Protoplasma (1998) 204: 119–127

PROTOPLASMA

Immediate disruption of spindle poles and induction of additional microtubule-organizing centres by a phenylcarbamate, during plant mitosis

M. I. Giménez-Abián[1], F. Panzera[2], J. F. López-Sáez[3], J. F. Giménez-Abián[1], C. De la Torre[1,*], and G. Giménez-Martín[1]

[1]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas, Madrid, [2]Genética Evolutiva, Facultad de Ciencias, Universidad de la República, Montevideo, and [3]Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid

Received July 19, 1998
Accepted September 7, 1998

Summary. The herbicide carbetamide [(R)-1-(ethylcarbamoyl) ethylphenylcarbamate], in the 0.4 to 0.8 mM range, efficiently induced multipolar mitoses in *Allium cepa* L. The frequency of multipolar anaphases rose earlier and reached higher values when both concentration and time of treatment increased, up to a maximum of 90% after 1 h of treatment. To identify the physiological target, the kinetics of induction of multipolar mitoses were followed during recovery from very short treatments (5, 10, and 15 min). Tubulin immunodetection showed that phenylcarbamate immediately disrupts the cohesion between the different bundles of microtubule minus ends which converge at the pole. The spindle was rendered multipolar about three times more efficiently in metaphase than in anaphase. The observations do not support any effect of the herbicide on the tubulin polymerization-depolymerization cycle, and suggest that the minus ends of the microtubules remained stabilized in carbetamide. Thus, the density of kinetochore microtubules and their lengths were unmodified in the individual chromosomes which became detached from both spindle poles in response to the herbicide. Extra microtubule-organizing centres for the assembly of both preprophase band and phragmoplast (the tubulin arrays which characterize the microtubular cycle responsible for cytokinesis in plant cells) were also rapidly induced.

Keywords: Phenylcarbamates; Multipolar spindles; Microtubule-organizing centres; Aneuploidy; *Allium cepa* L.

Abbreviations: MTOCs microtubule-organizing centres.

Introduction

Carbamates are herbicides which decrease the rate of growth apparently by disrupting mitosis and cytokinesis in proliferating plant tissues while they do not affect the fully grown crops which mainly comprise differentiated cells (Ennis 1948, Doxey 1949, Scott and Struckmeyer 1955, Mann and Storey 1966). Carbamates are on clinical trial as antitumoral drugs (Waud et al. 1990) as they seem selectively to disrupt proliferating cells when in mitosis. Carbetamide activates silent signals left by the preprophase band of microtubules and by phragmoplasts of previous mitoses which behave as additional microtubule-organizing centres (MTOCs) (Giménez-Abián et al. 1997, 1998). The main target of carbamates seems to be not the microtubule itself but the MTOCs in mitosis.

It is possible to induce abnormal poles either directly with carbamates (Coss and Pickett-Heaps 1974, Coss et al. 1975, Oliver et al. 1978, Giménez-Abián et al. 1997) or indirectly with c-mitotic agents, either colchicine (Hervás et al. 1974) or γ-hexachlorocyclohexane (Hervás and Giménez-Martín 1974). All these drugs give rise to the formation of either a single cell with tetraploid DNA content or to three or four cells derived from a single metaphase. Some of the aneuploid cells derived from multipolar anaphases display monosomies or even nullisomies. More often than not, these cells would be nonproliferating because they lack some of the genes required for cycle progression. In fact, Wood (1982) and Roth et al. (1982) and Roth and Lark (1984) proposed the use of carbamates to induce rapid chromosomal loss to facilitate

*Correspondence and reprints: Centro de Investigaciones Biológicas, CSIC, Velázquez 144, E-28006 Madrid, Spain.
E-mail: cibtg7a@pinar2.csic.es

somatic-cell genetics. On the other hand, if the cytokinesis which follows the multipolar telophase is prevented by caffeine, the DNA content of the autotetraploid cell is distributed in different aneuploid nuclei. This is useful for elucidating the role in *cis* of specific chromosomes in DNA cycle transitions as well as dominance and epistatic rules between alleles and non-alleles genes, respectively (Giménez-Martín et al. 1992, Panzera et al. 1997).

The objective of this work was to analyze the response kinetics to very short (5–15 min) carbetamide treatments and their short-term recovery (45–90 min) assessing possible changes in tubulin distribution in order to detect the primary physiological target(s) of carbetamide in the acentrosomal mitosis of plant cells.

Material and methods

Meristems from the roots of *Allium cepa* L. bulbs were used after the roots had reached at least 2 cm in length. Root growth was produced by placing the bulbs so that only their bases remained submerged in 90 ml of filtered tap water at a constant temperature of 25 ± 0.5 °C in darkness. Oxygen was continuously provided by bubbling air at the rate of 10 to 20 ml/min.

Treatments

Roots growing from bulbs were immersed in the different treatment solutions in filtered tap water, without altering other environmental conditions. Carbetamide or the (R)-1-(ethylcarbamoyl) ethylphenylcarbamate from a 96% carbetamide-rich powder was diluted in filtered tap water. This carbetamide source was a gift from Rhône-Poulenc Agriculture, Lyon, France. The concentrations which produced multipolarity without being highly clastogenic or cytotoxic were in the range of 0.1 to 0.8 mM. For some experiments, the 0.5 mM 5-aminouracil (Sigma, St. Louis, Mo., U.S.A.) working solution was also prepared in filtered tap water.

