 and 8, thymus; lanes 2 and 9, yolk sac; lanes 3 and 6, bone marrow; lanes 4, 7, and 10, fibroblasts; lane 5, bursa; lane 11, macrophages; and lane 12, spleen.

RESULTS

Sizes of c-*onc* transcripts. To identify transcripts from the four c-*onc* genes, polyadenylated RNA from various cells and tissues was denatured and electrophoresed through formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized to the *onc*-specific probes described above. Figure 1 shows the results obtained from several representative cells and tissues. In all tissues and cells used in this study, the mature transcripts for c-*src* and c-*myc* were RNAs of 3.9 and 2.5 kb, respectively, as previously reported (31; R. Parker, H. E. Varmus, and J. M. Bishop, Proc. Natl. Acad. Sci. U.S.A., in press). The mature c-*myb* transcript was a 4.0-kb species, which was not detected, however, in all tissues (see below). This size is not consistent with the sedimentation coefficient of 21S reported by Chen (3) for the c-*myb* transcript, although the latter value was determined under nondenaturing conditions. The other, larger species detected by the *myb* and *myc* probes are found only in the nucleus (unpublished data) and thus are probably precursors of the smallest cytoplasmic species; we suspect this is also the case for the larger c-*src* transcript. The *erb*-specific probe, on the other hand, detected several major transcripts, of 12.0, 9.0, 4.5, and 3.0 kb (B. Vennstrom and J. M. Bishop, Cell, in press), the relative abundance of which varied among the cells and tissues examined; in particular, the 12.0- and 9.0-kb species were not detectable in some tissues. All four of these major species are cytoplasmic (B. Vennstrom and J. M. Bishop, Cell, in press) and thus are not precursor RNAs. Furthermore, the 12.0- and 9.0-kb species have been shown to contain sequences from the 3′ or "B" domain of *erb*, whereas the 4.5- and 3.0-kb species contain sequences from the 5′ or "A" domain (B. Vennstrom and J. M. Bishop, Cell, in press); hence, the two domains of *erb* appear

to be differentially expressed in different tissues. The nature of the other, minor species detected with the *erb* probe (Fig. 1) is currently unknown. Thus, c-*myb*, c-*myc*, and c-*src* each give rise to a single mature transcript; by contrast, c-*erb* gives rise to multiple transcripts, some of which vary in abundance among tissues.

Quantitation of c-*onc* transcription. (i) Use of the dot-blot assay. To estimate the amounts of c-*onc* RNAs present in the various tissues and cells, we used a dot-blot hybridization assay. Various dilutions of polyadenylated RNA were spotted and baked onto nitrocellulose sheets, which were then probed with radiolabeled *onc* cDNAs, washed, and autoradiographed. Quantitation was achieved by comparing the intensities of the autoradiographic spots corresponding to dilutions of the various RNAs with the spots of an appropriate standard; the amount of *onc* RNAs in these standards had previously been determined by analysis of solution hybridization kinetics (see above). Representative dot-blots, for most of the cells and tissues used in this study, are shown in Fig. 2.

To ensure the accuracy and reproducibility of the dot-blot assay, the following tests were carried out. RNA preparations were analyzed by formaldehyde-agarose gel electrophoresis followed by ethidium bromide staining, transfer to nitrocellulose, and hybridization to cDNA probes. This enabled us to determine that our RNA preparations contained no more than 25% rRNA (by comparison with stained rRNA standards) and that they were intact. Most of the experiments shown in Table 2 were repeated either on duplicate dot-blots of the same RNA preparations, on separate RNA preparations or, in most cases, both. The results of such replicates usually agreed to within a factor of 3; there were some larger variations, however, which we cannot presently explain.

(ii) Salient features of the data. Examination of the data of Table 2 reveals several features of c-*onc* transcription. First, transcription from each c-*onc* gene could be detected in a wide range of uninfected tissues and cells; in fact, c-*myc*, c-*erb*, and c-*src* RNA could be detected in nearly all cells and tissues examined. By contrast, significant c-*myb* transcription was detected only in certain hemopoietic cells. The limit of detection of c-*myb* here was about 0.0002% of polyadenylated RNA. Secondly, the amount of the c-*onc* RNAs varied in different cells and tissues. Relatively little variation was observed in c-*erb* levels; there was a greater range in the values for c-*myc* and c-*src*, whereas c-*myb* levels showed the greatest variations.

