

Brief Definitive Report

RELATIONSHIPS OF gp70 OF MuLV ENVELOPES TO gp70 COMPONENTS OF MOUSE LYMPHOCYTE PLASMA MEMBRANES*

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The term gp70 has been applied to the major envelope component of murine leukemia virus (MuLV), primarily on grounds of chemical characteristics and mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (1). However, antisera to MuLV or MuLV constituents react with certain cells that are not producing virus, yielding plasma-membrane molecules that are not distinguishable from viral gp70 by such criteria and so are also called 'gp70' (2-6). These gp70 species that compose part of the cellular plasma membrane may show mendelian inheritance (7) and genetic programming in the normal course of differentiation (8).

Three variants of the plasma-membrane gp70 class of molecules, — G_{IX}-gp70, X-gp70, and O-gp70—have been categorized by immunogenetic methods, and Table I shows the cell-surface gp70 phenotypes of thymocytes from several mouse strains. We have now made peptide maps of tryptic digests of gp70 bands obtained by PAGE of immunoprecipitated lymphoid cell lysates and compared these maps with maps of plasma-membrane gp70s from cultured cells infected by ecotropic or xenotropic MuLVs.

We used MuLVs from our own cloned stocks (9). AKR ecotropic virus 69E5 and AKR xenotropic virus 69X9 were isolated from the thymus of the same 6-mo-old AKR mouse. [³H]glucosamine-labeled viruses were prepared according to Tung et al. (4).

Cultured cells were grown in Dulbecco's modified Eagle minimal essential medium supplemented with 10% fetal calf serum. Productively infected cell cultures were washed, dispersed with EDTA, and harvested by centrifugation (9). Thymocytes were obtained from 2-mo-old female mice of our colonies. RADA1 and ASL1 are the transplanted A strain leukemias we studied earlier (5).

Cells were surface-iodinated with ¹²⁵I by the lactoperoxidase method and lysed with Nonidet P-40 (4). The lysates were reacted with antisera, and the antigen-antibody complexes precipitated with α -Ig. The immunoprecipitates were analyzed by SDS-PAGE on a Laemmli-type 5-17% gradient slab gel (10). The gp70 bands located by autoradiography were excised, and SDS removed by washing extensively with 50% methanol and 10% acetic acid followed by 10% methanol. The gel slices were dried by infra-red heat and subjected to tryptic peptide mapping (11). In brief, the gel slices containing gp70 were digested with trypsin (TPCK) (25 μ g in 1 ml of 1% NH₄HCO₃). The digests were lyophilized to dryness, dissolved in buffer I (acetic acid:formic acid:water, 15:5:80), spotted on to 10 cm \times 10 cm cellulose-coated thin layer plates, and electrophoresed at 900

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TABLE I
Cell-Surface gp70 Phenotypes of Mouse Thymocytes

Antigenic sub-species of gp70	Mouse strains						Reference
	A	AKR	G _{IX} congenic strains				
			B6	B6-G _{IX} ⁺	129	129-G _{IX} ⁻	
G _{IX} -gp70	+	+	-	+	+	-	4,7
X-gp70	-	+	-	-	-	-	5
O-gp70*	ND‡	ND	+	+	-	-	6

* O-gp70 was originally identified on normal B6 thymocytes as a species of gp70 with the following properties: (a) precipitation by group-specific α -gp70 serum (eg α -R-MuLV gp70), (b) characteristic electrophoretic mobility in SDS-PAGE vis-à-vis G_{IX}-gp70 of 129 thymocytes, and (c) absence of type specificities of G_{IX}-gp70 [recognised by antibody in ' α -NTD' rat serum, (W/Fu \times BN)F, α -W/Fu(C58NT)D (7), that is cytotoxic for G_{IX}⁺ thymocytes] and of X-gp70 [recognised by precipitating antibodies in α -X.1 serum, (BALB \times B6)F, α -BALB radiation leukemia RL3 1 (5)]. In serological terms, O-gp70 is thus defined negatively, because no antisera identifying its type-specificity (or its type-specificities if it comprises more than one sub-species of gp70) have yet been discovered; hence the designation 'O-gp70'.

‡ Not determined.

