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Rauscher Leukemia Virus Populations Enriched for "Immature" Virions Contain Increased Amounts of P70, the *gag* Gene Product

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Preparations of Rauscher leukemia virus (RLV) that had relatively low, intermediate, or high levels of P70 (the *gag* gene product) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis were examined by thin-section electron microscopy. A direct correlation was found between the number of immature virions in the RLV preparation and the amount of P70. The immature core subparticles isolated from these RLV preparations could themselves be further subdivided into two categories, based on their P70 content and negative stain morphology. Those immature cores containing a high P70/p30 ratio predominantly (85%) exhibited a highly coiled internal structure; those with a relatively low level of P70 exhibited less of an internal coiled structure.

We have previously observed that when Rauscher leukemia virus (RLV) or Friend leukemia virus was exposed to low concentrations of the nonionic detergent Nonidet P-40 (NP-40) (NP-40/virus ratios of 0.5 to 3.0, wt/wt) and centrifuged on a 10 to 40% (wt/vol) sucrose gradient, a band of core subparticles at 350*s* was obtained. The cores were enriched in a protein with an apparent molecular weight of 70,000 (P70, the *gag* polyprotein precursor [1, 3, 12]), as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13). Furthermore, when the cores were examined in an electron microscope, they predominantly (>70%) exhibited the internal morphology of "immature" or enveloped "A"-type virions (4-6); i.e., the interior of the core contained a concentrically coiled ribonucleoprotein component (11). These observations suggested to us that preparations of intact RLV particles enriched in P70 should, likewise, exhibit an increased number of immature virions. We thus examined several preparations of RLV by thin-section electron microscopy or SDS-PAGE and classified them as to relatively high, intermediate, or low levels of immature virions or P70. We observed that there was a direct correlation between these two variables, using different RLV preparations. The preparations had been prepared from the same cell line (JLS-V9) (RLV) and were provided to us by the Frederick Cancer Center, under the auspices of Jack Gruber (National Cancer Institute, Resources Division). This observation represents the first instance where a difference in polypeptide composition between immature and mature murine leukemia virions has been reported. In addition, since thin sections of immature virions from relatively high- and low-P70-containing RLV preparations showed some qualitative differences in internal core organization, we isolated the cores from these virions and examined them in detail both at a biochemical and at an ultrastructural level. A difference in internal coiled strand organization was also observed for the isolated cores, which was correlated with the amount of P70.

In Fig. 1A and B, we can see representative electron micrographs taken from thin sections of two different RLV preparations that contained either a relatively large (A) or a relatively small (B) number of immature virions. We quantitated the percentages of immature virions observed in thin sections for five such RLV preparations (Table 1) and arbitrarily separated them into three classes, I (high), II (intermediate), and III (low). In Fig. 2, the SDS-PAGE polypeptide patterns of RLV preparations containing a relatively large (preparation 681A) or small (preparation 622) number of immature virions is exhibited. As can be seen qualitatively, P70, the *gag* precursor polyprotein, is present in greater amounts relative to p30 in preparation 681A. The amounts of P70 and p30 are quantitated for these and five other RLV preparations in Table 2. By comparing the relative values from classes I, II, and III in Tables 1 and 2, we note that there is a direct correlation between the numbers of immature virions (Table 1) and the P70/p30 ratios obtained from densitometry

