these molecular weight studies, the polymerization solutions were diluted with tetrahydrofuran and injected directly into the GPC in order to avoid any fractionation of the polymer samples during isolation, which would artificially narrow the molecular weight distributions. This system can also be used for the synthesis of well-defined polyacrylates. For example, the bulk polymerization of methyl acrylate (initial concentrations [methyl α-bromopropionate]$_0$ = 0.03 M, [CuBr]$_0$ = 0.03 M, and [dHbipy]$_0$ = 0.06 M) after 1.5 hours at 110°C yields a polymer with M_n = 17,000 and M_w/M_n = 1.05.

Generally, homogeneous ATRP is useful for synthesizing various polymers up to a molecular weight of 50,000 to 80,000. Above this molecular weight range some limiting side reactions can be noticed. We have also used this system to polymerize other vinyl monomers; to prepare block, random, graft, and gradient copolymers; and to synthesize macromolecules with various topologies (branched, hyperbranched, and star polymers) (*16*).

Computer simulations of the experimental kinetic and molecular weight data indicate that <3% of the chains are terminated at >99% monomer conversions. In addition, because the polymerizations are fast, the proportion of chains formed by thermal self-initiation of styrene does not exceed 1%. Thus, nearly all of the chains are either in the dormant or active state throughout the polymerization, fulfilling the requirements for a living polymerization. However, because termination undoubtedly occurs, we prefer to use the term "controlled" or living polymerization.

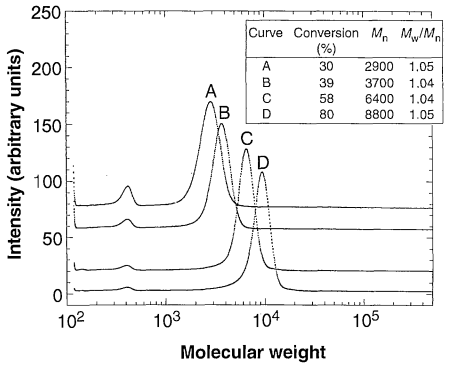

Fig. 2. Evolution of the GPC chromatograms (curves A through D) as a function of conversion for an ATRP of bulk styrene at 110°C using [CuBr]$_0$ = 8.4 × 10^{-2} M, [1-PEBr]$_0$ = 8.8 × 10^{-2} M ([monomer]$_0$/[initiator]$_0$ = 100), and [dHbipy]$_0$ = 17.5 × 10^{-2} M (1-PEBr = 1-phenylethyl bromide). An 80% conversion (GPC chromatogram D) was reached after 4 hours. Similar behavior has been observed for ligands dNbipy and dTbipy. The small signals in the low molecular weight range in each chromatogram are due to the residual catalyst used in these polymerizations (a result of injecting the diluted polymerization mixture directly into the GPC apparatus).

These simulations also show that the polydispersities are strongly affected by the ratio of the rates of deactivation to propagation. In these studies, the new ligands increase the solubility of both the Cu(I) and the Cu(II) species in the polymerization medium [commercial CuBr contains ~1 to 2% Cu(II)]. The latter species acts as a deactivator, and therefore its presence initially in the polymerization maintains a sufficiently high rate of deactivation relative to propagation throughout the polymerization. As a consequence, the experimentally observed polydispersities remain low. When polymerizations are conducted with very pure Cu(I) halide, containing no Cu(II) halide, the polydispersities of the resulting polymers increase (>1.10).

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Homologous DNA Pairing Promoted by a 20–Amino Acid Peptide Derived from RecA

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The molecular structure of the *Escherichia coli* RecA protein in the absence of DNA revealed two disordered or mobile loops that were proposed to be DNA binding sites. A short peptide spanning one of these loops was shown to carry out the key reaction mediated by the whole RecA protein: pairing (targeting) of a single-stranded DNA to its homologous site on a duplex DNA. In the course of the reaction the peptide bound to both substrate DNAs, unstacked the single-stranded DNA, and assumed a β structure. These events probably recapitulate the underlying molecular pathway or mechanism used by homologous recombination proteins.

