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REVIEW

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

Nuclear myosins – roles for molecular transporters and anchors

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ABSTRACT

The myosin family of molecular motors are well-characterised cytoskeletal proteins. However, myosins are also present in the nucleus, where they have been shown to have roles in transcription, DNA repair and viral infections. Despite their involvement in these fundamental cellular processes, our understanding of these functions and their regulation remains limited. Recently, research on nuclear myosins has been gathering pace, and this Review will evaluate the current state of the field. Here, we will focus on the variation in structure of nuclear myosins, their nuclear import and their roles within transcription, DNA damage, chromatin organisation and viral infections. We will also consider both the biochemical and biophysical properties and restraints that are placed on these multifunctional motors, and how they link to their cytoplasmic counterparts. By highlighting these properties and processes, we show just how integral nuclear myosins are for cellular survival.

KEY WORDS: Actin, DNA damage, Myosin, Transcription, Virus

Introduction

Myosins are actin-based molecular motors that require ATPase activity to cycle through actin attachment and detachment. Myosins can be divided into two subgroups, the conventional myosins, which comprises the class II muscle myosin group, and unconventional myosins (Sellers, 2000). The latter group makes up two-thirds of human myosin genes (Berg et al., 2001; Hartman et al., 2011). Unconventional myosins have been comprehensively characterised, with a plethora of roles being identified within the cell (Hartman and Spudich, 2012; Fili and Toseland, 2019). These roles include, among others, cargo trafficking (Li et al., 2016; Brawley and Rock, 2009), regulating membrane tension (Nambiar et al., 2009) and tethering of organelles to cortical filamentous actin (F-actin) (Wu et al., 1998). It is now known that one cell type can contain multiple non-muscle myosins (Hartman and Spudich, 2012). With this large array of myosins, it is not surprising that there are myosins found within the nucleus and that they can display novel functions; these myosins are nuclear myosin I, non-muscle myosin II, myosin Va and b, myosin VI (MVI), myosin X, myosin XVI and finally myosin MVIII (see Table 1 for a summary of the nomenclature) (de Lanerolle, 2012).

Over the past two decades, research into myosin functions has identified specific nuclear roles for some of these non-muscle myosins. The first of these nuclear myosins discovered was a myosin IC (MYO1C) isoform (Nowak et al., 1997), now commonly referred to as nuclear myosin I (NMI) (Pestic-Dragovich et al., 2000). This nuclear myosin has a significant role in RNA polymerase I (RNAPI) (Fomproix and Percipalle, 2004) and II (RNAPII) transcription (Pestic-Dragovich et al., 2000), which has been previously reviewed (de Lanerolle, 2012).

The number of known nuclear myosins has remained unchanged since previous reviews (de Lanerolle, 2012; de Lanerolle and Serebryannyy, 2011) with little being added to the roles of myosin II, which has been shown to be involved in differentiation of embryonic myoblasts (Rodgers, 2005) and in the preinitiation complex in RNAPII transcription (Li and Sarna, 2009), myosin X, which has been shown to aid in mitotic spindle formation (Woolner et al., 2008), myosin XVI, which is involved in cell cycle and proliferation (Cameron et al., 2013), and myosin XVIII, which is involved in myofibrillar development (Salamon et al., 2003).

Here, we will focus on myosins I, V and VI, which have been at the centre of the nuclear myosin field (shown in Fig. 1). These myosins are involved in the following processes, which will form the basis of this review – chromosome rearrangements (Chuang et al., 2006), DNA damage repair (Kulashreshtha et al., 2016), transcription (Zorca et al., 2015) and viral infections (Wilkie et al., 2018) (illustrated in Fig. 2). We will also highlight the recent work that has been carried out with regard to the regulation and activation (Lu et al., 2015), import and export control (Majewski et al., 2018), and characterisation of myosin-binding partners (Percipalle et al., 2006; Fili et al., 2017). Research on all these aspects of the biology of nuclear myosins is crucial in order to acquire a full understanding of the roles of myosins within the nucleus. To this end, we need to understand not only the implication of depleting a myosin from a nuclear process, but also the relevance of its specific biochemical and biophysical properties in this procedure, which will aid in defining its underlying mechanism of function. Specifically, this Review will begin to query what properties of myosins are required within the nucleus and how are these properties applied?

A diversity of properties leads to a diversity of roles

Before turning our attention to specific processes, we will first begin by focusing on myosin itself. The myosin structure consists of a motor, a neck region and a tail domain, all of which contribute to the specific function of a myosin (Fig. 1B). The motor domain is composed of the ATPase and actin-binding sites (Hartman et al., 2011), and the neck region can contain a single or multiple IQ motifs, defined by [I,L,V]QxxxRGxxx[R,K] where x is any amino acid (Shen et al., 2016; Li et al., 2017; Bahloul et al., 2004), which are responsible for binding to myosin light chains (Caride et al., 2010). These light chains, with the most common being the Ca^{2+}-binding calmodulin (herein referring to CALM1), can regulate the activity of the motor through modulating actin and ATP binding (Homma et al., 2000). They also provide mechanical support to the neck domain, which enables large step-sizes along actin filaments (Rock et al., 2005). Finally, there is a variable C-terminal tail domain enabling cargo and additional protein binding sites. In the case of nuclear myosins, this tail domain allows MVI and NMI to bind to DNA (Almuzzaini et al., 2015; Fili et al., 2017).

