Direct Observation of the Assembly of RecA/DNA Complexes by Atomic Force Microscopy

Bernie D. Sattin and M. Cynthia Goh
Department of Chemistry, University of Toronto, Toronto, Ontario, Canada

ABSTRACT The formation of the RecA/DNA nucleofilament on nicked circular double stranded (ds) DNA in the presence of ATPγS was studied using the atomic force microscope (AFM) at nanometer resolution. The AFM allowed simultaneous observation of both dsDNA substrate and RecA protein-coated sections such that they are highly distinguishable. Using a time series of images, the complex formation was monitored. AFM imaging provided direct evidence that assembly of the nucleofilaments occurs via a nucleation and growth mechanism. The nucleation step is much slower than the growth phase, as demonstrated by the predominance of naked dsDNA at early and middle time points, followed by the rapid appearance of partially then fully formed complexes. Observation of the formation of nucleation sites without accompanying growth on unnicked dsDNA enabled an estimate of the nucleation rate, of 5×10^{-5} RecA min^{-1} bp^{-1}. The published model for the analysis of RecA assembly on dsDNA deduces a single kinetic parameter that prevents the separate determination of nucleation rate and growth rate. By directly measuring the nucleation rate with the AFM, this model is employed to determine a growth rate of 202 min^{-1}. These AFM results provide the first direct evidence of previous results on complex formation obtained only by indirect means.

INTRODUCTION

RecA is a 38-kDa globular protein with a multitude of different cellular functions. It is ubiquitous in nature, occurring in almost all bacterial species, and it has structural and functional analogs ranging from archaeal Prec to eukaryotic Rad51 (Lusetti and Cox, 2002). RecA plays an important role in homologous recombination, SOS mutagenesis and repair, and double strand break repair (Roca and Cox, 1997; Lusetti and Cox, 2002), and has been shown to be a molecular motor, with key structural homology to the F_1-ATPase (Egelman, 1998; Cox, 2003).

RecA is well-known to have the capability of forming complexes with itself (Cox, 2003). These oligomeric states include a hexamer, a rod, and bundles of rods (Cox, 2003). RecA protein can also readily bind single-stranded (ss) DNA by itself, or in the presence of an ATP cofactor, to form a nucleofilament (Dicapua et al., 1982). The ATP is not hydrolyzed in the process of binding, and nonhydrolyzable analogs of ATP can also be used to carry out the reaction, although it is a matter of debate if the complexes formed are exactly the same (VanLoock et al., 2003). In the presence of ATP or one of its many nonhydrolyzable analogs plus a small amount of a divalent ion (preferably Mg^{2+}), RecA can also form a nucleofilament with double-stranded (ds) DNA (West et al., 1980).

Electron microscopy studies found that the binding of RecA to dsDNA elongates the DNA by 50% (Dicapua et al., 1982). The complex is between 9.5 and 11 nm in diameter (VanLoock et al., 2003), with a right-handed helical pitch of 9.5 nm (Egelman and Stasiak, 1986). Small angle neutron scattering determined that the RecA/DNA complex in solution has a radius of 3.3 nm, with a pitch of 9.5 nm (Timmins et al., 1991). Using carbon nanotube tips, the atomic force microscope (AFM) found complexes with a 10 nm pitch, a height of 4.2 nm, and a width of 27 nm, whereas with a more common TESP probe (Digital Instruments, Santa Barbara, CA) an 11-nm pitch, 4.4-nm height, and 19-nm full width at half height was reported (Seitz et al., 1998).

Studies of the RecA/DNA system using spectrophotometric monitoring of ATP hydrolysis, DNase resistance, and light scattering provided indirect information about the kinetics of its formation, which was found to depend on pH, temperature, and ionic strength of the solution (Pugh and Cox, 1987). A nucleation and growth mechanism was proposed for the formation of the nucleofilaments. The kinetics of assembly are dependent on pH, temperature, and ionic strength of the solution, and the overall rates are faster at pH 6.11, and slower at pH 7.5 (Pugh and Cox, 1987). It was inferred that the growth phase occurs on a time scale that is fast enough, such that occurrence of further nucleation events is negligible (Schutte and Cox, 1987). Under high concentrations of RecA, the generally-accepted mechanism is as follows. RecA initially binds ATP. This RecA:ATP complex weakly binds to DNA and elongates it locally. This binding is the nucleation event, after which is the polymerization into the filament (Pugh and Cox, 1988). Studies of ATP hydrolysis indicate that the propagation step is unidirectional, with RecA monomers assembling on the 3′ end of the extending RecA/DNA complex, and dissociating from the 5′ end (Lindsley and Cox, 1990). The rate of dissociation is much lower than the rate of association, yielding a net rate of assembly as high as 20 monomers/s on linear dsDNA at pH 7.5 under saturating RecA concentrations. Since ATP is hydrolyzed by the