Homologous recombination is the central phenomenon underlying genetic processes and the principal tool in genetic analysis, gene mapping, and gene targeting. The key reaction in homologous recombination is the pairing of any two arbitrary but homologous DNA molecules. Prokaryotic RecA protein plays an essential role in bacterial homologous recombination pathways and is the prototype for homologous DNA pairing proteins (*1*, *2*). RecA is a 38-kD polypeptide with diverse biochemical activities, including the promotion of DNA pairing in a reaction that involves three strands (*3*). RecA binds to single-stranded DNA (ssDNA), and the resulting nucleoprotein (presynaptic) filament is the homology-searching moiety that mediates the pairing with a target duplex. The DNAs that become paired reside within a hole 25 Å in diameter as seen in projection down the axis of a protein helix consisting of six protomers per turn (*4*). Lining this cavity and projecting toward the helix axis are two disordered or mobile loops, L1 and L2, that have been proposed as DNA binding sites (*5*).

Several lines of evidence indicate that loop L2 is a DNA binding domain. (i) Proteolysis of ssDNA-RecA complexes yields a unique 4-kD peptide protected by the DNA that spans this loop (*6*); (ii) photocrosslinks between a 5-iododeoxyuridine–containing ssDNA and RecA map to loops L1 and L2 (*7*); (iii) the fluorescence of a tryptophan substituted for the central phenylalanine in loop L2 is quenched in RecA-DNA complexes (*8*); and (iv) the 20–amino acid FECO peptide (Fig. 1) binds

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Fig. 1. Sequences of the synthetic peptides corresponding to amino acid residues 193 to 212 in the *E. coli* RecA protein. Underlined amino acids are highly conserved among RecA-like proteins. FECO, WECO, YECO, HECO, and AECO have phenylalanine (F), tryptophan (W), tyrosine (Y), histidine (H), and alanine (A), respectively, at the central 203 position. WT-14 includes those amino acids in loop L2 proper. WT-Scr is a peptide with wild-type amino acid composition but scrambled sequence. Abbreviations for the amino acid residues are as follows: A, Ala; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; T, Thr; V, Val; W, Trp; and Y, Tyr.

FECO	**NQIRMKIGVMFGNPETTTGG**
WECO	**NQIRMKIGVMWGNPETTTGG**
YECO	**NQIRMKIGVMYGNPETTTGG**
HECO	**NQIRMKIGVMHGNPETTTGG**
AECO	**NQIRMKIGVMAGNPETTTGG**
WT-14	**RMKIGVMFGNPETT**
WT-Scr	**IPEQTKGGRNTMNVFGMGIT**

to ssDNA, whereas three peptides that correspond to single-point mutations that abrogate the function of the whole RecA protein in vivo do not bind to ssDNA (6). Here we show that the FECO peptide not only binds to ssDNA but also has other activities, including the pairing of homologous DNAs. In addition, we explore how changes in the peptide sequence might enhance these activities.

A phenylalanine in the FECO peptide, corresponding to position 203 in the whole RecA protein, is the most conserved residue in the region corresponding to L2 (positions 195 to 209) among prokaryotic RecAs and their eukaryotic homologs, such as the DMC1 and RAD51 proteins (9). An aromatic amino acid in position 203 is important for binding, and peptides FECO, WECO, and YECO, spanning loop L2 (amino acids 193 to 212), can bind not only to ssDNA but also to double-stranded DNA (dsDNA), the other substrate of RecA, in the presence of Mg^{2+} ions (Fig. 2) (*10, 11*). Although only tyrosine and phenylalanine have been observed at this position in RecA-like proteins, the tryptophan-bearing WECO peptide binds most tightly to DNA. As with the whole RecA protein, the binding by these three peptides appears to be cooperative and presumably reflects peptide-peptide interactions on the DNAs. Binding could not be detected with peptides AECO (Fig. 2), HECO, WT-14, and WT-Scr (*12*).

