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Review

Actin is required for cellular death

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ABSTRACT

Actin is one of the most abundant cytoskeletal proteins, which takes part in many cellular processes. This review provides information on the history, forms and localization of actin and its role, in particular in cellular death processes. We discuss the relationships between reorganization of actin filaments and apoptosis, mitotic catastrophe and differentiation. Finally, we discuss the translocation and accumulation of actin in the nuclear area. Moreover, owing to the difficulties of F-actin localization by transmission electron microscopy (TEM), the phalloidin-based method of its detection using streptavidin-coated quantum dots is presented in this review.

Contents

Introduction

Actin is one of the most abundant proteins in nature and one of the most conserved throughout evolution. It is present both in muscle and in non-muscle cells and comprises 1–5% of all proteins in non-muscle eukaryotic cells. Actin was observed for the first time in 1887 by William Dobinson Halliburton who extracted proteins from muscle and found a structure which he named "myosin-ferment" (Halliburton, 1887). Even though Halliburton was the first to describe actin, the discovery of actin is attributed to Brunó Ferenc Straub, a biochemist working in Albert Szent-Györgyi's laboratory. In 1942, this young scientist used a novel technique for extracting muscle proteins and isolated relatively pure actin. His method closely resembles that used in laboratories today. The term "actin" was coined by Szent-Györgyi (Perry, 2003). In the years that followed, Brunó Straub continued his study of actin. He showed that actin binds ATP, which is hydrolyzed to ADP and phosphate during polymerization of the protein into filaments in muscle cells (Bárány et al., 2001). He also reported that actin exists in two different forms (Szent-Györgyi, 2004). One is a globular, monomeric protein called G-actin, with a molecular weight of 43 kDa that consists of a single polypeptide chain of 375 amino acids (Elzinga and Phelan, 1984; Sheterline and Clayton, 1995). The three-dimensional structure of G-actin was first described by Kabsch et al. (1990). In their report, the authors showed that the actin molecule consists of two domains and an ATP-binding site, in which hydrolysis or phosphorylation occurs (Kabsch et al., 1990; Kabsch and Vandekerckhove, 1992; Fujiwara et al., 2007; Graceffa and Dominguez, 2003). The second form is a long filamentous polymer called F-actin, which is formed by G-actin polymerization. The first step of F-actin formation is nucleation, which involves the oligomerization of three G-actin molecules (Fig. 1A). Actin filaments grow by the addition of monomers to both ends, but the plus end elongates much faster (Fig. 1B). As described above, G-actin binds ATP, which is hydrolyzed to ADP following filament assembly. Although ATP is not required for polymerization, hydrolysis plays a very important role in the regulation of assembly and dynamics of actin filaments (Janmey et al., 1990). Taking into account that actin polymerization

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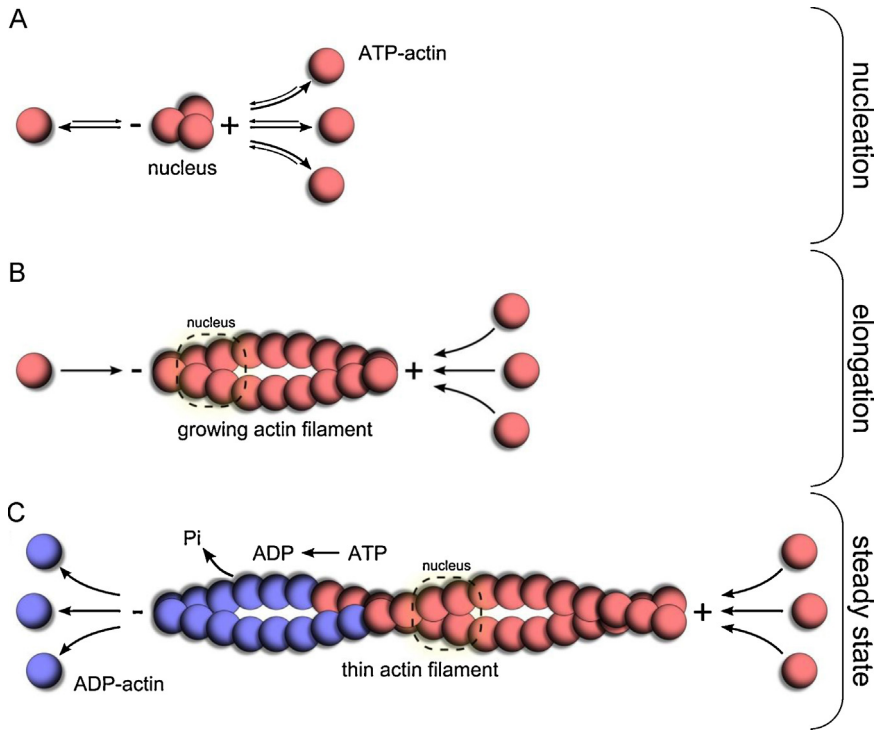

