Double-stranded DNA homology produces a physical signature

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DNA is found in the cell largely as a negatively supercoiled molecule. This high-energy form of the genetic material can engender sequence-dependent structures, such as cruciforms, Z-DNA, or H-DNA, even though they are not favored by conventional conditions in relaxed DNA. A key feature of DNA in living systems is the presence of homology. We have sought homology-dependent structural phenomena based on topological relaxation. Using two-dimensional electrophoresis, we demonstrate a structural transition in supercoiled plasmid molecules containing homologous segments. Atomic force microscopy (AFM) reveals a dumbbell structure in molecules whose linking difference is beyond the transition point. The position of the dumbbell shaft is a function of the site of homology, and its extent is proportional to the linking difference. Second-site-reversion electrophoresis data support the notion that the shaft contains PX-DNA. Predicted cross-linking patterns generated in vivo suggest that homology-dependent structures can occur within the cell.

atomic force microscopy | homology recognition | PX-DNA | supercoiling relaxation | unusual DNA motifs

DNA in the cell is a negatively supercoiled molecule (1). Supercoiled DNA corresponds to a high-energy state that can lead to the formation of structures other than traditional Watson–Crick DNA: It is well known that supercoiled molecules can undergo structural transitions as a consequence of sequence features: Palindromic sequences can result in the extrusion of cruciforms (2), alternating pyrimidine-purine sequences can produce Z-DNA (3), and mirror-symmetric oligo-purine:oligo pyrimidine sequences can lead to H-DNA formation (4, 5). In each of these cases, the transitions relax the DNA and simultaneously produce a distinctive structural feature. Here, we report a unique structural transition of superhelical DNA that occurs as a consequence of homology.

There have been recent reports that homologous DNA duplexes associate to form higher-order structures (6–8). We were motivated to investigate DNA-DNA recognition of homology by the notion that PX-DNA (9, 10) could be formed by homologous segments in superhelical DNA. PX-DNA (Fig. 1*A Right*) can be described formally as a four-stranded DNA motif wherein two parallel double helices are joined by reciprocal exchange (crossing over) of strands of the same polarity at every point where the strands come together (9). Nevertheless, no strand breakage and rejoining is needed, because two double helices can form PX-DNA merely by interwrapping. The PX motif has been used in DNA nanomechanical devices (11–14) and as the basis for intramolecular (15) and intermolecular (16) cohesion in structural DNA nanotechnology. Recently, it has been used to measure the free energy of forming a parallel crossover between double helices (17). In comparison with conventional B-DNA (Fig. 1*A Left*), its helical pitch is roughly twice as long, because each strand must wrap around two helices when executing a full turn around the structure; hence its twist is expected to average to about half that of conventional DNA. The actual change in linking number upon PX formation is not so simple: One must also treat the writhing of the double-stranded structures (blue and red in Fig. 1); its calculation is described in Fig. S1. The formation of PX-DNA from two double helices in a section of a circular plasmid is largely equivalent to unwinding the whole region.

In principle, the two double helices of PX-DNA are related by a central dyad axis parallel to their helix axes (Fig. 1*A Right*). Successive half turns of the two helices flank the central dyad axis, alternately exposing to it major (wide) groove (W) or minor (narrow) groove (N) surfaces (Fig. 1*A Right*). We find that minor groove separations of five nucleotide pairs and major groove separations of six, seven, eight, and nine nucleotide pairs (termed 6:5, 7:5, 8:5 and 9:5, respectively) yield the most stable structures in complexes containing four independent strands (10). An intramolecular 6:4 PX has been reported to form between hairpins (15); we note further species of PX here, in a system whose strands are even further constrained (*SI Text*).

The PX molecule is drawn in Fig. 1*A* as though it had been formed by the interwrapping of two double strands of DNA, one pair red and one pair blue. Base pairing is indicated as alternating between two paired red strands or two paired blue strands (in the half turns labeled **U**), and between paired red and blue strands (in the half turns labeled **H**). Homology is needed only in the half turns labeled **H** to form the PX structure; two double helices are said to be "PX-homologous" if they contain the same sequence in the half turns labeled **H** but not in the half turns labeled **U**. Of course, the ability to form PX-DNA from PX-homologous sequences does not prevent fully homologous molecules from assuming this structure. PX-DNA has been built in model systems (10) by using sequence-symmetry minimization approaches (18), similar to those used to produce immobile Holliday junction analogs (19); however, it has not been shown to form between relaxed homologous double helices.

Fig. 1*B* is a cartoon illustrating a supercoiled molecule consisting of negatively supercoiled B-DNA (*Left*), and a second molecule (*Right*) that has been relaxed by forming a PX-like structure. The prediction from this model is that PX-based shaft-like structures should occur between regions containing homology or PX-homology and that loops should form on the ends of a plasmid. In the resulting dumbbell-shaped molecules, the sizes of the loops and the length of the PX-like shaft would be a function of the location and extent of the homology in those cases where all of the homologous DNA is involved in forming a shaft.

