Protoplasma 114, 62–66 (1983)

PROTOPLASMA
© by Springer-Verlag 1983

Binucleation and Abnormal Chromosome Distribution in *Euglena gracilis* Cells Treated with Dimethyl Sulfoxide[1]

G. L. VANNINI* and F. POLI

Institute of Botany, University of Ferrara, Italy

Received April 5, 1982
Accepted May 24, 1982

Summary

The addition of 5% of dimethyl sulfoxide (DMSO) to cultures of *Euglena gracilis* in the logarithmic phase of growth caused an immediate inhibition of cell multiplication and motility without completely blocking nuclear division. Importantly, some 50% of the cells were 2–3 times larger than normal and were also binucleate after 24–48 hours of treatment. Evidently binucleation resulted from the lack of cytokinesis after mitosis was induced. Transmission electron microscopy, using serial sections, showed the occurrence of nuclei either with a normal or a reduced number of chromatin masses. Solvent withdrawal led to a rapid recovery of all the normal cell activities.

On the contrary, 2.5% of DMSO produced no effect during the entire period of treatment (48 hours), whereas a 1-hour exposure to 10% of the solvent was sufficient to provoke aspecific and irreversible cellular damage.

Since DMSO is known to produce alterations in actin-containing structures in a wide variety of cells types, an involvement of microfilaments in cell motility, cytokinesis and chromosome separation during mitosis in *Euglena* is proposed and discussed.

Keywords: Dimethyl sulfoxide; *Euglena gracilis;* Binucleation; Karyological abnormalities.

1. Introduction

The structures responsible for motive force development in cell aperiodic contraction and re-extension, cytokinesis and chromosome separation during mitosis in the unicellular alga *Euglena gracilis* have not yet been identified. Nevertheless, an involvement in all these activities of contractile proteins, chiefly actin, has been taken into account (HOFMANN and BOUCK 1976, LEFORT-TRAN *et al.* 1980, BRE *et al.* 1981, MURRAY 1981).

The idea that actin filaments play a role in a given process is often suggested by the observation that the process in question is affected by treatment with one of the cytochalasins, most commonly the B one (CARTER 1967, COPELAND 1974, BROWN and SPUDICH 1979). These mold metabolites, which usually disrupt microfilaments in a wide variety of cell types (BRAY 1979, BROWN and SPUDICH 1981, CASELLA *et al.* 1981, WEATHERBEE 1981), do not effect *Euglena*, probably because they do not penetrate into the organism (VANNINI, unpublished). Another substance useful in the study of microfilament organization and function is dimethyl sulfoxide (DMSO), a solvent that easily penetrates the membranes (JACOB *et al.* 1971, SZMANT 1975). It also induces, in several cell types, striking rearrangement of the actin-containing structures, such as the disappearance of microfilaments from the cytoplasm and the formation of actin bundles inside the interphasic nuclei (FUKUI and KATSUMARU 1980, OSBORN and WEBER 1980, SANGER *et al.* 1980, WEHLAND *et al.* 1980). When used at a suitable concentration, DMSO also causes multinucleation and inhibition of cytokinesis in the slime mold *Dictyostelium discoideum* (FUKUI 1980). These latter findings are of particular interest since the primary purpose of this study is to obtain, by means of DMSO treatment, some information on the contractile proteins involved in mitosis and cytoplasmic cleavage in *Euglena*.

[1] The study was supported by a grant from Italian Research Council (CNR), contract No. 80.00420.04.

* Correspondence and Reprints: Institute of Botany, University of Ferrara, Corso Porta Mare 2, I-44100 Ferrara, Italy.

