Circles: The replication-recombination-chromosome segregation connection

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Crossing over by homologous recombination between monomeric circular chromosomes generates dimeric circular chromosomes that cannot be segregated to daughter cells during cell division. In *Escherichia coli*, homologous recombination is biased so that most homologous recombination events generate noncrossover monomeric circular chromosomes. This bias is lost in *ruv* mutants. A novel protein, RarA, which is highly conserved in eubacteria and eukaryotes and is related to the RuvB and the DnaX proteins, γ and τ, may influence the formation of crossover recombinants. Those dimeric chromosomes that do form are converted to monomers by Xer site-specific recombination at the recombination site *dif*, located in the replication terminus region of the *E. coli* chromosome. The septum-located FtsK protein, which coordinates cell division with chromosome segregation, is required for a complete Xer recombination reaction at *dif*. Only correctly positioned *dif* sites present in a chromosomal dimer are able to access septum-located FtsK. FtsK acts by facilitating a conformational change in the Xer recombination Holliday junction intermediate formed by XerC recombinase. This change provides a substrate for XerD, which then completes the recombination reaction.

homologous recombination | Ruv/Xer recombination | dimer resolution | FtsK

Barbara McClintock, during her work on ring chromosomes in maize, inferred in 1932 that whereas crossing over between rod-shaped (linear) chromosomes does not alter their topology, crossing over between ring (circular) chromosomes generates larger ring chromosomes (circular dimers) that cannot be segregated normally at cell division (1). This topological complication arising from crossing over between circular chromosomes was largely ignored until the 1980s, when it was demonstrated that site-specific recombination systems act to convert dimeric plasmid molecules, formed by homologous recombination, to monomers, and thereby facilitate stable plasmid inheritance (2, 3). Subsequently, it was shown that one of these site-specific recombination systems, XerCD site-specific recombination, also functions in the conversion of dimeric *Escherichia coli* chromosomes to monomers (4–6). Xer recombination uses two related recombinases, XerC and XerD, belonging to the tyrosine recombinase family, each recombinase catalyzing the exchange of one pair of strands in a reaction that proceeds through a Holliday junction (HJ) intermediate (7–9). XerCD act at the recombination site *dif*, located in the replication terminus region of the *E. coli* chromosome and at related sites in multicopy plasmids, for example, *psi* in plasmid pSC101 and *cer* in ColE1 (3, 10).

Here we outline the processes that limit dimer formation by homologous crossing over in *E. coli*. We also discuss the mechanism that restricts Xer recombination at chromosomal *dif* to converting dimers to monomers by making a part of the recombination machine only accessible to *dif* sites when they are present in chromosomal dimers at the time of cell division.

Homologous recombination at stalled replication forks in *E. coli* is biased so as to minimize crossover events. Work with bacteriophages λ and T4 first identified the interdependence of homologous recombination and DNA replication (11, 12), although it has only recently been generally recognized that a major ubiquitous function of homologous recombination is to rescue and rebuild broken or stalled replication forks (refs. 13–18; also see this issue of PNAS). Even in meiotic cells where DNA is programmed to undergo crossing over, a strong interdependence of meiotic recombination and DNA replication exists (19, 20).

Crossover homologous recombination events form chromosomal dimers about once every seven cell generations in *E. coli* (21). This frequency of dimer formation is decreased in recombination deficient strains and is increased when elevations in the frequency of recombinational repair are predicted (21). About half of the dimers appear to arise through RecBCD-dependent recombination, whereas the other half arise from RecFOR-dependent events. This frequency fits well with the slow growth phenotype of *xer* mutant cells and the observation that ≈15% of cell divisions give no viable progeny (22). However, estimates of the frequency with which stalled or broken replication forks are repaired by homologous recombination in *E. coli* suggest that such events may occur as often as once or more per cell generation, thereby indicating a strong bias in the direction of recombination intermediate resolution (17, 18). Molecular mechanisms that can introduce such bias in HJ processing by the Ruv resolvasome have been uncovered in *E. coli* (23, 24). The ability of the Ruv proteins to bias HJ resolution toward noncrossovers is confirmed by the observation that increasing the frequency of replication fork stalling/breaking makes *E. coli* cells become dependent on Xer recombination for survival when the *ruv* genes are mutated (25).

The bias that limits homologous recombination at replication forks in *E. coli* to noncrossover events may be conserved in other organisms, because noncrossovers may also predominate during recombination in eukaryote mitotic cells (e.g., see ref. 26). In addition to mechanisms that bias resolution at HJs, the processing of asymmetric recombination intermediates, for example the inherent asymmetry of three-way junctions, may readily lead to biased resolution. It seems likely that novel mechanisms of resolution of recombination intermediates remain to be characterized, because many prokaryote and eukaryote cells lack known HJ resolving enzymes yet are competent for homologous recombination.

An outline of known and proposed pathways that are used to repair stalled or broken replication forks is shown in Fig. 1, which

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Abbreviation: HJ, Holliday junction.