Fig. 1. (A) Actin polymerization is inhibited by unfavorable kinetics of actin oligomer formation (different thickness of arrows). (B) After actin nucleus is formed, the actin filament grows by the addition of monomers to both ends, but the plus end elongates much faster. (C) G-actin binds ATP, which is hydrolyzed to ADP following filament assembly. Moreover, actin polymerization can be reversed and filament depolymerization by the dissociation of actin subunits at the minus end.

can be reversed, filaments usually depolymerize by the dissociation of actin subunits at the minus end (Fig. 1C) (Nishida and Sakai, 1983). The actin filament (also called microfilament) is a polar structure in the form of a double helix. The microfilament measures approximately 7 nm in diameter and its helix repeats every 37 nm (Holmes et al., 1990). It has been known for many years that actin filaments (thin filaments) are a part of the contractile apparatus (sarcomere) in muscle cells. In the 1970s, it was observed that actin is present not only in muscle cells but also in non-muscle cells.

In mammalian cells, six isoforms of actin coded by separate genes are known. They are divided into three classes according to their isoelectric points. The first of these is composed of four alpha actins, which are found in muscle cells, whereas beta and gamma isoforms are characteristic of non-muscle cells (Vandekerckhove and Weber, 1978; Skalli et al., 1988; North and Laing, 2008). It is known that mutations in genes encoding actin are present in many diseases. For example, a mutation of β-actin gene is associated with autosomal dominant developmental malformations, deafness and dystonia (Procaccio et al., 2006) Studies have also shown that changes in the cytoplasmic F- and G-actin ratios reflect the risk of bladder cancer development or that increased actin content in basal cell carcinoma (BCC) may contribute to its local invasiveness, but it is lost in the metastatic phenotype (Rao et al., 1997; Uzquiano et al., 2008). Owing to its central role in the cell, actin cytoskeleton seems to be well exposed to different stimuli that promote its rearrangement and thus may be a potential target for antitumor agents. Here, we present a summary of our studies and others on an actin involvement in mechanisms of cellular death induced by drugs and other factors. In this article, we also provide a review of actin translocation and accumulation in the nucleus and its influence on cell death processes.

Nuclear actin

G-actin is scattered throughout the cytoplasm and nuclei, but localization of actin filaments depends on the cell type and their distribution. Actin filaments are the main components of the microvilli located in the brush border of intestinal epithelial cells (Bretscher and Weber, 1978; Bretscher, 1983). At the ultrastructural level, F-actin present in microvilli forms large, parallel bundles, which are arranged perpendicular to the long axis of the cell. The same organization of actin filaments is also found in stereocilia (Tilney and Tilney, 1986). Loose bundles of F-actin are seen in filopodia and lamellipodia (Defilippi et al., 1999). Grzanka and coworkers demonstrated that F-actin is present in the form of short fibers in the cortical cytoplasm in HL-60 and K562 cell lines (Grzanka et al., 2003, 2007; Izdebska et al., 2009a,b). In adherent cells (CHO AA8, A549), they observed stress fibers in the cytoplasm (Grzanka et al., 2010a,b; Szczepański et al., 2010). It is known that G- and F-actin are permanent elements of the cytoplasm of eukaryotic cells and are involved in many important cellular processes. They participate in cell division (cytokinesis), differentiation and death. Actin filaments take part in intracellular transport, secretion, cellular motility (lamellipodia, filopodia) and cancer transformation (dos Remedios et al., 2003; Winder and Ayscough, 2005; Firat-Karalar and Welch, 2011). These processes largely depend on the polymerization and depolymerization of actin filaments, and the organization of actin into functional networks is regulated by actin-binding proteins (ABPs) (dos Remedios et al., 2003; Méré et al., 2005).

For many decades, the role of the actin cytoskeleton has been intensively investigated. However, its presence in the nucleus is still controversial and not all functions and mechanisms of nuclear actin and actin-related proteins are well understood.

The presence of actin in the nucleus was first reported by Nancy Lane (Lane, 1969). Clark and Merriam (1978) observed actin in hand-isolated nuclei of *Xenopus laevis* oocytes, but these studies were treated with skepticism and the presence of actin in the nucleus has been questioned for many years (Clark and Merriam, 1978). The main problem was the failure to detect actin filaments in the nucleus by fluorescence staining or at the ultrastructural level. In addition, cytoplasmic actin is often associated with myosin,