Monoclonal Antibody Specific for Human Nuclear Proteins
IEF 8Z30 and 8Z31 Accumulates in the Nucleus a Few Hours after
Cytoplasmic Microinjection of Cells Expressing These Proteins

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Abstract. A monoclonal antibody (mAB 1C4C10) that reacts specifically with human nuclear proteins IEF 8Z30 and 8Z31 (charge variants; HeLa protein catalogue number; Bravo, R., and J. E. Celis, 1982, Clin. Chem., 28:766–781) has been microinjected into the cytoplasm of cultured cells that either express (primates) or lack these proteins (at least having similar molecular weights and pIs; other species), and its cellular localization has been determined by indirect immunofluorescence. Nuclear localization (nucleolar and nucleoplasmic) of the antibody was observed only in cells expressing these antigens, suggesting that a determinant present in IEF 8Z30 and 8Z31 is required for cytoplasm–nuclear translocation. Nuclear migration was not inhibited by cycloheximide, implying that these proteins may shuttle between nucleus and cytoplasm. The results assumed to support the signal rather than the free diffusion model are further supported by microinjection experiments using antibodies (proliferating cell nuclear antigen/cyclin, DNA) that react with nuclear components but do not recognize cytoplasmic antigens. Furthermore, they raise the possibility that some nonnuclear proteins may be transported to the nucleus by interacting with proteins harboring nuclear location signals.

Two models have been proposed to account for the specific localization of nuclear proteins. The first assumes that proteins diffuse freely into the nucleus (albeit at different rates), but only some are retained because of their affinity to a nondiffusible nuclear component (2, 3, 22). The second model, which applies particularly to larger proteins, assumes that certain proteins are selectively translocated across the nuclear membrane (12–14). The latter model implies the presence of a signal in a nuclear protein that identifies it as a component destined for the nucleus.

Even though there is now abundant evidence that supports the signal model (at least for large proteins; 15–18, 23–26), a recent report by Bennett et al. (1) suggested that large proteins such as IgG molecules (C23 antibodies) can enter the nucleus when microinjected by the erythrocyte-mediated microinjection. Since nuclear entry was not inhibited by cycloheximide, it was concluded that antibodies penetrate the nuclear membrane (limited rate of entry) and bind nuclear proteins (C23) with the affinity characteristic of antibody–antigen reactions.

Here we present experiments in which we have microinjected a monoclonal antibody (mAB 1C4C10) that reacts specifically with nuclear proteins IEF 8Z30 and 8Z31 (charge variants; HeLa protein catalogue number [4]) (10) into the cytoplasm of cells that either express (primates) or lack these proteins (at least having similar molecular weights or pIs; other species) (10). The results, which are similar to those reported by Bennett et al. (1), are interpreted however to support the signal model. This conclusion is further supported by microinjection experiments involving proliferating cell nuclear antigen (PCNA)1/cyclin and DNA antibodies.

Materials and Methods

Cells
All cultured cells used in this study were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and antibiotics (100 U/ml penicillin, 50 μg/ml streptomycin). The SV-40 transformed human keratinocytes (K14) were a kind gift of E. B. Lane of the Imperial Cancer Research Laboratories.

Antibodies
The preparation of mouse mAB 1C4C10 (IgG) has been described in detail elsewhere (10). Specificity data are given in the Results section. The Ig fraction was purified from ascites fluid by ammonium sulfate precipitation (50%) followed by dialysis of the redisolved precipitate against Hanks' buffered saline. Human PCNA antibodies (IgG) specific for cyclin (11) were a kind gift from E. Tan. Human anti-DNA autoantibodies (IgG) were obtained from the Aarhus Kommune Hospital. Immunofluorescence staining with this antibody was abolished by DNase I treatment of the monolayers.

Microinjection of Somatic Cells with Glass Capillaries
Microinjection of cultured cells grown attached to glass coverslips was carried out essentially as described by Celis (8). Approximately 30 cells were injected per experiment and each experiment was repeated at least five times. Viability was higher than 90%.

1. Abbreviation used in this paper: PCNA, proliferating cell nuclear antigen.
Figure 1. Localization of microinjected mAB 1C4C10. Antibodies were microinjected (direct microinjection) into the cytoplasm of human K14 cells and subsequently localized by immunofluorescence at 1 (A), 2½ (B), and 18 h (C), respectively. (D) Fluorescence micrograph of K14 cells fixed with methanol and reacted with mAB 1C4C10. Bar, 10 μm.