To pursue our finding of a high level of c-*myb* transcription in the thymus, we prepared single-cell suspensions from the thymus and the other lymphoid organs, bursa, and spleen. The suspensions were prepared to dissociate the loosely

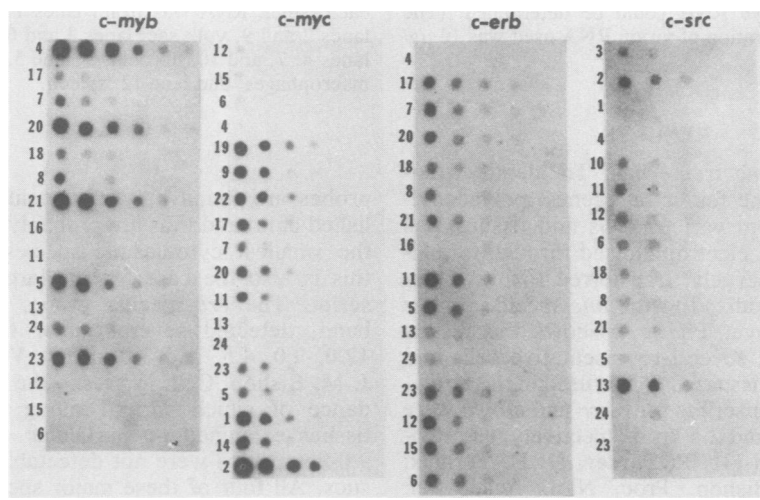

FIG. 2. Autoradiograms of representative dot-blots, hybridized with *onc*-specific DNA probes, as indicated at the top of each panel. The number at the left of each row of dots indicates the source of the RNA in each case: 1, AEV-transformed erythroblasts; 2, ALV-induced lymphoma cells; 3, AMV-transformed myeloblasts; 4, AMV 70S virion RNA; 5, bone marrow; 6, brain; 7, whole bursa; 8, bursa cell suspension; 9, bursal lymphocytes; 10, chicken embryo; 11, fibroblasts; 12, liver; 13, macrophages; 14, Marek's disease virus-transformed T cells; 15, muscle; 16, peripheral leukocytes; 17, whole spleen; 18, spleen cell suspension; 19, splenic lymphocytes; 20, whole thymus; 21, thymus cell suspension; 22, thymic lymphocytes; 23, yolk sac cells; 24, nonadherent yolk sac cells.

