Novel Polymorphism of RecA Fibrils Revealed by Atomic Force Microscopy

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Abstract. RecA fibrils in physiological conditions have been successfully imaged using Tapping Mode atomic force microscopy. This represents the first time images of recA have been obtained without drying, freezing and/or exposure to high vacuum conditions. While previously observed structures – the monomer, the hexamer, the short rod – were seen, a new type of fibril was also observed. This protofibril is narrower in diameter than the standard fibril, and occurs in three distinct morphologies: aperiodic, 100-nm periodic, and 150-nm periodic. In addition, much longer rods were observed, and appear curved and even circular.

Key words: RecA, recombination, assembly, AFM

The protein recA and its associated analogues are critically involved in a number of biological processes, centering around strand exchange activity: in the model organism *E. coli*, it serves as an essential enzyme in both the SOS mutagenesis pathway and in homologous recombination [1, 2]. There has been renewed interest in this prokaryotic protein due to the discovery of many analogues in a wide diversity of systems, e.g., Rad51 in eukaryotes [3], and pk-REC in archaea [4]. These analogues were shown to perform much the same function in these organisms as recA does in *E. coli*. In addition, recA has a strong sequence homology to typical motor proteins, such as the F₁ATPase, and dmc1 [1]. High concentrations of aggregated recA have been found in the bacterial spliceosome – a postulated cellular centre for genetic repair and recombination [5]. Hence, the study of the recA protein has assumed an increased importance, and a multitude of different techniques have been employed in order to elucidate its structure and function.

The atomic structure of the *E. coli* recA/ADP complex has been resolved by X-ray crystallography to 2.3 Å resolution, showing the atomic monomer structure, and proposed dimensions for the hexamer structure [6]. Electron microscopy (EM) [7–12], scanning tunnelling microscopy [13, 14], light scattering (LS) [15] and small angle neutron scattering (SANS) [16] have been utilised to show the gross morphology and aggregation of recA monomers with each other, and with DNA. A variety of structures have been observed: hexamers, short rods, bundles of rods

and nucleofilaments. Light scattering [7, 15, 17, 18] studies have been employed to follow the assembly, postulating that the hexamers and rods are intermediates in the formation of the nucleofilaments. From EM studies, it was ascertained that the diameter of the recA filament is 12 nm, and that the filament has a periodicity of 8 nm [7, 11, 19, 20].

The aggregation states of this protein are of great importance in determining the relevant pathways of physiological function, and what states of recA exist to interact with other constituents of the bacterial cell. These aggregation states have been probed with a variety of techniques. Structural data has been primarily gathered by electron microscopy and light scattering, while further details have been elucidated via small angle neutron scattering, and confirmed with fast protein liquid chromatography (FPLC). The first LS study indicated the Mg^{2+} sensitivity of assembly, and models for the aggregation process show the presence of fibrils greater than 1 micron in length [15]. Early EM and LS investigations confirmed a Mg^{2+} requirement for the assembly of aggregates [7]. A 0.5 μM solution of recA assembled to a maximum of LS intensity in maximum 15 min. This assembly could be dissipated by the addition of ATP, and was minimally affected by the manipulation of buffer and the addition of NaCl. The protein was fixed, diluted, then stained and imaged via EM to show a predominance of monomers and hexamers at lower concentrations of recA, and the presence of elongated fibrils at higher recA concentrations, for the given short period incubation time (30 min). The length of the fibrils was not characterized in detail [7]. Further examination using quick freeze/deep etch EM found recA to collapse into bundles in the presence of spermidine [19]. FPLC found recA also to exist in distinct aggregation states [9]. The presence, and proportions, of the various aggregates were also shown to be dependent on the concentration of recA and the incubation temperature; the fibrils at the highest concentrations were typically up to 200 nm in length [21]. The fixation and immobilization process may have contributed to the shortened observed length of the recA fibrils, by breaking of the fibrils [21]. One single report has been made of the investigation of solution-like structures of recA aggregates by cryo-EM [20]. By examination of the pitch of the fibrils, the fixation techniques used in previous works were found to alter the results of the investigation. For example, fixation shortens the pitch of the recA fibrils by 20 percent [20]. The length and aggregation states were not described in detail. SANS data was best used to determine the pitch of the compact form of the recA fibril, which was found to be 70 to 75 Å [22]. LS provides model-based information on the solution structure of recA. Initial work found a biphasic kinetic model whereby recA first aggregates linearly, and then the linear aggregates collide to form bundles. This process occurs in the presence of Mg^{2+} , and is dependent on the concentrations of Mg^{2+} , recA, and temperature [17].

