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# The DNA exonucleases of *Escherichia coli*

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## Abstract

DNA exonucleases, enzymes that hydrolyze phosphodiester bonds in DNA from a free end, play important cellular roles in DNA repair, genetic recombination and mutation avoidance in all organisms. This article reviews the structure, biochemistry and biological functions of the 17 exonucleases currently identified in the bacterium *Escherichia coli*. These include the exonucleases associated with DNA polymerases I (*polA*), II (*polB*) and III (*dnaQ/mutD*), Exonucleases I (*xonA/sbcB*), III (*xthA*), IV, VII (*xseAB*), IX (*xni/xgdG*) and X (*exoX*), the RecBCD, RecJ, and RecE exonucleases, SbcCD endo/exonuclease, the DNA exonuclease activities of RNase T (*rnt*) and Endonuclease IV (*nfo*) and TatD. These enzymes are diverse in terms of substrate specificity and biochemical properties and have specialized biological roles. Most of these enzymes fall into structural families with characteristic sequence motifs, and members of many of these families can be found in all domains of life.

### Keywords

DNase; DNA repair; mutation; homologous recombination; genome rearrangements; DNA polymerase; abasic sites; mismatch repair; proofreading; double-strand break repair; dsDNA; ssDNA; metal binding; mutator; lagging strand; flap endonuclease; base excision repair; replication gap repair; tandem repeats

## INTRODUCTION TO DNA EXONUCLEASES

Nucleases are enzymes that hydrolyze phosphodiester bonds of nucleic acids. Early in the classification of nuclease activity, a distinction was made whether such enzymes cleaved internally in the chain (“endonucleases”) or from the end in a step-wise manner (“exonucleases”), borrowing a system analogous to that for proteases. In practice, a nuclease can be tested for its requirement for an end by comparing linear vs. circular DNA substrates, with exonucleases cleaving the former but not the latter. This classification has been complicated by the fact that certain nucleases require an end to initiate degradation, but cleave DNA internally to yield oligonucleotide products. By the practical definition, these are exonucleases; formally, they are endonucleases—the field has used the term “endo/exonucleases” to refer to this class of nucleases. Some endonucleases that will cleave circular DNA also possess intrinsic exonuclease activity, hydrolyzing linear substrates in a stepwise fashion (for example Exonuclease III/Endonuclease II and Endonuclease IV) and are therefore both true exonucleases and true endonucleases.

This article will focus on DNA exonucleases from *E. coli*, including the endo/exonucleases, and their roles in DNA metabolism. For information about endonucleases and their activities, the reader is directed to the articles on DNA repair. Prior to the discussion of the biochemistry, structure and function individual *E. coli* exonucleases, I will introduce some concepts and terminology regarding the discovery and classification of exonucleases from *E. coli*.

## Discovery of Exonucleases

In the current literature, there are 17 exonucleases identified in *E. coli* (Table 1). These exonucleases were discovered by several means. Beginning in the early 1960s, efforts were made to purify exonuclease activities biochemically and to categorize their properties. As the nucleases were discovered, they were identified by numbers (Exonuclease I, II etc.). The genes corresponding to these were, in some cases, found by reverse genetics and named corresponding to the activity name (*xon* for exonuclease one, *xth* for exonuclease three, etc.). Some of these nuclease activities are associated with subunits of DNA polymerases: for example, Exonuclease II is the 3′ to 5′ proofreading activity of DNA polymerase I (the product of the *polA* gene); Exonuclease VI is associated with the 5′ to 3′ exonuclease of the same enzyme. Other activities were initially identified for their genetic function as in specific biological pathways and later discovered to be exonucleases. These are often named for the phenotypic properties they affect, for example the RecBCD and RecJ nuclease, involved in recombination, and SbcB and SbcCD nuclease, playing antirecombinational roles, named for "suppressor of RecBCD. In the post-genomic era, a number of nuclease activities have been identified from predictions based on sequence similarity to known exonucleases (Exonucleases IX and X).

## Biochemical properties of exonucleases

Exonucleases can be further distinguished by substrate specificity, reaction products and other enzymatic properties. Some enzymes show strong specificity; others are more ambivalent. Most of the exonucleases that degrade DNA will not degrade RNA, although there are examples of enzymes with dual specificity (e. g. RNase T found later to have robust DNase activity). Many double-strand DNA (dsDNA) specific exonucleases will only degrade one of the two strands of the duplex and therefore show a distinct polarity of degradation (3′ to 5′ or 5′ to 3′). Most single-strand DNA (ssDNA) specific-exonucleases also exhibit polarity of digestion. Some dsDNA exonucleases require 5′ phosphates; others will degrade molecules with 5′ OH ends.

Exonucleases are classified by the products of the reaction (mononucleotides vs. oligonucleotides) and whether released products contain 5′ or 3′ phosphate residues. Some exonucleases will bind substrate and execute a series of hydrolysis events before dissociation; this is termed "processivity", which can be quantified by substrate competition experiments that assay how many nucleotides are released per a single binding event. Processivity in the many thousands of nucleotides is not uncommon. On the other hand, other exonucleases are "distributive" and release only a single nucleotide for each binding.

## Structural families and exonuclease mechanism

In their function as hydrolases, exonucleases bind metal cation cofactors that assist catalysis. Most *E. coli* enzymes employ $Mg^{2+}$ in this role. These metal ions are coordinated by conserved aspartate, glutamate or histidine residues that define certain structural families of evolutionarily related enzymes. Most of these families can be found in all three domains of life, archaea, eubacteria and eukaryotes. Except for the metal-binding, architectural residues, the overall sequence conservation among nucleases in these families is weak. Although it is possible to assign an unknown function to a structural family by iterative sequence searches, note that this is not predictive of substrate specificity or other enzymatic properties, nor even of nuclease activity. Indeed, as is described below, nuclease superfamilies include non-nuclease members such as phosphatases.

The largest structural family, with 8 members in *E. coli*, are the 3′ to 5′ exonucleases in the DEDD family, named for conserved aspartate and glutamate residues in the active site; this family has also been termed the DnaQ superfamily (134) (Figure 1A). This family is highly diverged and found in all domains of life, with little sequence conservation except the metal binding sites. In this family within *E. coli* and Salmonella are the proofreading exonuclease activity of DNA polymerase III, encoded by the DnaQ, epsilon subunit, and the exonuclease domain of DNA polymerases I and II. Exonuclease I, X and RNase T are likewise in this family, as is RNase D and oligoribonuclease (ORN).

The 3′ exonuclease domain of DNA polymerase I was one of the first DNA exonuclease structures determined by X-ray crystallography. The exonuclease site was surprisingly distant from the polymerase site and appeared to interact with single-strand DNA. Two metal cations were revealed in the active site (14, 15), establishing a new paradigm for phosphodiester bond hydrolysis. One metal was deduced to promote formation of the attacking hydroxide ion that will hydrolyze the phosphodiester bond; the other stabilizes the pentacoordinated transition state and/or oxyanion leaving-group. The amino acid residues interacting with the two metal ions are those seen conserved in this family, which serve to establish the architecture of the attacking water molecule, single-strand DNA substrate and metal ions.

Although the primary sequences of this family are highly diverged, their overall topologies and structures are quite similar (Figure 1B). There are X-ray crystallographic structures for DnaQ (103), ExoI (22), RNase T (292), RNase D (291) ORN (39), Polymerases I (14, 15) and II (Brunzell, PDB ID1q8i, unpublished). The common structural feature can be incorporated in a variety of quaternary arrangements: this group includes monomeric proteins (ExoI, RNaseD, the Pol I, Pol II exonucleases), homodimers (RNase T, ORN, ExoX) as well as members of larger complexes (DnaQ).

A second superfamily of nucleases includes two of *E. coli*'s exonucleases (RecB of the RecBCD nuclease and Exonuclease VIII) in addition to bacteriophage lambda exonuclease, Mrr-like restriction endonucleases and the archaeal Holliday junction resolvase from *Pyrococcus furiosus*. Note that this group includes true endonucleases, true exonucleases and endo/exonucleases as well as structure-specific enzymes. This family is defined by three motifs, with conserved glutamate and aspartate residues, defining the "endonuclease fold"

(10). This fold is also found in defunct, inactive nucleases such as RecC, where it may play a structural role (210).

A number of exonucleases share structure with phosphatases and comprise a larger family of phosphoesterases. This includes RecJ, a member of the DHH family (for a conserved motif in the protein). The DHH family, which includes the polyphosphatase PPX1, is found in all three domains of life (9, 204, 239). This group particularly expanded in the archaeal species, which harbor multiple members, including *Methanococcus jannashii* with six DHH family members and *Archaeoglobus fulgidus* with seven. Likewise, SbcCD, a coiled coil protein with endonuclease and exonuclease activity is related to a family of repair enzymes such as the eukaryotic Rad50/Mre11 and T4 bacteriophage gp46/47. SbcD, the nuclease subunit, shares conserved residues (DXH-(25)-GDXXD-(-25)-GNHD) with a family of serine/threonine phosphatases, including lambda phosphatase (226, 284). In both nucleases and phosphatases, the conserved motifs of these phosphoesterases define metal binding sites involved in catalysis or substrate binding.

### Biological role of exonucleases

Exonucleases play key cellular roles in mutation avoidance and genome stability, DNA repair and recombination, which will be discussed as each exonuclease is introduced below. Although no two exonucleases in *E. coli* are exactly alike in their properties, the biological roles of exonucleases are often obscured by functional redundancy. This is especially a problem with the single-strand DNA-specific exonucleases, with some phenotypes not apparent until four members are knocked out.

## E. COLI EXONUCLEASES: PROPERTIES, STRUCTURE AND FUNCTION

### The proofreading exonucleases associated with DNA polymerases I, II and III

Three of *E. coli* DNA polymerases have associated 3′ to 5′ exonuclease activities. In DNA polymerases I and II, the exonuclease activity resides on a domain within the polymerase subunit; in DNA Polymerase III, the exonuclease is encoded by a separate subunit, epsilon, the product of the *dnaQ* (*mutD*) gene. These associated 3′ to 5′ activities increase the fidelity of DNA synthesis (reviewed in (137)).

DNA polymerase III (Pol III) performs the bulk of DNA replication of *E. coli*. The holoenzyme it is composed of 10 distinct subunits, including the polymerase core, processivity clamp and clamp loader complex (see module 4.4.2). The DNA polymerase III core (176, 179, 218) consists of three subunits: 130 kD alpha, encoded by *dnaE*, in which the polymerase activity resides, 27 kD epsilon, encoded by *dnaQ*/*mutD* gene with the 3′ to 5′ exonuclease activity and the small 9 kD theta protein, encoded by the *holE* gene. The *holE* gene is non-essential (229) and the role of the theta in the complex is still incompletely understood, although theta does appear to stabilize the epsilon subunit in vitro and in vivo (237, 240). The DnaQ protein consists of two domains connected by a flexible linker: the N-terminal domain possesses the exonuclease activity and the C-terminal domain interacts with alpha and theta (197). The structure of the DnaQ exonuclease domain shows similarity with other members of the DEDD superfamily (103). The alpha and epsilon subunits interact not only physically but also mutually stimulate each other's activities in vitro (175, 237).