The myosins within the nucleus, as for those in the cytoplasm, are in principle capable of a variety of functions owing to their

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Table 1. Summary of nuclear myosins

Name	Gene	Isoform
Nuclear myosin I	*MYO1C*	B
Myosin I	*MYO1C*	A and C
Non-muscle myosin IIA	*MYH9*	1
Non-muscle myosin IIB	*MYH10*	1
Myosin Va	*MYO5A*	1
Myosin Vb	*MYO5B*	1
Myosin VI	*MYO6*	5 (also known as non-insert)
Myosin X	*MYO10*	1
Myosin XVI	*MYO16*	1
Myosin XVIIIB	*MYO18B*	1

biochemical and biophysical properties. In fact, a single myosin is capable of different functions depending on its local environment, such as mechanical load, the local conditions (e.g. Ca^{2+} concentrations) and binding partners, as will be discussed below. With the ongoing debate on whether nuclear actin is present in the nucleus as either monomeric (Schoenenberger et al., 2005), polymeric or, under certain conditions, even filamentous forms (Kalendová et al., 2014; Belin et al., 2015), it is hard to ascertain the exact state of actin that interacts with the nuclear myosins. Undoubtedly, these states will impact on the potential roles of myosins. Since the debate about filamentous and monomeric actin in the nucleus has been already extensively reviewed (Hendzel, 2014; Moore and Vartiainen, 2017; Kristó et al., 2016; Kyheröinen and Vartiainen, 2020; Percipalle and Vartiainen, 2019), this Review will discuss actin only where it directly relates to the function of nuclear myosins.

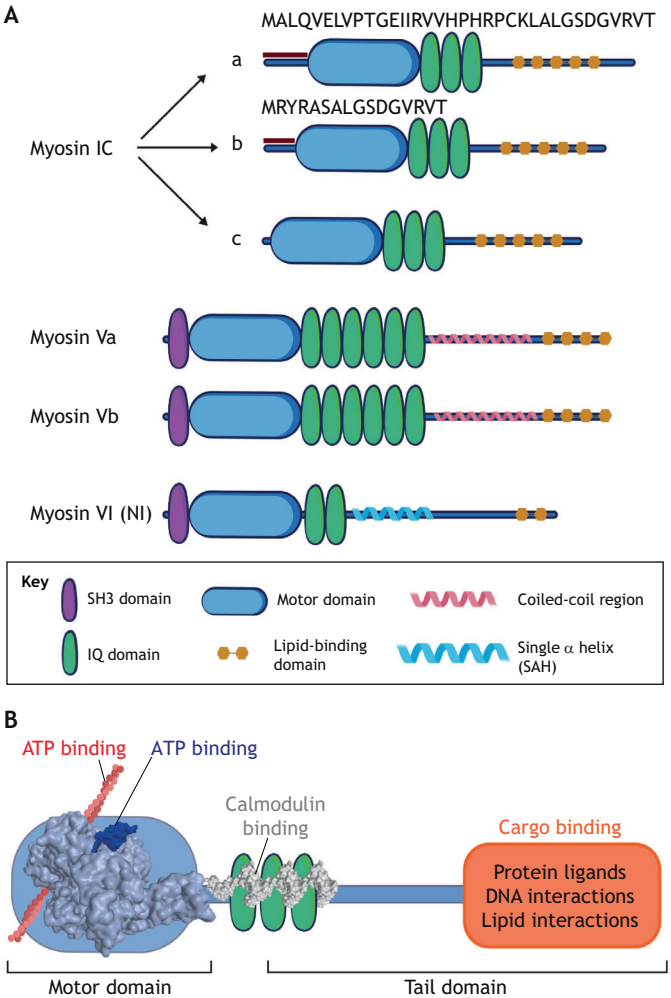

Fig. 1. Structural domains of nuclear myosins. (A) Overview of domain composition of the nuclear myosin heavy chain isoforms nuclear myosin 1 (NMI) with three variable N-terminal isoforms, myosin V (MV) and myosin VI (MVI) non insert (NI). (B) Generic myosin schematic, including the light chain (calmodulin)-binding site, and other interactions.

Certain myosins have a force-sensing capability; this occurs when a myosin binds to actin via its motor domain and a cargo through its tail domain (Houdusse and Sweeney, 2016). The cargo can act as a strain on the myosin, while it is still bound to actin, and depending on the cargo, this can generate different forces that the myosin has to withstand. In some cases, this variation of forces that is generated on the myosin can allow for the myosin to act as either an anchor (holding the cargo) or transporter (moving the cargo). For example, myosin IC is more likely to act as a slow transporter by generating power over a range of loads (Greenberg et al., 2012), compared to MVI, which at low forces of less than 2 pN acts as a transporter, but at forces above 2.5 pN acts as an anchor (Chuan et al., 2011). These differences in force sensing are not only important for the cytoplasmic functions of these myosins, but also for their nuclear roles. Therefore, it is important to consider these biophysical restraints on nuclear myosins in order to dissect the mechanisms underlying their nuclear function. For example, this raises the question of whether RNAPs are transported as a cargo by a myosin, or whether this interaction triggers the myosin to act as an anchor (Fig. 2D).

As mentioned above, the light-chain-binding partners also regulate myosin motors. Calmodulin, is a well-characterised light chain, whose affinity for myosin depends on the presence of Ca^{2+}, which in turn impacts on myosin motor activity (Adamek et al., 2008). This mode of regulation may also occur in the nucleus, as both Ca^{2+} and calmodulin are known to be present (Bachs et al., 1994). Therefore, Ca^{2+} signalling could impact on myosin function in the nucleus. Furthermore, calmodulin could impact on the nuclear localisation of myosin, as discussed in the next section.

Whereas calmodulin binds specifically to the IQ domains, other proteins can bind to the cargo-binding domains of myosins, which also affects their function. An example of this is the binding of the nuclear dot protein 52 (NDP52; also known as CALCOCO2) to the cargo-binding domain of MVI (Fili et al., 2017). This binding leads to the dimerization of MVI, which exposes DNA-binding sites, and subsequently promotes its ability to interact with the RNAPII complex (Fili et al., 2017). So far only a few binding partners of nuclear myosins have been characterised, but due to their potential to impact on nuclear myosins and the nuclear processes they regulate, their further discovery and characterisation is important. Moreover, understanding these interactions is likely to be crucial for identifying the role of myosins in a specific process, such as has been shown for that of MVI and NDP52 in transcription (Fili et al., 2019, 2017).

