MICROTUBULAR PROTEINS AND CONCANAVALIN A RECEPTORS

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ABSTRACT

The inherent topographical distribution of Con A binding sites (CABS) is disperse or random in all cell types studied using hemocyanin to mark CABS in surface replicas. In virally transformed cells, the addition of Con A leads to the formation of clusters (CABS). A role for microtubules is suggested in this process since colchicine treatment of transformed cells and Con A addition lead to the aggregation of Con A into a "cap." During phagocytosis CABS are selectively removed from the surface. This selective movement is abolished by drugs that disrupt microtubules. Binding of Con A or RCA to intact cells at 37°C leads to the removal of their receptors from the surface, presumably by "micropinocytosis."

I. INTRODUCTION

Important differences in the biological behavior of tumor cells and normal cells are reflected in altered surface properties. For tumor cells in vitro, these include the loss of contact inhibition of cell movement and division, altered membrane transport, and decreased surface adhesiveness (Hakamori, 1973). Since the work of Aub (1963), it is recognized that in general transformed cells are considerably more agglutinable by Con A and other lectins than their normal counterparts. Studies of temperature sensitive mutants have shown an intimate correlation between the transformed state (established at permissive temperatures) and agglutinability by lectins. It is assumed that agglutination results from the cross-linking of receptors on adjacent cells by the multivalent lectins. Initially it was supposed that transformed cells had increased numbers of...
receptors, but it was found that the numbers of receptors are essentially identical (Ozanne, 1971; Cline, 1971). This does not necessarily signify, of course, that the lectin receptors are chemically identical. However, it suggests that factors other than available binding sites determine agglutinability. Nicolson (1971), using ferritin-conjugated antibody to Con A, found that the topographical distribution of Con A binding sites (CABS) was clustered in the transformed cell surface and yet disperse (or random) in the normal cell surface. Presumably the clustering of sites would facilitate the formation of multiple linkages between cells at points of contact.

This paper presents a review of the evidence developed in our laboratory concerning the topographical distribution of Con A binding sites and the mechanisms that control topography, with particular emphasis on the possible role of microtubular proteins.

II. METHODS

The morphological distribution of CABS was determined by the technique of Smith and Revel (1972) in which Keyhold limpet hemocyanin (a high molecular weight blood pigment) that contains carbohydrate receptors for Con A is used to identify Con A adsorbed to the cell surface. CABS were enumerated by saturation with 125I-Con A.

Phagocytosis and membrane isolation in rabbit polymorphonuclear leukocytes have been described recently in detail (Oliver et al., 1974).

III. RESULTS

In accord with Singer and Nicolson (and using an entirely different technique which allows an analysis of larger areas of the surface) we found that CABS are dispersed in normal fibroblasts (Fig. 1), whereas in transformed fibroblasts CABS are drawn into clusters (Fig. 3). Moreover, the clusters in turn assume a characteristic distribution; they are withdrawn from the edges and pseudopods of the cell (Fig. 4) (Rosenblith et al., 1973). These experiments emphasize the possibility that it is the topographical arrangement of membrane constituents and not their chemical identities - or perhaps factors in addition to them - which differentiate normal from transformed cells.

The clustered topography is closely correlated with increased agglutinability and associated biological properties. Thus, brief trypsinization of normal fibroblasts increases their agglutinability and at the same time alters the topography of CABS from dispersed to