These results are not entirely consistent with one another. Particularly pressing is the observation of short (maximally 200 nm long) rods in EM preparations, that other experiments find to have greater length. In addition, a detailed LS study found three distinct aggregation states ($recA_{18}$, $recA_{42}$, and $recA_{18/42}$) never observed by

EM [23]. This report gave a very detailed investigation into the long term equilibrium aggregation state in the presence of Mg^{2+} . This has led to speculation that the conditions of EM sample preparation are slightly biasing to the outcome of the experiments [20, 21]. Clearly, the equilibrium aggregation state of the recA consists of six or more discrete variants.

While high resolution structural studies of recA exist, they are primarily conducted under high vacuum or dessicated conditions because of the nature of the techniques employed. In conjunction, studies that probe the aggregation states under physiological conditions, fail to provide precise details required to investigate the aforementioned issues. In order to remedy this situation, we perform atomic force microscopy (AFM) on solutions of recA aggregates in order to determine unambiguously the solution structure of the aggregates.

Over the last decade, atomic force microscopy (AFM) has been gaining momentum as a valuable tool for the observation of biological systems, precisely because of its ability to image biological materials under biological conditions – critically free of vacuums and desiccation. With the advent of Tapping Mode AFM (TM-AFM) in fluid, sample perturbation has been reduced further, and a number of high resolution imaging studies under conditions that mimic *in vivo* situations have been performed. For example, 2D crystal arrays of proteins have been examined for determination of protein conformation changes [24, 25], the number of sub-units in a protein structure has been resolved at sub-nanometer resolution [26], and the B-DNA double helix has been seen under physiological conditions [27].

In this paper we use TM-AFM to examine the recA protein and its aggregation states in solutions containing buffers that begin to replicate the cell's interior (neutral pH, and 0.1 M salt). Additionally, we introduce an extremely delicate process of sample preparation that eliminates all sample damage, allowing an accurate visualisation of the structures that exist in solution. By employing this methodology, we not only observed recA fibrils, similar to those seen previously by studying dried samples, but we also find other distinct types of recA aggregates.

Materials and Methods

SAMPLE PREPARATION

RecA protein was purchased from Sigma Chemical Company (Mississauga, Ontario, Canada), or New England Biolabs (Beverly, MA, USA), and used as received. No difference was found between the two sources. The protein was received in high purity as confirmed by multiple independent assays. The protein was spin-dialyzed using a 30000 molecular weight filter (Millipore corporation, Etobicoke, Ontario, Canada) until it reaches the pH of the desired buffer. Different imaging conditions were explored. The solution was buffered between pH 7 and 8 using various buffers (10 to 20 mM TRIS-HCl, HEPES, or MOPS, all from Sigma Chemical Company), and contained 10 mM MgCl_2 and 0.1 mM DTT, and sometimes, 0.1 mM

EDTA. Once dialyzed, the protein was maintained at the concentration at which it was obtained ($2 \text{ mg/ml} = 53 \mu\text{M}$), and stored at 4°C for no more than 2 days before use. Samples were then diluted (from 1 to 1000 times, $50 \mu\text{M}$, $5 \mu\text{M}$, 500 nM , 50 nM) to the desired concentration for imaging about one to six hours before a $60 \mu\text{l}$ aliquot was spread on a 1 cm^2 piece of freshly cleaved mica (Ted Pella). The sample was allowed to reach thermal equilibrium in a high humidity environment until it was imaged between one and 48 h later. Optimal imaging conditions typically occur in three to six hours. Since imaging occurs such a long time after deposition, this allows fibril formation without incubating the sample at 37°C .

AFM IMAGING AND MEASUREMENTS

All imaging experiments were performed with an extended multimode Nanoscope IIIa (Digital Instruments, Santa Barbara, CA, USA) operating under TM in fluid. A triangular Si_3N_4 with a nominal spring constant of 0.06 N/m was used and oscillated at a resonance peak between 16 and 36 KHz. The sample was imaged in a fluid cell (Digital Instruments, Santa Barbara, CA, USA) but without that O-ring that makes the seal. Instead, the edges of the mica square were surrounded by a thin layer of Teflon tape, which held the sample droplet in place. If necessary, buffer was allowed to flow into the cell using a hose and syringe attachment.