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Address reprint requests to M. Cynthia Goh, Dept. of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6 Canada. Tel.: 416-978-6254; Fax: 416-978-4526; E-mail: cgoh@chem.utoronto.ca.

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complex optimally at pH 6.2, it is expected that the dissociation rate will be higher at this pH. Using a dye displacement assay, the maximum half-life of the complex was determined to be 165 s in the presence of ATP (Zaitsev and Kowalczykowski, 1998). From the kinetics of the process, it was inferred but not proven that one single continuous RecA/DNA filament complex occurs instead of multiple short ones (Roca and Cox, 1990).

Experiments using optical tweezers on RecA binding to a single dsDNA molecule have also been performed. Bennink et al. (1999) monitored the elongation of DNA molecules by RecA in the presence of ATP, and found, but did not quantify, that the complex formed more quickly under an applied force. They reported that the complex dissociated slower under a higher applied force. The rate of filament growth increased with increasing applied force, up to 65 pN, the known point of conversion from B-DNA to S-DNA. At forces higher than this value, the polymerization continued to occur at a higher rate, but was more difficult to observe since the DNA is fully elongated at this point (Bennink et al., 1999). Shivashankar et al. (1999) compared the polymerization in the presence of ATP, and of ATPγS and found no difference in the force versus extension behavior of fully formed complexes. These complexes were found to have larger persistence lengths than naked DNA. In the presence of ATP, a nucleation rate of 10^{-6} nuclei min^{-1} $basepair^{-1}$ and a maximal growth rate of 12 monomers/s were reported. The observed linear behavior of the DNA extension with time was interpreted to mean growth proceeded from a single nucleus. In the presence of ATPγS, however, they reported that growth of the complex occurred from multiple (estimate = 6) nuclei. The kinetic model used in the analysis of this latter data did not allow for separate estimates of the nucleation and growth rates because their rates were deemed to be coupled.

In this article, we describe a detailed study of the time resolved formation and structure of RecA/DNA complexes using the AFM. The AFM is a powerful tool in biological studies because it allows high resolution visualization of systems with minimal sample preparation under a variety of relevant conditions, such as ambient, or under water or buffer. In the case of RecA/DNA, the AFM is able to resolve both protein and DNA. In this work, we perform AFM imaging on samples at time steps before the complete coverage of the plasmid DNA. From this time series, we are able to gain insight, and provide direct support for current views of the RecA/dsDNA assembly process. Further, our AFM study allows for the determination of the nucleation rate, thus we are able to explicitly determine the nucleation rate *and* growth rate using the kinetic model of Shivashankar et al. (1999).

MATERIALS AND METHODS

Reagents

RecA protein (*Escherichia coli*) was purchased (New England Biolabs, Beverly, MD) at a concentration of 2 mg/ml. The protein is supplied in high purity, free of contaminating nucleic acids and other proteins, as determined by multiple independent assays. A 20-μl aliquot was purified with a 100-kDa molecular weight spin filter. The glycerol was then removed, the buffer changed to the reaction buffer (10 mM Tris, pH = 7, 7mM $MgCl_2$, 100 mM NaCl) and the sample diluted 10 times with a 30 kDa spin filter. This diluted portion was used immediately, or stored for short periods at −20°C before use. ΦX174 RFII plasmid DNA was also purchased (New England Biolabs) at a concentration of 1 mg/ml. A 5 μl aliquot of this was added to 95 μl of storage buffer (20 mM Tris, pH = 7, 50 mM KCl, 5 mM $MgCl_2$, and 1 mM DTT) and stored for short periods of time at −20°C. ATPγS (Sigma Aldrich, St. Louis, MO) was solubilized and diluted in reaction buffer to 2 mg/ml. Mica sheets were from Ted Pella (Redding, CA).