Cytological procedures

For cytological analysis, roots were fixed in a 3 : 1 ethanol-acetic acid mixture. Their apical meristems were stained with acetic orcein according to Tjio and Levan (1950). The meristematic region of each root was isolated (1.0 to 2.0 mm from the root apex) and then squashed.

Tubulin detection

Cells of root tips were prepared for indirect immunofluorescence localization of tubulin by a modification of the method described by Wick et al. (1981). The second apical millimeter of the root was cut off and fixed for 50 min in freshly prepared 3.7% paraformaldehyde in Pipes buffer (50 mM Pipes, 5 mM EGTA, and 5 mM $MgSO_4$, pH 7.0) at room temperature. Root segments were subsequently washed in the buffer for at least 30 min before treatment with 1% (w/v) cellulase in the following medium: 5 mM EGTA, 0.3 mM phenylmethylsulfonylfluoride, 0.4 M mannitol, and 0.1% Triton, at pH 5.6, for 10 min at 37 °C. These root segments were then washed for a further 45 min in Pipes buffer. Six or seven such meristem segments were squashed between an acetone-washed coverslip and a subbed slide.

The coverslips were removed and the cell suspensions taken from them were returned to the slide preparation. Visible cell clusters were then taken off the slides. The preparation was air-dried. Afterwards, the slides were rinsed in phosphate-buffered saline (PBS) for 10 min. These preparations were stained by indirect immunofluorescence with either mouse monoclonal anti-α- or anti-β-tubulin antibodies (Amersham International, Little Chalfont, Bucks., U.K.) diluted 1/200 in PBS for 75 min at 37 °C. Fluorescein-labelled anti-mouse Ig antibody was also from Amersham. It was diluted (1/20) in PBS and applied for 45 min at 37 °C in darkness. During the final wash, 1 µg of 4′,6-diamino-2-phenylindole (DAPI; Sigma) per ml was added for 3 min in order to stain chromatin. Slides and coverslips were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, Calif., U.S.A.). For all washes between incubations with antibodies PBS was used for at least 20 min each, at room temperature and in darkness.

Microdensitometry of microtubules

To assess the density of kinetochore microtubules in chromosomes attached and unattached to the canonical poles, the negative films of 20 control and 2-carbetamide-treated metaphases taken under the same light conditions and magnification were processed. A similar square window was overlaid on the films so that only the area of the microtubular region closest to kinetochore was left uncovered. Tubulin content was quantified by recording light transmission throughout these regions, in a Quicksem Junior scanning densitometer (TLC; Helena Laboratories, London, U.K.).

Results

Microtubular assemblies in normal plant mitosis

Under control conditions, the successive microtubular assemblies which appear throughout mitosis are as follows: the preprophase band of microtubules (Fig. 1 a), the transient prophase spindle (Fig. 1 b), and the bipolar spindle which characterizes metaphase (Fig. 1 c). Its half spindles shorten and separate towards the poles throughout anaphase (Fig. 1 d). Finally, phragmoplast microtubules appear. The cytokinetic plate forms in the phragmoplast central region. A growing phragmoplast at late telophase is seen in Fig. 1 e. Shortly before both sister cells are fully separated, the nuclear envelope again operates as an MTOC (Fig. 1 f).

Induction of multipolar mitosis by carbetamide and the formation of cells with aneuploid DNA contents

After 15 min of continuous 0.6 mM carbetamide treatment, some early anaphases in which separation of the divided centromeres was just taking place displayed chromosomes that were apparently oriented in different directions (Fig. 2 a). However, multipolar metaphases usually remained undetected when view-