0033-183X/83/0114/0062/$ 01.00

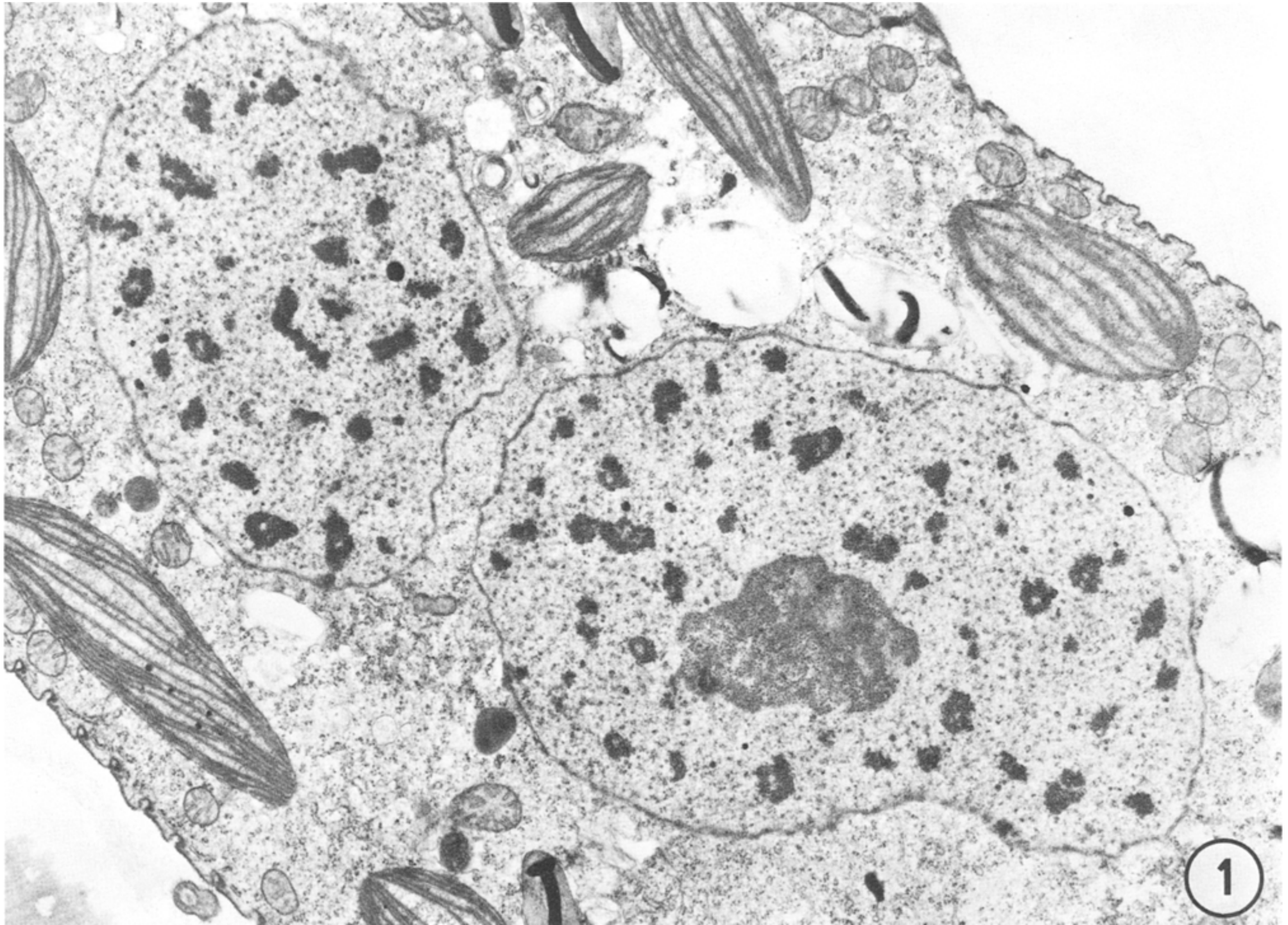


Fig. 1. Part of an *Euglena gracilis* cell treated for 24 hours with 5% of DMSO. Two normally structured nuclei are evident. × 13,500

