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A Blinkova, M F Burkart, T D Owens and J R Walker

Conservation of the *Escherichia coli dnaX* Programmed Ribosomal Frameshift Signal in *Salmonella typhimurium*

ALEXANDRA BLINKOVA, MARK F. BURKART, TRACY D. OWENS,† AND JAMES R. WALKER*

Microbiology Department, University of Texas at Austin, Austin, Texas 78712

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*Escherichia coli* DNA polymerase III subunits τ and γ are produced from one gene, *dnaX*, by a programmed ribosomal frameshift which generates the C terminal of γ within the τ reading frame. To help evaluate the role of the dispensable γ, the distribution of τ and γ homologs in several other species and the sequence of the *Salmonella typhimurium dnaX* were determined. All four enterobacteria tested produce τ and γ homologs. *S. typhimurium dnaX* is 83% identical to *E. coli dnaX*, but all four components of the frameshift signal are 100% conserved.

The *Escherichia coli dnaX* gene produces two DNA polymerase III (Pol III) subunits, τ and γ. The τ subunit, the full-length translational product of the *dnaX* reading frame, is the replisome organizer (40); it dimerizes the holoenzyme through contact with the core (13, 20, 26) and binds and stimulates both DnaB helicase and primase (1, 37). A mutant which produces wild-type DnaB helicase and primase (12, 40). The shorter C-terminal is generated by a programmed ribosomal frameshift which generates the C terminal of γ within the τ reading frame (3, 8, 34). Although the exact role of γ is unclear, it is generally considered to function in the γ complex (10, 18, 24, 27) to load processivity clamp β (14, 32) in an ATP-dependent reaction (27, 35). Two lines of evidence suggest that γ functions in clamp loading in vivo. First, the γ complex can be isolated from cellular extracts (18). Second, Xia et al. (38) assembled Pol III* (holoenzyme minus β [21, 36]) containing wild-type or ATP-binding site mutant τ or γ. The mutated τ or γ carried a single-residue change which eliminated the β-clamp loading activity of mutant τ complex or mutant γ complex. When the nine-subunit Pol III* was prepared with mutant γ and wild-type τ, the complex was inactive in clamp loading; when prepared with mutant τ and wild-type γ, it was active. This strongly suggests that the γ complex is the clamp loader and that τ does not normally participate in this process (38).

On the other hand, several observations indicate that γ might not be essential in normal replicative polymerization. First, β can be loaded in vitro by purified τ and δ or τ and δ’ (24, 25, 35) or by τ complexes (τ₂₋₅δδχδ) assembled in vitro (6, 26). Second, a mutant which produces wild-type τ but no detectable γ is viable and has no obvious phenotype (2). Third, Dallmann et al. (7) compared holoenzyme reconstituted with purified τ or γ with native holoenzyme and found that holoenzyme reconstituted with τ more closely resembled native enzyme than that reconstituted with γ. Specifically, (i) τ complex-dependent β loading required much less core than did γ complex-dependent loading; (ii) τ-containing holoenzyme resembled native holoenzyme in ability to use adenosine 5’-O-(3-thiotriphosphate) (ATP₈S), whereas the γ-containing holoenzyme was almost completely inhibited by ATP₈S; and (iii) holoenzyme could be reconstituted in the presence of a high salt concentration only if τ was present. Dallmann et al. (7) conclude that γ is not a necessary component of Pol III holoenzyme. Fourth, τ in crude extracts is extremely sensitive to proteolysis (16) by the OmpT protease, which cleaves between the two lysines at positions 429 to 430 to generate a γ-like fragment called γ₇ (28). It is possible therefore that earlier preparations of holoenzyme or subassemblies prepared from *ompT* strains and which were thought to contain γ actually contained some γ₇.