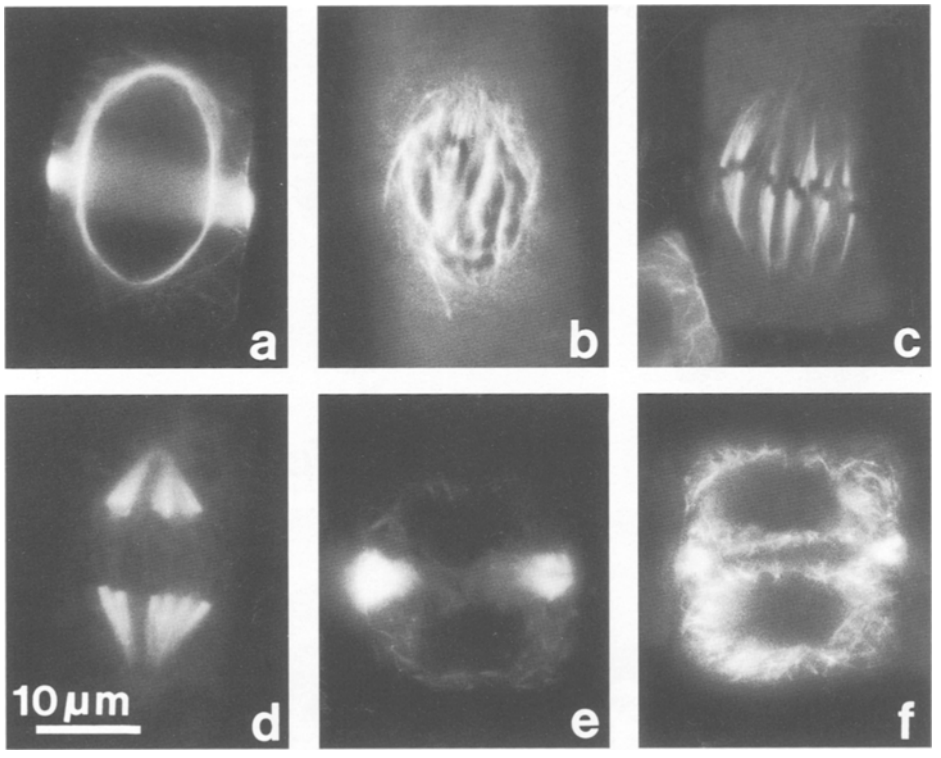

Fig. 1 a–f. The sequential microtubular assemblies which occur throughout mitosis under control conditions after tubulin immunodetection. **a** Preprophase band of microtubules. **b** Prophase spindle formed shortly before nuclear envelope breaks down at prometaphase. **c** Metaphase spindle, with its two halves keeping chromosome kinetochores between them, in the equatorial plate of the cell. **d** The anaphase spindle, both halves are shorter and more separated than during metaphase. **e** Phragmoplast in late telophase. **f** Nucleation of microtubules around the nuclear envelope of both nuclei at the late telophase/early interphase fringe. Bar: 10 µm

ing chromosomes rather than microtubules. On the other hand, multipolarity in anaphase and telophase was always evident (see a tripolar telophase in Fig. 2 b and tetrapolar one in Fig. 2 c). Finally, after completion of cytokinesis, three or four cells resulting from a single previous diploid metaphase (2n = 4C) were easily distinguished (Fig. 2 d). Some of these cells (if not all, as when four cells were formed) are obviously aneuploid, and probably unable to proliferate.

Disruption of the spindle poles in ana-telophase after short carbetamide treatments

The efficiency of short treatments (5, 10, and 15 min) was estimated by recording the frequency of multipolar ana-telophases which were formed at the end of each treatment, as well as during recovery. The maximum value recorded was larger after 15 min carbetamide treatment than after only 10 or 5 min treatment. Moreover, the frequency of multipolar ana-telophases increased in the recovery period, reaching maximum values after 20–30 min of growth in control conditions (Fig. 3).

Under continuous carbetamide treatment, at 0.6 mM concentration, a maximum of 90% of the anaphases were rendered multipolar after 1 h. The frequency of multipolar anaphases decreased after 2, 4, 8 h, and became zero at 12 and 24 h in the continuous treatment with caffeine.

Sensitivity of the mitotic spindle to carbetamide

In order to see whether there was any specific phase during which the effect of carbetamide was enhanced in mitosis, immunodetection of tubulin was carried out after 5, 10, and 15 min treatments, with 0.4 mM, 0.6 mM, and 0.8 mM carbetamide followed by up to 20 min of recovery. In this way, primary effects on mitotic phases could be detected.

When studying the frequency of multipolar metaphases and ana-telophases in relation to the total number of each of these mitotic stages after short treatments, it was found that without any recovery, after the 10 min treatments, the efficiency of carbetamide for inducing

