Single molecule detection of direct, homologous, DNA/DNA pairing

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Using a parallel single molecule magnetic tweezers assay we demonstrate homologous pairing of two double-stranded (ds) DNA molecules in the absence of proteins, divalent metal ions, crowding agents, or free DNA ends. Pairing is accurate and rapid under physiological conditions of temperature and monovalent salt, even at DNA molecule concentrations orders of magnitude below those found in vivo, and in the presence of a large excess of nonspecific competitor DNA. Crowding agents further increase the reaction rate. Pairing is readily detected between regions of homology of 5 kb or more. Detected pairs are stable against thermal forces and shear forces up to 10 pN. These results strongly suggest that direct recognition of homology between chemically intact B-DNA molecules should be possible in vivo. The robustness of the observed signal raises the possibility that pairing might even be the "default" option, limited to desired situations by specific features. Protein-independent homologous pairing of intact dsDNA has been predicted theoretically, but further studies are needed to determine whether existing theories fit sequence length, temperature, and salt dependencies described here.

dsDNA | sequence-dependent

Pairing of homologous DNA/chromosome regions is a central feature of many biologically important processes. Recombinational double-strand break repair and programmed homologous recombination during meiosis all involve complex series of biochemical reactions in which single-stranded DNA (ssDNA) plays a prominent role. There also exist homologous pairing reactions that seem to involve interactions between chromosomal regions whose DNAs are chemically intact double-stranded DNA (dsDNA) (1–15). In some instances, whole chromosomes pair via multiple interactions all along their lengths (1–9) or via any region present in duplicate copies (10). In other cases, pairing occurs preferentially or exclusively in particular localized regions ("pairing sites"), which tend to involve repeated sequences, specific proteins (for establishment and/or maintenance of pairing) and/or heterochromatic regions (characterized by a paucity of genes and a less "open" chromatin structure) (1, 11–15).

In contrast to recombination-related processes that are known to involve protein-mediated Watson-Crick basepairing interactions between a ssDNA and a ssDNA or dsDNA partner, the fundamental basis for "recombination-independent" pairing remains mysterious. The most obvious possibility is direct DNA/DNA interactions. Theoretical models have proposed that homology recognition arises from non-Watson-Crick hydrogen bond interactions between bases in the major or minor grooves (16). Local melting could also occur, permitting recognition via standard Watson-Crick base pairing. Other theories suggest that homology recognition can occur due to interactions between sequence-dependent charge distributions associated with neighboring DNA helices, where the charge distributions include not only the phosphates in the DNA but also other monovalent and/or divalent ions that are bound to or very near the neighboring DNA molecules (17–22). Interaction-induced correlations between the spatial distribution of charges can result in energy minimization when sequence-matched helices are in close proximity (17–21, 23–26) (*SI Text*). Despite its a priori attractiveness, acceptance of direct DNA/DNA pairing has been impeded by the lack of conclusive experimental evidence that such a pairing process occurs in biologically-relevant conditions. Encouragement is provided, however, by recent experiments showing evidence for preferential interactions between DNA molecules with like sequences (27, 28).

Two other general scenarios for homology recognition have been envisioned. First, information might come from local sequence information that is read out indirectly by mediating factors, e.g., by site-specific binding proteins, which then "dimerize" in *trans,* or by interaction of transcription complexes and/or RNAs that then carry out the inter-chromosomal interaction (29). Alternatively, homology might be recognized along the length of a chromosome via the spatial pattern of particular inter-chromosomal snaps, which, in the most extreme case, could be locally identical at every position in the array (30).

The present study investigates direct, homology-directed protein-independent dsDNA/dsDNA pairing. We provide multiple lines of evidence for homology-dependent pairwise interactions between chemically intact DNAs under biologically sensible conditions and begin to explore the dependence of pairing on sequence length, temperature, and monovalent salt concentration to provide a good basis for comparison with proposed theoretical models for pairing.

Results

Assay System. Pairing of homologous DNAs was determined using a parallel single molecule magnetic tweezers approach, similar to a sandwich assay (Fig. 1 and *SI Materials and Methods*). Two DNAs of interest are differentially labeled at "opposite" termini, one with biotin and the other with digoxigenin (Dig) (green circles and red diamonds, respectively). The Dig-labeled DNA can attach specifically to an anti-Dig labeled capillary, and the biotin labeled DNA can attach specifically to a streptavidin coated magnetic bead. No single DNA molecule can specifically bind a magnetic bead to the capillary surface, but if a DNA molecule labeled with Dig pairs with a DNA molecule labeled with biotin, then a paired molecule can specifically bind to both a bead and the capillary. After imposing a modest force to extend the paired molecules, the number of paired molecules is determined by counting the magnetic beads tethered to the capillary. This system has the potential to detect up to approximately 1,000 tethered beads in parallel in a single assay (31). Our apparatus is not calibrated at forces below 2 pN; however, previous measurements suggest that 0.4 to 0.7 pN are required to extend unpaired dsDNA to between 12 and 13 μm (32), so the pairing assay measures the number of pairs that withstand at least 0.4 pN.

