

**X-gp70: A THIRD MOLECULAR SPECIES OF THE ENVELOPE  
PROTEIN gp70 OF MURINE LEUKEMIA  
VIRUS, EXPRESSED ON MOUSE LYMPHOID CELLS\***

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It is now apparent that the gp70<sup>1</sup> envelope glycoprotein of murine leukemia virus (MuLV) is frequently expressed in mice that are not producing detectable amounts of MuLV (1-4). Furthermore, at least two species of the gp70 molecule have been distinguished (5). These may be referred to provisionally as G<sub>IX</sub>-gp70, found on 129 thymocytes, and 0-gp70, found on C57BL/6 (B6) thymocytes, on the basis of the presence or absence of the G<sub>IX</sub> type-specificity. These gp70 variants are coded or regulated by different unlinked genes; the thymocytes of an inbred mouse may express either, both, or neither (5). The recognition of distinct variants of gp70 offers new opportunities for exploring the role of C-type viral genomes in oncogenesis. Thus, it should become possible: (a) to relate particular gp70 species to particular types of ecotropic and xenotropic C-type viruses, whose oncogenic potentials may differ, and (b) to assess whether susceptibility to oncogenesis of any sort may be associated with constitutive expression of one or another gp70 species. For these reasons, as well as the general interest in this class of molecules, it seems important to identify other distinct gp70 variants of the mouse as a basis for assessing their biological significance. We report here the recognition of a third variant which we tentatively name X-gp70.

The three antisera used in this study are named and briefly described in Table I. All three are standard typing sera used in serological tests in this laboratory. All three are known to label virions in immunoelectronmicroscopy (6), although in the case of  $\alpha$ TL (the usual serum used for TL typing;  $\alpha$ , anti) this reaction with virions is almost certainly due to  $\alpha$ gp70 antibody generated by the TL<sup>+</sup> leukemia cells used for immunization and presumably has no relation to the TL antigens themselves.

The technique of surface iodination of cells bearing gp70 and detergent (Nonidet P-40 or NP-40) lysis of labeled cells, followed by immunoprecipitation of gp70 and subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as before (4).

Fig. 1 shows the results obtained with the three antisera in tests with three kinds of A-strain mouse cells: (a) normal thymocytes (which are G<sub>IX</sub><sup>+</sup> (7) and produce few or no virions visible in electromicrograph), (b) the transplanted G<sub>IX</sub><sup>+</sup> (E. Stockert, personal communication) X-radiation leukemia RADA1 (as-

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<sup>1</sup> MuLV glycoprotein of molecular weight in the range of 69,000-71,000 (sometimes called gp69/71).

TABLE I  
The Three Antisera

Abbreviation in text	Reference	Immunization	Description
$\alpha$ NTD	5, 7, 8	(W/Fu $\times$ BN)F <sub>1</sub> rat anti-W/Fu rat leukemia NTD induced by MuLV	Standard antiserum for G <sub>IX</sub> -typing by cytotoxicity assay; also contains antibody to group-specific antigen of gp70
$\alpha$ X.1	10	(BALB/c $\times$ B6)F <sub>1</sub> antiradiation leukemia BALB/c RL $\delta$ 1	Standard typing serum for X.1 leukemia-associated antigen in the cytotoxicity assay
$\alpha$ TL	13	(B6 $\times$ A-TL <sup>-</sup> )F <sub>1</sub> anti-A strain spontaneous leukemia ASL1	Standard typing serum for TL antigen on normal thymocytes and leukemia cells (TL.1,2,3) by cytotoxicity assay

cites cells), and (c) the transplanted G<sub>IX</sub><sup>-</sup> (E. Stockert, personal communication) spontaneous leukemia ASL1 (cells obtained from spleens of passage mice). Strain A thymocytes are G<sub>IX</sub><sup>+</sup>, and both leukemias are producing virions; thus, as expected,  $\alpha$ NTD (which contains antibody to gp70 group-specific antigen [5, 8]) precipitated gp70 from all three cell types (Fig. 1 a-c). But  $\alpha$ X.1 (Fig. 1 d-f) and  $\alpha$ TL (Fig. 1 g-i) precipitated gp70 only from the two leukemia lines, not from thymocytes.<sup>2</sup> Therefore the two leukemias express a gp70 variant which is not detectable on A-strain thymocytes. It cannot be G<sub>IX</sub>-gp70, and it is identified by both  $\alpha$ X.1 and  $\alpha$ TL. Provisionally, we propose to call this gp70 variant 'X-gp70.' A more systematic nomenclature for members of the gp70 molecular species will be called for in due course. In the meantime we chose the arbitrary and neutral prefix 'X' because X-gp70 was first recognized with X.1 antiserum. The X.1 system (10) was so named because antibodies to X.1 antigens are formed by mice (i.e., within the species), and the X.1 immune response is under the control of *Ir* genes, which suggested an interpretation of the 'X' antigens which Gorer et al. (11) demonstrated serologically in mouse leukemia cells many years ago. The presence of  $\alpha$ X-gp70 antibody in the  $\alpha$ TL antiserum is explained by the fact that the TL<sup>+</sup> leukemia used for producing this particular TL antiserum is ASL1.