Import and export of nuclear myosins

In recent years, research has begun to shed light onto the mechanisms that regulate the import and export of nuclear myosins. It is important to determine whether there are distinct nuclear populations, myosin-specific localisation signals or binding partners that allow their nuclear recruitment, as well as specific signalling pathways for the exchange between cytoplasmic and nuclear myosins.

So far, a large body of research has been performed on myosin IC, mainly its nuclear isoform NMI (myosin IC isoform B), which

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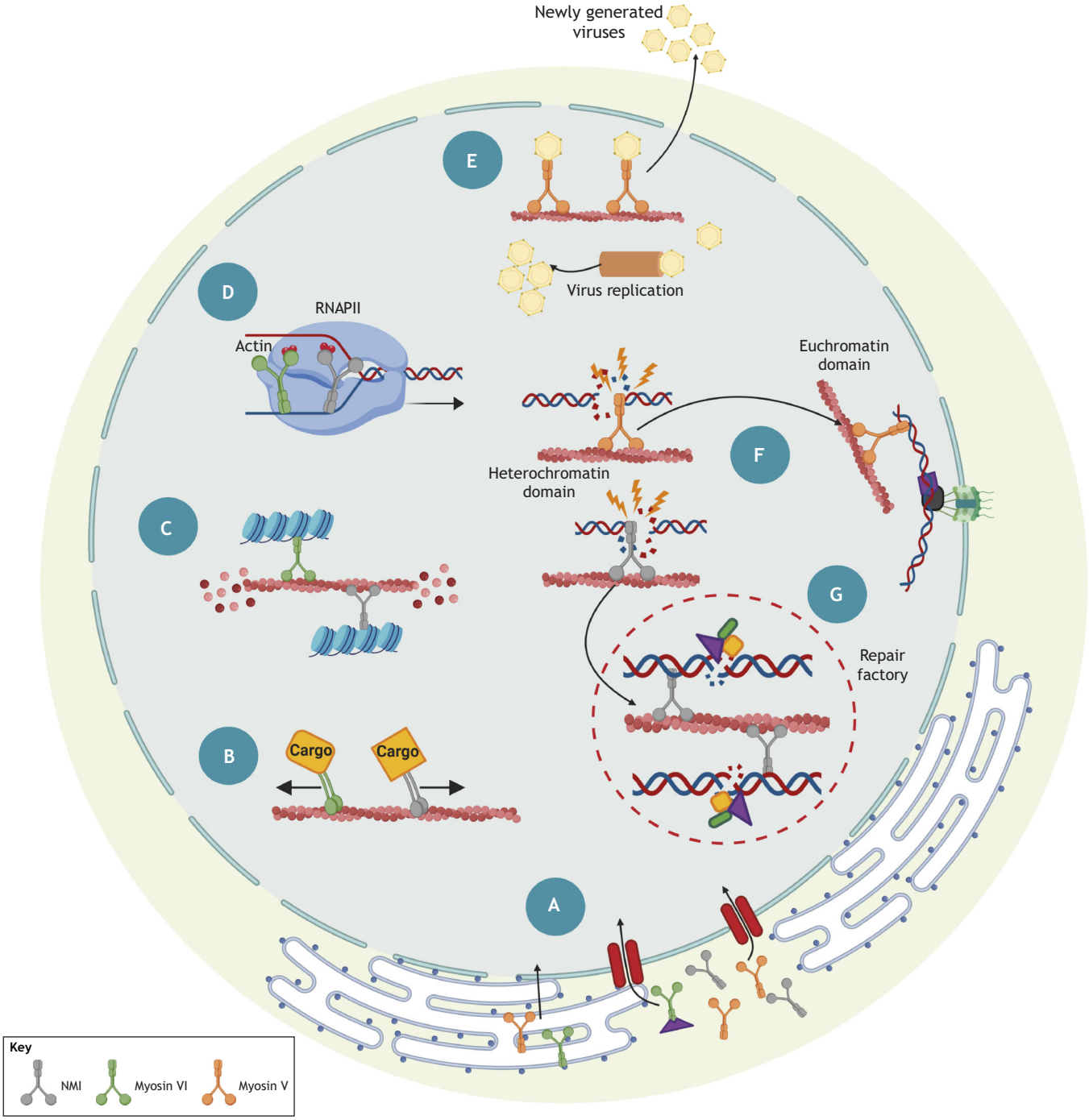

Fig. 2. Overview of the roles of nuclear myosins. Several mechanisms for the transport of nuclear myosins into the nucleus have been proposed, including transport through the endoplasmic reticulum and entry through nuclear pores, either with or without binding partners such as NDP52 (A). A possible role of nuclear myosins is act as a transporter and carry different cargoes through the nucleus (B), as well as to reorganise chromatin during transcription (C). Nuclear MVI and NMI bind to RNA polymerase II through actin and to DNA, thereby enabling transcription elongation (D). Nuclear myosin Va functions as a motor to transport newly produced viral capsids out of the nucleus. Here, myosin Va might transport the capsids along actin filaments to which it binds through its motor domain (E). Nuclear myosins also have a role in DNA repair and have been shown to transport damaged DNA to the nuclear pore along actin filaments (F), or bring together multiple damaged DNA strands into so-called repair factories; this facilitates the assembly of repair proteins at DNA breaks in an orchestrated fashion (G).