**Other Procedures**

The procedures for labeling cells with [35S]methionine (5), two-dimensional gel electrophoresis (7), and indirect immunofluorescence (21) have been described in detail elsewhere.

**Results**

**Nuclear Migration of mAB 1C4C10 after Cytoplasmic Microinjection of Cultured Cells Expressing Its Antigens**

Fig. 1 shows immunofluorescence micrographs of SV-40 transformed human keratinocytes (K14 cells) microinjected into the cytoplasm with a monoclonal antibody (mAB 1C4C10) that reacts specifically with nuclear proteins IEFs 8Z30 and 8Z31 (Fig. 2, see also Fig. 3 A) (10). Cells were processed for immunofluorescence at 1 (Fig. 1 A), 2½ (Fig. 1 B), and 18 h (Fig. 1 C) after microinjection. Cytoplasmic localization of the antibody was observed during the first hour after injection, but a distinct nuclear localization (nucleolar and nucleoplasmic) was detected as early as 2½ h. Thereafter, there was increasing nuclear migration (not shown) and at 18 h most of the antibody was localized in the nucleus (Fig. 1 C), revealing a fluorescence pattern very similar to that observed in methanol-fixed K14 cells reacted with mAB 1C4C10 (Fig. 1 D). Similar results were obtained with African green monkey kidney cultured cells (BS-C-1) injected under comparable conditions (Fig. 4 A, 1 h after injection; Fig. 4 B, 19 h after injection). These cells express both IEF 8Z30 and 8Z31 as determined by two-dimensional gel electrophoresis (Fig. 3 B).

**Cytoplasmic Localization of mAB 1C4C10 after Microinjection of Cultured Cells Lacking Its Antigens**

In contrast to the above results, microinjection of mAB 1C4C10 into the cytoplasm of cultured cells that do not express proteins having similar molecular weights or PIs as human or monkey IEFs 8Z30 and 8Z31 (Figs. 3, C-F) did not result in nuclear migration even after prolonged periods of time. Fig. 5, A–D shows immunofluorescence micrographs of dog (dog thymus, Fig. 5 A), goat (goat synovial, Fig. 5 B), mink (mink lung, Fig. 5 C), and mouse (3T3, Fig. 5 D) cultured cells injected with mAB 1C4C10 and processed for immunofluorescence 23 h after injection. Representative two-dimensional gels (isoelectric focusing, only the pertinent area of the gel is shown) of [35S]methionine-labeled proteins from these cells are shown in Fig. 3, C–F, respectively. Furthermore, one-dimensional gel immunoblots of proteins extracted from these cells showed no detectable
Figure 2. Identification of the antigens reacting with mAB 1C4C10 by two-dimensional gel electrophoresis and immunoblotting. K14 cell proteins were separated by two-dimensional gel electrophoresis (isoelectric focusing, non-equilibrium pH gradient electrophoresis) and transferred to nitrocellulose sheets. Antigens were identified by superposition of immunoblots with their corresponding (identical) autoradiograms.

reaction with mAB 1C4C10 (Fig. 6, lanes B-D; control K14 and BSC-1 extracts are shown in lanes A and E, respectively).

Antibodies Do Not Enter the Nucleus by Free Diffusion

Even though the experiments presented above suggested that mAB 1C4C10 may be transported to the nucleus by interacting with its antigens, the possibility could not be eliminated that it penetrated the nuclear envelope by diffusion. To approach this question we carried out microinjection experiments using antibodies that react with nuclear components but do not recognize cytoplasmic antigens. PCNA/cyclin antibodies (20) react specifically with S-phase nuclear cyclin (Fig. 7 A) (6, 9, 11, 19) but do not recognize newly synthesized cytoplasmic cyclin (II). Accordingly, if these antibodies enter the nucleus by diffusion they should be retained by interacting with nondiffusible cyclin present in the S-phase cells (40% of the cell population, see also Fig. 7 A). Fig. 7, B and C shows double-immunofluorescence micrographs of asynchronous K14 cells microinjected with a mixture of PCNA/cyclin antibodies (Fig. 7 B) and mAB 1C4C10 (Fig. 7 C). Of 40 injected cells analyzed in this particular experiment, none showed nuclear localization of the PCNA/cyclin antibodies (Fig. 7 B), while all cells showed nuclear localization of mAB 1C4C10 (Fig. 7 C). In line with the above observations, cytoplasmic microinjection of asynchronous K14 cells with DNA antibodies that react only with nuclear DNA (Fig. 8 A) also failed to reveal nuclear accumulation of the immune IgG molecules even after prolonged periods of time (Fig. 8 B).