Fig. 2 summarizes studies with RADA1 leukemia cells which demonstrate that two distinct variants of gp70 are carried by the A-strain leukemias. This conclusion is based on the argument that: (a) if  $\alpha$ X.1 and  $\alpha$ TL identify a type-specific gp70 (X-gp70) then removal of this gp70 by immunoprecipitation with either antiserum should leave in solution the other gp70, precipitable in turn by reaction with  $\alpha$ NTD; and (b), on the other hand, precipitation with  $\alpha$ NTD should leave nothing that can subsequently be precipitated by  $\alpha$ X.1 and  $\alpha$ TL. Both  $\alpha$ X.1 and  $\alpha$ TL remove gp70 precipitable by either antiserum (Fig. 2 e,f,h,i), but leave gp70 precipitable by  $\alpha$ NTD (Fig. 2 b,c), which confirms (a) above;  $\alpha$ NTD removes all gp70 precipitable by any of the three antisera (Fig. 2 a,d,g), illustrating that (b) also is correct. Thus RADA1 leukemia cells carry two species of gp70, one of which can be accounted for as the G<sub>IX</sub>-gp70, which is present on A-strain thymocytes, and another which is not present on A thymocytes and which we call X-gp70. The gp70 discovered on B6 thymocytes in

<sup>2</sup> The "tumor-associated antigen" which Yu and Cohen (9) observed with  $\alpha$ TL serum is doubtless this gp70.