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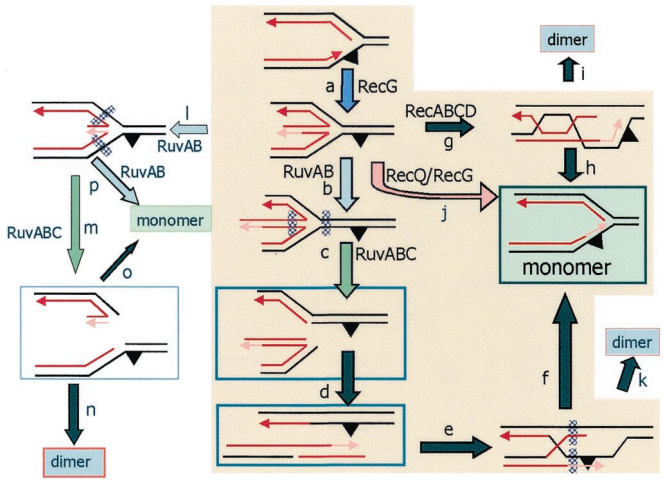

Fig. 1. A scheme to illustrate stalled replication fork rebuilding by replication fork regression, by using recombination enzymes in the *E. coli* chromosome (adapted from refs. 17, 18, 24, and 25). Pathways that lead to a single crossover (or an odd number of crossovers) generate dimeric chromosomes, and those that act without crossing over (or even numbers of crossovers) retain the monomeric status of the chromosome. Pathways considered to be major routes to retaining the monomeric chromosome status are overlaid onto a beige background. Similarly, the arrows bounded by a bold line are intended to indicate major pathways, with relative contributions being indicated by arrow breadth. Those arrows in dark green indicate pathways that would be expected to be RecABCD-dependent, whereas those in light green indicate RuvC cleavage from within a RuvABC complex. Arrows in light blue indicate RuvAB helicase action, whereas that in pink indicates similar action by RecG or RecQ helicases. Black lines are unreplicated DNA, and red/pink lines newly replicated daughter strands. (*a*) Reannealing of daughter strands at a stalled replication fork (closed triangle indicates a nontemplate lesion on the leading strand). Reannealing can be mediated by RecG (18) and facilitated by positive supercoiling ahead of the fork (27). RuvAB and RecA may also promote the growth of the reversed fork once fork reversal has been initiated. Lagging strand synthesis is indicated as proceeding beyond the lesion, thereby providing an opportunity to replicate past the lesion by copying of the switched template in a reaction that uses recombination proteins, but not recombination (pink line; ref. 18). (*b*) Productive RuvAB branch migration to extend the four-way HJ (RuvB is cartooned as a pair of cylinders on opposed arms of the HJ). Note that RuvB binding to the other two arms of the junction (step *l*) will lead to abortion of the four-way junction by branch migration (step *p*). (*c* and *m*) Action of RuvABC to cleave the strands 3′ of the bound RuvB on the branch point side, to generate broken forks (corresponding to single strand lesions in the leading and lagging parental template strands respectively; ref. 24). RecABCD-mediated reinvasion of the broken ends leads to rebuilt replication forks that most readily yield noncrossover (*d–f*) and crossover (*n*) chromosomes, respectively. Note that after reinvasion, a further round of RuvABC action is required; in each case the ''productive'' orientation of RuvB binding gives the majority species shown (*f* and *n*). Binding of RuvB in the abortive configuration either will act to reverse the invasion or will lead to RuvC cleavage to give the minority products, dimers and monomers, respectively (*k*, *o*). (*g–i*). RecABCD-mediated invasion of the end created by fork reversal into its homologous region to generate a molecule containing two HJs (or a HJ and a three-way junction). Such an intermediate can be processed by crossover (*i*) or noncrossover (*h*) pathways by several ways that involve the simultaneous or sequential action of proteins at each of the branch points; we predict dimers to predominate over monomers in these events. (*j*) Processing of the reversed replication fork intermediate by RecG or RecQ helicases (equivalent to step *p*, promoted by RuvAB).

also indicates which pathways are most likely to give crossover recombinant products and thereby generate dimers in cells with circular chromosomes. The key initiating step for the pathways shown is the conversion of a replication fork into a four-way HJ (step *a*), a process that can be promoted by *E. coli* RecG helicase and positive supercoiling ahead of the replication fork (17, 18, 27), although RecA and other helicases may also participate in the process. Once formed, a HJ can be processed by a number of different pathways (*b-f/k*; *g–i/h*; *l-o*, *n*), which include the RecBCD-mediated degradation of the extruded junction (not shown; ref. 17) or the simple reversion of the four-way junction back to a replication fork by helicase action (*j*, *p*). In the cartoon here, the latter reaction allows the bypass of a lesion in the absence of recombination (18). RecFOR-dependent gap repair provides an alternative mechanism to process stalled replication forks without regression of the fork into a HJ (not shown). Despite evidence suggesting that RecFOR-dependent events occur predominantly in the absence of crossing over (24), other work has indicated that ≈50% of dimeric chromosomes in *E. coli* arise from RecFOR-dependent processes (21). Although the mechanism by which RuvB can bias homologous recombination outcome to noncrossover events is established (Fig. 1; refs. 23–25), the pathways of recombination intermediate resolution in the absence of Ruv, and their lack of apparent bias, remain unclear. Nevertheless, the importance of recombination proteins, with or without the recombination process in replication fork restart is now well established.