Relaxation of supercoiling by forming PX-DNA in the presence of double-stranded homology would provide a physical signature for the cell to exploit in the recognition of homology. Here, we examine whether the presence of homology can lead to the relaxation of supercoiling in a plasmid. We demonstrate by 2D gel electrophoresis that the presence of homology leads

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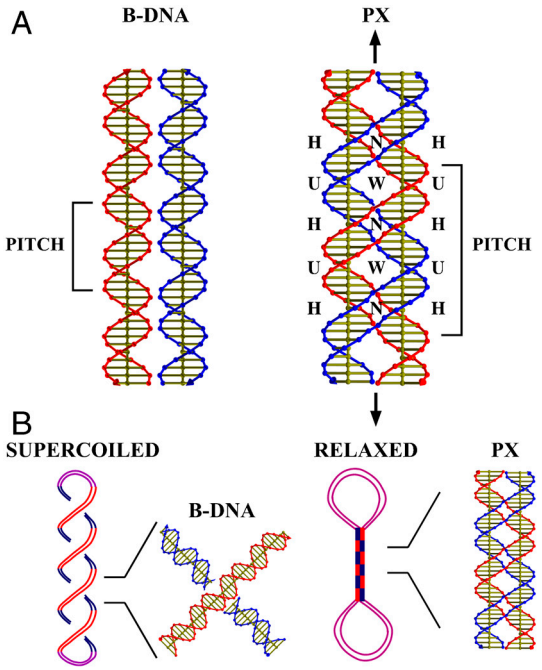

Fig. 1. Schematic drawings of the DNA species used. (*A*) A comparison of B-DNA and the PX motif. Two DNA double helices are on the left, one with a red backbone, and one with a blue backbone. Helix axes and bases are indicated. The PX motif is shown at right. It appears to consist of interwrapped red and blue double helices. Note that strands going from lower left to upper right pass in front of those going from lower right to upper left. The motif dyad axis is indicated by black arrowheads above and below the drawing. For any given half-turn of DNA, either a major (wide) groove separation (indicated by **W**) or a minor (narrow) groove separation (indicated by **N**) will face the dyad axis. The helical pitches of both structures are indicated. Half turns labeled **U** do not require complementarity between the blue and red strands for the PX structure to form, but those labeled **H** do require it, so the structures must be homologous in those regions. (*B*) Cartoons of supercoiled DNA. The left panel contains an interwound structure that has not contorted itself to relax; red parts of the backbone are closer to the reader than blue parts. B-DNA is drawn to the right in color coding that corresponds to the molecule on the left. The right panel contains a molecule that has relaxed by forming a PX structure, drawn as alternating red and blue boxes on the left, with the molecular structure at right. Relaxation will lead to a dumbbell-like structure, with a long PX shaft flanked by loops on either end. The position and extent of the homology will affect the position and length of the shaft and both the absolute and relative sizes of the loops.

to a structural transition in plasmids containing homology or PX-homology. We are able to restore transitions by second-site reversion. Using atomic force microscopy, we find dumbbell structures with shaft lengths that are a linear function of the linking difference; for those molecules in which the shaft-length is as long as the homology permits, the loop sizes are those predicted from the placement of the homology. We demonstrate by psoralen cross-linking that this relaxation occurs both in vitro and in vivo; the proximity of strands that are not otherwise required to be paired with each other is a prerequisite for this cross-linking. A single PX turn of full homology (22 nucleotides) is sufficient to yield a relaxation signal. We find that PX-homology, as well as full homology, is capable of producing relaxation and dumbbell structures.

Results

The Experimental System. To examine the effect of homology on DNA supercoiling, we have prepared pUC19 plasmids in *Escherichia coli* XL1-Blue cells (Stratagene), a RecA-deficient strain. We have excised specific lengths of DNA from one position and replaced them by the exact sequence of another segment contained within the molecule. For this purpose, we have used multiples of the 6:5 PX repeat unit, 22 nucleotide pairs. The positions of the homologous segments are either symmetric or asymmetric, i.e., equal or unequal size spacers flank the sides of the homologous regions. Exact species used are described in *Materials and Methods*.

Two-Dimensional Gel Electrophoresis of Molecules with Full Homology. Two-dimensional agarose gel electrophoresis is a standard technique for the examination of supercoiled DNA molecules (e.g., 20, 21). Fig. 2*A* shows that in the first dimension, molecules migrate according to the extent of their (negative) supercoiling, and molecules that have been relaxed somewhat through a structural transformation will migrate along with molecules whose linking difference, ΔLk, is smaller. In the second dimension, a moderate concentration of chloroquine is added, so that the supercoiling of all molecules is shifted to the positive direction, thereby eliminating the structural relaxation. Thus, molecules with the largest absolute linking numbers will migrate most rapidly across the gel, and those that are relaxed at the chloroquine concentration used will migrate most slowly. Structurally relaxed molecules in the first dimension now lead to a second arc, discontinuous with the first, producing a kink in the pattern.

