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V-2 Micromanipulation

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Since the success of recording intracellular action potentials in nerve and muscles (Hodgkin and Huxley, Nature *144*, 710, 1939; Nastuk and Hodgkin, J. Cell. Comp. Physiol. *35*, 39, 1950), a number of investigations using microelectrodes have been carried out, to determine membrane potentials, membrane resistance and membrane capacity and to apply electric current to cells. In other fields of cell biology, however, research with micrurgical techniques was carried out only by a small number of specialists before 1970. Within the recent decade, the importance of micrurgical techniques in studies of cellular phenomena has been understood by cell biologists, so that research using these techniques has been increasing in various fields of cell biology.

Although some pioneer research was done by the Japanese investigators using micrurgy before 1950 (*e.g.* the determination of membrane potentials in *Paramecium* by Kamada, 1934 and the induction of muscular contraction by intracellular injection of calcium ions by Kamada and Kinosita, 1943), most of the important research using micrurgical techniques in Japan has been done since after 1950. In the present article, cell-biological studies using micrurgical techniques done by Japanese investigators within the recent decade are reviewed with special reference to technology. Works in the field of electrophysiology are excluded because most of them are covered in Chapters II-6 and II-7.

Micromanipulators and related instruments for micromanipulation

Micromanipulators and various instruments for micromanipulation and micro-electrophysiology are manufactured in Japan, and some unique types have been developed by Japanese makers. In 1975 and 1976, Narishige Scientific Instrument Laboratory developed oil-pressure-controlled micromanipulators (MO-102 and MO-103) on my suggestion. These micromanipulators, in which three-dimensional movements of microtools held with the operation head are controlled with a joy-stick and/or screw knobs in the control unit, with oil pressures transmitting forces through a set of three Teflon tubings connecting the operation head with the control unit, have some advantages as compared with previous micromanipulators: The movement of microtools is free from mechanical vibrations and electric disturbances due to the manipulation of the joy-stick and/or knobs by hand, and the creep due to the external force applied to the microtool, which is inevitable in pneumatic micromanipulators, are eliminated. The operating head can be fixed directly to the microscope stage, so that a conventional microscope, in which focusing is made by moving the stage, can be used for micromanipulation.

Following the idea of Yamamoto and Furusawa (1978), Olympus Optical Co. Ltd.

manufactured the Injectscope (MIT-F), an instrument combining an Olympus inverted microscope (MIT) and a special mechanism to hold a micropipette. The micropipette is inserted into cells on the stage of the microscope, passing through a hole drilled along the optical axis of the condenser lens, while the cells are illuminated with light passing through the annular stop of the condenser lens of the phase contrast optics. This instrument is useful for qualitative microinjection experiments in cultured cells although failure of penetration of the micropipette into the cell is sometimes overlooked because the tip of the micropipette is not always in focus under the microscope.

Microinjection, nuclear transplantation and other microvivisections

Hiwatashi, Koizumi and their co-workers carried out extensive genetic and cell-biological investigations of *Paramecium* using a nuclear-and cytoplasmic-transplantation technique similar to the classic Chambers' (Chambers and Kopac, 1950) one (cf. Koizumi, 1974; Knowles, Exp. Cell Res. *88*, 79, 1974). They isolated and purified a substance characteristic of immature cytoplasm (Haga and Hiwatashi, 1981), found a protein regulating the function of the Ca^{2+} channel in the cell membrane and analyzed the roles of macro- and micro-nuclei, (Fujishima and Hiwatashi, 1978, 1981; Fujishima and Watanabe, 1981; Mikami, 1980; Harumoto and Hiwatashi, 1982; Karino and Hiwatashi, 1981). They also succeeded in the conversion of mating types by transplantation of macronucleus (Koizumi and Mikami, 1981).