The distance between the tethered beads and the surface of the capillary provides additional confirmation that the pairing is homologous (Fig. 1). Pairing was examined either between a Dig-

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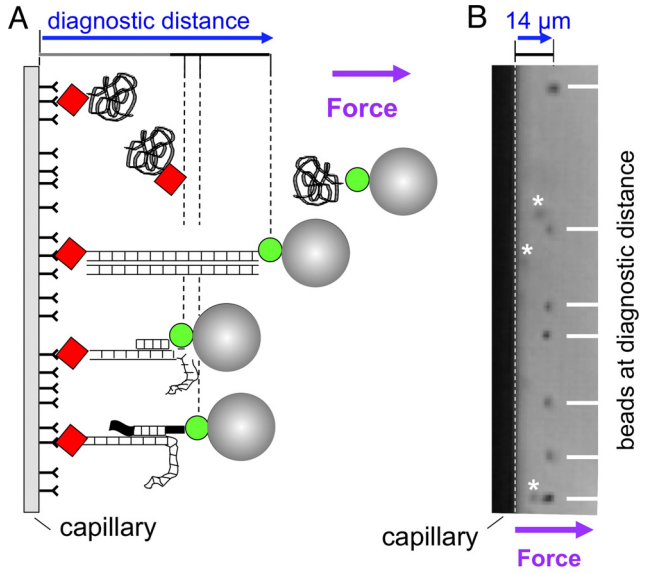

Fig. 1. Pairing of homologous DNAs using the parallel single molecule, magnetic tweezers-based assay. (*A*) Experimental approach. The black lines represent dsDNA molecules and the two samples are distinguished by red diamonds or green circles attached to their ends corresponding to Dig- and biotin labels, respectively. The dsDNA samples after incubation are mixed with superparamagnetic beads (gray circles) and incubated inside a capillary. (*B*) Image showing a black region that corresponds to the capillary. The distance between the inner capillary surface and the center of the bead is approximately 14 μm, corresponding to a DNA extension of approximately 12.5 μm. The asterisks show beads that are out of focus because their corresponding molecules are tethered to positions other than the edge of the (round) capillary that is in focus. Out-of-focus beads are not counted in the assay.

labeled lambda and a biotinylated lambda [λDNAs (λ × λ pairing)] or a Dig-labeled λ and a smaller molecule that includes a subregion of λ and a biotin tail. In the second case, since the shorter molecules have lengths less than 1/9 of lambda, the distance between the bead and the capillary should correspond to the sequence-matched position of the biotinylated end in an unpaired λ molecule stretched by the same force. Standard conditions for pairing analysis were 150 mM NaCl phosphate buffer (PBS) at 25 °C with equal volumes of the two differently labeled molecules, always at equal concentrations, which ranged from approximately 1–3 nM (in molecules) and 30–100 μg/mL.

A Signal Diagnostic of Homologous λ × λ Pairing Emerges with Time of Incubation. Coincubation of differentially-labeled λDNAs under standard conditions generates an appropriate diagnostic signal that initially increases with time and then plateaus (Fig. 2*A*). At very short (10-min) incubation times, no pairing signal is observed above nonspecific background, confirming that no pairing is occurring during the tethering steps; thus, the signal observed after longer incubation times does not involve artifactual colocalization of DNAs on the beads or other features of the detection system. In most experiments, the number of DNA-DNA pairs formed as a function of incubation time approaches an asymptotic value (e.g., Fig. 2*A*), but the values at times longer than 5 h are less reproducible than the data for shorter times. Some experiments even show a significant decrease in bound pairs at very long times, possibly due to the onset of interactions between more than two dsDNA.

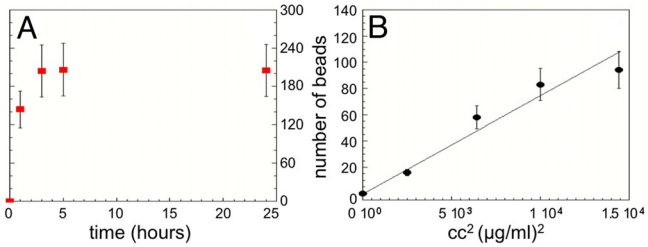

Fig. 2. Pairing of Dig-labeled λ DNA and biotin labeled λ DNA. (*A*) Number of tethered beads vs. time in 87 μg/mL λ DNA in PBS and incubated at 37 °C. (*B*) Number of tethered beads vs. square of the DNA concentration.

