

Research Article

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Desmin's conformational modulation by hydrophobicity

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published online March 1, 2024**Abstract**

Objectives: Nucleocytoplasmic transport is one of the key features in regulation of cellular physiology. Developing a better understanding of the molecular mechanism underlying the nucleocytoplasmic shuttling of proteins can broaden our perspective and understanding on the elaborate sorting mechanisms within cells. Desmin is a muscle specific intermediate filament with amphiphilic properties and has interactions with the components of the nuclear pore complex which facilitates the transport between the cytoplasm and nucleus. The study aims to develop a better understanding of the amphiphilic nature of desmin and its relation to nucleocytoplasmic transport.

Methods: We conducted a proteomic analysis of desmin-immunoprecipitates to identify the nuclear partners of desmin. Additionally, we analysed the amphiphilic nature of desmin using a hydrophobicity assay to determine if it can undergo conformational changes to adapt to a hydrophobic environment.

Results: Using proteomic and *in silico* analysis we demonstrated that desmin interacts with several nups. The hydrophobicity assay results showed that desmin can increase its surface hydrophobicity in a hydrophobic environment.

Conclusions: Our findings suggest that desmin has the ability to undergo conformational changes under favourable conditions and possibly can be transported through nucleus via direct interaction with nups. Further analysis is required to

understand the functional implications of this conformational change *in vivo*. Data are available via ProteomeXchange with identifier PXD047121.

Keywords: desmin; nuclear transport; nuclear pore complex proteins; proteomics; hydrophobicity

Introduction

Intermediate filaments (IFs) are a large and diverse protein superfamily in eukaryotes that form the basic architecture of the cell. Except for the nuclear lamins, IFs are primarily located in the cytoplasm. IFs are associated with a broad range diseases (more than 119 according to data from the Human Intermediate Filament Database in 2021), which underscores the pivotal role that IFs play in cellular maintenance.

A considerable body of literature has been published on the nuclear presence and functions IFs [1–3]. However, what remains to be elucidated is how IFs are transported between the cytoplasm and nucleus. Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), a complex protein structure comprising over 30 nucleoporins (nups) and measuring approximately 120 MDa in size [4]. The NPC consists of two functional regions, namely central and peripheral regions. There are four types of nups that form the NPC: transmembrane nups, which span the pore membrane and anchors the NPC to the nuclear envelope; core scaffold nups, forming the inner and outer rings of the NPC, Phe-Gly (FG) nups, responsible for the transport through NPC; and linker nups, which connects the core scaffold and FG nups [5].

Small molecules, ions and metabolites can freely pass through the NPC, but the NPC is impermeable to larger macromolecules. The transportation of macromolecules through the NPC requires energy, transport factors (karyopherins) and a transport signal. What remains unclear, however, is how certain macromolecules, lacking a transport signal, can be transported into and out of the nucleus.

There are several published studies [6–8] suggest a karyopherin-independent transport of cytoplasmic proteins into the nucleus. What these proteins share in common is a

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structural domain with amphiphilic features [1]. Amphiphilic proteins can increase their surface hydrophobicity by altering their conformation, allowing them to adapt to a hydrophobic environment and pass through the NPC [1].

Desmin is a muscle-specific type III IF located in the cytoplasm with amphiphilic properties. Desmin serves as a bridge connecting the myofibrils to nucleus, mitochondria and sarcolemma. While extensive previous research has focused on desmin's cytoplasmic functions, there have been relatively few studies examining its nuclear presence and functions [3, 9–12] and none have explored its transport mechanism. Although a potential non-classical nuclear localization signal (NLS) on desmin has been pointed out before [13], to date, no study has demonstrated whether this signal is actually functional.

We previously demonstrated that desmin and one of the FG nups, nup214 co-precipitate [14]. This finding is intriguing because Lockard's study (1993), illustrated that desmin could extend through nuclear pores to interact with a component of the nuclear lamina [15]. The authors argued that this interaction could occur with the help of anchorage proteins within the NPC. Given that desmin has an amphiphilic rod domain and may potentially interact with a FG nup, we hypothesised that desmin can be translocated into and out of the nucleus via hydrophobic interactions.

