Brief Definitive Report

AMPLIFIED *env* AND *gag* PRODUCTS ON AKR CELLS

Origin from Different Murine Leukemia Virus Genomes*

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Products of both the *env* (envelope) and *gag* (core) genes of murine leukemia viruses (MuLV) are present on the plasma membrane of AKR mouse thymocytes (1, 2). The *env* product is gp70. The *gag* products are the polyproteins gP85 and gP95. The expression of antigens associated with both gp70 and the polyproteins on AKR thymocytes becomes amplified before the onset of overt leukemia (3). In this report, we give evidence that the *env* information for the amplified gp70, and the *gag* information for the amplified gP85 and gP95, are derived from different proviral genomes.

The serum anti-X.1 (4) precipitates one of at least two varieties of gp70 present on AKR thymocytes (5). The type variant precipitable by anti-X.1 serum was called X-gp70 (5), but we now propose to name it Ec^+ gp70 because anti-X.1 serum recognizes gp70 only from ecotropic virus (6), and also because peptide maps of X-gp70 of the plasma membrane resemble peptide maps of gp70 of ecotropic virus (6). Accordingly, the gp70 that remains after Ec^+ gp70 has been eliminated by precipitation with anti-X.1 serum is referred to below as Ec^- gp70.

The peptide maps of Fig. 1 emphasize the contrast between AKR cellular Ec^+ gp70 (maps b and e), which resembles ecotropic viral gp70 (6), and AKR cellular Ec^- gp70 (maps c and f), which resembles xenotropic viral gp70 (6). The peptide map for total gp70 (a and d) is clearly composed of Ec^+ gp70 and Ec^- gp70. In qualitative terms, the maps for thymocytes of 2-mo-old AKR mice (Fig. 1 A) and those for AKR leukemia cells (Fig. 1 B) are rather similar.

Fig. 2 illustrates that Ec^- gp70 is amplified with age but Ec^+ gp70 is not, implying that the *env* gene involved in amplification does not belong to ecotropic virus.

With regard to the polyproteins, the following evidence implies that the polyproteins gP85 and gP95 are products of one *gag* gene, and not of alternative *gag* genes: First, they have never been observed independently of one another. Secondly, as shown in Fig. 1 C, gP85 and gP95 have similar peptide maps (g and h) with the exception of a prominent gP95 spot (compare g and h) that is also found in the map of p10 purified from virions (i); we do not find this spot in maps of the other processed *gag* proteins, p30, p15, and p12. These observations are in accord with our finding that no type-specific or group-specific anti-*gag*-protein serum other than anti-p10, can distinguish gP85 from gP95. Because the p10 sequence is encoded at the 3′-end of the *gag* gene (7), we conclude that both polyproteins are derived from the same viral gene, and