The λ × λ Pairing Signal Results from Pairwise Association. In a pairwise interaction, the rate of formation of beads bound at the appropriate diagnostic distance should increase with the square of the (equal) concentrations of the two differentially labeled DNAs. We measured the number of bound beads formed at various DNA concentrations after a 1h pairing incubation because at 1 h the level of pairing is still increasing linearly with time (Fig. 2*A*), so the number of beads corresponds to the rate of pairing. Fig. 2*B* shows that this rate increases with the square of the concentration of molecules for DNA(s) concentrations of 30–100 μg/mL (1–3 nM in molecules). A possible slight deviation from this relationship occurs at the highest concentration where, even at 1 h the number of bound beads may have begun to saturate due to almost complete pairing (below). At concentrations below 30 μg/mL (1 nM in molecules) the number of tethered beads is of the order of the nonspecific binding signal. Thus, the observed bead-tethering signal represents a pairwise DNA/DNA interaction.

Homologous λ × λ Pairing Is Very Efficient. To assess the efficiency of homologous pairing we compared the number of tethered beads observed in a standard λ × λ pairing reaction with that observed for a single one with biotin attached at one end and Dig attached at the other, where tethering does not require interaction with another molecule. The two samples contained the same total amounts of DNA [80 μg/mL (2.4 nM)], and were incubated in parallel in 150 mM NaCl at 37 °C for 1 h, where the 1 μL of the sample was added to 49 μL of buffer before the fluid was inserted into the capillary, whereas in most experiments 3 μL of sample were added to 47 μL of buffer. The lower sample volume was used so that most of the bound beads in the doubly-labeled sample were not touching another bound bead, but the number of bound beads in the paired sample was also reduced. Thus, errors associated with counting noise and uncertainties in sample volume were worse in these experiments than the other experiments reported in this work. One experiment gave 820 bound beads for the doubly labeled control DNA sample and 42 bound beads for the pairing DNA sample, where the nonspecific binding is subtracted from the pairing total. Similarly, a 2-μL and a 3-μL sample had 79 bound beads and 125 bound beads after the nonspecific binding was subtracted; therefore, a linear scaling of the 2- and 3-μL samples would predict 39.5 and 42 bound beads, respectively. The three different pairing samples were taken on different days with significant differences in the fractional nonspecific binding, but for all three cases the pairing efficiency is approximately 20%, since perfect pairing would correspond to a bound bead number that is 25% that seen for the doubly labeled DNA*. At 45 °C, the number of beads for a 3-μL sample 93 μg/mL (3 nM) of DNA exceeded 400 after the nonspecific binding was subtracted, corresponding to an efficiency of approximately 50%. We conclude that homologous pairing of two 48-kb λDNAs is an extremely efficient process, with essentially complete pairing readily achievable.

*(*i*) If all moleules were present in homologous pairs, half of all pairs would contain either two biotin-labeled molecules or two Dig-labeled molecules and would not be detected. (*ii*) Each tethered bead represents two λDNAs rather than a single DNA as in the control sample.

