MOLECULAR AND CELLULAR BIOLOGY, Mar. 1985, p. 474–483
0270-7306/85/030474-10$02.00/0

Vol. 5, No. 3

Intracisternal A-Particle Gene Expression in Normal Mouse Thymus Tissue: Gene Products and Strain-Related Variability

EDWARD L. KUFF* AND JOSEPH W. FEWELL

Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20205

Received 24 August 1984/Accepted 5 December 1984

Intracisternal A-particle (IAP)-specific sequences were 5- to 10-fold enriched in polyadenylated RNA from BALB/cJ thymus as compared with RNAs from liver, spleen, and kidney. The major transcripts of 7.2 and 5.4 kilobases were the same size as those found in an IAP-rich neuroblastoma cell line. The absolute levels and proportions of these transcripts varied in thymuses from mice of different inbred strains. With antiserum prepared against p73, the main IAP structural protein, several size classes of IAP-related proteins were immunoprecipitated from extracts of thymus cells incubated with [^{35}S]methionine; these included p73 itself and a group of polypeptides in the size range of 114 to 120 kilodaltons (p114-p120). The inbred strains showed marked characteristic differences in the electrophoretic patterns of their IAP-related proteins. Earlier studies showed that the 7.2-kilobase RNA from neuroblastoma IAPs coded for p73 in a cell-free translation system. Correlations between the RNA and protein patterns in thymuses of the different inbred strains indicated that 5.4-kilobase RNA gives rise to the p114-p120 polypeptides. Metabolically labeled p120 was found to include methionine-containing tryptic peptides of p73 plus additional peptides consistent with its larger size. In vivo labeling kinetics showed that the p114-p120 polypeptides were not major precursors of p73 in intact neuroblastoma cells. This study shows that IAP gene expression in mouse thymus is genetically determined and that a novel class of IAP-related polypeptides can be expressed independently of the major particle structural protein.

Intracisternal A-particles (IAPs) are retrovirus-like entities found in many types of mouse tumor cells (20, 45). They contain a group-specific internal structural protein, p73 (20, 32), and a DNA polymerase activity with properties of reverse transcriptase (42, 43). The isolated particles also contain specific polyadenylated [poly(A)] RNAs (30) ranging in size from 7.2 to about 3.5 kilobases (kb) (34, 36, 41). These RNA species, which are related to one another in sequence, vary in relative proportion in particles isolated from different tumor sources. Endogenous provirus-like elements homologous to the IAP-associated RNAs ("IAP genes") are reiterated 1,000-fold per haploid genome in *Mus musculus* (28). The full-size genetic unit is 7.3 kb in length and is colinear with the 7.2-kb transcript (21). Shorter elements with internal deletions are also found (21, 34), and one group of these, the so-called type II elements (40), are known to give rise to some of the shorter IAP-specific RNAs (34, 41). The IAP genetic elements share many structural properties with integrated retroviral proviruses and certain transposable elements in *Drosophila* spp. and yeasts (8a). Recently, IAP genes have been found to appear in novel locations in the genome of certain IAP-rich BALB/c mouse myeloma and hybridoma cell lines and to affect the function of genes at the various target sites (5, 7, 9, 10, 18, 19, 41). Thus, on occasion they can act as mobile elements to provide a source of genetic variability in mouse cells.

IAPs are regularly observed by electron microscopy in mouse oocytes and early embryos (reviewed in references 15, 46, 47), and both IAP-specific proteins (12–14) and RNA (37) have been demonstrated in preimplantation embryos. IAPs were seen only occasionally in a systematic survey of normal mouse tissues that included adult BALB/c thymus (45). However, in the present study we have detected substantial levels of IAP-specific poly(A) RNAs in thymus glands of BALB/c and certain other inbred mouse strains, together with the active synthesis of several IAP-related protein species. IAP gene expression appears to be constitutive in the mouse thymus but varies both quantitatively and qualitatively among the different strains.

* Corresponding author.

MATERIALS AND METHODS

Tissue and cell sources. Inbred mice of the following strains were obtained from the Jackson Laboratory: BALB/cJ, AKR/J, C58/J, NZB/J, SJL/J, DBA/2J, SWR/J, and RIIIS/J. The BALB/c myelomas MOPC-104E (33) and MOPC-21 line Pe-X63 (16) were maintained by subcutaneous transplant and cell culture, respectively. The N4 neuroblastoma cell line was derived from the C1300 tumor originating in an A/J mouse (1).

RNA extraction. For each mouse strain tested, thymuses were collected into liquid nitrogen from 20 to 22 1-month-old mice of both sexes. Separate collections of thymus tissue were also made from 2-week- and 2-month-old BALB/c animals. Other organs were collected from 1-month-old BALB/c mice. RNA was extracted by a modification of the method of Auffray and Rougeon (3). With a tight-fitting Dounce homogenizer, approximately 1 g of frozen tissue was rapidly dispersed in 25 ml of freshly prepared 6 M urea–3 M LiCl and homogenized vigorously at room temperature until the solution poured easily and stringing of DNA was no longer evident. The preparation was stored overnight in an ice bath and then centrifuged at 0°C for 1 h at 8,000 rpm in a JS21 rotor of the Beckman model J21B centrifuge. The supernatant fluids were discarded, and the pellets (from two 30-ml Corex tubes) were taken up in 10 ml of 50 mM Tris-hydrochloride (pH 7.9)–100 mM NaCl–0.5% sodium dodecyl sulfate (SDS). The suspensions were briefly warmed to 37°C and pipetted vigorously to disperse the pellet material, which was then extracted with phenol-chloroform-isoamyl alcohol (50:48:2) and precipitated with 67% ethanol at −28°C. The RNA pellets were washed with cold 80% ethanol and stored in moist form at −28°C. Cytoplasmic

RNA was prepared from MOPC-104E tumor tissue by an SDS-phenol procedure (30).

Isolation of poly(A) RNA. Poly(A) RNA was isolated by chromatography on oligodeoxythymidylate-cellulose (type 7, P.-L. Biochemicals) as previously described (30). All preparations were cycled twice over the column to ensure complete removal of DNA. Fractions were stored as aqueous solutions at −70°C. Samples of poly(A) RNA from the T-cell lymphomas P1758 and 5AKR13, prepared in similar fashion, were kindly provided by F. Mushinski, National Cancer Institute.

Preparation of RNA "dot blots." For quantitative estimate of IAP sequence content, dilutions of poly(A) RNA were applied to nitrocellulose membranes (BA85; Schleicher & Schuell), using a filtration manifold (SRC-96/0 Minifold; Schleicher & Schuell). The poly(A) RNAs were first diluted into formaldehyde dilution buffer: 4.4 M formaldehyde (prepared from freshly opened Fischer 37% formaldehyde solution brought to pH 4.5 with NaOH), 10× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate, pH 6.4), and 6 μg of *Escherichia coli* tRNA per ml, heated for 15 min at 65°C. Serial twofold dilutions of the denatured samples into formaldehyde dilution buffer were prepared in 96-well microtiter plates and then transferred to the manifold containing BA85 membrane previously wet with 20× SSC. The samples were pulled through under gentle vacuum and the wells were washed with 20× SSC. The filters were air dried and baked for 2 h at 80°C. Controls for DNA contamination were routinely performed because of the high concentration of IAP sequences in the mouse genome. Duplicate samples of each poly(A) RNA were added to alkaline dilution buffer: 50 mM Tris-hydrochloride (pH 7.5), 0.2 N NaOH, 6.67× SSC, and 5 μg of calf thymus DNA per ml. The samples were heated at 75°C for 15 min to hydrolyze the RNA and diluted serially into alkaline dilution buffer in microtiter wells. Just before transfer to the manifold wells, the dilutions were neutralized by addition of 1 N HCl (30 μl added to 150 μl of alkaline dilution buffer). The rest of the procedure was as given above.