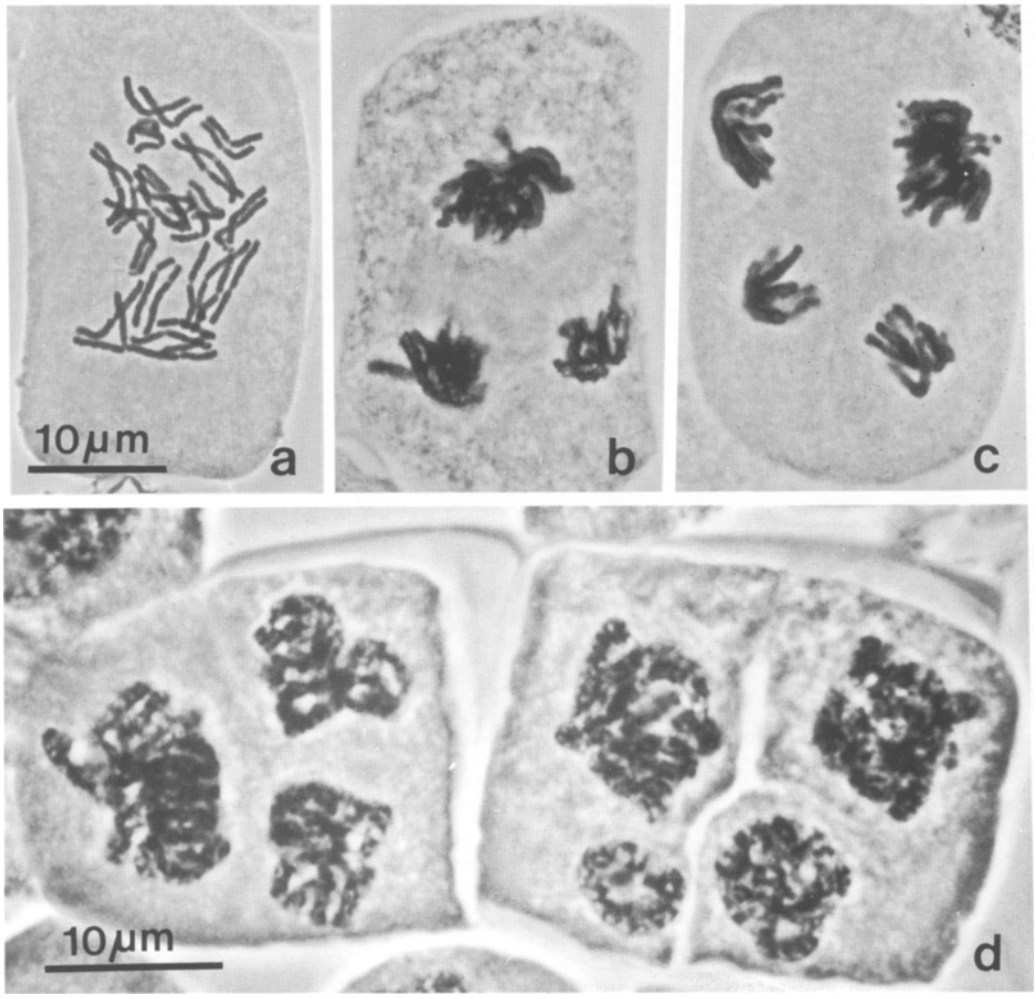

Fig. 2 a–d. Orcein-stained mitoses after 0.6 mM carbetamide (15 min treatment and 45 min recovery). **a** Early anaphase, **b** tripolar telophase, **c** tetrapolar telophase, **d** a tripolar late telophase (left) and the four sister nuclei resulting from a single metaphase (right). Bars: 10 μm

multipolarity was 2.7 times higher in metaphase (22.3%) than in anaphase (8.3%). The total time for metaphase, anaphase, and telophase under our experimental conditions is 1.5 h (data not shown).

In order to allow displacement of the chromosomes which became detached from the spindle poles, the cells were made to enlarge by pretreatment with 0.5 mM 5-aminouracil for almost a cycle time (18 h of treatment vs. 20 h). In this way, the meristematic cells became elongated, as cellular growth (measured by cell length) was maintained, while cell progression was prevented by blocking replication (Navarrete et al. 1984). During recovery from this treatment, these cells reached mitosis with double the length of the control cells. Moreover, they reached mitosis rather synchronously. 10 h after the withdrawal of 5-aminouracil, the mean mitotic index in 12 different roots from 3 different bulbs reached a value of 21.1%.

When looking at the effect produced in previously elongated cells by 5 min carbetamide treatments 10 h after recovery from 5-aminouracil, followed by a 15 min recovery period, the pattern of response was similar to that obtained in native cells of the control meristems. Thus 23.4% of the metaphases, 4.6% of the anaphases, and 3.9% of the telophases were multipolar, i.e., their chromosomes were oriented towards more than two poles (Fig. 4 a–c). In some cases, a single chromosome accompanied by its kinetochore microtubules was randomly located at the periphery of one of the spindles (Fig. 4 d, c). If there were additional spindle poles in meta- and anaphases they were not detected. Thus, no preferential clustering of minus ends of microtubules was observed in any particular spot, apart from the two main spindle poles. The multipolarity at anaphase was always obvious (Fig. 4 e, f). Unattached chromosomes behaved as if