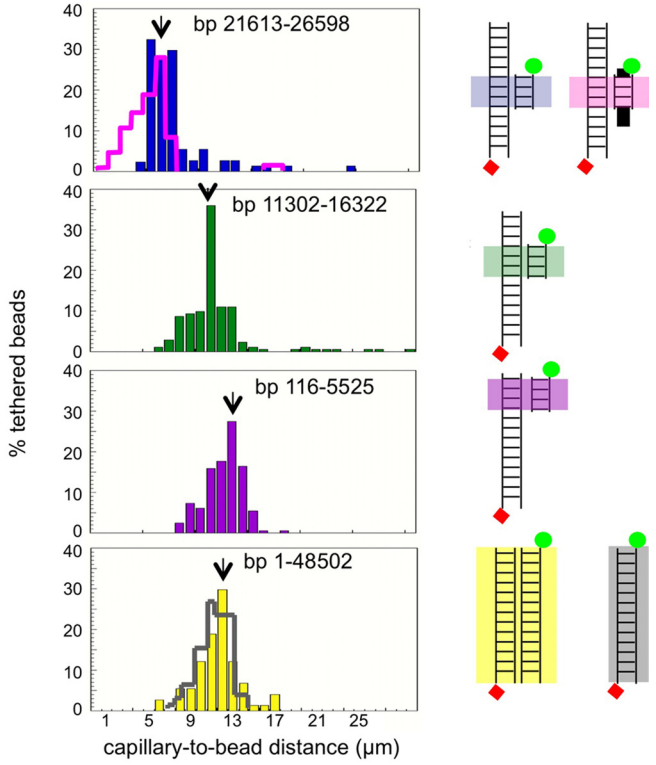

Fig. 3. Effect of sequence on DNA pairing. Distribution of extensions for about 100 beads at 0.4 pN for Dig-labeled λ phage dsDNA incubated with biotinylated molecules subregions from λ phage: 5-kb fragment bp #21613 (blue) and 5 kb fragment presenting tails (magenta outline); 5-kb fragment bp #11302 (green); 5 kb fragment bp #116 (purple); biotinylated λ phage (yellow). λ phage molecules with both labels are shown for comparison (gray outline). The schematic representations are shown to the right of each panel.

Five-Kilobase DNAs Pair with the Homologous Regions of Full Length λ DNA. We have also examined the interaction between λ DNA labeled with Dig and a series of biotinylated DNAs carrying 1–5 kb subregions of λ corresponding to selected positions along the length of the λ genome. Histograms of the fraction of the detected beads located at particular distances after 1 h incubation were determined for three different λ subregions. In each case, pairing occurs at exactly the appropriate position (Fig. 3, blue, green, and purple histograms). For comparison, the distribution of bead positions is also shown for the standard λ × λ pairing reaction and for a control sample in which biotin and Dig-labels are present at the two ends of each individual DNA (Fig. 3, yellow and gray-outlined histograms, respectively). The overlap of the yellow and gray histograms suggests that pairing does not significantly alter the extension at the applied force.

The above-5-kb DNAs included only the sequences of interest. To exclude the possibility of Watson-Crick pairing at the ends of molecules, we also examined pairing of Dig-labeled full-length λ with a biotinylated molecule in which 5 kb of λ sequence was embedded in flanking nonhomologous sequences of 400-bp and 2-kb at the bead-proximal and bead-distal ends, respectively. Pairing at the appropriate position still occurs with comparable efficiency (Fig. 3, compare magenta outline histogram with underlying blue histogram in top panel). Importantly, no pairing signal is observed between full-length λ and a nonhomologous 5 kb DNA, from pcDNA3.1, where there is no increase in the number of bound beads as a function of the incubation time (Fig. S1*A*). Further: (*i*) the number of beads bound (per unit length of capillary) is similar to that seen in control samples; and (*ii*) the distribution of bead positions peaks at the length of λ (≈12–13 μm), which is also the dominant bead position for nonspecific binding of a control sample containing only λ DNA end-labeled with Dig (Fig. S1 *B* and *C*).

Pairing Can Be Mediated by Regions of Homology Shorter than 5 kb. We also compared pairing between full-length λ DNA and DNAs sharing 1- to 5-kb regions of homology with or without long flanking nonhomologous tails on either side. In PBS, in the absence of tails, the pairing of a 1-kb λ DNA by full length λ does not result in bead binding that significantly exceeds a nonhomologous control (λ× pcDNA3) (Fig. S2). In contrast, molecules with 1 kb of homology flanked by long nonhomologous tails (Fig. S3 *A* and *B*) exhibit high levels of tethered beads, comparable to those for long-tailed molecules with 5 kb of homology (compare second and fourth rows in Fig. S3*C*). The same results are seen for analogous substrates with 2-kb of λ homology (compare second and third rows in Fig. S3*C*). However, for long-tailed molecules, the resulting position distributions increase in width as the length of the homologous region decreases (from 1–5 kb) and some distributions do not even peak at the homologous position. Importantly, these pairing signals are absolutely dependent on the presence of homology: the number of bound beads observed in these reactions significantly exceeded those for a biotin-labeled molecule containing only the nonhomologous tail regions and no λ DNA (Fig. S1) or in standard control samples.

The data above suggests the following: (*i*) formed pairs are significantly unstable; (*ii*) in the presence of long nonhomologous tails, loss of homology-dependent contacts leads to "sliding" of the smaller duplex, which preserves the interaction at nonhomologous positions; (*iii*) in the absence of adjacent nonhomologous tails, loss of homologous contact results in unbinding. These observations imply the existence of a short-range homology-independent attractive interaction, which becomes important only after homologous interactions have brought the nonhomologous portions into sufficient proximity. Such a short–range, homology-independent interaction is consistent with experiments showing that a single dsDNA can form a tightly coiled toroid where toroid formation can be initiated by kinks that bring adjacent parts of the dsDNA molecule sufficiently close to one another (33).

