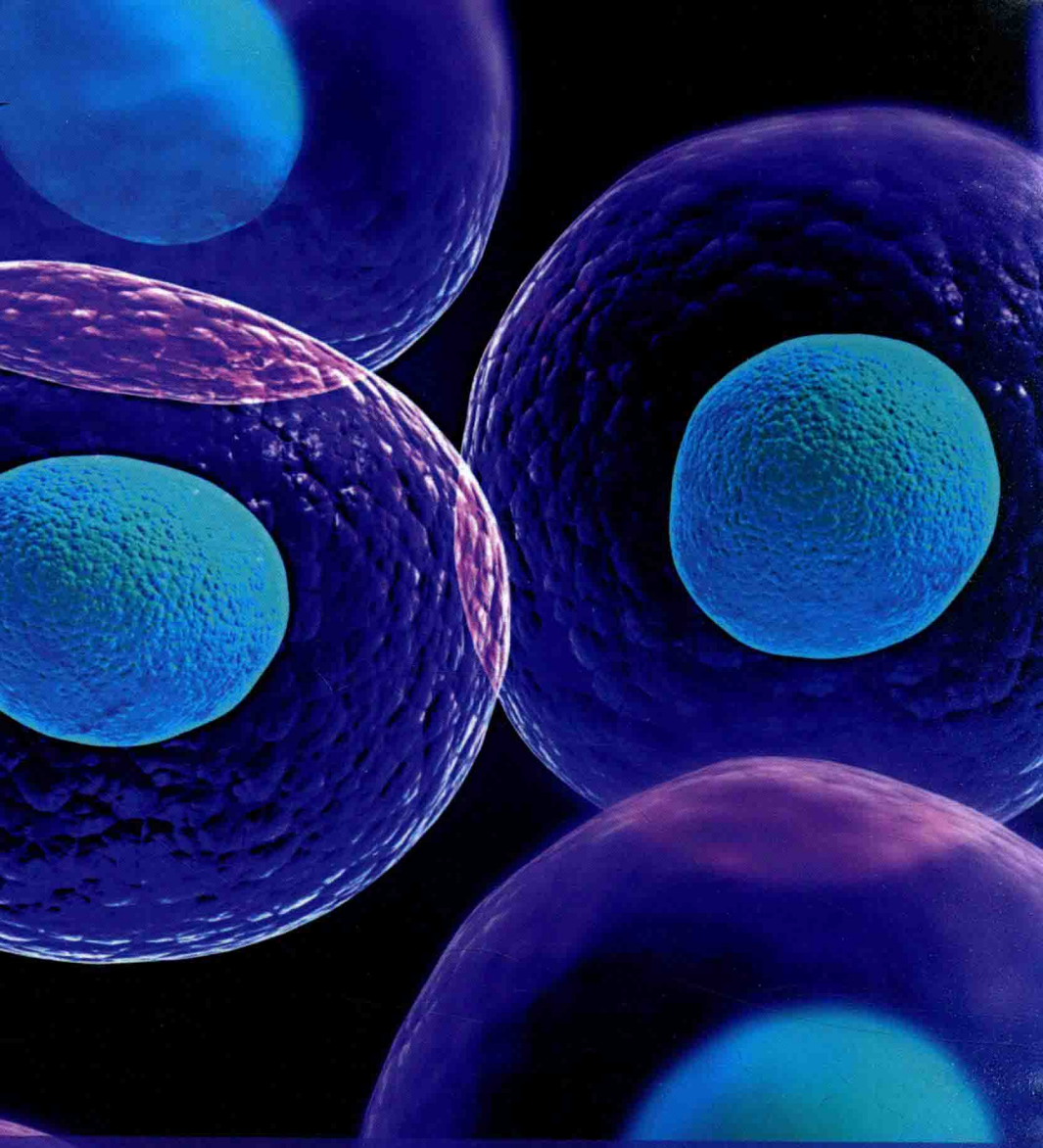

Encyclopedia of Cell Biology

Ralph Becker

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Edited by **Ralph Becker**

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Preface

This book provides an extensive analysis of the present knowledge regarding cell biology. The text consists of multiple chapters which can broadly be divided into categories such as regulatory mechanisms, cellular therapy and new methods in biology. However, due to the interdisciplinary approaches utilized by the authors this categorization is not rigid. The current frontiers explored within this book, sets the foundation for further research. Also, the views represented are visible in various areas of fundamental biology, biotechnology, biomedicine and other applications of the information regarding cell biology. This book will help beginners to gain interest and provide experts with new information in the field.