Mutations isolated in the *dnaQ* gene, such as the classic *dnaQ49* and *mutD5* "mutator" alleles, cause a greatly elevated spontaneous mutation rate due to increased replication errors and saturation of mismatch repair capabilities (217). The *dnaQ49* allele, which causes temperature-sensitivity on rich salt-free media (110) appears to affect the interaction with alpha and theta (117), whereas these interactions are preserved in *mutD5*. Mutations in two of the metal-binding carboxylates, *dnaQ926*, in the first conserved motif of the exonuclease active site, produces a strain that is essentially inviable due to the catastrophic accumulation of mutations (so-called "error catastrophe"). This strain survives only in combination with compensating antimutator alleles in the alpha, *dnaE*-encoded, polymerase subunit or with overproducers of the MutL mismatch repair protein (85).

The DNA Polymerase I (Pol I) 3′ to 5′ exonuclease activity, was purified as Exonuclease II from *E. coli* and later found to reside within the same 103 kD protein as the polymerase activity (148). Polymerase I is the most abundant DNA polymerase in *E. coli* (at approximately 400/cell) and is the polymerase believed to mature Okazaki fragments produced by Polymerase III-mediated lagging strand synthesis, including the removal of the RNA primer. Two domains in Polymerase I can be separated by proteolysis (126): a small N-terminal fragment containing the 5′ to 3′ exonuclease activity (see below) and the large C-terminal fragment (also known as the Klenow fragment) which possesses both polymerase and 3′ to 5′ exonuclease activities. The 3′ to 5′ exonuclease activity of DNA polymerase I bound to single-strand DNA was one of the first exonucleases to be characterized structurally ((14, 15), see discussion above). The polymerase and 3′ to 5′ active sites are quite distant at 35 Angstroms. The exonuclease binds to 3′ single-strand DNA, and 3 nucleotides are displaced from the polymerase active site. Two metal ions occupy the active site, coordinated by conserved residues that comprise the motifs that define the DEDD/DnaQ superfamily (16, 69).

Although by analogy to other proofreading activities associated with DNA polymerases, the 3′ exonuclease in Polymerase I should contribute to replication fidelity, this has been difficult to establish genetically. Inactivation of the Pol I 3′ exonuclease causes a modest, 2–4 fold increase in specific *lacZ* reversion rates. This is apparent in an orientation-specific manner, confirming the expectation that Pol I errors arise primarily on the lagging strand (177). In this study, deficiency in the 3′ exonuclease of Pol I did not strongly enhance the mutator phenotype conferred by defects in the Polymerase III holoenzyme, suggesting that Pol I does not readily replace Pol III in the processing of misincorporation errors.

DNA Polymerase II (Pol II) of *E. coli* also possesses an associated 3′ proofreading exonucleases. The *polB* gene is induced seven-fold by DNA damage via the SOS response (20), although is found at levels comparable to Polymerase III (about 30–50 molecules per cell) in uninduced cells (203). Of the three SOS DNA polymerases (Polymerases II, IV and V), the 90 kD Polymerase II is the only one with an intrinsic proofreading exonuclease. Pol II has a low rate of misincorporation in vitro and the loss of the proofreading activities elevates mutations 13–240-fold (29). In vitro, Polymerase II can polymerize DNA opposite abasic sites (21) and is required for efficient replication restart in UV-irradiated *E. coli* (206). Pol II is also involved in error-prone bypass of acetylaminofluorene lesions in vivo (191). In vivo, in the absence of mismatch repair and in the presence of an antimutator allele

of DNA polymerase III (*dnaE915*), inactivation of Polymerase II's exonuclease activity increases mutagenesis, as assayed by mutation to rifampicin resistance in *rpoB*; this is especially apparent at one specific G to A mutational hotspot (205). Mutations in F′ *lacZ* that occur during lactose selection (so-called "adaptive mutations") are also elevated by inactivation of Pol II proofreading (87). Loss of Pol II proofreading has a synergistic effect on mutation rates in combination with mutator Pol III alleles in *dnaQ* and *dnaE*, indicating that Polymerase II proofreading can process mispairs created by Polymerase III during chromosomal replication (12). The spectrum of mutations in *rpoB* exhibited by *polB* mutants also supports the idea that there is polymerase switching between Pol III and Pol II during DNA replication, in which Pol II plays a largely antimutagenic role (60).

## The 5′ exo/endonuclease activity of DNA polymerase I

A second hydrolase activity, associated with DNA polymerase I, specific for 5′ ends, was discovered by two groups (72, 127) and was known initially as Exonuclease VI. Proteolysis showed this activity resides in the small N-terminal domain of the protein, separated from the large fragment (also known as the Klenow fragment (126)) containing the 3′ exonuclease and polymerase activity of enzyme (221, 222). The 5′ nuclease activity is distributive (247), and limited relative to 3′ exonuclease (221, 222). The 5′ exonuclease, coupled to the DNA polymerase activity, affords Pol I its "nick translation" activity, wherein 5′ to 3′ polymerization at a nick is accompanied by 5′ to 3′ digestion of the DNA ahead of the polymerase (149). Mutations in the 5′ to 3′ exonuclease produce lethality under certain growth conditions but not those in the DNA polymerase or 3′ to 5′ exonuclease activities (reviewed in (149)).

The 5′ exonuclease activity of Pol I from *Thermus aquaticus* and *E. coli* is not a simple exonuclease but rather a structure-specific endonuclease activity, which cleaves the junction between a 5′ single-strand and duplex (172, 173). In the intact enzyme, polymerase activity I at a nick could displace DNA ahead of the enzyme, resulting in a 5′ flap that is then cleaved by the 5′ endo/exonuclease. The nuclease activity, although endonucleolytic in nature, requires a free 5′ end to thread through the enzyme (173). This "flappase" activity is rather inefficient on long 5′ ssDNA tails, assuring that is specific to the types of substrates encountered during its role in Okazaki fragment maturation during DNA replication (see below). Both *Taq* and *Eco* Pol I cleave between the two paired bases closest to the 5′ end (173, 270) and *Taq* Pol I cleavage is most efficient when a primer strand is juxtaposed to the flap as would be presented during Okazaki fragment processing. DNA polymerase I binds to the beta processivity clamp (163), but the consequences of this interaction on 5′ cleavage activity is not yet reported.

The 5′ exonuclease has motifs characteristic of a large group of nucleases found in prokaryotes and eukaryotes, the Rad2/XPG family and is structurally and functionally equivalent to the DNase IV/Fen-1 ("Flap endonuclease 1") enzyme of mammals (156, 211), RAD27/RTH1 of yeast (231) and bacteriophage T4 RNase H (187) and T5 5′ exonuclease (32). The structure of *Taq* DNA polymerase I (Figure 2A) indicated three metal binding sites with two of these in close proximity (125). The residues that define these two sites are highly conserved (99) and when mutated are defective in cleavage activity (269). Evident in

the structures of this class of enzymes is the presence of a helical arch in the protein that creates a hole that can accommodate single-strand but not double-strand DNA (32).

Mutants in the 5′ exonuclease activity of Pol I, *polA*ex*1*− (133) were originally isolated as hyper-recombinational and subsequently found to be mildly sensitive to UV and methyl methanesulfonate (MMS) and temperature-sensitive for growth on rich medium. In this mutant, polymerase activity is normal and the 5′ to 3′ exonuclease activity is specifically reduced. Okazaki fragment maturation is delayed in these mutants (252), suggesting a role for PolI in removal of RNA primers and formation of ligatable DNA chains (Figure 2B). Supporting the idea that the PolA 5′ exonuclease is the functional equivalent of the eukaryotic FEN1/RAD27 function, the N-terminal domain of *polA* can partially complement *rad27* mutants of *Saccharomyces cerevisiae* (238) and the yeast gene can partially complement *polA107*Ex-mutants (196).

The *polA* gene, under certain growth conditions, can be completely deleted but such mutants are lethal in combination with those in the *xni/xgdG* gene, a paralogous gene to the 5′ exonuclease domain of polymerase I (91), see below.

Mutants in the 5′ exonuclease of DNA polymerase I are also sensitive to peroxide (19). The 5′ exonuclease activity has also been implicated in the repair of daughter strand gaps that are formed after UV irradiation, a process known as “post-replication repair”. Gap-filling is defective in *polA546*, defective in 5′ to 3′ exonuclease activity (225).

Deficiency in the 5′ exonuclease of DNA polymerase I causes an increase in certain types of mutation (185, 190), as was established first for its yeast counterpart, *RAD27* (*RTH1*). This includes expansions of simple sequence repeats (116), expansions of CTG/CAG trinucleotide repeats (89) and duplication mutations at short sequence repeats (250). Displacement of 5′ DNA ends by DNA polymerase during Okazaki fragment maturation, coupled with the failure to be cleaved by the 5′ flappase activity and ligation to the growing chain can lead to the observed duplication mutations.

## Exonuclease I

Exonuclease I (ExoI) was the first exonuclease to be purified and characterized from *E. coli* (146, 147); it was identified as a $Mg^{2+}$-dependent 3′ to 5′ exonuclease, digesting DNA to mononucleotides. It has potent activity, degrading up to 10,000 nucleotides/min and is strongly specific for ssDNA. ExoI dissociates upon encountering dsDNA (25). It is active as a 55 kD monomer (200). ExoI is a member of the DnaQ superfamily and its structure (22) is highly similar to the 3′ to 5′ exonucleases active site of the Klenow fragment of DNA polymerase I. ExoI is not widespread in bacterial genomes; it appears to be restricted to the gamma-proteobacteria.

Exonuclease I is a processive exonuclease (247). Its structure indicates an extended binding site encompassing 12 residues (22, 23) and appears have the potential to encircle its substrate, a simple way to achieve processivity (Figure 3). The active site is contained in a deep positively charged groove in the structure that is wide enough only to contain ssDNA, explaining its strong specificity for single-strands.

The single-strand DNA binding protein, SSB, stimulates digestion of DNA by ExoI (169, 214), partly by removal of substrate secondary structure that would impede the enzyme, but also by recruitment through specific physical interactions (169, 215). ExoI binds to the C-terminal domain of SSB, the site of its interactions with a number of DNA metabolic enzymes (169). A peptide derived from the C terminal domain of SSB binds to ExoI through interactions in the pocket between its exonuclease and SH3-like domain (169).

ExoI is a part of a network of replication and repair proteins interacting with SSB (27), which includes RecJ exonuclease, Topoisomerase I, RecO recombination protein, RecG and RecQ helicases. Preparations of SSB are notoriously contaminated with trace amounts of ExoI. This led to the discovery that ExoI can stimulate RecA-mediated DNA strand exchange between linear duplex and circular single-strand molecules, when the 5′ ends are heterologous (13, 132). ExoI processively digests the displaced 3′ strand from the linear duplex and drives the reaction forward by degradation of the competitor strand for pairing. (RecJ exonuclease, with opposite polarity to ExoI, has similar properties, see below.) The recruitment of ExoI, and other repair factors, to gaps in DNA through interactions with SSB may constitute a tool-kit for gap-repair.