Next, we demonstrated that these aromatic amino acid–bearing peptides not only bind to ssDNA but also unstack it. RecA extends both ssDNA and dsDNA by 50% (*13*). The unstacking of the ssDNA has been proposed to be essential for RecA to facilitate the three-stranded interactions between ss- and dsDNA (*3*). In addition, other RecA-like proteins, including eukaryotic RecA homologs and the bacteriophage T4 UvsX protein, have been shown to extend DNA (*14*). Thus, this distortion of the substrates constitutes a structural signature for the mechanism of homologous pairing mediated by this class of proteins. Unstacked DNA bases were more accessible to modification by potassium permanganate (PP), an agent that attacks thymines in a direction perpendicular to the base plane; modification was monitored by strand cleavage at the modified base (*15, 16*). A ssDNA oligonucleotide was much more reactive to PP in a complex with RecA; DNA binding peptides changed the reactivity of the thymines in ssDNA in a similar manner (Fig. 3A). Thus, whereas the shuffled non–DNA binding WT-Scr peptide had no effect, binding by WECO and FECO resulted in a pronounced modification of the thymines. As a result,

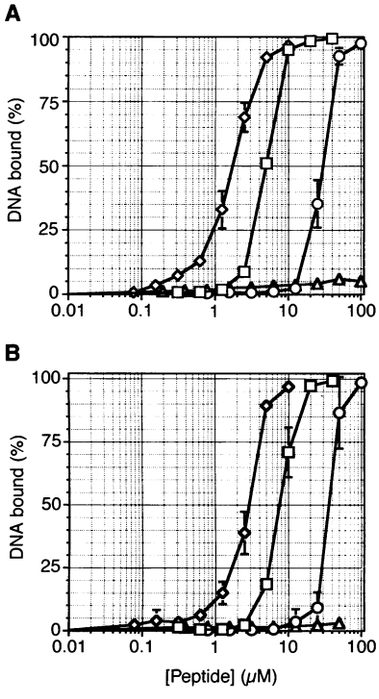

Fig. 2. Aromatic amino acid containing peptides derived from the region of amino acid residues 193 to 212 in the *E. coli* RecA protein bind both ss- and dsDNA. Binding of FECO (□), WECO (◇), YECO (○), and AECO (△) to ssDNA (**A**) and dsDNA (**B**) is shown.