A Highly Conserved Gene Whose Function May Influence Homologous Recombination Outcome. During our examination of *E. coli* genome organization, we noted that downstream of *ftsK* (and possibly cotranscribed with it) lies a gene of uncharacterized function, *ycaJ*, whose 447-aa protein product has substantial similarity to the HJ helicase RuvB and to DnaX, which encodes the τ and γ components of the DNA polymerase III replisome (Fig. 2). γ is a component of the β-sliding clamp loader complex, whereas the longer translation product of DnaX, τ, is involved in dimerization of the core polIII polymerase as well as being an ''organizer'' of the replisome complex (28, 29). The existence of this gene through its homology to *ruvB* and *dnaX* has also been noted by others (28, 30). Homologs of YcaJ are widely distributed in eubacteria and in eukaryotes (Table 1) but appear to be absent in archaea, consistent with the gene entering eukaryotes via a mitochondrial lineage. Remarkably, there is very high amino acid sequence conservation between the whole of YcaJ and its eukaryote homologs (Table 1; ≈40% identity over a region of greater than 400 aa). YcaJ is more closely related to RuvB and DnaX (≈25% identity to each) than the latter proteins are to each other (less than 15% identity). Furthermore, the similarity of YcaJ to DnaX is retained throughout the whole length of τ, perhaps suggesting that YcaJ is able to interact with the polIII core in addition to having the helicase-like present in γ and τ. Finally, the identity relationships of the YcaJ homologs with DnaX and its eukaryote counterpart, replication factor C, and with RuvB are retained in each of the organisms analyzed (Table 1).

Inactivation of *ycaJ* by replacing the 5′ part of the coding sequence with a chloramphenicol resistance (Cm^R) cassette gave no obvious mutant phenotype in cells competent for homologous recombination and DNA repair. In contrast, combination of this mutant *ycaJ* gene with a range of mutants deficient in recombination or dimer resolution has resulted in a number of novel phenotypes that indicate a possible role of YcaJ in influencing recombination outcome. For example, combination with *xer* or *dif* mutants leads to slower growth and cell morphology properties consistent with increased dimer formation.

As a consequence of the association of YcaJ with homologous recombination and with a replicative protein, and the likely association of homologous recombination with replication, we redesignate *ycaJ* as *rarA* and its protein product as RarA (*r*eplication *a*ssociated *r*ecombination gene/protein A). We tentatively conclude that the wild-type RarA protein influences the events that lead to recombination intermediate processing at a replication fork, thereby having an effect on the frequency of dimer formation. The high sequence conservation of the protein from bacteria to humans suggests that it has a highly conserved function that may involve interaction with a con-

