Severe Myopathy Mutations Modify the Nanomechanics of Desmin Intermediate Filaments

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Received 16 July 2008; received in revised form 20 October 2008; accepted 30 October 2008
Available online 11 November 2008

Mutations in the intermediate filament (IF) protein desmin cause severe forms of myofibrillar myopathy characterized by partial aggregation of the extrasarcomeric desmin cytoskeleton and structural disorganization of myofibrils. In contrast to prior expectations, we showed that some of the known disease-causing mutations, such as DesA360P, DesQ389P and DesD399Y, are assembly-competent and do allow formation of bona fide IFs in vitro and in vivo. We also previously demonstrated that atomic force microscopy can be employed to measure the tensile properties of single desmin IFs. Using the same approach on filaments formed by the aforementioned mutant desmins, we now observed two different nanomechanical behaviors: DesA360P exhibited tensile properties similar to that of wild-type desmin IFs, whereas DesQ389P and DesD399Y exhibited local variations in their tensile properties along the filament length. Based on these findings, we hypothesize that DesQ389P and DesD399Y may cause muscle disease by altering the specific biophysical properties of the desmin filaments, thereby compromising both its mechanosensing and mechanotransduction ability.

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Keywords: atomic force microscopy; coiled-coil interactions; molecular slippage; desminopathy; myofibrillar myopathy

Introduction

Desmin is the major muscle-specific intermediate filament (IF) protein (for a review, see Ref. 1). Its sequence and expression pattern is evolutionarily well conserved from “primitive” fish to man.2 In myocytes, desmin IFs form a well-ordered three-dimensional extrasarcomeric cytoskeletal network involved in several cellular functions such as maintenance of Z-disc registration, positioning of the nuclei and mitochondrial activity.3,4 To date, more than 40 disease-causing mutations have been reported for the human desmin gene†. All these mutations are causing a distinct subgroup of myofibrillar myopathy, namely, desminopathy, which is characterized by disintegration of Z-discs and myofibrils as well as by the accumulation of desmin, α-crystallin, plectin, ubiquitin and other proteins into large intracellular aggregates.5 Patients become symptomatic around the second to third decade of life with affection of striated as well as smooth muscles.5 However, it is cardiac involvement, which results in dilated, restrictive or even hypertrophic cardiomyopathy as well as cardiac arrhythmias,6 that eventually causes death in these patients. The detailed molecular mechanism linking desmin mutations to the distinctive histological phenotype remains unclear. In in vitro assembly studies and in cell transfection studies, mutations in the desmin gene were found to lead to either (i) assembly incompetence, protein aggregation and segregation of mutant from wild-type desmin or (ii) the formation of mixed filamentous networks that seem very similar to those formed by wild-type desmin alone.8 Surprisingly, most of the desmin mutants analyzed to date fall into the second category; that is, they exhibit a preserved assembly competence.6,9 As both groups of desmin mutations ultimately lead to the same clinical and histopathological phenotype, this astounding finding calls for a better understanding of the potential impact of desmin mutations on structural and functional properties of the seemingly bona fide IFs formed by some desmin mutants.

Desmin, like other IF proteins, exhibits a tripartite secondary structure combining unfolded N- and C-
terminal domains with a double-stranded central α-helical coiled-coil domain interspersed in its middle by a non-α-helical flexible linker.11 In this study, we have focused on the biophysical characterization of three filament-forming desmin mutants, all harboring point mutations located towards the C-terminus of desmin’s central rod domain, namely, DesA360P, DesQ389P and DesD399Y.8 The in vitro assembly properties of these mutants have been analyzed extensively and compared to that of wild-type desmin.8,9 The assembly starter unit is a well-defined tetramer for DesA360P and wild-type desmin, whereas under identical buffer conditions, DesQ389P and DesD399Y form a heterogeneous population of larger complexes (Table 1).9 Although in vitro all three mutants form filaments on their own and in combination with wild-type desmin, all three mutants were found to differ considerably from wild-type desmin with regard to filament radius and mass per length (MPL) (Table 1).9