Significant researches are present in this book. Intensive efforts have been employed by authors to make this book an outstanding discourse. This book contains the enlightening chapters which have been written on the basis of significant researches done by the experts.

Finally, I would also like to thank all the members involved in this book for being a team and meeting all the deadlines for the submission of their respective works. I would also like to thank my friends and family for being supportive in my efforts.

Editor

Contents

Section 1

Molecular and Cellular Regulatory Mechanisms

1

Exploring Secrets of Nuclear Actin Involvement in the Regulation of Gene Transcription and Genome Organization

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1. Introduction

Actin is one of the most abundant proteins in eukaryotic cells. It is a 43-kDa protein that was originally identified and purified from skeletal muscle. Once thought to be simply a component of muscle cells, actin has later been shown to be a highly conserved and ubiquitiously distributed protein in eukaryotic cells. It has been extensively studied as a cytoplasmic cytoskeletal protein that is involved in a wide range of cellular processes, including cell motility, growth and cytokinesis; endocytosis, exocytosis and secretion; signal transduction, synaptic transmission as well as intracellular trafficking (Ascough, 2004;Brakebusch and Fassler, 2003;Suetsugu and Takenawa, 2003). In the cytoplasm, actin exists in equilibrium between monomers (globular- or G-actin) and polymers (filamentous- or F-actin). The dynamics of actin, the coordinated assembly and disassembly of actin filaments in response to cellular and extracellular signaling, is critical for the diverse functions of actin and is tightly regulated by a plethora of actin-binding proteins (ABPs) in the cytoplasm (dos Remedios *et al.*, 2003). To date, over 70 distinct classes of ABPs have being identified and the inventory is still far from been completed (Pollard and Borisy, 2003).

While the cytoplasmic functions of actin are well established, the findings obtained from studies on nuclear actin have encountered consistent skepticism for many years. Presence of actin in the nucleus was considered to be cytoplasmic contamination from extraction or fixation procedures, or antibody cross-reactivity (Pederson and Aebi, 2002;Shumaker *et al.*, 2003). In addition, many known functions of actin in the cytoplasm are associated with the polymerization of actin into filaments, which can be detected by phalloidin staining. However, under normal conditions, nuclei cannot be stained by phalloidin. Nevertheless, in the past decade, there has been convincing data demonstrating that actin, actin-related proteins (Arps) as well as ABPs are not only present in the nucleus but also play important roles in diverse nuclear activities. Actin has been localized to specialized subnuclear compartments such as the nucleoli, splicing speckles and Cajal bodies (Fomproix and Percipalle, 2004;Gedge *et al.*, 2005;Saitoh *et al.*, 2004). In these subnuclear compartments, actin proves to be involved in almost all the processes associated with gene expression, from chromatin remodeling via transcription to ribonucleoprotein (RNP) assembly and maturation, as well as mRNA nuclear export (Blessing *et al.*, 2004;Chen and Shen, 2007;Olave *et al.*, 2002). Other nuclear processes in which actin is implicated, include

assembly of the nuclear structure (Krauss *et al.*, 2003;Krauss *et al.*, 2002;Olave *et al.*, 2002), genome organization, and regulation of transcription factor activity (Olave *et al.*, 2002;Vartiainen *et al.*, 2007).

In this chapter, the several aspects related to the nuclear actin presence and its importance in the regulation of gene expression will be reviewed.