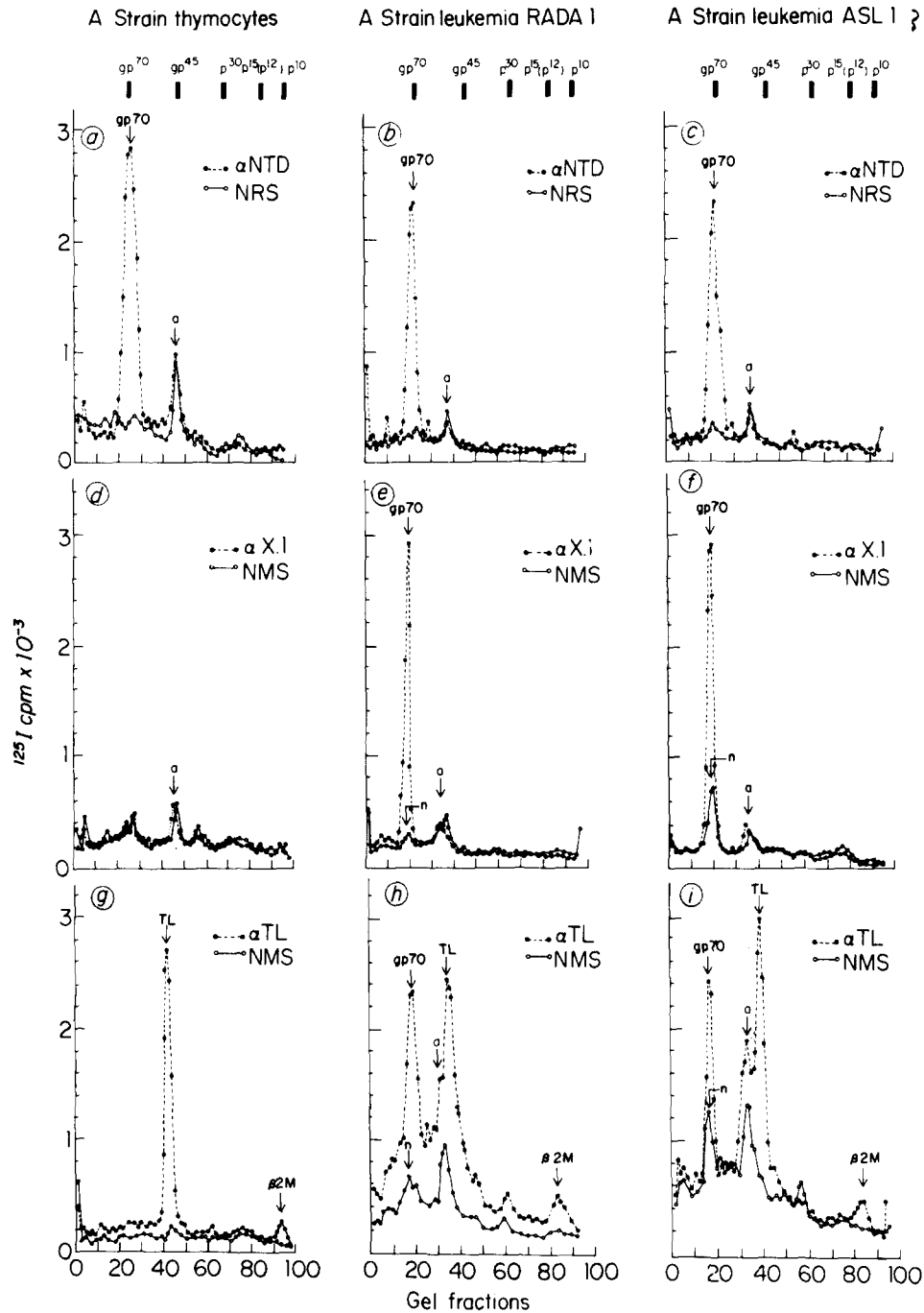


FIG. 1. SDS-PAGE patterns of immunoprecipitates from NP-40 lysates of surface-iodinated cells (A thymocytes, RADA1, and ASL1) formed by different antisera ( $\alpha$ NTD or normal rat serum, NRS: a,b,c;  $\alpha$ X.1 or normal BALB/c mouse serum, NMS: d,e,f; and  $\alpha$ TL or NMS: g,h,i). The patterns are plotted together from parallel gels. The electrophoresis conditions were previously described (4). The anode is on the right. The markers used for estimation of molecular weights were  $^{14}$ C-amino acid-labeled Gross-MuLV proteins, and their locations in parallel gels are indicated at the top of each column.  $^{125}$ I-labeled cell-surface components are identified by arrows: gp70, cell-surface gp70; TL, thymus leukemia antigen (14); a, probably a nonspecifically precipitated protein of the actin type which is a major host cell constituent (15); n, probably a cell-surface gp70 precipitated by  $\alpha$ gp70 occurring naturally in the NMS pool;  $\beta$ 2M,  $\beta_2$ -microglobulin associated with TL antigen (16).

