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ScienceDirect
European Journal of Cell Biology 86 (2007) 461–471
European Journal of Cell Biology
www.elsevier.de/ejcb

TPA-induced multinucleation of a mesenchymal stem cell-like clone is mediated primarily by karyokinesis without cytokinesis, although cell–cell fusion also occurs

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Received 5 August 2006; received in revised form 3 April 2007; accepted 13 April 2007

Abstract

The 5F9A cell, which is a mesenchymal stem cell-like clone established from rat bone marrow substrate adherent cells, can differentiate into adipocytes and osteoblasts in vitro under the appropriate conditions. Multinucleated cells could be also induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 5F9A cells. This effect was mediated by protein kinase C. Possible mechanisms of multinucleation by TPA were hypothesized to be either karyokinesis without cytokinesis or cell–cell fusion. By observation using time-lapse phase-contrast microscopy, we determined that the multinucleated cells were generated mainly by karyokinesis without cytokinesis. Cell fusion was studied using time-lapse photography, and confocal laser scanning microscopy using two differentially labeled cells. These techniques demonstrated that multinucleated 5F9A cells could be produced by cell fusion, albeit at a low frequency. We conclude that multinucleated 5F9A cells are formed primarily by karyokinesis without cytokinesis, although some cells are also formed by cell–cell fusion.

Keywords: Plasmodium; Syncytium; Mesenchymal stem cell; Cell–cell fusion; Phorbol ester; TPA; Multinucleation; Protein kinase C; Cytokinesis; Nuclear division

Introduction

Although most cells of the body are diploid, some cells (such as osteoclasts) are multinucleated and contain a greater number of chromosomes. Multiplication of the genome in a cell occurs either by endomitosis or by multinucleation (Fig. 1). An endomitotic cell has a multiple set of genomes in one nucleus. In this process, DNA duplication occurs without karyokinesis or cytokinesis (Vitrat et al., 1998). Megakaryocytes are typical endomitotic cells, and some leukemia cells endoreplicate following 12-*O*-tetradecanoylphorbol 13-acetate (TPA) stimulation (Bermejo et al., 2002; Murate et al., 1991).

Multinucleation can be divided into two categories: syncytium and plasmodium (Fig. 1, Bannister, 1995). A syncytium is formed by cell fusion (Anderson, 2000). Syncytium formation is well known among

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doi:10.1016/j.ejcb.2007.04.003