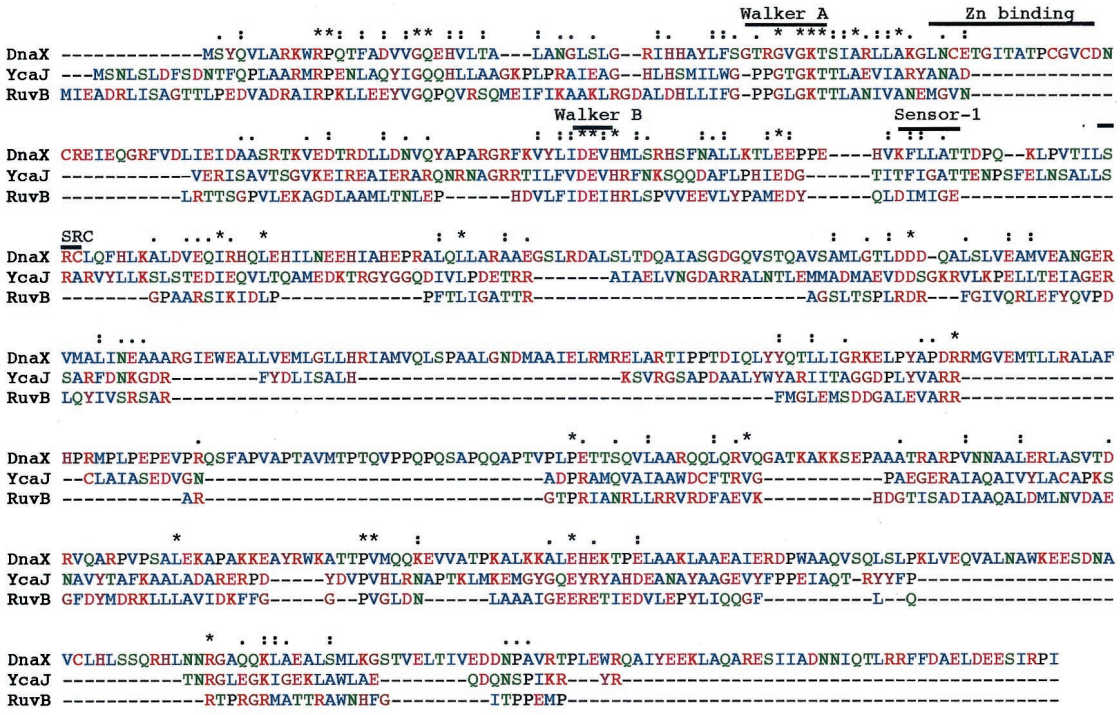

Fig. 2. Alignment of *E. coli* DnaX, YcaJ, and RuvB (CLUSTAL X multiple sequence alignment program, v. 1.8, http://www.ebi.ac.uk/clustalw/). The sequences of YcaJ, RuvB, and DnaX all contain well-conserved nucleotide-binding sites with Walker A (GxxxxGKT/S) and Walker B (Dexx) motifs. The Zn-binding motif of DnaX is absent in YcaJ, but the putative ATPase sensor motifs (29) are present. Colors represent types of amino acids.

served molecular machine involved in processing recombination and/or replication intermediates. In the absence of RarA, cells remain recombination proficient and apparently are as capable of repairing DNA damage as wild-type cells, by a pathway that appears not to be significantly mutagenic as judged by the frequency of spontaneous mutation to rifampicin resistance (B.S. and D.J.S., unpublished observations). We suspect that RarA may act as part of the replisome complex,

Table 1. Homologies of *E. coli* YcaJ to YcaJ-like sequences from other organisms

	E. coli YcaJ		*E. coli* RuvB		*E. coli* DnaX		Cognate RuvB		Cognate DnaX/Replication Factor C	
YcaJ from	Identity, %	Positives, %	Identity, %	Positives, %	Identity, %	Positives, %	Identity, %	Positives, %	Identity, %	Positives, %
E. coli	100	100	26	43	24	43	26	43	24	43
H. influenzae	73	85	27	44	25	43	25	41	24	41
Vibrio cholerae	73	83	24	39	25	43	24	39	24	42
Coxiella burnetii	58	74	26	43	25	42				
Neisseria meningitidis	54	69	28	47	27	44	31	47	32	51
Xylella fastidiosa	53	71	26	45	27	45	26	42	27	47
Deinococcus radiodurans	50	66	29	44	33	46	27	37	32	44
Arabadopsis thaliana	46	62	25	40	25	43			24	42
Streptomyces coelicolor	43	59	28	45	24	38	25	39	22	36
Mycobacterium leprae	42	61	24	40	28	44	28	41	25	38
Mycobacterium tuberculosis	42	61	27	48	26	42	28	43	26	40
Schizosaccharomyces pombe	42	59	30	51	26	45			21	41
Saccharomyces cerevisiae	40	58	31	48	30	48			27	44
Neurospora crassa	40	56	27	45	27	41				
Homo sapiens	40	56	26	43					23	39
Thermatoga maritima	38	56	21	40	23	42	24	44	26	42
B. subtilis	38	55	26	54	25	41	29	52	24	41
Campylobacter jejeuni	32	50	38	56	25	45	24	42	22	42
Heliobacter pylori	31	54	36	50	26	40	30	44		
Ureaplasma urealyticum	31	48	29	56	25	43	23	43	24	42

Amino acid identities and positives (conserved plus similar amino acid residues) were determined by the BLAST program over 400 amino acid residues for YcaJ alignments and over 200 amino acids for the YcaJ versus DnaX/RuvB/Replication Factor C alignments. Spaces are left blank where sequences are not available or where no significant homologies were evident. Eukaryotic species are underlined.