Fig. 2*B* shows a control of unmodified pUC19; no anomalies are evident in its migration pattern, where successively negative topoisomers migrate more rapidly in the first dimension. Fig. 2*C* illustrates a molecule with 220 nucleotides of full homology, asymmetrically placed head-to-head across from each other in the plasmid; Fig. 2*D* shows molecules containing 220 nucleotides of symmetrically placed full head-to-head homology. Both gels

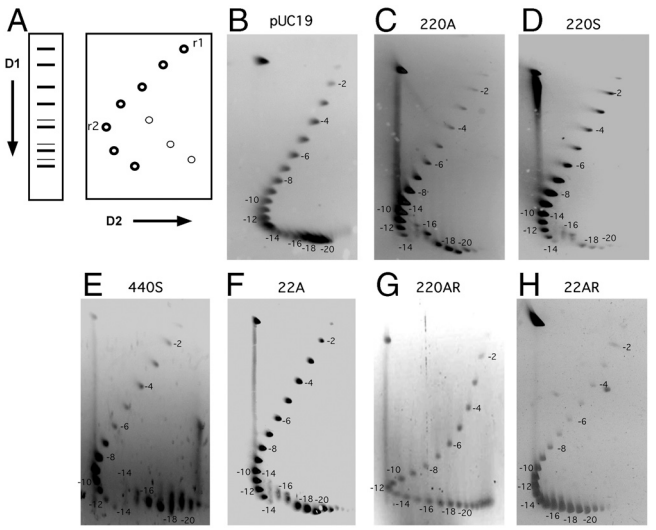

Fig. 2. Two-dimensional gel evidence for the relaxation of supercoils by the presence of segments containing exact homology. (*A*) A cartoon of a gel containing superhelical DNA that has undergone a relaxing structural transition. The most relaxed molecules in each dimension (r1 and r2) migrate the slowest. Three bands (drawn with a thinner line) migrate more slowly in the first (vertical) dimension because they have undergone relaxation and are less writhed. When a moderate amount of chloroquine is added, the relaxation is removed, and they move as molecules that are somewhat positively supercoiled; this behavior produces a kink in the pattern (adapted from ref. 21). In other panels, numbers indicate the negative topoisomer (ΔLk) corresponding to the band. The band or smear at the upper left of each gel results from the presence of nicked DNA. (*B*) A pUC19 control. This gel lacks any kinks. (*C–F*) pUC19 molecules with head-to-head homology. 220 nts of homology are placed asymmetrically (220A) (*C*) and symmetrically (220S) (*D*); 440 nts of symmetrical homology (440S) are seen in *E*, and 22 nts of asymmetric homology (22A) (*F*). In all cases, kinks are visible in the patterns. (*G* and *H*) pUC19 molecules with head-to-tail (reversed) homology. 220 and 22 nts (220AR and 22AR) of asymmetric homology are shown in *G* and *H*, respectively. No kink is visible in *G* or in *H*.

contain kink-like features, similar to that shown in the schematic, around topoisomer −14. Fig. 2*E* illustrates the gel pattern produced by a molecule with 440 nucleotides of full head-to-head homology; the kink also begins at −14. Fig. 2*F* shows the effect with homology as short as 22 nucleotide pairs, a single 6:5 PX turn; the kink is again clearly discernable at −14. Gels for numerous other species examined are shown in Fig. S3. They show features similar to those seen in Fig. 2. Thus, in each case, the presence of homology leads to a kink in the pattern around ΔLk = −14. The position of the kink is independent of the size of the homologous segment, suggesting that initiation of the transition need not involve the entire range of homology. There are usually four bands at the start of the kink. These may correspond to relaxation of different numbers of superhelical turns and the formation of turns of PX-DNA, ranging from zero to three. These differences are in agreement with the cohesion lengths seen in the AFM data presented below. The long homologous segment examined in *E* may have a second (perhaps independently initiated) transition at ΔLk = −18.

What about polarity? Fig. 2*G* shows the gel pattern from a molecule similar to that in Fig. 2*D*, except that the polarity of the sequences is head-to-tail. It is evident that a single dominant arc is visible here, suggesting the lack of a transition. The plasmid is large enough to form a figure-eight structure to reorient one of the homologous domains. However, if the structure involves the plectonemic winding of the two double helices about each other, the crossing point of the figure eight is expected to interfere with this winding. Fig. 2*H*, where a single PX turn of 22 nucleotides is present in head-to-tail orientation, unsurprisingly shows no kink either, although there is some trailing to the bands present. The head-to-tail arrangement requires the formation of a figure-eight shape to align the homologous segments; this topological impediment prevents the interwrapping that is apparently a component of the relaxed structure.