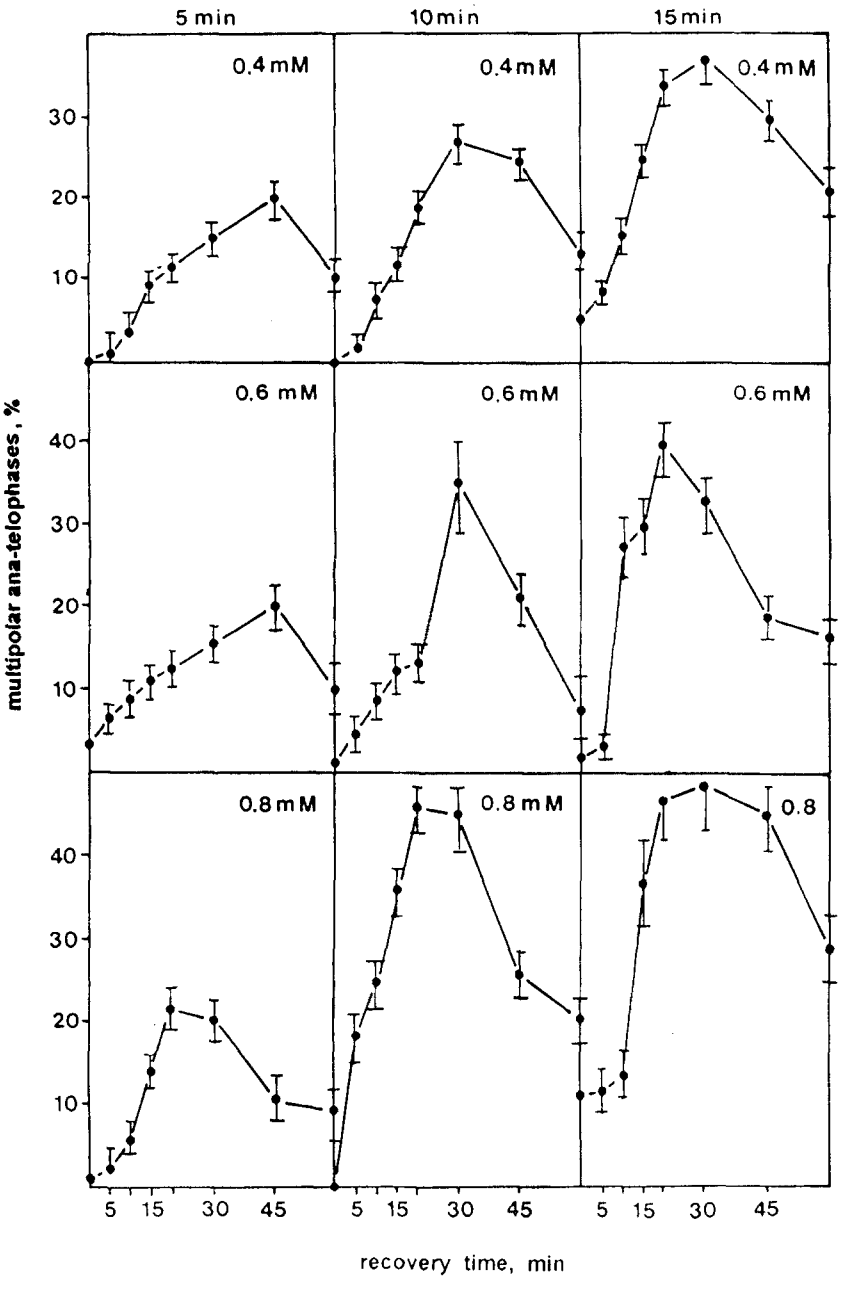

Fig. 3. Frequency of multipolar anaphases and telophases obtained during recovery from short treatments at different concentrations of carbetamide (top, 0.4 mM; center, 0.6 mM; bottom, 0.8 mM). Values were obtained after only 5 min of treatment (left), after 10 min (center), and after 15 min treatment (right). Between 3,000 and 4,000 mitoses were studied. 8–10 roots from at least 3 different bulbs were included in each individual sample. Vertical bars show 95% confidence limits

they had functional poles independent of the main ones of the bipolar spindle.

The length of the microtubules accompanying single chromosomes (n = 29) which were unattached to the regular spindle poles as well as that of kinetochore microtubules in the chromosomes of bipolar spindles (n = 80) were recorded. Microtubular lengths (7.7 ± 0.6 and 7.8 ± 0.7 μm, respectively) were not significantly different ($P < 0.001$). The density of microtubules reaching the kinetochore of chromosomes not attached to the conventional spindle poles was 1.1 of that in the normal bipolar spindles. Thus, carbetamide had no effect on the length and density of microtubules whether or not they connected the chromosomal kinetochore to the regular MTOCs. Multipolarity in anaphase was also obvious (Fig. 4 e, f).

Sensitivity of the microtubular cycle responsible for cytokinesis to carbetamide

Division of the cytoplasm is accomplished by an additional microtubular cycle. In plants, it is also based on tubulin and runs in parallel to the spindle cycle which is responsible for the segregation of chromosomes

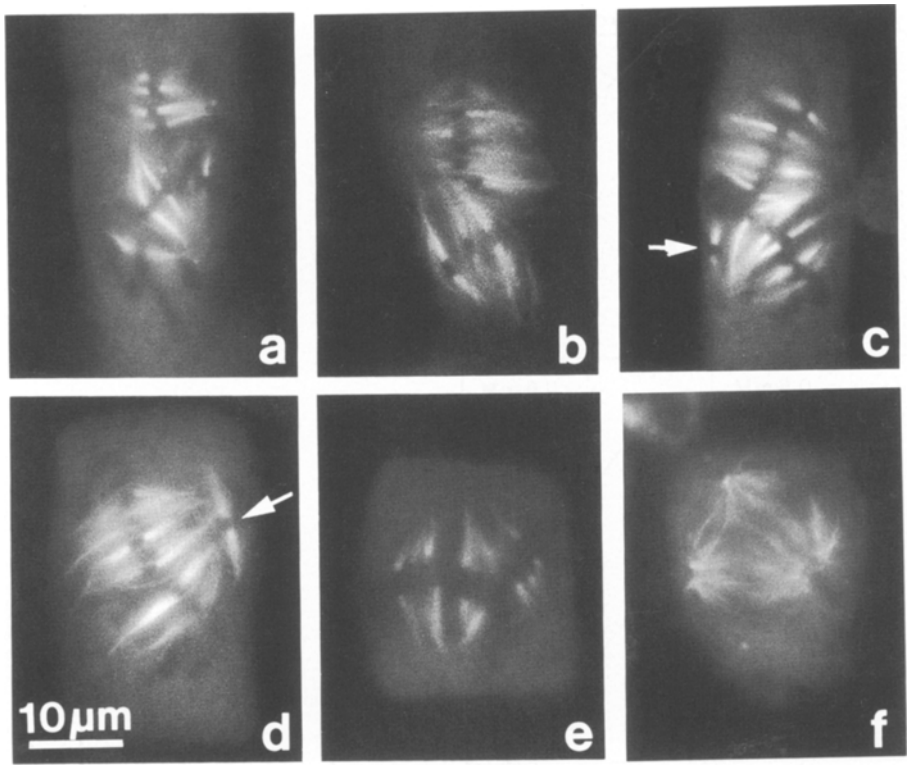

Fig. 4 a–f. Multipolar metaphases after 0.6 mM carbetamide: **a** and **c** 5 min treatment, no recovery; **b**, **d**, and **e** 10 min of treatment, no recovery. **f** Multipolar anaphase after 5 min treatment without any recovery. Arrows in **c** and **d** point to single chromosomes whose MTOCs are prominently displaced in relation to the main bipolar MTOCs. Bar: 10 μm

(Utrilla et al. 1993). The cycle which controls cytokinesis relies on two microtubular arrays: the preprophase band (present until the nuclear envelope breaks down during the prophase of these meristematic cells) and the phragmoplast (in late telophase). Carbetamide affected both arrays in a similar fashion: small extra aborted structures containing tubulin appeared in the cytoplasm alongside the conventional preprophase band and phragmoplast (Fig. 5 a, b, respectively).

