SHORT NOTE

Inhibition of Nuclear Accumulation of Karyophilic Proteins in Living Cells by Microinjection of the Lectin Wheat Germ Agglutinin

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The lectin wheat germ agglutinin (WGA), which has been reported to inhibit nuclear protein uptake *in vitro* by isolated nuclei (Finlay *et al.* (1987) *J. Cell Biol.* **104**, 189), also blocks, on microinjection into living cells, the migration of proteins into the cell nucleus. Radioactively labeled nuclear proteins were injected into the cytoplasm of *Xenopus* oocytes and their reentry into the nucleus was analyzed in the presence or absence of WGA by two-dimensional gel electrophoresis. In another set of experiments, fluorescently labeled nucleoplasmin was injected, alone or together with WGA, into the cytoplasm of rat hepatoma cells, and its nucleocytoplasmic distribution was studied by quantitative laser fluorescence microscopy. The results indicate that WGA inhibits the uptake of karyophilic proteins in general, independent of their sizes. Since the nucleocytoplasmic flux of a dextran with M_r 10,000 was not affected it can be excluded that WGA acts by a general blockade or constriction of the functional pore channel. At reduced WGA concentrations, the rate but not the final extent of nuclear protein accumulation was decreased. These findings support the concept that the O-glycosidically bound carbohydrates of certain nuclear pore complex proteins are exposed to the pore interior and that these regions are probably involved in nucleocytoplasmic translocation processes.

The exchange of macromolecules between nucleus and cytoplasm which takes place predominantly, if not exclusively, through the nuclear pore complexes [7] is a highly selective process. Thus, only a defined subset of the total soluble cellular proteins migrates into and accumulates in nuclei ("karyophilic proteins") whereas others are excluded from the nuclear compartment (reviewed in [1, 6, 15]). Recent evidence suggests that specific amino acid sequences act as "nuclear accumulation signals," thus specifying nuclear targeting of a given protein (for Refs. see [6]). However, the mechanism by which karyophilic proteins are selectively sequestered into the nucleus and the role played by the pore complexes in this process are largely unresolved. It was therefore of particular interest when Finlay and co-workers [8] recently reported that wheat germ agglutinin (WGA), a lectin binding to *N*-acetylglucosamine residues, binds to nuclear pore complexes and inhibits the uptake of the nuclear protein nucleoplasmin in an *in vitro* system, using isolated nuclei suspended in a *Xenopus* egg extract. In view of the importance of this finding for understanding the involvement of pore complexes in protein transport mechanisms, we have examined this question in living cells and extended these investigations in several directions. In the present study we show that WGA, upon microinjection into rat hepatoma cells and *Xenopus*

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oocytes, acts as a potent inhibitor of nuclear protein transport in living cells. Using laser fluorescence microscopy, we have quantitated intracellular mobility and nucleocytoplasmic distribution of nucleoplasmin, thus allowing us to define more precisely the primary effects of WGA. In addition we present evidence that WGA does not selectively inhibit the nuclear uptake of nucleoplasmin but interferes generally with the nuclear transport of karyophilic proteins.

Material and Methods

WGA, tetramethyl rhodamine-labeled WGA, *N, N', N''*-triacetyl chitotriose, and *N*-acetylglucosamine (GlcNAc) were obtained from Sigma (Deisenhofen, FRG).

Analysis of protein transport into nuclei of Xenopus oocytes. Proteins of *Xenopus laevis* oocytes were metabolically radiolabeled with [^{35}S]methionine as described [4]. After incubation for 24 h nuclei were manually isolated, gently homogenized, and dialyzed against 3:1 medium and finally the solution was concentrated by vacuum dialysis [2]. Following centrifugation (3000*g*, 5 min), the supernatant derived from 50 nuclei was injected into the cytoplasm of full-grown oocytes [2]. For each experiment 20 oocytes were used, which had received 3 h previously an injection with a WGA solution (2 mg/ml) with or without GlcNAc (500 m*M*). After an incubation period of 6 h at 20°C, nuclei and cytoplasmic portions were manually separated and the proteins were analyzed by two-dimensional gel electrophoresis essentially as described [3].

