SHORT NOTE

Detection of the 125-kDa Nuclear Protein Mitotin in Centrosomes, the Poles of the Mitotic Spindle, and the Midbody

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Mitotin is a nuclear protein detectable in all proliferating cells investigated so far, including human and plant cells. In interphase cells the protein is localized mainly in the nucleoplasm. In G2/M phase it displays a characteristic redistribution and a marked increase which initiated the name mitotin. This study presents the precise localization of mitotin in cytoplasmic structures in two cell types, the potoroo rat kangaroo PtK2 cell and the human lung cancer EPLC 65 cell. In addition to its nuclear localization the antigen is detectable in centrosomes, in the poles of the mitotic spindle, and along spindle fibers. During the last mitotic stages, cytokinesis and reconstitution of nuclei, mitotin displays a rapid decrease and another redistribution. A significant amount of the antigen is retained in the bridge connecting the dividing cells, the midbody. © 1992 Academic Press, Inc.

INTRODUCTION

Mitotin is a 125 kDa/p1 6.5 proliferation-related nuclear protein identified by the monoclonal antibody N-9D2 [1]. Mitotin has been detected in a variety of proliferating cells [1, 2], but not in quiescent cells or in in vitro differentiated HL60 cells [3]. The protein was found to display a speckled distribution in the nucleoplasm of interphase cells and it showed a characteristic redistribution and marked quantitative increase in mitosis [4]. Recently, it was found that the increase of mitotin during mitosis is due to its phosphorylation-related stabilization just prior to mitosis, while the protein is synthesized throughout the cell cycle at a constant level [5].

In the present study we took advantage of the fine morphology of the potoroo rat-kangaroo PtK2 and the human lung cancer EPLC 65 cell lines, where we could accomplish a precise localization of mitotin during the cell cycle.

MATERIALS AND METHODS

Cells. Potoroo rat-kangaroo kidney PtK2 cells (ATCC CCL56) and human lung carcinoma EPLC 65 cells [6] were grown to about 50% confluence on coverslips in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum.

Immunofluorescent microscopy. EPLC 65 cells were fixed first with methanol for 5 min at −20°C followed by three dips in acetone at room temperature. The preparations were treated with the N-9D2 anti-mitotin monoclonal antibody [1] and FITC-conjugated rabbit anti-mouse immunoglobulins (DAKOPATS, Glostrup, Denmark). PtK2 cells were fixed with methanol for 5 min at −10°C and rehydrated at 4°C with 70, 50, and 30% ethanol in 50 mM 3-(N-morpholino)propanesulfonic acid (Mops), pH 7.2 (see also [7]). The cells were preincubated for 20 min at room temperature in 0.5% bovine serum albumin, 50 mM Mops and were covered with the antibody solution (20 µg/ml anti-mitotin antibody N-9D2) for 1 h at room temperature. After intensive washing with PBS the samples were incubated with FITC-conjugated anti-mouse IgG (Jackson Laboratories, West Grove, PA), washed with PBS and water, mounted, and examined by means of a Zeiss fluorescent microscope. For double immunofluorescence labeling a rabbit-derived anti-tubulin antibody (Polysciences Inc., Warrenton) was used. It was applied at a 1:300 dilution immediately after removing the anti-mitotin antibody. After this incubation a mixture of equal amounts of FITC-labeled anti-mouse IgG and RITC-labeled anti-rabbit IgG was applied for 1 h.

RESULTS

The immunofluorescence analysis of PtK2 cells with the anti-mitotin monoclonal antibody reveals a speckled fluorescence pattern in nuclei of all interphase cells (Fig. 1). In addition, bright single spots could be seen in the cytoplasm at different levels when focusing up and down (arrows). To identify these spots we performed a double label immunofluorescence assay with anti-tubu-
FIG. 1. Localization of mitotin and tubulin in PtK2 cells. Cells are double labeled with anti-mitotin and anti-tubulin antibodies. (A, C, E, G) Anti-mitotin immunofluorescence; (B, D, F, H) anti-tubulin immunofluorescence. Arrows indicate the positions of centrosomes (A–D) and spindle poles (E–H). Bar, 10 μm.
lin antibodies. In this assay (Figs. 1B and 1D) we localized the cytoplasmic mitotin-specific spots at the site of the major microtubule-organizing center, i.e., the centrosome [8]. In the time course of the cell cycle the intensity of the centrosomal mitotin fluorescence reaction increases markedly and in parallel with the increasing nuclear mitotin fluorescence intensity, reaching highest levels in prophase cells (Fig. 1A, left). In more advanced prophase two centrosomes could be detected (Fig. 1C).