TABLE 2. Levels of c-*onc* RNAs in normal and transformed cells

Cell or tissue	% ($\times 10^{-3}$) c-*onc* RNA in total polyadenylated RNA of:[a]			
	c-*myb*	c-*myc*	c-*erb*	c-*src*
Normal				
Fibroblasts	0.2 (0, 0.34)	5 (std)[b]	2 (std)	2 (std)
Whole embryo	2 (NV)[c]	5 (std)	11 (2, 20)	2 (std)
Yolk sac cells	56 (34, 85)	5 (5, 6)	4 (3, 6)	0 (NV)
Bone marrow cells	34 (NV)	4 (3, 5)	2 (1.2, 3.4)	0.4 (0.2, 0.6)
Nonadherent yolk sac cells	10 (NV)	0.8 (0.5, 1)	0.7 (0.4, 1.2)	1 (0.6, 2)
Macrophages	0 (NV)	1 (NV)	0.7 (0.4, 1.2)	6 (4, 10)
Whole thymus	89 (51, 102)	5 (1.5, 8.5)	1 (0.6, 2)	2 (0.6, 2)
Whole bursa	5 (3.4, 5.5)	3 (1.5, 5)	1 (0.6, 2)	1 (0.4, 2)
Whole spleen	3 (1.7, 5.1)	4 (1.5, 5)	1 (0.6, 2)	6 (NV)
Thymus cell suspension	190 (170, 255)	8 (5, 15)	1 (0.6, 2)	3 (1.2, 6)
Bursa cell suspension	4 (3.4, 5.1)	3 (1.5, 5)	0.9 (0.6, 1.2)	0.3 (0.2, 0.6)
Spleen cell suspension	3 (1.7, 3.4)	7 (5, 8.5)	0.9 (0.6, 1.2)	2 (1.2, 2)
Thymic lymphocytes	31 (10, 51)	12 (8.5, 15)	0.3 (0.2, 0.4)	0.4 (—)[d]
Bursal lymphocytes	17 (—)	10 (—)	0.2 (—)	ND[e]
Splenic lymphocytes	17 (—)	15 (—)	0.2 (—)	ND
Peripheral leukocytes	3 (—)	8 (—)	0.9 (0.6, 1.2)	2 (—)
Brain	0 (NV)	0.3 (0.1, 0.5)	2 (1.2, 3.4)	0.7 (0.4, 1.2)
Liver	0 (NV)	0.5 (NV)	2 (1.2, 3.4)	0.6 (0.2, 1.2)
Muscle	0 (NV)	0.5 (NV)	2 (1.2, 3.4)	0 (—)
Transformed				
Marek's disease virus-transformed cells (MSB-1)	18 (5.1, 31)	7 (5, 8.5)	0.6 (NV)	0.3 (0.2, 0.4)
AEV erythroblasts	77 (51, 102)	10 (5, 15)	[>600][f] (NV)	0 (NV)
*ts*34 AEV erythroblasts	170 (—)	9 (—)	ND	ND
AEV fibroblasts	2 (—)	3 (—)	ND	ND
ALV-induced lymphoma cells	34 (NV)	[130] (100, 150)	2 (NV)	3 (0.3, 3)
AMV myeloblasts	[1,700] (—)	5 (—)	0.6 (—)	0.6 (0.4, 0.6)

[a] Numbers in parentheses represent the range of values obtained in replicate determinations.
[b] std, Standard.
[c] NV, No variation between replicate determinations.
[d] —, Only one determination carried out.
[e] ND, Not done.
[f] Brackets indicate increased *onc* RNA level known to be due directly to viral transformation.

bound hemopoietic cells from the epithelial and connective tissues of these organs (and any cells which bind tightly to the latter tissues). In general, the c-*onc* RNA levels in these suspensions, which appeared to be comprised mainly of lymphocytes (plus erythrocytes in the spleen), were similar to those in the whole organs. Transcription from c-*myb* and c-*myc* was also detected in cell preparations from these organs which were further enriched for lymphocytes (see above).

To complement our studies on uninfected cells, we also determined the levels of c-*onc* RNAs in several transformed hemopoietic cell lines (Table 2). Although such cells may not correspond precisely to normal cell types, they comprise homogeneous populations which can unambiguously be assigned to particular hemopoietic cell lineages (see below). Most of these cells were transformed by oncogenic retroviruses; thus, the level of RNA transcribed from the c-*onc* gene homologous to the viral oncogene responsible for transformation could not be determined by the dot-blot assay. The high level of c-*myc* transcription in ALV-induced B-lymphomas appears to be due to integration of an ALV provirus in the vicinity of the c-*myc* gene (16; G. Payne, J. M. Bishop, and H. E. Varmus, Nature [London], in press) and will not be further dealt with here. The highest c-*myb* levels were found in two AEV-transformed erythroblast lines, and moderately high levels were detected in the B-lymphoma cells. Hayward et al. have also reported that a B-lymphoma cell line showed an elevated c-*myb* RNA level (16).

DISCUSSION

Quantitative aspects of the data. The results presented in Table 2 are expressed as the percentage of each c-*onc* RNA present in total polyadenylated RNA, rather than as copies per cell. We chose to do this because we observed

marked but consistent variations in the amount of polyadenylated RNA obtained from different cell types, which usually reflected variations in cell size. Therefore, it seemed to us that the data should be expressed as a concentration rather than an amount per cell. Furthermore, cell numbers could not be determined for whole organs, and copies per cell is a misleading term in cases where the cell populations are clearly heterogeneous. This heterogeneity also requires that interpretation of the data be qualified in some instances, since in cases where low c-*onc* RNA levels were observed, it could be argued that these low levels reflect the small proportion of cells which express the particular c-*onc* gene. However, a positive result, i.e., the detection of significant c-*onc* RNA levels, clearly indicates that at least a proportion of the cell population is expressing that c-*onc* gene. Furthermore, some of the normal cell populations studied here were essentially homogeneous, e.g., macrophages and fibroblasts, and the transformed cell lines represent highly homogeneous populations. Additional studies utilizing in situ hybridization techniques are required to determine the proportion of cells within each population which express the various c-*onc* genes.

