Catalytic Residues of the Telomere Resolvase ResT

A PATTERN SIMILAR TO, BUT DISTINCT FROM, TYROSINE RECOMBINASES AND TYPE IB TOPOISOMERASES

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ResT is a member of the telomere resolvases, a newly discovered class of DNA breakage and reunion enzymes. These enzymes are involved in the formation of covalently closed hairpin DNA ends that are found in linear prokaryotic chromosomes and plasmids. The hairpins are generated by telomere resolution, where the replicated linear DNA ends are processed by DNA breakage followed by joining of DNA free ends to the complementary strand of the same molecule. Previous studies have shown that ResT catalyzes hairpin formation through a two-step transesterification similar to tyrosine recombinases and type IB topoisomerases. In the present study we have probed the reaction mechanism of ResT. The enzyme was found to efficiently utilize a substrate with a 5′-bridging phosphorothiolate at each cleavage site, similar to tyrosine recombinases/type IB topoisomerases. Using such a substrate to trap the covalent protein-DNA intermediate, coupled with affinity purification and mass spectroscopy, we report a new, non-radioactive approach to directly determine the position of the amino acid in the protein, which is linked to the DNA. We report that tyrosine 335 is the active site nucleophile in ResT, strengthening the link between ResT and tyrosine recombinases/type IB topoisomerases. However, a distinct pattern of catalytic residues with similarities, but distinct differences from the above enzymes was suggested. The differences include the apparent absence of a general acid catalyst, as well as the dispensability of the final histidine in the RKHRHY hexad. Finally, two signature motifs (GRR(2X)E(6X)F and LGH(4–6X)T(3X)Y) near the catalytic residues of aligned telomere resolvases are noted.

Telomere resolvases are a unique family of recently discovered DNA breakage and reunion enzymes (Refs. 1, 2, and see Refs. 3 and 4 for recent reviews). Telomere resolvases promote the formation of covalently closed hairpin ends or “telomeres” on DNA through a two-step transesterification (2, 5); they are required for the replication of linear DNA molecules with hairpin ends found in several phage and bacterial species (3, 4, 6–8).

Only four enzymes of this class have been identified by biochemical analysis: the phage encoded TelN of N15 (1), the bacterial protein ResT of Borrelia burgdorferi (2), Tel PY54 of the Yersinia enterocolitica phage PY54 (9), and TelK of the Klebsiella phage dKO2 (10). ResT, which is the subject of this study, is essential for B. burgdorferi (11). This bacterium is the causative agent of Lyme disease and has a segmented genome that includes a linear chromosome and 12 linear extrachromosomal elements, all terminated by covalently closed hairpin ends (12, 13). Replication of these linear molecules is believed to occur through bidirectional replication from a centrally located origin (14) followed by telomere resolution (15) mediated by ResT (2, 16), as shown in Fig. 1. Telomere resolution involves breakage of two phosphodiester bonds in a double-stranded DNA substrate (one in each strand) and joining of each end with the opposite DNA strand to form a covalently closed hairpin. The reaction is more complex than the breakage and reunion catalyzed by topoisomerases but simpler than the strand exchange promoted by site-specific recombinases.

The catalytic region of telomere resolvases, which have also been referred to as telomerases or protelomerases (17), has been shown to possess limited sequence homology to the family of tyrosine recombinases (2, 17). Telomere resolvases have also been shown to use a covalent protein-DNA intermediate (2, 5, 10, 18) demonstrating mechanistic similarity with tyrosine recombinases (19–23) and type IB topoisomerases (24, 25).

The catalytic mechanism of the tyrosine recombinases/type IB topoisomerases has been studied extensively through sequence alignments, genetic, biochemical, and structural approaches. These studies have revealed an active site tyrosine nucleophile, which becomes covalently linked to the DNA through a 3′-phosphotyrosyl linkage as an essential reaction intermediate in both enzyme families.

Activation of the nucleophilic attack is promoted by the side chains of five residues in tyrosine recombinases (22, 23) and four residues in type IB topoisomerases (24, 26). In the tyrosine recombinases this pentad is usually RKHR(H/W), whereas in the type IB topoisomerases it is RKR(H/N) with the third residue from the recombinases missing (see also Fig. 2). The role of the basic residues at positions 1, 3, and 4, and 1 and 3, respectively, is to provide a series of hydrogen bonds with the scissile phosphate and to stabilize the transition state intermediate. The lysine at the second position plays a role in leaving group expulsion during cleavage through protonation of the 5′-OH, apparently with the help of the first arginine in the topoisomerases (27, 28). The final position in each set of catalytic residues may have a variable role in different proteins.