differs from the primarily cytoplasmic isoform C and the uncharacterised isoform A by the presence of a unique 16 amino acid sequence at the N-terminus (Fig. 1A). Interestingly all three of these myosin IC isoforms contain a nuclear localization sequence (NLS) embedded within their IQ domains (Dzijak et al., 2012), which allows for redundancy between the isoforms for its nuclear role, as shown when NMI is specifically removed from cells, the myosin IC C isoform is recruited to the nucleus instead (Venit et al., 2013). It is possible that both the unique N-terminal region and NLS are required for the role of NMI in the nucleolus as neither of the other two isoforms (Schwab et al., 2013), missing this unique N-terminus, can be found localised within the nucleolus or to be

interacting with RNAPI (Ihnatovych et al., 2012). The importins that recognise this embedded NLS within NMI were found to be importin 5, importin 7 and importin β (Dzijak et al., 2012; Maly and Hofmann, 2016). These authors showed that importin β is able to recognise the NLS following the dissociation of calmodulin, caused by an increase in cellular concentrations of Ca^{2+}, from the IQ regions. This involvement of calmodulin links nuclear recruitment to Ca^{2+} regulation (Maly and Hofmann, 2016). However, subsequently, another study has disputed this mechanism by providing evidence that NMI follows a phosphoinositide-dependent pathway for nuclear entry that utilises the pleckstrin homology domain (PH) present within the tail-end of the protein (Nevzorov et al., 2018). This route entails the binding of NMI to the endoplasmic reticulum, from where it diffuses through the centre of the nuclear pore complex, suggesting that its NLS only has a function once NMI is inside the nucleus. This study also found that NMI shuttles between the nucleus and the cytoplasm in the order of minutes (Nevzorov et al., 2018). Because strong experimental evidence supports both mechanisms for nuclear shuttling, we believe the cell might be able to adapt to both pathways as needed. In this context, a lipid-based localisation mechanism could allow for transportation into the nucleus under basal conditions, whereas a Ca^{2+}-regulated recruitment pathway might allow for a rapid response to signalling or stress pathways.

Building on previous findings that MVI is found in the nucleus, specifically at sites of actively transcribed genes as part of the RNAPII complex (Vreugde et al., 2006), it has been shown that MVI also NLSs for its import into the nucleus (Majewski et al., 2018). These authors have also identified seven potential NLSs using Psort II bioinformatics (Nakai and Horton, 1999), with one being embedded within the IQ region, as occurs in NMI, and one potential export sequence within the coiled-coil domain of the protein, suggesting that MVI might also be able to shuttle (Majewski et al., 2018). However, although these signals have been identified, it has yet to be experimentally proven if any of them contribute to localisation.

Another possibility is that nuclear localisation may not only be controlled by localisation signals within myosins, but rather by interactions with other proteins. Indeed, it has been shown that splice isoforms of MVI can impact upon their nuclear localisation (Fili et al., 2017). MVI has four isoforms in total, described as the long insert (LI), defined by a 31-amino-acid addition immediately before the cargo-binding domain, the small insert (SI), defined by an addition of 9 amino acids, both inserts together, or no inserts at all (Wollscheid et al., 2016). In this case, splicing of MVI controls the accessibility of the RRL – arginine, arginine, leucine – motif that binds to NDP52, which has both cytoplasmic and nuclear localisation. Splice variants that lacked access to this site caused a loss of binding partners, which resulted in MVI being unable to localise to the nucleus. This finding indicates that nuclear recruitment of MVI is indeed controlled by the interaction between MVI and NDP52 via its RRL motif (Fili et al., 2017). Furthermore, two new MVI-binding partners have been recently identified (Majewski et al., 2018); hnRNPU, a protein that is responsible for pre-mRNA transport (Yugami et al., 2007), and nucleolin, a multifunctional protein found to regulate rDNA transcription, chromatin and rRNA maturation (Tajrishi et al., 2011). The authors suggest that both factors may provide insights into the nuclear recruitment and roles of MVI; however, they provided no explanation of how this finding can be applied to nuclear import (Majewski et al., 2018). Another known binding partner of MVI is the androgen receptor, which is vital for the survival of human prostate cancer cells (Loikkanen et al., 2009). Here, knockdown of MVI has been shown to perturb genes that are under the control of androgen response elements (Loikkanen et al., 2009). A similar relationship has been seen with the estrogen receptor, where a knockdown of MVI leads to a reduction in the expression of genes modulated by estrogen receptor response elements (Fili et al., 2017). We put forward the idea that MVI may either bind to cytoplasmic hormone receptors and transport them into the nucleus, or that this interaction drives the nuclear recruitment of MVI (Fili et al., 2017). Further experiments are required to address whether either of these scenarios are true and are important for nuclear recruitment.

Taken together, it is clear from these studies that there is not a single mechanism of nuclear myosin import and export, and each myosin may follow several pathways. Therefore, in order to fully understand the nuclear localisation of myosins, we need to consider both the myosins themselves and their interacting partners. As research focuses on the role of nuclear myosins in specific processes, understanding the mechanisms that control their nuclear import and export may provide important insights into the signalling pathways they are involved in. Furthermore, knowledge of nuclear localisation pathways may allow specific myosins, or myosin-specific processes, to be future therapeutic targets such as during tumorigenesis (Li and Yang, 2016).

Nuclear myosins and their role in chromosome organisation

Interphase chromosomal arrangements within the nucleus were originally assumed to be random. However, it is now becoming clear that chromosomes occupy distinct regions within the nucleus, known as chromosome territories (Cremer et al., 2003; Fraser and Bickmore, 2007; Cremer and Cremer, 2010). Broadly, these territories can be found either at the interior of the nucleus or at the nuclear periphery (Cremer and Cremer, 2010), and territories are not always distinct owing to the dynamic nature of the nucleus, which can often result in territories inter-mingling (Branco and Pombo, 2006).

Within each chromosome territory, chromatin can either be densely packed (heterochromatin) or loosely packed (euchromatin). Heterochromatin has been found to localise towards the nuclear periphery, and euchromatin to the centre of the nucleus (Vanrobays et al., 2017). During interphase, large-scale chromosome movements can occur, and one of the mechanisms that is thought to facilitate this movement is the binding of NMI to transcriptional start sites (Chuang et al., 2006). These chromosome sites have been visualised travelling long distances across the nucleus after transcription stimulation with rapamycin, and faster and with more direction than general diffusion would allow (Chuang et al., 2006).

