Mengovirus-induced Capping of Virus Receptors on the Plasma Membrane of Ehrlich Ascites Tumour Cells

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SUMMARY

Mengovirus particles adsorbed to Ehrlich ascites tumour cells are redistributed into patches and caps without the action of antibodies. Thus capping may be an early signal in picornavirus infection.

The lateral displacement of lipid-protein complexes in the plasma membrane of mammalian cells, induced, for example, by lectins that interact with glycoproteins or by antibodies that are directed against certain cell surface proteins, has become the subject of intensive research (Nicolson, 1976). In most systems this movement is primarily caused by binding of the respective ligand to a macromolecular constituent of the plasma membrane. The binding of ligand entails the formation of 'patches' by cross-linkage of membrane proteins. As a rule, the patches are concentrated at one pole of the cell through an active process. The term 'capping' has been generally accepted for this phenomenon; the fluorescence pictures resemble those of lymphocytes interacting with anti-gammaglobulin antibodies (Taylor et al. 1971). Independent capping of two different antigens has been demonstrated; e.g. cellular proteins and virus-specific proteins of membrane-borne viruses move to different poles of the cell if they are cross-linked by their respective antibodies. Furthermore, the detailed investigation of the capping process revealed a transmembranal connection of cell surface proteins with intracellular structures, especially with contractile elements (Rutter & Mannweiler, 1973, 1976, 1977). A close morphological neighbourhood of patches with actin-myosin filaments and the co-capping of cell surface antigens with actin-myosin have been demonstrated (Ash et al. 1977; Gabbiani et al. 1977). Thus the patching and capping phenomena could be looked at as part of a transmembranal information system which may trigger intracellular events at the moment of adsorption of a multivalent ligand to the cell surface.

In the present communication we show that one of the earliest events in the course of mengovirus infection of Ehrlich ascites tumour (EAT) cells is the patching and capping of mengovirus absorbed to the plasma membrane of the host cell. Employing the indirect immunofluorescence technique, we were able to demonstrate the redistribution of mengovirus particles which were located in small clusters evenly distributed on the surface of the EAT cells when the virus was allowed to adsorb at 0 °C (Fig. 1a). Raising of the temperature to 20 °C caused the redistribution of the virus antigens into a 'patchy' pattern within 20 min (Fig. 1b). At 37 °C the process was much quicker: patching was observed within 5 min and, on a few cells, already complete capping was detected. After 60 min at 37 °C, capping was complete in practically all cells (Fig. 1c). It was completely inhibited in the presence of 10 mM-NaNO₃; under these conditions only patching could be observed (Fig. 1e), as has been the case in other systems (Taylor et al. 1971; Rutter & Mannweiler, 1973). Since the specimen had been fixed before the addition of antisera, the demonstrated cross-linkage
Pig. 1. EAT cells in exponential growth phase were pelleted and resuspended in cold phosphate-buffered physiological salt solution without divalent ions; 200 infective units of mengovirus per cell were allowed to adsorb for 30 min at 0 °C. Cells washed three times in cold buffer were shifted to 20 and 37 °C respectively. A parallel series contained 10 mM-NaNO₃ in all solutions. Incubation was stopped by cooling to 0 °C and immediate sedimentation of the cells and resuspension in freshly prepared 4% formaldehyde solution. The fixed cells were treated with a potent rabbit antiserum directed against mengovirus (dilution 1:40). Bound antibody was stained with a fluorescein-labelled IgG fraction of swine antiserum directed against rabbit IgG (SEVAC, Prague). Cells were embedded in glycerolpolyviol and observed with a Zeiss fluorescence photomicroscope. (a) Shows the distribution of mengovirus in a dispersed and clustered pattern after adsorption at 0 °C; (b) shows the redistribution into patches at 20 °C; (c) and (f) show an EAT cell with mengovirus capped after 30 min at 37 °C; (d) and (e) are controls with unlabelled cells and cells treated with 10 mM-NaNO₃ respectively.

obviously was not caused by antibodies but by the virus particles or capsomers themselves. The virus-induced patches were collected into caps through an active cellular process. A possible transmembranal control mechanism involving the cytoskeleton is currently under investigation in our laboratory.

The experiments shown in Fig. 1 were done in isotonic salt solution, in the absence of amino acids and serum but in the presence of glucose. Cells incubated in complete growth medium show patching at 20 °C, but at 37 °C patching and capping are so quick that these phenomena could be observed only on a few single cells. At higher temperature, the capacity of the virus-charged cells to be marked with fluorescing antibodies is reduced very quickly due to the transport of the virus antigen away from the cell surface. The virus particles seem to enter the cell by endocytosis, since virus antigen was found inside the cell close to the nucleus (not shown). The fate of this virus antigen will be further investigated.

Mengovirus, like other picornaviruses, contains 60 identical capsomers, each of which can potentially bind to a receptor. Thus a virus particle bound to one receptor will pick up other receptors which float in the lipid layer of the membrane. Since the virus particles are found in patches, there must be more than one binding site on each receptor protein. It is our suggestion that the cross-linkage of membrane protein non-specifically triggers
certain reactions of the cell. If it is not inhibited by NaN3 or other toxic drugs, the cell responds by capping followed by endocytosis of the receptors together with the adsorbed material. This internal material (in our case virus coat protein and/or the virus genome) might then directly induce or repress cellular processes, e.g. the shutoff of host-specific macromolecular synthesis after picornavirus infection of mammalian cells.

However, it is also possible that the cessation of host-specific macromolecular synthesis is the result of structural changes in the plasma membrane which in turn are due to the cross-linkage of the virus receptor proteins. In this respect, the redistribution of material in the plasma membrane may alter its permeability for ions and small molecules and thus lead, for example, to the distortion of the gradient of monovalent ions which is actually observed shortly after mengovirus infection of EAT cells (Carrasco & Smith, 1976; Carrasco, 1977; Egberts et al. 1977; Hackett et al. 1978). Based on the results of in vitro experiments, the selective translation of virus RNA in picornavirus-infected mammalian cells was suggested to be the consequence of the virus-induced alteration of the intracellular ionic conditions (Carrasco, 1977; Egberts et al. 1977; Hackett et al. 1978). That is, the pre-conditioning of the host cell by adsorption of more than one virus particle to the plasma membrane and cross-linkage of the corresponding receptor molecules may secure the successful expression of the infecting virus genome. The observed one-hit kinetics in infectivity tests and in u.v.-light inactivation kinetics are not contradictory to our model, since the inactivated virus particles would still be able to cross-link receptors, alter the physicochemical properties of the plasma membrane and thus prepare the cell for the expression of one or a few active virus genomes. Experiments to challenge our hypothesis are in progress.

In this context it is noteworthy that immediately after adsorption of infective encephalomyocarditis virus particles to baby hamster kidney cells, the fluidity of the plasma membrane is significantly enhanced (Levanon et al. 1977). These changes in membrane fluidity are virus-dose dependent and occur only at higher temperature (37 °C). Furthermore, they can be almost prevented by blocking the virus receptors on the cell surface. The identity of the requirements for both capping of picornavirus receptors on the cell surface and increase in plasma membrane fluidity suggests a common mechanism underlying both phenomena, namely the lateral rearrangement of cell surface proteins. We are currently elucidating its effect on host-specific macromolecular synthesis.

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REFERENCES


Short communications


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