Mutations in structural gene for exonuclease I, *sbcB*, were originally isolated by their ability to suppress UV-sensitivity and recombination deficiency of *recBC* mutant strains (named for suppressor of recBC)(139, 141). The suppressor is attributed to the accumulation of long 3′ ssDNA tails (that would normally be degraded by ExoI) that permit the alternative recombination pathway, the RecFOR pathway, to operate. In these strains, co-suppressor mutations in the endo/exonuclease SbcCD have accumulated and contribute to suppressor phenotype (see below). Null mutations in the gene for Exo I (alternatively named "*xonA*") were isolated by loss of nuclease activity directly and are not as effective for suppression of *recBCD* (139, 248, 271). SbcB-type alleles have been found to retain ability to bind 3′ ends and protect from degradation (248); presumably they act as better suppressors that XonA-type alleles by blocking the access of other exonucleases to 3′ ssDNA ends. Supporting this idea, an *sbcB* allele mimics phenotypes of double mutants in Exonuclease I and VII (258).

In addition to this antirecombinational activity, ExoI can promote recombination by the RecBCD pathway, by "end-blunting" (248, 249), trimming 3′ ends to provide blunt ends required for RecBCD digestion and RecA loading. It has also been implicated in postsynaptic DNA processing of recombination intermediates (90, 256).

The absence of ExoI also promotes a number of mutations and genetic rearrangements including frameshifts in repetitive sequence runs (261), mutations templated by synthesis in a quasi-palindrome (81, 258), deletions at short direct repeats (2, 28, 84), and RecA-independent recombination reactions between short regions of homology (82). These can be explained by the ability of ExoI to scavenge displaced 3′ ssDNA ends that are intermediates of misalignment reactions that lead to mutations or genetic rearrangements. ExoI is one of the four exonucleases that promote mismatch repair (26, 50, 256). In many of the functions of exonuclease I listed above, the enzyme is often redundant with other exonucleases, including RecJ, Exonuclease VII, Exonuclease X, and SbcCD (see below).

## Exonuclease III

Exonuclease III (ExoIII) was identified as an exonuclease activity associated with a phosphatase (208, 209). Exonuclease III is a $Mg^{2+}$-dependent 3′ to 5′ exonuclease on dsDNA, releasing 5′ phosphomononucleotides as degradation products. The enzyme will not degrade ssDNA. ExoIII will degrade only one strand of the duplex in the 3′ to 5′ direction such that extensive ssDNA-tailed molecules or fully ssDNA is produced. Exonuclease III does not require a dsDNA blunt end and will degrade from a nick on a circular molecule in the 3′ to 5′ direction, producing single-strand gaps. ExoIII is a distributive enzyme, dissociating frequently during the course of digestion (247).

ExoIII will remove a 3′ phosphate in a $Mg^{2+}$-dependent manner, releasing inorganic orthophosphate. It will remove other 3′ residues damaged by radiation or oxidation, such as 3′ phosphoglycolate, 3′ phosphoaldehyde and urea N-glycosides (68, 106, 138, 183, 193). Exonuclease III also possesses endonuclease activity on apurinic/apyrimidinic DNA (265), an activity originally purified as “endonuclease II”. The incision at abasic sites is 5′ to the abasic residue. ExoIII is the most abundant abasic endonuclease in *E. coli*, accounting for 80% of the activity (266). ExoIII also possesses RNase H activity, degrading the RNA residues of a RNA:DNA hybrid molecule (266). Each of these activities is believed to be manifest from a single active site. ExoIII is therefore a key enzyme for the repair of depurination events caused by spontaneous cleavage of the N-glycosidic bond (158) and for repair of the abasic sites that are the intermediates in the base excision repair (BER) pathways of damaged nucleotide bases.

Exonuclease III orthologs are found in many organisms including mammals (67). The crystal structure of *E. coli* Exonuclease III (Figure 4A) shows a single metal ion active site with catalytic aspartate and histidine residues, suggested to be the single site for all the activities of the enzyme (184). Conserved residues among ExoIII and other AP endonucleases cluster in this region and the overall structure of the protein is similar to DNase I and *E. coli* RNase H.

Mutants in exonuclease III, *xthA (*or *xth)*, were isolated by direct microassay screens of *E. coli* lysates (181) and shown to co-defective in endonuclease II activity (272). Mutants in *xthA* were defective in repair of strand breaks produced by gamma-irradiation (219) and in base excision repair, a process whose intermediate is abasic sites. Mutants in dUTPase, (*dut*) that incorporate large amounts of uracil into DNA are inviable in the presence of additional mutations in exonuclease III; this inviability can be relieved by a mutation in uracil N-glycosylase (*ung*), that convert uracil residues to abasic sites (245). Mutants in *xthA* are hypersensitive to hydrogen peroxide (66) and near -UV light (213). Exonuclease III is controlled by the stationary phase and general stress response sigma factor, RpoS (212), and is required for the resistance to peroxide that is induced as cells begin to enter to stationary phase. Exonuclease III is also required for resistance to chain-terminating residue, 3′ azidothymidine, and is most likely the enzyme that removes this blocked 3′ residue in vivo (Cooper and Lovett, unpublished).

## The exonuclease activity of Endonuclease IV

Endonuclease IV (EndoIV) is a $Zn^{2+}$-dependent apurinic endonuclease, cleaving DNA at abasic sites (153, 159). Exonuclease III, also a potent AP endonuclease, accounts for about 80% of the cleavage activity, whereas EndoIV constitutes the bulk of the remaining activity (59, 266). Unlike ExoIII, EndoIV can also incise 5′ to oxidized residues such as 5-hydroxyuracil, 5,6-dihydrothymine and 2,6-diamino-4-hydroxy-5-N methylformamidopyrimidine (“Fapy”) residues (114). In a process known as “nucleotide incision repair” (NIR), this incision would allow DNA polymerase I to displace and cleave a flap with the damaged residue. Although it had been reported to have negligible exonuclease activity, EndoIV does indeed possess an intrinsic 3′ to 5′ exonuclease activity, in addition to its endonuclease activity, detected for both *E. coli* and *Thermotoga maritima* enzymes (124). Substrates with recessed 3′ ends were found to be preferred substrates for its exonuclease, which is highly sensitive to ionic strength, metal ions, EDTA and reducing agents (124) expaining why early attempts to assay exonuclease may have failed. This activity can process nicks to gaps but is reduced when the 5′ residue is an abasic deoxyribose, as would be produced by EndoIV AP endonuclease activity (95). Endo IV can release 3′ phosphates and phosphoglycoaldehydes with a $K_m$ of 50–100 nM (152).

The crystal structure of Endonuclease IV (Figure 4B) indicates the presence of three $Zn^{2+}$ atoms, which generate the attacking nucleophile by deprotonation of water, stabilize the transition state and the leaving group (111, 115). Mutational analysis shows that incision and exonuclease depend on common residues (95). This exonuclease activity, as that of ExoIII, mostly likely plays a role in the removal of 3′ blocking lesions in bacterial cells, including 3′ phosphates and phosphoglycolates (152). Although EndoIV plays a secondary role to ExoIII in the processing of hydrogen peroxide-induced lesions, Endonuclease IV appears to be the primary processor of 3′ lesions induced by bleomycin treatment (151), tert-butyl hydroperoxide, and nitric oxides (59, 151, 195).

Mutants in *nfo*, the gene for EndoIV, are sensitive to alkyating agents such as MMS, mitomycin C, and to the oxidants tert-butyl hydroperoxide and bleomycin. The sensitivity to tertbutyl hydroperoxide and bleomycin is more severe than that demonstrated by mutants in Exonuclease III (*xthA*) suggesting some specialization of EndoIV for the processing of free radical lesions. In combination with mutations in *xthA*, an *nfo* mutation further enhances the killing by hydrogen peroxides, MMS and gamma rays (59). Endonuclease IV is induced by superoxide generators such as paraquat, plumbagin, menadione and phenazine methosulfate; induction was more extensive in *sodAB* mutants lacking superoxide dismutase (33). The *nfo* gene is regulated as part of the SoxRS-dependent response to superoxide stress (3, 97, 251).

## Exonuclease IV

Exonuclease IV activity has been described in one paper (118) and the gene encoding this activity has never been identified. Exonuclease IV was characterized by its stronger activity on short DNA oligonucleotides than on native or denatured DNA of longer length. Its products were 5′ phosphorylated mononucleotides.

One likely possibility is that Exonuclease IV is identical to oligoribonuclease (ORN), the product of the *orn* gene, an $Mg^{2+}$-dependent 3′ to 5′ RNA exonuclease with preference for short oligonucleotides of 5 nucleotides or less in length (63, 275, 281, 286). ORN is a member of the DEDD DnaQ superfamily of 3′ exonucleases, which includes Exonucleases I, X, RNase T, RNase D and the proofreading exonucleases of DNA polymerases I, II and III. The *orn* gene is essential for viability of *E. coli* (92) and is presumed to be required for mRNA decay and nucleotide recycling. ORN is functional as a dimer (281), similar to RNase T (155) and Exonuclease X (Viswanathan and Lovett, unpublished). Activity on DNA has not been reported, but ORN, like RNase T, may degrade both RNA and DNA oligomers. The likelihood that ORN is DNA Exonuclease IV is encouraged by the fact that *E. coli* ORN will hydrolyze a 5′ nitrophenol ester of thymidine (deoxyribose) monophosphate (274) and the human homolog of ORN, Sfn, degrades both RNA and DNA oligonucleotides (192).

## RecBCD (Exonuclease V)

The RecBCD nuclease, originally termed Exonuclease V, is a multifunctional enzyme combining ATP-dependent DNA helicase activity with $Mg^{2+}$-dependent endonuclease activity (see review (76)). It is a heterotrimer composed of two large subunits, RecB (134 kD) and RecC (129 kD) and one small subunit, RecD (67 kD). RecBCD requires a blunt end to initiate unwinding and degradation (123, 201, 244) and in the presence of $Mg^{2+}$ and ATP is an extraordinarily potent exonuclease, degrading hundreds to thousand of bases per second. Products of digestion are oligonucleotides and RecBCD digests both strands of the duplex. In limiting $Mg^{2+}$ conditions, the nuclease activity is suppressed and the enzyme acts primarily as a helicase, nicking the DNA occasionally. The nuclease active site is present in the C-terminal domain of RecB that catalyzes cleavage of both strands (264, 276, 277) and both the RecB and RecD subunits act as motors to translocate the complex on ssDNA (77, 78, 234, 242). In the absence of the RecD subunit, the nuclease activity is diminished; the RecBC complex retains weak helicase activity, although it is less processive (136, 198).

Considerable interest in RecBCD concerns its ability to react to specific octomeric DNA sequences, known as Chi sites, which alter the properties of the enzyme in vitro and in vivo. When the enzyme encounters Chi (5′ GCTGGTGG 3′), its helicase activity becomes attenuated, the strandedness of its nuclease activity is altered and a RecA-loading function is revealed. DNA is unwound by two helicase motors, in the RecB and RecD subunits, operating on the 5′ and 3′ ended strands, respectively (77, 242). Initially, when RecBCD loads on a linear duplex molecule, the RecD motor translocates faster than the RecB motor, which extrudes a loop from the complex as the enzyme moves along DNA. Forward progress of the complex is arrested when the RecC subunit recognizes the Chi site; the RecD motor slows or disengages and a slow-moving complex is powered predominately by the RecB subunit (232, 233). Interaction with Chi switches the dispensation of the DNA strands relative to the nuclease active site in RecB (6, 79, 80). In the absence of Chi, the nuclease activity is directed predominantly to the 3′ ended strand, whereas after Chi recognition, the 5′ ending strand is targeted. After Chi recognition, the product of the reaction is a 3′ single-strand tailed duplex molecule. Encountering of Chi also causes RecBCD to load RecA onto the emergent 3′ single strand (7). The RecB subunit C-terminal domain, which by itself

makes stable complexes with RecA, is required for RecA loading (5, 43, 235) and this activity is essential for recombination in vivo (11).