Assembly of RecA/DNA complexes

RecA protein, ΦX174 DNA and ATPγS were mixed in various proportions, and complex formation was monitored by agarose gel electrophoresis using a mobility shift assay. As seen in Fig. 1, a ratio of 1 μl DNA solution, 6 μl ATPγS, and 8 μl RecA incubated at 37°C for 60 min resulted in the formation of complete RecA/DNA complexes, as evidenced by a mobility shift of the DNA bands; this composition was thus used in further experiments. The 0.6% agarose gel was run at 100 V for 130 min in TBE. It was then stained with a dilute ethidium bromide solution for 30 min, and destained with 10 mM $MgCl_2$ for 5 min. The stained gel was imaged using a UV illumination at 320 nm, and documented using a commercial gel documentation system (AlphaImager, Alpha Innotech, San Leandro, CA).

AFM imaging

After incubation at 37°C, 2 μl of the reaction mixture was added to 13 μl of deposition buffer (20 mM TRIS, pH=7.5, 5 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 2 mM $ZnCl_2$), then deposited of a 1 cm × 1 cm square of freshly

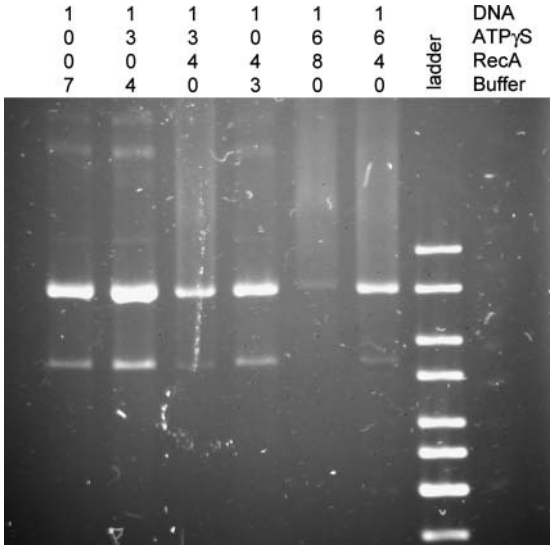

FIGURE 1 Mobility shift gel electrophoresis of RecA binding to DNA and various concentrations of RecA, and ATPγS. Complexes were incubated at 37°C for 60 min before addition to gel. Numbers above lanes indicate volume (μl) of DNA (0.05 mg/ml), ATPγS (1mg/ml), RecA (0.2 mg/ml), and buffer added. Kilobase marker ladder in right lane contains DNA of 10, 8, 6, 5, 4, 3.5, 3, and 2.5 kb. The 0.6% agarose gel was run at 12 V/cm for 130 min in TBE. Absence of a band in lane 5 at 8 kb indicates the formation of complete RecA/DNA complex.

cleaved mica. The deposition buffer was optimized to allow binding of complexes to the mica surface (Thomson et al., 1996). The solution was allowed to incubate on the mica for two minutes before mild rinsing with less than 5 ml deionized mOhm water, and drying for up to one minute with a stream of compressed nitrogen. Samples were inserted in a Nanoscope IIIa (Digital instruments, Santa Barbara, CA) or Solver (NT-MDT, Russia) AFM and imaged by tapping mode in air with beam-shaped silicon cantilevers (NT-MDT, Moscow, Russia). Imaging conditions were chosen to minimize sample damage and optimize image clarity.

AFM measurements

For contour length measurements, the AFM images with calibrated scale were imported into Scion Image (Scion Corp., Frederick, MD). The contour lengths of at least 10 separate RecA/DNA complexes were determined for each data point. Results are reported as mean ± SE. To ensure good sampling, complexes were selected from three unique samples. The results were consistent between different experimental runs.

For nucleation rate measurements the number of nucleation events was estimated by counting areas of high topographical density found on a topologically constrained plasmid. This value is divided by the incubation time and plasmid length that are known.