2. Materials and Methods

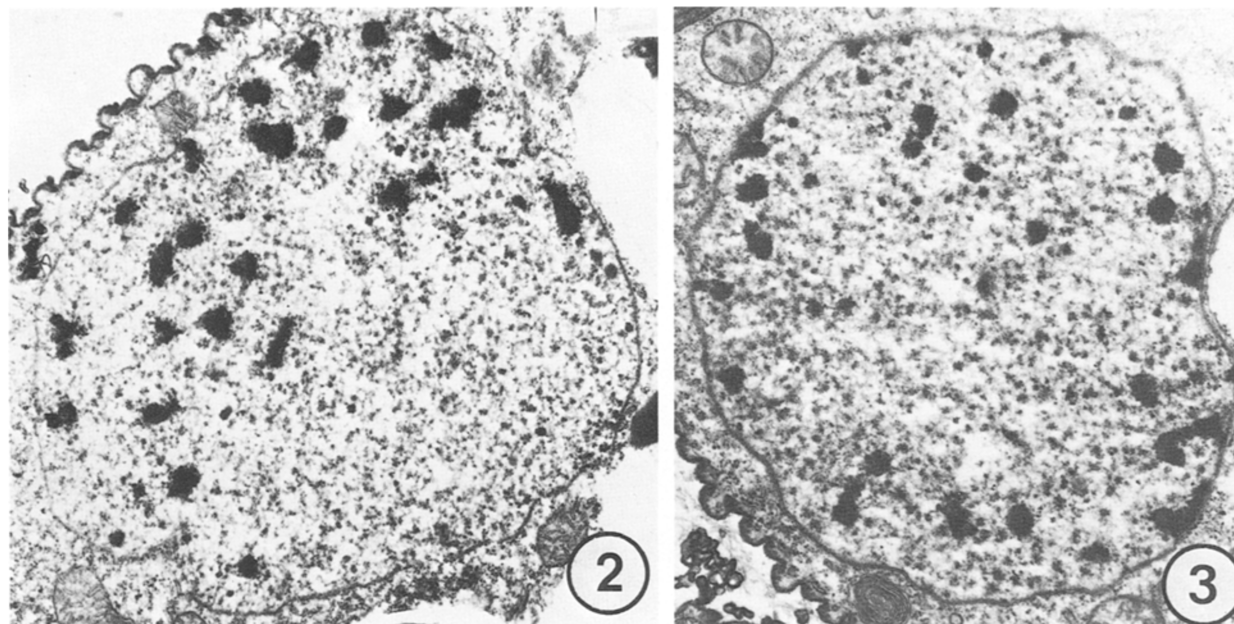
Logarithmically growing cells of *Euglena gracilis* Klebs, strain Z, cultured in organotrophic conditions at 26 °C under constant shaking and illumination (130-150 ft-c) (VANNINI 1981), were treated with 2.5, 5 and 10% DMSO. After exposure for 1, 6, 12, 24 and 48 hours, samples taken from each culture type served for direct cell counting, for conventional and phase microscope examination *in vivo* (VANNINI *et al.* 1977), as well as for routine preparation of specimens for transmission electron microscope observation (VANNINI *et al.* 1978). In the attempt to obtain a better preservation and visualization of cytoskeletal components, 1-2% tannic acid was added to the prefixative solution (BURTON *et al.* 1975, SEAGULL and HEATH 1979) in a part of the samples. Serial sections, obtained with a diamond knife, were doubly stained with uranyl acetate and lead citrate and then viewed and photographed with a Siemens Elmiskop 101 (Electron Microscopy Center of Ferrara University).

3. Results

After 1 hour in 10% DMSO, most cells of *Euglena gracilis* were rounded-up and did not display motile activities. At the ultrastructural level, about 60% of the algae presented deep, unequivocal signs of lysis. In less damaged organisms, however, the nuclei were apparently the same as in the control group and, in particular, no filament bundle was visible in their interior. Treatments of 6 hours or longer led to cell death in the entire population.

On the contrary, 2.5% of DMSO produced virtually no consequences in cell shape, motile activities and division rate. All the interphasic algae presented a single nucleus without inclusions and were normally structured except for some irregularities in the arrangement of the lamellar system in the chloroplasts (not shown).

The most interesting findings were noted from 24-48 hours after the addition of 5% of DMSO to the cell cultures. At this concentration, which completely prevented cell proliferation over the entire period of treatment (48 hours), most algae were 2-3 times larger than normal, round-shaped and with scarce cytoplasmic movements; flagellar locomotion was, how-



Figs. 2 and 3. Nuclei of cells as in Fig. 1, containing reduced chromatin clumps. ×13,000, ×13,000

ever, completely inhibited. Phase contrast microscope examination revealed that about 50% of the treated organisms were binucleate. Under the electron microscope, serially sectioned algae showed nuclei either with a normal (Fig. 1) or reduced number of chromatin masses (Figs. 2 and 3).