The nuclease domain of RecB is a member of structurally related endonucleases, including restriction enzymes (EcoRV and PvuII), the Vsr endonuclease involved in very short patch excision repair, lambda bacteriophage exonuclease, *Pyrococcus* Holliday junction resolvase and eukaryotic *DNA2* protein (10). The RecB and RecD subunits possess motifs characteristic of Superfamily 1 (SF1) helicase proteins (228).

The structure of *E. coli* heterotrimeric RecBCD complex with partially unwound duplex oligonucleotide (Figure 5A) provided key insights in the complex behavior of the enzyme (227). The RecC protein contributes a number of architectural features including two channels for the 5′ and 3′ unwound DNA strands and a pin structure, which appears to split the duplex at the entrance to the two channels. The RecB and RecD subunits are disposed to provide motors to feed the 3′ and 5′ strands, respectively, to a single nuclease activity site in the rear of the complex, with a single $Ca^{2+}$ ion bound. Residues that contribute to Chi recognition in RecC cluster in the 3′ channel preceding the nuclease domain. The RecC subunit itself surprising resembles a defunct SF1 family helicase, and is proposed to use these features as a Chi-scanning activity. Presumably, an encounter with a Chi-containing 3′ strand causes it to be bound in the Chi-scanner region, preventing its access to the nuclease site; this allows the 5′ strand to contact the nuclease site more freely (Figure 5B). RecC also possesses a defunct nuclease domain, similar to the active nuclease domain in RecB; this is proposed to act as a hoop into which the 5′ single-strand is fed (210).

In vivo, one of the roles of the RecBCD nuclease is to defend against viral infection and replication. Bacteriophage with linear genomes or which possess linear ends during their replication (such as during rolling-circle replication) commonly overcome RecBCD digestion by encoding a specific inhibitor of RecBCD (the most well-studied of which is the Gam protein of bacteriophage lambda (188). RecBCD also inhibits rolling circle replication of bacteriophage or plasmids that lack Chi sequences. The presence of Chi sequences promotes the accumulation of high molecular weight DNA, linear concatemers whose ends are protected by RecA (61, 279).

Chi sequences are over-represented in the *E. coli* genome in one orientation relative to the progression of the replication fork (8, 18) and occur about once every 4 kb. The probability that RecBCD will recognize a single Chi element is about 30% in vitro (80, 243); in vivo, multiple Chi sites can additively protect linear DNA from degradation in a RecA-dependent fashion (143). Chi sequences not only aid in the protection of DNA but also stimulate homologous recombination in their vicinity (144). RecBCD-dependent recombination is proposed to repair spontaneous double strand breaks that occur in chromosomes during replication (129, 142). Cultures of mutants in *recBC* have lower viabilities; only about 30% of the population will form visible colonies (30).

RecBCD is required for double-strand break repair and recombination that arises from ds-ended substrates, such as those presented during conjugation and generalized transduction. (See (199)). Mutants in *recB* or *recC* have 100–1000 fold reduced inheritance of genetic

markers after congugation or transduction (112). Mutants in the third subunit, *recD*, do not show reduction in recombination (4, 17)--in fact, they are hyper-recombinational--adding to confusion about the role of this subunit in the function of the complex. In *recD* mutants in which the nuclease activity of RecBCD is lost (4, 17), recombination becomes dependent on alternative 5′ to 3′ exonucleases such as RecJ exonuclease or exonuclease VII (71, 161, 167). Loss of RecJ, ExoI and ExoVII causes lethality in *recD* strains (70). In *recD* mutants, recombination still requires function of *recB* and *recC* (167) presumably to unwind DNA and to load RecA on the emerging ssDNA. In the absence of RecD subunit, RecBC loads RecA constitutively, even in the absence of Chi sequences (42).

## RecJ exonuclease

The properties of RecJ exonuclease are similar to Exonuclease I, with reverse polarity: it degrades ssDNA in a 5′ to 3′ polarity in a reaction that requires $Mg^{2+}$ (166). It is a processive monomeric exonuclease of 60 kD, degrading DNA to mononucleotides at a maximal rate of several thousand nucleotides/min (104, 166) and a $K_d$ of 10 nM. RecJ does not require a terminal 5′ phosphate and will digest equally well DNA terminating in 5′ OH. RecJ has no activity on blunt dsDNA and requires at least 6 unpaired bases to bind and to initiate degradation (104). Once bound to a ssDNA -tailed molecule, RecJ can digest into a dsDNA region to a limited extent but most often terminates digestion at the ds/ssDNA boundary (104). As with ExoI, SSB stimulates the ability of RecJ to bind and to digest DNA (104). Although interactions between *E. coli* RecJ and SSB proteins have not been yet demonstrated, the heterologous T4 gp32 ssDNA binding protein did not stimulate nuclease activity, suggesting a specific interaction is responsible for stimulation. Direct interaction between *Haemophilus influenzae* RecJ and SSB has been established, between the catalytic core domain of RecJ and the C-terminal domain of SSB (224).

RecJ is the best studied member of the DHH family of phosphoesterases (9, 239) and RecJ orthologs are found in virtually all bacterial genomes with the notable exception of Mycoplasmas and Mycobacteria. Initially five (9)) and later seven motifs (204, 239) were found to define this class and mutational studies have verified the essentiality of residues in the seven motifs for nuclease activity in vitro and biological function in vivo (239). Motif 5 of seven (204), (not identified in the earlier five-motif comparison (9)), with conserved asparagine, appears to be present specifically in nuclease members of this family. The structure of a C-terminally truncated *Thermus themophilus* RecJ shows a C-shaped molecule, with a narrow cleft into which ssDNA but not dsDNA can access the metal ion active site at the wall of this cleft (273). Only one $Mn^{2+}$ ion was found in the structure, but the location of conserved aspartate residues in its vicinity suggest the possibility of a second metal binding site, with the metal recruited or stabilized by DNA binding. Indeed, the recent structure of the fully intact Tth RecJ (263), with four domains shows two metal-ions (Figure 3). Domain III, partially lacking in the earlier structure, has similarity to the OB-fold, explaining the higher affinity of the intact enzyme for ssDNA (263). The O-structure of the enzyme, with the hole bounded by Domain I (containing the active site metal ions), Domain II (with nuclease-specific motif 5 residues at its periphery) and OB-fold Domain III would allow it completely encircle DNA, accounting for the processivity of RecJ.

RecJ was identified as a gene required for the so-called “RecFOR” and “RecE” pathways of recombination (94, 109, 165), pathways that operate independently of the RecBCD nuclease. Both RecBCD-independent pathways employ many of the same functions (RecFOR, RecJ, RecQ and RuvABC). The RecFOR pathway is believed to be specialized for recombination at gaps in DNA (230). Although normally inefficient in catalyzing recombination from double-strand ends in *E. coli*, increasing the levels of 3′ DNA ends by inactivation of 3′ exonucleases, ExoI and SbcCD, or increasing expression of 3′ end-generating RecE/ExoVIII makes it more active on dsDNA substrates. (Note that in most other bacterial species, which naturally lack ExoI, the RecFOR pathway may operate more efficiently on dsDNA substrates for recombination.) In the genetic backgrounds *recBC sbcBC* (eliminating ExoI and SbcCD) or *recBC sbcA* (activating expression of RecET), deficiency in *recJ* leads to a 1000–10,000-fold reduction in conjugational recombination; *recJ* deficiency also eliminates the residual recombination seen in *recBC* mutants with a concomitant loss of cell viability (165). In wild-type cells, *recJ* is required for recombination of plasmid alleles via a pathway that also includes RecFOR (130). The interpretation of these genetic properties suggest that RecJ provides presynaptic DNA processing activity equivalent to RecBCD, for production of recombinogenic ssDNA. In wild-type strains, RecBCD is specialized for degradation from a linear end; RecJ is specialized for degradation from a single-strand gap. In combination with a helicase like RecQ, RecJ can lengthen ssDNA gapped regions or produce 3′ ssDNA tailed recombinogenic molecules from double-strand ends, in a manner comparable to the RecBCD nuclease. In a reconstituted in vitro system containing RecA and RecOR, RecJ provides the nuclease activity to permit recombination between homologous dsDNA linear and dsDNA supercoiled circular molecules; RecF and SSB are nonessential, although stimulatory to the reaction (105). Inclusion of RecQ stimulates RecJ’s exonucleolytic processing but destablizes heteroduplex production formation. Unlike RecBCD, RecJ does not possess RecA loading activity, which is contributed by the RecFOR proteins for both the RecFOR and RecE pathways of recombination.

RecJ is required, along with ExoI, for efficient recombination via the RecBCD pathway, with the double mutant reduced about 10-fold for Hfr conjugal inheritance in *E. coli* (261), a 30-fold reduction in Salmonella for transduction with limiting homologies (180) and approximately 4-fold reduction for lambda recombination (207) in *E. coli*. Like ExoI, it may also promote recombination by the RecBCD pathway by presynaptic end-blunting (248, 249) and by post-synaptic stabilization of joint heteroduplex molecules (90, 261). In vitro, RecJ can accelerate RecA-promoted branch migration, by removal of the competing strand for pairing, and can help RecA bypass regions of nonhomology in strand exchange (53).

After UV irradiation, the combined action of RecJ exonuclease and RecQ helicase activities can result in degradation of nascent DNA. This is more pronounced in mutants of RecA (40) and its loading factors RecFOR, which fail to protect gapped regions of the chromosome (56). Both RecJ and RecQ are required for rapid recovery of DNA synthesis following UV-irradiation and prevent potentially mutagenic bypass by error-prone polymerases (54). This degradation may also help to prevent replication fork reversal (55).

As a ssDNA-specific exonuclease, RecJ shares overlapping functions with other such enzymes including Exonucleases I, VII and X. Any one of these four enzymes that can

catalyze the excision reaction during mismatch repair of replication errors (26, 50, 256). RecJ plays a redundant role with other single-strand DNA exonucleases for UV survival, particularly with fellow 5′ to 3′ ssdNA exonuclease ExoVII (248, 261). Either RecJ or ExoI are required to process lesions produced by gamma-irradiation to activate the regulatory SOS response to DNA damage (220). RecJ is also implicated, along with the other ssDNA exonucleases, in avoidance of a number of genetic rearrangements and recombination between short homologies. Over 90% of the deletions between short direct DNA repeats are aborted by the combined action of RecJ, ExoI and ExoX (84). Gene conversion between 80 bp homologies, which occurs independent of RecA, is stimulated almost 1000-fold by the loss of both RecJ and ExoI. The mechanism of these recombinational events is not known but appears to be targeted to the replication fork, since recombination is more efficient with exogenous ssDNA that is complementary to the lagging-strand replication template relative to that of the leading strand (82). The quadruple RecJ, ExoI, ExoVII and ExoX mutant is cold-sensitive for growth and the lethality can be alleviated by blocks in the early steps in the mismatch repair pathway, suggesting that mismatch repair in the absence of ssDNA degradation is somehow toxic (26). The quadruple mutant also shows a massive increase in RecA-independent crossover recombination at very short homologies at permissive temperature, leading to the suggestion that the cold-sensitive lethality is caused by catastrophic genetic rearrangements that result from the abnormal accumulation of ssDNA in these cells (82).