In order to prove a potential disease-causing mechanism for these filament-forming mutants, we thought to seek alterations in their biophysical properties and to correlate those with the previously observed structural differences (Table 1).9 To achieve this goal we have developed a single-filament approach based on atomic force microscopy (AFM).11 This method allows the characterization of the tensile properties of single filaments adsorbed to a solid support. A first set of experiments with wild-type desmin filaments indicated an unusual extensibility up to 240% extension associated with an abrupt increase of the mechanical stress necessary to stretch the filaments above 50–100% extension, a phenomenon also known as “strain hardening” or “strain stiffening”.12 This behavior was associated with a strong decrease of filament diameter upon stretching, as observed by AFM imaging of single stretched filaments and by electron microscopy (EM) of sheared filamentous networks.12

In this study, we now employed the same approach to investigate the desmin mutants described above (Table 1) and demonstrate that these mutants could be separated in two categories according to their tensile properties. Filaments formed by DesA360P behaved similarly to filaments from wild-type desmin, whereas filaments formed by DesQ389P and DesD399Y exhibited heterogeneous tensile properties along the length of individual filaments. Interestingly, this mechanical heterogeneity that we now observed at the single-filament level correlates with the fact that DesQ389P and DesD399Y form a heterogeneous population of assembly starter units that assemble into irregular filaments with segments of different widths and heights (Table 1).9

### Results

#### Morphology of mutant desmin filaments

At protein concentrations around 0.1 mg/ml used for previous EM investigations,9 the three mutants analyzed in this study formed extensive networks that did not adsorb well on the mica substrates used for AFM. However, by lowering the protein concentration to 0.05 mg/ml, we obtained populations of either short or aggregated mutant desmin filaments on the substrate (Fig. 1a–c). The former were suitable for subsequent nanomechanical analysis. DesA360P filaments exhibited a smooth height profile. However, they appeared knotted and some even ring-shaped (Fig. 1a, arrowheads and arrows, respectively). In contrast, filaments formed by DesQ389P and to a lesser extent by DesD399Y appeared irregular with segments of different widths and heights (Figs. 1b and c and 2a and b). The average height $H_0$ and the average full width at half maximum (FWHM) values for the different variants are compared to corresponding values for wild-type desmin filaments imaged under the same conditions (Table 1).12 Among the mutants analyzed, only filaments formed by DesA360P exhibited an average height significantly higher than that of wild-type desmin filaments, corresponding well to the average radius $R_n$ as previously estimated by scanning transmission EM (STEM).9

#### Tensile behavior of mutant desmin filaments

We used the AFM tip to push laterally on single filaments.12 For the three mutant desmin variants, we performed a total of 321 lateral pushing experiments with a vertical tip force fixed between 4 and 8 nN. In

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### Table 1. Structural parameters of desmin filaments

<table>
<thead>
<tr>
<th>Desmin</th>
<th>Wild type</th>
<th>DesA360P</th>
<th>DesQ389P</th>
<th>DesD399Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assembly starter units</td>
<td>Tetramers (A$_{11}$)</td>
<td>Tetramers (A$_{11}$)</td>
<td>Octamers and 12-mers</td>
<td>Octamers and 12-mers</td>
</tr>
<tr>
<td>$R_0$ (nm)</td>
<td>6.3±0.65</td>
<td>7.25±0.65</td>
<td>5.6±0.85</td>
<td>5.7±0.45</td>
</tr>
<tr>
<td>MPL (kDa/nm)</td>
<td>59±11</td>
<td>70±11</td>
<td>45±12</td>
<td>42±14</td>
</tr>
<tr>
<td>Height (nm)</td>
<td>4.5±0.8 (n=60)</td>
<td>6.7±2 (n=79)</td>
<td>5±1.1 (n=40)</td>
<td>5.1±1 (n=26)</td>
</tr>
<tr>
<td>FWHM (nm)</td>
<td>45±15 (n=60)</td>
<td>60±10 (n=79)</td>
<td>40±10 (n=40)</td>
<td>50±10 (n=26)</td>
</tr>
</tbody>
</table>

*a Assembly starter units formed by the proteins after refolding in 5 mM Tris–HCl, pH 8.4, 1 mM DTT as assessed by analytical ultracentrifugation.9

*b These assembly starter units were longer than typical A$_{11}$ tetramers, indicating the involvement of A$_{12}$ and A$_{13}$ interaction modes.9