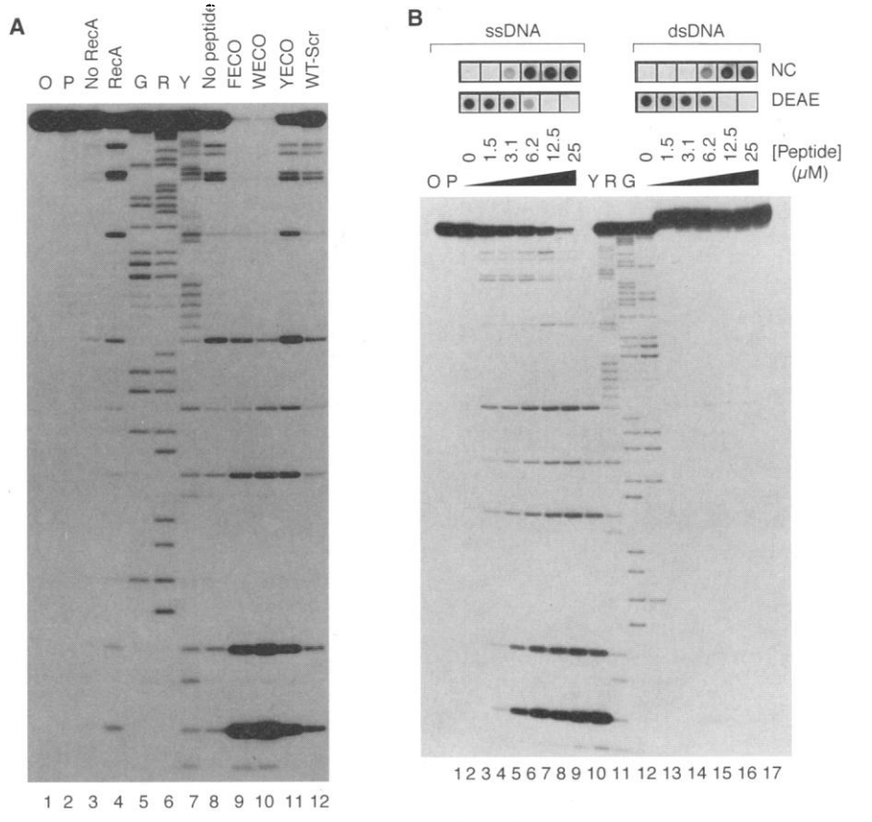

Fig. 3. DNA binding peptide unstacks ss- but not dsDNA. Reactivity of thymines toward potassium permanganate. Lanes G, R, and Y are standard Maxam-Gilbert sequencing ladders; lane O, oligonucleotide BS-S1; lane P, BS-S1 treated with pyrrolidine. (**A**) Reactivity of ss oligonucleotide toward PP in complex with RecA, FECO, WECO, YECO, and WT-Scr. The RecA and peptide concentrations were 2.7 μM and 25 μM, respectively. (**B**) (**Top**) Autoradiographs of filters showing that with both ss- and dsDNA, FECO forms complexes that are retained by nitrocellulose. (**Bottom**) Increase in FECO concentration results in a hyperreactivity of thymines in ss- but not dsDNA.

little if any full-sized fragment remained, and most of the radioactivity was detected in very short oligonucleotides, suggesting multiple hits (Fig. 3A). YECO, which binds to ssDNA less tightly, induced less unstacking. These results indicate that, as with the whole RecA protein, WECO, FECO, and YECO unstack ssDNA upon binding.

With the wild-type FECO peptide, the extent of modification of the thymine residues in ssDNA increased with FECO concentration in a manner that paralleled the binding profile (Fig. 3B). However, whereas the binding of FECO to dsDNA was similar to that for ssDNA, no distinct modification of dsDNA was observed at the peptide concentrations tested (Fig. 3B) (*17*). That we were unable to detect changes in the structure of dsDNA suggests that the peptide uses different modes to bind ss- and dsDNA and that binding to dsDNA does not occur at single-stranded regions in the duplex.

Just as the structure of the DNA is altered by peptide binding, the conformation of the peptide might change on binding to DNA. Indeed, binding of FECO to ssDNA induced a conformational transition (*18*) from a random coil to a predominantly β structure (Fig. 4A). Whereas the dominant negative circular dichroism (CD) peak at ~198 nm at lower peptide concentration represents a random-coil structure, the amplitude increase at both 190 and 215 nm at higher peptide concentrations indicates a concentration-dependent building of a structure with a high β-structure content (*19*) on the DNA. In the absence of DNA, the peptide remained a random coil even at higher peptide concentrations (for example, 0.6 mM). The isodichroic point at about 207 nm is consistent with a transition between two states. WECO formed an even more complete β structure than FECO on binding to DNA (Fig. 4B). All the other peptides tested (with the exception of YECO, which showed less β structure than FECO) remained predominantly random-coil structures in the presence of ssDNA, as indicated, typically, by the HECO CD (Fig. 4D). In addition, the reduction in the 278-nm DNA band for the WECO-ssDNA complex (Fig. 4B, inset) supports the chemical modification data showing that the ssDNA is unstacked upon complex formation (*20*). Binding to dsDNA also induced the formation of a β structure in WECO but, consistent with the chemical modification data, resulted in only minor changes in the 278-nm band (Fig. 4C). This result suggests that the conformational change of the peptide from random coil to β structure is important in binding to ss- and dsDNA and unstacking of the ssDNA. The cooperative behavior observed in the CD experiments and in the binding profile (Fig. 2) is suggestive of a β structure resulting from interpeptide as well as intrapeptide interactions. In support of these interpeptide interactions is the finding that the β structure can be induced at higher pH values in a concentration-dependent manner (*21*) and is thus an intrinsic property of the peptide that can be stimulated by, but is not dependent on, DNA.