2. Nuclear architecture and distribution of actin

The cell nucleus is a complex and multi-functional organelle, which displays a high degree of spatial organization and structural integrity. The most well characterized structural component of the cell nucleus is the nuclear lamina, mainly composed of A- and B-type laminas as well as lamina-associated proteins (Stewart *et al.*, 2007). The laminas are evolutionarily conserved nuclear-specific intermediate filaments that are essential for many nuclear functions, including the maintenance of nuclear shape, DNA replication, transcription, chromatin organization, cell cycle regulation and apoptosis (Andres and Gonzalez, 2009;Vlcek and Foisner, 2007;Wiesel *et al.*, 2008). Actin has been shown to interact with the c-terminus of A-type laminas (Sasseville and Langelier, 1998). A-type laminas are connected to the cytoskeleton by a linker of nucleoskeleton and cytoskeleton (LINC) complex found in the nuclear envelope. Connecting the A-type laminas to the cytoskeleton is necessary for nuclear migration and positioning within the cell as well as for transmitting mechanical signals from the cytoplasm to the nucleus (Starr, 2009;Tzur *et al.*, 2006;Worman and Gundersen, 2006).

Two important components of the LINC complex are Sun domain proteins and Nesprins. Located on the outer nuclear membrane, Nesprin 1 and 2 can interact with F-actin as well as Sun 1 and Sun 2 located on the inner nuclear membrane. Sun proteins, in turn, bind to lamina A (Crisp *et al.*, 2006;Ostlund *et al.*, 2009). Emerin, a lamina-associated protein, is also important for nuclear structure and has been shown to bind to actin. The interactions between actin, lamina and emerin indicate that an actin-containing structural network exists at the nuclear envelope and is involved in maintaining the nuclear structure and nuclear functions (Fairley *et al.*, 1999;Holaska and Wilson, 2007;Lattanzi *et al.*, 2003). The importance of actin in nuclear assembly was demonstrated using *Xenopus* egg extracts in which nuclear assembly is initiated after fluorescence-labelled actin is added. Moreover, the nuclear assembly gets blocked by Latrunculin A, which binds to G-actin and inhibits F-actin formation, suggesting that F-actin is required for nuclear assembly. In addition, the interaction between actin and protein 4.1 is implicated in this process (Krauss *et al.*, 2003;Krauss *et al.*, 2002).

Actin has also been associated with the nuclear matrix (Capco *et al.*, 1982;Okorokov *et al.*, 2002;Valkov *et al.*, 1989;Verheijen *et al.*, 1986). The nuclear matrix is a network of proteins throughout the inside of nucleus, which provides a structural framework for maintaining spatial order within nucleus and for proper nuclear functions , such as DNA replication and repair, gene transcription, RNA splicing and transport (Berezney, 2002;Berezney *et al.*, 1996;Hancock, 2000). It is tempting to speculate that nuclear actin acts as a component of intranuclear filament network (or nucleoskeleton) that is analogous to cytoskeleton. This was supported by a study showing a colocalization between actin and EAST (enhances adult sensory threshold), a structural protein of the nucleus. In a Drosophila model, EAST has been shown to be a ubiquitious nuclear protein forming a network throughout the

nucleus (Wasser and Chia, 2000). A number of studies have also confirmed that an actin-containing filament network exists in the nucleus. Studies of the *Xenopus* oocyte nuclei using electron microscopy have found that filaments containing actin and protein 4.1 form a network that attach to Cajal bodies and other subnuclear organelles (Kiseleva *et al.*, 2004). In this manner, the meshwork of actin-containing filaments might contribute to the nuclear compartmentalization.

3. Regulation of nuclear actin

3.1 The form of actin in the nucleus

Actin has been shown to be involved in diverse nuclear processes; but how and in what form actin takes part in these events remains to be elucidated. It has been suggested that nuclear actin coexists as a monomer (G-actin), short oligomer and polymer structure (Gieni and Hendzel, 2009;McDonald *et al.*, 2006). These different forms of nuclear actin are believed to be required for a variety of processes in the nucleus. There has been a great body of evidence in support of the presence of G-actin in the nucleus (Pederson and Aebi, 2002;Pederson and Aebi, 2005). Firstly, a number of G-actin binding proteins have been identified in the nucleus, including cofilin, profilin, β-thymosin, gelsolin and gelsolin-like protein (Huff *et al.*, 2004;Pendleton *et al.*, 2003;Percipalle, 2009;Prendergast and Ziff, 1991;Skare *et al.*, 2003). Secondly, using DNase I affinity chromatography, actin can be copurified with RNA polymerase I and II machinery (Fomproix and Percipalle, 2004;Kukalev *et al.*, 2005;Obrdlik *et al.*, 2008). DNase I binds to G-actin with very high affinity and F-actin with low affinity (Zechel, 1980). This suggesting that actin co-precipitated with RNA polymerase I and II is likely to be present in its monomeric or short oligomeric form. Thirdly, monoclonal antibodies directed against epitopes which are unique to monomeric or dimeric actin, display distinctive immunostaining of the nucleus (Jockusch *et al.*, 2006). Fourthly, the nuclear lamina proteins, such as lamina A (Sasseville and Langelier, 1998), emerin (Lattanzi *et al.*, 2003), and nesprin (Zhang *et al.*, 2002a) form complexes with actin. Biochemical evidence reveals that G-actin is present in these complexes.