Okada *et al.* (1974a) developed a unique technique for the transplantation of cytoplasm between *Drosophila* eggs. The eggs were dechorionated on double-stick Scotch tape by rolling each egg. These dechorionated eggs were fixed to a glass slide by double-stick Scotch tape, and put in a desciccator for 10 min or so to reduce the high turgor pressure. Transplantation of the cytoplasm was made using a pressure injection system. Gum damar in heptane was used to seal the wound in the egg at the part of insertion of the micropipette. Using this method, they revealed that sterility which results from UV irradiation could be prevented by the injection of polar cytoplasm. This provided the first demonstration of transplantation of agents causing determination in an insect. They (Okada *et al.*, 1974b) could produce adults by transplantation of genetically labeled nuclei from the anterior of donor embryos to the posterior pole of host embryos, supporting the idea that nuclei at the stage of nuclear multiplication are totipotent. Okada and his co-workers (Okada, Komatsu and Okumura, 1980; Togashi and Okada, 1982; Ueda and Okada, 1982) extended their research and obtained a subcellular fraction responsible for the induction of pole cells in sterilized embryos.

Hiramoto (1974) devised a method for precisely controlling a small volume of liquid for microinjection with a "braking micropipette", a micropipette with a constriction near its tip (Fig. 1a). Using a viscous liquid (paraffin oil or silicon oil) put into the constricted part of the micropipette, the flow of the liquid for microinjection can be easily controlled. Because the volume of the liquid to be injected into the cell is generally far smaller than the volume which can be controlled with a commercially-availabled microsyringe, this method is useful for microinjecting a controlled amount of substance and for precisely controlling the removal and/or transplantation of a nucleus or cytoplasm, as is the method previously reported by Hiramoto (1962) (see also Kiehart, 1982), in which the flow of the liquid in the micropipette is controlled by the surface tension of the mercury (cf. Fig. 1b).

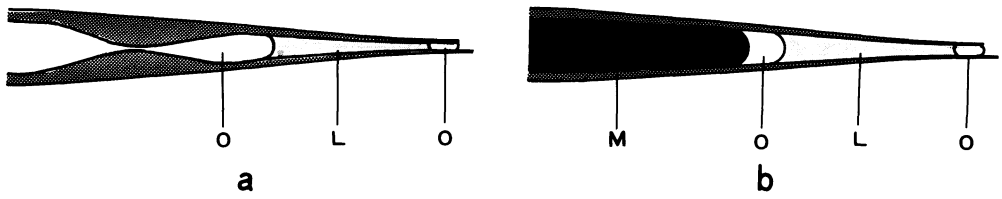

Fig. 1. Micropipettes for quantitative microinjction
L: injection liquid. M: mercury. O: oil.

Hiramoto (1956, 1974) devised a manipulation chamber with a wedge-shaped space in which the cell is supported. Because the micropipette to be inserted into the cell is moved in the plane of focus of the microscope in this case, manipulation can be carried out precisely and easily as compared with the traditional hanging-drop or lying-drop method (Chambers and Kopac, 1950).

Hiramoto and his co-workers carried out some microinjection experiments using echinoderm eggs and *Paramecium*: By microinjection of EGTA-Ca buffer into *Paramecium*, Saiki and Hiramoto (1975) confirmed Naitoh and Kaneko's conclusion (1972), obtained with *Paramecium* demembranated with Triton X-100, that ciliary reversal is induced by the increase in the Ca^{2+} concentration in the cell. By a similar experiment with sea urchin eggs, Hamaguchi and Hiramoto (1981) found that the egg is activated when the intracellular Ca^{2+} concentration is raised and that activation by insemination is suppressed when the intracellular Ca^{2+} concentration is clamped at low levels, suggesting that the first step of activation in sea urchin eggs is the increase in the intracellular Ca^{2+} concentration. Hamaguchi and Mabuchi (1978) measured intracellular Ca^{2+} concentrations in some echinoderm eggs by the luminescence intensity of aequorin that had been microinjected into the cell. Hamaguchi (1982) revealed the increase in intracellular pH in sea urchin eggs shortly after fertilization by the tint of the pH-buffer injected into the egg and the inhibition of development by clamping the intracellular pH to a low value by injecting pH buffer, suggesting an important role for the intracellular pH increase in early development. Hirano and Ishikawa (1979), Hirano (1982), and Hamaguchi and Kuriyama (1982) demonstrated the formation of sperm asters around centrioles microinjected into echinoderm eggs.