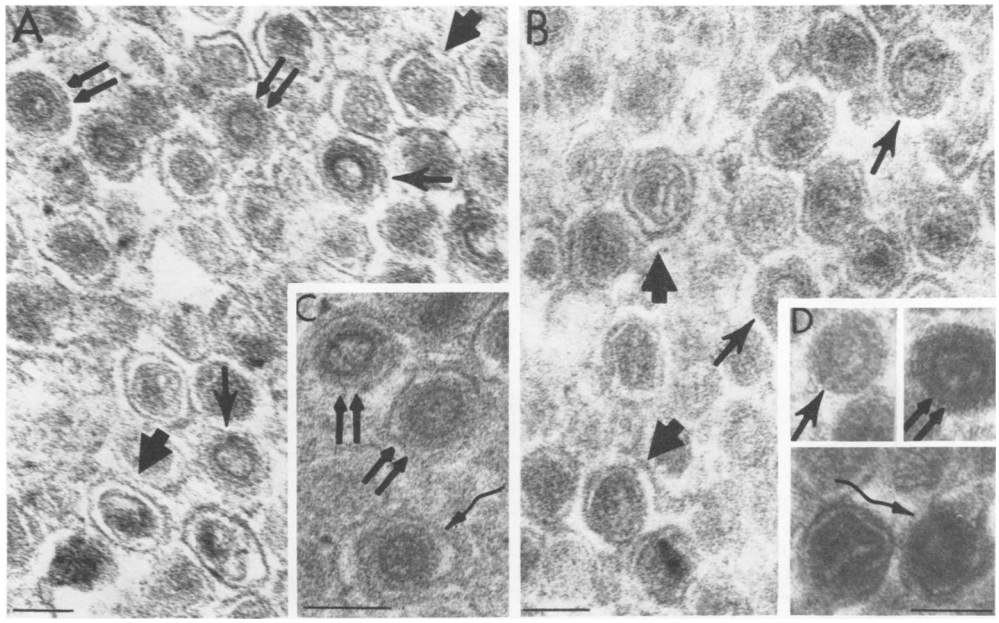

FIG. 1. *Thin section of two different RLV preparations, 681A (A, C) and 622 (B, D). Two milliliters of purified RLV (1.5 to 2 mg/ml) was pelleted by centrifugation for 45 min at 40,000 rpm with a 50 Ti rotor in a Spinco L3-5 ultracentrifuge. The pellets were overlaid with 3 ml of 0.15 M sodium cacodylate (pH 7.4) buffer containing 5% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) and then postfixed with 1% OsO_4, embedded, sectioned, and stained as described (13). Arrowheads point to mature virions, arrows point to immature virions, and double arrows point to immature virions with a highly organized central core region. (A, B) ×80,000; (C, D) ×120,000.*

of the electropherograms after SDS-PAGE. This confirms our suggestion that RLV preparations enriched in P70 are also enriched in the number of immature virions.

The explanation as to why certain RLV preparations have a two- to threefold greater number of immature virions when compared with other preparations is not clear. Based on observations made by a number of authors that the immature virions are virtually identical in thin-section morphology to nascent budding particles (4–6), it had been suggested that they represented newly released virions (6, 7). In support of this contention, we had previously observed (i) that RLV purified from relatively early harvests (3 h) had 2.5 times more immature forms than did virus from 24-h harvests (11) and (ii) that "early" virus seen at 2 h after release of Moloney-infected, synchronized JLS-V9 cells from serum starvation exhibited an immature morphology (40%) and were frequently observed budding from the outer membrane surface (R. B. Luftig, E. P. Bedigan, M. P. Paskind, and R. A. Weinberg, J. Cell Biol., p. 250a, 1975). However, none of the RLV preparations used in this study were early harvests. All were purified in essentially an identical manner, viz., using 24-h harvests of JLS-V9 (RLV) cells grown to near confluency (9; C. Benton, Frederick Cancer Center, personal communication). Although harvests of some lots were started at different times, viz., days 4 to 6 or 6 to 8 after the cells were initiated, there appeared to be no correlation with that time and the amount of P70; e.g., preparations 701 and 622 were started at the same time, yet they differed in P70 content. Thus, the age of the culture after seeding did not have an effect on P70 content.

Since it had been reported earlier that P70 occurs to a variable extent in RLV produced by related but different cell lines (1, 8), it is entirely possible that such a variability reflects the availability of some host factor. We thus suggest that the variable level of P70 observed in the different JLS-V9-produced RLV preparations (Table 2) depends on the amount of a host protease present in the vicinity of the budding virions. A candidate for such a protease would be the P70-specific proteolytic factor that is found in the virion (8, 14).