Range of tissues expressing c-*onc* genes. Polyadenylated transcripts from c-*myc*, c-*erb*, and c-*src* were detected in almost all of the cells and tissues examined, whereas c-*myb* transcription was detected only in certain hemopoietic tissues and cells; however, these tissues and cells (both normal and transformed) belong to several different lineages. Thus, it appears that none of the c-*onc* genes is uniquely expressed in any one cell lineage. These results suggest that c-*myb* may function in normal hemopoiesis (see below), whereas c-*myc*, c-*src*, and c-*erb* may function in a wider range of cell types. However, there may be some selective utilization of these genes by different cell types since the various tissues and cell populations display different relative levels of c-*src*, c-*myc*, and c-*erb* RNA.

c-*myb* expression and hemopoiesis. The organs which express the highest levels of c-*myb* (i.e., bone marrow, yolk sac, and thymus) are major hemopoietic organs of the chicken (24). This suggests that c-*myb* may be expressed, and hence function, in immature hemopoietic cells. Since high levels of v-*myb* expression, like high levels of expression of other oncogenes, result in the continued replication (i.e., transformation) of at least some cell types, it may be that c-*myb* expression is responsible for the capacity of some hemopoietic precursor cells for replication. Chen (3) also found that c-*myb* RNA was present at higher levels in hemopoietic tissues and that these levels varied with age during and immediately after embryogenesis in such a way as to suggest a correlation with hemopoietic activity. (The major discrepancy between our results and those of Chen [3] is the level of c-*myb* in the bursa; however, we believe that this may be a function of the age of the chickens used in the two studies, because the hemopoietic activity of the bursa declines rapidly after hatching [24]; Chen did, in fact, find that the level of c-*myb* RNA in the bursa decreases in older chickens [3].) On the basis of his results, Chen suggested a correlation between c-*myb* expression and granulopoiesis. Although granulopoiesis does occur in the embryonic bursa, as well as, to a smaller extent, in the thymus and bursa of hatched chickens, the thymus and bursa are predominantly lymphoid organs. For example, Dodge and co-workers (6, 7) have measured the relative numbers of myelomonocytic (macrophage) progenitor cells in various organs. The myelomonocytic progenitor cells were predominantly in yolk sac and bone marrow, with very few in thymus and bursa. Thus, if c-*myb* expression were restricted to granulocytic precursor cells, we might expect higher levels in the yolk sac and bone marrow compared with the thymus.

The high level of c-*myb* expression found in the AEV-transformed erythroblasts also suggests that c-*myb* function is not restricted to myelomonocytic cells. This argument assumes that the high c-*myb* level is a reflection of the properties of the AEV target cell, an early erythroid progenitor cell, the BFU-E (11), and is not a consequence of AEV transformation. This is supported (but not proven) by the absence of c-*myb* expression in AEV-transformed fibroblasts.

A moderately high level of c-*myb* expression was also found in an ALV-induced B-lymphoma cell line (in which c-*myc* transcription appears to be activated by the integration of an ALV provirus [16; G. Payne et al., in press]). These cells clearly belong to the B-lymphocyte lineage (4) and appear to resemble mouse myeloma cells in that they exhibit an aberrant, but relatively mature, phenotype (L. Chen, S. Courtneidge, and J. M. Bishop, unpublished data). This observation is consistent with the detection of c-*myb* expression in enriched normal lymphocyte preparations. The evidence discussed above, then, indicates that c-*myb* expression is not confined to any single hemopoietic cell type, but occurs in many or all immature, actively replicating hemopoietic cells. The c-*myb* gene may not be the only c-*onc* gene expressed at high levels in immature or dividing hemopoietic cells. For example, Scolnick et al. (27) have recently reported high levels of $p21^{c\text{-}ras}$, the gene product of the c-*ras* gene, in a pluripotential hematopoietic cell line. It is not certain, however, that this

apparently immortal cell line is entirely equivalent to a normal stem cell, and it is even possible that the elevated level of $p21^{c\text{-}ras}$ is an abnormality that either sustains or is consequent to the continued growth of the cells.