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The line bisecting the head-to-head (telL-telR) and tail-to-tail (telR-telL) telomere junctions in the replication intermediate is an axis of 180° rotational symmetry. The telomere breakage and reunion reaction is referred to as telomere resolution (15). This figure has been adapted from Ref. 2. A similar mechanism of replication has been found in the phage N15 (41, 42).

Structural studies have shown hydrogen bonding of this residue to the scissile phosphate or 5'-OH, however, recent studies have revealed a purely structural role for this residue in the Fip enzyme (23).

The current study begins with a sequence alignment of known and predicted telomere resolvases to identify putative catalytic residues. The active site tyrosine nucleophile of the B. burgdorferi telomere resolvase, ResT, has been directly determined and a mutagenic and biochemical analysis of ResT was performed. Our results firmly establish a similarity of catalytic residues between telomere resolvases and tyrosine recombinases/tyr I topoisomerases, but also indicate distinct differences.

**EXPERIMENTAL PROCEDURES**

**Generation of Oligonucleotide ResT Substrates—**Wild-type replicated telomere substrates corresponding to the left end of Ip17 were constructed from oligonucleotides as noted previously (29) and as described below. The sequences of these oligonucleotides can be found in Table II of the Supplemental Materials. They are labeled ResT substrates A–E. Three variants were made: first, an asymmetric substrate of 63 bp, using oligonucleotides A and B; second, a symmetrical substrate of 56 bp, with a 5'-bridging phosphodiester (OPS)1 at the cleavage site of ResT, using oligonucleotides C and D; third, a 56-bp substrate containing an OPs at the cleavage site on one strand (C) and a 3'-biotinylated oligonucleotide (E) on the complementary strand. Each of these substrate variants was prepared by mixing equimolar amounts of the two desired oligonucleotides and annealing them by heating to 95 °C for 3 min, followed by slow cooling to room temperature over 1.5 h. In the 56-bp substrates, the shorter oligonucleotide was phosphorilated at its 5' end, whereas the longer oligonucleotide was not. The annealed 56-bp substrates were joined through their sticky ends near the axis of symmetry and ligated with T4 DNA ligase (New England Biolabs) for 2 h at 16 °C. All three substrates were purified by gel electrophoresis on a 4% MetaPhor (Cambrex)-agarose gel (70 V, 3 h, 4 °C). All subsequent steps were carried out at 4 °C. After electrophoresis, the gel was stained with ethidium bromide and the substrate band was excised from the gel. The gel slice was then washed with at least 20 min, then thawed. The substrate was recovered by placing the gel slice in a Costar Spin-X column (Corning Inc.) and centrifuging for 8 min at 8000 × g at 4 °C. The recovered substrate was precipitated with ethanol and resuspended in TE buffer (10 mM Tris (pH 8.0), 1 mM EDTA) to a final concentration of 10 pmol/μl, as determined by fluorescence staining (Ficogreen, Molecular Probes). The substrates were stored at −20 °C.

**Preparation of a ResT Phosphopeptide, Containing the Tyrosine Nucleophile—**Covalent protein-DNA intermediate (CPD) was prepared by incubating ResT with a 3'-biotinylated substrate that carried an OPS at the cleavage site. The reaction conditions were as described above, except that the reaction volume was scaled up to 600 μl. After incubation, 5 μg of porcine trypsin (sequencing grade, Princeton Separations Inc.) was added to the reaction, followed by incubation at room temperature overnight. Afterward, 150 μl of paramagnetic streptavidin beads (Miltenyi Biotech) were added to the reaction. After a 5-min incubation at room temperature, the reaction mixture was applied to a μMacs column (Miltenyi Biotech) in a strong magnetic field, as per the manufacturer’s instructions. The DNA was retained in the column, which then was washed sequentially with 250 μl of reaction buffer, 250 μl of sodium acetate (1 M, pH 7.0), 250 μl of sodium acetate (25 μM, pH 7.0), and 500 μl of water. The column was washed with 5% acrylamide (acrylamide-bisacrylamide = 40:1), 1 M Tris (pH 8.4), 0.1% (w/v) SDS, and 0.5% glycerol (w/v). The electrophoresis buffer contained 0.1 M Tris base, 0.1 M Tricine, and 0.1% (w/v) SDS. The gel was run at 75 mA for 4 h at room temperature. Vacuum-dried gels were analyzed using a Packard Cyclone (Packard Bioscience) and band intensities were quantified as noted above.