Measuring the dimensions of any object by AFM is simple and routine. By examining a single scan line of an image, one can determine the difference in height of two objects on the same line with a resolution of $0.1 \text{ \AA}$. Unfortunately, the lateral dimensions of these same objects are subject to some convolution. This is mostly due to a well documented tip-broadening effect [28]. In our studies, we utilise the high accuracy of the height measurement for our determination of a fibril's diameter, assuming it is perfectly round in shape. This removes the need to deconvolute the image to obtain fibril diameters from the fibrils lateral dimensions. Since we rely on this measurement only in a single direction, we must be sure of its accuracy – as TM-AFM in fluid has been known to introduce artefactual measurements. In order to do this, close attention was paid to the parameters of tapping in commonly accepted methods of minimising height artefacts [29]. This being the case, our measurement's precision can vary between images. In general, the error in our measurements ranges from 0.5 to 2 nm , mostly due to a difficulty in ascertaining where the baseline sits.

Results

AFM imaging of recA in solution is more difficult than imaging dried samples, but provides more physiologically relevant information. While the AFM is capable of imaging materials under solution, it can only do so if the sample of interest remains reasonably immobilized at the substrate/solution interface. Thus, the first

challenge in any solution imaging is to determine the conditions that will enable this. In general, one wishes to obtain images of the protein at neutral pH and physiological ion concentrations. Unfortunately, the caveat that the sample needs to be at least partially immobilized on the surface often limits the ease of finding these conditions. For example, by increasing the pH one unit on either side of a protein's isoelectric point, one can drastically alter the net charge of the protein. This can lead from no surface coverage to complete surface coverage over a very limited pH range. In addition, the ions in solution play a large role in the interaction between the protein and the surface upon which it must sit. Depending on the concentration of the ions, the protein may see anything from a fully negatively charged mica surface, to a fully screened neutral surface. The second imaging issue to address is the reproducibility of the experiment. The results reported in this paper have been observed on multiple occasions, and occur regardless of the commercial source of pure protein.

In order to generate a sampling that is truly typical of the solution, the sample should be attached to the imaging substrate without any perturbation. This was achieved by introducing a novel method of AFM sample preparation whereby the sample solution is incubated over the substrate, and allowed to slowly deposit over time. The sample is then imaged under the mother liquor – no rinsing, no drying, no fixation, and no change in conditions. By employing this method, the sample reaches an equilibrium in solution; then, as evidenced by the images, a good sampling of the aggregates in solution deposit on the mica surface for imaging. In order to maximise the sample contrast, different buffers and ions were added to the solution at millimolar concentrations; this did not affect the types of aggregates observed.

We were able to observe recA protein under a small range of ionic strengths (10 to 20 mM) and neutral pH buffers (MOPS, HEPES, or TRIS). The results obtained were similar for experiments conducted under physiological strength (PBS), and for those performed at lower salt and buffer concentrations. However, due to the great technical difficulty of interpreting our images under true physiological conditions, images acquired in a more controlled reproducible manner are used for this paper. A typical image, in Figure 1, shows fibrils of 12 nm diameter and protofibrils of 6 nm diameter, along with a large number of monomers, hexamers and various non-specific aggregates in the background. Notice the smearing that is present in the image. This serves to emphasize one of the difficulties of AFM imaging of proteins in solution: the proteins that are loosely immobilized to the substrate can be moved or dislodged easily, while those in solution may adhere to the probe tip, effectively distorting it. The resultant smearing and other tip artefacts are typically more pronounced than under ambient conditions and are subject to some interpretation. Our interpretations are discussed in the Methods section. In addition, changes in the conditions of the solution can serve to make the imaging more difficult, which we found to occur at salt concentrations higher than 100 mM. This is attributable either to a decrease in interaction between the protein and the surface, or an increase in interaction between the protein and the probe tip.

(A)

(B)

Figure 1. Atomic force micrographs of recA under physiological conditions. Contrast is generated by topography – with brighter being higher and darker being lower. Four distinct morphologies are shown: the fibril, the protofibril, the hexamer and the monomer. In addition, there are a large number of non-specific/specific aggregates which may be intermediates in the assembly process. The fibril is 12 nm in height. The protofibril is 6 nm in height. Scale bar = (A) 1000 nm, (B) 500 nm.