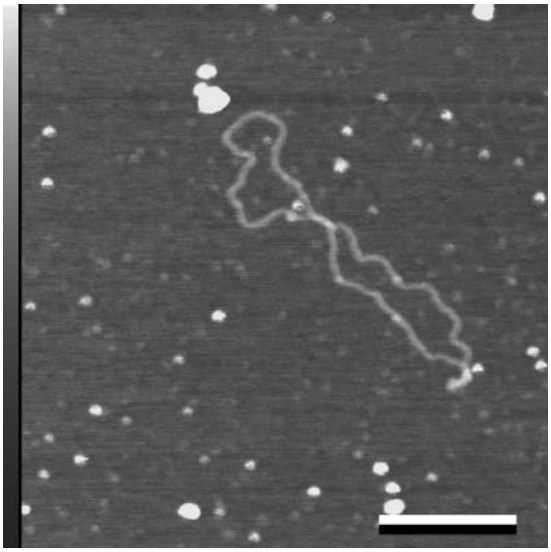

FIGURE 2 Only naked DNA were found after 0 and 15 min incubation time. Topographic image acquired in air: scale bar = 250 nm; *z* scale = 2 nm (*dark* to *light*).

RESULTS AND DISCUSSION

We examined the formation of the RecA/dsDNA complex as a function of time using the AFM. RecA was incubated with nicked double stranded plasmid DNA and ATPγS at 37°C, and aliquots were taken at 0, 15, 30, 45, and 60 min after incubation and prepared for AFM imaging. The use of ATPγS allows us to study the assembly process independently of the disassembly process.

The success of the reaction was confirmed by gel electrophoresis of samples incubated for 60 min (Fig. 1). At this point, the images we observed should consist of RecA/DNA complexes. To further confirm that the objects observed are RecA/DNA complexes, we measured the contour lengths of these objects and compared them with the expected value for the bare B-DNA. According to other experiments (Stasiak and Dicapua, 1982), RecA extends the pitch of dsDNA by 50%, from its canonical B-DNA pitch of 3.4 nm to 5.1 nm; the 5386 bp ΦX174 nicked plasmid DNA used here is thus expected to have a contour length of 1.83 μm. Contour length measurements of multiple complexes from multiple different images taken from three different samples gave an average value of 2.74 ± 0.03 μm, which is 50% longer than the bare DNA.

Figs. 2 and 3 show AFM images of RecA/DNA complexes at intermediate stages of assembly.

In samples incubated for 0 and 15 min at 37°C, the dominant species was bare plasmid DNA (Fig. 2). The height above the substrate, which is a measure of the diameter, is between 1 and 2 nm, and the contour length is 1.83 ± 0.02

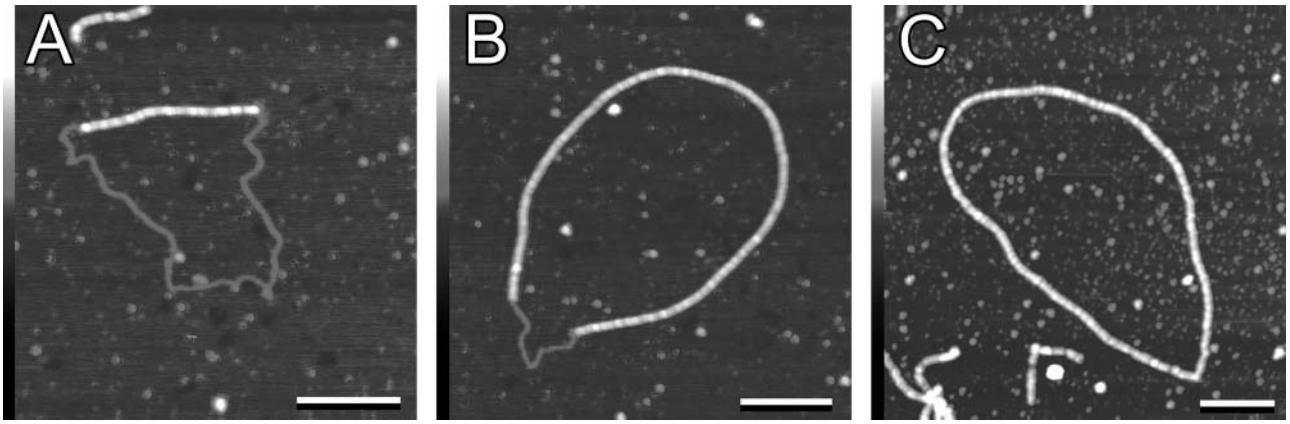

FIGURE 3 Typical RecA/DNA complexes observe at different incubation times, at 37°C: (*A*) after 30 min—~38% coverage of DNA by RecA; (*B*) 45 min—~88% coverage; and (*C*) 60 min—DNA fully coated by RecA. Topographic images acquired in air: scale bar = 250 nm; *z* scale = 10 nm (*dark* to *light*).