The aim of this research is to identify the interactors of desmin within the NPC and determine whether desmin's amphiphilic nature is sufficient to adapt to the hydrophobic conditions inside the NPC, facilitating its translocation into the nucleus.

In the presented research, we have demonstrated that desmin interacts with multiple nups and is capable of exposing its hydrophobic regions with increased alcohol concentration.

Materials and methods

Identification of proteins by mass spectrometry

Protein lysates from zebrafish skeletal muscle tissue was used for proteomic analysis. All procedures performed in studies involving animals were in accordance with the ethical standards of the Hacettepe University Animal Experimentations Local Ethics Board (18.02.2104; 2014/07-08). Mass spectrometry analysis was performed at Koç University, Proteomics Facility (İstanbul, Türkiye). Briefly, lysates from co-immunoprecipitation assays, one using an antibody against desmin (D8281, SIGMA, Missouri, USA) and one without non-specific IgG [11] were run and excised from Coomassie blue stained gels. Protein bands were digested in-gel. Samples were analyzed with Q Exactive Quadrupole-Orbitrap Mass Spectrometer with nano Liquid Chromatography (n-LC MS/MS) (Thermo Fisher Scientific, Massachusetts, USA) in duplicates with 300 nL/min flow rate for 50 min. False Discovery Rate (FDR) was 0.01 %. Raw data was analyzed using MaxQuant [16]. Peptides

identified using zebrafish protein list downloaded from UniProt (UP000000437) [17]. The final protein list analyzed on PantherDB using statistical overrepresentation test on GO cellular compartment [18, 19]. The proteins listed under the term 'nuclear' than analyzed on Enrich R [20–22]. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [23] partner repository with the dataset identifier PXD047121.

Cell culture

Human cervical cancer cells (HeLa) cells were grown in DMEM (Capricorn, Germany) containing 10 % foetal bovine serum (FBS) (Capricorn, Germany). Immortalized human skeletal myoblasts (T0033) were grown in proliferation media as described by Thorley (2016) [24]. Immortalized human skeletal muscle cells were differentiated as described by Thorley (2016) [24].

Plasmid construction and transient transfections

For construction of eGFP tagged desmin expression vector (pcDNA3.1(+)-hDES-eGFP), desmin sequence of human origin was used as template. Total RNA was extracted according to Pattern (2019) [25]. RNA concentrations quantified using NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA).

cDNA was synthesized from total RNA isolated with iScript cDNA synthesis kit (Bio-RAD, California, USA) according to the manufacturer's instructions.

The open reading frame (ORF) of *DES* was amplified by PCR with a 5' primer 5'-acagcgGAATTCCTcgcgccgcgcgtaccatga-3' and a 3' primer 5'-acagcgGCGGCCGcggtctctggtggcagagggtc-3', which creates restriction sites for KpnI and BamHI enzymes at the 5' and 3' ends of *DES*, respectively. The product was digested with the respected enzymes and cloned into the pcDNA3.1(+) eGFP to create pcDNA3.1(+)-hDES-eGFP. All enzymes used for cloning procedures were purchased from New England Biolabs (Massachusetts, USA).

For transfection, cells were grown to 70–80 % confluency and transfected with pcDNA3.1(+)-hDES-eGFP with Lipofectamine 3000 Transfection Kit (Invitrogen, Massachusetts, USA) according to the manufacturer's instructions. After 24 h, media was changed into fresh media.

Confocal imaging

Images were acquired using Zeiss LSM 880 laser scanning confocal microscope equipped with a 63×/1.4 Apochromat Oil DIC objective (Carl Zeiss, Germany). Cells were imaged using an argon laser with an excitation at 488 nm for eGFP and a diode laser at 405 nm for DAPI. Z-stacks were acquired with 1–2 µm intervals. Colocalization analysis was performed using FIJI with Coloc 2 plug in [26].