Under the buffer conditions of the experiment, the recA structures we observed can be classified into five categories: (i) the *monomer*, with cross-sectional area of 3 nm^2 ; (ii) the *hexamer*, a ring-like structure which is 12 nm diameter and 6 nm high; (iii) the *fibril*, which is 12 nm in diameter and hundreds of nm to μm in length; (iv) the *protofibril*, which is fibrillar in nature and of smaller diameter than the fibril, and microns in length; and (v) other small aggregates, ranging in dimension up to 20 nm in diameter, and 150 nm in length. The first three structures – monomer, hexamer and fibril – have been reported and discussed in previous literature [1], while the last two have not been directly observed, although there is some indirect evidence of their existence from LS, SANS and FPLC. They will be discussed in detail below.

The monomer and hexamer are identified by matching the dimensions with those reported from X-ray crystallography [6]. Small objects of the appropriate dimensions can be observed in the background of all of the images presented herein. Due to tip convolution effects, which effectively broadens an image, it is difficult to ascertain the exact structure of such small objects. However, the AFM has an excellent vertical resolution and we can determine the height of a sample above the substrate quite routinely. In our images, the typical observed height of 6 nm corresponds well with the thickness of a proposed hexamer, and a height of 1 to 3 nm is observed for monomers, as expected from X-ray studies [6]. However, the individual sub-units of the hexamer are not clearly defined, nor is a hole in the middle observed, as would be expected from the postulated hexamer structure [11]. This lack of fine resolution is likely a consequence of imaging conditions and tip size. Additionally, the force applied by the probe tip in TM-AFM is very slight, and may be inadequate to break through strongly-bound water layers.

Figure 2 shows a typical AFM image of the recA fibril. The fibrils were found to be 10 to 12 nm in cross-sectional height, and are curvilinear. These curved fibrils have a uniform diameter, and continue consistently for up to $6 \mu\text{m}$. In fact, fibrils shorter than $1 \mu\text{m}$, whether straight or bent, are rarely seen, in slight disagreement with the EM results. In addition, no periodicity is resolved. EM studies have found the fibril to be of uniform 12 nm diameter, 200 nm in length, and possess a pitch of 8 nm [1].

In addition to the 12 nm fibril, many fibrils occur that are significantly smaller in diameter. For the purposes of this paper, these narrower fibrillar structures will be considered under the broad category “protofibril”. There are a variety of morphologies within this class, falling into two subcategories: aperiodic and periodic. The most common aperiodic protofibrils are of 6 nm diameter, which is exactly half that of the fibrils. Several examples can be found in Figures 1 and 3. Like the fibrils, the protofibrils can extend for quite a distance (over $3 \mu\text{m}$). Some of them, like those in Figure 1, are generally straight with a few bends. However, they can also be strongly curved, as illustrated in Figure 3. Examples of the two varieties of periodic protofibrils are shown in Figures 4 and 5, with either 100-nm or 150-nm period. As was the case for the fibrils, no 8-nm period was found among the protofibrils.

(A)

(B)

Figure 2. Solution topographic AFM images of recA fibrils. These fibrils are seen amongst a background of monomers and hexamers. The length of the fibrils extends up to $4\ \mu\text{m}$; the height is 12 nm. Fibrils with this length have not been observed by other imaging methods. Scale bar = 500 nm.

Figure 3. AFM images of recA circular-shaped fibrils, and protofibrils. Under similar conditions, these two morphologies of recA fibrils in solution have co-deposited with (A), and without (B) a background of hexamers, monomers, and other non-specific aggregates. Complete strands of the protofibril (6 nm height) are observed, along with strands of both fibril (12 nm height) and protofibril (as seen in cross-section (C)). Continuous fibrils double in height, as protofibrils form fibrils. (C) Cross-section of image (position indicated with line on image). Scale bar = 200 nm and the scan size is 1.0 μm .

Shown in Figure 3 are long curved/circular structures, some of which are 6 nm in diameter (corresponding to aperiodic protofibrils), and some are 12 nm (corresponding to fibrils). Notice the different forms present in this figure: completely closed circles are seen side-by-side with open full-sized circles, and some incomplete semi-circles and arcs. These curved forms exhibit a wide range of sizes, which correspond to fibril lengths ranging from 1 to 4 μm . Even if these were stretched out, these fibril lengths are much longer than expected from other reports.

Figure 4. (A) 100 nm period protofibril observed in buffer by atomic force microscopy. (B) The section emphasizes the periodicity of the fibril. This protofibril is approximately 6 nm in height. Scale bar = 250 nm.