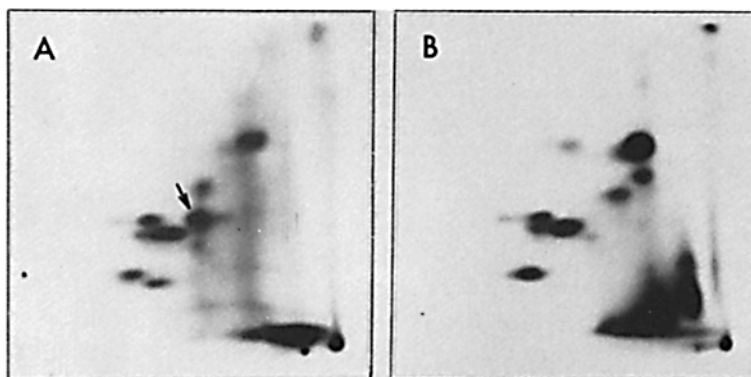


FIG. 1. Autoradiographs of ¹²⁵I-tyrosine-containing peptide maps of plasma-membrane gp70s of MuLV-infected tissue culture cells: (A) SC-1 mouse cells infected by AKR ecotropic virus 69E5, and (B) CCL64 mink cells infected by AKR xenotropic virus 69X9. Arrow indicates the 'A-spot'.

V for 20 min. The plates were dried, subjected to chromatography in buffer II (butanol:pyridine:acetic acid:water, 32.5:25:5:20), then dried and analyzed by autoradiography on Kodak XR-2 X-ray film with Cronex intensifying screens at -70°C .

Fig. 1 shows peptide maps from gp70s immunoprecipitated from MuLV-infected cultured cells. Goat α -R-MuLV gp70 antiserum was used for precipitation because of its broad group-specific reactivity. The two patterns shown, for (A) mouse fibroblasts infected with ecotropic AKR virus (AKR 69E5), and for (B) mink cells infected with xenotropic AKR virus (AKR 69X9), are readily distinguishable. The prominent 'A-spot' marked with an arrow on map A and missing from map B is a useful distinguishing feature. Maps from gp70s immunoprecipitated from virions gave patterns similar to those for infected cells. Thus virions of two ecotropic viruses (AKR-L1 CLG12 and BALB N-tropic WN1802N, reference 9) gave gp70 maps like pattern A (including the A-spot) whereas virions of two xenotropic viruses [AT124 and S16CL10(I), reference 9] gave gp70 maps like pattern B (no A-spot).

The two gp70 patterns in Fig. 1 (cultured cells infected with ecotropic or

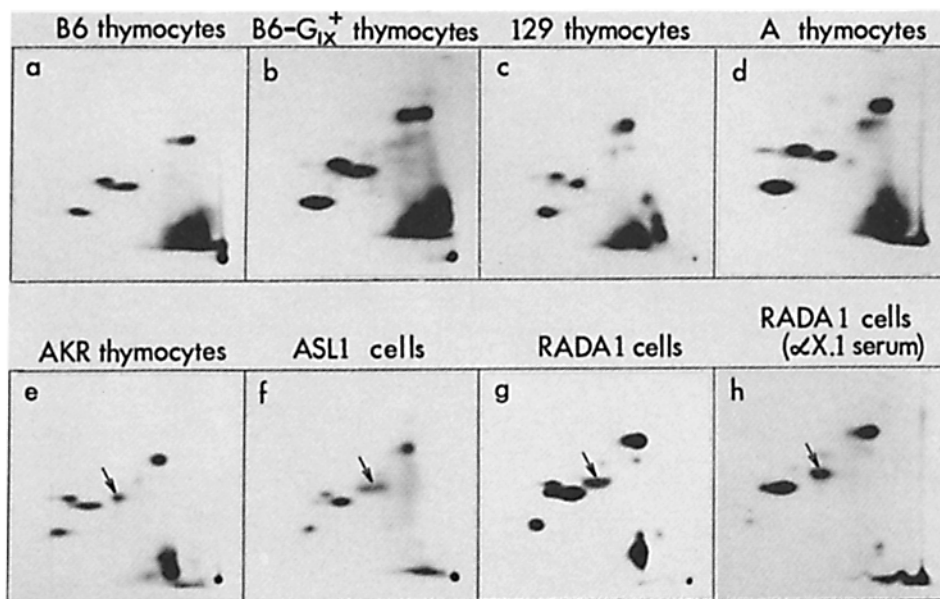


FIG. 2. Autoradiographs of ^{125}I -tyrosine-containing peptide maps of mouse cell plasma-membrane gp70s. Arrows indicate the A-spot.

xenotropic virus) were compared with maps of gp70 from seven mouse cell populations, comprising thymocytes of five mouse strains (Table I), and two A strain leukemias, RADA1 and ASL1, known to be X-gp70-positive (5). The seven maps are shown in Fig. 2.

(a) Maps *a* to *d*, for thymocytes of strains B6, B6-G_{IX}⁺, 129 and A, show no pronounced differences. Thus G_{IX}-gp70 and O-gp70, though serologically distinct (Table I), are not distinguished by differences in peptide maps under the conditions used (which reveal only tyrosine-containing peptides).

(b) The same four maps (*a* to *d*), resemble map B of Fig. 1 (xenotropic virus gp70) more closely than map A (ecotropic virus gp70), notably in lacking the A-spot. Thus G_{IX}-gp70 and O-gp70 of thymocytes of these four strains seem related to xenotropic virus.

