EXTENSIVE BINDING OF CONCANAVALIN A TO THE NUCLEAR MEMBRANE

Ariane MONNERON and Dominique SEGRETAIN
Unité de Microscopie Electronique, Institut Pasteur, 25, Rue du Docteur Roux, Paris 75015, France

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1. Introduction

Membrane glycoproteins are involved in the processes of cell–cell recognition, cell adhesiveness and cell mobility, and as such are mostly studied in plasma membranes. However, glycoproteins are constantly found in intracellular membranes as well, especially so in nuclear membranes [1–4]. Plant lectins have been used to demonstrate the presence of specific sugar residues on whole nuclei [2]. Their significance on the surface of such structures being as yet largely unknown, more data concerning their localization and amount are needed. In this paper, we study the distribution and relative number of Concanavalin-A (Con A) binding-sites on the nuclei of thymocytes, as compared with the surface of whole cells.

2. Materials and methods

2.1. *Preparation of cells and nuclei*

Calf thymocytes were obtained by incubating small thymus fragments in cold Hanks' medium with 10% foetal calf serum. Viability, as judged by trypan blue exclusion was 90%. Cells were collected by centrifuging the filtered supernatant (10 min, 600 *g*) and washed several times in glucose-free medium. Well preserved nuclei with their inner and outer nuclear membrane were prepared by disrupting the cells suspended in dense sucrose in a Potter homogenizer [5]. The homogenate was treated according to Blobel and Potter [6], using 2.2 M sucrose at the bottom of the sucrose gradient. The nuclear pellets, checked for purity by electron microscopy, were gently resuspended, filtered, and washed four times in TKM buffer. The last wash did not contain detectable sucrose. Washed nuclei and whole cells were fixed in glutaraldehyde (1.8% in 0.1 M cacodylate buffer, pH 7.4, for 10 min at 4°C), washed and either used at once, or dialyzed overnight in a large volume of cacodylate buffer.

2.2. *Spectrophotometric determination of the binding of lectin*

Identical numbers of nuclei and cells, counted in a hemocytometer, were suspended in a Con A (Sigma type IV, 50 μg per ml per 10^8 cells or nuclei) solution in cacodylate buffer, or in a solution of lectin made up in 0.2 M α-methyl-mannoside. After 20 min of incubation in the cold (4°C), the cells and nuclei were washed, reacted for 20 min in a solution of peroxidase (Sigma type VI, 1.25 mg per ml, per 10^8 cells or nuclei), washed again. Aliquots of cells and nuclei were reacted with *o*-dianisidine and the lectin-bound peroxidase was spectrophotometrically determined [8].

2.3. *Electron microscopy*

The rest of cells and nuclei were reacted with diaminobenzidine [9], washed, dehydrated in acetone and embedded in Epon. Ultra-thin sections, unstained or lightly counterstained with lead citrate, were examined in a Siemens 101 electron microscope.

2.4. *Binding of triatiated Con A*

Cells and nuclei were reacted with tritiated, acetylated Con A (7.6 Ci/mm, prepared by Amersham according to Agrawal et al. [11], and extensively dialyzed), for 4 or 20 min at 4°C. They were then