The most fascinating detail visible in Figure 3 is the existence of fibrils that double in cross-sectional diameter. Here we observe several circles that are 6 nm on one side (corresponding to protofibril), and 12 nm along the other side (corresponding to fibril). This region would thus appear to be the nucleation point for conversion from protofibril to fibril. This type of structure, wherein an aggregate doubles in diameter, was observed in many different samples. We also noted that while complete fibrils of 6-nm cross-sectional height were observed regularly, a complete fibril with 12 nm cross-sectional height is more seldom seen. These observations lead us to infer that the doubling in cross-sectional height corresponds to the build up of fibrils, and not their disassembly.

RecA periodic protofibrils (i.e. protofibrils that show distinct long periodicities) are also observed regularly. We can classify them into two morphological classes:

Figure 5. (A) 150 nm period protofibril observed in buffer by AFM. (B) The cross-section emphasizes the periodicity. This striking fibril with high rigidity extended for over $10\ \mu\text{m}$ in length. The height of the nodules is 12 nm while the rest of the protofibril is only 4 nm tall. In the background, a number of aggregates can be seen. (C) A magnified view of the nodules shows that the fibril may be turning over itself at a regular interval. Size bar = 150 nm for figure and 75 nm for inset, (the image has been rotated 90° for clarity of presentation).

a short 100 nm period (Figure 4) and long 150 nm period (Figure 5) protofibril. These periodic protofibrils are clearly different than structures reported by EM: their dimensions and periodicities are quite distinct. In all the cases examined, the cross-sectional heights are significantly less than those of the protofibrils in Figures 3. The protofibril in Figure 4 has a period of ~ 100 nm, and is slightly less than 6 nm in cross-sectional height. The protofibril in Figure 5 has a longer period of ~ 150 nm, and a substantially smaller cross-sectional height of 4 nm, while the height of the nodules is 12 nm.

Both types of periodic protofibrils extend for several microns in length, and have been observed repeatedly in a number of samples. However, as one can see by comparing Figures 4 and 5, the environment of the long-period protofibril differs from that of the short-period one. The short-period protofibril (Figure 4) is found in a situation where there is a great deal of non-specific debris in the background, while the long-period protofibril (Figure 5) occurs with a relatively clean background with just a few monomers and hexamers.

We have seen the various protofibril morphologies by themselves, or accompanied by other structures, such as other types of protofibrils, fibrils and hexamers. However, there was no distinct correlation between the various imaging conditions used and the types of fibrils found: all structures could be seen in all salt conditions and all buffer conditions utilized. In some instances there may be some trends, however, such as the fact that increasing the allotted time for deposition and assembly to occur (thus causing an increase in the concentration of all the different types of structures) increased the surface coverage and obscured the details seen. Eventually, everything in the solution ends up adsorbing to the mica surface, and if samples are allowed to sit for a prolonged period of time (but still kept moist) complete surface coverage occurs. Under these conditions, one would expect the next level of assembly, the formation of recA bundles, to occur. We do not observe such structures, presumably because no details can be resolved with the AFM at this point.

Discussion and Conclusion

The AFM of recA fibrils in solution has confirmed some previously known facts, and gone on to demonstrate a wider polymorphism of the fibril. The classical picture of the recA fibrils derived from EM shows them to have a diameter of 12 nm, less than a micron in length – typically only two or three hundred nanometers [19, 30] – and possessing a periodicity of 8 nm. Our AFM results indicate that these fibrils can actually extend many microns (Figures 2), with a constant height above the substrate of 12 nm over such range. It is possible that this is due to a bias in the sampling, that is, only long fibrils adsorb to the mica substrate under the conditions of the experiment. However, we do not believe this to be the case, since in all cases we see the fibrils in conjunction with smaller aggregates such as hexamers in the background: if hexamers and longer fibrils can adsorb under these

conditions, then so should shorter fibrils. It may also be the case that, with such a long incubation time, the aggregates have grown to much longer sizes. In addition, the 8 nm periodicity is not observed. This is beyond a doubt a resolution issue. We believe that in buffer solution, the recA fibrils are surrounded by a tightly bound water layer. This water layer is impenetrable to the mild forces of a TM-AFM tip, obscuring the 8 nm periodicity. Unfortunately, any increase in the tapping force would result in loss of image quality, by dislocation of the recA from the surface, or destruction of the sample.