Electrophoresis of poly(A) RNA and transfer to nitrocellulose membrane (Northern blots). The poly(A) RNAs were denatured in the presence of formaldehyde and formamide and electrophoresed in formaldehyde-containing 1% agarose gels as described by Maniatis et al. (31). For size markers, a mixture of DNA restriction fragments which would react with the final hybridization probe was denatured by heating to 75°C for 15 min in 80% formamide and then diluted into the formaldehyde running buffer. After electrophoresis, the gels were prepared for transfer as follows: a trimmed gel (150 ml) was soaked twice in 500 ml of water for 10 min and then serially for 30 min each in 500-ml quantities of (i) 50 mM NaOH; (ii) 5× MOPS buffer (1× MOPS buffer is 0.2 M morpholinopropage sulfuric acid [pH 7], 50 mM sodium acetate, 1 mM Na EDTA [pH 8.0]) containing 1.1 M formaldehyde, and (iii) 1× MOPS buffer containing 1.1 M formaldehyde (electrophoresis buffer). For transfer, the gel was laid face down on a thick bed (six sheets) of Whatman 3MM paper soaked in 20× SSC, overlaid with a sheet of BA85 nitrocellulose membrane and two additional sheets of paper all wet with 20× SSC, and then with additional paper to a height of 2 to 3 cm. Transfer was allowed to proceed overnight, after which the membranes were air dried and baked at 80°C.

Preparation of radioactive probe. We used a recombinant plasmid, pMIA1, containing 5.2 kb of internal sequence from a genomic IAP gene copy (29). The plasmid was labeled by nick translation to a specific activity of 1×10^8 to 2×10^8 cpm/μg of DNA, using [α-^{32}P]dATP and [α-^{32}P]dCTP (50 μCi of each at 800 Ci/mmol in a 25-μl reaction mixture containing about 0.5 μg of DNA).

Hybridizations. Dot blots and Northern transfers were prehybridized for 16 h at 42°C in hybridization buffer: 50% (vol/vol) formamide, 1× Denhardt solution, 5× SSC, 50 mM sodium phosphate (pH 6.8), 0.1% SDS, 100 μg of denatured calf thymus DNA per ml. The blots were transferred to fresh hybridization buffer containing heat-denatured ^{32}P-labeled probe at 10^6 cpm/ml and hybridized for 40 to 48 h at 42°C with shaking. Before autoradiography, the blots were washed at room temperature for 15 min each time with two changes of 2× SSC–0.1% SDS and two changes of 0.1× SSC–0.1% SDS.

Protein labeling of N4 and thymus cells. N4 monolayer cultures in 100-mm dishes were used at about two-thirds confluence. The usual medium (Dulbecco minimal essential medium [DMEM] containing 10% fetal calf serum) was removed, and the cells were washed thoroughly with DMEM lacking methionine. The cultures were then labeled for 4 h with [^{35}S]methionine (800 Ci/mol; Amersham Corp.), 100 μCi/ml in methionine-free DMEM containing 10% dialyzed fetal calf serum. Individual thymuses were placed in 60-mm culture dishes and teased to single-cell suspensions in methionine-free DMEM. The cells were pelleted (1,500 rpm, 10 min), suspended in methionine-free DMEM plus 10% dialyzed fetal calf serum, and labeled as above.

Preparation of extracts. Labeled cells were collected and washed once with cold phosphate-buffered saline. The cell pellets were suspended in 100 μl of lysing buffer: 8.5% (wt/vol) sucrose, 100 mM NaCl, 100 mM Tris-hydrochloride (pH 7.4), 10 mM EDTA, 0.6% Nonidet P-40. The cells were disrupted by vigorous vortexing, and the nuclei were pelleted by centrifugation at 1,500 rpm for 10 min at 5°C. The supernatant fluid was removed and used for immunoprecipitation. Protein concentrations were determined by the Folin procedure (25).

Immunoprecipitation of IAP-related proteins. Portions of thymus and N4 extracts containing 250 and 40 μg of protein, respectively, were generally used. The extract proteins were denatured in a volume of 100 μl by heating at 100°C for 3 min in 0.5% SDS–1 mM dithiothreitol. The samples were cleared of small shreds of insoluble material by centrifugation for 5 min at 12,000 × *g*. Excess dithiothreitol was eliminated and the proteins were alkylated by addition of 5 mM iodoacetamide. The volume was brought to 450 μl with 10 mM sodium phosphate (pH 6.8)–0.2% Triton X-100. Rabbit antiserum A1.3, prepared against electrophoretically purified IAP structural protein p73 (17), was added in the amount of 3 μl. An equal volume of preimmunization serum A1.0, or of rabbit anti-goat immunoglobulin G, was added to control tubes. After 15 min, the antigen-antibody complexes were collected on *Staphylococcus aureus* (Pansorbin; Calbiochem) and washed twice with RIPA buffer (1% Triton X-100), 1% sodium deoxycholate, 0.1% SDS, 0.1 M sodium phosphate [pH 7.2], 10 mM EDTA) and twice with a solution containing 0.05% Nonidet P-40, 0.15 M NaCl, 50 mM Tris-hydrochloride (pH 7.4), and 5 mM EDTA. The proteins were then eluted with SDS at 100°C and analyzed by electrophoresis in SDS-containing 8.5 or 10% polyacrylamide gels, using the buffer system of Laemmli (22). Proteins were detected by autoradiography.

Tryptic peptide mapping. N4 cells in six 150-mm culture dishes were labeled for 6 h with [^{35}S]methionine, and the IAP-related proteins were immunoprecipitated with anti-

serum A1.3 as described above. The proteins were separated by polyacrylamide gel electrophoresis on a preparative scale, and the labeled bands representing the p120 and p73 polypeptides were located by autoradiography and extracted by repeated tryptic digestion as previously described (36). Samples of the tryptic digests were applied to precoated thin-layer silica gel plates and fractionated in the first dimension by electrophoresis at pH 3.5 (glacial acetic acid-pyridine-water, 50:5:945) and in the second dimension by ascending chromatography in *n*-butanol–glacial acetic acid–pyridine–water, 30:6:24:24 (36).

RESULTS

Concentration of IAP-related sequences in normal and tumor cells. Poly(A) RNA fractions were prepared from a number of normal and tumor sources and assayed for IAP-related sequences, using a dot blot procedure as shown in Fig. 1. The immobilized RNAs were hybridized with a radioactive probe prepared from cloned IAP DNA (29), and the assay was calibrated with authentic IAP poly(A) RNA extracted from gradient-banded myeloma particles (30). IAP sequence levels were first calculated as a proportion of the total poly(A) RNA and then converted to absolute tissue concentrations (Table 1).

The relative IAP sequence content of MOPC-104E myeloma cytoplasmic poly(A) RNA measured by the dot blot technique was 8%, in good agreement with the value of 7.8%

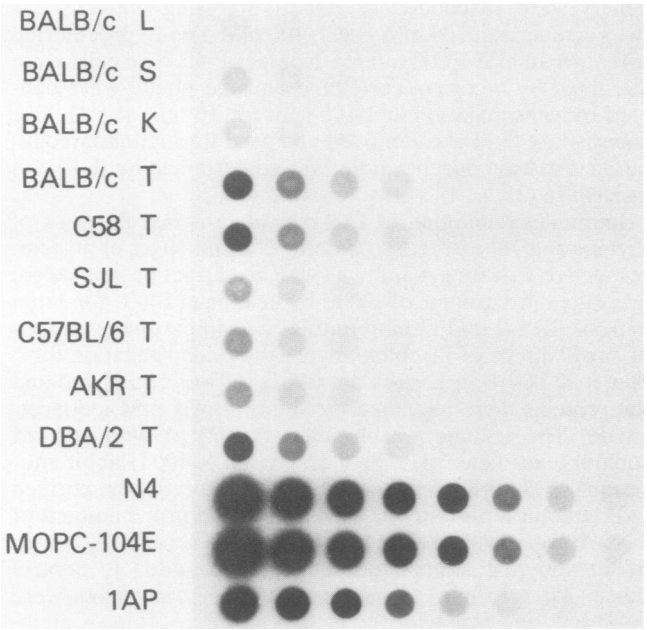

FIG. 1. Dot blot assay of IAP sequence levels in poly(A) RNAs from various mouse tissues. Sources are indicated on the left: L, S, K, and T refer to liver, spleen, kidney, and thymus, respectively; N4 refers to a neuroblastoma cell line; and MOPC-104E refers to a transplantable myeloma. Poly(A) RNAs were denatured in formaldehyde and applied to nitrocellulose membranes in twofold dilutions; the most concentrated dots contained 1 μg of total poly(A) RNA. The IAP standard (bottom row) was prepared from purified MOPC-104E particles and contained 20 ng of IAP-specific sequence in the most concentrated dot. The immobilized RNAs were hybridized with ^{32}P-labeled pMIA1, a recombinant plasmid containing 5.2 kb of IAP-specific sequence (29). Hybridization conditions are specified in the text.