Surface hydrophobicity measurements

BSA (Thermo Fisher Scientific, USA) and recombinant desmin (GenScript, New Jersey, USA) proteins were dissolved in PBS containing 0–55 % v/v ethanol (EtOH) and 10 µM 4,4'-Bis(phenylamino)-[1,1'-binaphthalene]-5,5'-disulfonic acid dipotassium salt (Bis-ANS) (R&D Systems, USA) and equilibrated for 15 min at room temperature.

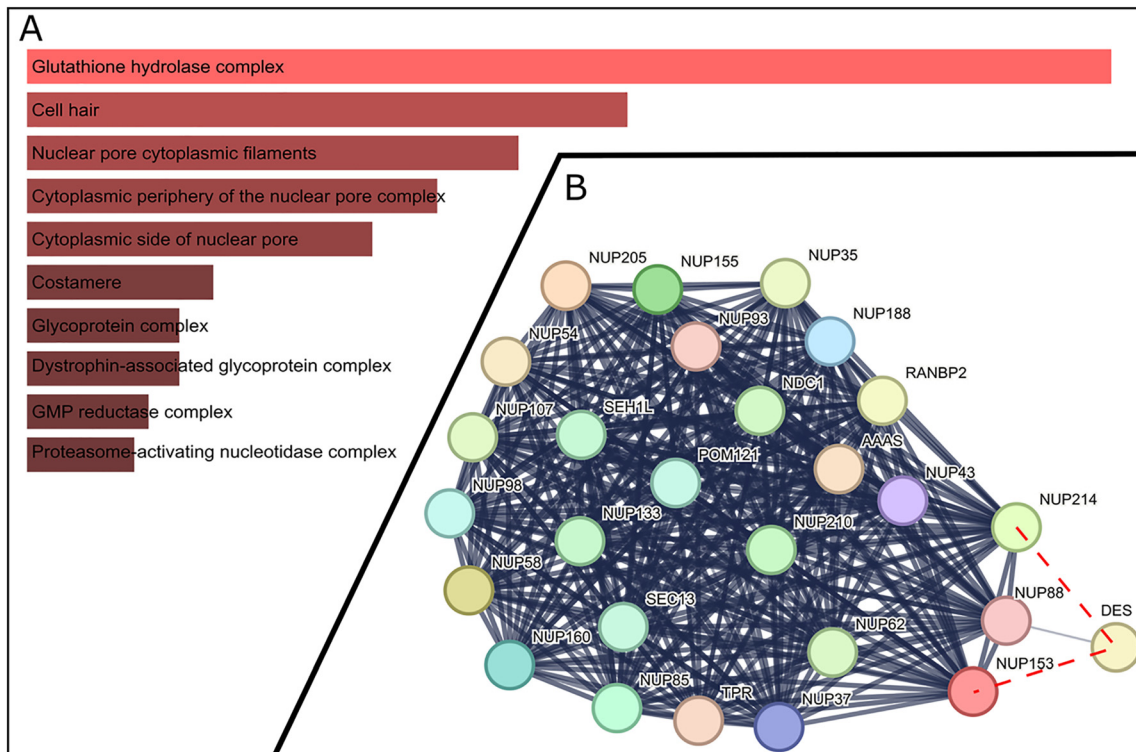


Figure 1: Analysis of the proteomic data. (A) Ontology analysis on EnrichR representing the cellular localization of nuclear partners of desmin. The X-axis (the length of the bar) represents the significance of the term, and the lighter color indicates more significant results. Starting from the top, the p-values are: 7.87E-04; 3.50E-03; 4.89E-03; 6.28E-03; 7.67E-03; 1.25E-02; 1.39E-02; 1.39E-02; 1.53E-02; 1.60E-02, respectively. (B) The relationship between desmin and nups as generated by STRING. Red dashed lines indicate the interactions of desmin that we identified.