Similarly, the recA monomer and hexamer are also observed at dimensions known by X-ray crystallography and EM. For the hexamer, a height of 6 nm is observed, but all other information is lost. The lateral dimensions cannot be resolved due to tip-broadening effects, and the subunit structure remains obscured by tightly bound water molecules as in the recA fibril.

The structures that we call protofibrils have been directly observed for the first time. These protofibrils come in three distinct varieties: aperiodic (Figure 3), with a short period (Figure 4) and with a long period (Figure 5). The aperiodic protofibrils are usually 6 nm in cross-section, and always exactly half the diameter of any fibril present in the same image. They appear to share all the features of fibrils. We have found both fibrils and aperiodic protofibrils as straight or curved, and some even form circles. In Figure 3, we even see them coexist in the same aggregate. These observations suggest that there exists an equilibrium between these two structure types, and it is likely that the protofibril is a stable intermediate in the formation of fibrils. Why then are protofibrils not observed in EM studies? We can offer a few possibilities. They may be prone to quick disintegration during sample preparation, or their dimensions may be below the resolution of routine EM imaging, as has been alluded to in previous reports [20, 21]. A close examination of some reported EM micrographs of recA show that there are features that may correspond to protofibrils in the background, although they have not been discussed in the reports. Our attempts to perform EM have been unproductive: the samples were found to be quite sensitive to perturbations, including drying.

There is a wide body of evidence from non-imaging techniques that lend support to the existence of alternative aggregation states of recA. Light scattering studies have found alternative species that have been formed in the presence of Mg^{2+} ions [17, 23]. Additionally, FPLC measurements detect alternative states of aggregation that are not explained by results from EM [9]. Without corroboration from an imaging technique, the observation of these aggregates has been set aside in many reports on recA, these results take on a renewed importance in light of the true physiological result obtained here by AFM.

One observation common among the various EM micrographs of recA fibrils is their apparent rigidity. In previously published studies, the only images of recA-DNA complexes were curvy in nature, presumably dictated by the morphology of the plasmid DNA around which the recA protein wraps. Contrary to this, our AFM studies of recA without DNA show a variety of bent, curved and circular

fibrils (Figures 2, and 3). However, it should be pointed out that while we see many curved fibrils, the features manifested in Figures 2 and 3 do not necessarily imply a higher flexibility of the fibril. That is, unlike a flexible coil, the curved fibrils are mostly in rings or semi-circles, which are determinate structures, and in fact may be reasonably rigid, only in a different way. Furthermore, if one was to simply examine a short (200–300 nm) section of any of these curved fibrils, they appear the same as the short rods seen in the previous literature. The short-period protofibril, which has a larger diameter, appears to be slightly flexible, while the long-period protofibril is highly rigid. This is exactly opposite to what one might expect: for the same material, the larger-diameter fibril would be much less easily deformed. Thus, while we cannot determine ultrastructural details, their trend in rigidity implies that there are fundamental differences between the short- and long-period protofibrils.

The most puzzling aspect of the periodic protofibrils is the scale at which periodicity occurs. The monomer and hexamer units, which are postulated to be important intermediates to the fibril assembly, are only a few nanometers in any dimension. The observed periodicity of 100 or 150 nm are an order of magnitude larger by comparison. How can the sub-units maintain correlations this far? The simplest way is by a very slow twist propagating along the axial direction, as is observed in a number of examples [31]. However, we find no evidence for such a twist. In particular, examination of a longitudinal section along the long-period protofibril in Figure 5 shows that the protofibril may actually fold over itself at regular intervals for an apparently unknown reason. This is clearly demonstrated in the inset of Figure 5, in which we see a typical example of such an event.

The wide variety of polymer structure seen in all varieties of protofibril leads us to propose an additional step in the mechanism of formation of recA nucleofilaments. We propose that these protofibrils are a more versatile form of recA polymer. Their reduced height likely means a longer pitch than that of fibrils, conferring an added element of mobility. It is possible that all fibrils are actually in equilibrium with one another, and just at various states of a pitch extending/relaxing, waiting to encounter a nucleic acid molecule and begin the nucleofilament assembly process. Unfortunately, we have not been successful in directly observing this process. What we did find is that the structures reported here are no longer found when DNA is added to the mixture. Rather, we produce typical nucleofilaments of recA-coated DNA [32].

In the future, we hope to further exploit the abilities of the AFM to allow us to perform studies of recA/DNA filaments, eventually leading to a real-time study of homologous recombination under physiological conditions.

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