TABLE 1. Total and IAP-specific poly(A) RNA in normal mouse tissues and IAP-rich tumor lines

Source	Total poly(A) RNA (μg)[a]	IAP poly(A) RNA	
		% of total[b]	μg[a,c]
BALB/c			
Liver	248	0.05	0.12
Kidney	122	0.1	0.12
Spleen	83	0.1	0.09
Thymus	220	0.5	1.1
C58, thymus	164	0.4	0.65
SJL, thymus	206	0.2	0.4
C57BL/6, thymus	268	0.2	0.5
AKR, thymus	281	0.2	0.6
DBA/2, thymus	190	0.4	0.8
MOPC-104E[d]	200	8	16
N4[e]	200	8	16

[a] Values for 1 g (wet weight) of tissue or cells.
[b] Derived from dot blot (Fig. 1).
[c] Calculated from percentage of total poly(A) RNA.
[d] Myeloma line of BALB/c origin.
[e] Neuroblastoma line of A/J origin.

previously obtained for this same preparation by a liquid hybridization assay (30). Total poly(A) RNA from the particle-rich N4 neuroblastoma line had a similarly high content of IAP-specific sequence. Earlier, we showed that cytoplasmic poly(A) RNAs from mouse liver and several IAP-negative cell lines contained much lower but still measurable proportions of IAP sequence, in the range of 0.02 to 0.05% (30). In the present study, the IAP-specific fraction of total cellular poly(A) RNA was 0.05% for liver and 0.1% for spleen and kidney.

There was a marked preferential accumulation of IAP-specific RNA in BALB/c thymus as compared with the other normal tissues from this mouse strain. IAP-related sequences accounted for 0.5% of the total poly(A) RNA in BALB/c thymus. On a tissue weight basis the concentration of IAP-specific RNA in the 1-month-old BALB/c thymus was 10 times greater than in the other normal tissues and >5% of the very high levels found in the two tumor lines. IAP sequences were also relatively enriched in the poly(A) RNAs from C58 and DBA/2 thymus glands.

IAP-related transcripts in BALB/c thymus and several tumor lines. Poly(A) RNA fractions from 0.5-, 1-, and 2-month BALB/c thymus and from several tumor lines were electrophoresed under denaturing conditions, transferred to nitrocellulose membrane, and hybridized with IAP-specific probe (Fig. 2). The thymus RNAs (lanes a, b, c) contained two major reactive species with measured sizes of 7.2 and 5.4 kb. These components were more concentrated in thymus RNA at 1 month than at the other ages tested. Hybridizing species of similar size appear in the neuroblastoma pattern (lane d), just as earlier (36) they were observed as the main ethidium bromide-staining components in IAP-associated RNA obtained from this tumor cell source.

Variations in the total amount, relative proportion, and mobility of the IAP-related RNA species were evident in preparations from the different tumors. Although the larger RNA species had the identical apparent size (7.2 kb) in each case, there were minor variations in the mobility of the "5.4-kb" component (Fig. 2, lanes e and h). The neuroblastoma and MOPC-21 patterns (lanes d and e) show that IAP RNA expression can be very similar in tumors of different histological type and strain of origin. Conversely, the two BALB/c myelomas (lanes e and f) show large differences in

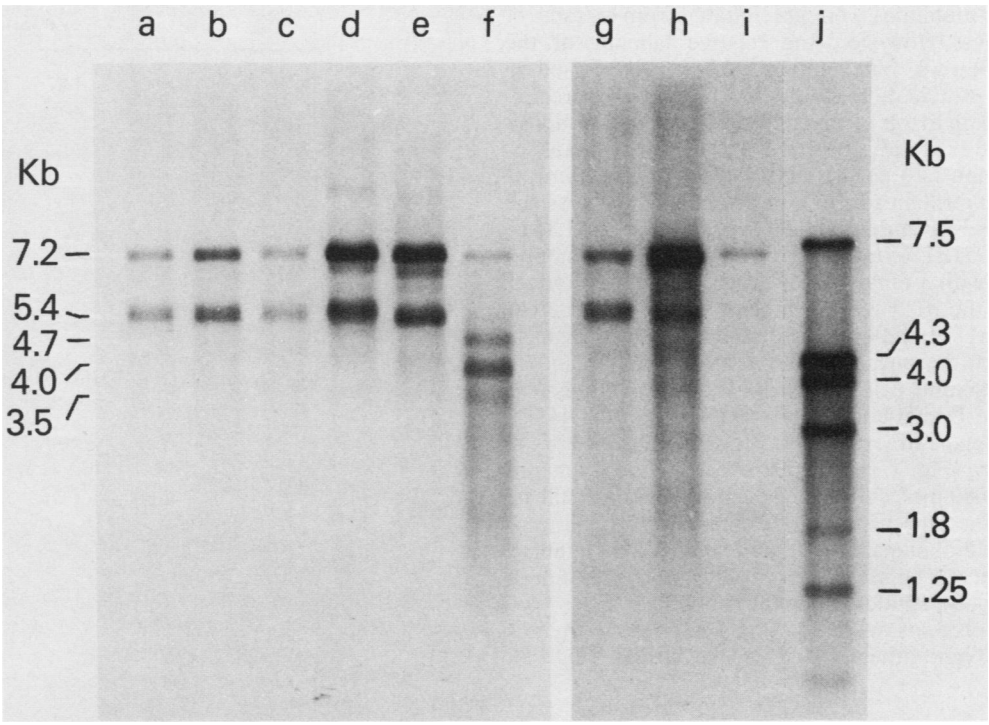

FIG. 2. Electrophoretic analysis of IAP-specific transcripts in poly(A) RNA fractions from BALB/c thymus and various mouse tumors. The RNA fractions were denatured by warming in formaldehyde and formamide, electrophoresed in 1% agarose gels in the presence of formaldehyde, and transferred to nitrocellulose membrane (see text). The lanes represent: a, b, and c, 4 μg each of poly(A) RNA from 2-week, 1-month, and 2-month BALB/c thymus, respectively; d, e, and f, 0.5 μg each of poly(A) RNA from the N4 neuroblastoma of A/J origin and the BALB/c myelomas MOPC-21 and MOPC-104E, respectively; g, 4 μg of poly(A) RNA from 1-month BALB/c thymus; h and i, 5 μg each of poly(A) RNA from the T-cell lymphomas P1758 and 5AKR13 of BALB/c and AKR origin, respectively; j, DNA size standards derived by restriction endonuclease cleavage of recombinant plasmids containing cloned IAP gene fragments. The blots were hybridized with ^{32}P-labeled pMIA1.

their IAP-RNA patterns. The MOPC-104E RNA components with sizes of 4.7 and 4.0 kb are transcripts of type II IAP genes (40, 41), as determined by hybridization with an authentic type II probe, kindly provided M. Cole (data not shown). Transcripts of this type are poorly represented in poly(A) RNAs from the other cell sources, including thymus. The RNA from the BALB/c T-lymphoma P1798 (lane h) contained the same two major bands as the normal BALB/c thymus preparation, but a greatly enhanced level of the 7.2-kb component. IAP-specific transcripts were much less concentrated in the AKR lymphoma (lane i) than in P1798, a difference which parallels the relative content of IAP poly(A) RNA in the normal thymus glands of these two strains (see below).

IAP-related transcripts in thymus of different inbred mice. We compared the electrophoretic patterns of IAP-specific RNAs in the thymus glands of seven inbred mouse strains (Fig. 3). The absolute intensity of hybridization varied widely among the different strains, in general accord with the dot blot results. A 5.4-kb band was the predominant reactive species in each case. The ratio of hybridization in the 7.2- and 5.4-kb bands ranged from near equivalence in the BALB/c pattern to very low levels in the cases of C58 and C57BL/6. Since each thymus RNA preparation was derived from at leat 20 mice of both sexes, we believe that the observed differences reflect real strain variations in the absolute amounts and relative proportions of the main IAP-related RNA species.

Immunoprecipitation of IAP-related protein products. In an earlier study (36) the 7.2- and 5.4-kb components in RNA from isolated neuroblastoma IAPs were tested individually for their coding capacities in a cell-free translation system. The 7.2-kb species directed synthesis of a 73,000-dalton product whose identity as the p73 IAP structural protein was established by tryptic peptide mapping. The 5.4-kb RNA coded for a heterogeneous group of products which we did not attempt to analyze by tryptic digestion. The products, among which only minor amounts of p73 could be identified, showed particular abundance in the size range above 100,000 daltons.