Similar observations were made when human EPLC 65 cells were inspected using a different protocol for immunofluorescent staining (results not shown). Bright centrosomes were seen in most of the cells at different levels of focusing. The mitotin fluorescence in these spots was also generally strongest in prophase cells.

In metaphase (Fig. 1E), just after the breakdown of the nuclear envelope, the entire nuclear mitotin fluorescence became distributed uniformly in the extrachromosomal area (see also [4]). On this level, the intensity of the fluorescence signal around the condensed chromosomes was found to be much higher than in interphase cells present in the same specimen. Despite the strong and diffuse overall fluorescence, bright spots representing the poles of the mitotic spindle could be distinguished in metaphase (Figs. 1E and 1F) as well as in anaphase cells (Figs. 1G and 1H). In some cells the spin-
dle microtubules were also positively stained, which points to some mitotic accumulation along the spindle fibers.

In telophase and during cytokinesis the diffuse mito-
tin immunofluorescence decreases rapidly (see also [4]) and large fluorescent speckles are left in the cytoplasm (Fig. 2A). These spots become integrated into the re-
constituting nuclei (Fig. 2C). The brightest mitotic fluo-
rescence during cytokinesis is seen in the midbody. At the
beginning of cytokinesis the fluorescence staining is
found over the whole length of the midbody (Fig. 2A).
Later the extension of this fluorescent rod is reduced
and only a small fluorescent spot is retained in the
midbody structure at the time of reappearance of the mito-
tin fluorescence in nuclei (Fig. 2C). In rare cases we
could detect fluorescent speckles on the midbody re-
mainng between interphase cells (Fig. 2E). The mito-
tin-specific fluorescence in the midbody was generally
found in the region where the tubulin fluorescence was
faunter than in other portions of this structure (Figs. 2B,
2D, and 2F). This discontinuity of the microtubular sys-
tem was observed in all midbodies inspected (Fig. 2).

DISCUSSION

The results indicate that the proliferation-associated
nuclear protein mitotin is not exclusively localized in
the cell nucleus and that it can be found also in cyto-
plasmic structures such as centrosomes, mitotic spindle
poles, and midbodies. Due to this widespread distribu-
tion it is not possible to deduce the specific role of this protein on the basis of the presently available data.
However, it is evident that mitotin accumulates at
structures where premitotic or mitotic morphological
changes occur. For example, in early prophase, in parallel with its total increase, the protein increases
markedly in centrosomes at the time when the centrosomes
move to form the mitotic spindle poles. In metaphase, it
is accumulated (over the extrachromosomal back-
ground) in the spindle poles and during cytokinesis it is
concentrated in the midbody. During the reconstitution
of nuclei, the number of mitotin speckles increases first
in the cytoplasm and later mitotin becomes integrated into the reconstituted nuclei where it is expectedly com-
mittted in an unknown nuclear function.

Mitotin is enriched during G2/M-phase, which is a
result of a phosphorylation-related stabilization in this
stage [5]. It is unknown which protein kinase(s) trans-
forms it into the stabilized and most likely activated
state. Possible candidates are protein kinases that are
also found in centrosomes, for example the cAMP-de-
dependent protein kinase II [9], the p34/cdc2 protein
kinase [10, 11], and the protein kinase specified by the
antibody MPM-2 that is associated with nuclei, centros-
omes, kinetochores, and the midbody [12–14]. In addition,
the similar immunofluorescence patterns of mitotin
and p34/cdc2 as well as p13/suc1 (see [10, 11]) could
suggest a possible relation of mitotin with the cdc2/cy-
clin system. However, the place of mitotin in the cas-
cade of protein phosphorylations/dephosphorylations
and in the reorganizations accompanying mitosis (see
[15, 16]) remains to be elucidated. It is tempting to
assume that mitotin is activated by one of the above-men-
tioned protein kinases and that the activated mitotin
plays an important role in the progress of mitosis.

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