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Review Article

Transportation Course of Macromolecules to the Nucleus from the Extracellular Environment: Steroid Hormones' Cellular Entry Mode Revisited

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Abstract: Polyomavirus virions such as simian virus 40 (SV40), antinuclear antibodies such as immunoglobulin G (IgG) and steroid hormones all enter the nucleus from the extracellular environment. Testosterone-bovine serum albumin conjugate labeled with 2 nm colloidal gold (testosterone-BSA-gold) is taken up by endocytosis into target cells, and enter the nucleus through a similar route as SV40 nuclear migration. Upon injection into the vascular system of rats, IgG coupled with hydrocortisone also enters the hormone-target cell nuclei with intact antigenicity. These results suggest that steroid hormones could act as transporters to deliver exogenous macromolecules, e.g. drugs, into their target cell nuclei *in vivo*, although further studies are required on whether steroid hormones coupled with proteins exert genomic actions in the nucleus, etc. Finally, testosterone-BSA-gold seems to be isolated from the cytosol in the processes of nuclear entry. Together, these findings challenge the popular belief that steroid hormones mostly enter the cell in unbound form via uncontrolled passive diffusion.

Keywords: Vesicular Trafficking, Macromolecules, Transportation Course, Nuclear Diaphragm, Target Cell Nuclei, Exterior of Cells, Steroid-Protein Conjugates, Intact Antigenicity

1. Introduction

Various large molecules destined for cell nucleus arrive from the extracellular environment. Many of the mechanisms by which such molecules enter the cell and its nucleus are still unclear. For example, antinuclear antibodies can enter the nucleus from the exterior of a cell. However, native IgG does not pass freely the cell- or nuclear membrane; IgG introduced to the cytoplasm did not enter the nucleus [1, 2]. Polyomavirus virions such as simian virus 40 (SV40) comprise proteins and DNA, and are able to enter the nucleus from the extracellular environment [3]. Substances such as SV40 tumor antigen and nuclear proteins migrate into the nucleus from the cytoplasm [4, 5]. IgG with synthetic peptides containing nuclear localization signal such as that of SV40 tumor antigen could enter the nucleus from the cytoplasm by active transport through nuclear pore complexes (NPCs) [2, 6]. This review reports about vesicular trafficking of macromolecules to the nucleus from extracellular environment.

2. Transportation Course of Macromolecules to the Nucleus from the Extracellular Environment

In some infectious processes, virus-containing vesicles fuse with the outer nuclear membrane, delivering the virus particles into the perinuclear cisterna (Figure 1a) [7, 8]. In our search for other entryways to the nucleus, migration of SV40 was pursued in cultured cells, using ferritin and concanavalin A as cell membrane markers. Ferritin particles introduced into the cytoplasm did not enter the nucleus by themselves. In contrast, SV40-containing vesicles with ferritin particles were observed close to a single-bilayer nuclear membrane or a diaphragm (Figure 1b, c) [9, 10]. The nucleoplasmic side of the diaphragm was covered with electron-dense materials, and cell membrane markers were localized along the

nucleoplasmic side of the inner nuclear membrane [9, 10]. These results suggest that SV40-containing vesicle membrane fuses to a single-bilayer diaphragm in the nuclear envelope in order to transport virus particles into the nucleoplasm, and that the exogenous macromolecules used here as cell membrane markers were transported into the nucleus in this manner (Figure 1b) [9].

3. Diaphragms in Nuclear Envelope

A question then arose whether diaphragms in nuclear envelope are formed by hemifusion between the outer- and inner nuclear membrane, or by deletion of the outer membrane. Complete fusion of two membranes such as pore formation occurs through the process of hemifusion (Figure 1d) [11]. The hemifused area then enlarges [12]. To validate the presence of pores other than NPCs in the nuclear envelope,