Homologous Pairing Is Increased by Crowding Agents and Is Not Affected by the Presence of Competitor DNA or BSA. We studied pairing under reaction conditions closer to those present in vivo.

Molecular Crowding. Our standard pairing reactions contain approximately 0.003 pmol of each type of DNA in a 2-μL volume, for an overall total molar concentration of homologous molecules of 3 nM. This is approximately the molar concentration of two homologous DNAs in a single yeast cell nucleus (two molecules in nucleus of 1 μm diameter, i.e., a volume of 10^{-15} L). Since excluded volume effects associated with molecular crowding in vivo might increase the pairing level, we added polyethylene glycol (PEG; average molecular weight 8,000 Da), often used as a crowding agent in DNA experiments. Adding PEG increases pairing during short incubation times (1–5 h) (Fig. 4*A*), implying an increase in the initial pairing rate that is consistent with indications of homology-dependent dsDNA associations under crowding created by osmotic stress (27, 28).

Nonhomologous Competitor DNA. Pairing between λ and a 5 kb λ fragment (lacking tails) is not reduced by the presence of unrelated DNAs of several types including: (*i*) a 5 kb fragment of pcDNA3.1 at three times the concentration of the 5 kb fragment (in molecules and base pairs) (Fig. 4*B*); 40- to 300-kb (average 200-kb) human genomic DNA fragments at one quarter the concentration of the 5 kb fragment in molecules and a 10-fold excess in base pairs (Fig. S4); or (*iii*) fish sperm DNA, average length approximately 400-bp, at 250 times the concentration of the 5-kb DNA in molecules and 20 times in base pairs (Fig. 4*B*). Indeed, in the latter case, both the rate and final level of pairing are *increased* by nonhomologous DNA, a

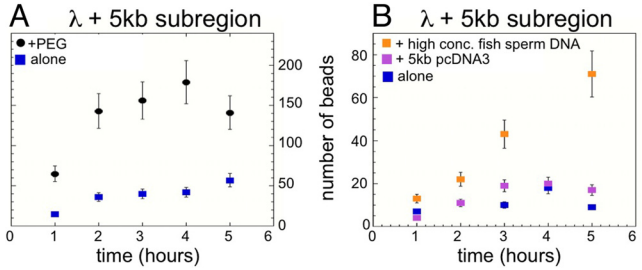

Fig. 4. Intermolecular pairing between 6 μg/mL biotinylated 5-kb fragments and 60 μg/mL Dig-labeled λ phage in the presence of crowding agent and nonspecific competitors. (*A*) Pairing of Dig-labeled λ DNA with 5 kb subregion bp #21613 with and without 15% p/v PEG 8000. (*B*) Pairing of Dig-labeled λ DNA with 5 kb subregion bp #21613 in the presence of high concentration of fish sperm DNA (orange), pcDNA3.1 (purple) and control without competitor (blue).

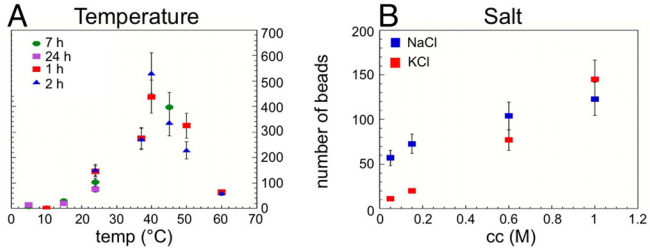

Fig. 5. Effect of temperature and salt on the DNA-DNA interaction. (*A*) Number of tethered beads vs. temperature for biotinylated λ phage and Dig-labeled λ phage, 83 μg/mL in PBS incubated during several time intervals at 37 °C; the values on the *y* axis correspond to an incubation time of 24 h. The data for other incubation times has been normalized so that all data sets have the same value at 37 °C. (*B*) Number of tethered beads vs. salt concentration for 60 μg/mL biotinylated λ phage and 60 μg/mL Dig-labeled λ phage incubated for 1 h at 37 °C in phosphate buffer 10 mM: NaCl (blue) and KCl (red).

result we attribute to excluded volume effects. We also note that the total DNA concentration (in base pairs) present in that situation, approximately 2 mg/mL, approaches the range of DNA concentrations found in a eukaryotic nucleus in vivo (10–50 mg/mL). Thus, homologous pairing is not decreased by competitive nonhomologous dsDNA at concentrations substantially exceeding those of the homologous dsDNA, for competitors of varying lengths relative to the 5-kb homologous DNA partner.

Protein. No evident effect on pairing is observed by inclusion of 0.1% BSA (approximately 15 μM) (Fig. S4).