Although FECO and WECO bind to both of the substrate DNAs (ss- and dsDNA) used by RecA to mediate homologous pairing, assume a well-defined structure as a result of this binding, and unstack ssDNA, one would not expect 20–amino acid peptides to pair a ssDNA with a dsDNA in a homology-dependent manner. When we incubated a ss 53-nucleotide ssDNA with supercoiled plasmid DNA in the presence of the peptides and displayed the deproteinized products on an agarose gel (*22*), we observed the targeting of the ssDNA to its homologous site on the duplex DNA and the formation of joint molecules similar to those formed by RecA (Fig. 5) (*23*). FECO and WECO promoted the formation of stable joints between a ^{32}P-labeled ss oligonucleotide and a plasmid bearing a homologous duplex target; the joint molecules were detected as a comigration of radioactivity with the plasmid. We recovered 10 to 20% as many joint molecules from reactions with these two peptides as with the whole RecA protein. As with the whole RecA protein, no joints were observed when the ss oligonucleotide was replaced with a duplex of the same sequence or when a target plasmid without homology to the ss oligonucleotide was used. YECO and peptides that do not bind to DNA did not form joint molecules. The formation of stable joint molecules is not dependent on the sequence targeted; in a separate experiment, another plasmid was targeted with a different ss oligonucleotide (*12*). Although the results shown are from peptide-promoted reactions carried out at 45°C, reactions at 37°C and room temperature also resulted in the formation of joint molecules with yields of 50% and 5%, respectively, of those at 45°C.

Joint molecule formation can be assayed with two types of duplex targets, linear

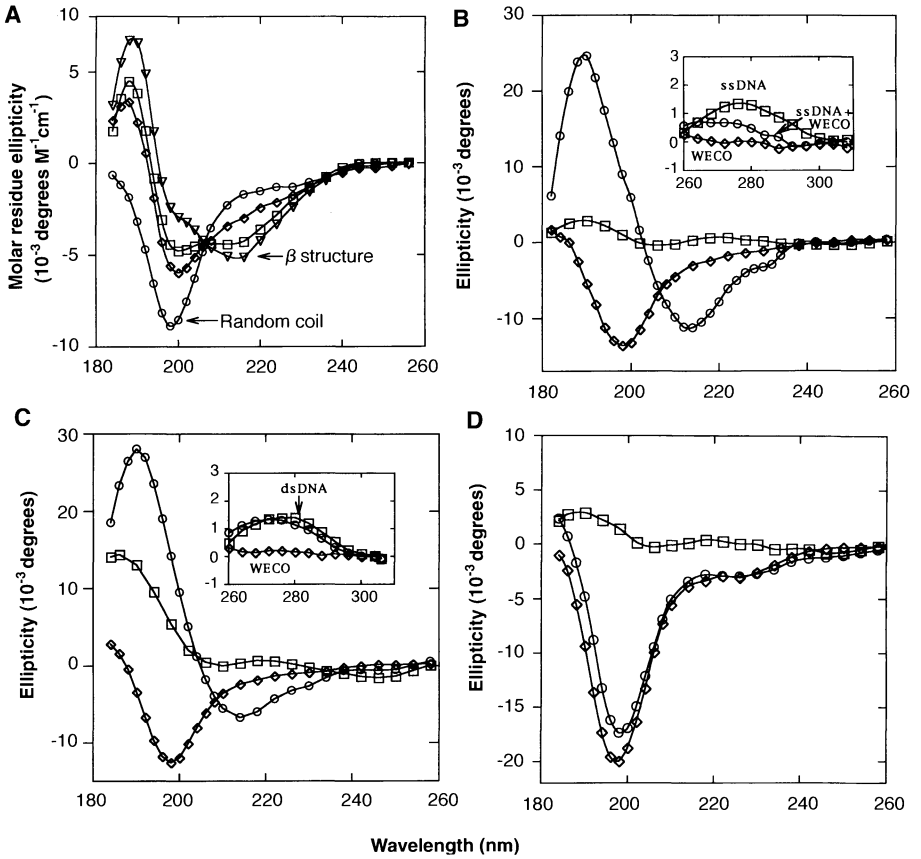

Fig. 4. The ss- and dsDNA binding peptides assume a β structure upon complex formation. A 57-nucleotide ssDNA (CTGTCTACTCTCGAGGTTAACCCGTGCGAATTCTACGATTGGTGCGGCCGGTATATC) was purified by SDS-PAGE, and the Hae III digestion products of pUC18 were used as dsDNA. All the solutions were buffered by 10 mM NaH_2PO_4. (**A**) FECO titration of 0.30 mM ssDNA at pH 7.5 ± 0.2. FECO concentrations were 0.10 mM (○), 0.20 mM (◇), 0.25 mM (□), and 0.40 mM (▽). (**B** and **C**) CD of WECO in the presence and absence of ssDNA or dsDNA, respectively, at pH 6.0 ± 0.2. (□) 0.2 mM ssDNA or dsDNA, (◇) 0.10 mM WECO, and (○) a mixture of 0.20 mM ssDNA or dsDNA plus 0.10 mM WECO. (B and C **insets**) Enlargement of the spectral region from 260 to 310 nm to emphasize the intensity change at the DNA 278-nm band. (**D**) CD of HECO in the presence and absence of ssDNA at pH 7.5 ± 0.2. (□) 0.4 mM ssDNA, (◇) 0.40 mM HECO, and (○) a mixture of 0.40 mM ssDNA and 0.40 mM HECO.