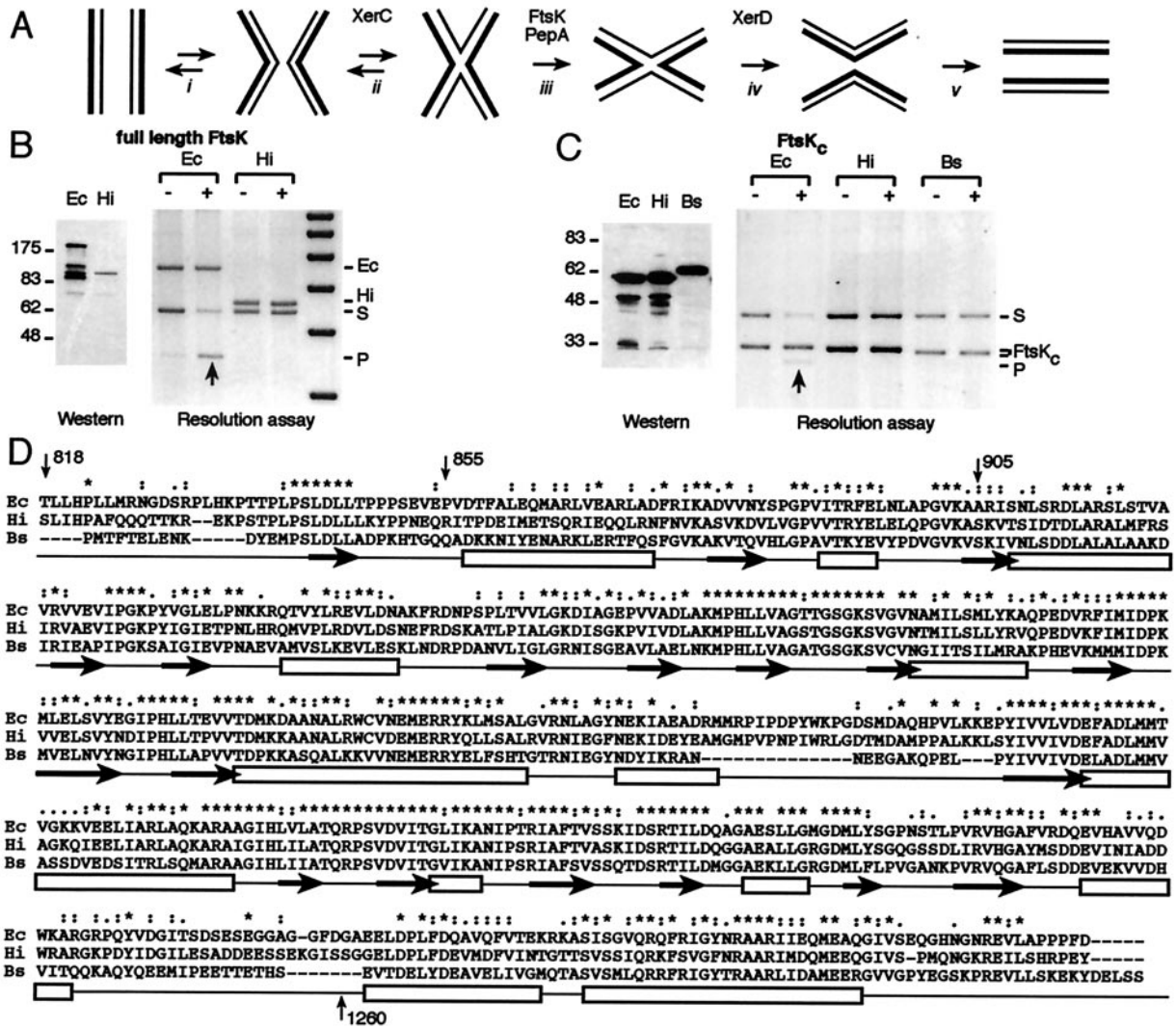

Fig. 3. (*A*) An outline of the Xer recombination reaction. XerCD bind cooperatively at *dif*, *psi*, or *cer* recombination sites, ensuring synapsis (with the help of accessory sequences and proteins in the case of *psi*/*cer*) (*i*). XerC initiates catalysis (*ii*) to form a HJ intermediate, which undergoes a conformational change (*iii*) to provide a substrate for catalysis by XerD, which can then complete the recombination reaction (*iv*). There is normally a barrier to this conformational change, and XerC frequently catalyzes the conversion of the HJ back to substrate (*ii*). In recombination at *psi*, the proteins PepA and ArgA-P facilitate the HJ conformational change, whereas in recombination at *dif*, $FtsK_c$ is thought to facilitate this change (34, 35). (*B*, *C*) Species specificity of FtsK action. $FtsK_c^-$ cells (DS9041) were transformed with pBAD expression vectors (48) carrying full-length FtsK proteins (*B*) or the C-terminal domains (*C*) of different species. To assay for Xer recombination, they were transformed with a plasmid containing two *dif* sites and grown in conditions of repression (−; 0.2% glucose) or induction (+; 0.2% arabinose) of the expression vectors (34). Induction was checked by Western blot analysis by using an antibody directed against a FLAG epitope fused to the N termini of the constructs, after resolution of the protein extracts on a 6% (*B*) or an 8% (*C*) SDS/PAGE. (*D*) Alignment of the C-terminal domains of FtsK homologues. Identical residues are indicated by stars, conservative substitutions by dots. Open boxes underline regions predicted to adopt an α-helix conformation by PREDICTPROTEIN PHD software v. 1.96, http://www.embl-heidelberg.de/predictprotein/predictprotein.html whereas black arrows underline those predicted to form β sheets. Ec: *E. coli* FtsK, Hi: *H. influenzae* FtsK and Bs: *B. subtilis* SpoIIIE.

at least during replication fork rebuilding. It could also facilitate RuvB loading and/or influence the preferred orientation of RuvB loading onto a four- or three-way junction, thereby biasing resolution outcome.

Chromosome Dimer Resolution by Xer Recombination. Despite the bias toward formation of chromosome monomers during homologous recombination, dimers still arise in the bacterial cell population at a substantial frequency. It is therefore not surprising to find that the Xer recombination system is so highly conserved (31).