μm. These values correspond well to those expected for a relaxed B-DNA plasmid. In the background of this image, a variety of sizes of round objects were observed. These were likely monomers, hexamers, or other aggregates of RecA protein only. Occasionally, we observed a spot of higher topography on a small percentage of the naked plasmids. These could be RecA protein monomers, hexamers, or very short protomers bound to the DNA, but they could also simply be unbound protein lying on top of or underneath the plasmid; AFM is not able to distinguish between these two cases. It is clear, however, that there was no extensive polymerization of the RecA along the DNA, unlike those seen at later times.

After 30 min of incubation at 37°C, RecA has polymerized around approximately a third of the plasmid DNA, a typical image of which is shown in Fig. 3 *A*. For every nicked plasmid observed, there were two distinct sections: one of continuous higher topography, indicating a section of plasmid coated in RecA, and one of continuous lower topography, indicating a section of bare DNA. All coated plasmids had only a single continuous section of RecA coating. The overall contour lengths of the coated plasmids were extended, but a range of values was observed, from 1.9 μm to 2.3 μm, with an average of 2.1 $\pm$ 0.1 μm. There is still a plethora of small round objects in the image background. Again, these are likely monomers and small aggregates of RecA protein only.

After 45 min of incubation at 37°C, the nicked DNA plasmids were 90% covered with RecA protein (Fig. 3 *B*). As in the previous time interval, the RecA coverage of the plasmid DNA was continuous, and the RecA/DNA complex had an extended pitch, as indicated by the elongated contour length. As before, there was a distribution of extensions, with the average contour length at 2.6 $\pm$ 0.2 μm. In the background of the images, small objects that correspond to monomers and short polymers of RecA were visible.

For these time intervals, when RecA coating on DNA was not complete, the section of the DNA coated with RecA was always continuous. We found no instance of a plasmid with more than one continuous stretch of RecA coverage. This observation indicates that the binding of a RecA to DNA—the nucleation step—is the slow step in the process. Once nucleation has occurred, the rest of the polymerization step is rapid. Based on the delay time for the appearance of continuous RecA coatings, we can estimate that the nucleation event takes place between 15 and 30 min of incubation. Further support to this view, is the occasional observation of poorly covered plasmids even at 45 min of incubation. This serves to reinforce the view that the nucleation event must be a slow process, and only once this occurs can the RecA polymerize along the rest of the DNA molecule.

By the 60th minute, the plasmids are completely coated with RecA (Fig. 3 *C*), consistent with the results of the gel electrophoresis (Fig. 1). Partially coated complexes are rarely observed at this point (<2% of the complexes). The average contour length of the coated plasmids is 2.74 $\pm$ 0.03 μm, which is 1.50$\times$ the contour length of the original plasmid. A fully formed complex is shown in Fig. 3 *C*. That the plasmid DNA is completely coated with RecA protein is also indicated by the height of the complex above the substrate, which is substantially higher than that of naked DNA (1–2 nm, shown in Fig. 2). We further confirmed that the images are of RecA/DNA complexes by looking for the pitch of ~10 nm that was observed by others (Egelman and Stasiak, 1986; Timmins et al., 1991; Umemura et al., 2001; Cox, 2003). There are several aspects of this AFM experiment that make this difficult. First, the forces that hold the complexes on the mica surface are known to stretch the molecule. Second, the nominal radius of the tip is between two and five times the expected pitch, and tip convolution effects can obscure the topography. Third, distance measurements that go across multiple scan lines are prone to further artifactual lengthening. Fourth, it is not adequate to simply measure the distance between two adjacent RecA monomers; it is necessary to take an average number of monomers over a much longer distance to accurately compute the pitch. The strong curvature of the complexes makes this particularly difficult. However, if one is highly diligent one can observe portions of the sample that clearly demonstrates the 10 nm pitch, an example of which is shown in Fig. 4.