The observed variations in quantity and proportion of IAP-specific RNAs suggested that thymuses of the inbred mouse strains might differ significantly in their content of IAP-related polypeptides. Accordingly, cell suspensions from individual thymus gland were metabolically labeled for 4 h with [^{35}S]methionine, and extracts prepared from them by detergent lysis were immunoprecipitated with a rabbit antiserum (A1.3) raised against purified p73. Figure 4 shows the electrophoretic patterns of labeled polypeptides immunoprecipitated from the IAP-rich neuroblastoma cells and from thymus cells of 1-month-old C57BL/6 and BALB/c male mice. Controls using a preimmunization serum (A1.0) from the same rabbit are included. Approximately four times more thymus than neuroblastoma extract protein was used for immunoprecipitation.

The main immunoprecipitated component in neuroblastoma extracts had a molecular weight of 73,000 and corresponded to the major IAP structural protein. Two components with molecular weights of 114,000 and 120,000 (p114 and p120) were next in abundance. Polypeptides of about 160,000 and 100,000 daltons were also specifically immunoprecipitated in lesser amounts. Components of identical

electrophoretic mobilities were precipitated from extracts of BALB/c thymus. However, the relative labeling of the p114-p120 doublet was much greater in these cells. Based on the amounts of protein radioactivity used for immunoprecipitation and a comparison of the autoradiographic film densities at different exposure times, we estimate that the absolute incorporation into p114-p120 was roughly one-third as much in BALB/c thymus as in neuroblastoma, whereas the ratio of p73 labeling in the two cell types was about 1:15.

Extracts of C57BL/6 thymus gave one major immunoprecipitable band with a molecular weight of 117,000, intermediate between the p114 and p120 components in the other patterns. The p117 band appears disproportionately light in the C57BL/6 pattern because the overall protein labeling in this particular thymus cell suspension was considerably less than that in the BALB/c preparation (27,000 versus 44,000 cpm per μg of total extract protein). Longer exposures of the C57BL/6 pattern (Fig. 4, right lane) revealed a small amount of labeled 100,000-molecular-weight component but still no p73. We examined the patterns of immunoprecipitable proteins in two additional males and two females of each inbred strain and observed no sexual dimorphism in expression of the individual polypeptides (data not shown).

IAP-related proteins were examined in labeled thymus extracts from eight other inbred mouse strains (Fig. 5).

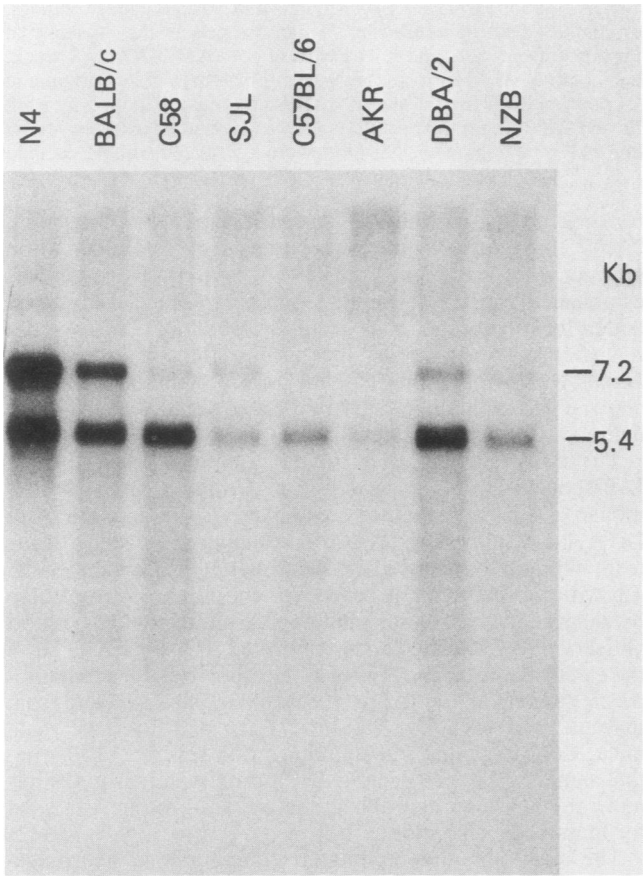

FIG. 3. IAP-specific transcripts in poly(A) RNA fractions from thymuses of 1-month-old mice of the indicated inbred strains and from the N4 neuroblastoma. A 4-μg amount of each thymus poly(A) RNA and 0.5 μg of the N4 preparation were applied. Conditions for electrophoresis and hybridization were as described in the legend to Fig. 2.

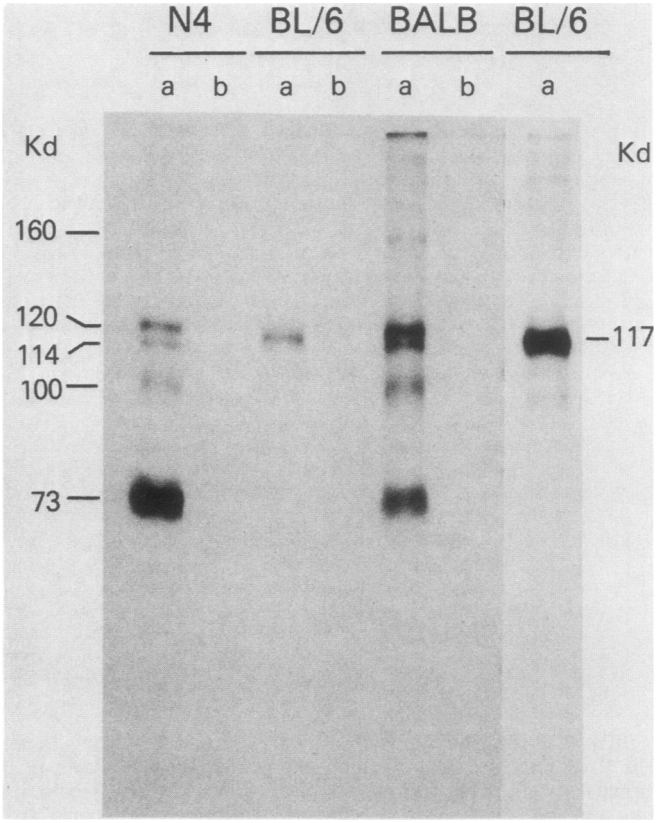

FIG. 4. Electrophoretic patterns of IAP-related proteins in N4 neuroblastoma cells and 1-month-old male C57BL/6 (BL/6) and BALB/c thymus cells that had been metabolically labeled with [^{35}S]methionine for a period of 4 h. N4 cells were incubated as monolayer cultures, and thymus cells were incubated as suspensions prepared from individual mice. The labeled cells were lysed with Nonidet P-40, and proteins were immunoprecipitated from cytoplasmic extracts with antiserum A1.3 prepared against the main IAP structural protein p73 (lanes a) or with preimmunization serum A1.0 from the same rabbit (lanes b). The amounts (micrograms) and specific radioactivities (counts per minute per microgram) of the extract proteins used for immunoprecipitation were: N4, 63 and 50,000; C57BL/6, 250 and 27,000; BALB/c, 250 and 44,000. The immunoprecipitated proteins were electrophoresed in polyacrylamide gels under denaturing conditions. The autoradiographic exposure time was 16 h except for the right lane, in which the C57BL/6 a pattern was exposed for 48 h. Details of the procedures are given in the text.

Where tested (six strains), the patterns of immunoprecipitated proteins were reproducible in two individuals of the same strain (Fig. 5, left and right panels). Differences among the strains in the labeling of IAP polypeptides far exceeded the differences in general protein labeling of the various extracts (see legend, Fig. 5). For example, the C58 and SWR patterns in the left panel represented the high and low extremes of IAP-specific labeling, yet the specific activities of the whole-extract proteins were not greatly different in the two cases (85,000 and 71,000 cpm per μg of protein, respectively).