The molecular basis of the strand exchange reactions mediated by XerCD and related tyrosine recombinases is now well understood (9, 32, 33). XerCD form HJs between *dif* sites *in vivo* and *in vitro* independently of any other cellular process (34). These HJs result predominantly from XerC-mediated catalysis and are unstable, being rapidly converted back to substrate by XerC-mediated strand exchange (34). A conformational change of the HJ formed by XerC appears to be required to block XerC-strand exchanges and provide a substrate for catalysis by XerD, thereby completing a recombination reaction (refs. 9, 34, 35; Fig. 3*A*).

Much less is known about the mechanisms that ensure that Xer site-specific recombination at chromosomal *dif* is restricted to converting chromosome dimers to monomers and that facilitate synapsis and recombination between *dif* sites located 4.6 Mbp apart in a chromosomal dimer. Clues to understanding this are given by the observations that Xer recombination is normally restricted to *dif* sites located in the replication termination region of the chromosome (22, 36), and that complete Xer reactions at

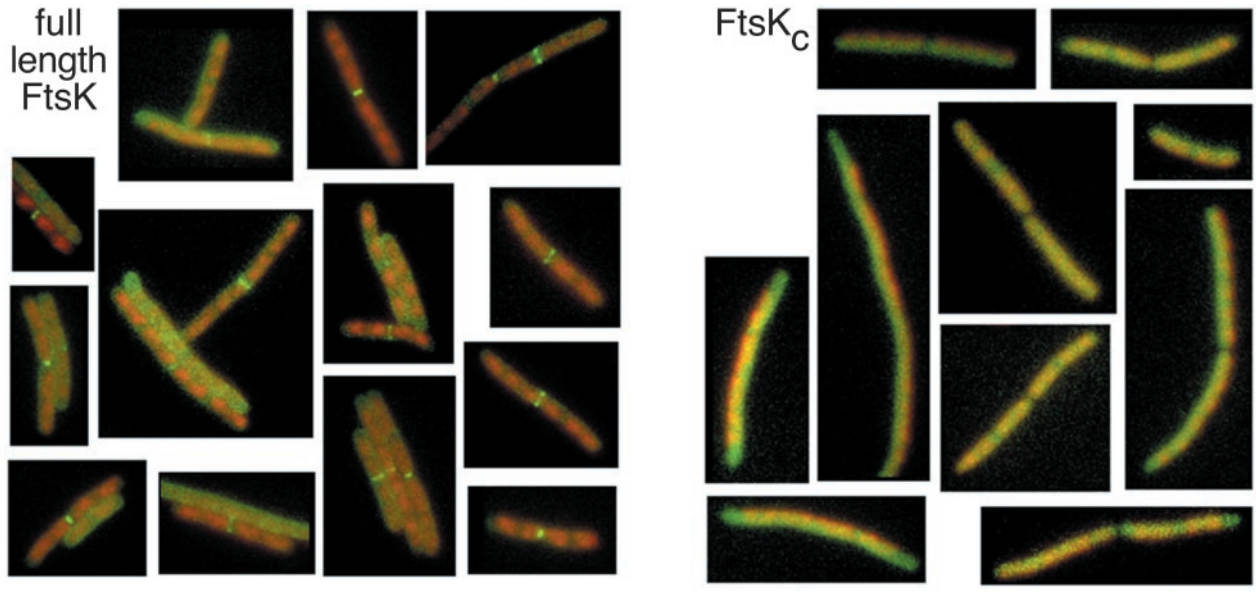

Fig. 4. The C-terminal domain of FtsK is randomly distributed throughout the cytoplasm. FtsK$_c^-$ cells (DS9041) were transformed with pBAD expression vectors carrying an N-terminal fusion of the green fluorescent protein (GFP) to full-length FtsK or to the C-terminal domain of FtsK (FtsK$_c$). Cells were grown to midexponential phase in LB supplemented with 0.1% (full-length) or 0.2% (FtsK$_c$) arabinose. Nucleoids were stained by using 4′,6-diamidino-2-phenylindole (DAPI). Phase-contrast and fluorescent images were acquired by using a cooled charge-coupled device camera (Princeton Instruments, Trenton, NJ) and METAMORPH IMAGE ACQUISITION software (Universal Imaging, Media, PA) from an Olympus BX50 (New Hyde Park, NY) fluorescence microscope. Shown are overlays of the DAPI image in red and the GFP image in green. Full-length FtsK frequently localizes to the septum, whereas FtsK$_c$ is always distributed throughout the cytosol and never found at the septum.

dif require the presence of the FtsK protein (34, 35, 37). *E. coli* FtsK is a three-domain 1,329-aa protein. The 200-aa N-terminal domain is necessary for cell division (38, 39), contains four transmembrane domains, and localizes to the septum in an FtsZ-dependent manner (40–42). The 500-aa C-terminal domain, FtsK$_C$, is implicated in chromosome segregation (43, 44) and is homologous to the C-terminal domain of the *Bacillus subtilis* SpoIIIE protein that functions in DNA transfer from the mother cell to the prespore (45). Therefore, FtsK appears well suited to coordinate cell division with chromosome segregation. Whereas the N- and C-terminal domains of FtsK are highly conserved in eubacteria, the region between these domains is of highly variable size and sequence. In *E. coli*, this 600-aa region is abundant in proline and glutamine residues. Cells lacking FtsK$_C$ form filaments and chains with mispositioned nucleoids (35, 43, 44) and are defective in Xer recombination at chromosomal and plasmid *dif* (34, 35, 37), but not at *cer* and *psi* sites (35). Most of the chromosome segregation defect is suppressed in a *recA* background (35), consistent with a major part of the chromosome segregation function of FtsK$_C$ being in its role in promoting Xer recombination at *dif*.