In general, myosins need actin filaments to travel long distances (Fili and Toseland, 2019), and interestingly, when actin polymerisation is perturbed by a G13R mutation (which prevents actin polymerisation), chromosome movement is inhibited (Chuang et al., 2006). This was not an isolated observation, as a previous study also used the G13R mutation to assess chromosome re-arrangement and transcriptional stimulation, and loss of movement was also observed (Dundr et al., 2007).

Chromosome movement also occurs during transcription, during which there is a pairing of two genes on separate chromosomes. For example, if cells are stimulated with 17β-estradiol, two genes containing estrogen promoter elements have been observed to travel through the nuclear space where they then colocalise and become paired (Hu et al., 2008). There is strong evidence that this process involves NMI, as two NMI mutations, R353C in the actin-binding site and S397L in the ATPase domain, have been shown to disrupt

the pairing of genes (Hu et al., 2008). However, this study has been disputed and it has now been suggested that long-distance chromosome movements do not occur after estrogen stimulation (Kocanova et al., 2010). Therefore, it remains to be defined whether this process does indeed occur and if so, whether NMI is involved.

Finally, chromosome territories have been mapped before and after serum stimulation, and serum removal has been found to cause a rapid repositioning of chromosomes that could only be reversed upon reintroduction of serum (Mehta et al., 2010). Furthermore, repositioning of chromosomes 10, 18 and X did not change upon serum introduction when actin polymerisation was blocked by using the drug latrunculin A, or when siRNAs specific to myosin IC were used (Mehta et al., 2010).

These data suggest that the motor activity of NMI, as well as multiple actin subunits, are required for correct chromosomal arrangements. We are hopeful that future work within the field will provide a clearer picture of actin filament formation, which would allow the observations of a nuclear myosin acting as a traditional transporter.

Role of nuclear myosins in the DNA damage response

The DNA damage response involves multiple pathways and varies depending on the type of the damage (Giglia-Mari et al., 2011). DNA damage can range from single nucleotide modifications (Schärer, 2013) to breaks in single-strand (Abbotts and Wilson, 2017) or double-strand DNA (Li and Xu, 2016). All these forms of damage require the DNA repair response, or instead trigger apoptosis in order to reduce damage to the cell or organism. In the past few years, nuclear myosins have been shown to play critical roles in the DNA damage response, including in the movement of chromosomes after DNA damage and their direct interactions with well-characterised DNA damage repair factors (Fig. 2G) (Kulashreshtha et al., 2016; Evdokimova et al., 2018; Izidoro-Toledo et al., 2013; Caridi et al., 2018; Jung et al., 2006; Cho and Chen, 2010).

As described above, NMI has been shown to be involved in the direct movement of chromosomes after stimulation; this is also the case after cisplatin-induced DNA damage (Mehta et al., 2013). Depending on the location of the damage site, upon treatment with cisplatin, a complete relocation of chromosomes can occur; in Mehta et al., it was shown that chromosomes 12, 17, 19 and 20 in human fibroblast cells travel from the nuclear periphery to the nuclear interior, or vice versa (Mehta et al., 2013). Such a DNA damage-induced repositioning of chromosomes does not occur after knockdown using a siRNA that targets all myosin IC isoforms, thus implicating this myosin family in chromosome movement (Kulashreshtha et al., 2016). These authors also noted an upregulation of NMI in the nucleus after DNA damage, as well as an increased amount of NMI directly bound to chromatin (Kulashreshtha et al., 2016). Interestingly, in the presence of a mutated γ-H2AX, the histone modification responsible for signalling double-strand DNA breaks, there is no observed increase in NMI recruitment to the chromatin, providing further evidence that NMI is required for chromosome movement through the γ-H2AX double-strand break signalling pathway (Kulashreshtha et al., 2016). The motor function of NMI is also required during the repair of double-strand breaks, when homologous chromosomes need to make contact in order to provide a template for the repair of the damaged chromosome (Evdokimova et al., 2018).

Myosin V isoforms have been identified as inducing apoptosis in DNA-damaged melanoma cells (Izidoro-Toledo et al., 2013), and, more recently, as being involved in the relocalisation of heterochromatic breaks in *Drosophila* cells (Caridi et al., 2018). Heterochromatin predominantly consists of satellite repeats and transposons, therefore, once a double-strand break occurs, it is vital the chromatin rearranges promptly to prevent unintended recombination (Fortuny and Polo, 2018). To initiate recombination, myosin V and myosin IA and IB are first activated by the myosin activator Unc45. Then, the myosins are recruited to the double-strand breaks by Mre11, a double-strand break repair protein, and heterochromatin protein 1a (HP1a). From here, it has been suggested that the myosins drive the movement of these heterochromatin breaks towards the nuclear periphery (Fig. 2F) (Caridi et al., 2018). This movement of damage sites also relies on the presence of actin filaments that originate from the points of damage and form in the direction of the nuclear periphery; this resembles the common feature of cytoplasmic myosins travelling along actin (Caridi et al., 2018). These data suggest that points of damage may be simultaneously moved to produce localised repair foci, where simultaneous repair of multiple points of damage can occur.

MVI has also been described to have a role in the DNA damage response, where MVI expression is increased after the cell cycle controller p53 is activated in RKO and LS147T cells (Jung et al., 2006). Furthermore, MVI localisation changes upon DNA damage, from the cells periphery to both the Golgi and nucleus (Jung et al., 2006). Subsequently, the increase in MVI stabilises activated p53, which, in turn, inhibits further production of MVI, representing a feedback loop (Jung et al., 2006). The authors claim that this places MVI within the prosurvival p53 pathway; however, we suggest that it still remains unknown whether this interaction with p53 is a nuclear or cytoplasmic function of MVI, and why there is an increase of MVI within the nucleus. Furthermore, such a role for MVI in the prosurvival pathway has since been defined as being cell line dependent and not reflecting a global function, as in LNCaP and MCF-7 cell lines, an opposite effect has been shown, in that DNA damage causes a decrease of MVI expression (Cho and Chen, 2010). This also leads to the question of what are the advantages of altering MVI abundance.