Fluorescence was quantified using a microplate reader (SpectraMax M2, Molecular Devices, California, USA) with excitation and emission wavelengths of 405 and 460 nm, respectively.

Statistical analysis

Student's t-test performed on Microsoft Excel, and graphics created on GraphPad Prism v.5 (GraphPad Software Inc., California, USA). The results were considered significant when p-value < 0.05. All error bars were presented as mean ± SD.

Results

Desmin's binding partners at the nuclear periphery

We previously co-precipitated desmin and nup214, a nup located at the cytoplasmic filaments of the NPC, and that desmin and nup214 co-precipitates [14].

To further explore the extends of this relationship and identify additional interactors, we conducted a proteomic analysis. The protein list obtained after initial analysis was subsequently analyzed on EnrichR [20–22] (Figure 1).

Intriguingly, the terms nuclear pore cytoplasmic filaments, cytoplasmic periphery of the nuclear pore complex and cytoplasmic side of the nuclear pore were found to be enriched (Figure 1). Among other findings, a protein was particularly of interest: nup153. Nup153 resides in the nuclear basket of the NPC, known to shuttle between the nuclear and cytoplasmic faces of the NPC, and plays a role in protein transport through the NPC [27]. The results of proteomic analysis strongly suggest a functional relationship between the NPC and desmin.

Additionally, we performed a protein interaction network analysis on The Biological General Repository for Interaction Datasets (BIOGRID) [28, 29]. This analysis revealed that desmin also has an interaction with nup88 [30].

We then use STRING [31] to search for a pattern between nups and desmin (Figure 1). The human nups [5] and desmin were analysed for interaction. As expected, nups displayed strong interconnections, and our analysis revealed the interaction between desmin and nups.

When this data is combined with our interaction data (PXD047121) of nups and desmin it forms a strong case for nup assisted karyopherin-independent nucleocytoplasmic transport of desmin.