Hamaguchi and Mabuchi (1982) revealed the intracellular distribution of F-actin by fluorescence microscopy in echinoderm eggs injected with fluorescence-labeled phaloidin. Hamaguchi and Iwasa (1980) clearly demonstrated the intracellular distribution of calmodulin by fluorescence microscopy in sand-dollar eggs injected with fluorescence-labeled calmodulin. Mabuchi and Okuno (1977) found the inhibition of cleavage in starfish blastomeres by intracellular injection of anti-myosin, suggesting an important role for the actomyosin system in the process of cleavage. Hiramoto and Shôji (1982) analyzed the location of the motive force for chromosome movement by microvivisection of dividing sand-dollar eggs.

Following Kanatani and Hiramoto's work (1970), which showed by microinjection technique that the maturation-inducing hormone (1-methyladenine) acts on starfish oocytes from outside the cell, Kishimoto and Kanatani (1976) revealed, by transplantation of the oocyte cytoplasm, that a maturation promoting factor (MPF) is produced in oocyte stimulated by 1-methyladenine. MPF is amplified in the cytoplasm

under the participation of germinal vesicle materials (Kishimoto *et al.*, 1981). MPF is species non-specific in a wide range of invertebrates and vertebrates (Kishimoto and Kanatani, 1977; Kishimoto *et al.*, 1982).

Iwamatsu, Ohta and their co-workers (Iwamatsu and Ohta, 1974; Iwamatsu, Miki-Noumura and Ohta, 1976; Ohta and Iwamatsu, 1974, 1980, 1981) injected various biological materials; sperm homogenates from medaka, loach, toad and rat, flagella and centrioles obtained from sea urchin sperm and eggs, and microtubules from sea urchin and oyster sperm, into eggs of medaka (teleost). Cleavage initiating activity was found in the above materials, especially in the flagellum- and centriole-fractions.

Since the 1960s, Nishioka and her co-workers have carried out extensive studies of the production of amphibian inter-species hybrids using nuclear transplantation techniques that they developed by improving the original Briggs and King's technique (Proc. Natl. Acad. Sci. USA *38*, 455, 1952). They succeeded in producing frogs with reproductive functions having two or three sets or diploid chromosomes originating from different species. Most of their research has been published in the Scientific Reports of the Laboratory for Amphibian Biology, Hiroshima University vols. 1–6.

Using an "Injectscope" (Yamamoto and Furusawa, 1978), Kondoh *et al.* (1983) succeed in demonstrating the expression of the δ-crystalline gene of chick in mouse lens epithelium cells by injecting the cloned gene into cultured mouse somatic cells. Similar microinjection experiments with cloned genes and cultured cells are being carried out by other Japanese investigators (*e.g.* Kudo *et al.*, 1982).

Yamamoto *et al.* (1981, 1982) devised a "pricking method" for introducing substances into cultured cells, in which cells are pricked with a microneedle. According to them, a fixed quantity of material contained in the culture medium is introduced into each pricked cell as tested using diphtheria toxin, although the generality of such a quantitative relation in other kinds of cells should be examined critically.

Mechanical properties of single cells

Micrurgical techniques are useful in measuring the physical properties of single cells. In the present chapter, measurements of the force generated by single cells and their mechanical properties are discussed. Research on electrogenesis and the electric properties of cells are discussed in Chapters II-6 and II-7.

Using a method for measuring small forces by the bending of a glass fiber (Yoneda, 1960; Hiramoto, 1963), Yoneda, Hiramoto and their co-workers determined the relation between the force applied to the cell and its deformation in echinoderm eggs at various stages of development (Hiramoto, 1976a, b, 1979; Ikeda *et al.*, 1976; Nemoto *et al.*, 1980; Yamamoto and Yoneda, 1983; Yoneda, 1976, 1980; Yoneda *et al.*, 1978; for reviews, see Hiramoto, 1981, 1982). Hiramoto (1979) determined the force exerted by the cleavage furrow in sea urchin eggs by the same method.

Mechanical properties of the cell also have been determined from the relation between the negative pressure applied to a part of the cell surface with a capillary closely in contact with it and the deformation produced by applied negative pressure in echinoderm eggs (Nakamura and Hiramoto, 1978, Hiramoto, 1979) and in amphibian eggs (Sawai, 1979; Sawai and Yoneda, 1974).