Although we cannot fully reproduce the complex in vivo nuclear environment, these results show that homologous pairing is not impeded by nonhomologous sequences; homologous pairing is not suppressed in complex environments; and the rate and efficiency of pairing is increased by the crowded conditions characteristic of the cell nucleus.

Comparison with Watson-Crick Pairing. The above observations show that pairing can occur in regions far from the ends of either interacting molecule. Thus, pairing is not attributable to melting/fraying that gives rise to open ends and therefore not attributable to simple Watson-Crick reannealing at such ends. Four additional experimental probes further argue against possible involvement of ssDNA in the observed pairing as described below.

First, we studied the effect of T4 gene32 ssDNA binding protein on $\lambda \times \lambda$ pairing. Given that T4 gene32 can bind to as few as eight bases of ssDNA occlusion by gene 32 protein should inhibit the reaction if ssDNA plays a significant role; however, the protein has no effect on the pairing.

Second, we measured the ability of base pairing interactions to mediate pairing by examining the formation of λ phage DNA dimers created from one λ labeled with biotin and a second λ labeled with Dig at its "opposite" end where the two carried complementary 12-bp ssDNA overhangs at their respective unlabeled ends. After a 2h incubation under standard pairing conditions, [60 μg/mL ($\approx$2 nM) of each DNA)], the number of dimers was only 20% the number of DNA-DNA pairs measured in parallel in our standard $\lambda \times \lambda$ reaction under the same conditions.

Third, we heated a $\lambda \times \lambda$ pairing mixture to 50 °C, and then quickly quenched it, before incubation for pairing. This procedure should create ssDNA bubbles located preferentially in AT-rich regions. These ssDNA bubbles could pair with other open bubbles or interact with duplex DNA; however, no increase in the rate or level of pairing was observed.

Fourth, regions of Watson-Crick duplex as short as 12-bp are resistant to shear forces up to 25 pN (34). In contrast, molecules linked by homologous pairing over regions of 5- or 48.5-kb shear at forces of 10–20 pN. Thus, homologous associations are not maintained by single or multiple Watson-Crick base-pairing interactions of 12-bp or longer, although involvement of even shorter regions of base-pairing is not excluded.

Finally, we note that the long-tailed DNA segments that pair efficiently with full-length λ DNA were generated from in vivo amplified plasmid DNA, and terminally biotinylated subfragments were prepared without the use of ethanol or any chemical (e.g., phenol) known to generate deformations (Fig. S3*A*). Thus, pairing is unlikely to involve deformed structures along the DNAs.

Pairing as a Function of Temperature and Monovalent or Divalent Salt.

Temperature. The pairing rate and saturated pairing level increase significantly as the temperature is raised from 5–40 °C, and then decreases strongly at higher temperatures. (Fig. 5*A*). Preliminary experiments further suggest that the width of the bead location distribution does not increase for temperatures between 40 and 60 °C, even though the number of paired molecules decreases dramatically. Thus, the reduced higher temperature signal still corresponds to regular homologous pairing and higher temperature does not promote nonhomologous pairing. The latter result was confirmed by measurements of the pairing between lambda and pcDNA3.

Monovalent and Divalent Salt. The rate of pairing, defined by pairing levels after a 1h incubation, increases monotonically with the concentration of either NaCl or KCl over a range from 50 mM to 1 M, with slight differences for Na^+ versus K^+ (Fig. 5*B*). Below 50 mM (15 mM), pairing is of the order of the nonspecific signal. Divalent salts are well-known to promote aggregation of DNA (35). We therefore also examined homologous pairing in reaction mixtures where $MgCl_2$ is present instead of a monovalent salt. We find significant pairing between λ and a 5-kb subregion of λ in 10 mM $MgCl_2$ during a 10-min incubation time, whereas pairing is almost absent in 10 mM NaCl even after 1-h incubation. Further, no pairing was observed when pcDNA3.1 was paired with full λ in 10 mM $MgCl_2$, further demonstrating that, even in $MgCl_2$, pairing is homology-dependent.

Implications. These findings are compatible with occurrence of homologous DNA/DNA pairing in vivo. Pairing occurs robustly throughout the range of temperatures encountered by most living organisms and at salt concentrations corresponding to those generally thought to occur in vivo: 150 mM monovalent salt, with K^+ predominant over Na^+; and approximately 10 mM total Mg^{2+}, with the concentration of free Mg^{2+} being considerably lower (36).