The various strains also showed distinctive differences in the relative labeling of individual IAP-related polypeptides. Thus, p73 was not detected in thymus extracts from SWR, AKR, 129, RIII, and, as noted above, C57BL/6 mice. This component was labeled at relatively low levels in thymus extracts from the other strains shown in Fig. 5 and in two

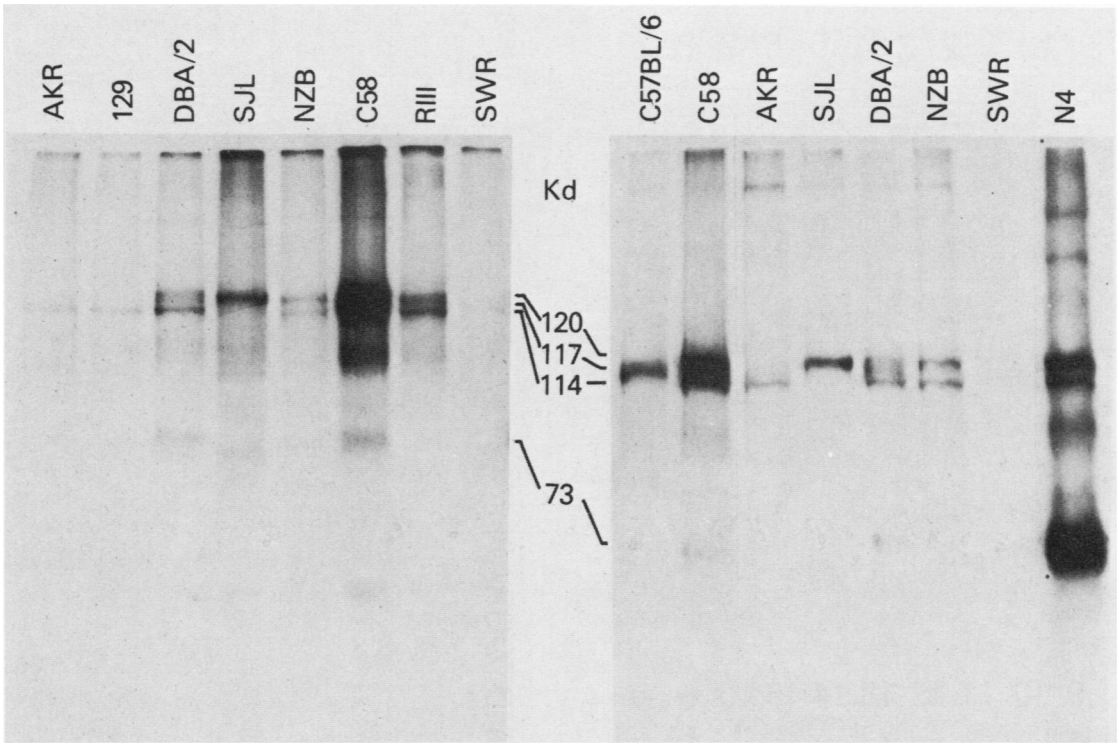

FIG. 5. Labeling patterns of IAP-related proteins in thymus cells from individual 1-month-old male mice of the indicated inbred strains. The left and right panels represent separate experiments and include different mice from six of the inbred strains. A reference pattern from N4 neuroblastoma cells is included. Labeling with [^{35}S]methionine, immunoprecipitation with antiserum A1.3, and electrophoresis were as described in the legend to Fig. 4 and detailed in the text. A 250-μg amount of thymus extract protein was used for the immunoprecipitations. The extracts used for the left-hand panel had specific activities (counts per minute per microgram of extract protein) of: AKR, 64,000; 129, 51,000; DBA/2, 74,000; SJL, 59,000; NZB, 64,000; C58, 85,000; and SWR, 71,000. Specific activities of the extracts used for the right-hand panel are not available; however, labeling of the cell suspensions was carried out under identical conditions.

cases, SJL and DBA/2, exhibited variant electrophoretic mobility. All of the extracts contained one or more labeled components in the 114,000- to 120,000-molecular-weight range, but the number and relative proportion of the different size species varied from one strain to another. All of the inbred strains except AKR and 129 could be distinguished from one another on the basis of the electrophoretic patterns of their IAP-related proteins.

IAP-related proteins in progeny of matings between BALB/c and C57BL/6 mice. The strain-related differences in RNA and protein patterns indicated that IAP gene expression is genetically determined in mouse thymus. As the first step in studying the inheritance of these expression patterns, we examined the immunoprecipitable polypeptides in labeled thymus glands from progeny of crosses between BALB/c and C57BL/6 mice. These strains differ clearly with regard to both the synthesis of p73 and the selective expression of proteins in the p114 to p120 size group (Fig. 4). The electrophoretic patterns from thymuses of 16 F_1 animals are presented in Fig. 6. They show that, among the larger polypeptides, the C57BL/6 component p117 was generally expressed, as well as one or the other BALB/c-specified proteins p114 and p120. A striking aspect of the patterns was the generally low labeling of proteins in the size range of p73, both on an absolute basis and relative to the labeling of the larger polypeptides. In only 1 of the 16 progeny was p73 synthesis at all commensurate with the expected contribution of a BALB/c parent. These results are not readily interpretable in terms of the simple Mendelian inheritance of active genes from the BALB/c parents, but rather suggest the operation of additional regulatory processes in the progeny animals.

Tryptic peptide analysis of the p120 polypeptide. The high-molecular-weight polypeptides p114 and p120 were previously observed as minor components of neuroblastoma IAPs (17), but their structural relationship to p73 was not defined. We carried out a tryptic peptide analysis of [^{35}S]methionine-labeled p73 and p120 isolated by immunoprecipitation from neuroblastoma cells, with the results shown in Fig. 7. It is seen that p120 contains labeled peptides found in p73 plus additional peptides consistent with its higher molecular weight. A rigorous electrophoretic separation of p114 from the predominant p120 species was not possible. In one experiment where we attempted such a separation, the peptide maps of the two fractions were indistinguishable (data not shown).

Labeling kinetics of IAP-related proteins in neuroblastoma cells. We considered the possibility that in intact cells p73 is generated principally through the processing of higher-molecular-weight precursors such as the p114-p120 group of polypeptides. To examine this possibility, we determined the proportion of label associated with various immunoprecipitable components in neuroblastoma cells after incorporation periods of 5 min, 5 min followed by a 15-min puromycin block, and 4 h (Table 2). The relative labeling of p114-p120 was no greater at 5 min than at 4 h, and there was no apparent transfer of label from these components to p73 during the puromycin chase. These data, together with the results of a previous study that could find no evidence for metabolic precursors of p73 in neuroblastoma cells (27),