dsDNA (with ds ends) or superhelical DNA (*1*). Although the use of superhelical DNA as the target DNA may facilitate the formation of joint molecules, the absence of dsDNA ends, particularly overhanging ends, in this target greatly minimizes artifactual joint molecule formation. Thus, whereas assays that use dsDNA ends show joint molecule formation in protein-free solutions (*24*) or with proteins lacking a known role in homologous recombination [such as fatty acid synthase (*25*)], no other proteins, except RecA or members of the RecA family of proteins [for example, the bacteriophage T4 UvsX protein (*26*)], have been shown to form joint molecules with superhelical DNA as the duplex target. Even the *Escherichia coli* SSB protein—a DNA binding protein with a role in homologous recombination (*1*)—fails to form joint molecules on its own with this assay (*27*). We have confirmed this negative result with our substrates and reaction conditions and have shown that histones do not form joint molecules with this assay (*12*). Thus, DNA binding per se is insufficient for joint molecule formation when superhelical DNAs are targeted. In addition, in classical experiments (*28*) describing the D-loop assay with filter binding, substantially longer single strands gave rise to joint molecules at increased temperatures in the absence of protein. However, using our substrates, we were unable to detect any joint molecules in the absence of peptides even at 65°C (*12*).

Although nonprotein polymers such as poly (ethylene oxide) can promote joint molecule formation in other assays (*24*), they do so predominantly by inducing an increase in the effective concentration of the DNAs (*29*), thereby resulting in the annealing of the ssDNA to the dsDNA through "breathing" of the ends or the presence of overhanging ends. Subsequent nonenzymatic branch migration can then result in the formation of joint molecules (*30*). Unlike what we have observed for the FECO and WECO peptides, however, the nonprotein polymers have not been shown to bind to the substrates, distort (unstack) the ssDNA, or form a geometrically well-defined polymer–nucleic acid structure. In addition, the peptides promote joint molecule formation at concentrations less than 1000 times that of nonprotein polymers. We propose that DNA binding, unstacking of the ssDNA, and formation of a well-defined polymer–nucleic acid structure provide a benchmark for how the pairing domain of homologous recombination proteins, including RecA, specifically promotes joint molecule formation and distinguishes this molecular pathway or mechanism from less-specific mechanisms.

The finding that DNA binding peptides derived from RecA can form joints between homologous DNAs indicates that this domain comprises at least part of the active site of the whole protein responsible for DNA pairing. That the reaction proceeds efficiently in the absence of the rest of the protein and in the absence of nucleotide cofactors or their analogs suggests that the rest of the protein, in addition to its other biochemical activities, functions to modulate access to this pairing domain in a nucleotide cofactor–dependent manner—an idea supported by the adenosine 5′-triphosphate (ATP)–dependent induction of the "high-affinity binding state" of RecA (*1*) for both ss- and dsDNA. One means of regulating access is to use another protein domain as an intrasteric regulatory or autoinhibitory domain (*31*)—a role ideally suited to loop L1 (*5*).

The mechanism of joint molecule formation by either RecA or these peptides has yet to be determined. Defining the part of the domain of RecA responsible for the homologous pairing of DNAs should facilitate understanding of the structures and mechanisms responsible for this quintessential step in homologous recombination. In addition, the ability to carry out homologous pairing with such well-defined small molecules suggests a variety of applications for these peptides or peptide mimetics—applications for which RecA or its homologs have been used (*32*) or proposed, including the delivery of such proteins inside mammalian cells. For example, the recent success in blocking transcription in vivo through use of specific peptides designed to bind to DNA at a single site (*33*) in a cell may be extended to the use of a single general peptide designed to target oligonucleotides to any arbitrary but specific cognate genomic sequence (*32*).

