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MEMBRANE LIPID ACYL GROUP ALTERATIONS IN CELLS INFECTED WITH A TEMPERATURE-CONDITIONAL MUTANT OF ROUS SARCOMA VIRUS

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Summary

The increased percentage of oleic acid and decreased percentage of arachidonic acid which occurs in the lipids of chicken emrbyo fibroblasts transformed by Rous sarcoma virus was shown to be transformation specific rather than a consequence of virus infection. Cells infected with a temperature conditional mutant of Rous sarcoma virus (RSV-T5) had a normal type fatty acid composition when held at the restrictive temperature of 41°C, but had a transformed type fatty acid composition when held at the permissive temperature of 36°C. However, these acyl group changes occurred slowly in the course of transformation, suggesting that they are not a primary event in the genesis of the transformed phenotype.

There exists extensive correlational evidence that alterations in cell membrane structure and function play a role in malignant transformation (see ref. 1 for a recent review). Among the membrane alterations is a decrease in the arachidonic acid (20:4) content of cellular lipids and a corresponding increase in oleic acid (18:1) following malignant transformation. This change in the ratio of 18:1/20:4 occurs in chicken embryo fibroblasts transformed by Rous sarcoma virus [2,3], 3T3 and WI38 cells transformed by SV40 [4,5] and rat hepatomas [6,7]. Thus, it is a biochemical alteration common to a number of malignant cell types. Membranes which have an increased ratio of 18:1/20:4, in the absence of other alterations, might be expected to display a decreased lipid bilayer "fluidity" (or increased "microviscosity") since the decreased percentage of double bonds might allow better stacking of the phospholipid acyl groups [14]. In fact, both SV40 transformed 3T3 cells and Rous transformed chicken embryo fibroblasts have been reported to have somewhat increased membrane "microviscosity" measured by

fluorescence depolarization (ref. 8 and Friis, personal communication) and electron spin resonance [3]. (However, there is some contradictory evidence on the "microviscosity" of transformed fibroblast membranes [9]. Moreover, it is important to note that comparisons of lymphomas with normal lymphocytes reveal a decreased microviscosity of the membranes of the malignant cells, due to decreased cholesterol content of these cells [15].

In making comparisons between normal and malignant cells, it is important to be certain that the differences observed are specific for transformation if deductions about oncogenesis are to be made. For example, biochemical comparisons between 3T3 cells and their SV40 transformants are sometimes difficult to interpret since these established cell lines may not have had identical histories and thus may have accumulated numerous biochemical differences secondary to the transforming event. Moreover, since the efficiency of transformation by SV40 is so low (typically, fewer than 10% of the infected cells grow into transformed clones) the possibility exists that the virus selects a pre-existing variant type. Similar criticisms can be made of comparisons between tumors and normal tissues. On the other hand, the RNA tumor viruses, such as Rous sarcoma virus, although they can rapidly transform 100% of a culture, generally replicate productively in the cells they transform, and thus may cause cellular alterations as a consequence of replication rather than just transformation.

To determine whether lipid acyl group changes following Rous sarcoma virus infection of chicken embryo fibroblasts were transformation specific, we have taken advantage of RSV-T5 [10], a mutant of Rous sarcoma virus which is temperature-conditional for transformation, but not for replication. Cells infected with this mutant are phenotypically normal by a number of criteria when held at 41°C, but rapidly become transformed when shifted to 36°C [10,11]. Conversely, cells transformed by RSV-T5 at 36°C rapidly revert to a normal phenotype when shifted to 41°C. Since 100% of a culture can be rapidly and reversibly transformed, and since normal, high titers of infectious virus are produced at both temperatures, this system is ideal for determining whether virus-induced changes in cellular biochemistry are due to selection or infection or are transformation-specific.

In Table I is shown the acyl group composition of total cellular lipids from normal, exponentially growing uninfected cells, cells transformed by the wild-type Rous sarcoma virus, cells infected by RSV-T5 and held at 41°C, (where they are phenotypically normal), and cells transformed by RSV-T5 at 36°C. It can be seen that the RSV-T5 infected cells held at 36°C which had a transformed morphology and a high rate of hexose transport (which is characteristic of transformed fibroblasts) also had a fatty acid composition typical of the wild-type transformed cells, with a high ratio of 18:1/20:4. The increased ratio of 18:1/20:1 in Rous-transformed cells occurs in all the separated phospholipids [2] and in isolated plasma membranes as well as whole cells [3]. But when RSV-T5 infected cells were held at 41°C, they had a normal type fatty acid composition, with a lower 18:1/20:4 ratio. Thus, this cellular change is dependent on the expression of viral oncogenic activity, and is not due to infection or selection.