(c) Maps *e* to *g*, for AKR thymocytes and the two A strain leukemias, are broadly similar to one another and present a second pattern for gp70 of lymphocyte plasma membranes. This is more like A of Fig. 1 (ecotropic virus gp70), including the A-spot.

(d) Sequential precipitation (5) indicates that each of the leukemias expresses at least two gp70s. Thus gp70 patterns from these cells could be composite if these two gp70s give distinguishable maps. Map *h* shows this is probably so. For map *h* the lysate of RADA1 leukemia cells was precipitated with α -X.1 serum, which reacts with only one of the gp70s on this cell population (5). Some components of map *g* are missing from map *h*, and the missing elements therefore belong to a second gp70, other than X-gp70.

(e) Map *h* retains the A-spot which distinguishes ecotropic MuLV gp70 from xenotropic MuLV gp70 in the materials tested. The A-spot can therefore be assigned in this instance to X-gp70. The resemblance of map *h* to gp70 of ecotropic rather than xenotropic MuLV accords with the precipitation of gp70s

TABLE II
Discrimination of MuLV gp70 Types by α -X.1 Serum

³ H-Glucosamine-labeled MuLV (reference 9)	gp70 Precipitated by α -X.1 (% total gp70)*
Ecotropic:	
Gross Passage A	92†, 98, 100
AKR	92, 95, 101
WN1802N (BALB Endogenous)	95, 102
WN1802B (BALB Endogenous)	65, 70
B6N (B6 Endogenous)	82, 90, 93
B6B (B6 Endogenous)	89, 92
RL1 (From a BALB radiation-induced leukemia)	98, 102
Rauscher	7, 8, 10
Xenotropic:	
AT124 (From NIH/Swiss mice)	<1, <1, <1
S16 CL10(D) (From BALB/3T3 cell line)	<1, <2, <1

* cpm of gp70 PAGE gel band from α -X.1 precipitate \div cpm of the same band from α -R-MuLV gp70 precipitate \times 100. The quantities of labeled virus used were equivalent, and the same dilution of each antiserum was used for all determinations with that antiserum.

† Individual values represent separate experiments.

of several ecotropic but not xenotropic virions by α -X.1 serum (Table II).

There is further evidence that G_{IX} -gp70 is associated with xenotropic MuLV: mouse strain 129 and its congenic partner 129- G_{IX} are genotypically similar except for the locus *Gv-1*. G_{IX} -gp70 is present on thymocytes and in the serum of 129 but not 129- G_{IX} mice (12, 13). Thus *Gv-1* controls expression of G_{IX} -gp70 in both situations. Map *c* of Fig. 2 represents the G_{IX} -gp70 molecule recognized in the 129 thymocyte plasma membrane by the G_{IX} cytotoxicity assay. Also Elder et al. (11) report that the tryptic peptide map of gp70 found in 129 but not 129- G_{IX} serum resembles that of xenotropic virus from NZB mice. And radioimmunoassays of McClintock et al. (14) and Stephenson et al. (personal communication) similarly indicate that G_{IX} -gp70 belongs to xenotropic virus.

Summary

The family of glycoproteins called gp70 includes molecules that are the main constituent of murine C-type viral envelopes, and some that are expressed as mendelian constituents of thymocyte plasma membranes in the absence of virions. To investigate further the relation of viral gp70s to plasma-membrane gp70s we compared peptide maps of gp70s derived by immunoprecipitation from cells infected with chosen viruses and from various thymocytes and leukemia cells known to express one or more of three immunogenetically defined gp70 types: G_{IX} -gp70, X-gp70, and O-gp70. Maps of gp70 from cultured cells infected with ecotropic and xenotropic viruses were distinguishable from one another, and in general resembled gp70 maps prepared directly from ecotropic and xenotropic virions respectively. Maps of gp70s immunoprecipitated from thymocytes of five mouse strains and from two A strain T-cell leukemias also fell into two distinguishable and generally corresponding patterns. Thus peptide-mapping substantiates earlier conclusions that viral gp70s and plasma-membrane gp70s inherited independently of virus-production are highly related or identical molecules. The gp70 maps of thymocytes from B6, B6- G_{IX}^+ , 129, and A mice formed a group resembling the map from cultured cells infected with xenotropic virus. Thymocytes from AKR mice, and the two A strain leukemias, gave gp70 maps conforming more to the second pattern, that of cultured cells

infected with ecotropic virus. This second pattern probably comprises at least two gp70 types, one of which is X-gp70. Our data indicate that the G_{IX}-gp70 and O-gp70 sub-species of gp70 expressed in the cell populations we have studied are coded by xenotropic viral genomes, and X-gp70 by ecotropic viral genomes.

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