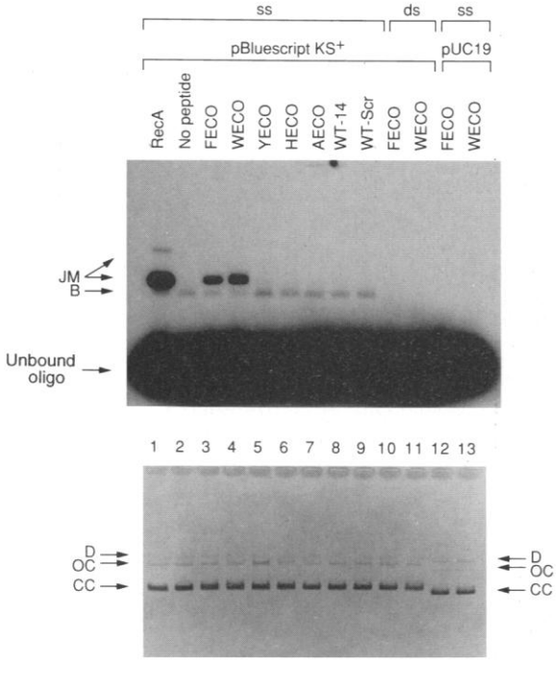

Fig. 5. The DNA binding peptides, like RecA protein, promote formation of joint molecules between ss oligonucleotide and a homologous dsDNA target. JM, joint molecules; D, dimeric plasmid; OC, open circular plasmid; CC, covalently closed plasmid. Autoradiograph (top) of ethidium bromide–stained agarose gel (bottom). Lanes 1 to 9 (top) contain an additional band B, which is a result of hybridization of the ss oligonucleotide with irreversibly denatured supercoiled DNA, the "Birnboim band" (*35*).

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10. The oligonucleotide BS-S1 GGCCGCTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCT, spanning positions 742 to 690 in the polylinker region of the plasmid pBluescript SK+, and complementary oligonucleotide BS-S2 were synthesized on an Applied Biosystems model 380B synthesizer and purified by SDS–polyacrylamide gel electrophoresis (PAGE). The peptides were made on an Applied Biosystems model 431A synthesizer, purified by reversed-phase chromatography on C-18, and dissolved in 20 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), pH 10.6. dsDNA was prepared by annealing of 5′-^{32}P–labeled BS-S1 and unlabeled BS-S2 followed by purification of the duplex on SDS-PAGE. The binding reactions were carried out in a volume of 40 μl and contained 0.5 μM ^{32}P-labeled ssDNA or 1 μM ^{32}P-labeled dsDNA (expressed as phosphate concentration), 40 mM tris-borate (pH 7.5), 10 mM $MgCl_2$, 20 mM NaCl, bovine serum albumin (BSA, 10 μg/ml), 10 mM CAPS (pH