Quantitative laser fluorescence microscopy. Cultivation of hepatoma tissue culture (HTC) cells, generation of polykaryons by incubation of confluent monolayer cultures with polyethylene glycol, microinjection, video-enhanced fluorescence microscopy, and the measurement of intracellular mobility and nucleocytoplasmic transport by fluorescence microphotolysis were as described [12]. Nucleoplasmin was isolated and labeled with fluorescein isothiocyanate as described [14]. The concentration of nucleoplasmin in the fluid injected was 1 mg/ml in all experiments. The injected volume amounted to approximately 5–10% of the total cellular volume; therefore injected substances are diluted to the same percentage. All experiments were performed at 37°C.

Results

We injected radioactively labeled soluble nuclear proteins into the cytoplasm of *Xenopus* oocytes and studied their nuclear accumulation in the presence or absence of WGA at various times after injection by manual separation of nuclei from the ooplasm, followed by two-dimensional gel electrophoresis. The nuclear proteins used for injection are shown in Fig. 1*a*. Among the numerous polypeptides some such as protein N1/N2 (for nomenclature see [1, 2, 5]), nucleoplasmin [10, 13], high mobility group protein HMG-A [9], and actin were identified. On microinjection the majority of the proteins disappeared, within 6 h, from the cytoplasmic phase and appeared in the nuclei (Figs. 1*b* and 1*c*; see also [1, 5]). Injection of WGA (cellular concentration approximately 100 μg/ml) largely inhibited the migration of the proteins into the nucleus since 6 h later most of them were still recovered in the cytoplasm (Figs. 1*d* and 1*e*). It is important to note that the WGA-induced nuclear exclusion of proteins occurred independently of their molecular mass; i.e., large proteins such as N1/N2 (110 kDa) were affected as well as small proteins (e.g., HMG-A which occur *in vivo* as free 25-kDa monomers [9]). While karyophilic proteins such as N1/N2 and nucleoplasmin remained almost quantitatively in the cytoplasm in the presence of WGA, some other proteins were apparently able to enter the nucleus (Fig. 1*d*, arrowheads; upon prolonged exposure of the X-ray films actin could also be detected in the nuclei). In control oocytes, the same polypeptides were identified in both the cytoplasm and the nucleus, indicating that they behaved as "amphiphilic" pro-

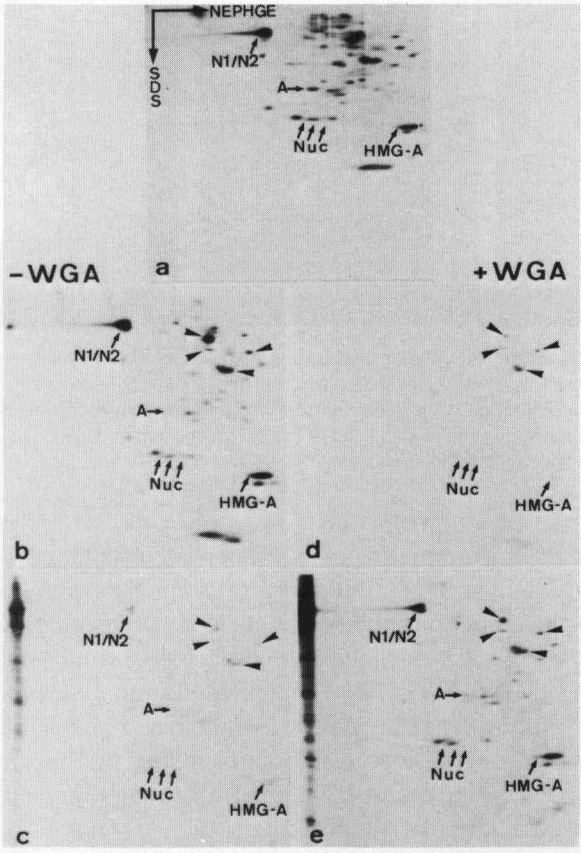

Fig. 1. Nucleocytoplasmic distribution of radioactively labeled nuclear proteins 6 h after microinjection into the cytoplasm of *Xenopus* oocytes as revealed by 2-D gel electrophoresis (first dimension NEPHGE; second dimension SDS–15% PAGE) and autoradiography. (*a*) Total injected proteins. Nuc, nucleoplasmin; A, actin. (*b, c*) Control without WGA. Most of the injected proteins have migrated from the cytoplasm (*c*) into the nucleus (*b*). (*d, e*) In the presence of WGA most of the injected proteins remain in the cytoplasm (*e*) and are excluded from the nucleus (*d*) except for a group of amphiphilic proteins which occur in both compartments (denoted by arrowheads in *d, e*; the same proteins are also marked in *b, c*).

teins (Figs. 1 *b* and 1 *c*, arrowheads). When WGA was injected together with the competing sugar GlcNac, the inhibitory effect of the lectin was completely abolished.

