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ARTICLE ADDENDUM

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Molecular motors and nuclear movements in muscle

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ABSTRACT

Muscle fibers have the particularity of containing numerous nuclei evenly distributed and positioned next to the plasma membrane. This unique disposition is the result of sequential events of nuclear movements that start when myoblasts fuse together and end with the clustering of few nuclei under the neuromuscular junction. Nuclei are mispositioned in multiple muscle disorders therefore the mechanisms of nuclear positioning can be novel targets for muscle disorders therapies. The 2 first nuclear movements that occur upon myoblast fusion require different microtubule motors. We performed a siRNA screen against all the microtubules motors and quantified nuclei behavior after fusion and inside the myotube. The different motors we found to be involved in the nuclear behaviors and the analysis of motors expression suggest a competition between both movement mechanisms, which potentially relies on the discrepancy between myoblast and myotube microtubules stability.

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Nuclear positioning in muscle fibers involves 4 successive nuclear movement events.[1] The 2 initial steps, centration and spreading, involve the microtubule cytoskeleton and associated motors.[2,3] It is noteworthy that the microtubule network is profoundly modified during muscle cell differentiation as its organization switches from being centrosome to nuclear envelope-based.[4] This rather unique feature creates an anti-parallel array of microtubules between nuclei on which proper nuclear movement relies. In the attempt to better understand the mechanisms involved, we performed a siRNA screen against all known microtubule motor proteins together with live imaging of the muscle cell line C2C12 and quantified nuclear behavior during centration and spreading movements. Our primary focus has been on nuclear spreading and we showed that multiple motors are involved.[5] Kif5b, an ubiquitous microtubule (+) end kinesin, is particularly important as its downregulation affects speed, time in motion and alignment of nuclei in the multinucleated myotube. Here, we further show the differences between centration and spreading movements after knockdown of microtubule motors affecting these movements, and compare their respective expression during cell differentiation (Fig. 1, Table 1). Interestingly, by analyzing the expression profile performed by Chen and colleagues[6] (GDS2412), we found that the expression of several motors involved in nuclear movement, and particularly Kif5b, are upregulated during differentiation supporting their role in nuclear movements during muscle fiber formation. We and others have shown kif5b implication in moving the nucleus by cross-linking and sliding anti-parallel microtubules between nuclei as well as through its localization at the nuclear envelope to rotate nuclei along microtubules.[3,7] Interestingly, Kif5b is not involved in the initial, faster centration movement; a movement that relies almost exclusively on the microtubule (–) end motor Dynein,[2] also localized at the nuclear envelope (Fig. 1, Table 1). After fusion, the nucleus from the myoblast moves rapidly toward the closest nuclei cluster in the myotube. It is therefore tempting to propose the existence of a competition between the mechanisms involved in centration and spreading, a possible tug-of-war.[8,9] Compliant with this hypothesis is that absence of Dynein reduces nuclear speed of centration but increases significantly the speed of nuclear spreading inside the myotube (Fig. 1, Table 1).

The molecular motors involved in one nuclear behavior, centration vs spreading, are usually not involved in the other, except Dynein heavy chain, supporting a complete separation between the mechanisms regulating these movements. A particular kinesin, Kif13b also known as GAKIN, is upregulated upon differentiation.

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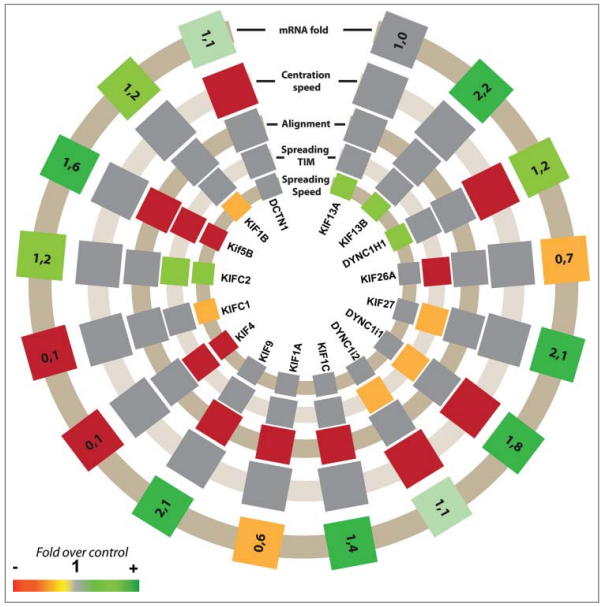