The *recJ* gene is found in an operon downstream of the disulfide bond isomerase gene, *dsbC* (182). Mutations in *dsbC* (originally denoted *xprA*) have polar effects on *recJ* expression (164). Expression of the operon appear is activated by cell envelope stress, in a sigmaE-dependent manner (98). RecJ levels are very low in the cell, estimated at 5 molecules per cell and expression is kept low by poor translation (100, 166).

### SbcCD exo/endonuclease

The two subunit SbcCD nuclease is a combined ATPase/endo/exonuclease protein in a family that includes bacteriophage T4 gp46/47 nuclease and the eukaryotic Mre11 Rad50 Xrs2/Nbs1 complex (57). The SbcC subunit is an SMC-like coiled coil protein with an intrinsic ATPase; the nuclease activity resides in the SbcD subunit (48, 49). In vitro, SbcCD is an ATP-independent single-strand DNA endonuclease and an ATP-dependent 3′ to 5′ exonuclease (45, 47, 48). SbcCD is a structure-specific nuclease, showing preference for hairpin secondary structures in DNA, which it cleaves in an ATP-dependent fashion close to the unpaired tip (48). SbcCD can also cleave 4-strand cruciforms and can open hairpin-capped ends.

In vivo, SbcCD will cleave at secondary structures formed by inverted repeats, or palindromic DNA sequences, producing double-strand breaks (83). These breaks can be repaired by recombination between sister chromosomes (83) or by single-strand annealing at homologies flanking the break (28). SbcCD is also required to repair breaks made by restriction endonuclease activity (58) and its exonuclease activity may process the ends of broken chromosomes. In addition, its SMC character may bind and coordinate the two ends of broken DNA molecules to assist in their repair. SbcCD can remove a streptavidin/biotin

moiety at a 5′ end and may therefore remove, by its endonuclease activity, covalently attached or tightly bound proteins that interfere with end processing (46). SbcCD's 3′ exonuclease activity may digest 3′ tailed substrates in vivo, explaining its discovery as a function inhibitory to the RecFOR-mediated recombination of double-strand ended substrates (160). Mutations in SbcCD produce synergistic UV-sensitivity in combination with other ssDNA-specific exonucleases, particularly with the 3′ to 5′ exonucleases, ExoI and ExoVII (248). Loss of SbcCD and Exonuclease I blocks the "reckless" DNA degradation and anucleate cell formation provoked by RecBCD in UV-irradiated *recA* recombination mutants (278). This confirms that Exonuclease I and SbcCD can remove single-stranded tails ("end-blunting") on DNA in vivo to permit RecBCD loading and subsequent DNA degradation.

SbcCD expression increases as cells starve and enter the stationary phase of growth and is at least partially under RpoS regulation, although this may be indirect (62). The SMC-like SbcC proteins localize to the replication fork as visualized as a GFP fusion, whereas the SbcD nuclease subunits appear evenly distributed through the cytoplasm (62).

## Exonuclease VII

Exonuclease VII (ExoVII) possesses two subunits, a large catalytic subunit (XseA) at 53 kD and a smaller subunit (XseB) at 10 kD, with the stoichometry 1: 4 (254). The genes in *E. coli* are encoded by unlinked loci, *xseA* and *xseB* (253); in other bacteria, the genes are found in a single operon. Catalytic activity is found in the large subunit, whereas the small subunits are believed to regulate the activity of the enzyme. Exonuclease VII activity is strongly single-strand DNA-specific and possesses dual polarity, degrading both 3′ and 5′ ends, in a processive reaction (37, 38). It can digest DNA containing thymine-dimers. It can degrade a 3′ end as short as 4 nucleotides, and can digest into the duplex region, probably the result of its processivity and "breathing" of the duplex to form single-stranded termini. The products of the reaction are oligonucleotides, primarily 4–12 nucleotides in length (37), indicating an endonucleolytic mechanism, although the enzyme is classified as an exonuclease for its requirement for a free ssDNA end. ExoVII is the only DNA exonuclease activity from *E. coli* that does not require exogenous $Mg^{2+}$ ion; it is active even in 8 mM EDTA (38). Because of the seeming absence of metal ion requirement and stimulation by phosphate, it is possible that ExoVII is a phosphorylase rather than a hydrolase enzyme, with phosphate rather than water attacking the phosphodiester bond. However, the Thermotoga enzyme does require $Mg^{2+}$ and conserved aspartate residues, reminiscent of a metal binding site (145) and found invariant among *xseA* orthologs, including *E. coli*. Presumably, the *E. coli* enzyme is purified with a very tightly bound metal ion and is a true hydrolase. In contrast to ExoI and RecJ, ExoVII is inhibited by SSB (Cooper and Lovett, unpublished results).

Exonuclease VII is a very well conserved enzyme and *xseA* orthologs are found in almost every bacterial genome, as well as a few archaeal species. (Because of their small size and lack of catalytic activity, *xseB* orthologs are more difficult to identify.) Despite its prevalence, Exonuclease VII is the one of the most poorly understood exonucleases of *E. coli*. A number of residues in both subunits are conserved and are similar to the charged

residue motifs found conserved in other hydrolytic metal-dependent exonucleases. The N-terminal domain of ExoVII resembles the OB fold, characteristic of single-strand nucleic acid binding proteins (145). There is no crystal structure for XseA to provide clues about the mechanism; a XseB structure can be found (Protein Data Bank, PDB ID:1VP7 “Crystal structure of Exodeoxyribonuclease VII small subunit from *Bordetella pertussis* at 2.40 A resolution”, Joint Center for Structural Genomics) resembling a “paper clip” of alpha helices. The sedimentation coefficient and Stokes radius of the enzyme suggests an elongated structure (38). It is not clear how the XseB subunits interact with the larger XseA catalytic subunit, nor is it known how they regulate the activity of the enzyme.

Mutants in exonuclease VII are hyper-recombinational and weakly sensitive to nalidixic acid and UV irradiation (36). For UV irradiation, exonuclease VII deficiency is strongly synergistic with *recJ* (261). It has been suggested that ExoVII may participate in the mechanism of “long-patch excision repair” of UV damage, suggested to be recombinational in nature (51). Specifically, a role for RecJ and/or ExoVII in widening gaps caused by UV blocks to DNA synthesis on the lagging strand been suggested, which would promote nucleation of RecA and recombinational gap filling repair (261). In contrast with the redundant role of ExoVII and RecJ for repair, there is no normal requirement for ExoVII in homologous recombination measured after conjugation (36, 261). This may be because recombinational substrates are SSB-bound; ExoVII is inhibited by SSB (Cooper and Lovett, unpublished results), whereas RecJ is not (104). Mutants in exonuclease VII are hyper-recombinational (36), perhaps due to the accumulation of lesions normally repaired by ExoVII. Mutations in *xseA* modestly enhance the temperature-sensitivity promoted by 5′ exonuclease-deficient alleles of Polymerase I, *polA480* and *polA546* (35), suggesting a potential role in processing displaced ends during Okazaki fragment maturation.

ExoVII is one of the four ssDNA exonucleases that can mediate mismatch repair in *E. coli* (26, 256). ExoVII seems to abort frameshift and template-switch mutations in a manner partially redundant with 3′ exonuclease, ExoI (81, 258, 261). By itself, deficiency in Exonuclease VII stimulates frameshift mutations 6–7 fold; in combination with ExoI frameshift mutations are stimulated 12–30 fold. Stimulation is seen for both +1 and −1 frameshift mutations. Some of this effect may be due to constitutive SOS induction and induction of mutagenic polymerase, DinB, in these strains, since accumulation of −1 frameshifts is alleviated by mutations that block SOS induction such as $lexA^{ind-}$ and *recA* and by *dinB* (107). However, DinB is highly selective for effects on −1 frameshifts (262) and the ExoI and ExoVII effects are equally strong on assays detecting +1 frameshifts (261), suggesting an additional effect on mutagenesis.

Similar effects are seen for a mutational hotspot in *thyA* in which mutations are templated by synthesis within an inverted repeat structure (81, 258). Loss of ExoVII stimulates these events about 7-fold, which is enhanced to 40-fold by the additional loss of ExoI. These effects have been explained by the role for the exonucleases in scavenging displaced 3′ ends that result from stalled replication. In the absence of degradation, these strands misalign, causing the template-switch that leads to mutation in the presence of an inverted DNA repeat nearby.

Expression of *xseA* in *E. coli* appears to be repressed by cAMP response protein, CRP, during nutritional downshifts (113) and may be regulated by the macrophage response transcriptional regulator, SlyA, in Salmonella (236). Interestingly, in *Neisseria meningitidis*, *xseB* is up-regulated when the bacterium encounters human host cells, leading to an increase in resistance to UV light and alkylating agents (186).

## Exonuclease VIII/RecE

Exonuclease VIII is encoded by the *recE* gene of the cryptic lambdoid Rac prophage of *E. coli* K-12. It possesses processive $Mg^{2+}$-dependent 5′ to 3′ exonuclease activity on dsDNA (119, 120, 140) and is the functional equivalent of the lambda exonuclease (“Exo”, Red alpha) required for bacteriophage recombination. RecE is a much larger protein than lambda Exo (866 compared to 226 amino acids), although the exonuclease activity appears to reside in its approximately 300 amino acid C-terminal domain (34, 41, 170, 189). RecE belongs to an evolutionary-related extended group of nucleases, including restriction endonucleases, RecB and lambda exonuclease (10, 34). Although there is little overall sequence similarity, RecB, lambda Exo and RecE share similar residues in their active sites (Figure 6B), with RecE more closely related to RecB than to lambda Exo. Unlike lambda Exo, which requires a 5′ phosphate for its exonuclease activity, RecE exonuclease is not affected by the absence of a 5′ phosphate (120). Like lambda exonuclease, RecE is highly processive, degrading the 5′ strand dsDNA to mononucleotides (120), with a catalytic rate of 19 nucleotide/second and a $K_d$ of 70 nM (282). RecE has no activity on ssDNA nor on dsDNA circles (120, 140).

The crystal structure of RecE C-terminal domain shows it to be a tetrameric toroid (Figure 6A), with a central cavity large enough to surround dsDNA substrate at its front and a ssDNA product at its rear (280). This structure nicely explains RecE’s processivity on linear dsDNA substrates and lack of endonuclease activity. Similar to lambda Exo, which associates with the recombination annealing protein, Red beta, RecE protein functions in conjunction with the RecT annealing protein (44, 101, 102, 194). Both functions are required for recombination (44), even if the DNA was pre-resected (189). Specific protein interactions exist between these pairs and the heterologous pairs (Red alpha/RecT; RecE/Red beta) do not support recombination (189). This interaction allows coupling of RecE degradation of the 5′ strand to the loading of RecT on the resulting ssDNA tail to promote annealing of complementary strands (101) or strand invasion of a homologous DNA duplex (102). RecET-orthologs can be seen in many bacteriophage genomes; the Gifsy-1 prophage of *Salmonella enterica* serovar *typhimurium* LT2 strains contains RecET orthologs (150).