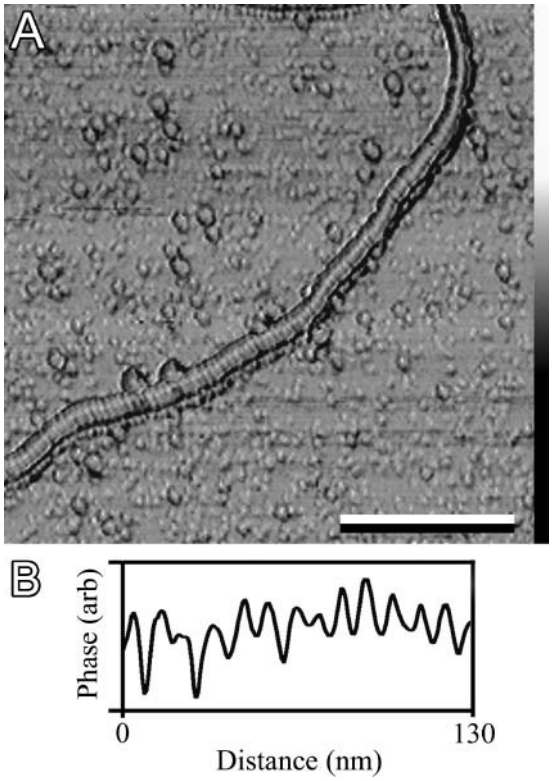

FIGURE 4 (*A*) High-resolution AFM phase image of RecA/DNA complex, clearly showing the 10 nm periodicity of the RecA/DNA complex. Tapping mode phase image acquired in air: scale bar = 250 nm; *z* scale = 25°. (*B*) Short line-section of nucleofilament.

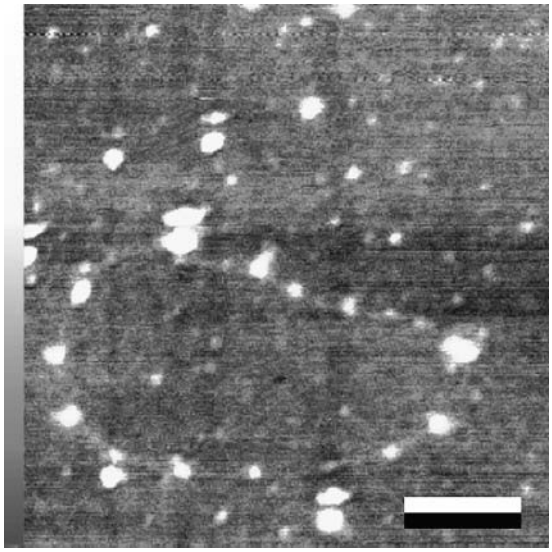

FIGURE 5 Unnicked plasmid DNA with RecA binding at intervals, but not able to polymerize. This image was collected from a sample incubated at 37°C for 60 min. Topographic image acquired in air, size bar = 250 nm; z scale = 5 nm.

At this point, we found a few instances of DNA that were not complexed with RecA. In Fig. 5, for example, one can clearly observe a continuous circle with height that is between 1 and 2 nm, that is, corresponding to plasmid DNA. However, there are spots of high topographic density that are about evenly spaced around this plasmid, denoting sporadic binding of RecA that does not lead to polymerization. This observation gives insight to interaction between RecA and plasmid DNA: RecA will only fully complex and polymerize on a nicked plasmid because RecA binding has to be accompanied by DNA elongation and change in twist. If the DNA is nicked, it is conformationally free to extend and change twist. The unnicked plasmid, however, is constrained, and thus can only support a limited amount of torsional strain. In these experiments, the closed circular plasmid DNA were nicked on a single strand by neutral thermal hydrolysis. The nicking reaction is successful in ~90% of the plasmids. The remaining plasmids are either nicked on both strands, thus producing linear DNA, or not nicked at all, thus remaining circular and constrained. In our samples, we do see linear DNA fully covered with RecA, even if the starting material were all circular plasmids. The bare plasmids, as exemplified by that on Fig. 5 must have remained unnicked.