One approach to determining the significance of γ is to study its distribution among other genera. We report here that τ and γ homologs are conserved among all four species of *Enterobacteriaceae* tested and that the four-component, *E. coli* programmed ribosomal frameshift signal is 100% conserved in *Salmonella typhimurium*. This degree of conservation suggests that the production of the τ-γ pair serves some physiological function. τ and γ homologs in other genera. Extracts of five gram-negative species were probed by Western blots for proteins detectable by polyclonal antibody to *E. coli* τ and γ. Extracts of *Shigella flexneri*, *S. typhimurium*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, and *Vibrio cholerae* were electrophoresed, transferred to nitrocellulose, and probed with antibody which reacts with both τ and γ (Fig. 1). All the enterobacteria, *Shigella*, *Salmonella*, and *Enterobacter*, produced cross-reacting proteins similar in molecular mass to those of *E. coli*. The antibody to *E. coli* τ and γ did not detect *Aeromonas* or *Vibrio* proteins similar in mass to the enterobacterial τ and γ. However, it did cross-react with an intermediate-size protein of 58 kDa in extracts of both organisms; these proteins might be τ and γ homologs.

Two other organisms, *Caulobacter crescentus* and *Bacillus subtilis*, did not synthesize homologs detectable with anti-*E. coli* antibody (data not shown).

Sequence of the *S. typhimurium dnaX* gene and comparison to *E. coli dnaX*. The *S. typhimurium dnaX* region had been cloned from a *Sal*I partial digest of chromosomal DNA into vector pL1059 by Maurer et al. (19), generating λRM159. From this phage DNA, a 7-kb dnaX*–*SacI fragment was cloned into pCCL1920, generating pAB100. PstI fragments of 3.9 and 2.9 kb were subcloned from pAB100 into pCCL1920 (generating pAB101 and pAB102, respectively); neither provided wild-type complementing activity (Fig. 2).

Sequencing about 2.2 kb around the PstI site which forms the right and left boundaries of pAB101 and pAB102, respectively, revealed regions homologous to the *E. coli apt, dnaX,*...
All organisms were grown in yeast extract (1%)–tryptone (0.5%) plus 0.5% NaCl.

The deduced DnaX amino acid sequences of \(E. \) coli and \(S. \) typhimurium \(\tau\) and \(\gamma\) are 91% identical (Fig. 4), although the (deduced) \(S. \) typhimurium DnaX protein consists of 642 residues, compared to 643 in \(E. \) coli. The 91% identity is not uniform over the entire protein. For example, the first 206 residues are identical in all but one position, whereas positions 387 to 408 (\(E. \) coli) are identical in only 8 residues (36%). \(E. \) coli residue 403 is not present in \(S. \) typhimurium. Homology of the N-terminal region of \(\tau\)-\(\gamma\) to other clamp loading factors has been reported (23).

Functions of several regions might be deduced from known motifs. ATPase/helicase motifs I, Ia, and II (4, 11, 31) are indicated below the sequence in Fig. 4. The cysteine-rich region from residues 59 to 92 is identical in both organisms. \(C \ X_8 \ C \ X_2 \ C \ X_2 \ C\) might represent a novel form of zinc finger, although the spacing is not typical (29). The \(\tau\)-\(\gamma\) pairs of both organisms contain proline-rich regions, including 15 prolines within a 45-residue region (residues 361 to 405 in \(E. \) coli) or a 44-residue region in \(S. \) typhimurium (361 to 404). Twelve of the 15 prolines are conserved in both organisms. This region probably constitutes a domain which binds another protein(s), such as in \(P \ X_2 \ P\) sequences form helices which intercalate between aromatic residues on hydrophobic surfaces; other prolines appear to promote helix formation, and nonprolines in that region determine specificity (1, 17, 37, 39).