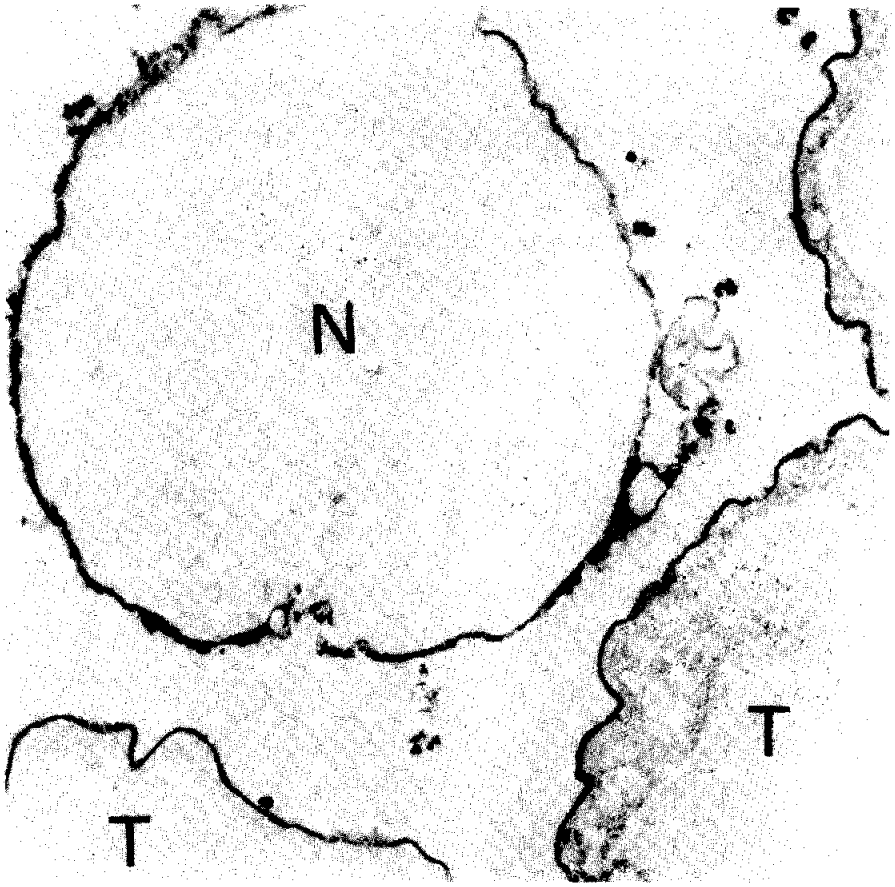

Fig. 1. A free nucleus N floating among thymocytes in suspension, T. The nuclear envelope is at least as heavily contrasted as the surface of whole cells. Unstained section. X 10 500.

either collected by membrane filtration and briefly rinsed, or washed 3 times by centrifugation and dissolved in a tissue solubilizer. Samples were counted in an Intertechnique scintillation counter.

3. Results and discussion

3.1. *Distribution of Con A binding sites*

Electron microscopy revealed in cell suspensions a small percentage of free nuclei originating from damaged cells, thus allowing the study of nuclei not obtained through sucrose gradients. Fig. 1 shows that the nuclear envelope of such nuclei is at least as stained by the lectin as the surface of neighbouring cells. Such nuclei, as well as isolated nuclei prepared through sucrose gradients and washed as described, showed a strong positive reaction all along the inner and outer nuclear membrane (figs. 2a, and 3). The pore lumen were free of reaction (fig. 2b) as well as other nuclear structures. The binding of the lectin occurred only on the cisternal side of the inner nuclear membrane, but stain was seen on both sides of the outer nuclear membrane, cisternal and cytoplasmic (fig. 2a). On the cytoplasmic surface, only the ribosomes were stained. Nuclei treated with detergents according to Penman [10] (1 ml of detergent mixture per 5×10^9 unfixed nuclei in 5 ml) and then with the lectin, were completely unstained.

In order to investigate the possibility of capping in nuclear membranes, we incubated washed, unfixed cells and nuclei with the lectin at 37°C for 30 min.