The current models of the assembly of RecA on both ssDNA and dsDNA are based on experiments monitoring ATP hydrolysis and light scattering (Pugh and Cox, 1988; Lindsley and Cox, 1990), and optical tweezers (Bennink et al., 1999; Hegner et al., 1999; Shivashankar et al., 1999). These are all indirect methods for probing structure. It is generally believed that there is a slow nucleation step followed by a growth step that is orders of magnitude faster. The assembly is unidirectional moving 5′ to 3′, involving an association step in the 3′ end, and a slower dissociation step in the 5′ end.

The assembly is effected by the presence of ATP although ATPγS can also be used. In the presence of ATP, the overall kinetics of the assembly process was found to be slower than in the presence of the nonhydrolyzable ATPγS (Cox, 2003). This is consistent with the fact that RecA is a DNA dependent ATPase (Cox, 2003). Although the hydrolysis of ATP has not been directly proven to be coupled to any precise function of RecA, it is known that the ATP must be converted to ADP for its dissociation from DNA to occur (Cox, 2003). Explicitly this means that once an ATP:RecA complex binds to dsDNA, it has a chance of unbinding, whereas in the case of ATPγS:RecA, it does not have that option (Lindsley and Cox, 1990). Once elongation starts to occur, some of the nucleofilament will disassemble during the process in the presence of ATP; this dissociation will not occur in the presence of ATPγS. Once the entire dsDNA is coated, no further association can occur, and thus it will start to disassemble in the presence of ATP, but not in the presence of ATPγS. Importantly, this allows us to study RecA assembly on dsDNA independently of the disassembly process.

Our experiments using AFM imaging of the intermediate steps in the formation of the nucleofilaments provide the first direct evidence to support this picture and to properly elucidate the nucleation and growth stages. All the RecA/DNA complexes observed at all time points contain only a single continuous section of bound RecA protein. This means that the binding to DNA is the bottleneck in the assembly process—the slow nucleation step. Once this event happens, the nucleofilament elongation proceeds until the plasmid is completely coated. This model is further supported by the measurements of contour lengths of the complexes, which increase monotonically with time from an initial value of 1.83 μm: at 30 min, the average contour length is 2.1 $\pm$ 0.2 μm; at 45 min, it is 2.6 $\pm$ 0.1 μm; and at 60 min, it is 2.74 μm.

That the nucleation step is slow is consistent with our observation of the occasional presence of plasmid DNA with a few spots of bound RecA at late times of the assembly (Fig. 5). Since we use ATPγS in our experiments, the RecA can bind to an unnicked plasmid, but it cannot detach. However, the growth step also cannot happen since that would require DNA elongation, which is not allowed by the constrained plasmid conformation. The use of ATPγS in this manner can enable the separation of the nucleation step from the growth step. The calculated nucleation rate is 5×10^{-5} min^{-1} bp^{-1}.

Shivashankar et al. (1999) developed a quantitative model of RecA/DNA nucleation and growth for a single molecular under tension, as was the case for their experiments using optical tweezers. This analysis is derived for a single DNA molecule under tension, but we believe the analysis can still be used to describe our data. They explicitly considered two different cases: (i), when there is a single nucleus for growth; and (ii), when there are multiple nucleation points. Our

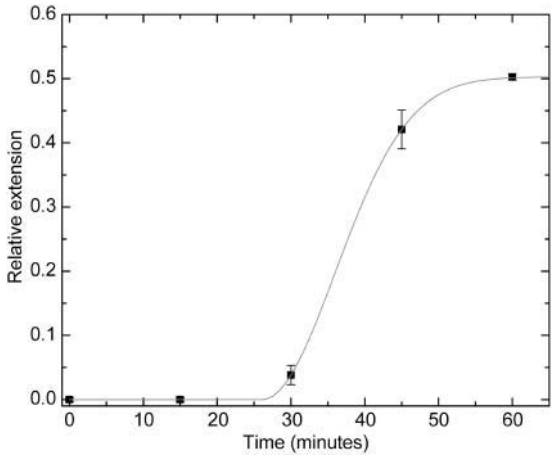

FIGURE 6 Average relative extension as a function of time, with best fit of nucleation and growth data by Eq. 1.

observations indicate a single nucleus as in (i), for which they obtained a polymerization (growth) rate of 12 RecA/sec whereas the DNA is held under 6 pN of tension (Shivashankar et al., 1999). In our case, assuming a linear growth rate between the 30- and 45-min points of the experiment (that is, after nucleation has taken place), we obtain a value of 47 ± 14 nm/min. Assuming a single RecA is bound to every three bp of DNA, this translates to a rate of 1.5 RecA/sec, about an order of magnitude less than the result of Shivashankar et al. However, it has been reported that having an applied force increased the rate of assembly (Bennink et al., 1999).

