Microinjection of Proteins into Somatic Cells: Needle Microinjection and Scrape Loading

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I. Introduction

Microinjection has been used since the 1920s as a means for delivering foreign molecules into cells. The greatest value of the approach is in allowing direct experimentation on live cells (see also articles by M. Graessmann and A. Graessmann and by Rainer Pepperkok, Rainer Saffrich, and Wilhelm Ansorge). The technique is receiving renewed interest in conjunction with considerable technical improvement and large expansion in the spectrum of probes. In addition, a number of new approaches, such as scrape loading (McNeil, 1989) and electroporation (Glogauer and McCulloch, 1992), have been introduced to complement the direct microinjection approach. To probe different aspects of cells, various types of molecules have been microinjected, including (caged) second messengers, fluorescent probes and indicators, drugs and antibodies, active genes, and antisense nucleic acids. This article focuses on the needle microinjection and scrape loading of protein molecules into cultured somatic cells; however, similar approaches should apply to other types of molecules and cells.

II. Materials and Instrumentation

Collodion bags (Cat. No. 25310 or 25320) and apparatus for vacuum dialysis (Cat. No. 27240) were purchased from Schleicher and Schuell. Centricon filters (Cat. No. 4205 or 4208) were obtained from Amicon. Cells for microinjection were plated in special chamber dishes, with acid-washed No. 2 glass coverslips (Cat. No. 12-543F) as the substrate, as described by McKenna and Wang (1989). In some experiments the medium was covered with a layer of mineral oil (Cat. No. 400-5, Sigma) to minimize evaporation. See McKenna and Wang (1989) for details of culturing cells on the microscope stage.

Microinjection was performed on a Zeiss Axiovert 10 inverted microscope, equipped with a 10× Achrostigmat objective (Cat. No. 44 01 31), a 40× phase-contrast Plan-Neofluar objective (Cat. No. 44 03 51), and 10× eyepieces. Vibration of the table was dampened by installing small tires under the legs. Needles were pulled from Omega-Dot capillary tubing (Cat. No. 30-31-0, Friderick & Haer), using a vertical pipette puller (Model 720, David Kopf). Needles were mounted on a microinstrument collar (Cat. No. 520-145, Leitz) and a micromanipulator (Cat. No. 520-137, Leitz) with a microinstrument holder assembly (Cat. No. 520-142, Leitz). Pressure for microinjection was generated with either an air-filled 10-ml glass

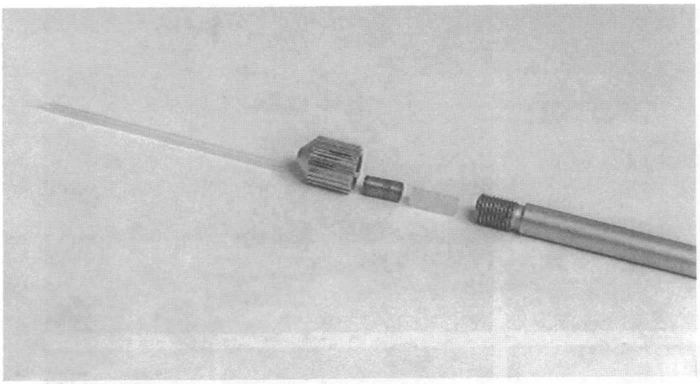

FIGURE 1 Assembly of microneedle and microinstrument collar. The parts from left to right are microneedle, captive nut, brass guide piece, silicon rubber sealing tubing, and metal cylinder. The brass guide piece has one end beveled, which should face toward the sealing tubing. The needle is first passed through the captive nut and the guide piece, and is attached to the silicon rubber tubing. The assembly is then screwed onto the metal cylinder with a gentle tightening pressure.

syringe, coated with a small amount of immersion oil on the inside surface as sealer and lubricant, or with an electronic regulator (custom designed) which maintains a steady air pressure in the range 0.3–1.0 psi. Additional details of the equipment are described in Wang (1992).

A rubber policeman for scrape loading was purchased from Fisher Scientific (Cat. No. 14-105A).

III. Procedures

A. NEEDLE MICROINJECTION

Solutions

1. *Buffer:* Proteins to be microinjected were dialyzed overnight into 2 m*M* Tris–acetate, pH 7.0, or a buffer compatible with both the cell (e.g., low calcium, low buffering capacity, near-neutral pH, limited salt and magnesium, nontoxic) and the protein. Collodion bags were used for the dialysis of small volumes and for the concentration of protein solution by vacuum dialysis. Amicon filters were used for concentration of some proteins. The solution was clarified by centrifugation at 25,000 rpm for 20 min in a Beckman type 42.2 Ti rotor.

2. *Culture medium at 36–37°C.*

Steps

1. Plate cells for 12–48 hr.

2. Scan the dish at a low magnification (e.g., 100×) to choose an area for microinjection. For most experiments, the cell density should be somewhat below confluency to provide adequate space for cells to spread and for maneuvering of microinjection needles.

3. Load protein solution from the back end of the needle with a drawn-out Pasteur pipette. Mount the loaded microneedle on the microinstrument collar (Fig. 1) and the micromanipulator.

4. Apply some pressure to prevent backflow of medium into the needle.