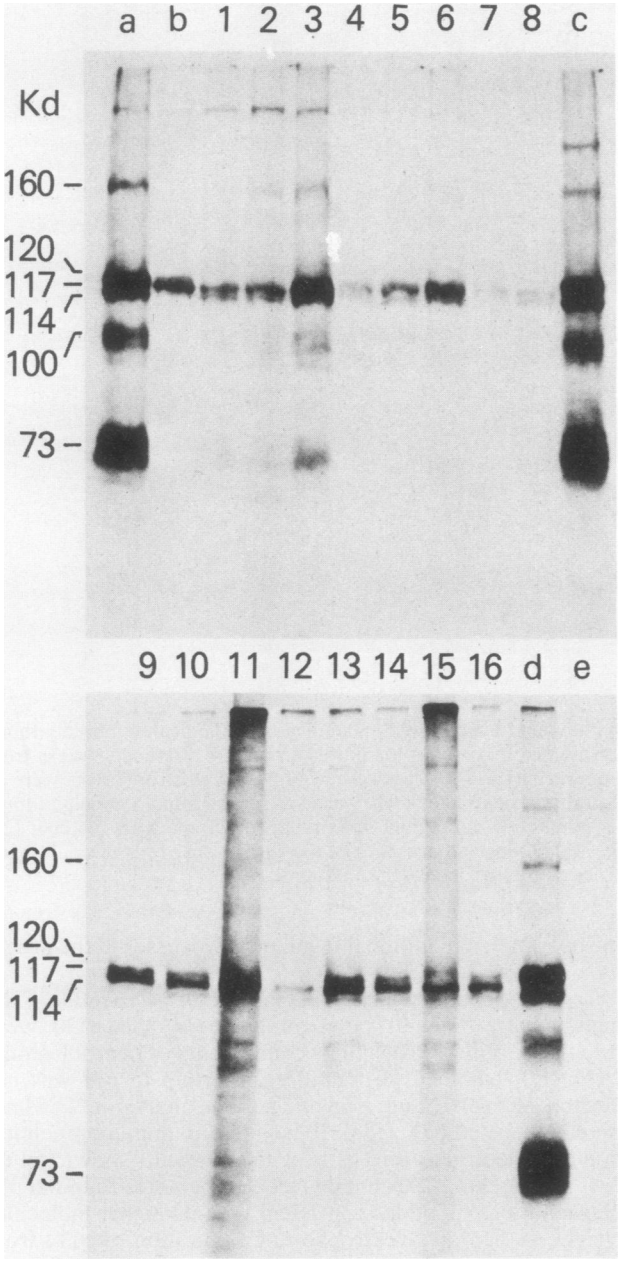

FIG. 6. Labeling patterns of IAP-related proteins in thymuses of 1-month-old progeny from crosses between BALB/c and C57BL/6 mice. Labeling of cells with [^{35}S]methionine, preparation of extracts, immunoprecipitation, and electrophoresis were as described in the legend to Fig. 4 and detailed in the text. Lanes a and b, proteins from BALB/c and C57BL/c thymuses, respectively; lanes c, d, and e, from N4 neuroblastoma cells; and lanes 1 to 16, from thymuses of individual F_1 progeny. A control antiserum, A1.0, was used for lane e; otherwise all lanes show proteins immunoprecipitated with the specific antiserum A1.3. Progeny in the upper panel (lanes 1 to 8) were from a single (C57BL/6 × BALB/c) mating. Offspring from two (BALB/c × C57BL/6) matings are represented in the lower panel. Lanes 8 to 12 represent male mice. A 250-μg protein of thymus extract protein was used for immunoprecipitations. Specific activities of the extract proteins used in the upper panel are not available; for the lanes in the lower panel they were (counts per minute per microgram) 9, 24,000; 10, 19,000; 11, 47,000; 12, 32,000; 13, 59,000; 14, 23,000; 15, 26,000; and 16, 40,000

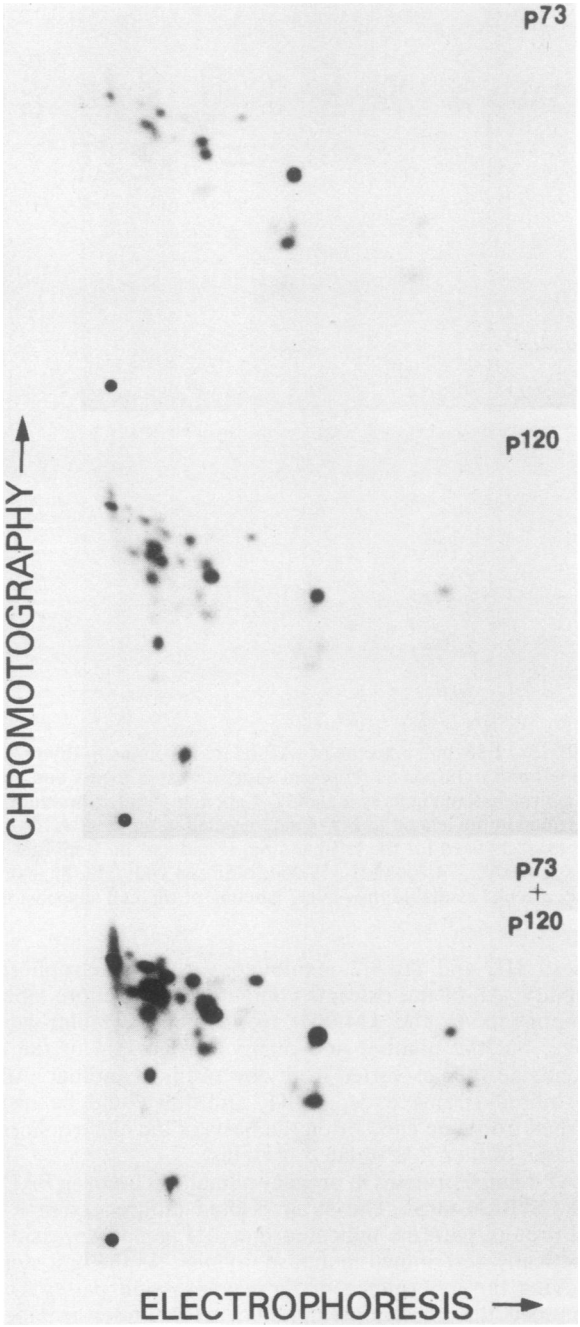

FIG. 7. Tryptic peptide mapping of [^{35}S]methionine-labeled p73 and p120 from N4 neuroblastoma cells. Cell monolayers were labeled for 6 h, collected, and lysed with Nonidet P-40. Cytoplasmic extracts were prepared, and the IAP-related proteins were immunoprecipitated with antiserum A1.3. The immunoprecipitated proteins were separated by electrophoresis in polyacrylamide gels and digested with trypsin. The tryptic peptides were displayed by autoradiography after sequential thin-layer electrophoresis and chromatography on silica gel plates. Details of the procedures are given in the text.

suggest that formations of p73 and the p114-p120 group of proteins are largely independent synthetic events. They do not exclude the possibility that a small fraction of p73 is generated by cleavage of the larger polypeptides.

TABLE 2. Distribution of radioactivity among IAP-related proteins from neuroblastoma cells labeled for various periods of time[a]

Electrophoretic component	% of total film density		
	5 min	5 min plus puromycin, 15 min	4 h
p114 + p120	15	13	24
p100	5	7	5
p73	80	80	70

[a] N4 monolayer cultures at 50% confluence were incubated with [^{35}S]-methionine (1,000 Ci/mmol, 160 μCi/ml; Amersham Corp.) in methionine-free DMEM and 10% dialyzed fetal calf serum. One culture was harvested after 5 min of labeling; a second, after an additional 15 min in the presence of 0.5 mM puromycin to block further protein synthesis (26); and a third, after 4 h of labeling in the absence of puromycin. The cells were lysed with Nonidet P-40, and IAP-related proteins were immunoprecipitated from the detergent extracts and fractionated by electrophoresis on SDS-containing polyacrylamide gels. Details of the procedures are given in the text. An autoradiograph was prepared under conditions of linear film response, and the lanes were scanned with an Ortec model 4310 densitometer. Values are expressed as the percentage of total film density at each time point that was represented in the indicated protein component.

DISCUSSION

IAP-related poly(A) RNA was detected in thymus, liver, spleen, and kidney of BALB/c mice. In additional experiments not illustrated here, we have used Northern blots provided by R. Callahan, National Cancer Institute, to detect 7.2- and 5.4-kb IAP-related transcripts in BALB/c brain, salivary gland, epididymis, and mammary gland. A degree of IAP gene activity thus appears to be widespread in normal adults tissues of this strain, although accumulation of specific poly(A) RNA is much greater in the thymus than in the other tissues examined. IAP sequences were nearly as highly enriched in the poly(A) RNA of C58 and DBA/2 thymuses as they were in the BALB/c preparations, but neither in these two instances nor in the additional strains studied have we made a direct comparison between the IAP-RNA levels in the thymus and the other normal tissues. Therefore, we cannot yet say whether enhanced accumulation of IAP transcripts in the thymus relative to other mouse tissues is a general phenomenon.

IAP genes are abundantly reiterated in the mouse DNA (28, 34) and have the capability of transposing to new locations in the genome (5, 7, 9, 10, 18, 19, 40). Strain-specific transpositions could bring different individual IAP genes or groups of genes under control of tissue regulatory elements, with consequent variation in expression of IAP-related RNA and protein products. In addition, or as part of the above process, IAP gene activity could be influenced by strain- and tissue-specific patterns of DNA methylation. Lasneret et al. (23) showed a dramatic enhancement of IAP production in Kirsten murine leukemia virus-transformed BALB/3T3 cells treated with 5-azacytidine and suggested that DNA methylation may have a role in regulating IAP gene expression in that system. Hojman-Montes de Oca et al. (11) studied the methylation of IAP sequences in the DNA of IAP-producing teratocarcinoma lines. They found a high degree of methylation which they felt was consistent with the concept that only a few of the genes were transcriptionally active. Recently, we have been studying the methylation state of a specific *MspI*/*HpaII* site located within 100 base pairs of the RNA start site in the IAP 5′ long terminal repeat sequence (19, 26; A. Feenstra and E. Kuff, manuscript in preparation). On the order of 100 gene copies are demethylated at this site in the DNA of IAP-rich neuroblastoma and myeloma cells, whereas in the DNA of BALB/c liver and thymus demethylation is below the limit of detection (less than about 10 copies per haploid gene). Thus, the observed variations in thymus IAP-related RNA and protein patterns may be related to the differential expression of a very minor fraction of the total IAP gene population. Since mouse IAP genes are polymorphic in a number of respects, the number and identity of the active genes in thymuses of different strains could possibly be established by detailed comparison of cDNA clones from the respective tissues.