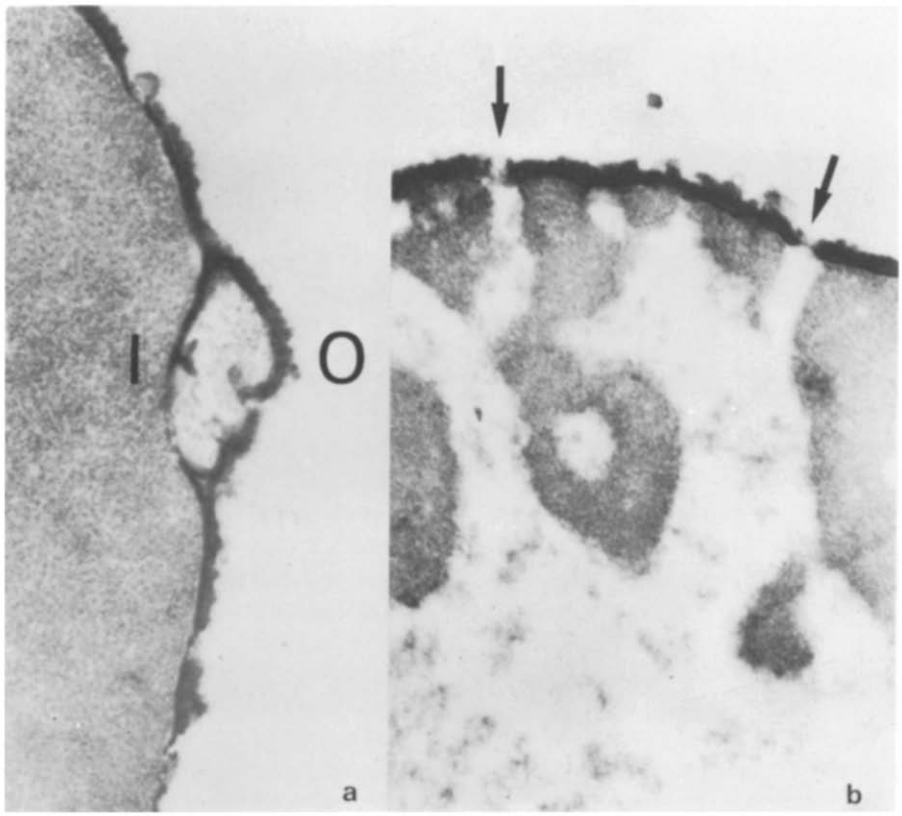

Fig. 2. Nuclei isolated in sucrose gradients and washed: a) both inner (I) and outer (O) nuclear membranes are stained. × 21 000; b) pores (arrowed) are free of precipitate. × 15 200. Sections slightly contrasted with lead.

Typical capping was observed on the cell surface, nothing similar could be seen on nuclear membranes.

3.2. *Spectrophotometric determination of the binding of lectin*

The amount of lectin-bound peroxidase was related to the number of reacted cells or nuclei. Mild glutaraldehyde fixation has been shown not to interfere with agglutinin binding, but to prevent cell aggregation induced by Con A [7]. In our material, aggregation occurred only to a slight extent, permitting an easy counting of cells and nuclei. Nuclei contained 80–150% as much lectin-bound peroxidase as that found in an equivalent number of cells. Nuclei treated with detergents before fixation bound only 2–5% of that amount. Nuclei treated with detergents after fixation bound 40–50% of the lectin-bound to intact nuclei, indicating that glutaraldehyde might cross-link part of the molecules bearing the lectin-binding sites.

3.3. *Binding of tritiated Con A*

It was first checked that neither glutaraldehyde fixation, nor dialysis, noticeably changed the binding of Con A to cells and nuclei, as compared to unfixed, undialysed organelles. Experimental variations, especially concerning nuclei, were rather large, as shown by table 1. Assuming that the molecular weight of Con A is 10^5 [12] and that one site per molecule of lectin had reacted with the membrane lectin-binding site [13], we found an average of 3 to 9 × 10^6 Con A binding sites per fresh, unfixed whole thymocyte or nucleus. By comparison, human lymphocytes were assumed to bear from 10^7 such sites per cell [14,15] to half or less that number [13]. Unlike B lymphocytes, thymocytes have a smooth surface [16].