An ancillary observation that we made on the ultrastructure of these immature virions after a detailed examination of thin sections from preparations 681A and 622 was that the immature

TABLE 1. *Quantitation of the percentage of immature virions observed by thin-section electron microscopy in several different preparations of RLV*[a]

Class	Preparation	Immature virions (%)	No. of virions examined[b]
I	681A-1	22.2 ± 1.4	1,384
	681A-2	29.2 ± 2.8	1,438
II	650-1	18.1 ± 3.1	1,635
	650-2	16.3 ± 3.6	1,966
	701-1	16.8 ± 2.7	1,894
	701-2	16.7 ± 3.5	1,725
III[c]	622-1	9.3 ± 1.2	1,658
	622-2	10.3 ± 1.4	1,449
	529-1	8.3 ± 2.0	1,424
	529-2	7.8 ± 2.9	1,053

[a] The RLV preparations used in this study were all derived from JLS-V9 (RLV). They had undergone two zonal purifications and were free from contaminants, with the exception of 681A and 701, which had a yellow color. We further purified these two preparations by layering 5 ml on a 10 to 60% sucrose gradient for 150 min at 24,000 rpm in a Spinco SW27 rotor. These viral bands were collected and, when examined by SDS-PAGE, showed the same general polypeptide pattern as that found for the other RLV preparations used in this study (see Fig. 2A).

[b] Each line represents an independently embedded sample. Areas were randomly selected so that, for each sample, a minimum of two grids and six fields per grid were observed. A determination was made on the screen at a magnification of ×27,000 as to whether a virus was mature or immature (see Fig. 1). About 10% of the time, a decision could not be made, and such particles were not tallied. The validity of using this rapid, visual counting approach was confirmed when we counted particles from electron micrographs (×81,000) and found, respectively, for preparations 681A-1, 650-1, and 622-1, the following percentages of immature virions: 24.3 ± 2.5% (n = 1,079), 17.0 ± 0.2% (n = 771), and 7.9 ± 1.0% (n = 806). Recounting of particles on the same micrograph or the same field gave an error of <2%.

[c] Two other preparations were also examined, viz., 594 and 628. They both exhibited low (<10%) numbers of immature virions and would be classified in class III.

virion core region internal structures could also be different for different RLV preparations. Specifically, we observed that the core region of 681A virions qualitatively appeared to have a better defined dark concentric ring and a more highly organized outer shell substructure than those of 622 (compare the double arrows of Fig. 1A and the single arrows of 1B; the insets, Fig. 1C and D, show particles at a higher magnification). This apparently higher degree of organization and less amorphous core structure was a commonly observed feature of immature virions in the 681A preparation and an infrequently observed feature for 622; however, it is difficult to quantitate these values, since ~30% of the

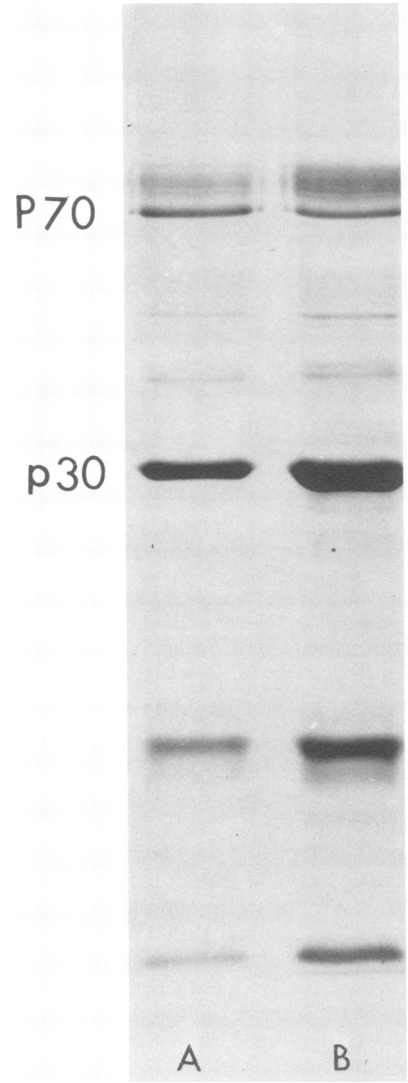

FIG. 2. *SDS-PAGE (12.5% gels) of total virus from 681A (A) or 622 (B). A 50-μg amount of virus in 25 μl was mixed with an equal amount of 2×-concentrated electrophoresis sample buffer containing 2% SDS and 10% 2-mercaptoethanol. The combined sample was boiled for 1 min, and then an aliquot containing 25 μg of protein was placed on the 12.5% slab gel made by the procedure of Laemmli (10). The gels were run on a Hoefer SE500 vertical slab gel system for 5 h at 15 mA. Apparent molecular weights of RLV polypeptides were determined relative to bovine serum albumin (68,000), egg albumin (43,000), chymotrypsinogen (25,000), and cytochrome c (12,500). The numbering of polypeptides (p), and precursor polypeptides (P) by apparent molecular weight is according to convention (2).*