The partial suppression of p73 production in thymuses of F_1 progeny between BALB/c and C57BL/6 mice was not consistent with the uncomplicated expression of constitutively active genes inherited from presumably homozygous parents. Although more detailed analysis of this phenomenon is clearly required, including examination at the level of RNA expression, the results suggest the operation of a *trans*-operating regulatory factor(s) contributed by the C57BL/6 parent. It seems likely that DNA methylation will prove to be only one level of control of IAP expression and that the demonstrated promoter activity of fully demethylated IAP long terminal repeats (26) can be influenced by other factors.

Strain-related variations in thymus IAP-specific RNAs were reflected in the patterns of protein expression. We believe that the 7.2-kb RNA codes for p73 and the 5.4-kb RNA codes primarily for proteins in the p114-p120 size range. This supposition is based not only on the earlier in vitro translation studies, but also on the correlation between the relative proportions of the two RNAs in various tissues and the in vivo labeling of the two classes of protein. For example, the 7.2 kb RNA predominates in neuroblastoma, and p73 is the major immunoprecipitated protein in these cells. Nearly equivalent levels of 7.2- and 5.4-kb transcripts are found in BALB/c thymus, and in this tissue p73 and p114-p120 label with approximately equal intensity. BALB/c thymus is unique among the strains tested in containing a high proportion of 7.2-kb transcript and at the same time is the only thymus in which p73 is a major synthetic product. Recently, we have found that a 120,000-molecular-weight protein was the major immunoprecipitable product when 5.4-kb poly(A) RNA from neuroblastoma IAPs was translated in a cell-free reticulocyte system (data not shown).

The IAP p73 is the chief structural component of the inner particle shell (32, 44) and may be regarded as analogous to the unprocessed *gag* polypeptides of conventional retroviruses (8). It is likely that the coding region for p73 lies towards the 5′ end of the IAP genome (36), and there is indirect evidence that it is followed by a polymerase-coding region as in the usual retroviral genetic order (4). The p120 protein contains methionine-labeled tryptic peptides of p73 plus additional protein sequence of unknown nature. We are presently trying to define the genetic content of the 5.4-kb RNA species which codes for the p114-p120 polypeptides. On Northern blots, this RNA reacts with cloned probes covering the IAP genome for a distance of at least 2.5 kb downstream from the p73 coding region (data not illustrated), showing that the additional sequence is in fact IAP related. However, it is not yet clear whether this additional sequence is derived entirely from the putative polymerase (*pol*) region of the IAP genome or includes information from the more 3′ region which in conventional retroviruses contains the envelope (*env*) coding sequence but whose function in the IAP genome has not been defined (no envelope protein

having been identified for the IAPs). Polymerase precursor polypeptides incorporating both *gag* and *pol* sequences are common to all functional retroviruses, although these are synthesized from full-size viral transcripts (8) rather than from a subgenomic form of RNA, such as the 5.4-kb species. A *gag-env* fusion polypeptide would be unusual among the retroviruses, whose envelope proteins are synthesized from specific subgenomic mRNAs formed by splicing the 5′ leader region of the viral RNA to the 3′ envelope coding region (8). Analysis of R-loops formed between isolated 5.4-kb RNA and cloned IAP genes should help to clarify the origin of this RNA.

The present study shows that the p114-p120 group of polypeptides can be expressed, sometimes abundantly, in the virtual absence of the main IAP structural protein. It may be questioned whether typical IAPs can be assembled under such circumstances. In this respect, IAPs have been observed by electron microscopy in BALB/c thymus (45), but were not detected after prolonged search in glands from C57BL/6 animals (E. Leiter, personal communication).

Our results are relevant to the known IAP expression in early mouse development. Yotsuyanagi and Szollosi (46) have recently shown that large IAPs typical of those seen in mouse tumors are present in ovarian oocytes but disappear progressively during oocyte maturation, fertilization, and early cleavage. After a minimum at the two- to four-cell stage, there is some increase in this type of IAP with further development, but the numbers remain small in the embryos of most of the mouse strains examined. (A second population of morphologically distinct intracisternal particles, the "small IAPs" [6] or "ε-particles" [46], shows a reciprocal time course of appearance, reaching a peak of abundance at the two-cell state and diminishing thereafter. At all periods after cleavage begins, the ε-particles far outnumber the larger IAPs. The genetic relationship between these two types of intracisternal particle remains at issue [46, 47].) Piko et al. (37) measured levels of IAP-related RNA in oocytes and early embryos derived from mating (BALB/c × DBA/2)F_1 mice. They found that the RNA molecules per "embryo" fell 15-fold during maturation of the oocyte to the unfertilized egg and then increased during cleavage in a manner indicative of renewed and continuous synthesis. IAP-related RNA from late blastocysts, consisting almost entirely of molecules synthesized after fertilization, was chiefly 5.4 kb in size. Our findings raise the possibility that this RNA might support comparatively little synthesis of the p73 structural protein, a supposition consistent with the observed paucity of large IAPs during the post-fertilization period. Some IAP formation does occur, however, particularly in Swiss mice (46), and metabolically labeled peptides with molecular weights between 67,000 and 73,000 have been precipitated from two- to eight-cell embryos of the ICR (random-bred Swiss) line with antiserum prepared against purified myeloma IAPs (12). It would be of interest to determine the relative expression of the 7.2- and 5.4-kb RNA species in mouse embryos of different genetic backgrounds and to examine these embryos specifically for the higher-molecular-weight IAP-related polypeptides. Proteins of this type might account for the observed positive immunocytochemical reactions for IAP-related antigen at the embryo cell surfaces (13), where formed IAPs are absent, and over the ε-particles themselves (14).

Although IAP genetic information is constitutively expressed in the thymus and other normal tissues of adult and embryonic mice, it is not possible to ascribe a programmed physiological role to the IAP-related protein products. The intracellular localization and possible polymerase activity of the p114-p120 group of polypeptides are currently under study. Rapid cell proliferation in the young thymus is associated with extensive cell death, a process which may entail the release of IAP-related polypeptides in extracellular and possibly immunogenic form. Immune responses to IAP polypeptides have been considered in the pathogenesis of a genetically determined insulin-resistant diabetes syndrome in mice, characterized by enhanced IAP expression in pancreatic B cells, B-cell necrosis, and pancreatic islet atrophy (24).

ACKNOWLEDGMENTS

We thank K. K. Lueders for many useful discussions and C. Ray for expert help in preparation of the manuscript.

ADDENDUM IN PROOF

Recent detailed hybridization studies, using probes derived from various regions of a 7.3-kb IAP gene, indicate that a majority of the 5.4-kb transcripts from both neuroblastoma and thymus are deficient in the sequence encompassing a 3′ portion of the *gag* (p73)-coding region and a 5′ portion of *pol*. Such transcripts may be derived from members of a recognized class of type I IAP genes that contain 1.8-kb deletions in this region (40), as recently suggested by Wujcik et al. (J. Virol **52**:29–35, 1985).

LITERATURE CITED

1. **Amano, T., E. Richelson, and M. Nirenberg.** 1972. Neurotransmitter synthesis by neuroblastoma clones. Proc. Natl. Acad. Sci. U.S.A. **69**:258–263.
2. **Aoki, T., B. Stück, L. J. Old, U. Hämmerling, and E. de Harven.** 1970. E antigen: cell surface antigen of C57BL leukemias. Cancer Res. **30**:244–251.
3. **Auffray, C., and F. Rougeon.** 1980. Purification of mouse immunoglobin heavy-chain messenger RNAs from total myeloma tumor RNA. Eur. J. Biochem. **107**:303–314.
4. **Callahan, R., E. L. Kuff, K. K. Lueders, and E. Birkenmeier.** 1981. The genetic relationship between the *Mus cervicolor* M432 retrovirus and the *Mus musculus* intracisternal type-A particle. J. Virol. **40**:901–911.
5. **Canaani, E., O. Dreazen, A. Klar, G. Rechavi, D. Ram, J. B. Cohen, and D. Givol.** 1983. Activation of the c-*mos* oncogene in a mouse plasma cytoma by insertion of an endogenous intracisternal A-particle genome. Proc. Natl. Acad. Sci. U.S.A. **80**:7118–7122.
6. **Chase, D. G., and L. Piko.** 1973. Expression of A- and C-type particles in early mouse embryos. J. Natl. Cancer Inst. **51**:1971–1975.
7. **Cohen, J. B., T. Unger, G. Rechavi, E. Canaani, and D. Givol.** 1983. Rearrangement of the oncogene c-*mos* in mouse myeloma NSI and hybridomas. Nature (London) **306**:797–799.
8. **Dickson, C., R. Eisenman, H. Fan, E. Hunter, and N. Teich.** 1982. Protein biosynthesis and assembly, p. 513–648. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

8a. **Finnegan, J. D.** 1985. Transposable elements in eukaryotes. Int. Rev. Cytol. **93**:281–326.