**Determination of the Specific Enzymatic Activity of ResT on a Plasmid Substrate—**The plasmid pYT1, which contains a 50-bp replicated telomere was used to measure the enzymatic activity of the purified ResT proteins. For this purpose the plasmid was linearized first with the restriction endonuclease PstI (New England Biolabs) and purified. Reaction mixtures containing 5% Tris Tricine polyacrylamide gels (15 × 15 × 0.1 cm). Thin gels contained 5% acrylamide (acrylamide-bisacrylamide = 40:1), 1 M Tris (pH 8.4), 0.1% (w/v) SDS, and 0.5% glycerol (w/v). The electrophoresis buffer contained 0.1 M Tris base, 0.1 M Tricine, and 0.1% (w/v) SDS. The gel was run at 75 mA for 4 h at room temperature. Vacuum-dried gels were analyzed using a Packard Cyclone (Packard Bioscience) and band intensities were quantified as noted above.

1 The abbreviations used are: OPS, 5'-bridging phosphodiester; CPD, covalent protein-DNA intermediate; MALDI-TOF, matrix-assisted laser desorption time-of-flight; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.
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RESULTS

Identification of Potential Catalytic Residues in ResT—To identify potential catalytic residues in ResT, a sequence alignment with other characterized and putative telomere resolvases was performed (Fig. 2). In addition to ResT, TelN from phage N15 and Tel from phage PY54 have been characterized previously (1–3, 5, 9), making them most valuable for comparison. The recently described enzyme from phage δKO2 (10) was not included in the alignment because of its very high degree of identity with N15 TelN. The putative telomere resolvases from Agrobacterium tumefaciens, Ectocarpus siliculosus, and Vibrio harveyi were recovered by BLAST searches against ResT. Because telomere resolvases are functionally related to tyrosine recombinases and type IB topoisomerases, consensus sequences for these two enzyme groups (31–34) were also added to the alignment. The initial alignment was performed using ClustalW (www.ebi.ac.uk/clustalw, see Ref. 35 for recent review), but manual correction was necessary to make all conserved regions and residues apparent.

The final alignment shows that the overall similarity between telomere resolvases, as well as telomere resolvases and tyrosine recombinases/type IB topoisomerases, is rather low. Only close to the potential catalytic residues can reasonable similarity be found. We observed a constellation of conserved residues in the telomere resolvases, a number of which correspond to known catalytic residues in either the tyrosine recombinases or type IB topoisomerases (marked white on a blue background in Fig. 2, with corresponding ResT positions above). The only exception is Tyr-293 in ResT, which corresponds to a catalytically active histidine in tyrosine recombinases but is not conserved among the telomere resolvases (see “Discussion”).

In addition to conserved residues that correspond to suspected catalytic functions, the group of telomere resolvases has several residues conserved among its members that are not found in the tyrosine recombinases or type IB topoisomerases. The N-terminal and C-terminal regions of the telomere resolvases have little or no similarity to each other and the alignment of these regions can be found under Supplemental Materials (see Fig. SF1).
Tyrosine 335 Is the Active Site Nucleophile in ResT—The putative tyrosine nucleophile at position 335 in ResT is conserved in all known and suspected telomere resolvases. Mutagenesis of this position in both *B. burgdorferi* ResT (2) and N15 TelN (5) resulted in inactive proteins, suggesting its identification as the tyrosine nucleophile. However, the weak alignment between the telomere resolvases and the tyrosine recombinases/type IB topoisomerases prompted us to directly identify the position in ResT that becomes covalently linked to the DNA substrate. To simplify this process compared with previous studies (38, 39) we developed a new approach based upon recent technological advances.

We first prepared a 56-bp suicide substrate that contained OPS at the cleavage site (Fig. 4A, step I). The substrate was also biotinylated to allow subsequent purification steps. Incubation with ResT resulted in the accumulation of the covalently linked protein-DNA intermediate or CPD (step II). The accumulated CPD was then digested with trypsin (step III) and the DNA-linked peptide separated from free peptides by virtue of its biotin tag (step IV). Subsequently, the DNA was digested to 5′ mono-nucleotides with nuclease P1, releasing the covalently linked ResT fragment as a phosphopeptide (step V). The eluted phosphopeptide was first subjected to MALDI-TOF mass spectroscopy to identify the phosphorylated tryptic fragment (step VI).