In the native short mononucleate cells, a second preprophase band was observed in 11.9 ± 0.7% of the prophases. Since the formation of an extra preprophase band could mean either the presence of a second set of alternative MTOCs or splitting of the single MTOC region of the cell, recording of the frequency of such cells with double preprophase band was carried out. Carbetamide did not increase the frequency of prophase cells with double preprophase band in the recovery from a 15 min treatment with 0.6 mM carbetamide (12.5 ± 0.8) relative to the control.

Finally, the appearance of more than one phragmoplast between contiguous chromosomal groups, as in cells with T (Fig. 5 c) or X-shaped phragmoplasts (Fig. 5 d) recorded 60 min after finishing any of the short carbetamide treatments, should not be ascribed to any direct response to drug treatments. Multiple phragmoplasts appear between nonsister chromosomal groups in plants, e.g., in endosperm.

Sensitivity of interphasic and mitotic cells to carbetamide

Mitotic and phase indices were followed during the first 4 h of continuous treatment with 0.6 mM carbe-

Table 1. Labelling and mitotic indices during continuous 0.6 mM carbetamide treatment

	Time (h)				
	0	1	2	3	4
Labelling index	43.7	44.3	43.7	42.3	42.7
Mitotic index	12.3	13.3	12.3	11.7	10.1
Prophase (%)	46.9	47.6	46.3	43.2	42.5
Metaphase (%)	15.0	17.8	17.6	18.1	19.1
Anaphase[a] (%)	9.1	9.1	8.8	9.5	9.4
Telophase[a] (%)	29.0	25.5	28.0	29.2	29.0

[a]Bipolar and multipolar

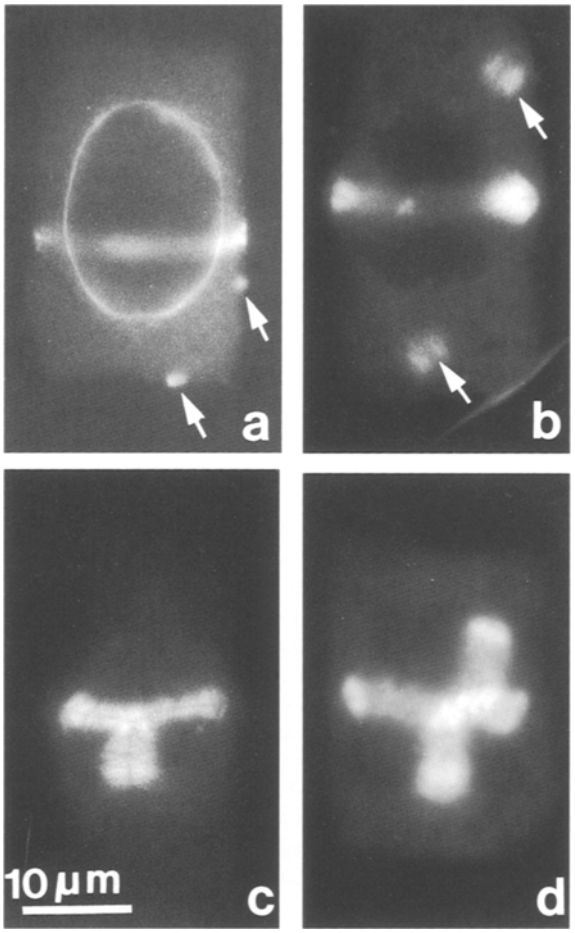

Fig. 5. a Prophase where the preprophase band is observed. The nuclear envelope maintains tubulin. Notice the two extra tubulin spots which are located in the longitudinal plasma membrane (upper arrow) and in the transversal plasma membrane (lower arrow). **b** A cell in late telophase, with its phragmoplast similarly developed to that of the control in Fig. 1 e. Two extra small phragmoplasts (arrows) which survived cytokinesis are at the upper right and bottom mid region of this cell. **c** and **d** Phragmoplasts in T (**c**) and X (**d**) shape formed after 10 min 0.6 µM carbetamide treatment followed by 45 min of recovery. Bar: 10 µm

tamide, in at least 2,000 cells per point. There was no preferential lengthening of any of the mitotic phases in the presence of this herbicide at that concentration (Table 1), apart from very slight lengthening of the relative time taken by metaphase (Table 1). The labelling index recorded after a single short treatment with ^{3}H-thymidine was also unmodified under the same set of conditions (Table 1).

Finally, the highest concentration of carbetamide tested (0.8 mM) was clastogenic, as chromosomal breaks or bridges were observed in 3% of the ana-telophases (after 30 min of recovery from a treatment lasting at least 15 min).