The mechanical properties of cilia and flagella and the forces exerted by them are discussed in Chapter III-6.

REFERENCES

CHAMBERS, R. and KOPAC, M. J. (1950). in *Handbook of Microscopic Technique*, 3rd ed. ed. Mc Clung, J. R. pp. 492–543, Haeber, New York.

FUJISHIMA, M. and HIWATASHI, K. (1978). *Exp. Cell Res.* **111**, 468–471.

FUJISHIMA, M. and HIWATASHI, K. (1981). *Exp. Cell Res.* **131**, 63–71.

FUJISHIMA, M. and WATANABE, T. (1981). *Exp. Cell Res.* **132**, 47–56.

HAGA, N. and HIWATASHI, K. (1981). *Nature* **289**, 177–179.

HAMAGUCHI, M. S. (1982). *Develop. Growth Differ.* **24**, 443–451.

HAMAGUCHI, Y. and HIRAMOTO, Y. (1981). *Exp. Cell Res.* **134**, 171–179.

HAMAGUCHI, Y. and IWASA, F. (1980). *Biomed. Res.* **1**, 502–509.

HAMAGUCHI, Y. and KURIYAMA, R. (1982). *Exp. Cell Res.* **141**, 450–454.

HAMAGUCHI, Y. and MABUCHI, I. (1978). *Cell Struct. Funct.* **2**, 259–263.

HAMAGUCHI, Y. and MABUCHI, I. (1982). *Cell Motility* **2**, 103–113.

HARUMOTO, T. and HIWATASHI, K. (1982). *Exp. Cell Res.* **137**, 476–481.

HIRAMOTO, Y. (1956). *Exp. Cell Res.* **11**, 630–636.

HIRAMOTO, Y. (1962). *Exp. Cell Res.* **27**, 416–426.

HIRAMOTO, Y. (1963). *Exp. Cell Res.* **32**, 59–75.

HIRAMOTO, Y. (1974). *Exp. Cell Res.* **87**, 403–406.

HIRAMOTO, Y. (1976a). *Develop. Growth Differ.* **18**, 205–209.

HIRAMOTO, Y. (1976b). *Develop. Growth Differ.* **18**, 379–388.

HIRAMOTO, Y. (1979). in *Cell Motility: Molecules and Organization*, eds. HATANO, S., ISHIKAWA, H. and SATO, H., pp. 653–663, Univ. Tokyo Press, Tokyo.

HIRAMOTO, Y. (1981). in *Cellular Dynamics: Mitosis/Cytokinesis*, eds. ZIMMERMAN, A. M. and FORER, A., pp. 397–418, Academic Press, New York.

HIRAMOTO, Y. (1982). *Bioheology* **19**, 71–78.

HIRAMOTO, Y. and SHÔJI, Y. (1982). in *Biological Functions of Microtubules and Related Structures*, eds. SAKAI, H., MOHRI, H. and BORISY, G. G., pp. 247–259, Academic Press, New York.

HIRANO, K. (1982). *Develop. Growth Differ.* **24**, 273–281.

HIRANO, K. and ISHIKAWA, M. (1979). *Develop. Growth Differ.* **21**, 473–481.

IKEDA, M., NEMOTO, S. and YONEDA, M. (1976). *Develop. Growth Differ.* **18**, 221–225.

IWAMATSU, T., MIKI-NOUMURA, T. and OHTA, T. (1976). *J. Exp. Zool.* **195**, 97–106.

IWAMATSU, T. and OHTA, T. (1974). *J. Exp. Zool.* **187**, 3–12.

KAMADA, T. (1934). *J. Exp. Biol.* **11**, 94–102.

KAMADA, T. and KINOSITA, H. (1943). *Jap. J. Zool.* **10**, 469–493.

KANATANI, H. and HIRAMOTO, Y. (1970). *Exp. Cell Res.* **61**, 280–284.

KARINO, S. and HIWATASHI, K. (1981). *Exp. Cell Res.* **136**, 407–415.