Atomic Force Microscopy of Molecules with Full Homology. Direct observation of the plasmids by atomic force microscopy (AFM) illustrates the structural nature of the relaxed molecules. Fig. 3*A* shows AFM images associated with the molecule of Fig. 2*C*, in which 220 head-to-head nucleotides of homology are separated by segments of unequal length. We show four sample images from 2,234 measured images of this molecule. We have isolated molecules from the 2D gel in four batches, those with ΔLk from near the transition to −17, those from −18 to −22, those from −23 to −29, and those with ΔLk larger than −29. The prominent feature of all of these molecules is the presence of a shaft connecting relaxed loops so that the molecule looks roughly like a dumbbell. The estimated length of a completely formed 220-mer PX segment is about 75 nm. We find a rough relationship of about 44 Å increase in shaft length per unit change in linking number, not far from what would be expected from a PX 6:5 or 7:5 molecule. The start of the kink (ΔLk = −14) in the 2D gel (Fig. 2*C*) shows four bands simultaneously; this finding suggests the formation of zero to three turns of PX-DNA, leading to cohesive lengths (not counting 0) of 7–22 nm, which are part of the first range in Fig. 3*A*. Those molecules with a shaft length near 75 nm have approximately the expected placement of the shaft relative to the ends and corresponding loop sizes. This is seen for all molecules in the series where the expected length of the shaft is found, regardless of the amount of homology or of the placement of the homology. Fig. S4 shows several molecules with fully homologous segments. Dumbbell structures are not seen in molecules isolated from bands before the transition (ΔLk less than −14). An apparent conundrum, however, is that the length of the shaft does not stop increasing when the homology is satisfied, but rather it continues to grow beyond the ends of the homologous region (see below).

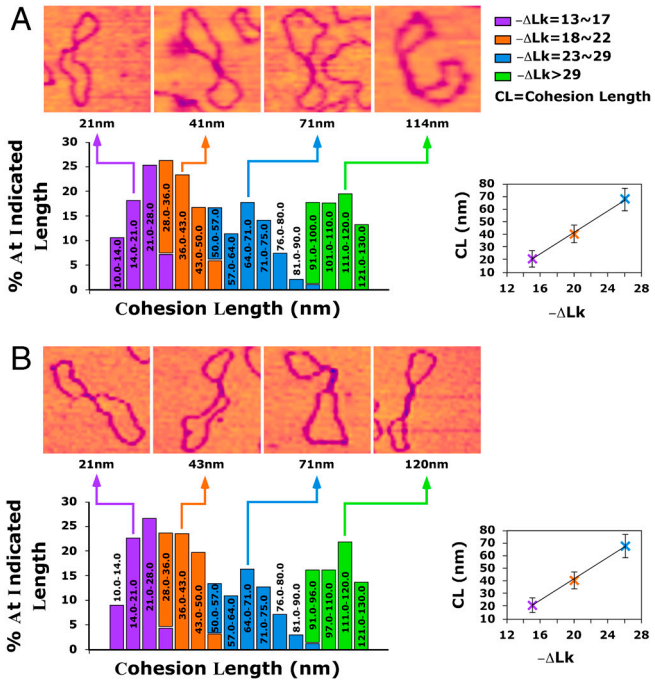

Fig. 3. AFM images of relaxed molecules containing segments of exact homology (*A*) or PX-homology (*B*). The molecules were isolated from the gel in four topoisomer groups, indicated by the color coding in the chart at the upper right. The bar graph indicates the shaft cohesion length along the abscissa, and the percent of such molecules seen with a given cohesion length for that topoisomer. The actual cohesion length is written on each bar. Typical molecules are shown at the top of each panel; arrow colors correspond to the group from which the image comes. The relationship between average ΔLk per group and average cohesion length is plotted for each group (except the last, green, group, whose ΔLk can only be estimated by a lower bound. The number of molecules examined in each category are the following [ΔLk range, 220A, 220A-PX6:5]: [(−13 to ~ − 17), 764, 698]; [(−18 to ~ − 22), 697, 632]; [(−23 to ~ − 29), 676, 813]; [> − 29, 97, 76].