It has been very challenging to document polymerization status of actin in the nucleus. Phalloidin staining is the most common method used for detecting actin filaments in the cytoplasm. Under physiological conditions, nuclear actin present in most of the cells cannot be detected by phalloidin staining, which specifically recognizes actin filaments of at least seven subunits in length. However, under certain cellular stress conditions, distinctive actin rods (also called bundles or paracrystals) can be induced in the nucleus in a variety of cell types. These conditions include dimethyl sulfoxide (DMSO) treatment (Sanger *et al.*, 1980a;Sanger *et al.*, 1980b), heat shock (Iida *et al.*, 1986;Welch and Suhan, 1985), Latrunculin B treatment and ATP deletion (Pendleton *et al.*, 2003) as well as viral infection (Charlton and Volkman, 1991;Feierbach *et al.*, 2006). Cellular stress-induced formation of actin filaments seems to be caused by an increased nuclear actin level because nuclear translocation and accumulation of actin are also observed at the same time. This is supported by the observation that actin filaments exist in the *Xenopus* oocytes, which have a very high concentration of actin (~2mg/ml) due to the lack of nuclear export receptor, exportin 6 (Bohnsack *et al.*, 2006;Clark and Rosenbaum, 1979;Roeder and Gard, 1994;Stuven *et al.*, 2003). In addition, some nuclear-actin dependent functions, such as nuclear export of RNA and proteins (Hofmann *et al.*, 2001), nuclear envelope assembly (Krauss *et al.*, 2003), transcription (McDonald *et al.*, 2006) and intranuclear movement of Herpes simplex virus-1 capsid

(Forest *et al.*, 2005) as well as movement of chromosome loci (Hu *et al.*, 2008) can be inhibited by Latrunculin B, a drug that binds G-actin with high affinity and prevents polymerization and thus F-actin formation (Spector *et al.*, 1989). These indirect evidence imply that some sort of polymerized actin exist in the nucleus to carry out corresponding nuclear functions. The presence of polymeric actin in the nucleus was also shown (McDonald *et al.*, 2006) in living cells using fluorescence recovery after photobleaching (FRAP) experiments. In that study, FRAP, which allows to analyze the dynamic properties of GFP-actin in the nucleus, shows that both a fast recovery and a slow recovery GFP-actin exist in the nucleus. Moreover, the latter type of actin is sensitive to actin mutants and Latrunculin B. Therefore, the slow species represents a polymeric form of actin with distinctive dynamics which is quite different from the actin dynamics observed in the cytoplasm. Interestingly, recent studies provided evidence that the nuclear polymeric actin is important for RNA polymerase I-mediated transcription and transcriptional activation of HoxB genes by RNA polymerase II (Ferrai *et al.*, 2009;Ye *et al.*, 2008).

3.2 Regulation of nuclear translocation of actin

Extracellular stress can induce nuclear translocation of actin. Sanger and colleagues demonstrated that a disappearance of stress fibers from the cytoplasm and a reversible translocation of cytoplasmic actin into the nucleus occur after treatment of PtK2 and WI-38 cells with 10% DMSO (Sanger *et al.*, 1980a;Sanger *et al.*, 1980b). Courgeon and colleagues showed that heat shock causes actin to accumulate in the nucleus of *Drosophila* cells (Courgeon *et al.*, 1993). In mast cells, entry of actin into the nucleus was induced by either treatment with Latrunculin B, or ATP depletion (Pendleton *et al.*, 2003). Most recently, nuclear translocation of actin was found in HL-60 cells and human peripheral blood monocytes when differentiated to macrophages by phorbol 12 myristate 13-acetate (PMA) (Xu *et al.*, 2010). These results suggest that actin is able to shuttle between the cytoplasm and the nucleus. To date, the molecular mechanism by which actin enters into the nucleus in response to cellular stress has not been established.