RecE was discovered by its ability to restore recombination function to Red mutants of lambda or to *E. coli* cells deficient in the RecBCD nuclease (141, 246). The *recE* and *recT* genes can restore function to lambda by recombination between the cryptic prophage and a $Red^-$ mutant lambda genome, producing a phage known as “lambda reverse” (96, 285). The Rac prophage has been lost in certain *E. coli* K-12 lineages, (e. g. AB1157) (121, 122). Although the *recET* genes are not normally expressed, zygotic induction of the genes transferred by conjugation into *E. coli* lacking the Rac prophage can suppress the recombination deficiency of *recBCD* mutants, explaining the prophage’s name (Rac for

"Recombination Activation") (168). Mutations in the Rac prophage known as *sbcA* ("suppressor of *recBC*"), produce a stable suppression of recombination deficiency of *recBC* mutants by driving constitutive expression of the RecET genes (41, 88, 94, 121, 131, 140, 246, 268). RecET will promote RecA-independent recombination of plasmids and lambda bacteriophage (86, 93, 241); for chromosomal recombination measured after conjugation, the RecET pathway requires additional recombination functions including RecA, RecFOR, RecJ and RecQ (94, 165, 171, 174). Interest in the RecET and lambda Red pathways has been revived because of their ability to catalyze recombination between short homologies and their practical use in bacterial genetic engineering ("recombineering" or "ET cloning") (reviewed in (52, 255, 283)). The RecET pathway is a recombination system capable of repairing of broken dsDNA molecules by recombinational, two-ended repair (128, 241) with a homologous partner.

## Exonuclease IX

Exonuclease IX (ExoIX) is encoded by the *ygdG* gene and possess sequence similarity to the 5′ exonuclease domain of DNA polymerase I (216). Despite its name, exonuclease activity has yet to be demonstrated for the *E. coli* protein. The reported 3′ to 5′ exonuclease activity of the protein (223) appears to be a consequence of Exonuclease III contamination of the preparation (108). Although the *Staphylococcus aureus* orthologous protein, *Sau* FEN, has flap endonuclease and 5′ exonuclease activity, the *E. coli* ExoIX enzyme does not (1). Because the *E. coli* protein lacks the conserved residues of the proposed metal binding site II (1), it may not possess cleavage activity or may required additional factors. *E. coli*Exonuclease IX protein binds single-strand DNA binding protein, SSB and to the histone-like protein H-NS (1), suggesting a potential role in DNA metabolism.

Mutations in *xni (ygdG)*, the structural gene for exonuclease IX, are synthetically lethal with those in polymerase I (91), implicating the protein in Okazaki fragment maturation. Mutations in *xni* do not have any detected synthetic phenotypes on recombination, UV or oxidative damage repair in combination with mutations in other single-strand exonucleases, ExoI, ExoVII, ExoX or RecJ (162).

## Exonuclease X

Exonuclease X was discovered as an open reading frame with predicted similarity to the DnaQ family of 3′ to 5′ exonucleases. Purification and characterization of the 25 kD protein demonstrated it possessed $Mg^{2+}$-dependent 3′ to 5′ exonuclease activity on both single-strand and dsDNA substrates, with a preference for the former (260). Despite the fact that the enzyme is distributive, it has a fairly rapid catalytic rate of 1400 nt/min/monomer on ssDNA. The $K_d$ of the protein for ssDNA is 2 nM, confirming a very strong affinity for ssDNA. RNA does not act as a competitive inhibitor of the exonuclease activity, suggesting the protein is specific for DNA substrates.

Exonuclease X is a member of the DnaQ or DEDD superfamily and is most homologous to the proofreading activity of PolC from gram-positive bacteria and to the mammalian TREX1 protein implicated in autoimmune disorders, with which it shares many mechanistic features (157). ExoX purifies as a homodimer, similar to RNase T, another member of the family

(Viswanathan and Lovett, unpublished). There is no crystal structure of the protein, although structures for TREX1 may provide some insights (24, 65).

A mutant in Exonuclease X had little phenotype on its own but effects can be seen in combination with other ssDNA exonucleases. Loss of ExoX with ExoI in *recD* mutants of *E. coli* causes a 100-fold decrease in recombinants detected after conjugation, suggesting a role in recombination secondary to ExoI, revealed when the nuclease activity of RecBCD is impaired (70). ExoX, in combination with RecJ and ExoI, plays a role in promoting genetic stability by aborting deletion between tandem repeated sequences. In the absence of these three exonucleases, deletion rate between adjacent 100 bp repeats is elevated about 20-fold (84). The quadruple mutant in RecJ, ExoI, ExoVII and ExoX is UV-sensitive, cold-sensitive for growth and exhibits a modest mutator phenotype, epistatic to MutS, suggesting a deficiency in mismatch repair (26). Confirming this, cell extracts from RecJ ExoI VII X mutant are devoid of mismatch repair capacity (256). Consistent with the known polarity of these enzymes, either RecJ or Exo VII can support mismatch repair from a nick 5′ from the mismatch whereas ExoI, ExoX and, to a lesser extent, ExoVII can support repair from 3′ direction (50, 256). The ssExo mutants are sensitive to 2-aminopurine, which increases the load of mismatches in the cell; this sensitivity can be suppressed by mutations blocking earlier steps in mismatch repair, including MutL, MutS or UvrD (26). This finding and the relatively weak mutator phenotype have been attributed to the notion that attempts at mismatch correction in the absence of exonucleases is lethal.

The mechanism of cold-lethality of the strain is not fully understood, but it can be relieved fully by mutations in the DNA helicase UvrD and partially by upstream mutations in the mismatch repair pathway, MutS, MutH and MutL (26). The displacement of ssDNA by UvrD, in part in response to mismatch correction, appears to be lethal in the absence of ssDNA exonucleases. It does not appear merely to be due to the induction of the SOS response since mutations in *recA*, *lexA* and *sulA* did not alleviate the lethality (Sutera and Lovett, unpublished results). The quadruple RecJ ExoI ExoVII ExoX mutant also exhibits extraordinarily high rates of *recA*-independent recombination at short sequence homologies, 20–70 fold elevated relative to wild-type strains at 25 bp of homology (82). The cold-sensitive phenomenon could be related to increased recombination rates, promoted by UvrD unwinding of DNA, contributing to a catastrophic destabilization of the genome.

## RNase T (Exonuclease T)

RNase T was initially identified as a RNA exonuclease removing the terminal AMP residue of tRNA molecules (73–75); it also removes the two terminal residues during the maturation of 5S rRNA (154). The gene encoding RNase T was identified as a high-copy suppressor of the UV sensitivity in mutants of ssDNA exonucleases, RecJ ExoI and ExoVII (259), implicating a potential role as a DNA exonuclease. RNase T appeared to compensate specifically for ExoI in such strains, since it did not suppress *recJ xseA* double mutants. RNase T overexpression also appeared to support UV survival of strains deficient in nucleotide excision repair, *uvrA* or *uvrC* (259).

RNase T has robust exonuclease activity on long ssDNA substrates, degrading in a 3′ to 5′ polarity dependent on $Mg^{2+}$ or $Mn^{2+}$ (257). Only very weak degradation was observed on

dsDNA. Its $K_m$ for ssDNA was 5 nM in contrast to a reported $K_m$ for tRNA of 14 μM. Although its mechanism was nonprocessive, RNase T could extensively degrade ssDNA, as much as 30,000 bases on a single molecule (257), in comparison to its limited degradation of tRNA or rRNA (290). (For RNA, cytosine residues in particular appear to inhibit degradation (290)). Higher affinity for ssDNA over RNA was confirmed in a second study using oligonucleotide substrates ((289). RNase T will produce blunt ends from 3′ ssDNA overhangs, such as those produced by restriction endonucleases (289). It has been reported that like its RNase activity (155), RNase T's DNase activity requires dimerization (289).

RNase T is a member of the DEDD DnaQ superfamily of 3′ exonucleases (135). Mutation in these conserved motifs are required for RNase activity (287). A crystal structure confirms the resemblance to proofreading 3′ exonucleases and to other members of this family such as ORN and RNase D (292). The catalytic and substrate binding residues are contributed by separate molecules at the dimer interface, explaining why dimer formation is essential for function (287, 288, 292).

### TatD (Exonuclease XI?)

TatD, the fourth gene in the twin-arginine transport system operon (Tat) of *E. coli*, has been reported to have $Mg^{2+}$-dependent DNase activity (267) and has been termed named "Exonuclease XI" in one study (31). Orthologs are found in all three domains of life, including organisms that lack the twin-arginine protein secretory pathway. *E. coli* possesses two paralogs to TatD, YjjV, and YcfH. TatD, but not the two paralogs, has been classified as a horizontally transferred gene of *E. coli* (64). The yeast Tat-D protein, expressed in *E. coli*, has been characterized as an endonuclease and exonuclease with a 3′ to 5′ polarity (202). It will cleave both single and double-strand DNA, with a preference for the latter, and has an acidic pH optimum (pH 5). Neither TatD nor its paralogs has any effect on the secretion of proteins by Tat pathway (267). However, another study found a role for TatD in quality control of FeS proteins that are translocated by the Tat pathway, promoting rapid turnover of misfolded substrates (178), a property difficult to reconcile with proposed nuclease activity of the protein. TatD mutants of *E. coli* have been reported to exhibit a two-fold increase in the number of constitutive RecA-GFP foci visualized in growing, a property shared by mutants of other 3′ exonucleases such as Exonucleases III, VII, and X (31).

## SUMMARY AND CONCLUSIONS

*E. coli* possesses 15 proteins with verified exonuclease activity and two others, Exonuclease IX (YgdG) and TatD, that are suspected exonucleases because they are orthologous to known exonucleases. All but one activity (Exonuclease IV) have defined genetic basis and there is reason to believe that Exonuclease IV is the product of the *orn* gene. Many of these functions are vital to mutation avoidance, genome stability, DNA repair or genetic recombination. Some exonucleases are essential for viability (DnaQ, Pol I 5′ exo and ORN), whereas loss of others is lethal in combinations. The biochemical properties of these enzymes including polarity, processivity, substrate specificity and protein interactions are suited to their biological role. Some exonucleases appear to have redundant functions in vivo and phenotypes are not evident until multiple exonucleases are eliminated. There is

structural information for most of the *E. coli* exonucleases or their orthologs in thermophilic eubacteria.

Most exonucleases fall into structural superfamilies with members in all domains of life, suggesting that these activities evolved quite early in the history of life and play important biological roles. The DEDD/DnaQ family has been especially prolific and *E. coli* possesses 8 members of this family, including DNA proofreading exonucleases associated with DNA polymerases and stand-alone DNase, RNase or dual DNase/RNase activities. RecJ, Exonuclease III, Endonuclease IV and SbcCD appear to be ancient activities. Is *E. coli* especially blessed with exonucleases? It is difficult to say whether *E. coli* possesses more exonucleases than other bacterial species since systematic biochemical characterization of exonuclease activities has only taken place with *E. coli*. There are likely to be other exonucleases in bacteria that remain to be discovered and certainly new exonuclease activities in *E. coli* will be forthcoming.