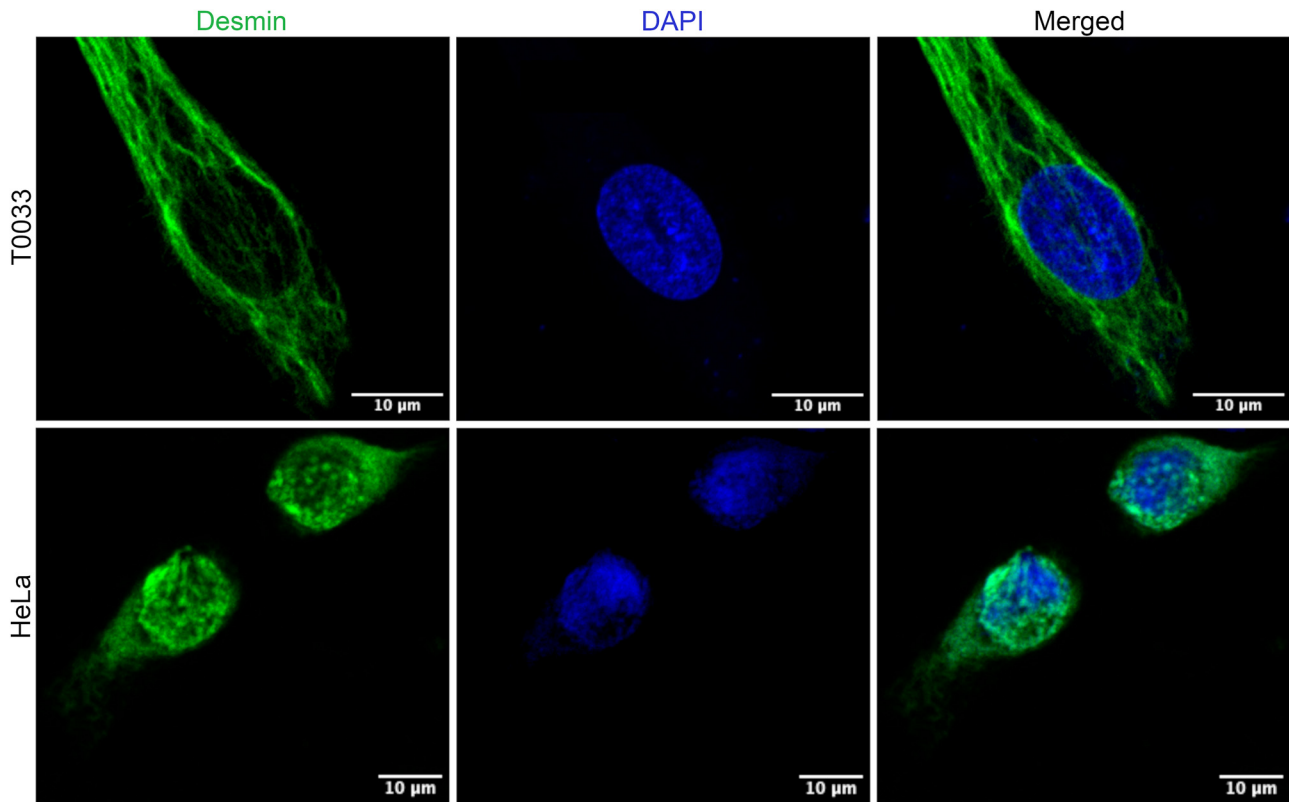


Figure 2: Localization of desmin in nuclei of myoblast (T0033) and epithelial (HeLa) cells. Representative confocal immunofluorescence micrographs of myoblast (T0033) and epithelial cells (HeLa). Desmin, green; nucleus, blue. Images at the right are merged images.

Desmin can be localized in the nucleus of human skeletal myoblasts and epithelial cells

The proteomic analysis was conducted using protein lysates from zebrafish skeletal muscle tissue. While previous studies have demonstrated the nuclear presence of desmin in cell lines from various origins, such as renal fibroblasts, skeletal myoblast of mouse origin, embryonic cardiac stem cells of mouse origin and others [3, 9, 32], there was no record of a study in human originated myoblasts. In addition to that, most of the studies dated before 2010s. Therefore, prior to investigating the hydrophobic features of desmin, which we believe to be a key feature that lets desmin into and out of the nucleus, we first demonstrated that desmin can be localized in the nucleus of human skeletal myoblasts and epithelial cells with taking advantage of the new imaging and analysing technologies. We transiently overexpressed the eGFP tagged desmin in two different cell lines one of which expresses (T0033) desmin and the other (HeLa) not 24 h after transfection, the skeletal myoblast proliferation media were changed to differentiation media and T0033 cells were

allowed to differentiate for two days and then prepared for confocal imaging. HeLa cells were fixed 24 h after the addition of fresh proliferation media for confocal imaging.

We performed colocalization analysis with nuclear staining (DAPI) and desmin signal to determine desmin's nuclear localization, and calculated the Pearson correlation coefficient [33]. According to colocalization analysis using FIJI [26], desmin was detected in 48 % nuclei of differentiating myoblasts and 68 % of epithelial cells (Figure 2, Supplemental Figures 1 and 2). We allow the myoblasts to start differentiate because desmin expression is relatively higher after the induction of differentiation [34]. Furthermore, we have showed that desmin enters the nucleus during early myogenesis (unpublished data), consistent with the literature. Inhibition or reduction of desmin interferes with the expression of muscle-specific genes, MYOD and MYOG, which are particularly important in the determination skeletal myoblast lineage [35] and differentiation [36, 37], respectively.

Whilst, desmin has been reported to be found in the nucleus of cells of various origins [3, 9, 32], our study presented here is the first report on desmin's nuclear localization in human skeletal myoblasts.