Molecules with PX-Homology. Our study was inspired by the notion that PX-DNA might lead to relaxation, so we have repeated the experiments described above with molecules containing PX-homology. Fig. 4*A* illustrates an asymmetric molecule with 220 nucleotides of major groove 8:5 homology. A second arc begins clearly at the −14 topoisomer. Using a high-chloroquine protocol, we found that the extent of the signal increased as we went from PX 6:5 to PX 8:5, so we continued through PX 11:5, when the signal got weaker (22); here we show data from the unambiguous low-chloroquine gels. Dramatic kinks are seen in a variety of molecules with PX-homology, again beginning around topoisomer −14. Fig. 4*B* shows the electrophoresis of an asymmetric molecule with 220 nucleotides of major groove 10:5 homology. The effect is seen in the 11:5 220 nucleotide asymmetric molecule, but it is somewhat weaker (see Fig. S3). A symmetric molecule with 220 nucleotides of major groove 9:5 homology (Fig. 4*C*) also shows a kink. The 220 nucleotide asymmetric major groove homology 6:4 PX molecule (not stable in four-stranded structures) (10) also displays a kink (Fig. 4*D*). Minor groove homology is also effective, as seen in Fig. 4*E*, showing a 220 nucleotide asymmetric molecule with 7:5 minor groove PX-homology. A 440 nucleotide symmetric major groove 6:5 PX-homologous molecule is seen in Fig. 4*F*, perhaps beginning around −13. A variety of other molecules, covering most of the combinations within this range, is shown in Fig. S3.

We demonstrate by second-site reversion (4) that the PX structure is likely to be involved here. Fig. 4*G* shows the 2D gel for an 88-nucleotide 6:5 PX-homologous segment, where the kink is evident; we use PX-homology rather than full homology to

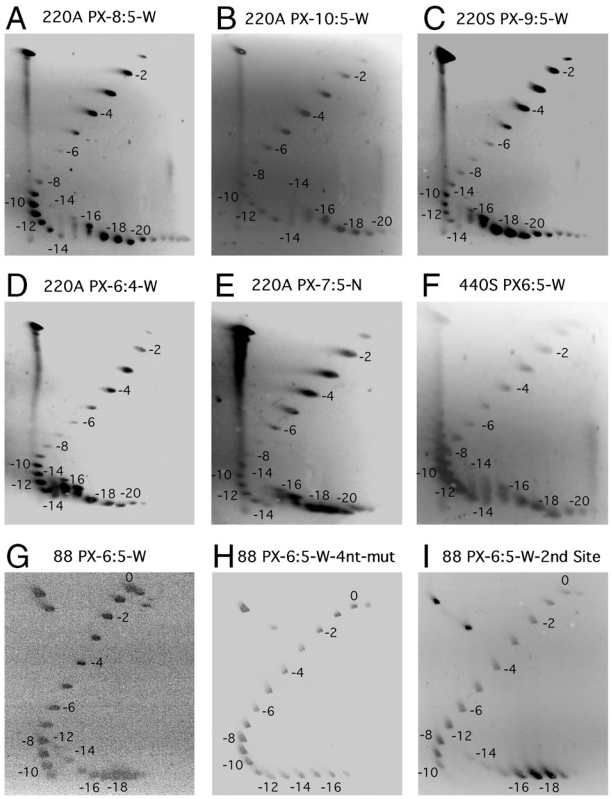

Fig. 4. Two-dimensional gel evidence for the relaxation of supercoils by segments containing PX-homology. Notation: Length of homology, Placement of Homology (Symmetric or Asymmetric), PX-Major:Minor groove sizes-Groove containing homology (Wide or Narrow); except for *E*, homology is always in the major groove. All arrangements are head-to-head (*A*) 220 nt 8:5 PX-homology, placed asymmetrically. (*B*) 220 nt 10:5 PX-homology, placed asymmetrically. (*C*) 220 nt 9:5 PX-homology, placed symmetrically. (*D*) 220 nt 6:4 PX-homology, placed asymmetrically. (*E*) 220 nt 7:5 minor groove PX-homology, placed asymmetrically. (*F*) 440 nt 6:5 PX-homology, placed symmetrically. Kinks are evident in all patterns. (*G*) 88 nt 6:5 major groove PX-homology. (*H*) The four middle nt of each homologous stretch mutated; the kink disappears. (*I*) The four middle nt reverted; the kink reappears.

maintain a constant phasing and to prevent branch migration. In Fig. 4*H*, we show the gel corresponding to changing the middle four bases of each of the 6-base homologous stretches in one duplex segment, and the kink clearly disappears. We can restore the kink (Fig. 4*I*) by changing the middle four bases in each of the 6-base stretches in the other segment, so that they match those of the first segment, thereby restoring homology by a second site reversion.

The AFM signatures of molecules with PX-homology (Fig. 3*B*) are similar to those of molecules with full homology. Fig. 3*B* is similar to Fig. 3*A*, but it shows a molecule with PX 6:5 major groove homology rather than full homology. The various isolated topoisomer groups produce the same dumbbell-like molecule distribution with a similar amount of average shaft development per unit of linking difference. Molecules with differently sited PX-homology whose dumbbells contain shafts with the expected full-length are seen to display them in the expected positions (Figs. S5 and S6). Thus, symmetrically sited molecules show loops of equal size, and asymmetrically sited molecules show loops of unequal size; in all cases, the sizes measured are those calculated from the dimensions of the components.