The majority of the nuclear myosins associated with DNA damage repair have been suggested to have a transportation role, specifically myosin I and myosin V, which can transport chromatin, and, at the same time, there is an influx of these myosins into the nucleus. Nevertheless, the role of MVI in the DNA damage response is not that well defined, and questions still remain with regard to its expression upon DNA damage, which may be cell line dependent. This could be advantageous for future experiments, as differences in cell lines and the role of MVI therein could be used to unpick the variety of roles of this myosin has both in the nucleus and cytoplasm.

The role of nuclear myosins in transcription

The role of nuclear myosins in transcription, in particular NMI and MVI (Vreugde et al., 2006; Percipalle and Farrants, 2006; Fili et al., 2017), are well established. Both NMI and MVI are required for complete mRNA or rRNA gene transcription, and both myosins are able to interact with their respective RNAP complexes – NMI with both RNAPI (Philimonenko et al., 2004) and RNAPII (Hofmann et al., 2004), and MVI with RNAPII (Vreugde et al., 2006).

Nuclear actin has also been shown to have a key role in transcription, monomeric actin is part of all RNAP complexes and the presence of longer actin polymers has been heavily discussed (Dundr et al., 2007; Hofmann et al., 2004; Hu et al., 2004; Kukalev et al., 2005; Philimonenko et al., 2010; Grosse and Vartiainen, 2013). More recently, actin polymerization has been

linked to clustering of RNAPII in response to transcription stimulation (Wei et al., 2020).

The nuclear myosin best characterised for its role in transcription are the myosin IC isoforms, in particular NMI (reviewed by Sarshad and Percipalle, 2014). ChIP-seq data clearly demonstrate that NMI is localised at class II promoters, where RNAPII transcription initiation occurs (Almuzzaini et al., 2015). In addition, two RNAPII subunits, Rpb6 and Rbp8, can be perturbed from binding to their promoters in the absence of all myosin IC isoforms (Almuzzaini et al., 2015). Taken together, these observations indicate that NMI, alongside actin, creates the transcription initiation complex needed for RNAPII to bind to the promoter regions (Almuzzaini et al., 2015). This reinforces the concept that NMI is an important cog in the polymerase machinery, as first suggested in 2000 (Pestic-Dragovich et al., 2000).

Analysis of ChIP-seq data also demonstrated that NMI stabilises the association between SNF2h, also known as SMARCA5 (Aihara et al., 1998), an ATP-dependent nucleosome remodeller (Erdel and Rippe, 2011), and its well-characterised binding partner Williams syndrome transcription factor (WSTF; also known as BAZ1B), which has been initially found to be involved in the replication of heterochromatin (Bozhenok, 2002). Together, they form the WSTF–ISWI chromatin remodelling complex, known as B-WICH, which rearranges nucleosomes for RNAPI transcription (Vintermist et al., 2011). NMI acts a molecular bridge between B-WICH and RNAPI; B-WICH induces acetylation of histones ahead of the transcription machinery, which loosens the nucleosome-wrapped DNA, thereby making it available for transcription and allowing cell cycle progression (Vintermist et al., 2011; Sarshad et al., 2013; Percipalle et al., 2006). In the case of RNAPII transcription, NMI also has a role in nucleosome organisation by binding to the B-WICH complex, which in turn results in acetylation of downstream nucleosomes, allowing for gene transcription (Almuzzaini et al., 2015).

MVI has been described as a potential auxiliary motor in RNAPII transcription that can be regulated by its binding partner NDP52 (Fili et al., 2017, 2019). Similar to what is seen with NMI, the cargo-binding domain of MVI can bind to DNA; this is controlled by unfolding of its tail domain, which is regulated by NDP52 (Fili et al., 2017). Upon inhibition of the motor activity of MVI with the small molecule TIP (Heissler et al., 2012), transcription by RNAPII was reduced by ∼75% *in vitro*, indicating that MVI is required for complete transcription (Cook et al., 2018). Based on their findings, the authors proposed a mechanism, whereby NDP52 enables the dimerization and thus activation of MVI in the nucleus. Following activation, the motor domain of MVI binds to actin present on RNAPII and its C-terminus to DNA. The authors hypothesise that both these binding events could enable anchoring of the polymerase on the DNA and/or movement of RNAPII along the DNA (Fili et al., 2017).

MVI also mediates the transition from a paused RNAPII complex to an elongating RNAPII complex, as shown through the re-stimulation of the tumour necrosis factor (TNF) allele expression within TH1 cells; yet, this observation on its own does not provide any evidence of MVI either acting as an auxiliary motor or an anchor (Zorca et al., 2015).

Given that NMI and MVI are present in the RNAPII complex, this raises the question of how do they function in relation to each other. Also, how do they interact with the protein-rich core at transcription sites containing transcription factors and other proteins involved in transcription? Interestingly, knockdown of MVI does not totally inhibit transcription (Fili et al., 2017), and a simple possible explanation is that there is some redundancy between these two myosins. Moreover, MVI is found to colocalise with nuclear speckles that are involved in pre-mRNA processing (Majewski et al., 2018). This, together with its localisation on promyelocytic leukaemia protein (PML) nuclear bodies (Majewski et al., 2018), which consist of chromatin and transcription activators, suggests that MVI is present in a number of transcriptionally active areas in the nucleus.

Furthermore, given the interaction of MVI with both the estrogen receptor (Fili et al., 2017) and the androgen receptor (Loikkanen et al., 2009), we speculate that MVI is required for the transcription of specific genes under certain conditions, for example, upon hormone stimulation. Such a specific role under certain conditions adds to the complexity of studying nuclear myosins, and therefore further highlights the importance of investigating nuclear myosins within the right context. With advances and cost reduction in genomic technologies, it will become more feasible to perform larger scale studies to dissect the roles of nuclear myosins in the context of differentiation, throughout the cell cycle and in response to various stimuli, such as DNA damage and hormones.