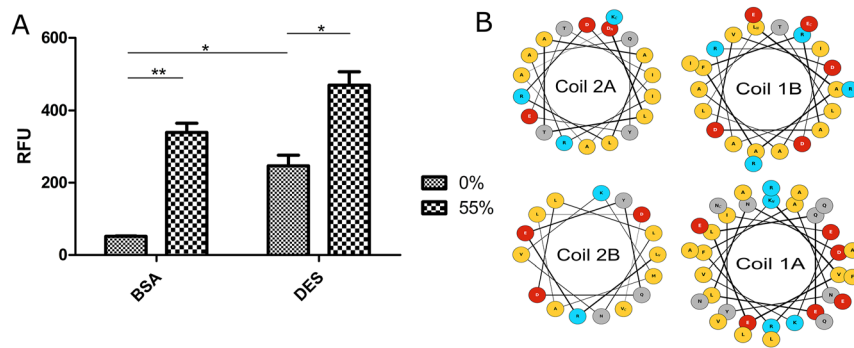


Figure 3: Conformational changes of desmin in hydrophobic environment. (A) Fluorescence signal intensities for desmin (DES) and BSA in the presence of 0 %, 55 % EtOH. Values of three different measurements are represented as the mean $\pm$ SD (*: $p \leq 0.05$; **: $p \leq 0.01$). (B) Conjectural helical wheel representations of the desmin coils[†] (RFU, relative fluorescence unit). Hydrophobic amino acids are indicated in yellow. Grey, red, cyan represents the polar, acidic, and basic amino acids, respectively. [†]Full amino acid sequence of coil 1a and coil 2a and, the hydrophobic regions according to Kyte–Doolittle [41, 42] of coil 1b (208–230 amino acids) and coil 2b (385–400 amino acids) was presented (Supplemental Figure 3).

Desmin can increase its surface hydrophobicity in hydrophobic environment

Proteins can enter the nucleus with direct interaction with nups via their amphipathic alpha helix [38] and they tend to increase their surface hydrophobicity in hydrophobic solutions [1]. Considering the relationship of desmin with the components of the NPC, we asked ourselves whether desmin has the ability to change its surface hydrophobicity to pass through nuclear pore complex. To investigate if desmin can change its surface hydrophobicity, we used Bis-ANS dye, which binds noncovalently to hydrophobic surfaces. Binding of Bis-ANS to a hydrophobic surface result in an increase in fluorescence. We used BSA as a control since its hydrophobic characteristics are well characterized [39, 40]. When equal amounts of BSA and desmin were diluted in PBS containing 10 μ M of Bis-ANS in the absence and presence of 55 % ethanol, the signal intensity for desmin was significantly higher, as is for BSA, indicating that desmin increases its surface hydrophobicity in a hydrophobic environment (Figure 3A). This means that desmin can modulate its conformation to adapt the hydrophobic nature of the NPC and to interact with nups. We also observed that the signal intensity of desmin in an alcohol-free environment was significantly higher than that of BSA, which is probably due to the exposed hydrophilic regions on the surface and may potentially be antigenic.

Next, we created a helical wheel representation of desmin's coils to illustrate the position of hydrophobic amino acids [43]. The amphiphilic rod domain of desmin consist of four coils namely coil 1a, coil 1b, coil 2a and coil 2b. To generate the helical wheel representations of the desmin coils, full amino acid sequence of coil 1a and coil 2a and, the hydrophobic regions according to Kyte–Doolittle [41, 42] of

coil 1b (208–230 amino acids) and coil 2b (385–400 amino acids) was used (Supplemental Figure 3). As shown in Figure 3, hydrophobic regions of desmin coils are directed inwards in such a manner that as if they form a corridor (Figure 3B, Supplemental Figure 3). These regions hidden inside the coil, can be exposed to interact with nups during translocation through the NPC.