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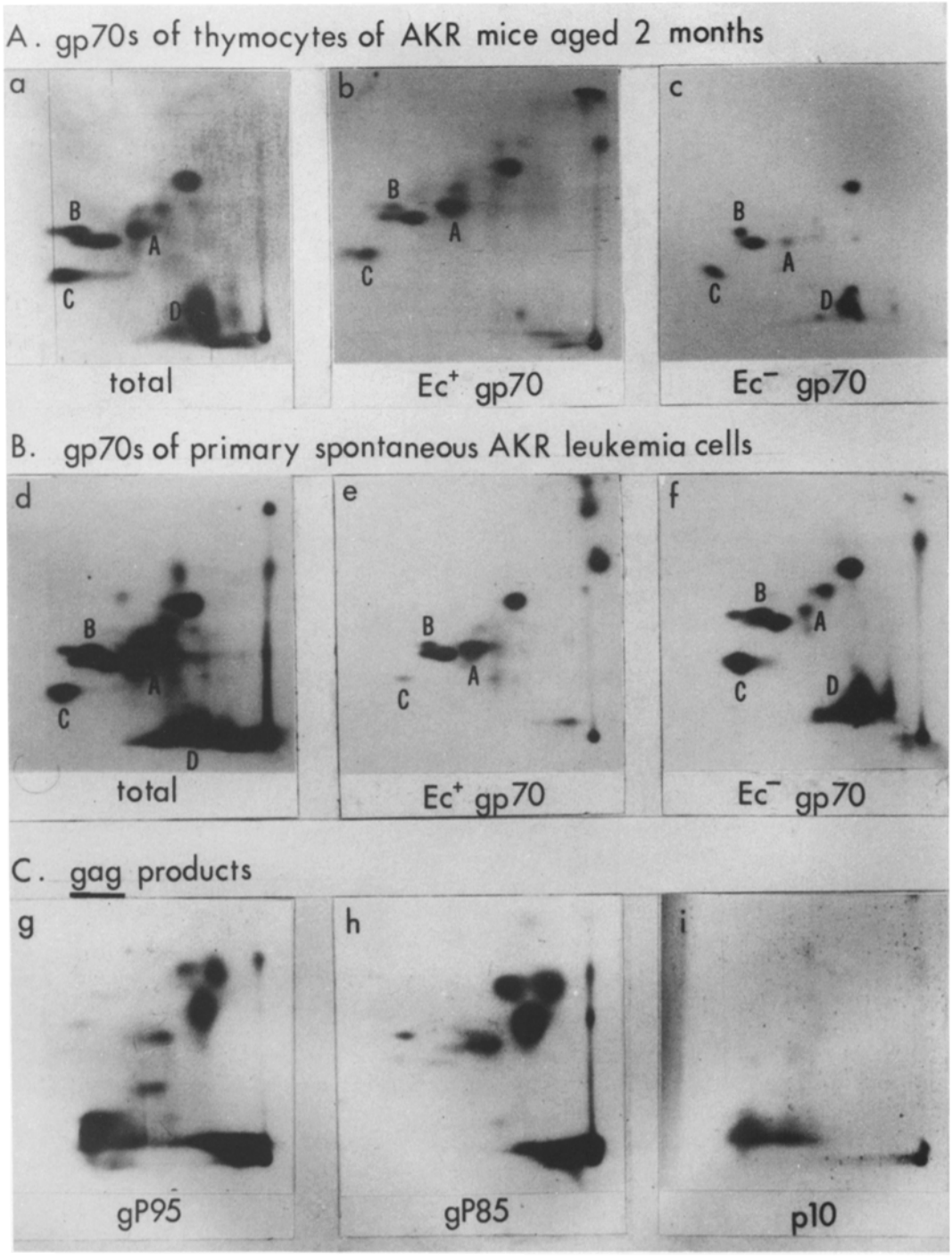

FIG. 1. Two-dimensional maps of tryptic peptides from ^{125}I-labeled *env* and *gag* products isolated by immunoprecipitation and slab gel electrophoresis (6). Total gp70: (a) and (d), was precipitated with the group-specific goat-anti-Rauscher-MuLV-gp70 serum (obtained from R. Lerner, Scripps Clinic and Research Foundation, La Jolla, Calif.). Ec^+ gp70: (b) and (e), was precipitated with type-specific mouse-anti-X.1 serum. Ec^- gp70: (c) and (f), was precipitated with group-specific gp70 antiserum after prior clearance-precipitation with anti-X.1 serum. gP95: (g), and gP85: (h), were precipitated from AKR leukemia cells with rabbit-anti-Rauscher-MuLV-p30 serum (obtained from W. Hardy, Jr., Memorial Sloan-Kettering Cancer Center). p10: (i), was precipitated with goat-anti-AKR-MuLV-p10 serum (obtained from R. Wilsnack, Huntingdon Research Center, Brooklandville, Md.). Some spots of maps a–f are designated as A, B, C, and D for the purpose of orientation. Spot A appears to be characteristic for Ec^+ gp70. Spots B and C represent peptides common to both Ec^+ and Ec^- gp70. Spot D may represent a mixture of several unresolved peptides; it is a feature of the Ec^- gp70 class, including gp70 of replicating xenotropic virus (6). Small amounts of spot A present in maps c and f are attributed to incomplete preclearing by anti-X.1 serum.