*c Average radius of the filaments estimated from STEM data.9

*d Mass per length of the filaments estimated from STEM data.9

*e Average height of the filaments measured by AFM. Data for wild-type desmin were taken from a previous study where the protein was analyzed under the same conditions.12

*f Average FWHM measured by AFM. Data for wild-type desmin were taken from a previous study.12
more than half of cases, the AFM tip had stretched a short segment of filament that was left adsorbed to the mica surface (Fig. 2a and b). Overall, we obtained 145 usable stretching events (Table 2) that were divided into two categories based on morphology: (i) filament segments that were stretched as one branch (n = 45; Fig. 2a and c) and (ii) filament segments that were stretched into two branches of similar lengths (n = 100; Fig. 2b and d). Each segment had a different length $L_0$, with a minimal value of around 35 nm being close to the length of a desmin dimer. The maximal values were $\sim 140$ nm for wild-type desmin, $\sim 235$ nm for DesA260P, $\sim 300$ nm for DesQ389P and $\sim 230$ nm for DesQ389P (Table 2 and Ref. 12). The maximal extensibility as measured on the AFM images (Fig. 2c and d) was different for each mutant but always equal or superior to that observed for wild-type desmin filaments (Table 2). Whereas wild-type desmin was stretched to a maximum of 240%, DesQ389P exhibited the most extreme behavior with a filament that was extended up to 360% extension (Table 2).

For wild-type desmin filaments, stretching was associated with a decrease in the filament’s radius as monitored by AFM after each lateral pushing experiment via the ratio of heights after ($H_S$) and before ($H_0$) deformation (Fig. 3a). The ratio of heights decreased faster than expected from a constant volume approximation (Fig. 3a, broken line). A similar behavior was observed for the three desmin mutants (Fig. 3b–d). For each type of filament the minimal value of $H_S/H_0$, i.e., 0.2–0.3, was obtained for filaments stretched around 150% extension (Fig. 3a–d). Above 150% extension, $H_S/H_0$ varied between 0.2 and 0.4 for wild-

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**Fig. 1.** Typical contact mode AFM images of mutant desmin filaments before stretching. (a) DesA360P, filaments are knotted (arrowheads) and even ring-shaped (arrow). (b) DesQ389P and (c) DesD399Y; these filaments are short and have a tendency to aggregate into filamentous clumps. Scale bars represent 1 μm.

**Fig. 2.** Mutant desmin filaments, at a concentration of 0.05 mg/ml, were assembled for 1 h at 37 °C in 25 mM Tris–HCl (pH 7.5), 100 mM KCl and adsorbed to freshly cleaved mica. (a) Contact mode AFM image of a stretched DesQ389P filament where one branch is visible. (b) Contact mode AFM image of a stretched DesD399Y filament where two branches are visible. (c and d) Diagrams presenting the geometrical parameters measured on the AFM images after stretching. $F$ represents the force vector applied by the AFM tip to the filament. $\bar{F}$ was always parallel to the fast scanning axis and oriented from left to right. $L_1$ and $L_2$ are the lengths of the stretched branches. $\gamma_1$ and $\gamma_2$ are the angles between the branches and $\bar{F}$. $\delta L$ is the distance by which the filament was moved by the tip.
Electron microscopy of stretched mutant desmin filaments

A very effective way to analyze the substructure of stretched IFs is shearing of a dense filamentous network with a carbon-coated EM grid. The shearing process leaves numerous stretched filaments on the grid that are then negatively stained and imaged by transmission EM. For DesA360P, we obtained EM pictures clearly showing distinct subfilaments (Fig. 4a). The filaments demonstrated a drastic reduction in their diameter over several hundreds of nanometers of filament length and a decrease in the number of subfilaments down to only two per cross section (Fig. 4a, arrowheads). In that case, the diameter was reduced to about one-third of that of unstretched filaments (Fig. 4a, inset). For DesQ389P and DesD399Y filaments, we obtained networks before shearing where filaments had a pronounced tendency to cling to one another (data not shown). This finding was compatible with the behavior of these mutants as revealed by AFM at a 10-fold lower protein concentration (Fig. 1b and c). Again, most of the filaments were stretched as bundles and it was impossible to distinguish individual ones. Very often the major deformation was restricted to only short filament segments (Fig. 4b, arrowhead). These circumscribed alterations of the filament’s morphology in response to mechanical stress are an indication of a structural heterogeneity within the filament, leading to areas of definite “weakness” along the filament axis.