Discussion

The presented results reveal that in the absence of proteins, two homologous DNA segments can efficiently and rapidly identify one

another, and interact to form complexes stable against thermal motion. These findings confirm and extend the results of two other studies pointing to such a possibility (27, 28). The most important conclusion from the current work is that direct DNA/DNA interactions occur under physiologically sensible conditions; therefore such DNA/DNA interactions may underlie recombination-independent pairing in vivo.

The Mechanism of Homology-Dependent dsDNA/dsDNA Pairing. Physical theories for homologous pairing are discussed briefly below and in more detail in *SI Text*. Extensive discussions of theories that predict sequence dependent pairing (see 27, 28) are also excellent sources of information on possible mechanisms.

General Aspects. Any process that leads to persistent homology-dependent association of two DNA duplexes will be characterized by certain features.

First, bringing two dsDNA molecules together in solution requires one or more attractive interactions that overcome strong intermolecular repulsion due to the negative charges on the phosphodiester backbones (37). We show that the pairing rate increases with salt concentration, consistent with a reduction in repulsion, although the pairing is not simply a function of the ionic strength of the solution. Of course, changing salt concentration may also alter other interactions; however, two further findings suggest that improved screening is important (*i*) an earlier work showed that, at low concentrations, Na^+ screens the intramolecule backbone repulsion better than K^+ (38) and this work shows that at low concentrations of salt, the pairing rate in Na^+ exceeds the pairing rate in K^+. (*ii*) Similarly, the melting temperature and unzipping force also depend on the intramolecular backbone repulsion and previous work has shown that measured values for both quantities are similar for 10 mM Mg^{2+} and 150 mM NaCl (39, 40), which is consistent with the observation that the pairing rates in 10 mM Mg^{2+} exceed those in 150 mM NaCl. We note that theories that predict homologous attraction is due to a spatial modulation of the charge distribution do not predict that the pairing will occur in monovalent salts at room temperature.

Second, the attractive interaction that brings pairs together must be dominated by homology-dependent forces because (*i*) we do not observe pairing between sequences without homology; and (*ii*) pairing of homologous DNA is not suppressed by the presence of nonhomologous competitors. Strong sequence dependence of the long-range attractive interaction avoids (unwanted) nonhomologous interactions that might result from the short range nonsequence dependent attractive interaction.

Third, interactions between homologous regions must be strong enough to allow correctly bound sequences to remain together but weak enough that unmatched sequences with small regions of accidental homology unbind rapidly, thereby avoiding kinetic trapping in nonhomologous interactions.

Specific Mechanisms for Homology Recognition. Homology recognition could occur by a direct mechanism, in which attractive interactions involve the bases themselves (16), or by indirect mechanisms where recognition and attraction occur through interactions involving the helical features of the molecules that vary in correlation with base pair sequence (17–19, 21, 24). Although extensive further theoretical and experimental work is required before firm conclusions can be formed, one observation presented above helps to discriminate among models proposed thus far: we find that the rate and extent of pairing increases progressively with temperature up to 40 °C and then decreases. In general, indirect pairing models depend on the matching of the conformations of two nearby dsDNA molecules, and it has been predicted that conformation-based models [e.g., including sequence-dependent binding of ions (41, 42)] should exhibit exactly the pattern of temperature dependence that we observe. At low temperatures, homologous molecules may get frozen in nonmatching conformations that do not result in a strong attraction. Higher temperatures may allow molecules to readjust conformations in response to their neighbors, permitting the two molecules to minimize their energy by pairing. At still higher temperatures, conformational fluctuations may be too large to allow the molecules to adjust and pair. We note that the observed sequence specificity of the pairing does not decrease with temperature.

It has been suggested that pairing may result from the displacement of specifically bound water into the disordered solvent in an entropy-driven process (20). This mechanism is especially intriguing because sequence dependent hydration effects are already known to play a role in sequence dependent DNA-protein interactions (43) and RNA folding (44). It would be reasonable to assume that hydration effects also play a role in homology-dependent dsDNA/dsDNA interaction and attractive to think that such diverse situations all have a common underlying physical basis.

Implications for Recombination-Independent Pairing In Vivo. Pairing as observed in the present study is fully compatible with occurrence of analogous interactions in vivo. Pairing occurs efficiently between relatively short regions of homology; independent of DNA ends; under physiological conditions of monovalent salt; in the presence of complex nonhomologous competitor DNA and nonspecific protein; over reasonable time scales; at molecule concentrations comparable to that of two homologous segments in a yeast nucleus; with higher rates (or comparable rates at lower DNA concentrations) achievable by inclusion of crowding agents and by optimization of temperature. Further, the DNA concentrations involved in the observed pairing are orders of magnitude lower than those required for collapse of DNA into toroids in NaCl via nonspecific attractive interactions (33).