The C-terminal domain of FtsK is sufficient for a complete XerCD-mediated recombination reaction between two *dif* sites *in vivo*. In the absence of FtsK$_C$, HJs at *dif* form *in vivo* and *in vitro* and are converted back to substrate by XerC (Fig. 3*A*; refs. 34, 35). To gain better insight into the features of the C-terminal domain of FtsK that are important for its activity in Xer recombination, we tested a number of different FtsK derivatives for their ability to complement the Xer recombination defect of FtsK$_c^-$ cells. The derivatives used were full-length *E. coli* and *Hemophilus influenzae* FtsK proteins, and the C-terminal domains of *E. coli*, *H. influenzae* FtsK and *B. subtilis* SpoIIIE proteins. Each protein had a FLAG epitope at its N terminus and was expressed from the arabinose promoter of a multicopy plasmid. Whereas all proteins were shown to be expressed when the arabinose promoter was induced, only the *E. coli*-derived proteins were able to promote efficient Xer recombination between two *dif* sites carried by a plasmid (Fig. 3 *B* and *C*). We confirmed that the *H. influenzae* FtsK$_c$ derivatives are active by demonstrating that they can catalyze Xer recombination mediated by *H. influenzae* XerCD acting at the cognate recombination site, *hif* (F.-X. B. and D. J. S., unpublished data).

The minimal complementing domain of FtsK starts between amino acids 855 and 905 and ends between amino acids 1265 and 1329 (34). The FtsK homologs are highly identical in that region (Fig. 3*D*). However, the overall sequence divergence rises significantly after amino acid 1260 and before amino acid 906, despite conservation of the predicted secondary structure. We therefore suspect that residues in those two regions are responsible for the species specificity of the different FtsK proteins. It seems likely that the C-terminal domains of FtsK and SpoIIIE encode similar biochemical activities and that the species specificity reflects the fact that these proteins interact with species-specific components of some macromolecular machine that function in DNA processing.

Xer Recombination at *dif* Is Restricted to Dimer Resolution as a Consequence of a Temporal and Spatial Cellular Location of Enzyme and Substrate. Overexpression of the C-terminal domain of FtsK renders the Xer recombination reaction independent of chromosome dimer formation by homologous recombination and independent of the chromosomal location of *dif* (34). However, expression of the C-terminal domain alone did not completely restore a wild-type phenotype to FtsK$_c^-$ cells (34). We checked the distribution of the full-length and C-terminal FtsK proteins inside cells by immunohistochemistry and by using N-terminal green fluorescent protein fusions (ref. 34; Fig. 4). Whereas the full-length protein localizes exclusively to the septum under low levels of expression, the C-terminal domain was distributed throughout the cytoplasm.

We conclude that the C-terminal domain of FtsK acts directly on the Xer recombination reaction by facilitating the HJ conformational change necessary to provide a HJ substrate for strand exchange by XerD (Fig. 3*A*; refs. 9, 34, 35). The accessory proteins, PepA-ArcA-P, facilitate the same HJ conformational change during Xer recombination at the plasmid site *psi* (Fig. 3*A*;

