occur *in situ* and showed that succinate externalization of AAT requires a more fluid membrane than for its internalization. As a matter of fact, internalization still occurs at temperatures below the thermotropic fusion point of the membrane for the externalization of the enzyme.

This asymmetrical recognition of both the externalizing signal and the molecule to be transitorily internalized was also suggested by cross-linking experiments. After treating mitochondria with dimethyladipimidate, externalization of AAT was no longer triggered by succinate, whereas in its absence, internalization of 70% of the enzyme still occurred on cross-linked organelles. This suggests that a greater rigidity or stability of the membrane could be required for the recognition and integration of protein signals as opposed to recognition of ionic signals (5). The driving force of this protein movement seems to be dependent on the chemical potential of both mobile protein and membrane as modulated by environment rather than by hydrolysis of an energy rich bond, or modification of the primary structure of the mobile protein (Table II).

Configurational energy and cognitive properties could thus be responsible for the externalization-internalization phenomenon.

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FORMATION OF THE ENVELOPE OF ROUS SARCOMA VIRUS AND VESICULAR STOMATITIS VIRUS FROM LOCALIZED LIPID REGIONS IN THE PLASMA MEMBRANE

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The phospholipid polar head group composition of chicken embryo fibroblasts and enveloped viruses grown on these cells can be altered by supplementing the culture media with polar head group analogues. Studies of the incorporation of analogues into host cell plasma membranes and virus envelopes have shown that the viruses bud from localized lipid regions which are different from the average composition of the plasma membrane (1). This selective incorporation of phospholipids into the virus envelope may result from a specific requirement of the virus to regulate the envelope membrane structure. To test this hypothesis, the effects of altering the host cell plasma membrane lipid composition and fluidity on the viral envelope lipid composition and fluidity were determined. Measurements were made with 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe of membrane structure and fluidity using steady-state anisotropy methods and differential polarized phase fluorometry (2).

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METHODS

Chicken embryo fibroblasts were cultured as previously described (3). RSV-T5-infected cells growing at 41°C were split into fresh medium, and half were incubated at 41°C and half at 36°C. After 32 h the medium was changed, and viruses and cells were harvested 12 h later. Polar head group modification of phospholipids was done on tertiary cultures using previously described methods (1). Cells were infected for 12 h with VSV (San Juan strain) and viruses and cells harvested after 36 h growth in choline- or *l*-2-amino-1-butanol-supplemented media. Virus purification, plasma membrane isolation, and phospholipid analysis were done as previously described (1). Membranes were labeled with DPH as previously described (4).

RESULTS

RSV-T5 is a Rous sarcoma virus mutant which infects cells and replicates equally well at 36°C and 41°C. At

TABLE I
STEADY STATE DPH ANISOTROPIES OF THE PLASMA MEMBRANE AND ROUS SARCOMA VIRUS FROM RSV-T5-INFECTED CELLS GROWN AT 36°C OR 41°C

Measurement temperature	Plasma Membrane		RSV-T5	
	36°C (trans-formed)	41°C (normal)	36°C (trans-formed)	41°C (normal)
10°C	0.259	0.244	0.293	0.291
25°C	0.217	0.200	0.265	0.262
37°C	0.187	0.179	0.236	0.235

41°C, the cells are not transformed, whereas at 36°C they are transformed (5). There were differences in the lipid compositions of the plasma membrane from RSV-T5-infected cells grown at either 36°C or 41°C which were similar to previous results comparing normal and wild-type RSV-infected cells (6). The phospholipid composition of the RSV-T5 was similar to that of the wild-type virus but very different from that of the host plasma membrane (1). A comparison of transformed and normal plasma membranes showed differences in the DPH anisotropies as shown in Table I. However, RSV-T5 isolated from these cells had similar lipid compositions and DPH anisotropies. Thus, the virions produced were identical, although the plasma membranes from which they budded were different.

Growth of chicken embryo fibroblasts in media supplemented with *l*-2-amino-1-butanol resulted in the formation of phosphatidylbutanolamine in the plasma membrane and VSV envelope. Overall, the changes in phospholipid composition were the same as previously described (1). The steady-state and limiting anisotropies of DPH in the supplemented plasma membranes and virus envelopes are compared in Table II. The *l*-2-amino-1-butanol- and choline-supplemented VSV envelopes have the same steady-state and limiting anisotropies. However, these parameters differ slightly between the respective plasma membranes, and thus the differences in the plasma membrane structure and fluidity are not reflected in the virus envelope. This suggests that the virus may be regulating its envelope structure and fluidity. The DPH rotational rates calculated from the steady-state anisotropies and differential polarized phase fluorometric data showed a large difference between the virus and plasma membrane: $R \sim 5.0 \times 10^7$/s for the virus and $R \sim 1.3 \times 10^8$/s for the plasma membrane. However, the error in the calculation of R was too large to distinguish differences between the choline- and *l*-2-amino-1-butanol-supplemented membranes. Other factors appear to be involved in determining the lipid composition of the envelope, and further experiments

TABLE II
STEADY-STATE (r) AND LIMITING ANISOTROPIES (r_∞) OF DPH IN PLASMA MEMBRANE AND VESICULAR STOMATITIS VIRUS (VSV) FROM SUPPLEMENTED CELLS

Sample	Supplement	r	r_∞
VSV	Choline	0.306	0.271
VSV	*l*-2-amino-1-butanol	0.304	0.274
Plasma membrane	Choline	0.261	0.242
Plasma membrane	*l*-2-amino-1-butanol	0.280	0.258

Limiting anisotropies were calculated as previously described (2) from measurements of the steady state anisotropies, lifetimes, and differential polarized phase lifetimes measured on an SLM 4800 subnanosecond phase fluorometer (SLM Instruments, Inc., Urbana, IL) with an excitation wavelength of 360 nm and a Corning 7-54 excitation filter (Corning Glass Works, Science Products Div., Corning, NY). Schott KV-418 emission filters were used (Schott Optical Glass Inc., Duryea PA), and readings were taken at 10°C.

are necessary to determine the conditions under which the virus regulates its envelope structure.

CONCLUSIONS

The results provide further evidence that the virus selectively incorporates host cellular phospholipids into the viral envelope. Moreover, the selection of lipids may partly reflect the need to regulate the structure and fluidity of the viral envelope.

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