In order to study the effect of WGA on nuclear protein uptake in somatic mammalian cells, fluorescein-labeled nucleoplasmin was coinjected with rhodamine-labeled WGA into the cytoplasm of HTC polykaryons. The intracellular distribution of nucleoplasmin and WGA was visualized 30 min later by video-enhanced fluorescence microscopy (Fig. 2). At a high concentration of injected WGA the nuclear uptake of nucleoplasmin was completely prevented (Fig. 2 *a*). Selective excitation of rhodamine fluorescence in the same cell showed WGA to be concentrated in the perinuclear cytoplasm (Fig. 2 *b*). When injected in the absence of WGA nucleoplasmin accumulated in the cell nuclei (Fig. 2 *c*).

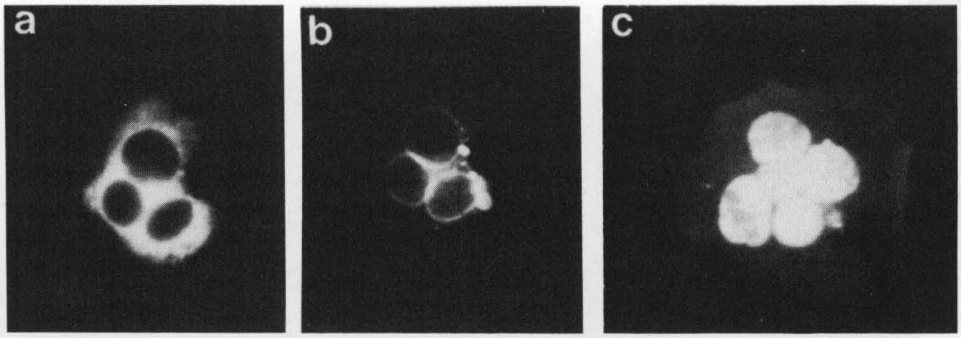

Fig. 2. Effect of WGA on the subcellular distribution of nucleoplasmin. Fluorescein-labeled nucleoplasmin and rhodamine-labeled WGA were coinjected into the cytoplasm of rat hepatoma polykaryons. Fluorescence micrographs were taken 30 min after injection with an image-intensifying video system. The typical distribution in the same cell of (*a*) nucleoplasmin and (*b*) WGA. A control cell into which nucleoplasmin was injected in the absence of WGA (*c*).

In order to establish the kinetics of nuclear protein uptake fluorescence was measured alternately in a small unit area of a nucleus and the juxtanuclear cytoplasm. From these measurements the nucleocytoplasmic fluorescence ratio F_n/c was calculated. Figure 3 plots F_n/c of nucleoplasmin versus time after injection. In the absence of WGA, F_n/c of nucleoplasmin rose within 20 min from an initial value of about 0.4 to a plateau of about 4.3 (Fig. 3, curve *a*; initial fluorescence derives from the cytoplasm surrounding the nucleus [12]). When WGA was added to the injectate at a high concentration (3 mg/ml) nuclear entry of nucleoplasmin was completely prevented (Fig. 3, curve *d*). Inhibition of nuclear uptake was almost completely abolished by *N, N′, N″*-triacetylchitotriose (1 m*M*) added to the injectate together with WGA (Fig. 3, curve *b*). At intermediate concentrations of WGA in the injectate (0.3 mg/ml), nuclear uptake was slower but not completely prevented (Fig. 3, curve *c*). The effect of the interme-