The effects of temperature on the fatty acid composition of these

TABLE I

ACYL GROUP COMPOSITION OF TOTAL LIPIDS FROM NORMAL AND TRANSFORMED CELLS

Each value is the average of two determinations, expressed as a weight percentage of the total. Error was less than 5%. Cell culture, virus infections and lipid analyses were as previously described [2,12].

Acyl groups	Normal, exponentially growing cells	Rous sarcoma virus-transformed cells	RSV-T5 infected cells, held at 41°C	RSV-T5 infected cells, held at 36°C
16:0	20.9	16.8	18.9	17.5
18:0	20.6	24.3	23.8	24.3
18:1	23.8	30.7	20.8	28.4
18:2	12.6	10.4	6.9	11.6
20:3	3.2	3.6	5.1	4.2
20:4	14.5	10.1	14.8	8.5
22:4	1.8	1.8	2.2	1.2
22:5	1.2	1.0	2.2	1.8
22:6	1.6	1.4	3.4	2.7
Total saturated fatty acids	41.5	41.1	42.7	41.8
Ratio 18:1/20:4	1.6	3.0	1.4	3.3

cultured cells are clearly related to the temperature-sensitive lesion in RSV-T5, since uninfected cells and cells infected with wild-type virus or with a non-transforming deletion mutant of Rous sarcoma virus (tdNY101) did not show temperature-conditional variations in the ratio of 18:1/20:4 in cellular lipids (Table II). Thus, this change in lipid composition is transformation specific.

The conclusion that the acyl group alterations are transformation-specific has been confirmed in another system: human cells, infected with either adenovirus 12 or Herpes viruses type 1 or type 2 (infections which do not lead to mass transformation of the culture), did not display alterations in the lipid acyl group composition (Yau and Ledinko, unpublished observations).

To determined whether the alterations in fatty acid composition could be a primary event in malignant transformation by Rous sarcoma virus, we compared the kinetics of appearance of the fatty acid changes to the virus-induced alterations in the rate of hexose transport, following a shift in the growth temperature of RSV-T5 infected cells (Fig. 1). It can be seen that cells infected with RSV-T5 and held at 41°C had a low ratio of 18:1/20:4 (panel b) and a low rate of hexose transport (panel c). When shifted to 36°C, they rapidly increased their rate of hexose transport until, after 12 h it had reached the level characteristic of transformed cells. On the other hand, the change in the ratio of 18:1/20:4 occurred much more slowly, requiring almost two days to go to completion. Similarly, cells held at 36°C, which had the transformed type fatty acid composition and a high rate of hexose transport, reversed their hexose transport rate much more quickly than their acyl group composition when shifted to 41°C. The kinetics of the acyl group changes occurred no more quickly in isolated plasma membranes (panel a) nor in individual phospholipids [3].

The results presented here indicate that the increased ratio of 18:1/20:4 seen in the lipids of Rous sarcoma virus-transformed chicken embryo fibroblasts is transformation-specific, depending on the expression of viral oncogenic activity in cells infected with a temperature-conditional virus mutant. However, the slow kinetics with which these compositional changes occur suggests that they cannot be a primary event in malignant transformation,

TABLE II

RATIO 18:1/20:4 IN CELLS GROWN AT DIFFERENT TEMPERATURES

Cultures were infected with virus and grown at either 41 or 36°C. 24 h before harvesting half the cultures were shifted to the opposite temperature.