The robustness of the observed pairing process also raises the strong suspicion that intrinsic homology-based pairing interactions might be the "default option" in vivo. This notion is diametrically opposed to the common view that homologous pairing is a rare and unfavorable condition that must be specifically promoted by appropriate molecular features. Instead, evolution may have specifically ensured that pairing between homologous chromosomes is usually precluded genome-wide, with restrictions lifted specifically in specialized cases where pairing is useful [or, in a few organisms, left in place and accommodated as "somatic" pairing (1)].

It has been argued that the most significant problem for a pairing process is not the need to find the correct partner, but rather the need to avoid getting kinetically trapped in stable nonspecific interactions, which in turn requires any general homology searching process to involve weak, transient "kissing" interactions that are then further stabilized (45). Recent theoretical work and modeling on macroscopic systems (46) also supports the usefulness of a pairing process that occurs in stages where, again, the first stage requires weak transient interactions to avoid trapping in incorrect pairs. These initial weak binding of short matching regions should be strong enough to allow neighboring regions to bind if they are matched, but weak enough that the two short matching regions will unbind if the neighboring regions do not match. Under these conditions, correctly aligned homologs will rapidly form strong bonds, but short regions of accidental homology will not trap pairs in false minima. Additional recognition stages can then promote sequence stringency over longer and longer lengths.

Such considerations could help to explain why, in several situations, in two of the most robust cases of "region-specific" pairing, for the XY chromosomes of Drosophila and for "pairing centers" in *C. elegans*, specificity is conferred by clusters containing multiple nontandem repeats of short sequences (1, 11). For Drosophila, where 50 copies of a <250-bp repeat are normally involved in pairing, it has further been shown that, when pairing site sequences are moved to locations where flanking homology is absent, eight copies are largely sufficient to confer detectable pairing but two

copies are not (1). The probability of an initial contact between two DNAs in a short region will be independent of the presence of related sequences nearby and will be weak but stringently dependent on strong homology; the presence of repeats allows the sequence dependent long range interaction to efficiently bring molecules together even when they are not in correct registration as long as they are aligned within the region of the repeats.

Conversely, sticky sequence-specific protein factors would seem unsuitable as primary mediators of homology recognition. Instead, proteins might either (*i*) enhance the susceptibility of underlying sequences to DNA/DNA interaction; (*ii*) further stabilize contacts made at the DNA level; and/or (*iii*) carry out unrelated functions, e.g., to tether paired regions to subcellular locations. Correspondingly, for Drosophila somatic pairing, on the scale of an entire chromosome, an effective pairing contact, once formed, is quite stable (47).

It is also interesting to consider the possibility of direct DNA/DNA pairing interactions as a factor in interactions not only between different (homologous) chromosomes but between sister chromatids. Such pairing might act before, or in concert with, the known factors of protein-mediated cohesion and topological linkages. Sister chromatids will automatically tend to emerge from a replication complex into a confined joint space, and at least transiently, lack a full complement of nucleosomes, features that could favor DNA/DNA interactions between sisters.

Conclusion

The current observations show that homologous dsDNAs can specifically recognize one another and pair stably enough for detection on a time scale of minutes/hours. These findings encourage future studies to assess the physical basis for such homology-dependent recognition and the relevance to, and rules for, DNA/DNA-mediate homologous pairing in vivo.

Materials and Methods

For pairing between two full-length λ DNAs (NEB;48,502 bp) two types of samples were prepared. (I) λ DNA was hybridized and ligated to an oligonucleotide complementary to the ssDNA tail at the left end of λ that contained a Dig-label and to another oligonucleotide without label at the right end. (*ii*) λ DNA was hybridized and ligated to a biotinylated oligonucleotide at the right end and to an oligonucleotide without label at the left end. Pairing between λ and smaller DNA molecules lacking nonhomologous tails was achieved with high fidelity PCR fragments.

In our apparatus, the magnets were held in a lateral position with respect to the microchannel on a 3-axis translation stage to exert a force perpendicular to the glass surface to which the DNA was bound. The assay system is discussed in detail in *SI Text*.

Pairing Reaction Protocol. Initially equal volumes of each sample are mixed and incubated for a chosen period after which an aliquot is: (*i*) incubated for 2 min at 37 °C with superparamagnetic (Dynal; 2.8-μm diameter) streptavidin-coated magnetic beads; (*ii*) placed for 10 min in a microchannel containing a round capillary, 0.55-mm diameter, previously coated with anti-Dig antibody. After incubating, a force is applied to the beads by bringing a permanent magnet close to the capillary to apply a constant force of less than 1 pN.

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