

STRUCTURAL ALTERATIONS IN THE SURFACE MEMBRANE DURING THE CELL CYCLE

C. A. PASTERNAK, A. M. H. WARMSLEY, and D. B. THOMAS. From the Department of Biochemistry, University of Oxford, Oxford, England, and the Department of Immunology, Middlesex Hospital Medical School, London, England

INTRODUCTION

Crucial to an understanding of the mechanism of cell replication is a knowledge of the manner in which intracellular and surface membranes develop between successive divisions. The application of zonal centrifugation to separate cells according to their size and hence their relative age (Warmley and Pasternak, 1970) has enabled us to study the timing of these events by measuring changes in four parameters characteristic of membranes: (a) the cellular content of phospholipid, (b) the activity of enzyme "markers" indicative of mitochondrial and microsomal membranes, (c) the activity of mitochondrial energy-coupling structures revealed by a fluorescent probe, (d) the cell volume. Each parameter was found to approximately double during the intermitotic period of P815Y neoplastic mast cells; the rate of increase is most rapid during the S period, though clearly distinguishable from that of DNA in that it commences already in G₁ and continues into G₂ (Warmley and Pasternak, 1970; Warmley, Phillips, and Pasternak, 1970).

The fact that P815Y cells contain the H-2 antigen has enabled us to study changes in the surface membrane in rather more detail, by carrying out immune cytotoxicity (Sanderson, 1964; Wigzell, 1965) of cells from different regions of the gradient with the appropriate antiserum. The recent report of cyclic changes in an agglutinin-binding site on 3T3 cells as revealed by immune fluorescence studies (Fox, Sheppard, and Burger, 1971) has now prompted us to report on some of our initial findings.

METHODS

Exponentially growing P815Y cells (Pasternak and Bergeron, 1970) (1×10^9 total) were concentrated (to 4×10^7 cells per milliliter) and separated by zonal centrifugation (Warmley and Pasternak, 1970); fractions from the gradient were pooled to give approximately 5×10^7 cells in each sample. Cell volume was measured as previously described (Warmley and Pasternak, 1970).

Cells ($1-2 \times 10^7$ per milliliter) were incubated at 37°C with Na⁵¹CrO₄ (The Radiochemical Centre, Amersham, Buckinghamshire, England) (100 μCi/ml) in Fischer's medium (Grand Island Biological Co., Grand Island, N. Y.) for 40 min. After three washes to remove extraneous Na⁵¹CrO₄, cells (4×10^6 per milliliter) were incubated in Fischer's medium at 37°C with antiserum 17/4 (kindly donated by Doctors D. A. L. Davies and O'Neill of Searle Research Laboratories, High Wycombe, Buckinghamshire, England) and fresh guinea pig serum (diluted 1 in 15) as complement source for 40 min. The cell suspension was centrifuged and released ⁵¹Cr in the supernatant, measured with a Packard Tri-Carb γ counter. The antiserum, which has activities directed against histocompatibility-2 (H-2) alloantigenic specificities 3, 4, 8, 13, and 31 (Davies, 1969), was used at a dilution of 1 in 200, so as to release approximately 75% of maximum releasable radioactivity from unfractionated cells. Release was linear up to 1 hr. Dilutions of 1 in 100 and 1 in 140 gave essentially similar results. Appropriate complement and antiserum controls were included in each determination and the resulting values were subtracted.

RESULTS AND DISCUSSION

Fig. 1 shows that expression of H-2 antigenicity, as measured by the sensitivity of cells to H-2 antiserum, decreases during the G₁-S period and is restored again in G₂ cells. Addition of unlabeled cells from each portion of the gradient to a reconstituted mixture of labeled cells showed that this pattern is paralleled by the capacity of cells to inhibit ⁵¹Cr release. Thus cytotoxicity is indeed a measure of antigenic expression in this system. The pattern of cytotoxic variation cannot be accounted for simply in terms of an increase in surface area (Cikes, 1970 *a*), since the cytotoxic titer varies independently of cell volume (Fig. 1).

One may conclude that the antigenic character of the cell surface does not reflect the increased amount of other membrane constituents, since its expression decreases (Fig. 1) when most macromolecular synthesis is maximal (Warmley and Pasternak, 1970; Warmley, Phillips, and Pasternak, 1970). This may imply a "masking" (Burger,

1969) or other rearrangement of antigenic determinants during the cell cycle; it is certainly unlikely that H-2 antigens are actually degraded during the G₁-S period in exponentially growing cells. The observed alteration in a component which is probably a glycoprotein (Nathenson and Davies, 1966; Shimada and Nathenson, 1969; Muramatsu and Nathenson, 1970) may explain some of the fluctuations in electrophoretic mobility—partly attributed to sialic acid residues—seen with synchronized cells of other species (Mayhew, 1966; Kraemer, 1967). Although our experiments to date have not shed any light on the timing of synthesis of H-2 antigen, it is interesting to note that membrane glycolipids of L5178Y cells are said to be synthesized exclusively during mitosis (Bosmann and Winston, 1970); it is therefore possible that the observed decrease in H-2 antigenicity during interphase is due to a cessation of H-2 synthesis which, in presence of a continuing increase in cellular size, leads to a lower H-2 density per surface area.

The fact that virally transformed 3T3 cells do not show the variation in surface architecture observed in untransformed 3T3 cells (Fox, Sheppard, and Burger, 1971), suggests that cultured P815Y cells may resemble normal cells rather

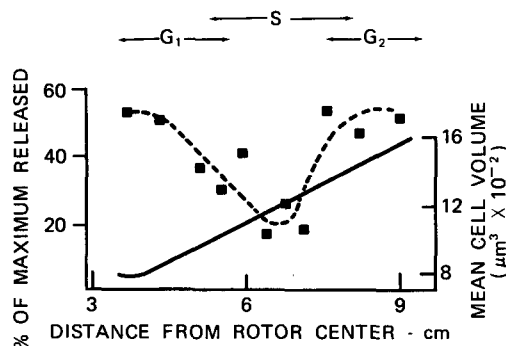


FIGURE 1 Expression of H-2 antigenicity during the cell cycle. Percentage of isotope released from ⁵¹Cr-labeled cells by incubation with antiserum. 100% represents the amount released at antibody dilution of 1 in 50, which is approximately 70% of the total ⁵¹Cr taken up by each cell fraction. Cell volume is shown as a continuous line. The approximate allocation of different parts of the gradient to the G₁, S, and G₂ periods was made on the basis of cell number, cell volume (Warmsley and Pasternak, 1970), thymidine incorporation (Pasternak and Bergeron, 1970), and radioautographic analysis of the cell cycle (Bergeron, Warmsley, and Pasternak, 1970). The mean values from two separate experiments are plotted.

than other neoplastic cells in yet another (Bergeron, Warmsley, and Pasternak, 1970), respect. On the other hand, experiments on the relation between growth rate and cytotoxicity in YCAB lymphoma cells suggest that H-2 antigenicity may be maximal in early G₁ in this case also (Cikes, 1970 b).

In summary, it appears that during the intermitotic period of P815Y cells, a structural change in antigenic determinants accompanies the assembly of other membrane constituents.

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