Figure 1. Schematic representation of the effects of knock-down of 16 microtubules motors on nuclear behaviors and their mRNA fold changes after differentiation. The color coding indicates an increase or decrease compare with a control situation as shown on the bottom left. Spreading speed corresponds to the speed when nuclei are in movement. Spreading TIM: Time In Motion during the spreading movement; in control situation, nuclei spend 55% of the time in movement. Alignment: In a control situation, 70% of myotubes have aligned nuclei. Centration speed: nuclear speed between the site of myoblast fusion and the first myotube nucleus.

However, its downregulation by siRNA induces an increase in nuclear movement within myotubes suggesting that this kinesin might act as a brake in myotubes, even though it is a plus-end directed motor like Kif5b. Kif13b is involved in cargo transport in several polarized cells,[10–17] it could also be involved in anchoring microtubules at the cell cortex.[17] Its role in nuclear movement is yet unknown and might impact the distribution of specific proteins important for nuclear spreading. Similarly, Kifc2 is upregulated during differentiation (although to a lesser extent than kif13b) and its depletion increases nuclear speed and time in motion. Initially described as a neuron-specific kinesin,[18] its elevated expression has been observed in muscle tissues[19] (and in Human Protein Atlas) and can therefore represent another level of nuclear movement regulation in the differentiated muscle cells. Kifc1 shares the same KIFC consensus sequence with Kifc2,[18] and its expression decreases during differentiation and its depletion decreases nuclear speed. This is a rather puzzling result, where depletion of a protein that is downregulated during differentiation has a negative effect on nuclear movement. However, it can be explained by the existence of a long lived protein whose action is required during the first event of differentiation or by a key role that is exerted even with low levels of protein. Interestingly, Kifc1 is able to interact with Kif5b[20] and is found at the nuclear envelope in spermatids[21] and might therefore be implicated in Kif5b-dependent nuclear movement in muscle cells.

Kif4, whose depletion induces a strong decrease in nuclear spreading movement, is downregulated during differentiation.[6] However, it is noteworthy that Kif4 has been reported to be implicated in stabilizing microtubules in migrating fibroblast.[22] Stable microtubules are required for proper muscle cell differentiation,[23,24] and they are probably involved in the tug-of-war between centration and spreading. As proposed by Mian et al.,[23] the difference between a myoblast and a myotube could reside in the differential amount of stable -detyrosinated-microtubules. A fusing myoblast with more unstable microtubules will have less chance to participate in an anti-parallel microtubule array between the myoblast nucleus and myotube nuclei thereby favoring the centration movement. In addition to this hypothesis is the existence of a preference of Kif5b for stable microtubules.[25–29] Therefore, we propose a model where the centration movement relies on the stable microtubules originating from the myotube nuclei that bind to the myoblast nucleus through Dynein motor. This will pull the new nucleus toward the myotube nuclei. Then, as soon as this new nucleus will have stabilized microtubules, it will

Table 1. Values of centration and spreading speeds after silencing of the indicated motors, and their increase of expression from myoblast to myotube.