Discussion

When used at high concentrations in mammalian cells, carbamates interact with α- and β-tubulins, inhibiting their in vitro polymerization. However, at lower concentrations, they do not inhibit tubulin polymerization. In vivo, the paradoxical observation that low carbetamide concentrations still produce a halt in proliferation (De Inés et al. 1994) can be explained by the preferential and selective effect carbamates have on MTOCs (Oliver et al. 1978) in mitosis. The range of concentrations which induces multipolar mitosis is nearly twenty times higher in *Allium cepa* than in *Arabidopsis thaliana* (Liu et al. 1995). The reason for this is unknown but carbetamide penetration or compartmentalization differs between species.

In this work, three facts were obvious. First, that carbetamide has the potential to induce rapidly the disruption of the spindle poles in metaphase, and to a lesser extent also in anaphase. Second, that carbetamide does not modify the density of the kinetochore microtubules or their length, even when single chromosomes become detached from the main bipolar poles. Third, that carbetamide over-induces MTOCs for the preprophase band of microtubules and the phragmoplast in sites preferentially, but not necessarily, related to sites supporting microtubular arrays in previous mitoses.

The present work detects the functional acentrosomal pole of the assembled mitotic spindle as the immediate and preferential physiological target of carbetamide, while the tubulin polymerization-depolymerization cycle was apparently unaffected. Thus, the range of concentrations of carbetamide tested is low by this latter criterion.

Spindle pole assembly is a dynein-dependent mechanism common to both centrosomal and acentrosomal mitoses (Heald et al. 1997, Gaglio et al. 1997). The rapid disruption of the bipolar spindle by carbetamide closely resembles that described after depletion of the nuclear/mitotic apparatus protein (NuMA). Thus, in both situations the spindles lost their fusiform shape because the microtubules did not converge at a pole (Gaglio et al. 1995, Merdes et al. 1996).

A second independent target for carbetamide exists in these meristematic cells, as the herbicide also induced the appearance of MTOCs other than the two expected ones. This effect was also immediate and direct. It is possible that the same NuMA-dynein-dynactin complex or its carbetamide-response element may also be involved in the induction of additional

MTOCs for the assemblies responsible for cytoplasm division, i.e., the preprophase band of microtubules and the phragmoplast. The timing of the appearance of both the spindle and phragmoplast microtubular cycles seem to be coordinated by an apparently previous trigger event, common to both. Though both cycles in plant cells are based on tubulin, they can be easily uncoupled. Hence, the preprophase band of microtubules appears when the completion of DNA synthesis and/or transcription is prevented (Mineyuki et al. 1988, Utrilla and De la Torre 1991) and the phragmoplast appears when the chromosome cycle is blocked in prophase (González-Fernández et al. 1971) or even in anucleate cell fragments (Bajer and Molè-Bajer 1986). These two microtubular arrays which incorporate tagged tubulins (Vantard et al. 1990, Zhang et al. 1990) are responsible for cytokinesis and, as a consequence, for plant morphogenesis.

As established in previous works (Giménez-Abián et al. 1997, 1998), the additional MTOCs stimulated by carbetamide usually corresponded to sites that supported microtubular assemblies in previous mitoses. The present report shows that this was not always the case, as sites of the internal face of the plasma membrane, apparently unrelated by their position to old microtubular structures, were also activated.

Finally, the present work stresses that potential carbamates have to selectively exclude the progeny of the proliferating cells in a tissue, as any multipolar mitosis ends with the formation of inviable aneuploid cells.

Acknowledgements

This work was partially supported by the Dirección General de Investigación Científica y Técnica, Spain (Project PB94-0167) and by the Dirección General de Enseñanza Superior from the Spanish Ministerio de Educactión y Cultura (PB96-0909) and by the European Union (Project BIO4-CT96-0275). We are also grateful to the Instituto de Cooperación Internacional del Ministerio de Asuntos Exteriores de España for the doctoral fellowship one of us (F. P.) enjoyed. We thank Mr. J. L. Marcilla and Ms. M. Carrascosa for their excellent technical assistance. We also thank Mrs. Beryl Ligus Walker for her revision of the English.

References

Bajer AS, Molè-Bajer J (1986) Reorganization of microtubules in endosperm cells and cell fragments of the higher plant *Haemanthus* in vivo. J Cell Biol 102: 263–281

Coss RA, Pickett-Heaps JD (1974) The effect of isopropyl-N-phenylcarbamate on the green alga *Oedogonium cardiacum* I: cell division. J Cell Biol 63: 84–98

– Bloodgood RA, Brower DL, Pickett-Heaps JD, McIntosh JR (1975) Studies on the mechanism of action of isopropyl N-phenyl carbamate. Exp Cell Res 92: 394–398

De Inés C, Leynadier D, Barasoaín I, Peyrot V, García P, Briand C, Rener GA, Temple C (1994) Inhibition of microtubules and cell cycle arrest by a new 1-deaza-7,8-dihydropteridine antitumor drug, CI 980, and by its chiral isomer, NSC 613863. Cancer Res 54: 75–84

Doxey D (1949) The effect of IPC upon mitosis in rye (*Secale cereale*) and onion (*Allium cepa*). Ann Bot 13: 329–335

Ennis WB (1948) Responses of crop plants to O-isopropyl-N-phenyl carbamate. Bot Gaz 109: 473–493

Gaglio T, Saredi A, Compton DA (1995) NuMA is required for the organization of microtubules into aster-like mitotic arrays. J Cell Biol 131: 693-708

– Dionne MA, Compton DA (1997) Mitotic spindles poles are organized by structural and motor proteins in addition to centrosomes. J Cell Biol 138: 1055–1066