10.6), and peptide at concentrations up to 100 μM (final pH, 8.3). After incubation for 30 min at room temperature, reaction mixtures were filtered by means of a double-filter system (*34*) with BA85 nitrocellulose and NA45 DEAE membranes (Schleicher & Schuell). All experiments were done at least in triplicate. Data were quantitated with a PhosphorImager (Molecular Dynamics).
11. Although the peptides bind slightly less tightly to DNA in the absence of Mg^{2+} (because 100 to 200 mM NaCl can substitute for 10 mM Mg^{2+}), this requirement is not a specific divalent ion effect.
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16. Peptide-DNA complexes were obtained as described (*10*), except that the volume was 100 or 140 μl. In the latter instance, 40 μl were used for filter binding and the rest was treated with PP. For binding of RecA protein to BS-S1, 2.7 μM RecA and 0.5 μM ^{32}P-oligonucleotide were incubated in a volume of 100 μl containing 25 mM tris-HCl (pH 7.5), 10 mM $MgCl_2$, 20 mM NaCl, 0.4 mM dithiothreitol (DTT), 0.5 mM EDTA, 0.3 mM ATPγS, 1.1 mM adenosine 5′-diphosphate (ADP), and BSA (10 μg/ml) for 30 min at 37°C. Complexes formed were subjected to 0.5 mM PP for 1 min at room temperature. Reactions were terminated by addition of dimethyl sulfate stop solution (*15*) followed by ethanol precipitation. After treatment with 1 M pyrrolidine (95°C, 20 min), the samples were separated on a 20% urea gel (Sequagel, National Diagnostic). Reactivity of ssDNA in the complex with RecA is less than that in the complexes with the peptides because of the presence of DTT, which is a quencher of the PP modification, in the RecA reaction.
17. In this assay, the binding of RecA to dsDNA [in the presence of either 0.3 mM ATPγS plus 1.1 mM ADP (*22*), or 0.3 mM ATPγS] does not induce a hyperreactivity of the thymines to PP (*12*).
18. All the CD spectra were measured on a Jasco 720 Spectropolarimeter. The instrument was calibrated with a 0.06% ammonium (+)-d_{10}-camphorsulfate solution, which generates a CD intensity of 190.4×10^{-3} degrees at 290.4 nm. A baseline of 10 mM NaH_2PO_4 buffer was subtracted out as background. Cells of 0.20-, 0.50-, or 1.00-mm pathlength were used and were maintained at 22 ± 1°C. The response and bandwidth for data collection were 2.0 s and 1.0 nm, respectively. Spectra were smoothed with the use of Savitsky-Golay smooth function and are presented in molar residue ellipticity (degrees M^{-1} cm^{-1}) or as raw data (10^{-3} degrees).
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HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein–Coupled Receptor

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A cofactor for HIV-1 (human immunodeficiency virus–type 1) fusion and entry was identified with the use of a novel functional complementary DNA (cDNA) cloning strategy. This protein, designated "fusin," is a putative G protein–coupled receptor with seven transmembrane segments. Recombinant fusin enabled CD4-expressing nonhuman cell types to support HIV-1 Env-mediated cell fusion and HIV-1 infection. Antibodies to fusin blocked cell fusion and infection with normal CD4-positive human target cells. Fusin messenger RNA levels correlated with HIV-1 permissiveness in diverse human cell types. Fusin acted preferentially for T cell line–tropic isolates, in comparison to its activity with macrophage-tropic HIV-1 isolates.

The primary receptor for HIV-1, CD4, supports viral entry only when expressed on a human cell type (*1–3*). Experiments with CD4-expressing hybrids of nonhuman and human cells have revealed that the defect in nonhuman cells is due to the absence of a human-specific cofactor required for membrane fusion (*4–10*). This cofactor is essential both for entry of HIV-1 virions into $CD4^+$ cell lines and for fusion between cells expressing the HIV-1 envelope glycoprotein (Env) and cells expressing CD4. Functional studies have suggested that the cofactor is present in a wide variety of human cell lines (*1–3*), though some exceptions have been noted (*3*, *11*). The identity of the fusion cofactor remains unresolved.

We previously reported a recombinant vaccinia virus–based transient expression and assay system in which fusion between Env-expressing and CD4-expressing cells leads to activation of a reporter gene (*Escherichia coli lacZ*) (*12*). We adapted this system for functional expression cloning of a fusion cofactor complementary DNA (cDNA) (*13*, *14*). The approach made no assumptions about the mode of action of the cofactor, except that it can allow a CD4-expressing nonhuman cell type to undergo fusion (*15*).

NIH 3T3 cells expressing vaccinia-encoded T7 RNA polymerase and CD4 were transfected with a HeLa cDNA plasmid library (inserts linked to the T7 promoter). These cells were mixed with NIH 3T3 cells expressing vaccinia-encoded Env and containing the *Escherichia coli lacZ* gene linked to the T7 promoter. After incubation, the cultures were stained for β-galactosidase (β-Gal) in situ. Consistently more β-Gal–positive cells were observed with the CD4-expressing cells transfected with the entire library compared to control CD4-expressing cells transfected with a single random plasmid from the library. For example, in one experiment we detected an average of 76 cells per well with the library compared to 16 cells per well with the single plasmid. In an additional negative control, we observed only background numbers of stained cells with the library when the partner cells expressed a mutant uncleavable (Unc) Env rendered nonfusogenic by deletion of the gp120/gp41 cleavage cite. These results suggested

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