Lack of correlation of expression of c-*onc* genes with susceptibility to v-*onc* transformation. It has been suggested that target cells for transformation by leukemia viruses carrying a given v-*onc* gene may be cells in which the corresponding c-*onc* gene is normally expressed (13, 14). The data of Table 3 (excerpted from Table 2) illustrate that there was no obvious correlation, direct or inverse, between susceptibility to transformation and c-*onc* expression. It could be argued that a low level (or absence) of c-*onc* expression in some cell populations which contain target cells for the corresponding v-*onc*, reflects the small proportion of target cells in these populations; for example, less than 1% of bone marrow cells can be transformed in vitro by DLVs (10, 15). However, this argument is false in at least one case. Several workers have reported that yolk sac macrophage cultures (prepared in a similar manner to those used in the present study) contain AMV target cells (2, 21); recently, Durban and Boettiger have shown that most or all of these target cells must be mature macrophages, not other cell types present in the cultures (8). Since these cultures are comprised of over 99.5% macrophages (8), we can safely conclude that these AMV target cells do not express c-*myb* at detectable levels (less than 0.0002% of polyadenylated RNA). Furthermore, expression of the c-*onc* genes homologous to the AMV, MCV, and AEV v-*onc* genes did not appear to be confined to the cell lineage to which the corresponding leukemic cells belong (cf. Tables 1 and 2). For example, AMV transforms macrophages and other cells in the myelomonocytic lineage (8, 10, 15) but, as discussed above, c-*myb* is expressed in a number of hemopoietic cell lineages. Similarly, the levels of c-*myc* RNA in the lymphoid cell preparations were as high as, or higher than, the levels in several target cells and organs, although MCV is not known to cause lymphomas or transform lymphoid cells in vitro.

The target cell specificity of oncogenic retroviruses appears to be dictated by the viral oncogene (25, 29) and not by restriction of viral replication (14). One explanation for this finding, originally suggested by Graf et al. (14), is that the presence or absence of a target protein for the v-*onc* gene product in a given cell determines susceptibility to transformation. However, the data presented here suggest that the determination of target cell specificity may be more complex. For example, as discussed above, it appears that some cells which are not susceptible to transformation by a particular v-*onc* gene nevertheless express the homologous c-*onc* gene. If it is assumed that the v-*onc* and c-*onc* gene products interact with the same target protein, we must conclude that either the target protein is absent in some cells in which the c-*onc* gene is expressed or that the interaction of the v-*onc* gene product with the target protein does not necessarily result in transformation.

TABLE 3. c-*onc* expression in target versus nontarget cells and organs

Oncogene	Target[a]		Nontarget[a]	
myb	Macrophage	(0)	Fibroblast	(0.1)
	Yolk sac cells	(56)	Thymus cells	(190)
myc	Macrophage	(1)	Brain	(0.3)
	Fibroblasts	(5)	Thymus cells	(8)
erb	Fibroblast	(2)	Macrophage	(0.7)
	Bone marrow	(2)	Liver	(2)
src	Fibroblast	(2)	Yolk sac	(0)
			Macrophage	(6)

[a] Percent c-*onc* RNA in total polyadenylated RNA ($\times 10^{-3}$) is given in parentheses.

ACKNOWLEDGMENTS

We thank Lois Henson for yolk sac and macrophage preparations, Sara Courtneidge for advice on the preparation of lymphocytes, and Bertha Cook for stenographic assistance.

This work was supported by grants from the National Cancer Institute (CA 12705B, CA 19287B, and the training grant 08T2 CA 09043A) and the American Cancer Society (MV48H); T.J.G. was supported in part by a postdoctoral fellowship from the American Cancer Society, California Division.

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