Conservation of the programmed ribosomal frameshift signal. In \(E. \) coli, the programmed ribosomal frameshift signal consists of four components: an –AAA AAG slippery sequence, a downstream stem-loop, use of the AAAG codon for which there is no \(E. \) coli lysine tRNA with the perfectly com-

![FIG. 1. Conservation of the \(\tau\)-\(\gamma\) pair among enterobacteria. Whole-cell extracts were electrophoresed, transferred to nitrocellulose, and probed with antibody to \(E. \) coli \(\tau\) and \(\gamma\) (2, 33). Lane A was loaded with 5 \(\mu\)g of extract of \(E. \) coli C500 containing the dnaX \(pAb47\); this strain overproduces both \(\tau\) and \(\gamma\). All other lanes were loaded with 200 \(\mu\)g. The lane labelled E. coli (\(\tau\)-only) contained extract of a strain mutated to eliminate the frameshift signal with the result that no detectable \(\gamma\) is produced (2). Other species and strains were \(S. \) flexneri SA101, \(S. \) typhimurium LT2, \(E. \) aerogenes AA62-1, \(A. \) hydrophila UT1, and \(P. \) cholerae O395. All organisms were grown in yeast extract (1%)–tryptone (0.5%) plus 0.5% NaCl.

![FIG. 2. The dnaX region of \(E. \) coli and \(S. \) typhimurium. (A) The \(E. \) coli dnaX region, adapted from the work of Chen et al. (5). The open bars represent reading frames; the filled areas are intergenic regions. (B) Inserts of \(S. \) typhimurium dnaX region DNA in pCL1920. The striped areas have not been sequenced. S, SacI; Sa, SalI; H, HincII; P, PstI; E, EcoRI. pAB101 and pAB102 were cloned from pAB100; pAB101 contains a short Pst-SacI polylinker segment from pAB100. The activity of each plasmid in complementing an \(E. \) coli dnaX(Ts) mutant is shown at the right: +, supports growth at 42°C; –, does not support growth at 42°C. Yeast extract-tryptone-NaCl medium was supplemented with 50 \(\mu\)g of spectinomycin per ml for growth of plasmid-containing strains, including complementation tests, and with maltose (0.2%) and MgSO

(5, 9). The intergenic region in \(S. \) typhimurium includes the sequence TCGCTG–17 nt–TAGCAT which is similar to the \(E. \) coli dnaX promoter TCGCCG–17 nt–TAGCAT. This promoter presumably drives transcription from the T at position 135, labelled +1 in Fig. 3, as in \(E. \) coli. The AGAG, located 7 nt from the initiation codon, is identical to the same region in \(E. \) coli and presumably serves as the Shine-Dalgarno sequence.

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complementary CUU anticodon, and the presence of an internal Shine-Dalgarno sequence upstream of the frameshift site (3, 8, 15, 33, 34). The *S. typhimurium dnaX* gene encodes the same four components, preserving the sequence and spacing. Over the 60-nt region, there are four differences between the two organisms, but none of the changes occurs in the specific components of the frameshift signal (Fig. 5).

What is the significance of \( \gamma \)? Does \( \gamma \) provide some useful function, or is it simply the result of an evolutionary mistake? Conservation of \( \tau \) and \( \gamma \) homologs among several species of

FIG. 3. Nucleotide sequence of the *S. typhimurium dnaX* region and deduced amino acid sequences of the C-terminal region of Apt, of DnaX, and of the N-terminal of Orf-12.

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**Notes:**

DNA sequencing (30) was done manually or with an ABI model 377 sequencer. Primers were obtained from Life Technologies, Inc.
Enterobacteriaceae and 100% identity of the frameshift signal components in both E. coli and S. typhimurium suggest that γ provides some useful function. Moreover, the frameshift signal consists of four components, alteration of any of which reduces the efficiency of shifting (3, 8, 15, 33, 34), and is so efficient that about half the ribosomes shift under normal growth conditions (16). Both Dallmann et al. (7) and Xiao et al. (38) have suggested that γ could be involved in other aspects of DNA polymerization or repair, such as unloading β clamps (22).

Nucleotide sequence accession number. The entire S. typhimurium dnaX sequence and portions of the apt and orf-12 sequences have been deposited with GenBank under accession no. U66040.

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REFERENCES