When mitotic nuclei were sectioned along the division axis, a normal array of spindle microtubules (MTs) was distinguishable (Figs. 4–6). In both interphasic and mitotic nuclei, actin bundles were never seen.

Another morphological alteration associated with the 5% of DMSO treatment was the reduction of the chloroplast internal structure, a phenomenon which became more and more evident as treatment duration increased (not shown).

After a 48-hours exposure, the withdrawal of DMSO led to an almost complete recovery of the characteristic shape, motile activities and cell division rate within 12 hours.

4. Discussion

This study demonstrates that, when applied at the suitable concentration of 5%, DMSO produces in *Euglena* the complete inhibition of cell proliferation and remarkable, but substantially reversible alterations.

Probably due to the existence of a mechanism operating to keep the cytoplasm/nucleus ratio constant (for this subject, see *e.g.* FUKUI 1980 and references therein), the increase of the cellular volume may be a determinant factor in inducing nuclear division in a considerable number of cells. Since cytoplasmic cleavage does not follow mitosis, the formation of binucleate cells occurs. Cytokinesis in *Euglena* requires a suitable growth and morphogenetic pattern of the pellicle, a complex structure in which the plasma membrane is in close association with a network of filaments probably of the elastic group (HOFMANN and BOUCK 1976, LEFORT-TRAN *et al.* 1980). Therefore it may be proposed that DMSO impedes the cytoplasmic split by affecting these filaments or their interaction with the plasma membrane.

The most relevant finding of this study, *i.e.* the abnormalities in the distribution of genetic materials, does not seem attributable to defects in spindle MTs because a characteristic set of these structures forms inside the nuclei undergoing mitosis. Therefore chromosome separation in *Euglena* probably requires some additional spindle components, which have not yet been identified by electron microscopy or other means; they could conceivably correspond to the actin microfilaments because they are sensitive to DMSO. On the contrary, the solvent does not affect the structures that are instrumental in nuclear constriction at the mitosis end.

Our data and conclusions are compatible with the view of FORER (1976) and FORER and JACKSON (1976)