	Temperature (°C)			
	36	41	41→36	36→41
Normal, uninfected cells	1.4	1.5	1.2	1.4
Cells transformed by wild-type RSV	2.6	3.2	2.9	3.3
Cells infected with non-transforming mutant tdNY101	1.2	1.6	1.5	1.3
Cells infected with RSV-T5	3.2	1.7	3.7	2.1

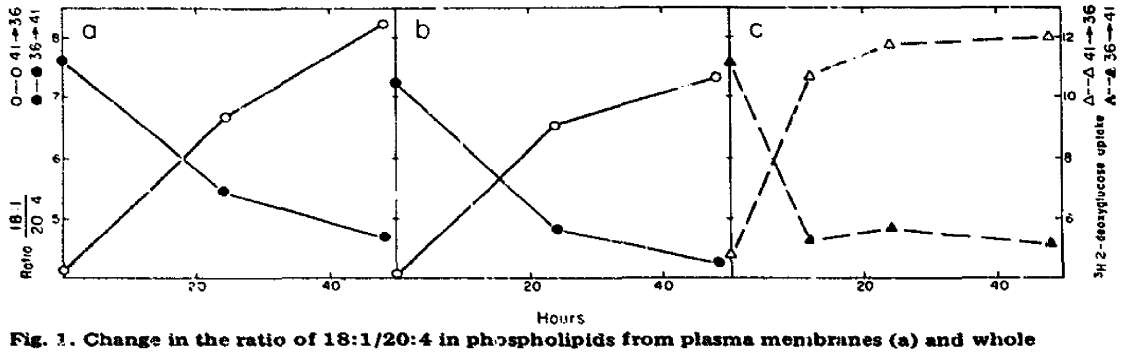

Fig. 1. Change in the ratio of 18:1/20:4 in phospholipids from plasma membranes (a) and whole cells (b) and the rate of transport of 2-deoxyglucose (c) in cultures infected by the temperature-conditional mutant of Rous sarcoma virus, RSV-T5. Cells were infected with RSV-T5 at either 36 or 41°C. After infection was fully established, cultures were shifted to the opposite temperature (time zero) and samples were withdrawn for lipid analysis and transport measurements. Lipid extraction, acyl group analysis and hexose transport were performed as previously described [2,3,12]. Plasma membranes were isolated by the method of Perdue et al. [13].

but most likely are a secondary consequence of some earlier metabolic alteration. Since the ratio of 18:1/20:4 changed slowly in both the plasma membrane and in whole cells, it is difficult to argue that a specific membrane fraction which might change composition rapidly is critical in precipitating the early events in malignant transformation, although it is conceivable that alterations in critical "micro-domains" or the induction of phase separations may cause changes which would not be detected by analysis of total cellular lipids and isolated plasma membranes.

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References

1 Robbins, J.C. and Nicolson, G.L. (1975) In Cancer, A Comprehensive Treatise, (Becker, F.F., Ed.), Vol. III, Plenum Press, New York
2 Yau, T.M. and Weber, M.J. (1972) Biochem. Biophys. Res. Commun. 49, 114—120
3 Yau, T.M., Buckman, T., Hale, A.H. and Weber, M.J. (1976) Biochemistry, in the press
4 Horwitz, A.F., Hatten, M.E. and Burger, M.M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3115—3119
5 Howard, B. and Kritchevsky, D. (1969) Int. J. Cancer 4, 393—402
6 Veerkamp, J.H., Mulder, I. and VanDeenen, L.L.M. (1962) Biochim. Biophys. Acta 57, 299—309
7 VanHoeven, R.P., Emmelot, P., Krol, J.H. and Oomen-Meulmans, E.P.M. (1975) Biochim. Biophys. Acta 380, 1—11
8 Fuchs, P., Parola, A., Robbins, P.W. and Blout, E.R. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3351—3354
9 Gaffney, E.J. (1975) Proc. Natl. Acad. Sci. U.S. 72, 664—668
10 Martin, G.S. (1970) Nature 227, 1021—1023
11 Martin, G.S., Venuta, S., Weber, M.J. and Rubin, H. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2739—2741
12 Weber, M.J. (1973) J. Biol. Chem. 248, 2978—2983
13 Perdue, J.F., Kletzien, R., Miller, K., Pridmore, A. and Wray, V.L. (1971) Biochim. Biophys. Acta 249, 435—453
14 Seelig, J. (1972) in Passive Permeability of Cell Membranes, Biomembranes, (Kreuzer, F. and Slegers, J.F.G., Ed.), Vol. 3, Plenum Press, New York
15 Shinitsky, M. and Inbar, M. (1974) J. Mol. Biol. 85, 603—615