The second case may be more applicable to fit our data. Although there is only one nucleation event within each plasmid, the are multiple nucleation events in the same solution with a time distribution; this is analogous to Shivashankar's multiple nucleation events on one long strand. Using the more detailed analysis of Shivashankar et al. (1999) we plot the relative extension of the plasmid DNA as a function of time (Fig. 6) and fit with the equation

$$\frac{L_t - L_0}{L_0} = \frac{L_{max} - L_0}{L_0}(1 - \exp(-\kappa(t - t_0)^2)), \qquad (1)$$

where L_t is the length of the (coated) plasmid at time t, L_0 is the B-DNA length of the plasmid, and L_{max} is the maximum length of the coated plasmid. The adjustable parameters for the fit are t_0 which is the lag time, and the rate constant $\kappa = n\nu/2$, where n is the nucleation rate, and ν is the growth rate. Using this analysis, the lag time is determined to be 26 ± 1 min, and the rate constant to be $5.05 \pm 0.05 \times 10^{-3}$ min^{-2}, which, as with the previous estimate of growth rate, is an order of magnitude less than Shivashankar's finding of 0.022 min^{-2}. In that study, however, the nucleation rate and growth rate could not be determined individually. In this work we have been able to measure the nucleation rate. Given our nucleation rate (n) of 5×10^{-5} min^{-1} bp^{-1}, the resulting directly measured growth rate (ν) is 202 min^{-1}.

Other experimental approaches can also provide kinetic information but in an indirect manner. Fluorescent dye displacement assay yielded a reaction half-time of 165 seconds (Zaitsev and Kowalczykowski, 1998). For the 7238 bp m13mp7 phage DNA used in the experiment, this corresponds to a maximum rate 8 RecA/s. On the other hand, using linear and circular dichroism, Takahashi et al. reported a half-time of 20 min (Takahashi et al., 1989).

There is thus a discrepancy in the reported values for the kinetics of the process. Aside from inherent differences on what is being measured in a specific experiment, the propagation kinetics is dependent on the concentration of the species involved, and there is variability in concentrations in these studies, presumably partly due to the constraints of the experimental approach utilized. In the optical tweezers experiments, for example, there is an inherent difficulty in determining the appropriate concentrations when one is dealing with a single molecule in a trap. In our work, we deliberately chose lower concentrations for ease of AFM imaging in terms of the time constraints, as well as in not having too much material that could obscure each other in the image. That all the data are in the same general range is a point of interest.

In studies of ATP hydrolysis, another important parameter measured is the lag time, which is the time it takes for the rate of ATP hydrolysis to reach a maximum constant value (Pugh and Cox, 1988). The reported value of 14.5 min is close to our result of how long it took to incubate the system before any sign of growth. Whether or not the lag time as measured in these two different ways correspond to a nucleation time in a nucleation-and-growth model needs further study and direct comparison of various experiments.

CONCLUSION

The time resolved imaging study of the assembly of RecA onto DNA gives several key observations not available in other methods of measurement. Foremost is the ability to resolve both the naked plasmid DNA and the RecA protein coated sections of the plasmid. The resolution we achieved without the use of specialized AFM probes, or dehydration in a vacuum, is comparable to previous studies. This leads to the observation that multiple nucleation points do not occur on plasmids of this length (5386 bp). This direct visual evidence of slow nucleation coupled to rapid growth is confirms previous indirect experiments of RecA binding to dsDNA. By implementation of a kinetic model for RecA/DNA nucleation and growth measurement by optical tweezers, we have determined both the nucleation rate and growth rate. These studies also show the potential capability of AFM imaging for providing insights about mechanisms of biomolecular interactions, and for elucidating results from other measurements.

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