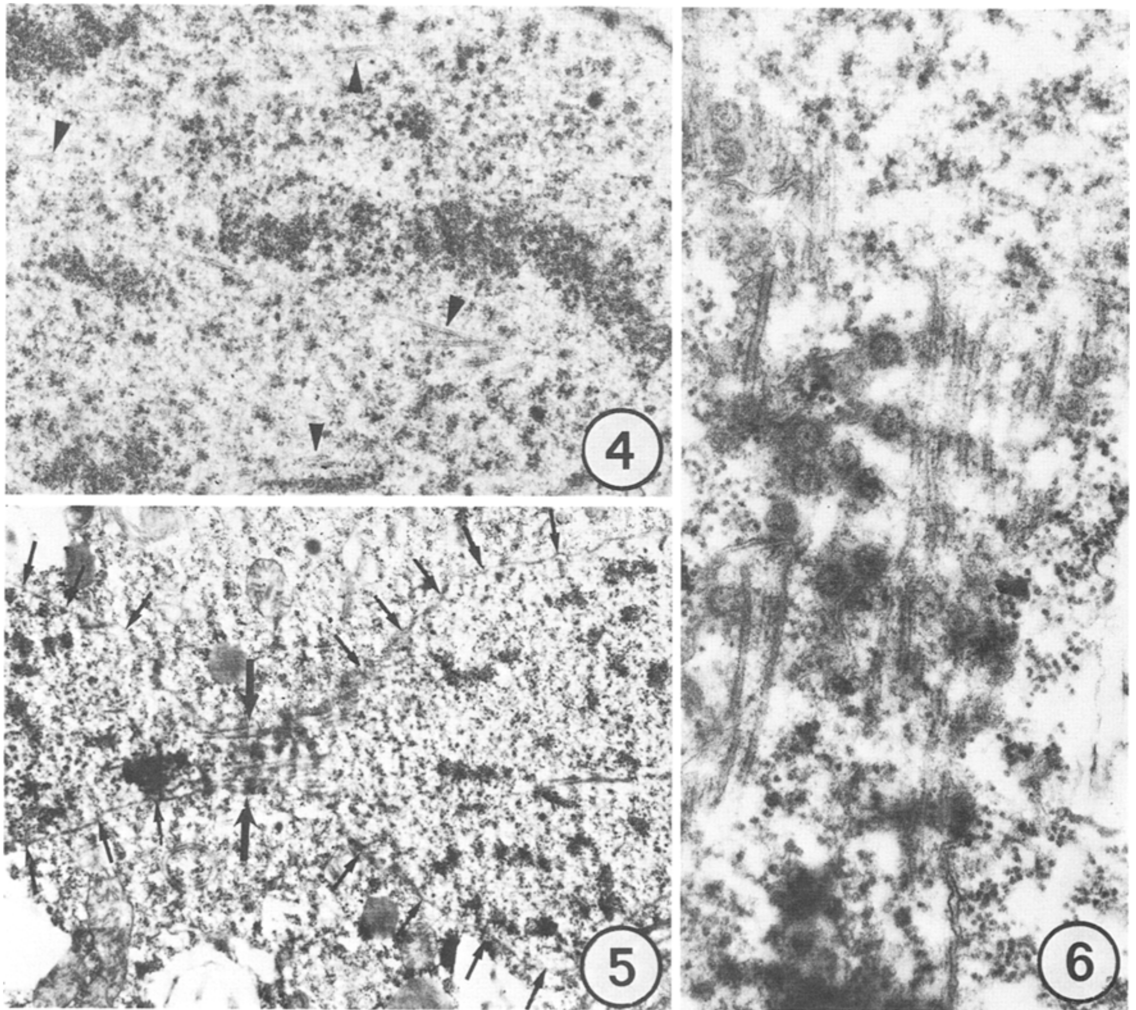


Fig. 4. Nucleus undergoing mitosis in a cell treated for 48 hours with 5% of DMSO. The arrow-heads point to four of the many visible spindle microtubules. ×25,000

Fig. 5. Low magnification of a cell as in Fig. 4. The small arrows point to the envelope of a nucleus during the final stage of mitosis. An highly constricted, equatorial region (large arrows) still joins the daughter nuclei. ×9,000

Fig. 6. At higher magnification, the equatorial region of Fig. 5 shows several spindle microtubules. The round structures are nuclear pore complexes. ×40,000

regarding the essential role of the actin filaments in the mitotic spindle. However, alternative explanations of the origin of nuclei with a reduced number of chromatin masses could be presented, since nuclear, contractile proteins sensitive to DMSO may be involved in other activities such as gene replication and control of chromatin structure (LE STOUGERON *et al.* 1975, FUKUI and KATSUMARU 1980, SANGER *et al.* 1980). Nevertheless, at the present stage of research, the interpretation in favour of Forer's hypothesis appears the most simple and probable.

Whatever the mode in which DMSO causes the nuclear abnormalities described, it is evident that all our results, taken together, do not confirm the argument that the induction of nuclear inclusion by DMSO is a general phenomenon (see OSBORN and WEBER 1980). Moreover, some consistent divergences exist with what was observed in *Dictyostelium* exposed to the same concentration of the solvent. In this cellular slime mold, in fact, several normally structured nuclei form in the same cell through a mitotic process which is unique in that the spindle MTs are not formed (FUKUI 1980). Therefore, it appears that consistent differences may occur in nuclear structure and activity in protistian cells. On the contrary, there are no significant divergences between *Euglena* and other cell types,

including *Dictyostelium*, with regard to the effects of DMSO on motile activities, cytokinesis and changes in cell shape (FUKUI 1980, SANGER *et al.* 1980).
A continuation of this study is planned using fluorescent actin antibody and heavy meromyosin.

Acknowledgements

We would like to thank Dr. A. DONINI for his skilful assistance and Dr. A. ALEOTTI for his help in the use of instruments of Electron Microscopy Center of Ferrara University.

References

BRAY, D., 1979: Cytochalasin action. Nature **282**, 671.

BRE, M. H., EL FERJANI, E., LEFORT-TRAN, M., 1981: Sequential protein-dependent steps in the cell cycle. Initiation and completion of division in vitamin B_{12} replenished *Euglena gracilis*. Protoplasma **108**, 301–318.

BROWN, S. S., SPUDICH, J. A., 1979: Cytochalasin inhibits the rate of elongation of actin filament fragments. J. Cell Biol. **83**, 657–662.