**Discussion**

The fact that mutations in the desmin gene can give rise to devastating muscle disease affecting skeletal as well as cardiac muscle is well recognized. Recently, we were able to show that in contrast to prior assumptions, it is not in general a simple assembly deficiency of the mutant desmin variants that gives rise to disease, but that in fact the mechanisms resulting in desmin aggregation are far more complex, at least for some mutants. Of the 27 desmin mutants analyzed to date, more than half are capable of filament formation in vitro and in living cells (Refs. 6, 8 and 14 and unpublished data). This surprising finding raises the question about the structural and functional properties of these mutant filaments and about the role of the desmin cytoskeleton for proper muscle function. In this context, we already documented that some mutations lead to profound alterations of the filament architecture (Table 1). However, the functional consequences of these architectural alterations regarding the filament’s mechanical properties were not yet analyzed.

**Comparative nanomechanics of mutant desmin filaments**

In this study, we have employed a simple nanomechanics approach to fingerprint subtle differences between wild-type and mutant filaments (Fig. 3). For

### Table 2. Tensile properties of desmin filaments

<table>
<thead>
<tr>
<th>Desmin</th>
<th>Wild type</th>
<th>DesA360P</th>
<th>DesQ389P</th>
<th>DesD399Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of stretching events</td>
<td>60</td>
<td>79</td>
<td>40</td>
<td>26</td>
</tr>
<tr>
<td>Range of L0 (nm)</td>
<td>35-140</td>
<td>55-235</td>
<td>45-300</td>
<td>45-230</td>
</tr>
<tr>
<td>$F_{\text{max}}$ (%)</td>
<td>3.4</td>
<td>290</td>
<td>360</td>
<td>250</td>
</tr>
<tr>
<td>of $T$ (nN)</td>
<td>(at 170%)</td>
<td>(at 160%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a** Data for wild-type desmin were taken from a previous study.12
- **b** For each stretching event $T$ is defined as $F_{\text{max}} \cos \gamma$ (see Materials and Methods).
- **c** Values correspond to the stretching event reaching $F_{\text{max}}$ or to another event at a smaller extension, indicated in parentheses.

Type desmin filaments (Fig. 3a), whereas the mutants exhibited a slightly wider variation, with some points being close to or even above the constant volume trend (Fig. 3b–d, broken lines).

In addition, we measured the lateral force on the AFM cantilever as a function of the tip displacement.12 As previously experienced with wild-type desmin filaments, a detailed analysis of individual force curves was not straightforward.12 Hence, we decided to focus on the behavior of the maximal force $F_{\text{max}}$ measured for each curve. In order to take into account the orientation of the filament branches with respect to the direction of the applied force (Fig. 2c and d), we computed the maximal tensile force $T = F_{\text{max}} \cos \gamma$. The data points were averaged with a bin size of 20% extension. For each bin, the mean and standard deviations are presented as well as the number of events per bin. For less than three events in a given bin, all the points are presented. The behavior of T as a function of extension enabled us to divide the mutants into two groups (Fig. 3e–h). For DesA360P filaments, T increased non-linearly with extension, indicating that these filaments require more and more tension in order to be stretched to a certain extension, the highest extensibility being 290% (Fig. 3f, broken line). This strain-hardening phenomenon is more obvious for DesA360P than for wild-type filaments, where less data points in the region between 150% and 200% extension provide evidence of the same behavior (Fig. 3e).12