Giménez-Abián MI, Giménez-Martín, G, Navarrete MH, De la Torre C (1997) Microtubular structures developed in response to a carbamate herbicide in plant mitosis. Protoplasma 200: 65–70

– Utrilla L, Cánovas JL, Giménez-Martín G, Navarrete MH, De la Torre C (1998) The positional control of mitosis and cytokinesis in higher plant cells. Planta 204: 37–43

Giménez-Martín G, Panzera F, Cánovas JL, De la Torre C, López-Sáez JF (1992) A limited number of chromosomes makes a nucleus competent to respond to inducers of replication and mitosis in a plant. Eur J Cell Biol 58: 163–171

González-Fernández A, Giménez-Martín G, López-Sáez JF (1971) Cytokinesis at prophase in plant cells treated with ethidium bromide. Exp Cell Res 62: 464–467

Heald R, Tournebize R, Habermann A, Karsenti E, Hyman A (1997) Spindle assembly in *Xenopus* egg extracts: respective roles of centrosomes and microtubule self-organization. J Cell Biol 138: 615–628

Hervás JP, Giménez-Martín G (1974) Measurements of multipolar anaphases production by γ-hexachlorocyclohexane in onion root tip cells. Cytobiology 9: 233–239

– Fernández-Gómez ME, Giménez-Martín G (1974) Colchicine effect on the mitotic spindle: estimate of multipolar anaphase production. Caryologia 27: 359–368

Liu B, Joshi HC, Palevitz BA (1995) Experimental manipulation of γ-tubulin distribution in *Arabidopsis* using anti-microtubule drugs. Cell Motil Cytoskeleton 31: 113–129

Mann JD, Storey WB (1966) Rapid action of carbamate herbicides upon plant cell nuclei. Cytologia 31: 203–207

Merdes A, Ramyar K, Vechio JD, Cleveland DW (1996) A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. Cell 87: 447–458

Mineyuki Y, Wick SM, Gunning BES (1988) Preprophase bands of microtubules and the cell cycle: kinetics and experimental uncoupling of their formation from the nuclear cycle in onion root-tip cells. Planta 174: 518–526

Navarrete MH, González-Gil G, Martín-Hurtado S, López-Sáez JF (1984) High sensitivity of DNA replication to 5-aminouracil in the middle of the S period. Protoplasma 121: 138–145

Oliver JM, Krawiec JA, Berlin RD (1978) A carbamate herbicide causes microtubule and microfilament disruption and nuclear fragmentation in fibroblasts. Exp Cell Res 116: 229–237

Panzera F, Giménez-Abián MI, López-Sáez JF, Giménez-Martín G, Cuadrado A, Cánovas JL, De la Torre C (1997) Competence for nuclear replication and the NOR-chromosomes of *Allium cepa* L. Eur J Cell Biol 72: 9–12

Roth EJ, Lark KG (1984) Isopropyl-n(3-chlorophenyl) carbamate (CIPC) induced chromosomal loss in soybean: a new tool for plant somatic cell genetics. Theor Appl Genet 68: 421–431

Weber G, Lark KG (1982) Use of isopropyl-n(3-chlorophenyl) carbamate (CIPC) to produce partial haploid cells from suspension cultures of soybean (*Glycine max*). Plant Cell Rep 1: 205–208

Scott MA, Struckmeyer E (1955) Morphology and root anatomy of squash and cucumber seedlings treated with CIPC. Bot Gaz 117: 37–45

Tjio JH, Levan A (1950) The use of oxiquinoline in chromosome analysis. An Estac Exp Aula Dei 2: 21–64

Utrilla L, De la Torre C (1991) Loss of microtubular orientation and impaired development of prophase bands upon inhibition of RNA synthesis in root meristem cells. Plant Cell Rep 9: 492–495

Giménez-Abián MI, De la Torre C (1993) Timing the phases of the microtubule cycles involved in cytoplasmic and nuclear divisions in cells of undisturbed onion root meristems. Biol Cell 78: 235–241

Vantard M, Levilliers N, Hill AM, Adoutte A, Lambert AM (1990) Incorporation of *Paramecium* axonemal tubulin into higher plant cells reveals functional sites of microtubule assembly. Proc Natl Acad Sci USA 87: 8825–8829

Waud WR, Leopold WR, Elliot WL, Dykes DJ, Laster WR, Temple C, Harrison SD, Griswold DP (1990) Antitumor activity of ethyl 5-amino-1,2-dihydro-2-methyl-3phenyl-pyrido[3,4-b]pyrazin-7-ylcarbamate, 2-hydroxyethanesulfonate, hydrate (NSC 370147) against selected tumor systems in culture and in mice. Cancer Res 50: 3239–3244

Wick SM, Seagull RW, Osborn M, Weber K, Gunning BES (1981) Immunofluorescence microscopy of organized microtubule arrays in structurally stabilized meristematic plant cells. J Cell Biol 89: 685–690

Wood JS (1982) Mitotic chromosome loss induced by methyl benzimidazol-2-ylcarbamate as a rapid mapping method in *Saccharomyces cerevisiae*. Mol Cell Biol 2: 1080–1087

Zhang D, Wadsworth P, Hepler PK (1990) Microtubule dynamics in living dividing plant cells: confocal images of microinjected fluorescent brain tubulin. Proc Natl Acad Sci USA 87: 8820–8824