– – 1981: Mechanism of action of cytochalasin. Evidence that it binds to actin filament ends. J. Cell Biol. **88**, 487–491.

BURTON, P. R., HINKLEY, R. E., PIERSON, G. B., 1975: Tannic and stained microtubules with 12, 13 and 15 protofilaments. J. Cell Biol. **65**, 227–233.

CARTER, S. B., 1967: Effects of cytochalasin on mammalian cells. Nature **213**, 261–264.

CASELLA, J. F., FLANAGAN, M. D., LIN, S., 1981: Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during plateled shape change. Nature **293**, 302–304.

COPELAND, M., 1974: The cellular responses to cytochalasin B. A critical review. Cytologia **39**, 709–727.

FORER, A., 1976: Actin filaments and birifrangent spindle fibers during chromosome movements. In: Cell motility (GOLDMAN, R., POLLARD, T., ROSENBAUM, J., eds.), pp. 1273–1293. Cold Spring Harbor.

– JACKSON, W. T., 1976: Actin in the endosperm spindles of the higher plant *Haemanthus katherinae* Baker. Cytobiologie **12**, 199–214.

FUKUI, Y., KATSUMARU, H., 1980: Dynamics of nuclear actin bundle induction by dimethyl sulfoxide and factors affecting its development. J. Cell Biol. **84**, 131–140.

– – 1980: Formation of multinuclear cells induced by dimethyl sulfoxide: inhibition of cytokinesis and occurrence of novel nuclear division in *Dictyostelium* cells. J. Cell Biol. **86**, 181–189.

HOFMANN, C., BOUCK, B., 1976: Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism. J. Cell Biol. **69**, 693–715.

JACOB, S. W., ROSENBAUM, E. E., WOOD, D. C., 1971: Dimethyl sulfoxide. New York: Marcel Dekker Inc.

LEFORT-TRAN, M., BRE, M. H., RANCK, J. L., POUPHILE, M., 1980: *Euglena* plasma membrane during normal and vitamin B_{12} starvation growth. J. Cell Sci. **41**, 245–262.

LE STOUGERON, W. M., FORER, A., YANG, Y., BERTRAM, J. S., RUSCH, M. P., 1975: Major components of nuclear and chromosome non-histone proteins. Biochim. biophys. Acta **379**, 529–552.

MURRAY, J. M., 1981: Control of cell shape by calcium in the *Euglenophyceae*. J. Cell Sci. **49**, 99–118.

OSBORN, M., WEBER, K., 1980: Dimethylsulfoxide and the ionophore A 23187 affect the arrangement of actin and induce nuclear actin paracrystals in PtK 2 cells. Exp. Cell Res. **129**, 103–114.

SANGER, J. W., SANGER, J. M., KREIS, T. E., JOCKUSCH, B. M., 1980: Reversible translocation of cytoplasmic actin into the nucleus caused by dimethylsulfoxide. Proc. nat. Acad. Sci. U.S.A. **77**, 5268–5272.

SEAGULL, R. W., HEATH, I. B., 1979: The effects of tannic acid on the *in vivo* preservation of microfilaments. Eur. J. Cell Biol. **20**, 184–188.

SZMANT, H. H., 1975: Physical properties of dimethyl sulfoxide and its function in biological systems. Ann. N.Y. Acad. Sci. **243**, 20–23.

VANNINI, G. L., FASULO, M. P., BRUNI, A., 1977: First observation on the cytological changes induced in *Euglena gracilis* by coumarin treatment. Z. Pflanzenphysiol. **84**, 183–187.

– – – DALL'OLIO, G., 1978: Structural and developmental aspects during the greening process of *Euglena gracilis* treated with myomycin. Protoplasma **96**, 335–349.

– 1981: Effects of concanavalin A on the growth phases of *Euglena gracilis*. FEMS Microbiol. Lett. **12**, 175–177.

WEATHERBEE, J. A., 1981: Membranes and cell movement: interactions of membranes with proteins of the cytoskeleton. Int. Rev. Cytol. **12**, 113–176.

WEHLAND, J., WEBER, K., OSBORN, M., 1980: Translocation of actin from the cytoplasm into the nucleus in mammalian cells exposed to dimethylsulfoxide. Biol. Cell. **39**, 109–111.