In Vitro Cross-linking Experiments. We have seen from AFM data that a shaft-like structure apparently containing four strands (two from each homologous section) is formed in molecules whose linking difference exceeds that of the −14 transition point. It might be that two helices are just adjacent to each other, but if a PX structure forms, a special probe is available. The half turns labeled **H** in Fig. 1*A* consist of a Watson–Crick double helix that contains strands from both sides of the plasmid, involving pairing between red strands and blue strands. If a PX molecule is formed, these strands have exchanged pairing partners and thus should be susceptible to being covalently bonded by a cross-linking agent such as psoralen. Following cross-linking, the plasmids can be restricted, and inspected by AFM; Fig. 5 shows the results of these experiments on molecules with 220 nucleotides of complete homology (*A*) or PX 6:5 homology (*B*). As in Fig. 3, we indicate the linking difference of the species from which the material was isolated. Following isolation, the material was restricted either singly or doubly, as indicated in Fig. 5.

The length of the cross-linked material cannot be a fixed quantity because it depends on the particular sites where cross-linking occurs. Given that caveat, the lengths of the restricted loop or loops of the plasmid are approximately what is expected. Nevertheless, the maximum length of cross-linking seen in the molecules with known linking differences reflects the maximum length in which a PX-like structure can form. In stark contrast to the AFM data on uncross-linked molecules, the maximum

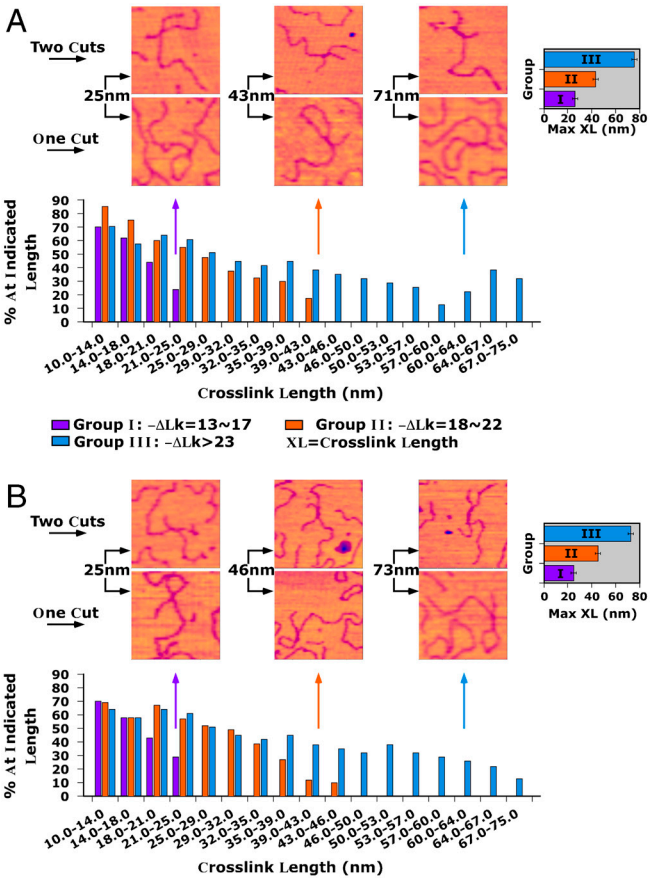

Fig. 5. AFM images of molecules cross-linked in vitro, containing full homology (*A*) or 6:5 PX-homology in the major groove (*B*). The three topoisomer groups for both panels are indicated at the middle, and the distribution of cross-linked lengths are shown for each group below it, coded by color. The maximum cross-linking length is plotted for each panel to the right of the AFM images. Singly and doubly cut molecules are shown for each group, as indicated, with the arrow colors corresponding to the group. It is key that the maximum length of cross-linking never exceeds that expected for the 220 nts of full or PX-homology. The number of molecules containing two cuts examined in each category are the following [ΔLk range, 220A, 220A-PX6:5]: [(−13 to ~ − 17), 3000, 4150]; [(−18 to ~ − 22), 2636, 4023]; [> − 23, 1871, 1886].

length of cross-linking never exceeds significantly the homology length of ~75 nm; this is true even though nearly three times the number of cross-linked samples have been examined for these experiments, relative to the uncross-linked AFM images. Thus, the unexpectedly long shafts noted in Fig. 3 for samples with very high linking differences appear to represent writhing not directly associated with the formation a PX-like molecule. Nevertheless the presence of a PX-like structure may nucleate the tight wrapping as a response to superhelical stress; we cannot exclude the possible impact of the mica support in producing this phenomenon.

The cross-linking observed for both sets of molecules demonstrates that re-pairing has occurred, consistent with the notion that the structure formed is PX-DNA. The experiments with fully homologous molecules do not exclude tetrahelical structures, such as those proposed by McGavin (23) or Wilson (24). However, a PX-homologous molecule is unlikely to yield the base pairing structures proposed for their models.