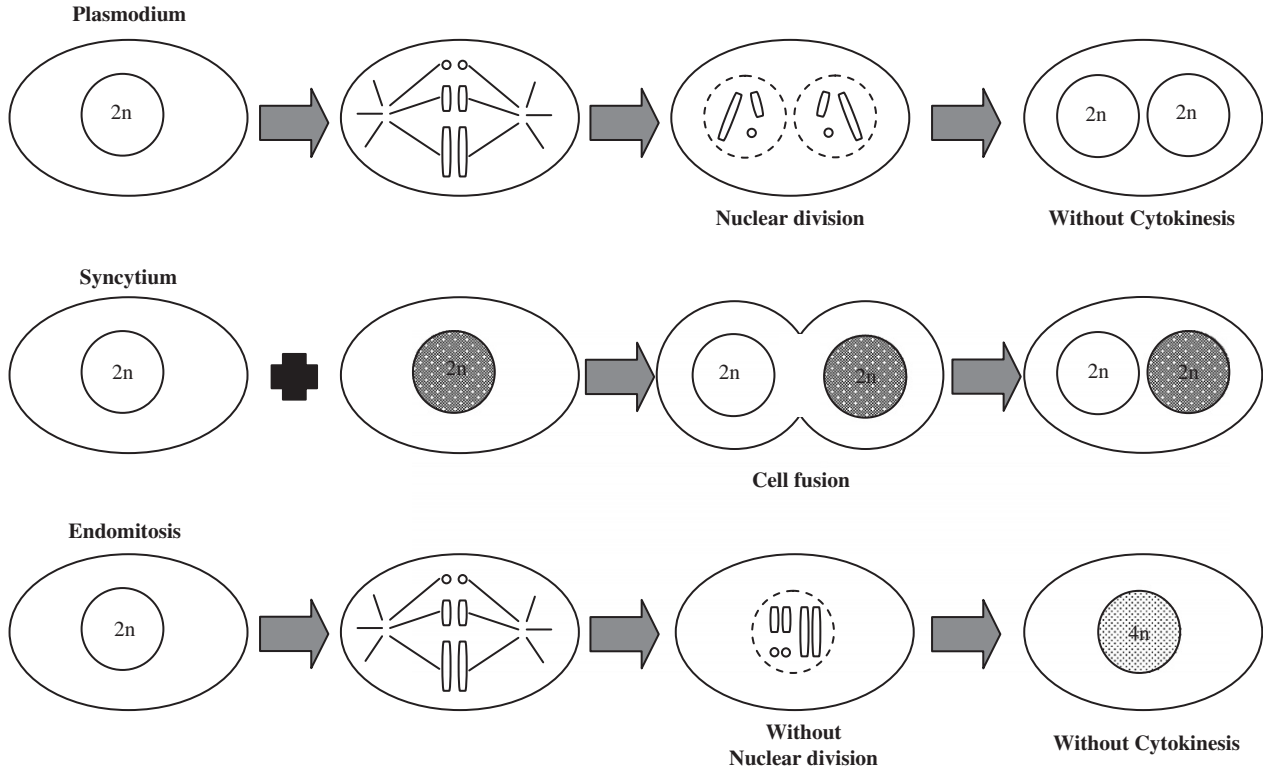

Fig. 1. Mechanisms of genome amplification.

macrophage-related cells, such as osteoclasts. Multinuclear striated skeletal muscle cells are also syncytia (Taylor, 2003). Many molecules have been suggested to induce the formation of a syncytium, although the mechanism of action of these compounds remains unknown (Ogle et al., 2005). A plasmodium is generated by karyokinesis without cytokinesis. Plasmodia are observed in some tumor cells (Menaya and Clemens, 1991) and hepatocytes (Guidotti et al., 2003).

It has been shown recently that lipid metabolism is required for cytokinesis. Thus, the inhibition of cholesterol biosynthesis leads to plasmodium formation (Fernández et al., 2004). Cytochalasin B has been reported to induce multinucleation by the disassembly of actin filaments and microtubules. This phenomenon seems to be due to the failure in the formation of the contractile ring and central spindle, both affecting each other's integrity (Cimini et al., 1998). Moreover, inhibition of the genes regulating cytokinesis results in plasmodium formation (Echard et al., 2004).

TPA can induce multiplication of the genome by a number of mechanisms that depend on the cell type. TPA has been reported to act by endomitosis (Bermejo et al., 2002; Murate et al., 1991), by plasmodium formation (Menaya and Clemens, 1991), and by syncytium formation (David et al., 1990; Hassan et al., 1989). The mechanisms underlying the variability of the action of TPA are unknown.

5F9A cells are a cell clone established in our laboratory from rat bone marrow (BM) stroma. These cells can differentiate into adipocytes and osteoblasts in vitro (Yoshida et al., submitted for publication). Thus, they exhibit oligopotentiality (Smith, 2006). This clone can also form multinucleated cells following TPA stimulation. This report discusses the results of our efforts to determine the mechanism by which TPA stimulates 5F9A cells to form multinucleated cells.

Materials and methods

Cell culture

Normal rat BM was obtained from the femur of DA/Slc rats (Japan SLC. Inc., Hamamatsu, Japan). The BM cells were cultured in minimum essential Eagle medium with alpha modification (α-MEM) (Sigma, St. Louis, MO) with 10% fetal calf serum (FCS, Lot. S04301S1820, BioWest, Miami, Fla.), 300 μg/ml L-glutamine (WAKO, Osaka, Japan), and 60 μg/ml kanamycin sulfate (WAKO) for 10 days at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The medium was changed twice a week. For cloning, the cells were removed with 0.03% trypsin (Type II, Sigma) and 2 mM ethylenediamine tetraacetic acid (EDTA) (WAKO) in phosphate-buffered saline (PBS) and subcultured in 96-well multititer plates at