subacrosomal nuclear envelopes (SNEs) of spermatids were observed under electron microscope, as SNE is devoid of NPCs. Two membranes of SNE are in close apposition. The continuity between the outer and inner nuclear membranes of SNE was observed successfully [13], indicating the possibility that there is hemifusion membrane in the SNE. Earlier, we found pores that are different from NPCs in nuclear export of baculovirus nucleocapsids. Recombinant baculovirus nucleocapsids (45 nm x 280-300 nm) are formed in the nucleoplasm, and migrate into the cytoplasm to bud through cell membrane. In the study using rapid cryofixation, we proposed that the nucleocapsids pass through small pores formed in the protrusion of double membranes derived from the nuclear envelope [14]. From our study on the nuclear entry of testosterone-BSA-gold discussed below, the diaphragm seems to be formed by hemifusion of both membranes.
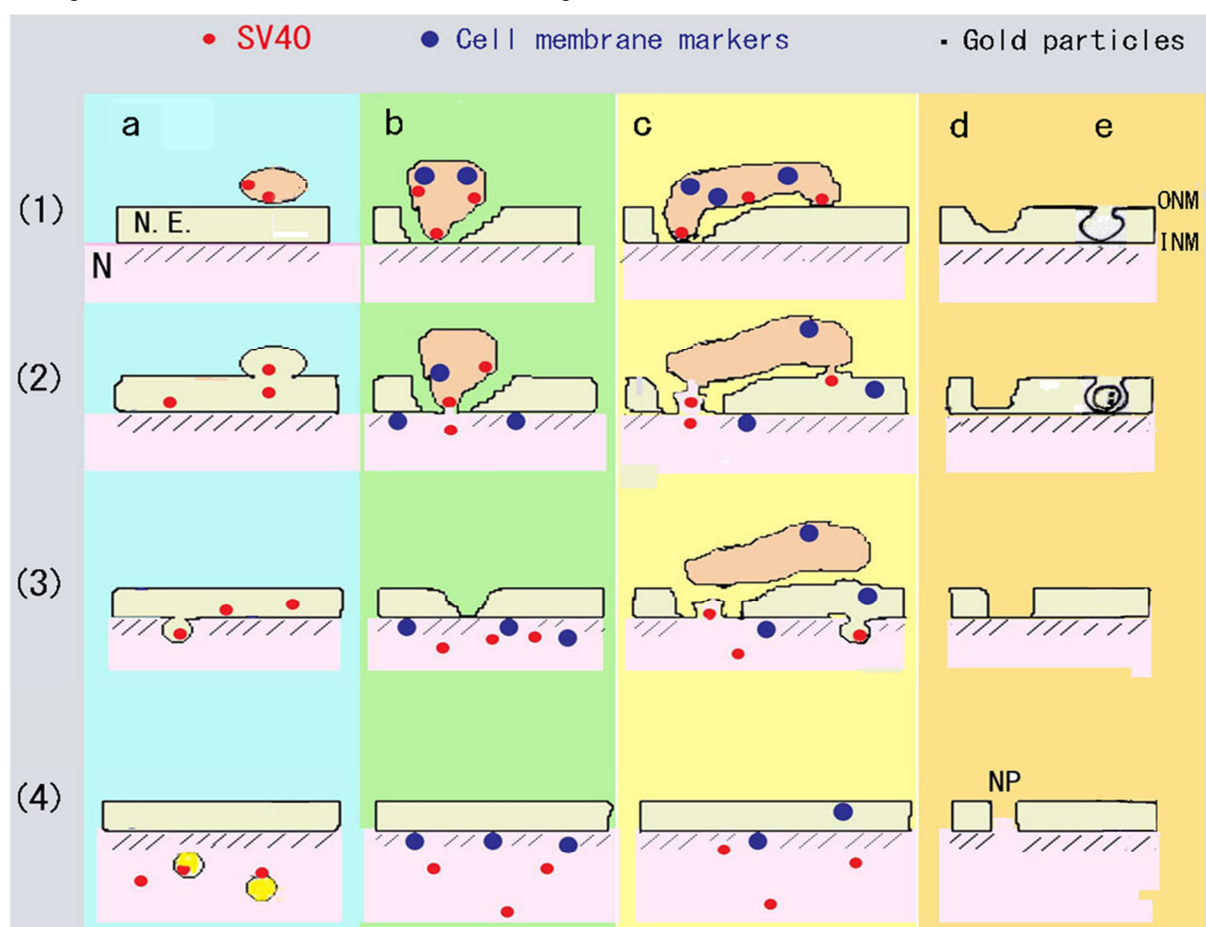
Figure 1. Schema of fusion between nuclear membranes and vacuole membrane.

a. Nuclear entry of SV40 proposed by Maul. (1, 2) Vacuoles containing SV40 fuse with outer nuclear membrane. (2) Virus particles enter perinuclear cisterna. (3) Virus particles are enveloped by inner nuclear membrane. (4) Membrane envelopes free SV40 in the nucleus.

b. (1) Vacuoles containing cell membrane markers and SV40 are located close to single membrane-like envelopes, or diaphragms. (2) Fusion between vacuoles' membrane and diaphragms. (3, 4) Cell membrane markers and SV40 enter the nucleus.

c. (1) Large vacuoles containing cell membrane markers and SV40 are located near the nucleus. (2) Parts of vacuoles' membrane fuse with diaphragms, while other parts fuse with the outer nuclear membrane. (3, 4) Cell membrane markers and SV40 enter the nucleus, and some markers remain in the perinuclear cisterna.

d. (1, 2) Invagination of the outer nuclear membrane toward the inner nuclear membrane. (3) Formation of hemifusion diaphragm. (4) Formation of pore.

e. (1) Invagination of the outer nuclear membrane of rat spermatogenic cells where testosterone-BSA-gold was injected. (2) A double-membrane-like vesicle with testosterone-BSA-gold in the nuclear envelope.