In Vivo Cross-Linking Experiments. Can homology lead to the transition within the cell? Cross-linking experiments similar to those in the last section can also be conducted in vivo. Although one might imagine that psoralen would relax the DNA on its own, it is important to recall that a PX structure is a multiple Holliday junction, a structure shown by Kallenbach and coworkers to be a hotspot for binding intercalating drugs (25).

The key AFM data from the cross-linking experiments are shown in Fig. 6. Cross-linked molecules, when seen, constitute about 10% or less of the sample. Fig. 6*A* shows a pUC19 control with no cross-linking. Cross-linked species are visible on denaturing gels, and none are detected from pUC19. Fig. 6*B* shows the cross-linking of the fully homologous asymmetric molecule with 220 nucleotide pairs of homology. It is clear that a long stretch of DNA has been cross-linked. The lengths of the cleaved loop fragments correspond to those expected from the sites of the restriction enzymes. Fig. 6*C* illustrates the fully homologous 22-nucleotide molecule. The cross-linking zone is short, as expected, but present nevertheless. Cross-linked 220- and 22-nucleotide full homology head-to-tail molecules can be detected rarely (Fig. 6 *D* and *E*, respectively), but the data of Fig. 2 suggest that that these are likely to be transient species, indicating the care that must be applied to the interpretation of cross-linking data. The cross-linking in Fig. 6*D* is only about one PX turn long.

Molecules with PX-homology are also cross-linked. Fig. 6 *F–J* show AFM cross-linking data from 220 nucleotides of asymmetric major groove 6:5, 7:5, 8:5, 9:5, and 10:5 PX major groove homology, respectively. The lengths of the cross-linked regions may vary from theoretical, depending on how extensively the region of homology was actually cross-linked in any given instance.

Discussion

Homology and PX-Homology Produce a Characteristic Response in Superhelical DNA. We have demonstrated by 2D gel electrophoresis that the presence of homology or PX-homology in a head-to-head orientation results in a characteristic relaxation transition evidenced by a kink. We have also demonstrated that the response can be destroyed by eliminating PX-homology but restored by reestablishing the PX-homology at a second site. AFM data show the presence of a shaft, which would be expected from the presence of PX-DNA formed in homologous regions. Both in vitro and in vivo cross-linking data support the notion that a PX-like molecule forms in the presence of homology or PX-homology in a supercoiled molecule. The in vitro cross-linking data suggest that there is a direct interaction between stretches of complementary DNA bases from opposite sides of