	CTR	KIF13A	KIF13B	DYNC1H1	KIF26A	KIF27	DYNC1I1	DYNC1I2	KIF1C
Centration speed (um/min)	0.757 +/– *0,035*	0.680 *+/–0,057*	0.712+/– *0,067*	0.539 +/– *0,051*	0.984+/– *0,075*	1.060+/– *0,079*	0.580+/– *0,035*	0.461+/– *0,058*	0.806+/– *0,083*
Spreading speed (um/min)	0.289 +/– *0,009*	0.388+/– *0,031*	0.359+/– *0,015*	0.268+/– *0,010*	0.258+/ *–0,011*	0.270+/– *0,009*	0.217+/– *0,006*	0.230+/– *0,011*	0.274+/– *0,011*
mRNA fold	1.000	1.004	2.233	1.212	0.725	2.098	1.769	1.126	1.394
	KIF1A	**KIF9**	**KIF4**	**KIFC1**	**KIFC2**	**KIF5B**	**KIF1B**	**DCTN1**	
Centration speed (um/min)	0.924 +/– *0,099*	0.809+/– *0,086*	0.957+/– *0,072*	1.024+/– *0,081*	0.640+/– *0,069*	0.831+/– *0,047*	0.617+/– *0,039*	0.566+/– *0,047*	
Spreading speed (um/min)	0.253 +/– *0,008*	0.282+/– *0,014*	0.227+/– *0,009*	0.241+/– *0,009*	0.388+/– *0,028*	0.182+/– *0,007*	0.268+/– *0,011*	0.215+/– *0,007*	
mRNA fold	0.635	2.130	0.133	0.126	1.228	1.641	1.210	1.099	

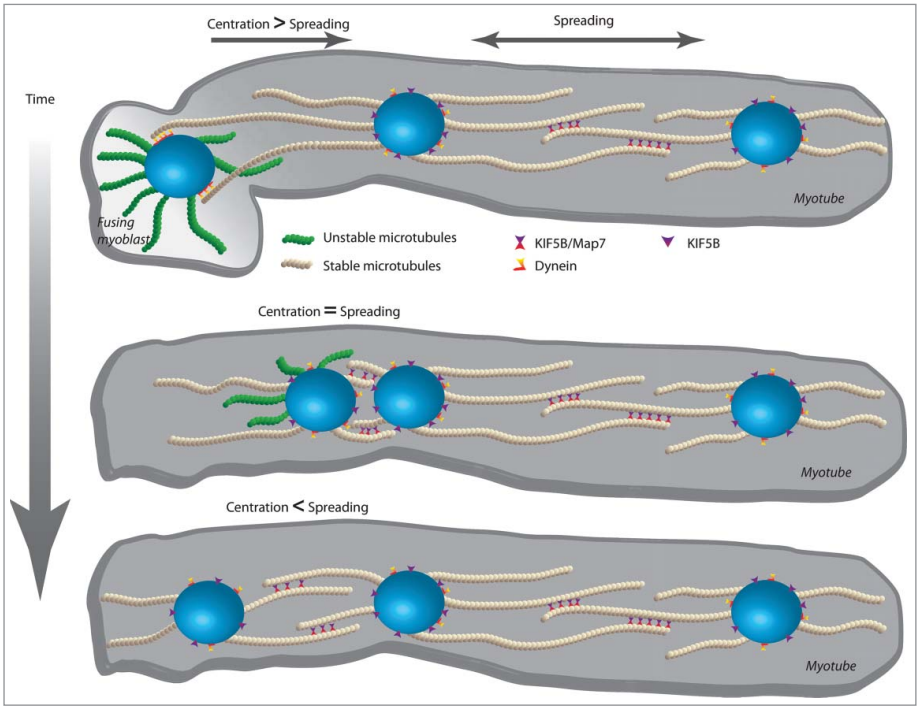

Figure 2. Model of the centration and spreading movements in the differentiating muscle cells. The minus end motor Dynein will promote the centration movement using longer and more stable microtubules emanating from myotube nuclei. With time the microtubules from the newly entered nucleus will become stable (acetylation, detyrosination), Kif5b expression will increase, and thus favor the spreading movement.

create an antiparallel array of microtubules with the other nuclei and separate from them through the spreading movement (Fig. 2).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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