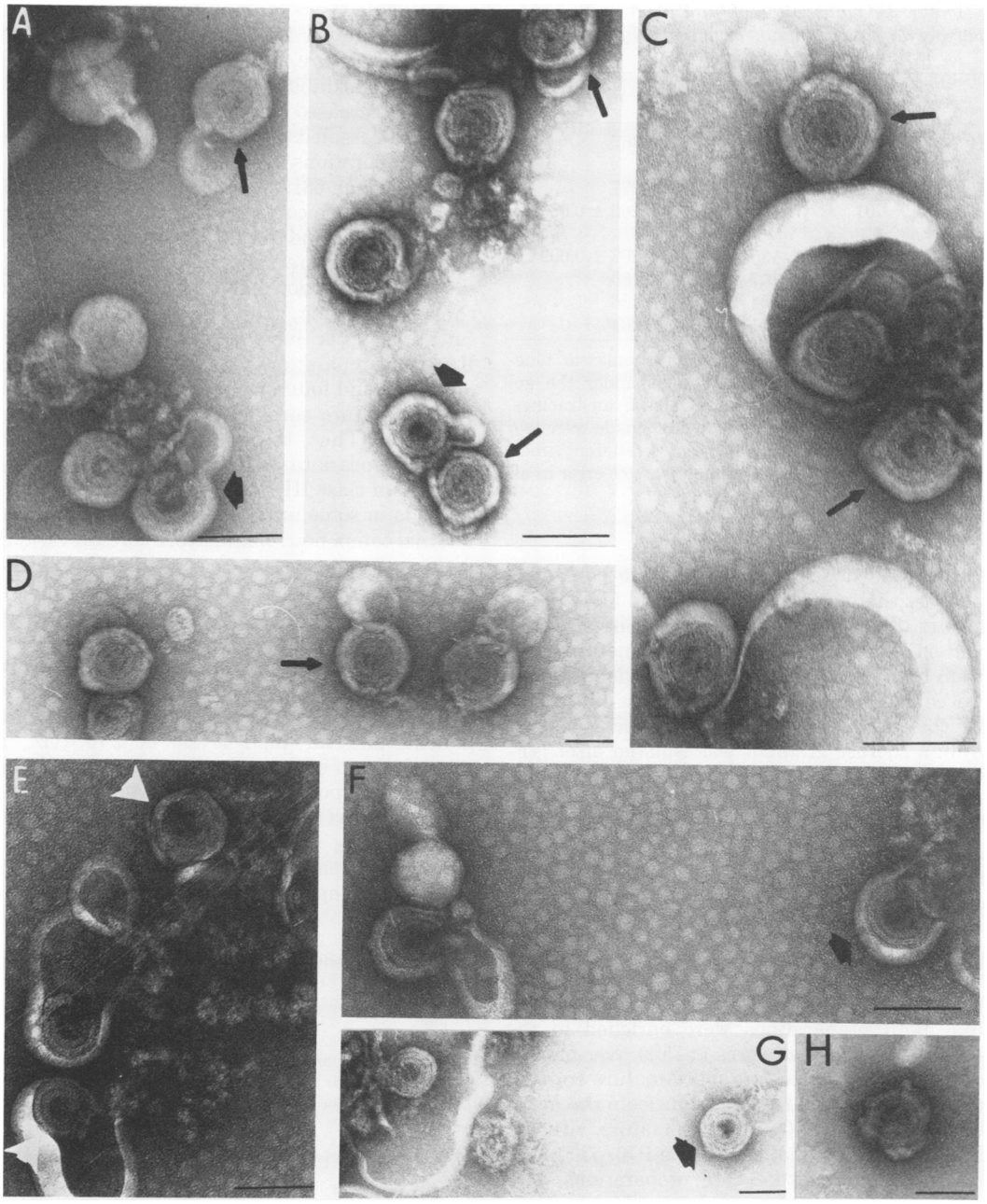

FIG. 3. *Morphology of immature cores from preparations 681A, (A–D) and 622, (E–H), isolated on sucrose gradients after low NP-40 (NP-40/virus = 3.0, wt/wt) exposure as described (13). Note the concentrically coiled nature of the ribonucleoprotein in both cases and the greater penetration of stain into the center of particles in (E through H) (arrowheads). Negative staining was done, using 5% glutaraldehyde (0.15 M sodium cacodylate, pH 7.4) and 2% uranyl acetate (pH 4.2) as described previously (11). We have classified those cores where stain has not penetrated into the center (A–D) as highly organized, since the concentric coil extends into the central region (arrows). For 681A, such forms constitute 93 ± 2% (n = 180) of the cores, whereas for 622 they represent 25 ± 14% (n = 400) of the cores. Each of these counts represents the sum obtained from two separate core isolations. Bar = 0.1 μm.*

TABLE 2. *Quantitation of the P70 and p30 SDS-polyacrylamide gel bands from several preparations of RLV*

Class	Preparation	P70[a]	P70/p30[a]
I	681A	108	0.367
II	650	164	0.277
	701	145 ± 15[b]	0.266 ± 0.003[b]
III	622	135 ± 3[b]	0.207 ± 0.009[b]
	529	107	0.180
	594	104	0.136
	628	111	0.204

[a] Units presented are arbitrary Coomassie blue-staining units calculated from areas under the gel band peaks make from Helena Quick Scan tracings.

[b] These averages are from tracings made over two identical sample preparations taken at different times. This suggests that there is probably a 10% error in all of the data points presented above.

virions had an indistinct immature core morphology that could not be classified (see curved arrows, Fig. 1C and D). These empirical observations did induce us to isolate immature cores from both of these preparations to determine if we could more clearly define such differences for the core region. In each case, the 350*s* peak from a sucrose gradient was isolated after NP-40 (NP-40/virus = 3, wt/wt) treatment of the virus. Specifically, to 1 mg of purified RLV in TNE (0.01 M Tris-hydrochloride–0.13 M NaCl–0.001 M EDTA [pH 7.2]) we added 3 mg (1%, wt/vol) of NP-40, loaded the suspension onto a 10 to 40% (wt/vol) sucrose gradient, centrifuged it at 30,000 rpm for 40 min at 4°C, and collected the visible core band (350*s*). The cores were then examined by negative-staining electron microscopy. We found, as expected (13), that both preparations were enriched in the number of immature cores (>75%) over mature cores. The failure to find more mature cores in the 350*s* band apparently results from the instability of most (>70%) of the mature virus to NP-40 treatment (13). When the P70/p30 ratios for the two immature core preparations were determined after densitometry of the SDS-PAGE patterns, we unexpectedly found that they were very different, viz., 0.71 for 622, and 6.54 for 618A. It should be noted, of course, that for mature cores, which do not have any concentrically coiled internal component, there is no P70 (Fig. 6 of reference 13). These observations then led us to reexamine the morphology of immature cores in greater detail, by negative staining (Fig. 3). When this was done, we observed that there were two morphologically distinguishable types of immature cores; one type (Fig. 3A through D) exhibited a highly coiled internal structure, whereas the other (Fig. 3E through H) exhibited less internal coiled structure and increased staining in the central region. We now classified the immature cores from the RLV preparations according to these two new categories; for 681A, we found that about 85% of the immature cores had a highly coiled internal component and that 15% had a less structural but still-coiled internal component. In contrast, the 622 preparation had 25% of the immature cores in the first, and 75% in the secondary, category. Other RLV preparations from class III, e.g., preparations 529 and 628, were also examined and found to have <20% (n = 100) of their immature cores in the highly coiled conformation. Thus, the observation made above for 622 appears to hold in general for preparations from class III. This suggests that P70 can function in some unknown way to maintain the internal component of immature cores in various degrees of organization. Specifically, we suggest that if there is no P70, then the cores have a mature morphology, where the internal strand is collapsed (13); for moderate levels of P70 (viz., a P70/p30 ratio of about 1 for the immature cores) the cores have an immature morphology, with a loosely structured, coiled internal component; and for high levels of P70 (viz., a P70/p30 ratio of 6 for the immature cores) the internal component is highly organized. This apparent correlation between P70 and the degree of coiling is clearly not stoichiometric and probably depends in some unknown way upon the other components, e.g., lipid and RNA, of the core.

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