The nuclear envelope is a lipid bilayer that forms a barrier between the nuclear and cytoplasmic spaces. The traffic between nucleus and cytoplasm is mediated through nuclear pore complexes (NPCs) embedded in the nuclear envelope. NPCs allow passive diffusion of small molecules (such as ion and protein smaller than 40 kDa) but restrict the movement of larger molecules across the nuclear envelope. Macromolecules usually carry specific signals allowing them to access the nucleocytoplasmic transport machinery. Monomeric actin has a molecular weight of ~43 kDa, therefore it is unlikely to enter into nucleus by diffusion. Actin lacks a classical nuclear localization signal (NLS) and to date, no specific import receptor for actin has been identified. Therefore it most likely relies on an active carrier which guides it into the nucleus. Cofilin, an actin-binding protein, is suggested to be involved in the regulation of nuclear import of actin. Cofilin contains a NLS and it has been recognized as a component of intranuclear actin rods in response heat shock and DMSO treatment (Nishida *et al.*, 1987). A study by Pendleton *et al.* showed that stress-induced nuclear accumulation of actin was blocked by an anti-cofilin antibody, demonstrating that cofilin is required for actin import into the nucleus (Pendleton *et al.*, 2003).

For nuclear export, actin seems to use an active transport mechanism. The actin polypeptide has two well conserved nuclear export signals (NESs). In yeast, these two sequences were specifically recognized by chromosome region maintenance 1 (CRM1, also known as exportin 1), a general export receptor for cargos bearing leucine-rich export signals, and

actin can then be rapidly removed from nucleus. Transfection of cells with mutant actin lacking NESs or inhibition of CRM1 by leptomycin B results in nuclear accumulation of actin (Wada *et al.*, 1998). Exportin 6, a member of the importin β superfamily of transport receptor, is responsible for nuclear actin export in mammalian cells (Stuven *et al.*, 2003). Knockdown of exportin 6 by RNA interference also leads to nuclear accumulation of actin and the formation of actin rods. Interestingly, exportin 6 recognizes the actin:profilin complex rather than actin or profilin individually, suggesting a difference in the form of actin being presented to CRM1 and to exportin 6.

So far, the exact roles of nuclear accumulation of actin in response to external signals remain to be understood. Nuclear actin controls transcription of its target genes through several different ways: (1) Actin specifically binds to a 27-nt repeat element in the intron 4 of the endothelial nitric oxide synthase gene to regulate its expression (Ou *et al.*, 2005;Wang *et al.*, 2002); (2) Actin participates in chromatin remodeling for gene activation as a component of the chromatin remodeling complex (Rando *et al.*, 2002;Song *et al.*, 2007;Zhao *et al.*, 1998); (3) Actin plays a direct role in RNA transcription by being part of the pre-initiation complex with RNA polymerase II (Hofmann *et al.*, 2004). (4) Actin participates in transcriptional elongation as a component of RNP particles. Therefore, it is tempting to speculate that under stress, actin translocates into nuclei to function as a transcriptional modulator, playing an important role in the regulation of gene transcription along with stress-activated transcription factor. This hypothesis is supported by recent studies showing that nuclear accumulation of actin is involved in transcriptional activation of SLC11A1 gene during macrophage-like differentiation of HL-60 cells induced by PMA (Xu *et al.*, 2011;Xu *et al.*, 2010).

3.3 Regulation of actin polymerization

It is believed that the concentration of nuclear actin is sufficient for spontaneous polymerization. Therefore, in order to have dynamic equilibrium of the different forms of actin, an active process preventing polymerization is required.