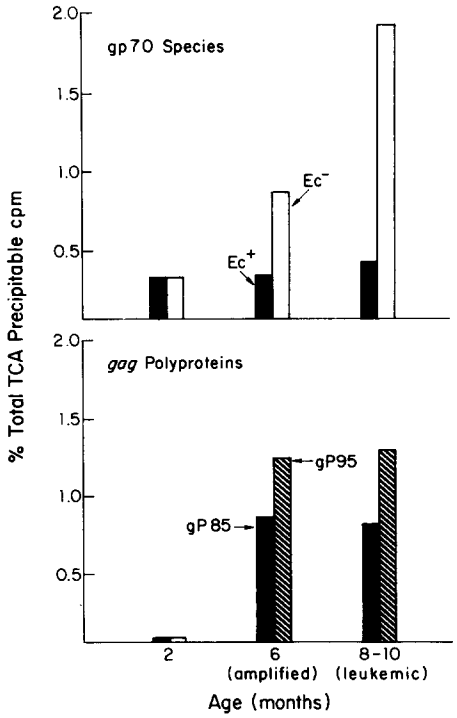

FIG. 2. Cell-surface MuLV proteins of AKR thymus. Top panel: bars represent counts per minute of ^{125}I of bands of gp70 excised from slab gels, presented as the percent of total trichloroacetic-acid-precipitable ^{125}I in the aliquots of surface-labeled cell lysate used for immunoprecipitation. Total gp70 and Ec^+ gp70 species were prepared by precipitation with excess group-specific and type-specific (anti-X.1) anti-gp70 sera, respectively. The amount of Ec^- gp70 was then calculated as the difference between the counts per minute in these two species (Ec^- gp70 = total gp70 − Ec^+ gp70). Values shown are the means of four experiments. Bottom panel: similar measurements of cell-surface gP85 and gP95 as percentages of total ^{125}I-labeled protein in the lysates. Values shown are means of three experiments.

that gP85 differs from gP95 by cleavage of a terminal amino acid sequence.[1] The following evidence links the premalignant amplification of gP85 and gP95 (Fig. 2) with ecotropic virus: (*a*) The polyproteins are known to carry determinants of the Gross cell-surface antigen (GCSA) antigen (2, 9, 10); (*b*) the full set of GCSA determinants are amplified (3); and (*c*) GCSA is fully encoded only by ecotropic endogenous MuLV and by recombinant viruses of the mink-cell-focus-inducing (MCF) category which have derived *gag* genes from Gross-type ecotropic MuLV (11, 12). Moreover, a monoclonal antibody against p30 (which constitutes part of the peptide sequence of gP85 and gP95) recognized p30 from ecotropic virus but not p30 from xenotropic virus, and precipitated both gP85 and gP95 from AKR leukemia cells (J.-S. Tung, P. V. O'Donnell, N. G. Famulari, and U. Hämmerling. Unpublished observations.).

[1] Ledbetter et al. (8) have found some arginine-containing tryptic peptides of p10 (an arginine-rich protein) to be present in gP85 as well as gP95. Our data would indicate that these peptides do not contain tyrosine. The deletion of only a portion of p10 could lead to a substantial molecular-weight change if this part of the sequence contains a glycosylation site.

We propose that the combination of major *env* and *gag* products observed on both premalignant and malignant AKR lymphoid cells reflects a genetic event bringing parts of two disparate proviral elements under coordinate transcriptional control. The simplest model would be a recombination event similar to that postulated in formation of MCF MuLV genomes (13).

Summary

Thymocytes of AKR mice express two species of gp70, the envelope glycoprotein of murine leukemia virus (MuLV), encoded by the *env* gene. One is denoted Ec^+ gp70 in reference to the type-antigen Ec and association with ecotropic virus. The other, Ec^- gp70, resembles gp70 found also on thymocytes of mouse strains that are not overt producers of MuLV, and has no evident relation to ecotropic virus. Expression of Ec^- gp70 type, but not of Ec^+ gp70 type, is amplified with age on AKR thymocytes. In contrast, viral core polyproteins, encoded by the *gag* gene and simultaneously amplified with age, appear to be related to ecotropic virus. These observations imply selective amplification of products of *env* and *gag* genes from two sorts of provirus, a phenomenon which may be connected to the dual genetic origin of recombinant mink-cell-focus inducing viruses in AKR mice.

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