N: nucleus; NE: nuclear envelope; NP: nuclear pore; ONM: outer nuclear membrane; INM: inner nuclear membrane.

4. Steroid Hormones as Carriers to Deliver Exogenous Proteins into the Target Cell Nuclei

Steroid hormones circulate in blood plasma in three different forms: albumin-bound, steroid hormone-binding globulin (SHBG)-bound, and free [15]. In the classical model of genomic steroid hormone action, lipophilic hormones are first released from their carrier proteins and cross the cell membrane by passive diffusion in their free form [16, 17]. Contrary to this belief, SHBG coupled with [^{3}H]-testosterone (testosterone-SHBG) is internalized by receptor-mediated endocytosis in spermatogenic cells, which are target cells of testosterone, and then enters their nuclei *in vitro* [18, 19]. Colloidal gold embedded in epoxy resin becomes visible as silver deposits on the sections after silver enhancement [20]. Upon injection into the vascular system of rats, testosterone-bovine serum albumin conjugate labeled with 2 nm colloidal gold (testosterone-BSA-gold) is taken up by endocytosis into the target cells of testosterones such as round spermatids, and then enters the nucleoplasm [10, 20, 21]. In contrast, the nuclei of cells that are not targeted by testosterone such as thymocytes and hepatocytes showed very few silver deposits implying the presence of testosterone-BSA-gold [20]. These results suggest that the nuclear entry of testosterone-BSA-gold is specific to the target cells of testosterone. From the distribution of silver deposits, it has become clear that hydrocortisone-BSA-gold conjugates injected into rats enter the target cell nuclei such as hepatocytes and thymocytes [22]. The target-specificity suggests that the fate of gold labeled-steroid-BSAs may be decided at the cell membrane level.

In the spermatogenic cells of rat injected with testosterone-BSA-gold, the silver deposits were present on the cell membrane, vesicles, Golgi region, acrosome, subacrosomal space, both the post-acrosomal and the subacrosomal nuclear envelope, and the nucleoplasm [10, 21]. In observations without silver enhancement, a single-bilayer nuclear membrane or a diaphragm was visible in the SNE [10, 13]. In post-acrosomal nuclear envelope, the outer nuclear membrane was invaginated toward the inner nuclear membrane, and was likely to interact with the latter (Figure 1e) [10, 21]. Furthermore, a double-membrane-like vesicle containing gold particles was observed in the pit formed by the invagination of the outer nuclear membrane (Figure 1e) [10, 21]. These results suggest that testosterone-BSA-gold is transported by vesicles from the extracellular environment to the nucleoplasm. This route resembles the entryway proposed for nuclear migration of SV40 (Figure 1b) [21].

We also investigated whether BSA in the steroid-BSAs remains intact in the cell nuclei. For this purpose, testosterone-BSA, hydrocortisone-BSA or corticosterone-BSA was injected into rats; it showed that BSA-steroid hormone conjugates enter the hormone-target cell nuclei while maintaining antigenicity [23]. Steroid-BSA binds to nuclear receptors [24, 25]. Bovine IgG coupled with hydrocortisone injected into adrenalectomized rat vascular system enters the hormone-target cell nuclei in the liver, maintaining the antigenicity [26]. Together, these findings support the idea that steroid hormones could be useful as target cell-specific carriers to deliver exogenous macromolecules into cell nuclei.

5. Conclusion

Cells have various mechanisms to control the substances and their quantity that can pass through the cell- and nuclear membrane, and countermeasures have been developed by viruses such as SV40. The popular belief that lipophilic molecules such as steroid hormones can simply diffuse into cells uncontrolled seems to go against this controlling nature. Moreover, why do steroid hormones not remain in the lipid layer of biomembrane like their precursor cholesterol, if they enter cells by simple diffusion? Mounting evidences such as those mentioned above and others indicate that the passage of steroids into cells and their nuclei is better regulated than previously believed. In addition, the notion that steroids only traverse the cell membrane in unbound form is also challenged. In the process of nuclear entry, steroid-BSAs seem to be isolated from the cytosol. It is unknown whether steroid hormones coupled with proteins exert genomic actions in the nuclei. Further studies are required to elucidate these processes.

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