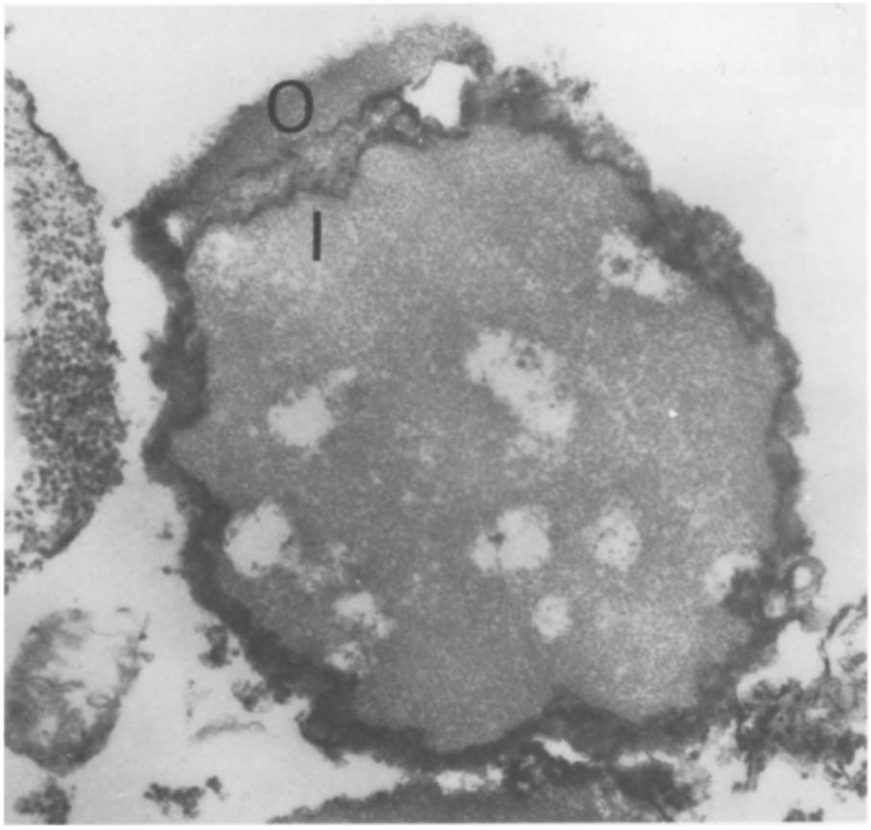

Fig. 3. Tangential section of a nucleus. The perinuclear space is free of precipitate, except on the inner (I) and outer (O) nuclear membrane. × 15 400. Sections contrasted with lead.

Table 1

	CPM per 10^5 units	Con-A binding sites per unit
C_1	3.6×10^3 (3.2×10^3)	4.3×10^6
N_1	8×10^3 (3.8×10^3)	9.6×10^6
C_2	3.2×10^3 (2.1×10^3)	3.8×10^6
N_2	3.4×10^3 (3.1×10^3)	4.1×10^6

C_1 and N_1 refer to fresh, unfixed cells and nuclei incubated in ^{3}H-labelled concanavalin A (25 μg/ml/10^8 cells C or nuclei N) or in ^{3}H-labelled concanavalin A prepared in 0.2 M α-methyl-mannoside (number in parentheses) for 4 min at 4°C, then poured on filter paper discs and washed for a few seconds. C_2 and N_2 refer to fixed, dialyzed (48 hr) cells and nuclei, incubated as above, for 20 min, washed by centrifugation and dissolved in tissue solubilizer. Note the relatively high level of labeling of cells and nuclei in 0.2 M α-methyl-mannoside.

We equated them with spheres of 115 μ^2, the nuclei being about 80 μ^2. The nuclear envelope has two membranes, inner and outer; however, in the nuclei isolation procedure, part of the outer nuclear membrane was lost, while the inner nuclear membrane was always present [17]. Therefore, the surface of the nuclear envelope per isolated nucleus was assumed to be roughly comparable to that of the intact cell. For both, the number of Con A binding-sites was of the order of $2.5–5 \times 10^4$ per square micron.

In summary, our data obtained by three different techniques show that in thymocytes there exist about as many Con A binding sites on the nuclear membrane as on the plasma membrane, whether taken as a whole, or per unit area.

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