In contrast, T varied randomly between 1 and 5 nN for DesQ389P and DesD399Y filaments over the whole extension range (Fig. 3g and h), indicating that different filament segments require different amounts of tension in order to be stretched to a given extension. For these latter two mutant desmin variants, the maximal tensile force was obtained for a filament stretched around 160–170% extension, a value well below the maximal extensibility (see Table 2). In addition, over most parts of the extension range recorded, the tensile force measured for these two mutants was equal or superior to the one measured for wild-type desmin filaments, i.e., between 1.0 and 5.0 nN (Fig. 3g and h).
Fig. 3. Tensile properties of wild-type (black), DesA360P (green), DesQ389P (blue) and DesD399Y filaments estimated from lateral pushing experiments. The plots of the wild-type data were adapted from Ref. 12. In each plot, one data point represents one stretching event. (a–d) The ratio of average heights before and after stretching $H_s/H_0$ as a function of the extension measured on the AFM images. In each case, the ratio decreases faster than expected from a constant volume approximation (broken lines). (e–h) Maximal tensile force $T=\max F \cos \gamma$ for each stretching event as a function of extension. The data points were averaged with a bin size of 20% extension. For each bin, the mean and standard deviations are presented as well as the number of events per bin. For less than two events in a given bin, all the points are presented. DesA360P filaments (f) show a non-linear behavior. The broken line is an exponential least-square fit through the data, $T=0.89 \exp(\varepsilon/163)$, $R=0.96$. In contrast, the two other mutants show a scattered plot (g and h) with no clear dependency between tension and extension.
In this purpose, we selected three filament-forming mutants that have been characterized in great detail with respect to their architecture. Based on filament morphology and assembly properties, these desmin variants can be separated into two groups: DesA360P, exhibiting an almost normal in vitro assembly, and DesQ389P and DesD399Y, with slight alterations of the morphology of the in vitro assemblies (Table 1). Wild-type desmin and DesA360P filaments differ by their average filament radius $R_0$ (Table 1) but their tensile behavior (Fig. 3a, e and b, f) is similar. The tensile behavior of DesA360P filaments confirms the strain-hardening process observed with wild-type desmin filaments. Since the mechanical properties of wild-type desmin filaments depend on their distinctive architectural design, we can assume that the overall intrafilamentous architecture is conserved between wild-type and Des A360P filaments. In other words, dimer–dimer as well as tetramer–tetramer interactions are supposed to be very similar for the two types of filaments in terms of strength and even spatial distribution.

The other two mutants, DesQ389P and DesD399Y, exhibit a smaller average radius $R_0$ compared to wild-type IFs (Table 1), but their filaments can withstand tensions well above the ones endured by wild-type desmin filaments for extensions smaller than 200% (Table 2 and Fig. 3g and h). For DesQ389P, filament segments stretched around 150% extension under tensions between 1.0 and 4.0 nN (Fig. 3g). Such a spread could be explained by structural variations along the filament length and by the static friction that increases linearly with the surface area of contact. However, the FWHM measured on the manipulated segments (Table 1) exhibits a narrow distribution. The same statement is valid for DesD399Y; thus, we propose that the observed variations in tension are mainly due to stronger intrafilamentous interactions in DesQ389P and DesD399Y filaments than in wild-type filaments. Since both mutations are located towards the end of coil 2B of desmin’s α-helical rod domain, DesQ389P and DesD399Y may exhibit more stable A12 and A22 dimer–dimer interaction modes than wild-type desmin. Interestingly, we previously observed that these mutants form elongated assembly starter units, most likely containing A12 and A22 interaction modes, in a buffer with low ionic strength and high pH where wild-type desmin only forms A11 tetramers (Table 1). It is also noticeable that for both mutants, the highest tensile force was reached for filament segments stretched around 150% extension (Table 2 and Fig. 3g and h). This corresponds to the maximal extensibility of a double-stranded α-helical coiled coil. We therefore propose that within some filament segments, all the stretching force is directly transferred to the coiled coils that unfold. Any segment that is stretched above 150% extension must combine unfolding of coiled coils with an additional sliding mechanism of dimers against one another.

Desmin mutations might result in defective filamentous stretch sensors and transducers within muscle fibers

In skeletal and cardiac muscle, desmin filaments have been proposed to serve as a physical link...
between neighboring myofibrils during large deformations.\textsuperscript{16,17} In contracted skeletal muscle, short desmin IFs have been observed to be stretched between costameres and neighboring Z-discs in order to maintain their proper alignment.\textsuperscript{16} Based on these observations and on the spatial organization of the desmin network within myocytes,\textsuperscript{9} it is most likely that individual desmin filaments are constantly submitted to stretching stresses in \textit{vivo}.