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A.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ExoI | 11 | LFH**D**Y**E**TFGTHPALDRPAQFAA | 66 | LGYNNVRF**D**DEVTRN | 64 | SNAHDAMA**D**VYAT |
| ExoX | 2 | RII**D**T**E**TCGLQGGIVEIASVDV | 52 | YVAHNASF**D**RRVLPE | 39 | LHHHRALY**D**CYIT |
| ORN | 31 | IWI**D**L**E**MTGLDPERDRIIEIAT | 73 | ICGNSIGQ**D**RRFLFK | 6 | AYFHYRYL**D**VSTL |
| RNase T | 19 | VVI**D**V**E**TAGFNAKTDALLEIAA | 75 | MVAHNANF**D**HSFMMA | 37 | CQTAGMDF**D**STQA |
| RNase D | 24 | IAL**D**T**E**FVRTRTYYPQLGLIQL | 30 | KFLHAGSE**D**LEVFLN | 55 | RQCEYAAA**D**VWYL |
| PolI | 123 | FAF**D**T**E**TDSLDNISANLVGLSF | 42 | KVGQNLKY**D**RGILAN | 62 | EAGRYAAE**D**ADVT |
| PolII | 353 | VWV**E**G**D**MHNGTIVNARLKPHPD | 75 | IGWNVVQF**D**LRMLQK | 91 | ALATYNLK**D**CELV |
| DnaQ | 8 | IVL**D**T**E**TTGMNQIGAHYEGHKI | 62 | LVIHNAAF**D**IGFMDY | 49 | RTLHGALL**D**AQIL |

B.

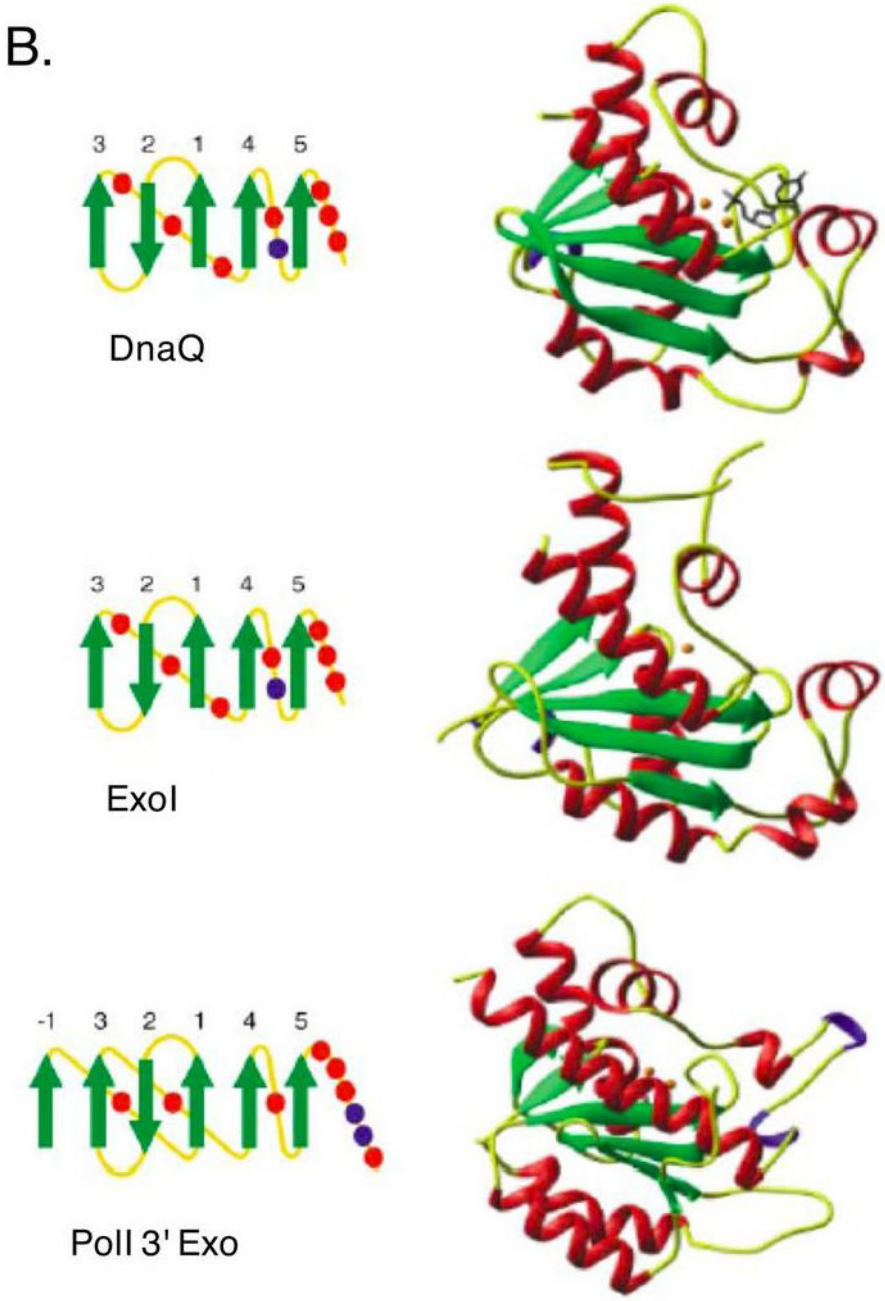


**Figure 1.**

A. *E. coli*'s DnaQ superfamily members. Shown are aligned amino acid sequences of exonuclease I, exonuclease X, oligoribonuclease, RNase D, RNase T, and the 3′ exonucleases of DNA polymerases I, II and III. Conserved acid residues are shown in bold and comprise metal coordination residues for those proteins with determined three-dimensional structure. Numbers refer to amino acid residues not shown. B. Structure of three members of the DnaQ/DEDD superfamily: DnaQ, ExoI and Polymerase I 3′ exonuclease domain. Figure republished with permission from Hamdan et al. 2002.

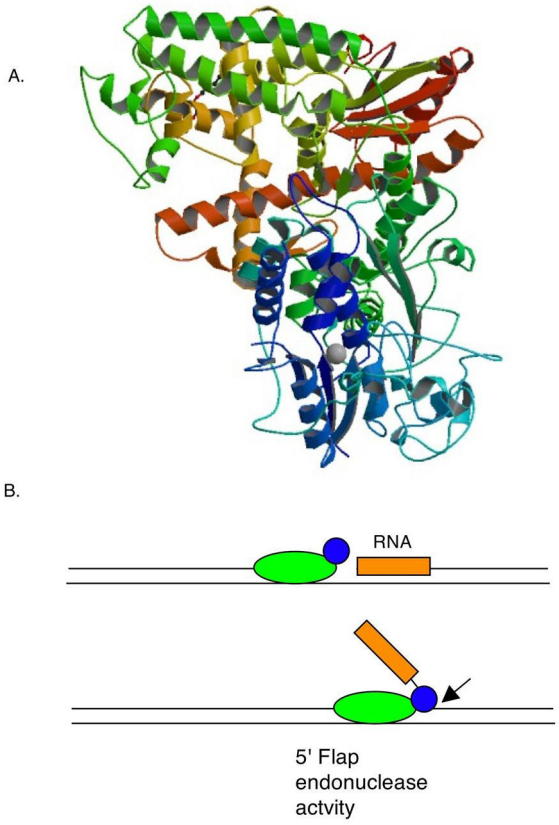


**Figure 2.**

A. Structure of Taq DNA polymerase I, showing 5′ exonuclease domain in blue and cyan. Image from RCSB PDB (www.pdb.org), PDB ID: 1TAQ (Kim, Y., S. H. Eom, J. Wang, D. S. Lee, S. W. Suh, and T. A. Steitz. 1995. Crystal structure of *Thermus aquaticus* DNA polymerase. Nature 376:612–616). B. Okazaki maturation by Polymerase I. Polymerization at a gap causes displacement of lagging strand RNA primer (orange), which is cleaved by 5′ flap endonuclease activity.

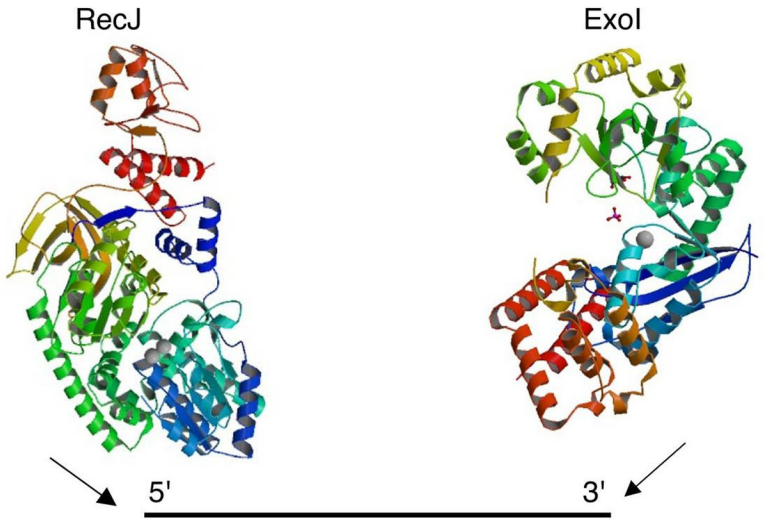


**Figure 3.**

Structures of single-strand DNA specific exonucleases RecJ exonuclease and Exonuclease I (Images from RCSB PDB (www.pdb.org) ID: 2ZXP Wakamatsu, T., Y. Kitamura, Y. Kotera, N. Nakagawa, S. Kuramitsu, and R. Masui. 2010. Structure of RecJ exonuclease defines its specificity for single-stranded DNA. J Biol Chem 285:9762–9769 and PDB ID: 1FXX Breyer, W. A., and B. W. Matthews. 2000. Structure of *Escherichia coli* exonuclease I suggests how processivity is achieved. Nat Struct Biol 7:1125–1128). RecJ digests ssDNA 5′ to 3′ and ExoI 3′ to 5′. Both produce mononucleotide products.