Many of the regulators known to control cytoplasmic actin dynamics have also been shown to be present in the nucleus (Table 1). These regulators include Arps such as Arp 2/3; and ABPs such as cofilin, profilin and CapG; and signalling molecules (see section 3.4). In humans, Arp2/3 represents a stable complex of two Arps (Arp2 and Arp3) and five other subunits including p16, p20, p21, p34, p41 (Deeks and Hussey, 2005;Welch *et al.*, 1997). The Arp2/3 complex is capable of initiating *de novo* polymerization of actin and stimulating the formation of branched actin filaments when activated by members of Wiskott-Aldrich syndrome protein (WASP) family (Higgs and Pollard, 2001;Machesky and Insall, 1998;Pollard and Borisy, 2003;Volkmann *et al.*, 2001). The WASP family members share a common C-terminal verprolin-cofilin-acidic (VCA) region. Polymerization of actin is initiated by the interaction of the VCA region with both Arp2/3 complex and an actin monomer, forming the first subunit of *de novo* actin polymer (Dayel and Mullins, 2004;Kim *et al.*, 2000;Prehoda *et al.*, 2000;Rohatgi *et al.*, 1999). The potential role of Arp2/3 in the regulation of actin dynamics in the nucleus was suggested based on the viral infection studies, for example infection with baculovirus, results in accumulation of Arp2/3 complex in the nucleus, where it becomes activated by WASP-like virus protein p78/83. This event in turn results in Arp2/3-mediated actin polymerization that is essential for virus replication (Goley *et al.*, 2006). Furthermore, it has been demonstrated that N-WASP and Arp2/3 complex associate with RNA polymerase II and regulate the efficiency of gene transcription.

Induction of actin polymerization through the N-WASP-Arp2/3 complex pathway has been shown to be required for efficient transcription by RNA polymerase II (Wu *et al.*, 2006;Yoo *et al.*, 2007). Importance of the Arp2/3 complex -mediated actin polymerization in other nuclear actin-dependent processes remains to be fully elucidated.

Protein	Roles in the nucleus	References
Arp 2/3	De novo actin polymerization	Higgs and Pollard, 2001
	Formation of Branched actin filaments	Pollard et al., 2003
	Associated with transcription by pol II	Wu et al., 2006; Yoo et al., 2007
N-WASP	Activating ARP2/3-mediated actin polymerization	Higgs and Pollard, 2001; Volkmann et al. 2001
	Regulating transcription by pol II	Wu et al., 2006; Yoo et al., 2007
Gelsolin	Serving actin polymers	Ocampo et al., 2005
	Androgen receptor co-activator	Nishimura et al., 2003
Flightless I	Chromosome remodelling	Archer et al., 2005
Supervillin	Nuclear receptor-induced transcription	Ting et al., 2002
Filamin	Androgen receptor action	Ozanne et al., 2000
CapG	Unknown	De Corte et al., 2004
Profilin	Nuclear export of actin mediated by exportin 6	Stuven et al., 2003
	Possible involvement in pre-mRNA splicing	Skare et al., 2003
Thymosin β4	Sequestering actin and blocking actin polymerization	Hannappel et al., 2007; Huff et al., 2004
Cofilin	Nuclear import of actin	Pendleton et al., 2003
	Repressor of the glucocorticoid receptor	Ruegg et al., 2004
	A component of nuclear actin-rods	Nishida et al., 1987
Emerin	Nuclear architecture	Holaska et al., 2004
Myo1c/NM1	Transcription	Hofmann et al., 2006 ; Ye et al., 2008
	Chromatin remodeling	Percipalle et al., 2006
Tropomodulin	Unknown	Kong and Kedes, 2004
Protein 4.1	Nuclear assembly	Krauss et al., 2003
Actinin	Nuclear receptor activator (actinin alpha 4)	Khurana et al., 2011
	Regulation of DNase Y activity (actinin alpha 4)	Liu et al., 2004
Spectrin II α	Involved in DNA repair	Sridharan et al., 2003
Paxillin	Stimulating DNA synthesis and	Dong et al., 2009
	Promoting cell proliferation	
CAP2	Unkown	Peche et al., 2007
CABP14	Possible role in cell division	Aroian et al., 1997