9. **Gattoni-Celli, S., W.-L. W. Hsiao, and I. B. Weinstein.** 1983. Rearranged c-*mos* locus in a MOPC-21 murine myeloma cell line and its persistence in hybridomas. Nature (London) **306**:795–796.
10. **Hawley, R. G., M. J. Shulman, H. Murialdo, D. M. Gibson, and N. Hozumi.** 1982. Mutant immunoglobulin genes have repetitive DNA elements inserted into their intervening sequences. Proc. Natl. Acad. Sci. U.S.A. **79**:7425–7429.
11. **Hojman-Montes de Oca, F., L. Dianoux, J. Peries, and R. Emanoil-Ravicovitch.** 1983. Intracisternal A particles: RNA ex-

pression and DNA methylation in murine teratocarcinoma cell lines. J. Virol. 46:307–310.
12. **Huang, T. T. F., Jr., and P. G. Calarco.** 1981. Immunoprecipitation of intracisternal A-particle-associated antigens from preimplantation mouse embryos. J. Natl. Cancer Inst. 67:1129–1133.
13. **Huang, T. T. F., Jr., and P. G. Calarco.** 1981. Evidence for cell surface expression of intracisternal A-particle-associated antigens during early mouse development. Dev. Biol. 82:388–392.
14. **Huang, T. T. F., Jr., and P. G. Calarco.** 1982. Immunologic relatedness of intracisternal A-particles in mouse embryos and neoplastic cell lines. J. Natl. Cancer Inst. 68:643–649.
15. **Kelly, F., and C. Hubert.** 1982. Tumor viruses and early mouse embryos. Biochim. Biophys. Acta 651:105–141.
16. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predetermined specificity. Nature (London) 256:495–497.
17. **Kuff, E. L., R. Callahan, and R. S. Howk.** 1980. Immunological relationship between the structural proteins of intracisternal A-particles of *Mus musculus* and the M432 retrovirus of *Mus cervicolor*. J. Virol. 33:1211–1214.
18. **Kuff, E. L., A. Feenstra, K. K. Lueders, G. Rechavi, D. Givol, and E. Canaani.** 1983. Homology between an endogenous viral LTR and sequences inserted in an activated cellular oncogene. Nature (London) 302:547–548.
19. **Kuff, E. L., A. Feenstra, K. K. Lueders, L. Smith, R. Hawley, N. Hozumi, and M. Shulman.** 1983. Intracisternal A-particle genes as movable elements in the mouse genome. Proc. Natl. Acad. Sci. U.S.A. 80:1992–1996.
20. **Kuff, E. L., K. K. Lueders, H. L. Ozer, and N. A. Wivel.** 1972. Some structural and antigenic properties of intracisternal A-particles occurring in mouse tumors. Proc. Natl. Acad. Sci. U.S.A. 69:218–222.
21. **Kuff, E. L., L. A. Smith, and K. K. Lueders.** 1981. Intracisternal A-particle genes in *Mus musculus*: a conserved family of retrovirus-like elements. Mol. Cell. Biol. 1:216–227.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.
23. **Lasneret, J., M. Canivet, F. Hojman-Montes de Oca, J. Tobaly, R. Emanoil-Ravicovitch, and J. Peries.** 1983. Activation of intracisternal A-particles by 5-azacytidine in mouse Ki-BALB cell line. Virology 128:485–489.
24. **Leiter, E. H., and E. L. Kuff.** 1984. Intracisternal type A particles in murine pancreatic B cells: immunocytochemical demonstration of increased antigen (p73) in genetically diabetic mice. Am. J. Pathol. 114:46–55.
25. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
26. **Lueders, K. K., J. W. Fewell, E. L. Kuff, and T. Koch.** 1984. The long terminal repeat of an endogenous intracisternal A-particle gene functions as a promoter when introduced into eucaryotic cells by transfection. Mol. Cell. Biol. 4:2128–2135.
27. **Lueders, K. K., and E. L. Kuff.** 1975. Synthesis and turnover of intracisternal A-particle structural protein in cultured neuroblastoma cells. J. Biol. Chem. 250:5192–5199.
28. **Lueders, K. K., and E. L. Kuff.** 1977. Sequences associated with intracisternal A particles are reiterated in the mouse genome. Cell 12:963–972.
29. **Lueders, K. K., and E. L. Kuff.** 1980. Intracisternal A-particle genes: identification in the genome of *Mus musculus* and comparison of multiple isolates from a mouse gene library. Proc. Natl. Acad. Sci. U.S.A. 77:3571–3575.
30. **Lueders, K. K., S. Segal, and E. L. Kuff.** 1977. RNA sequences specifically associated with mouse intracisternal A-particles. Cell 11:83–94.
31. **Maniatis T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual, p. 202–203. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Marciani, D., and E. L. Kuff.** 1973. Isolation and partial characterization of the internal structural proteins of murine intracisternal A-particles. Biochemistry 12:5075–5083.
33. **McIntire, K. R., R. M. Asofsky, M. Potter, and E. L. Kuff.** 1965. Macroglobulin-producing plasma-cell tumor in mice: identification of a new light chain. Science 150:361–363.
34. **Ono, M., M. D. Cole, A. T. White, and R. C. C. Huang.** 1980. Sequence organization of cloned intracisternal A particle genes. Cell 21:465–473.
35. **Paterson, B. M., and J. O. Bishop.** 1977. Changes in the mRNA population of chick myoblasts during myogenesis *in vitro*. Cell 12:751–765.
36. **Paterson, B. M., S. Segal, K. K. Lueders, and E. L. Kuff.** 1978. RNA associated with murine intracisternal type A particles codes for the main particle protein. J. Virol. 27:118–126.
37. **Piko, L., M. D. Hammons, and K. D. Taylor.** 1984. Amounts, synthesis and some properties of intracisternal A particle-related RNA in early mouse embryos. Proc. Natl. Acad. Sci. U.S.A. 81:488–492.
38. **Rechavi, G., D. Givol, and E. Canaani.** 1982. Activation of a cellular oncogene by DNA rearrangement: possible involvement of an IS-like element. Nature (London) 300:607–611.
39. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. 113:237–251.
40. **Shen-Ong, G. L. C., and M. D. Cole.** 1982. Differing populations of intracisternal A-particle genes in myeloma tumors and mouse subspecies. J. Virol. 47:411–421.
41. **Shen-Ong, G. L. C., and M. D. Cole.** 1984. Amplification of a specific set of intracisternal A-particle genes in a mouse plasmacytoma J. Virol. 49:171–177.
42. **Wilson, S. H., E. W. Bohn, A. Matsukage, K. K. Lueders, and E. L. Kuff.** 1974. Studies on the relationship between deoxribonucleic acid polymerase activity and intracisternal A-type particles in mouse myeloma. Biochemistry 13:1087–1094.
43. **Wilson, S. H., and E. L. Kuff.** 1972. A novel DNA polymerase activity found in association with intracisternal A-type particles. Proc. Natl. Acad. Sci. U.S.A. 69:1531–1536.
44. **Wivel, N. A., K. K. Lueders, and E. L. Kuff.** 1973. Structural organization of murine intracisternal A-particles. J. Virol. 11:329–334.
45. **Wivel, N. A., and G. H. Smith.** 1971. Distribution of intracisternal A-particles in a variety of normal and neoplastic mouse tissues. Int. J. Cancer 7:167–175.
46. **Yotsuyanagi, Y., and D. Szollosi.** 1981. Early mouse embryo intracisternal particle: fourth type of retrovirus-like particle associated with the mouse. J. Natl. Cancer Inst. 67:677–685.
47. **Yotsuyanagi, Y., and D. Szollosi.** 1984. Virus-like particles and related expressions in mammalian oocytes and preimplantation stage embryos, p. 218–234. *In* J. Van Blerkom and P. M. Motta (ed.), Ultrastructure of reproduction. Martinus Nijhoff, Boston.