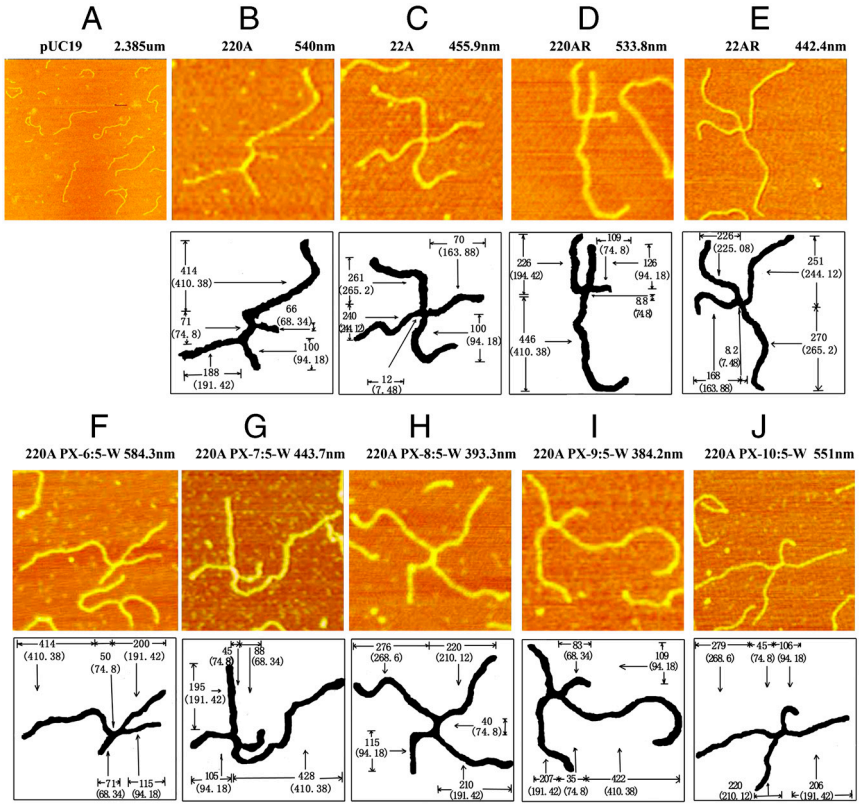

Fig. 6. In vivo cross-linking studies of molecules containing homology. Numbers compare measured and expected lengths of arms in nanometers. (*A*) A pUC19 control showing no X-like structures. (*B*) 220 nts of full head-to-head homology in an asymmetric molecule. (*C*) 22 nts of full head-to-head homology in an asymmetric molecule. (*D*) 220 nts of full head-to-tail homology in an asymmetric molecule. (*E*) 22 nts of full head-to-tail homology in an asymmetric molecule. (*F–J*) 6:5, 7:5, 8:5, 9:5, and 10:5 major groove PX-homology, respectively. In all cases, cross-linking is seen to occur, but only a single turn is seen in *D*.

the plasmid. The maximum lengths of in vitro cross-linked species are in agreement with a model resembling the PX molecule. The successful in vivo cross-linking of the relaxed molecules suggests that these structures can occur within the cell.

None of the pretransition molecules examined contain the characteristic shaft visualized in the AFM for posttransition molecules, formed both from homologous and PX-homologous molecules. As expected for a supercoiling-dependent transition, the length of the shaft increases with increasing linking difference. The average increase is approximately 44 Å per unit ΔLk, corresponding to ~13 nucleotides of B-DNA, about the length expected by forming one turn of 6:5 or 7:5 PX-DNA: Full shaft-length formation in the 10 PX-turn 220A molecule occurs in the topoisomers around 10 turns beyond the transition point. The inability to form the shaft in head-to-tail molecules further supports the notion that the shaft is an interwrapped molecule, such as PX-DNA.

Aspects of the Data that Remain to be Explored. There are three findings here that do not fit our conventional thinking about the behavior of superhelical DNA. First, other known alternative structures are facilitated by the increased length of the segment involved, owing to the positive free energy associated with the transition. That the transition point is independent of segment length suggests an unusual nucleation-free energy near zero. Second, there is a small change in mobility that is not a function of the transition region length. The transition described here differs from previously described transitions (e.g., 2–5); in those cases, the mobility change corresponds well to a relaxation of supercoiling (1). Here, the change in structure is not tightly localized but involves distant portions of the plasmid. We see differences in mobility that appear to correspond to $\Delta\Delta Lk = 3$, suggesting a small change in writhing were the transition tightly localized. However, because this transition is not localized, mobility changes do not fit the requirements for being directly related to the extent of relaxation. The mobility changes associated with forming this new type of structure are not easily predicted. Third, at very high linking differences, the shaft-like structure extends beyond the region of homology and beyond the cross-linked region. Its nature is unclear except for the possibility that some sort of interwound structure is nucleated by the presence of the PX-like shaft or by the presence of the mica support.

Role of Superhelical Relaxation in the Recognition of Homology. We have demonstrated that both complete homology and PX-homology generate a shaft-like relaxation structure in DNA of sufficient superhelical density (about −0.052). These results suggest both a structural and an energetic basis for interactions between homologous segments of DNA. This type of relaxation could provide a driving force and a structural context to enable homologous recognition. Furthermore, we have demonstrated that this phenomenon occurs at lengths as short as one PX turn. This is a key point, because a single turn could, with a little flexibility, interact with another molecule without the intervention of a topoisomerase. Thus, a turn of PX-DNA may well be able to serve as the type of recognition element postulated by Weiner and Kleckner (26).

It remains to be established whether the phenomenon described here is involved in homologous recombination, chromosome pairing, or other phenomena where homology is implicated. Nevertheless, the past half-century of molecular biology has demonstrated that nature is opportunistic and that structures that can be formed both in vitro and in vivo are likely to have a functional role in living systems.

Materials and Methods

Fully homologous molecules with 20 multiples are only symmetric, and those with one, two, three, four, and seven multiples are only asymmetric. We have made constructs in both relative orientations, head-to-head and head-to-tail for both 10- and 20-multiple molecules. Specific molecules are shown in Fig. S2.

Specifics of molecules with PX-homology (all head-to-head): For 10 multiples in an asymmetric plasmid, we have placed homology in the major groove of 6:4, 6:5, 7:5, 8:5, 9:5, 10:5, and 11:5 PX-homologous segments. We have also examined molecules with 5:6, 5:7, and 5:10 PX-homology in the minor groove in 10-multiple asymmetric molecules. PX-homology species 6:5, 7:5, 8:5, and 9:5 also have been studied in 20-multiple symmetric molecules.

All plasmids have been treated with ethidium and topoisomerase I, so as to give a complete set of topoisomers, starting from completely relaxed species (20). Molecules not described in Figs. 2, 3, 4, and 5 are shown in *SI Text*.

Experimental details of 2D gel electrophoresis, PCR, topoisomerase I treatment, transformation, psoralen cross-linking, and AFM are described in *SI Text*.

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