Table 1. Proteins known to modulate cytoplasmic actin dynamics exist in nucleus

Actin filaments capping proteins bind the barbed (or fast growing end) of an actin filament and therefore block filament assembly or promote disassembly at that end. In the cytoplasm, members of the gelsolin family are characterized by the ability to cap, sever and bundle actin filaments in a Ca^{2+}-dependent manner in the cytoplasm (Archer *et al.*, 2005). Several members of gelsolin family has been detected in nucleus, including gelsolin (Nishimura *et al.*, 2003;Salazar *et al.*, 1999), CapG (De, V *et al.*, 2004;Onoda *et al.*, 1993), flightless (Lee *et al.*, 2004) and supervillin (Wulfkuhle *et al.*, 1999). In the nucleus, gelsolin has been found to be involved in chromosome decondensation by severing actin (Ocampo *et al.*, 2005). Flightless I has been found to bind to actin and Arp BAF53, a subunit of mammalian chromatin remodelling complex, and negatively regulates actin polymerization (Archer *et al.*, 2005). It is currently unclear whether other members regulate actin dynamics in the nucleus. Interestingly, many of them appear to function as transcriptional coactivators for nuclear hormone receptors (Gettemans *et al.*, 2005).

Many G-actin binding proteins are also present in the nucleus. Thymosin β4 is the most abundant polypeptide of the β-thymosin family in the cytoplasm and regulates F-actin polymerization by sequestering polymers (Huff *et al.*, 2004). In the nucleoplasm, thymosin β4 is present at a high level and suggested to sequester nuclear actin and block actin polymerization (Hannappel, 2007;Huff *et al.*, 2004). In addition, it has been shown to interact with ATP-dependent DNA helicase II to regulate specific gene expression (Bednarek *et al.*, 2008). Despite its small size (~4.9 kDa), Huff et al. showed that passive diffusion of thymosin β4 through the NPC can be ruled out (Huff *et al.*, 2004), and its nuclear localization has been reported to be regulated by the DNA mismatch repair enzyme human mutL homolog 1 (hMLH1) (Brieger *et al.*, 2007). Profilin is a small protein that binds specifically with G-actin. It enhances the nucleotide exchange on actin to convert ADP actin into ATP actin, which can readily be incorporated in to a growing filament. In the nucleus, formation of profilin-actin complex is required for nuclear export of actin through exportin 6 (Stuven *et al.*, 2003), to avoid excess actin polymerization in the nucleus. This was supported by Bohnsack and colleagues' work (Bohnsack *et al.*, 2006).

ADP/cofilins represent a family of small actin-regulatory proteins that bind to both actin monomers and filaments, and remove actin filaments by severing and depolymerising (Maciver and Hussey, 2002). Using fluorescence resonance energy transfer assay, they have been shown to bind to actin directly in the nucleus and at levels much higher than in the cytoplasm (Chhabra and dos Remedios, 2005). As mentioned in section 3.2, actin accumulates in the nucleus and forms intranuclear actin rods under a variety of cellular stress conditions. Cofilin has been recognized as a component of the actin rods (Gettemans *et al.*, 2005). The high level of cofilin present in the nucleoplasm and in the actin rods might explain the reason why actin filaments appear to be restricted in the nucleus since the cofilin/actin structures cannot be stained with phalloidin (Nishida *et al.*, 1987). The formation of nuclear actin rods is highly dynamic and is reversible when the cellular stress conditions are removed (Gieni and Hendzel, 2009), suggesting that cofilin might play a role in restricting the excess accumulation of polymeric actin, which otherwise could affect the polymeric actin-mediated nuclear process.

3.4 Signalling molecules regulating actin dynamics

The activities of ABPs are tightly controlled through various signalling pathways to ensure proper spatial and temporal regulation of actin dynamics in the cells. Several signalling molecules, including small GTPases, Ca^{2+} and phosphoinositides which display well-characterized effects on actin dynamics in the cytoplasm, are also found in the nucleus.