Unlike the identified function in the DNA damage response, a long-range transport activity of myosins has not yet been observed directly during the progression of a polymerase, despite the fact that MVI is known to be required for transcriptional pause release as mentioned above (Zorca et al., 2015). Indeed, a loss of homologous pairing of TNF alleles was observed upon MVI knock-out and transcriptional pausing was maintained (Zorca et al., 2015).

Taken together, these findings once again implicate nuclear myosins in chromosome organisation and transcription, and we hypothesize that the motor function is required for chromosome movement and the anchoring ability of MVI to hold the chromosomes *in situ* (illustrated in Fig. 3).

Involvement of nuclear myosins in viral infections

Recently, nuclear myosins have been shown to be involved in processes mediated by viral infections. Viral genomes have to be maintained within the host cell nucleus, and their replication and expression needs to be regulated. NMI has been linked to these processes, although through roles that have already been described for transcription (Fuchsova et al., 2015; Sankovski et al., 2018; Oswald et al., 2017). The human papilloma virus has multiple E proteins that have a role in aiding viral infection proliferation (reviewed in Mattoscio et al., 2018). So far, three E proteins have been found to be capable of interacting with NMI, namely E2 (Sankovski et al., 2018), E6 and E7 (Oswald et al., 2017).

The E2–NMI complex has been shown to actively remodel chromatin through interactions with the WSTF–SNF2h chromatin remodelling complex (Sankovski et al., 2018). As discussed above, the NMI–WSTF–SNF2h complex allows for viable RNAPI transcription (Percipalle et al., 2006), which the viral protein E2 hijacks for viral transcription (Sankovski et al., 2018). The E2–NMI complex also interacts with Brd4 (Sankovski et al., 2018), another protein involved in chromatin remodelling (Devaiah et al., 2016). Upon addition of ATP, the E2–NMI complex falls apart owing to a conformational change in NMI (Sankovski et al., 2018). This conformational change in NMI occurs within the motor domain, from which actin is released upon the binding of ATP; as proposed by the authors, this experiment thus suggests that actin may also be part of this NMI–E2 complex (Sankovski et al., 2018).

Myosin Va has been shown to have a role in the transport of viral capsids during nuclear egress of the human cytomegalovirus (Wilkie et al., 2018). Here, nuclear myosin Va binds directly to the viral capsids, which are then found to be on filamentous actin that is directed towards the nuclear periphery (illustrated in Fig. 2E). However, a direct visualisation of myosin Va moving these capsids

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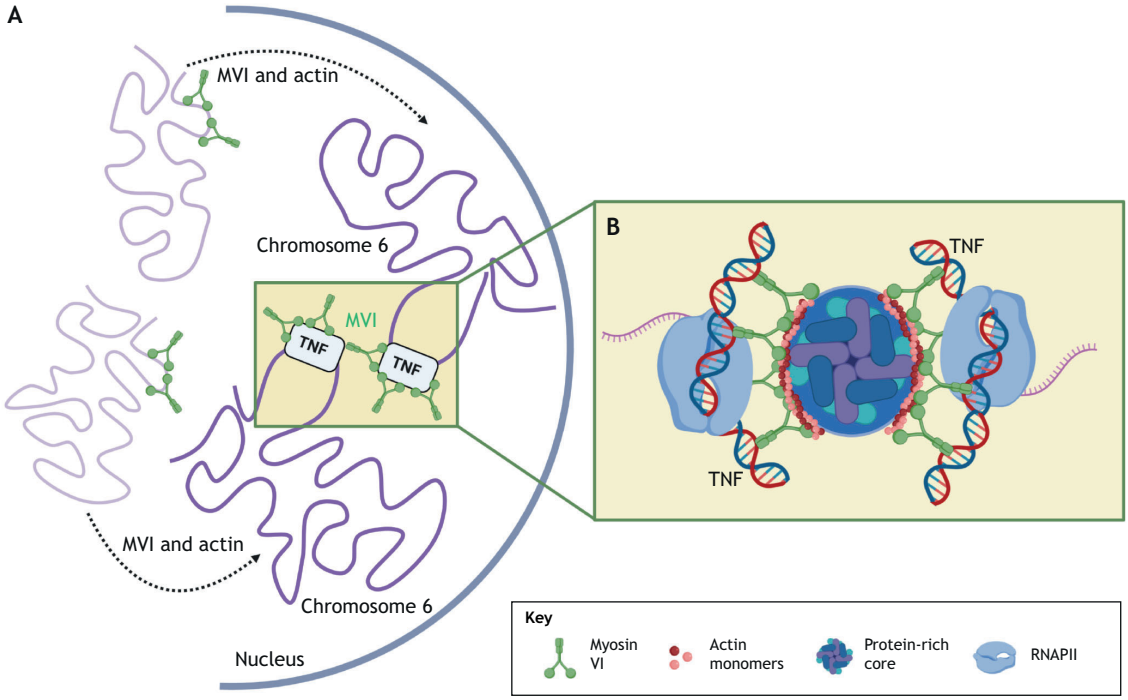

Fig. 3. Transcription pairing of genes by myosin VI. (A) Proposed mechanism of chromosomal movement to mediate transcriptional pairing of genes, which requires nuclear MVI and actin, suggesting that nuclear MVI acts as a molecular motor to guide the chromosome from one area of the cell to another. As illustrated here, the binding of nuclear MVI to the tumour necrosis factor (TNF) in T cells might anchor the TNF allele *in situ*, allowing the homologous pairing of TNF alleles to enable simultaneous transcription. (B) Schematic model of MVI-mediated anchoring of the two TNF alleles for simultaneous transcription around a protein-rich core, which consists of transcription factors, other transcriptional-related proteins and is decorated with actin.

along actin lacking thus far. This role of myosin Va was shown to be crucial for the virus, as a dominant-negative mutant of myosin Va results in defects in both capsid accumulation and localisation (Wilkie et al., 2018). This study also provided some evidence for a colocalisation between F-actin, myosin Va and capsid proteins, which suggests that nuclear myosin Va may work as a classical transporter (Wilkie et al., 2018), similar to its cytoplasmic role (Evans et al., 2014). This supports previous work showing that filamentous actin is required for nuclear egress of cytomegalovirus, as demonstrated by inhibiting actin polymerisation with the well-characterised drug latrunculin A (Coué et al., 1987), which halted the process (Wilkie et al., 2016).