In this context, our finding that two of the filament-forming mutants exhibit, \textit{in vitro}, heterogeneous tensile properties along their length may be a hint for potential mechanisms of disease development. Desmin filaments that run perpendicular to the myofibrils axis connecting Z-discs to costameres and other Z-discs\textsuperscript{3,18,19} will overstretched during contraction. Stretching could be a simple way of signaling large mechanical deformations to the nucleus where desmin filaments are attached to the outer nuclear membrane.\textsuperscript{3,4} If individual DesQ389P and DesD399Y filaments do not strain-stiffen with higher tension but instead are able to extend longitudinally (Fig. 3g and h), a vital mechanical signal could be dissipated without intracellular transmission, thus omitting one potential mechanical feedback mechanism of the contractile apparatus. Based on the nanomechanical behavior of the three disease mutants, we propose that the wild-type desmin network may play an active role in mechanosensing as well as mechanotransduction rather than only being a passive structural cytoskeletal element. The development of a mouse model with a knock-in mutation, either DesQ389P or DesD399Y, could offer the opportunity to test this hypothesis \textit{in vivo}.

\section*{Conclusion}

Point mutations in IF proteins are linked to a wide range of human diseases. In some cases, the mutations do not seem to drastically affect filament assembly \textit{in vitro} or in cell transfection studies.\textsuperscript{6,9,14,20} We have demonstrated that the mechanical properties of desmin are finely tuned and that a single amino acid change can alter filament architecture and nanomechanical properties significantly. Although the result mutant desmin filaments are not brittle—they do not rupture or disintegrate upon stretching (Fig. 2a and b)—they nevertheless either overstretched at low force or stiffen prematurely at low deformation (Fig. 3). In these mutant filaments, we propose that the intrafilamentous architecture is altered in a way that significantly modifies the force-bearing filament properties.

These results call for a detailed nanomechanical analysis of the entire IF protein family, especially the keratins, as they exhibit an assembly plan different from that of desmin IFs and are expressed in tissues that are also subjected to great physical forces.\textsuperscript{20} It also urges the community to study the exact role played by the various IF networks in mechanosensing and mechanotransduction throughout the human body.

\section*{Materials and Methods}

\subsection*{Desmin preparation and assembly}

Murine recombinant desmin mutants DesA360P, DesQ389P and DesD399Y were obtained as described and stored at \texttext{-80}°C in DP buffer (5 mM Tris–HCl, pH 8.4, 1 mM DTT) containing 9.5 M urea, 1 mM ethylenediaminetetraacetic acid, 0.1 mM ethylene glycol bis(\textbeta-aminoethyl ether) N, N\textprime-,tetraacetatic acid and 10 mM methyl ammonium chloride.\textsuperscript{21} The day before use, the protein was diluted to 1 mg/ml with the above storage buffer, followed by stepwise dialysis at room temperature into DP buffer containing 8, 4, 2, 1 and 0 M urea. Dialysis was continued overnight at 4 °C into fresh DP buffer without urea. Under this regimen, DesA360P forms stable tetramers in DP buffer, whereas DesQ389P and DesD399Y form higher order oligomeric structures.\textsuperscript{7} For AFM experiments, assembly of murine desmin was induced by adding an equal volume of 45 mM Tris–HCl (pH 7.0), containing 200 mM NaCl to a DP solution containing 0.1 mg/ml desmin. The final protein concentration was 0.05 mg/ml. After 1 h at 37 °C, assembly was terminated by adsorption of a 10-μl aliquot to a freshly cleaved mica disk for 5 s, followed by washing with 25 mM Tris–HCl buffer, pH 7.5, containing 100 mM NaCl. For EM, assembly of desmin was induced by addition of an equal volume of 45 mM Tris–HCl buffer, pH 7.0, with 100 mM NaCl to a desmin solution at a concentration of 1 mg/ml in DP, followed by incubation at 37 °C for 1 h.

\subsection*{Electron microscopy of sheared IF networks}

After assembly at a final protein concentration of 0.5 mg/ml, a 10- or 20-μl drop of mutant desmin filaments was adsorbed to a Teflon surface. A glow-discharged carbon-coated copper grid was placed on top of the drop and pulled horizontally 30 mm in 10 s in order to totally shear and rupture the desmin network. Then the grid was removed, washed with water and stained with 2% uranyl acetate. EM pictures were recorded with a Hitachi H-8000 transmission electron microscope (Hitachi, Ltd, Tokyo, Japan) operated at 200 kV.