Discussion

Presence of cytoskeletal elements in the nucleus has long been a subject of discussion within the scientific community. While they act as sensors and transmitters of extracellular signals in cytoplasm, their transportation into the nucleus plays a crucial role in regulation of the cellular responses. The nuclear/cytoplasmic localization of the shuttling proteins changes perpetually to adapt and can be affected by several factors from their partners (interaction with transport proteins) to cell cycle stage (proliferation/differentiation). Our previous work revealed a physical link between desmin and nuclear lamin B [11, 14, 44], which led us to investigate the extent of this relationship. Following the co-precipitation of desmin and nup214, we conducted proteomic analysis to further explore these interactions. The analysis of proteomic data revealed multiple interactors of desmin, many of which were found to be localized at the NPC. Notably, one of these interactors was another FG nup, nup153. Using various interaction databases, we generated a protein interaction map which identifies nup88 as a binding partner for desmin. Nup88 is another FG nup located at the cytoplasmic site of the NPC. These findings support the notion that desmin's nucleocytoplasmic transport can be regulated through direct interactions with nups.

Intriguingly, members of the signal transducing family, Smad2, Smad3 and Smad4 [45, 46] can be exported from nucleus in a karyopherin-independent manner. The distinguishing feature of these proteins is the presence of a hydrophobic corridor that allows direct interaction with nup214 and nup153, facilitating their nucleocytoplasmic transport. Our initial hypothesis on desmin's interaction with nups was centered around the nuclear import mechanism. However, various studies in the academic literature indicates that nup214, nup153 and nup88 are take part at the nuclear export of proteins [45, 47, 48]. Based on this information it is rational to consider that these interactions are related to nuclear export of desmin and maybe reevaluate the functionality of the potential NLS on desmin [13]. Yet, the possibility of nuclear import via direct interaction with nups for nuclear translocation of desmin should not be overlooked considering the functions of nup153 in nuclear import [49]. It has been shown that nup153 can directly interact with a transcription factor to facilitate its nuclear import [49]. Although desmin has a potential NLS, as suggested by Hobbs (2016), a protein can use alternative transport pathways to increase its adaptive skills and adapt to different physiological conditions [13].

Taken together, these results support the idea that a karyopherin-independent nucleocytoplasmic transport mechanism is a strong candidate to explain desmin's nucleocytoplasmic transport.

Since the quest for identifying desmin's nucleocytoplasmic transport started with the identification of its nuclear partner, lamin b, in skeletal myoblast, we first investigated the cellular distribution of desmin in skeletal myoblast. In accordance with the literature, we observed that desmin localizes in the nuclei of human skeletal myoblast. This research presents, for the first time, that desmin can be found in the nucleus of human skeletal myoblasts.

Hydrophobicity of a protein specifies its solubility, pre-disposition to aggregate and even protein–protein interactions. Surface hydrophobicity is one of the major factors contributing to the selectivity of the NPC. Previous studies showed that proteins capable of adapting to a hydrophobic environment are more likely to pass through the NPC [1, 45, 46]. Surface hydrophobicity analysis showed that with the increasing concentration of alcohol, desmin appear to be capable of revealing its hydrophobic residues. Returning to the question posed at the beginning of this study, it is now possible to state that desmin can reveal its hydrophobic regions to pass the selectivity barrier of the NPC.

We have showed that desmin has the ability to undergo conformational changes, yet further research is required to confirm whether this ability of desmin is applicable in

cellulo. Additionally, the potential NLS on desmin should be investigated to fully understand the nuclear transport mechanism of desmin.

Numerous published studies have described the role of desmin in the nucleus, suggesting that 'nuclear' desmin is a key player in myogenesis. Desmin's functions include transcriptional regulation [3], signal transduction, transportation of myogenic factors to the nucleus, or modulation of chromatin conformation [34, 50]. Preliminary results from another ongoing study of ours, focused on determining the cell cycle stage of desmin's nuclear translocation in skeletal myoblasts show that desmin localizes in the nucleus during early myogenesis (unpublished data), further reinforcing findings from previous studies.

Understanding the nucleocytoplasmic transport of desmin can broaden our perspective on how signals are sensed and transmitted by myoblasts, as well as the permeability limitations of the NPC.

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Research ethics: The research related to animals' use has complied with all the relevant regulations and institutional policies for the care and use of animals (Hacettepe University Animal Experimentations Local Ethics Board, 2014/07-08).

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

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