The results showed a phosphorylated tryptic peptide with a mass of 2063.8 Da (M + H), which corresponds to a non-phosphorylated tryptic peptide of 1983.8 Da (also M + H). The MALDI-TOF experiment was repeated 5 times and the signal at 2063.8 Da was detected every time and was the base peak in four of those experiments. The tryptic peptide carrying tyrosine 335 has an unphosphorylated theoretical mass of 1983.96 Da (M + H) and was the only possible match. This peptide is a 17-mer with the sequence VGHEPNDITTAFHYNR. The tyrosine at position 15 from the N terminus is the only tyrosine in the peptide. To identify the phosphorylated position and hence the point of attachment between the DNA and ResT, the phosphopeptide was sequenced by tandem mass spectroscopy (Fig. 4B). The sequencing confirmed the identity of the tryptic fragment (monoisotopic mass of 2062.92 Da (M only) and identified tyrosine 335 as the site of phosphorylation (see also Supplemental Materials Fig. SF2 for the original sequencing data).

Mutagenic Analysis of Potential Catalytic Residues in ResT—From the initial sequence alignment and the comparison with tyrosine recombinases and type IB topoisomerases, it was possible to identify five potential catalytic residues, in addition to Tyr-335, the tyrosine nucleophile. These residues were Arg-199, Lys-224, Tyr-293, Arg-296, and His-324 (see Fig. 2). With the exception of Tyr-293, the remaining residues were all conserved in the known and predicted telomere resolvases. The tyrosine at position 293 was also observed in the *Agrobacterium* enzyme, and replaces the histidine normally observed at this position in tyrosine recombinases (31, 32). This position in the other telomere resolvases was occupied by a lysine or a histidine. Also of interest were residues Arg-198, Arg-226, Arg-253, Tyr-299, and Thr-331, which are all conserved within the family of telomere resolvases, but not in tyrosine recombinases or topoisomerases IB. They were therefore included in this mutagenesis study.

To minimize changes in protein structure, the selected residues were changed to alanine (in the case of basic residues) or phenylalanine (for tyrosine residues). Purified proteins were tested for telomere resolution activity by monitoring reaction kinetics using the linearized plasmid substrate pYT1 (Fig. 5A).

The reaction rates per pmol of ResT were determined for each mutant protein using the linear region of the kinetic plots, and are shown in Fig. 5B.

The putative catalytic mutants R199A, K224A, and R296A did not display any detectable telomere resolution activity (see Fig. 5B). The lower limit of detection in our assay system was about 0.1 unit/pmol of ResT; these mutant proteins therefore displayed a greater than 200-fold reduction in activity when compared with wild-type ResT, as might be expected for catalytic mutants. The Y293F mutant did exhibit low but reproducible activity at about 2% wild-type levels, also consistent with a possible catalytic role (Fig. 5B). The one surprise from mutagenesis of the putative catalytic hexad was the H324A mutant. This protein displayed a telomere resolution activity at 6% of the wild-type level (Fig. 5B), suggesting that in contrast to tyrosine recombinases and type IB topoisomerases, this position is not required for catalysis in ResT.

As also shown in Fig. 5B, mutation of the conserved positions Arg-198, Arg-226, and Arg-253 resulted in a loss of telomere resolution activity, also making these positions possible candidates for catalytic residues. Mutation of the conserved Tyr-299 resulted in a protein that had a specific activity of about 5% of wild-type, and Thr-331 at about 28% of wild-type, adding Tyr-299 to the list of possible catalytic residues while eliminating Thr-331 for such a role.
To ensure that the loss of telomere resolution activity observed for the various mutant proteins was not the result of a global disruption of the protein structure, each of the purified proteins was tested for sequence independent DNA binding activity, the only other measurable activity of ResT. With the exception of R226A and R253A, the mutant ResT proteins exhibited near wild-type DNA binding activity (Fig. 6), indicating no global disruption of protein structure. Wild-type ResT protein, which had been inactivated by heating to 50 °C for 60 min served as control and showed almost no binding activity (Fig. 6). ResT mutant proteins R226A and R253A showed reduced binding activity, suggesting that these proteins might be structurally compromised or that they might be involved in DNA binding.