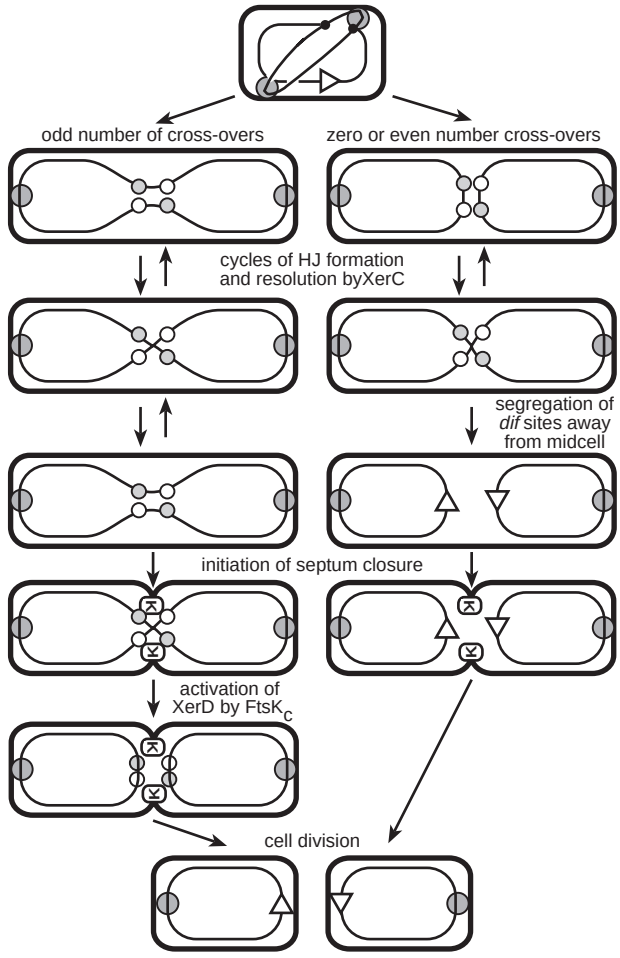

Fig. 5. A model for chromosome segregation in *E. coli*. In contrast to the replication origins (large dark gray circles), the replication terminus region, which contains the *dif* site (open triangle), stays localized at midcell (49). As a consequence, sister *dif* sites can be synapsed by the XerCD recombinases (small white and light gray circles) whether the chromosomes form a dimer or not. This leads to cycles of HJ formation and resolution by XerC. If the chromosomes are monomeric, segregation will eventually break the synaptic complex and move the sites away from midcell before septum closure. If the chromosomes form a dimer, the synaptic complex will stay trapped at midcell, which allows access to FtsK. FtsK mediates the HJ conformation change needed to activate catalysis by XerD, thus coordinating resolution of chromosome dimers to cell division.

M.R. and D.J.S., unpublished data). We propose that both of these mechanisms involve changes in the architecture of DNA in the vicinity of the HJ, with the effect of FtsK being DNA-sequence-independent, whereas that of PepA-ArcA-P is DNA-sequence-dependent. Consistent with this view, the C-terminal domain of SpoIIIE has been shown to alter the topology of a supercoiled substrate *in vitro* (46), whereas differences in DNA topology as a consequence of the presence or absence of catenation can change the direction of HJ resolution in a supercoiled plasmid (47).

Our current view of how chromosome dimer resolution is integrated into the bacterial cell cycle is cartooned in Fig. 5. After replication initiation, newly replicated origins move toward the cell poles at some point before cell division (49). It seems possible that the chromosome segregation process is facilitated by re-establishing DNA supercoiling condensation in the region of the pole-proximal replication origins. An opportunity for synapsis of *dif* sites cannot arise until the *dif* sites replicate. For correctly positioned *dif* sites, this will not happen until almost all DNA is replicated, when the two daughter *dif* sites appear to be able to synapse and form HJs efficiently, probably because the two sites remain in close proximity. In contrast, *dif* sites positioned elsewhere in the chromosome appear to synapse and form HJs less readily, presumably because the DNA segregation process separates the *dif* sites immediately after replication (34). Because FtsK is temporally and spatially restricted to the septum of the cell through its N-terminal domain, FtsK appears to allow completion of Xer recombination only between sister *dif* sites when they are located in the terminus region of a dimeric chromosome. Consistent with this view, the terminus region of the chromosome has been shown to remain at the position of the future septum throughout the cell cycle (49). In monomeric chromosomes, the two *dif* sites of daughter chromosomes will be segregated away from the invaginating septum before they can be accessed by septum-bound FtsK (Fig. 5). An essential feature of this model is that the amount or activity of FtsK in a cell should be normally limiting. This has been shown to be the case, at least for Xer recombination at *dif* on multicopy plasmids (34). Increases in the level of full-length FtsK lead to its deposition throughout the membranes of the cell, where it now supports efficient Xer recombination of ectopic *dif* sites (34). Presumably before septum formation, FtsK is distributed throughout the membrane, although it might remain associated with the old septum at the cell poles (for example, see ref. 34). Whether localization of FtsK to a new septum at midcell leads to changes in its activity remains to be determined. FtsK levels are at least partly under control of the SOS regulon, which is induced in response to cellular DNA damage (40); indeed, FtsK is the only known essential gene to be under SOS control. The reasons for this are unclear, because after SOS induction there may be no functional FtsZ septal rings to which even increased levels of FtsK can localize. Perhaps in this situation, FtsK may be playing other roles in chromosome processing.

Concluding Remarks and Perspectives. The results discussed here demonstrate a remarkable integration of DNA processing functions that act in concert to ensure efficient replication of chromosomes and their propagation through successive cell generations.

The interrelationships of DNA replication and homologous recombination are emerging from work in many laboratories, although further work needs to determine the different pathways that lead to recombination intermediate resolution and how these bias recombination outcome. Indeed, the identification and preliminary characterization of the highly conserved gene, *rarA*, which may provide a link between replication and recombination, underline our current ignorance of homologous recombination mechanisms and their relationship to replication fork rebuilding.

In the final steps of the *E. coli* cell cycle, FtsK, appears to play an important role in coordinating cell division with chromosome segregation by acting specifically at *dif* sites located in chromosomal dimers to provide a HJ substrate suitable for strand exchange by XerD, thereby completing the conversion of dimers to monomers.

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