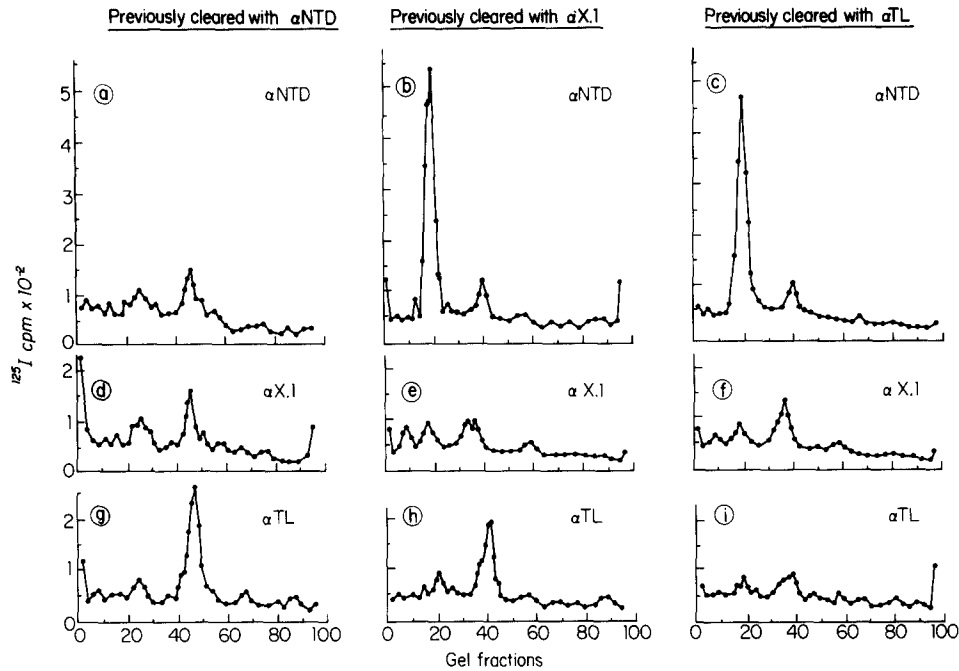


FIG. 2. Sequential immunoprecipitations. The three column headings ('Previously cleared with  $\alpha$ NTD,  $\alpha$ X.1, or  $\alpha$ TL') refer to the preparatory treatment of each antiserum. This consisted in reacting NP-40 lysates of surface-iodinated RADA1 cells with an excess of the antiserum indicated by the heading, followed by anti-immunoglobulin at equivalent concentration, both predetermined by titration. Control experiments indicated that such a 'clearing' step did not affect detection of a second antigen exemplified by precipitation of H-2 antigen before and after 'clearing' (data not shown). Peaks of gp70 are visible in panels b and c. In panels g and h precipitation of TL antigen is visible against a background peak of autoprecipitating cell protein near the same position in the gel (cf. Fig. 1).

previous work (5) is neither  $G_{IX}$ -gp70, because B6 thymocytes are  $G_{IX}^-$  (7), nor X-gp70, because  $\alpha$ X.1 precipitates no gp70 from B6 thymocytes (data not given). Provisionally, the notation 0-gp70 seems appropriate for this other gp70, the '0' signifying that no type specificity has yet been established for this molecule.

#### Comment

This report is limited to results with cells of A-strain origin because these findings, taken in conjunction with our earlier data, are adequate to establish the existence of the third type of MuLV gp70. It is known that  $G_{IX}$ -gp70 and 0-gp70 are coded or specified by unlinked loci and so constitute two discrete genetic systems, possibly two different integrated MuLV genomes. It would seem likely that X-gp70 constitutes a third independent locus, but proof of this is lacking. In studies of several mouse strains other than A, we have found X-gp70 only on leukemia cells which are producing virus or on cells from strains such as AKR which produce large amounts of virus; thus AKR thymocytes express X-gp70. But although the X-gp70<sup>+</sup> phenotype appears to be associated with productive infection, the X-gp70<sup>+</sup> genotype is clearly not so restricted. Thus for example

thymocytes of BALB/c mice do not have precipitable X-gp70; yet the BALB/c radiation leukemia RL ♂ 1 (Table I) is X-gp70<sup>+</sup> (data not shown). G<sub>IX</sub><sup>-</sup> mice similarly may produce G<sub>IX</sub><sup>+</sup> leukemias, and in this respect there is a resemblance between the X-gp70 and G<sub>IX</sub>-gp70 systems. However, G<sub>IX</sub>-gp70, unlike X-gp70 (on present evidence), can be expressed without productive infection. [Aoki and coworkers (6, 12) find that RADA1 leukemia cells produce three types of virions identifiable by immunoelectronmicroscopy with a set of typing sera including αX.1. This accords with the occurrence of at least two gp70 species on RADA1 cells, since these visual distinctions may well be largely due to antigenic variants of gp70.] The most pointed distinction between G<sub>IX</sub>-gp70 and 0-gp70 on the one hand and X-gp70 on the other is that expression of the former two variants is geared to physiological controls governing the differentiation of T cells and need not be accompanied by virus production. So far this has not been observed in the case of X-gp70.

### Summary

Three variants of the gp70 envelope component of MuLV are now recognizable serologically: G<sub>IX</sub>-gp70, 0-gp70, and X-gp70. The last of these, X-gp70, has so far been found only in mice or cells producing abundant C-type virus. This distinguishes X-gp70, provisionally, from the G<sub>IX</sub>-gp70 and 0-gp70 variants, each of which can be expressed on normal thymocytes without accompanying virus production, as exemplified by mouse strains 129 and B6, respectively. The X-gp70 genotype, however, is not limited to strains of mice-producing abundant virus, because X-gp70<sup>+</sup> leukemias occur in strains of mice which do not produce a great deal of virus and whose thymocytes and other tissues are X-gp70<sup>-</sup>; this is analogous to the appearance of G<sub>IX</sub><sup>+</sup> leukemias in G<sub>IX</sub><sup>-</sup> mouse strains.

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