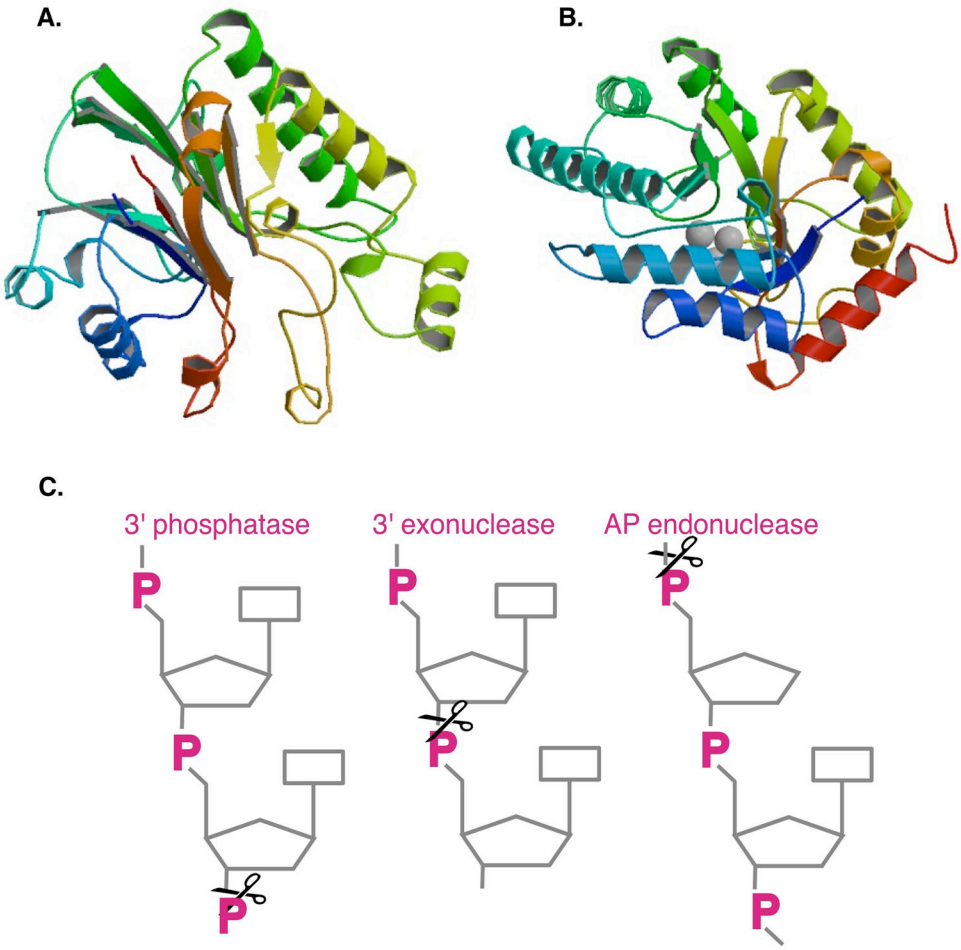


**Figure 4.**

A. Structure of Exonuclease III (Image from RCSB PDB (www.pdb.org) PDB ID: 1AKO Mol, C. D., C. F. Kuo, M. M. Thayer, R. P. Cunningham, and J. A. Tainer. 1995. Structure and function of the multifunctional DNA-repair enzyme exonuclease III. Nature 374:381–386). B. Structure of Endonuclease IV, showing 3 $Zn^{2+}$ ions (PDB ID: 1QTW Hosfield, D. J., Y. Guan, B. J. Haas, R. P. Cunningham, and J. A. Tainer. 1999. Structure of the DNA repair enzyme endonuclease IV and its DNA complex: double-nucleotide flipping at abasic sites and three-metal-ion catalysis. Cell 98:397–408). C. Activities of Exonuclease III and Endonuclease IV: 3′ phosphatase, 3′ to 5′ exonuclease, AP (abasic) endonuclease.

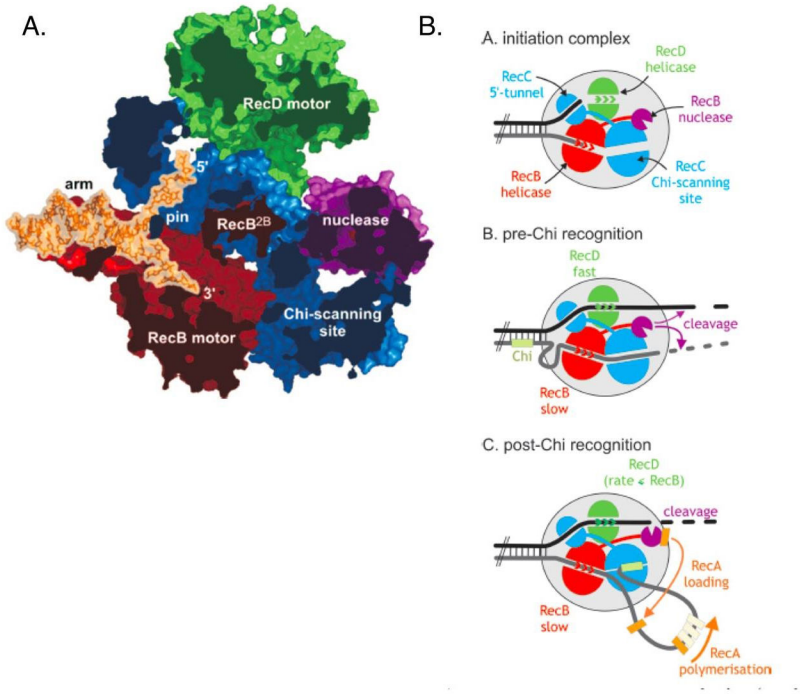


**Figure 5.**

A. Structure of RecBCD complex with DNA. B. Activities of the enzyme, before and after Chi recognition. Figure reproduced with permission from Dillingham and Kowalczykowski 2008.

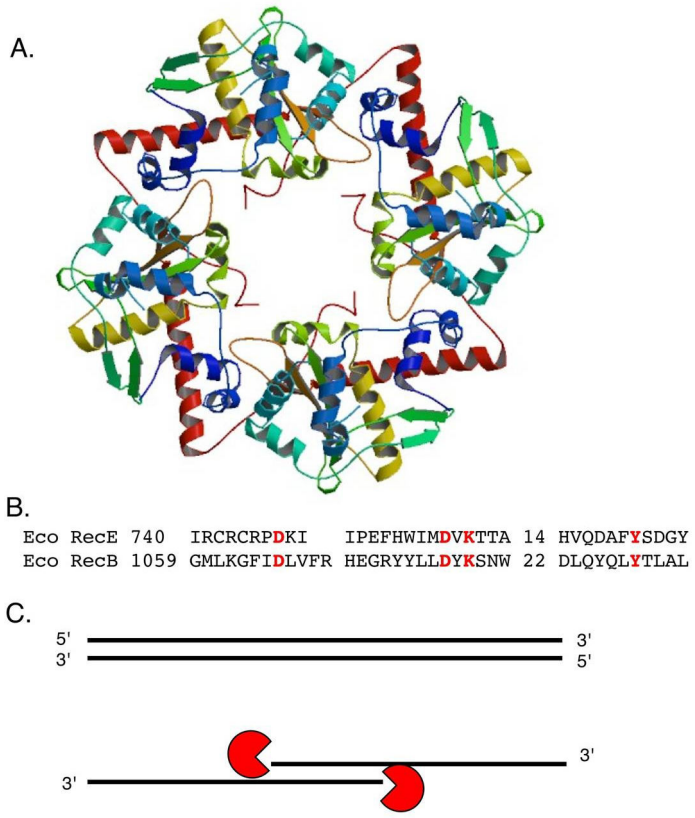


**Figure 6.**

A. Structure of RecE (Image from RCSB PDB (www.pdb.org) PDB ID: 3H4R Zhang, J., X. Xing, A. B. Herr, and C. E. Bell. 2009. Crystal structure of *E. coli* RecE protein reveals a toroidal tetramer for processing double-stranded DNA breaks. Structure 17:690–702). B. Alignment of active site regions of RecE and RecB nucleases. C. 5′ to 3′ exonuclease activity of RecE on dsDNA.

**Table 1**

DNA exonucleases of *E. coli*

| Enzyme | Gene(s) | Preferred substrate | Polarity | Other features | Biological role in DNA metabolism |
|---|---|---|---|---|---|
| ExoI | *xonA* (=*sbcB*) | ssDNA | 3′ -5′ | processive; stimulated by SSB | recombination, damage repair, mutation avoidance, MMR |
| ExoIII | *xthA (=xth)* | dsDNA | 3′ - 5′ | acts at nicks and ends, abasic endonuclease; 3′ phosphatase; can remove damaged 3′ residues | base excision repair, repair of oxidative and ionizing radiation damage |
| ExoIV | *orn*?[2] | Olignucleo- tides | 3′ -5′ | may be identical to RNA oligoribonuclease[2] | ? nucleotide recycling? |
| ExoVII | *xseA, xseB* | ssDNA | 3′ - 5′, 5′ - 3′’ | heterodimer, processive, oligonucleotide products, metal cofactor-independent | damage repair, mutation avoidance, MMR |
| ExoVIII | *recE* | dsDNA | 5′ - 3′ | processive, encoded by cryptic Rac prophage | recombination, damage repair |
| ExoIX | *ygdG (=xni)* | ? | ? | in 5′ nuclease/FEN-1 family[3] | ? |
| ExoX | *exoX* | ssDNA, dsDNA | 3′ - 5′ | distributive | mutation avoidance, MMR |
| DNA polI 5′ Exo (ExoVI) | *polA* | ssDNA | 5′ - 3′ | flap endonuclease | replication (Okazaki fragment maturation) |
| DNA Pol I 3′ Exo (ExoII) | *polA* | ssDNA | 3′ - 5′ | | proofreading |
| DNA Pol II 3′ Exo | *polB* | ssDNA | 3′ - 5′ | | proofreading |
| DNA Pol III 3′ Exo | *dnaQ* (=*mutD*) | ssDNA | 3′ - 5′ | subunit | proofreading |
| RecBCD | *recB, recC, recD* | dsDNA, ssDNA | 3′ - 5′, 5′ - 3′ | ExoV, highly processive, ATP-dependent, oligonucleotide products, helicase activity; Chi recognition | recombination, DSB repair, restriction of bacteriophage |
| RecJ | *recJ* | ssDNA | 5′ - 3′ | processive; stimulated by SSB | recombination, damage repair, mutation avoidance, MMR |
| RNase T (Exo T) | *rnt* | ssDNA | 3′ - 5′ | activity on DNA > RNA, homodimer, distributive | repair?[3] |
| SbcCD | *sbcC, sbcD* | dsDNA | 3′ - 5′ > 5′ - 3′ | ATP-dependent, processive | repair |
| Endonucleas e IV exonuclease activity | *nfo* | dsDNA | 3′ -5′ | $Zn^{2+}$ enzyme; can digest damaged 3′ residues; also possesses endonuclease 5′ to AP sites and other damaged residues | BER, NIR, repair of oxidative and ionizing radiation damage |
| TatD (Exonuclease XI) | *tatD* | dsDNA > ssDNA | 3′ - 5′ | $Mg^{2+}$ dependent DNase, unclear whether endonuclease or exonuclease activity | ?, *E. coli* YcfH and YjjV are paralogs; yeast Tat-D is orthologous, with 3′ to 5′ Exo/Endo activity. |

[1] Abbreviations: Pol, polymerase; Exo, exonuclease, dsDNA, double-strand DNA; ssDNA single-strand DNA; MMR, mismatch repair; DSB, double-strand break; BER, base excision repair; NIR, nucleotide incision repair

[2] Eukaryotic oligoribonuclease has activity on oligodeoxynucleotides making it likely that *E. coli* oligoribonuclease is identical to exonuclease IV.

[3] RNase T can act as a high-copy suppressor of some DNA repair defects of RecJ$^-$ ExoI$^-$ ExoVII$^-$ strains, has potent DNase activity and so could play a redundant function in DNA metabolism.