\subsection*{Atomic force microscopy}

All samples were imaged in contact mode in liquid using a Nanowizard AFM (JPK, Berlin, Germany) with a close-loop scanner. The rectangular silicon cantilevers were 220 μm long, 20 or 45 μm wide, with an oxide coated copper grid was placed on top of the drop and pulled horizontally 30 mm in 10 s in order to totally shear and rupture the desmin network. Then the grid was removed, washed with water and stained with 2% uranyl acetate. EM pictures were recorded with a Hitachi H-8000 transmission electron microscope (Hitachi, Ltd, Tokyo, Japan) operated at 200 kV.
Stretching protocol

Before laterally pushing on single mutant desmin filaments adsorbed to mica in buffer, we imaged an area of 100 or 400 μm² at a constant vertical force of 0.1 nN and a scanning speed of 1–2 Hz in order to preserve the filament architecture. The scanning angle was chosen such that the rectangular cantilever was perpendicular to the fast scan axis. Then the tip was withdrawn and stretching of single filaments was performed using the stored AFM image as a reference. This was possible due to the close-loop scanner that allowed us to reliably address every pixel of the images. For each stretching experiment, the tip was moved from left to right along a horizontal line crossing a desmin filament at a speed of 50 nm/s. During the experiment, the vertical force applied by the cantilever to the surface (the vertical deflection) was fixed between 4 and 8 nN in order to laterally push the filament. We have shown in a previous study on wild-type desmin filaments that a vertical force of at least 3 nN was necessary to physically move the filaments on mica under the buffer conditions described above. For each experiment, the vertical and lateral deflection of the cantilever and the height with respect to the surface were recorded as a function of the tip position at a sampling rate of 100 Hz. Upon completion of a lateral pushing experiment, the event area was imaged again at a constant vertical tip force of 0.1 nN and a scanning speed of 1–2 Hz in order to document the “irreversible” deformation caused by the lateral pushing event previously mediated through the action of the AFM tip.

AFM image analysis

Analysis of the AFM images was performed with Image SXM2. When a segment of filament was stretched to yield either one or two branches (Fig. 2a and b), we measured the filament displacement by the tip δl, the average height of the stretched branches Hν, the average height of the unstretched filament H0, the length L0 of the filament segment prior to deformation, the lengths L1 and L2 of the stretched filament branches, and the angle γ between the filament axis and the tip scan line (Fig. 2c and d). When the filament was stretched to yield two branches (Fig. 2d), we measured the two angles γ1 and γ2 and calculated the effective cos γ = (cos γ1 + cos γ2)/2. In cases where the two branches had different heights Hν, we always used the smallest value. The extension of a stretched filament segment was calculated as ε = 100 × [(L1 + L2)/L0 − 1]%.

Lateral force-displacement curve analysis

In parallel, the corresponding lateral deflection trace was analyzed using Origin 6.0. The lateral deflection was transformed into a lateral force using the lateral sensitivity and the lateral spring constant (see the section on Atomic force microscopy). The friction between the tip and the surface gave rise to a background noise with average amplitude 0.4–0.5 nN that was fitted by a straight line and subtracted. The initial contact point between the tip and the filament was estimated on the lateral force trace and used as origin. Then the lateral force traces were smoothened using a five-point averaging algorithm. The filament displacement by the tip, δl (Fig. 2c and d), was estimated a second time along with the maximum lateral force applied to the filament, Fmax. The values for δl (i) directly estimated from an AFM image of a stretched filament segment and (ii) determined from the corresponding lateral force versus tip displacement curve were very similar (data not shown). This indicated that little relaxation of the stretched filament segment occurred after lateral pushing by the AFM tip. Hence, the extension ε measured on the image is a good approximation of the maximum length increase experienced by a given filament segment.

Acknowledgements

We thank Ueli Aebi and Harald Herrmann for their constant support during the course of this study and for their comments on the manuscript. L.K. was supported by a grant from the Swiss Society for Research on Muscular Diseases awarded to Ueli Aebi and Serguei Strelkov. H.B. acknowledges a grant from the German Research Foundation (DFG; BA 2186/2-1).

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† http://www.liv.ac.uk/~sdb/ImageSXM/