Nuclear Migration of mAB 1C4C10 in Human K14 Cells Is Not Inhibited by Cycloheximide

Treatment of human K14 cells with cycloheximide under conditions in which >95% of protein synthesis was inhibited (10 µg/ml given 2 h before injection and kept in the medium for 23 h after injection) did not inhibit nuclear accumulation of mAB 1C4C10 (Fig. 9), indicating that protein synthesis in general and in particular newly synthesized IEFs 8Z30 and 8Z31 are not required for nuclear localization of the antibody.

Cytoplasmic microinjection of cultured cells of nonprimate origin in the presence of cycloheximide did not result in nuclear migration of the antibody (data not shown).

Discussion

We have shown that mAB 1C4C10 accumulates in the nucleus when injected into the cytoplasm of cultured cells expressing its antigens, the nuclear proteins IEF 8Z30 and 8Z31 (4, 10). Nuclear accumulation was not observed in cells lacking these proteins (at least having similar molecular weights and pIs), suggesting that a determinant present in IEF 8Z30 and
8Z31 may be required for cytoplasm–nuclear translocation. Furthermore, nuclear migration was not inhibited by cycloheximide, implying that these proteins may shuttle between nucleus and cytoplasm.

Similar results to those described here have been reported by Bennett et al. (1) who injected antibodies against the nucleolar protein C23 using the erythrocyte-mediated microinjection. Since nuclear entry was not inhibited by cycloheximide, they concluded that the antibodies penetrated the nucleus by diffusion and remained there because of their interaction with a nondiffusible nuclear component, most likely protein C23. Our results, however, using two antibodies (PCNA/cyclin, DNA) that react with nuclear components but do not recognize cytoplasmic antigens, clearly argue against the diffusion model as we did not observe nuclear localization of the immune IgG molecules even after prolonged

Figure 3. Two-dimensional gel electrophoresis (isoelectric focusing) of [35S]methionine-labeled proteins from: (A) K14, (B) BS-C-1, (C) dog thymus, (D) goat synovial membrane, (E) mink lung, and (F) mouse 3T3 cells. Only the pertinent area of the gel is shown. Actin and alpha- and beta-tubulin (αt, βt) are indicated for reference.

Figure 5. Localization of mAB 1C4C10 microinjected into cultured cells of nonprimate origin. (A) Dog thymus, (B) goat synovial, (C) mink lung, and (D) mouse 3T3 cells. Cells were processed for immunofluorescence 18 h after microinjection. Bar, 10 μm.
periods of time. Both antibodies react with nondiffusible nuclear antigens (Figs. 7A and 8A), and therefore, it was expected that if immune IgG molecules penetrated the nuclear envelope by diffusion at least some cells should have exhibited nuclear fluorescence. These results strengthen the notion that mAB 1C4C10 enters the nucleus by combining with its antigens (signal model) rather than by diffusion. Recently, Tsuneoka et al. (26) have presented evidence showing that anti-HMG-1 antibodies migrate into the nucleus when co-introduced with HMG-1 into the cytoplasm of F1 cells. No nuclear migration was observed when the antibody alone was injected into the cytoplasm.

Finally, our results raise the interesting possibility that some non-nuclear proteins may be transported to the nucleus by interacting with nuclear proteins harboring location signals. Further experiments will be necessary to assess this possibility.

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Figure 4. Localization of mAB 1C4C10 microinjected into the cytoplasm of monkey BS-C-1 cells. (A) 1 h after injection (B) 19 h after injection. Bar, 10 μm.
Figure 6. One-dimensional immunoblot analysis (mAB IC4C10) of proteins extracted from (A) K14, (B) dog thymus, (C) goat synovial, (D) mink lung, and (E) BS-C-1 cells.

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Figure 8. Localization of human anti-DNA autoantibodies microinjected into K14 cells. (A) Methanol-fixed K14 cells reacted with DNA antibodies. (B) K14 cells injected into the cytoplasm with variable amounts of DNA antibodies and processed for immunofluorescence 18 h after microinjection. Bar, 10 μm.

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