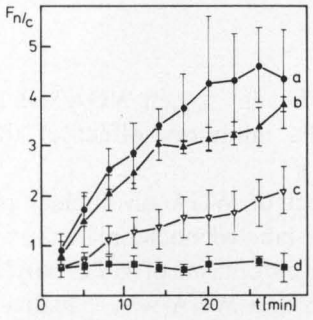

Fig. 3. Inhibition of nucleocytoplasmic protein transport by WGA. Fluorescein-labeled nucleoplasmin was injected into the cytoplasm of rat hepatoma polykaryons. The nucleocytoplasmic fluorescence ratio F_n/c of nucleoplasmin was measured and plotted versus time after injection. The mean values ±SD of 5–15 measurements are given. (*a*) Nucleoplasmin alone (1 mg/ml). Coinjection of nucleoplasmin with (*b*) WGA (3 mg/ml) and *N, N′, N″*-triacetylchitotriose (1 m*M*), (*c*) WGA (0.3 mg/ml), and (*d*) WGA (3 mg/ml).

diate WGA concentration was studied in a separate series of experiments on an extended time scale. At 2 h after injection the F_n/c value of nucleoplasmin in the presence of WGA was 3.6±0.4 and approached the value in the absence of WGA which was 4.2±0.6 (mean ±SD of 10–20 measurements in each case).

The nuclear envelope has properties of a molecular sieve. For instance, when dextrans are injected into the cytoplasm of HTC cells only molecular species with a mol wt of ≤17.5 kDa can enter the nucleus whereas larger dextrans are excluded [12]. The nuclear influx rate coefficient k provides a quantitative and very sensitive measure of the functional pore radius [11]. Therefore, the effect of WGA on both exclusion limit and influx kinetics was examined. However, within experimental accuracy no effect of WGA on these properties was observed. The exclusion limit was between the 17.5- and the 40-kDa dextran irrespective of the presence or absence of WGA (3 mg/ml) in the injectate. For a 10-kDa dextran (Stokes' radius about 28 Å [14]) the nuclear influx rate coefficient k as 0.019±0.004/s in the absence of WGA and 0.02±0.01/s in the presence of WGA (mean ±SD of 10–11 measurements).

Tight association with immobile cellular structures can profoundly influence the subcellular distribution of proteins. In order to test for this possibility, the effect of WGA on the intracellular translational mobility of nucleoplasmin was measured. Nucleoplasmin was found to be highly mobile. Its apparent translational diffusion coefficient D in the cytoplasm of HTC cells was 2.27±0.86 $\mu m^2/s$ with a mobile fraction R of 90±5% (mean ±SD of 10–15 measurements). Within experimental accuracy, WGA had no effect on D and R. For instance, at the high concentration of WGA in the injectate (3 mg/ml) D of nucleoplasmin was 2.77±0.54 $\mu m^2/s$ with R=85±5%.

Discussion

Our results confirm and extend the *in vitro* study of Finlay *et al.* [8] and show that WGA, when microinjected at sufficiently high concentrations into living rat hepatoma cells or *Xenopus* oocytes, blocks the uptake of nuclear proteins. In addition, quantitation of nucleocytoplasmic translocation and intracellular mobility by laser fluorescence microscopy, in conjunction with detailed gel electrophoretic analysis of the cytoplasmic–nuclear migration of proteins, has permitted us to obtain a clearer picture of the inhibitory action of WGA and to draw the following conclusions. (i) WGA does not influence nucleocytoplasmic protein transport by affecting the functional radius of the nuclear pore complexes. This excludes possible, more unspecific effects of WGA such as a blockade or constriction of diffusion channels presumably localized in the nuclear pore complex. (ii) WGA blocks the nuclear uptake of the whole spectrum of karyophilic proteins, independent of their molecular masses. (iii) The intracellular mobility of nucleoplasmin is not influenced by WGA; i.e., a possible association of nucleoplasmin with immobile cellular structures is not promoted by WGA. (iv) The primary action of WGA is to reduce the rate of nuclear protein uptake whereas the final exent of accumulation does not seem to be affected.

These observations should help us to discriminate between potential mechan-

isms of nucleocytoplasmic protein transport. Moreover, they lend further support to the importance of the recently identified nuclear pore complex-associated proteins which have been shown to carry cytoplasmically exposed, O-glycosidically linked GlcNac residues [17, 18] in the control of nuclear entry of proteins.

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