Small GTPases of the Rho family, such as Cdc42 and Rac1, have been found in the nucleus, (Williams, 2003). As discussed above, Arp2/3 is an important candidate for regulating nuclear actin polymerization and N-WASP, the most potent inducer of Arp2/3 -mediated actin nucleation remains to be the only member of the WASP family found in the nucleus (Suetsugu *et al.*, 2001;Zalevsky *et al.*, 2001). In the cytoplasm, N-WASP is activated by Cdc42, linking Rho family GTPase signalling with Arp2/3 -mediated actin polymerization (Rohatgi *et al.*, 1999). N-WASP is also activated by Rac1, and both Cdc42- and Rac1-mediated stimulation of N-WASP activity is further enhanced by the presence of phosphatidylinositol 4,5-bisphosphate (PIP2). The functional significance of the presence of Rho GTPases in the nucleus is not fully known. Some downstream effectors of Rho family GTPase, such as LIM

kinases (LIMK), has been shown to localize to the nucleus. LIMK can phosphorylate and inactivate cofilin, suggesting that Rho GTPase signalling pathway may play an important role in regulation of nuclear actin cytoskeleton. Rac1 was shown to shuttle in and out of the nucleus during the cell cycle and to accumulate in the nucleus in late G2 phase. In addition, GTP-bound Rac1 and a Rac1/Cdc42 GTPase activating- protein, MgcRacGAP, bind directly to phosphorylated transcription factors, STAT3 and STAT5, to mediate their translocation into the nucleus. Therefore, nuclear accumulation of Rac1 may also regulate actin polymerization influencing RNA polymerase II-mediated transcription.

Phosphoinositides (PIs) are major regulators of actin dynamics in the cytoplasm (Mao and Yin, 2007). PIs control actin polymerization by modulating the activity of regulatory proteins promoting actin assembly and inhibiting disassembly of actin filaments. For example, PIP2 activates nucleation of actin filaments induced by N-WASP-Arp2/3 complex and inhibits the actin-binding activity of cofilin (Hilpela *et al.*, 2004). PIP2 also binds and inhibits capping proteins, and seems to remove capping proteins from capped ends of actin filaments, which may help to stimulate actin assembly (Kim *et al.*, 2007). Based on the observations, one can speculate that PIs also modulate actin-binding activity of capping proteins in the nucleus. So far, the downstream targets of PI signalling remains poorly identified. Several studies have linked chromatin remodelling complexes with PIs. For example, PIP2 participates in the recruitment of mammalian chromatin remodelling complex, BRG1/BRM associated factor (BAF), to nuclear matrix-associated chromatin, upon activation of antigen receptor in T-lymphocytes (Zhao *et al.*, 1998). Further analysis has revealed that PIP2 can bind directly to BRG1, an ATPase subunit of the BAF complex, modulate the actin-binding activity of BRG1 (Rando *et al.*, 2002). Within the BAF complex, BRG1 is associated with β-actin and Arp BAF53 through two actin-binding domains. Interestingly, one of the acting-binding domains of BRG1 is required for PIP2 binding. Based on these findings, a model is designed in which interaction between PIP2 and BRG1 would essentially uncap β-actin or BAF53, thereby allowing them to interact with actin filaments in the nuclear matrix (Rando *et al.*, 2002).

In the cytoplasm, actin dynamics is also controlled by Ca^{2+} level. The activity of several ABPs, including members of gelsolin family, are regulated by Ca^{2+} influx (Archer *et al.*, 2005). For example, Ca^{2+} activates gelsolin to allow capping and severing of actin filaments. The importance of Ca^{2+}-regulated actin severing has been well-documented in platelet activation (Witke *et al.*, 1995). Gelsolin has six Ca^{2+} binding sites within domain S1-S6. When domains S5 and S6 are occupied by Ca^{2+} at submicromolar concentration gelsolin is activated to bind actin. However, for full activation of severing activity, higher Ca^{2+} concentrations are required most likely filling the sites on domains S1, S2 and S4 (Burtnick *et al.*, 2004;Choe *et al.*, 2002). It is clear that nuclear Ca^{2+} level is regulated which in turn regulates the activity of transcription factors, such as DREAM (Carrion *et al.*, 1999) and CREB (Chawla *et al.*, 1998). Likewise, it is possible that nuclear Ca^{2+} level could modulate the activity of actin-containing chromatin remodelling complex by controlling activity of certain nuclear ABPs.

4. Involvement of actin in chromatin remodelling

Eukaryotic DNA is tightly packaged into nucleosome repeats. Each nucleosome unit consist of a histone octamer core surrounded by a segment of 146 base pairs of double stranded