Owing to their similarities in structure and function with that of host proteins, these viral binding partners of nuclear myosins may help in the discovery of additional interactions that myosins undergo in the nucleus and to thus advance our understanding of their roles and means of regulation.

Conclusions and perspectives

Research into nuclear myosins is diverse and requires extensive input from cell biologists, geneticists, biochemists and biophysicists. Researchers with expertise in the field of myosins, DNA damage, gene expression, genome organisation and virology have so far contributed to the current state of the field. Within this relatively new area, it has become apparent that although effects of myosin knockdowns can be significant, such as the effect on transcription, they are unable to provide the details of the underlying mechanism. Therefore, communication across these diverse fields is critical, and researchers must start to question which of the functions of a myosin is required for a given process.

As discussed in this Review, nuclear myosins are involved in a variety of roles. However, the impact of force, the necessity for Ca^{2+} and calmodulin, and the association with binding partners, which have been well characterised for cytoplasmic myosins, remain unknown for many of their nuclear counterparts. Only when these aspects are linked to the roles of a single myosin in the nucleus, can we begin to probe its molecular and mechanistic details.

The tendency to directly attribute the outcome of the knockdown of a nuclear myosin to an observed defect is likely to be misleading owing to their overlapping roles within the nucleus. For instance, when studying the role of NMI in DNA damage, it should be kept in mind that perturbing this myosin not only affects the DNA damage response, but also transcription and any cytoplasmic role of myosin IC isoforms. Moreover, as highlighted in this Review, many studies have not specifically targeted NMI, but all three myosin IC isoforms, while attributing their study to NMI alone. Therefore, models need to consider the wider implications of myosin functionality because if a knockdown of MVI leads to a decrease in transcription, what genes are being affected by this and are they present in the process that is being studied? Another issue is the selective targeting of the nuclear myosin pool and not their cytoplasmic counterparts. Owing to the shuttling of myosins in and out of the nucleus, it is impossible to remove only the nuclear pool in knockdown or inhibition studies. This further highlights the importance of research into nuclear import and export mechanisms, as only by excluding a myosin from the nucleus while maintaining its cytoplasmic pool, will we be able to further elucidate its nuclear functions. In this regard, the identification of any additional binding partners might allow for the specific disruption of a nuclear myosin, without affecting its activity in the cytoplasm.

With the requirement of actin for many of these myosin–protein interactions, it is likely that certain myosins are acting as transporters, as exemplified for myosin Va in viral egress from the nucleus. Conversely, short actin polymers, or monomeric actin,

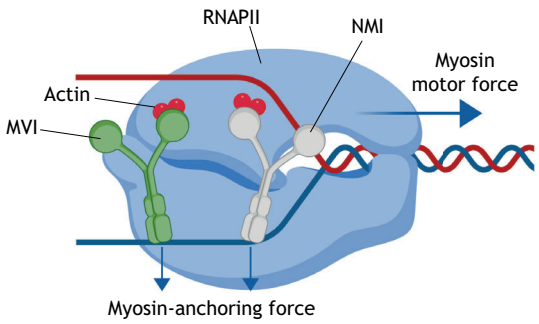

Fig. 4. Nuclear myosin-mediated anchoring or transport of RNA polymerase II. Nuclear MVI and NMI can bind directly to DNA through their C-terminal domain and indirectly to RNAPII through actin, which is bound by their motor domain. This suggests a possible model, in which myosin could either anchor RNAPII to the DNA, or move it along the DNA. Questions still remain as with regard to whether nuclear MVI and NMI are working alone, in tandem, or in opposing roles to each other when bound to RNAPII.

aid in the anchoring of NMI or MVI, resulting in stabilisation and initiation of the RNAPII complex (Miralles and Visa, 2006).

So far none of the publications discussed within this Review have addressed the role of the myosin light chains in the nucleus, such as calmodulin, in the discussion of their findings. This is worrying as the presence of calmodulin bound to a myosin can fine-tune the stability, cargo binding and actin-binding capabilities of the respective myosin (Heissler and Sellers, 2014). It is known that both Ca^{2+} and calmodulin play a role in nuclear actin formation based on studies of both T cell activation (Tsopoulidis et al., 2019) and chromosomal dynamics in NIH3T3 cells (Wang et al., 2019); therefore, the apparatus for myosin regulation is present. The key biochemical and biophysical properties of cytoplasmic myosins have been well characterised. Based on further studies and the application of biophysical and biochemical techniques, the mechanical properties of nuclear myosins might also become better understood, enabling us to answer questions, such as whether it is physically possible for NMI to withstand the forces exerted on it while it is bound to polymerases if MVI present on the RNAPII alongside NMI, and whether these two myosins work in tandem, or against each other. In addition, we also need to know whether MVI anchors the polymerase, or if both myosins work as motors (illustrated in Fig. 4).

Finally it is apparent that nuclear myosins are important regulators of fundamental cellular processes, which impact on diseases, such as deafness (Arden et al., 2016), Griscelli disease, (Hirokawa and Takemura, 2003) and a variety of cancers (Ouderkirk and Krendel, 2014). A greater understanding of this group of proteins will potentially also provide new avenues for therapeutics and highlight future biomarkers.

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Competing interests
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