A General Acid Catalyst Could Not be Identified in ResT—A general acid catalyst that donates a proton to the leaving group during the cleavage reaction by vaccinia virus topoisomerase has been identified (Lys-167), along with a second residue (Arg-130) believed to be involved in a proton relay (27, 28). These residues correspond to Lys-224 and Arg-199, respectively, in ResT. To probe for the presence of a general acid catalyst as well as any other residues that might be involved in leaving group expulsion, we tested our defective ResT mutants for the ability to promote the first transesterification (the cleavage step) on a substrate containing a 5'-bridging phosphothiolate at the cleavage site. Rescue of cleavage by catalytically impaired mutants involved in leaving group expulsion has been reported using OPS substituted substrates, as the lower pKₐ of the 5'-sulfhydryl leaving group (27, 28) allows the reaction to occur in the absence of an enzyme-derived proton donor.

Fig. 4. Determination of the position of the covalently linked tyrosine in ResT. A, schematic of the experimental plan. A palindromic, 56-bp resolution substrate for ResT was generated (I). The sequence was as in wild-type, but a phosphorothiolate (shown in the circle) was incorporated into the backbone (37) at the position of ResT cleavage (red dash), between bases three and four from the axis of symmetry (dotted line). The substrate was also biotinylated at the 3'-end. Following incubation with wild-type ResT protein (see “Experimental Procedures”), the resulting protein-DNA intermediate was treated with trypsin (III) at room temperature overnight. The resulting reaction mixture contained the peptide with the active site tyrosine covalently attached to the DNA (III), together with other free tryptic peptides, trypsin, ResT, and buffer. To purify the phosphopeptide bound to the biotinylated substrate, the mixture was incubated with streptavidin-covered paramagnetic beads. The beads were retained in a μMacs column that was placed in a strong magnetic field, whereas the unwanted components of the reaction were washed away (IV). To recover the peptide, the DNA on the column was digested to 5' mononucleotides with nuclease P1. This allowed the elution of the phosphopeptide (V), which was analyzed by MALDI-TOF spectroscopy. A peak of the expected size (2063.8 Da) was detected (VI), as was a small amount of the nonphosphorylated peptide. B, sequencing of the phosphopeptide, containing the active tyrosine, by tandem mass spectroscopy (MS-MS). The purified phosphopeptide was analyzed, using a Q-TOF tandem mass spectrometer. The results of the sequencing are shown. In the first dimension a monoisotopic peak of the expected size (2063 Da) was detected. Its sequence is shown. The line marked b (no) shows the amino acid sequence number, as counted from the N terminus. The line b (Da) shows the mass of the detected N-terminal fragment. The line, labeled y' (no) shows the amino acid sequence number as counted from the C terminus, y' (Da) shows the mass of the detected C-terminal fragment in daltons. The phosphotyrosine at position 15 of the tryptic peptide is boxed.
level rescue is real or not would be to compare the mutants on an OPS substrate and a non-OPS suicide substrate that traps CPDs. However, we have been unable to generate non-OPS suicide substrates in the ResT system.

**DISCUSSION**

**Similarities between ResT and Tyrosine Recombinases/Type IB Topoisomerases**—Sequence alignment of known and putative telomere resolvases has revealed only low levels of sequence homology; however, a small number of highly conserved residues within the region believed to contain the catalytic residues was found to exist. Some of these conserved residues can be aligned with the catalytic pentad of tyrosine recombinases and the catalytic tetrad of type IB topoisomerases. Similar to the observed properties of both of these classes of enzymes, ResT was found to efficiently utilize a substrate containing a 5′-bridging phosphorothiolate and to accumulate a covalently linked protein-DNA intermediate with this substrate. We made use of this observation to develop a new approach for mapping the active site nucleophile through an affinity based enrichment coupled with mass spectroscopic analyses. This new approach is highly sensitive, facile, does not require radioisotopes, and should be generally applicable to the study of other systems involving a covalent protein-DNA intermediate.

In ResT, the active site nucleophile was mapped to tyrosine 335, which validates the weak sequence alignments with the tyrosine recombinases/type IB topoisomerases and previous mutagenesis of this residue in ResT (2) and the phage enzyme TelN (5). Based upon sequence alignment Arg-199, Lys-224, Tyr-293, Arg-296, and His-324 were expected to constitute residues also involved in catalysis. As expected, changes at these positions (with the exception of His-324, to be discussed below) resulted in a loss or dramatic reduction in telomere resolution activity, but not in DNA binding activity, suggesting a catalytic role and strengthening the link with tyrosine recombinases/type IB topoisomerases.