KIEHART, D. P. (1982). *Methods in Cell Biology* **25**, 13–31.

KISHIMOTO, T., HIRAI, S. and KANATANI, H. (1981). *Dev. Biol.* **81**, 177–181.

KISHIMOTO, T. and KANATANI, H. (1976). *Nature* **260**, 320–322.

KISHIMOTO, T. and KANATANI, H. (1977). *Gen. Comp. Endocrinol.* **33**, 41–44.

KISHIMOTO, T., KURIYAMA, R., KONDO, H. and KANATANI, H. (1982). *Exp. Cell Res.* **137**, 121–126.

KOIZUMI, K. (1974). *Exp. Cell Res.* **88**, 74–78.

KOIZUMI, S. and MIKAMI, K. (1981). *Exp. Cell Res.* **131**, 441–446.

KONDOH, H., YASUDA, K. and OKADA, T. S. (1983). *Nature* **301**, 440–442.

KUDO, A., YAMAMOTO, F., FURUSAWA, M., KUROIWA, A., NATORI, S. and OBINATA, M. (1982). *Gene* **19**, 11–19.

MABUCHI, I. and OKUNO, M. (1977). *J. Cell Biol.* **74**, 251–263.

MIKAMI, K. (1980). *Develop. Biol.* **80**, 46–55.

NAITOH, Y. and KANEKO, H. (1972). *Science* **176**, 523–524.

NAKAMURA, S. and HIRAMOTO, Y. (1978). *Develop. Growth Differ.* **20**, 317–327.

NEMOTO, S., YONEDA, M. and UEMURA, I. (1980). *Develop. Growth Differ.* **22**, 315–325.

OHTA, T. and IWAMATSU, T. (1974). *Develop. Growth Differ.* **16**, 67–74.

OHTA, T. and IWAMATSU, T. (1980). *J. Exp. Zool.* **214**, 93–99.

OHTA, T. and IWAMATSU, T. (1981). *J. Exp. Zool.* **218**, 293–299.
OKADA, M., KLEINMAN, I. A. and SCHNEIDERMAN, H. A. (1974a). *Develop. Biol.* **37**, 43–54.
OKADA, M., KLEINMAN, I. A. and SCHNEIDERMAN, H. A. (1974b). *Develop. Biol.* **39**, 286–294.
OKADA, M., KOMATSU, H. and OKAMURA, I. (1980). *Develop. Growth Differ.* **22**, 599–610.
SAIKI, M. and HIRAMOTO, Y. (1975). *Cell Struct. Funct.* **1**, 33–41.
SAWAI, T. (1979). *J. Embryo. Exp. Morphol.* **51**, 183–193.
SAWAI, T. and YONEDA, M. (1974). *J. Cell Biol.* **60**, 1–7.
TOGASHI, S. and OKADA, M. (1982). in *The Ultrastructure and Functioning of Insect Cells*, eds. AKAI, H., KING, R. and MOROHOSHI, S., pp. 41–44, Society for Insect Cells Japan, Tokyo.
UEDA, R. and OKADA, M. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 6946–6950.
YAMAMOTO, F. and FURUSAWA, M. (1978). *Exp. Cell Res.* **117**, 441–445.
YAMAMOTO, F., FURUSAWA, M., TAKAMATSU, K., MIURA, N. and UCHIDA, T. (1981). *Exp. Cell Res.* **135**, 341–345.
YAMAMOTO, F., FURUSAWA, M., FURUSAWA, I. and OBINATA, M. (1982). *Exp. Cell Res.* **142**, 79–84.
YAMAMOTO, K. and YONEDA, M. (1983). *Develop. Biol.* **96**, 166–172.
YONEDA, M. (1960). *J. Exp. Biol.* **37**, 461–468.
YONEDA, M. (1973). *Advan. Biophys.* **4**, 153–190.
YONEDA, M. (1976). *Develop. Growth Differ.* **18**, 387–389.
YONEDA, M. (1980). *Develop. Growth Differ.* **22**, 39–47.
YONEDA, M., IKEDA, M. and WASHITANI, S. (1978). *Develop. Growth Differ.* **20**, 329–336.