**Differences between ResT and Tyrosine Recombinases/Type IB Topoisomerases**—During the course of these studies several differences between the catalytic residues of ResT and that of tyrosine recombinases/type IB topoisomerases were noted. The first was that His-324, an expected catalytic residue that is

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**Fig. 5. Reaction rates of mutant ResT proteins.** A, measurement of the enzymatic activity of ResT. The telomere resolution assay was performed as described under “Experimental Procedures.” The inset shows a representative ethidium bromide-stained 1% agarose gel in reverse contrast, used to monitor the reaction. Each lane contained 72.2 fmol of linear plasmid substrate incubated with 0.85 pmol of ResT. The amount of reaction product was determined for each lane and plotted as shown. The steepest slope in the graph represents the maximal rate and was used to calculate the specific activity of ResT for comparisons between different mutants. B, reaction rates of mutant ResT proteins. The bar diagram shows the specific enzymatic activity of ResT wild-type protein, as well as ResT proteins carrying single point mutations. A linear plasmid substrate (pYT1 linearized with PstI) was used. The specific activity of the proteins was determined as described (see Fig. 5A and “Experimental Procedures”). One unit is defined as the amount of enzyme converting 1 fmol of substrate per minute. Each experiment was repeated at least three times, the error bar shows the S.D. The corresponding catalytic amino acid residues in tyrosine recombinases and type IB topoisomerases are shown above each of the mutant ResT proteins. The position of the general acid catalyst has been shaded light gray and the position of the catalytic tyrosine dark gray.

**Fig. 6. Sequence-independent DNA binding activity of mutant ResT proteins.** ResT binding assays were performed as described under “Experimental Procedures.” The amount of substrate bound at a protein/DNA ratio of 22:1 is shown. Heat-inactivated (50 °C, 45 min) ResT WT-protein served as control for the inactivated protein.
part of the LGH motif (see Fig. 2), was not required for enzyme activity. Substitution of His-324 with an alanine resulted in a protein that displayed an activity of about 70% of wild type. The corresponding position is catalytic in tyrosine recombinases/type IB topoisomerases, with the single exception of the Trp-330 in Flp, which has recently been shown to be important for a structural rather than a catalytic role (23). The corresponding residue of the N15 telomere resolvase TelN (His-415) has also been found to be non-essential (18), supporting the lack of a requirement for this position in telomere resolvases in general.

The second major difference we observed was our inability to identify a general acid catalyst in ResT by rescue of catalytic mutants with an OPS substrate, where the lower $pK_a$ of the 5′-sulphydryl leaving group allows the reaction to occur in the absence of an enzyme-derived proton donor (27, 28). With vaccinia topoisomerase two active site mutants (equivalent to Arg-199 and Lys-224 in ResT) were rescued using this approach, defining residues involved in leaving group expulsion through a proton relay. In tyrosine recombinases the equivalent position to Lys-224 in ResT is also believed to function as a general acid catalyst (22, 40). Our inability to find a general acid catalyst is most likely explained by either a true absence of such a residue in the ResT active site or an as yet untested residue that performs this role. An alternative possibility is that either Arg-198 or Lys-199 performs this function, but has a dual essential role in catalysis; in this case rescue of the mutant ResT with an OPS substrate would not be successful.

A third difference in ResT compared with the tyrosine recombinases/type IB topoisomerases is the possibility of additional catalytic residues. Two positions conserved in other telomere resolvases (Arg-198 and Tyr-299), but not part of the catalytic residues in tyrosine recombinase/type IB topoisomerases, resulted in loss or pronounced decreases in activity when substituted, but did not loose their DNA binding activity. Therefore, R198A and Y299F are candidates for catalytic residues in ResT with no direct equivalents in tyrosine recombinases/type IB topoisomerases. An important feature of ResT that may necessitate differences in catalytic residues from the above enzymes is the presence of a composite active site made up of the catalytic residues investigated in this paper, and a hairpin binding module similar to that found in cut-and-paste transposases (29). Structural studies on telomere resolvases will be required to resolve the issues on catalytic similarities between telomere resolvases and tyrosine recombinases/type IB topoisomerases.

Finally, a small number of highly conserved residues within the catalytic region was found and defines two tentative signature motifs unique to telomere resolvases: GR$^{R2X}$E/6X$^{F}$ and LGH(−4)X$^{T}$X$^{Y}$, where the bold and underlined residues are positions 199 and 335 in ResT, corresponding to the first and last residues in the catalytic hexad of tyrosine recombinases. These motifs may be useful for the identification of additional telomere resolvases.

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