Noncanonical prokaryotic X family DNA polymerases

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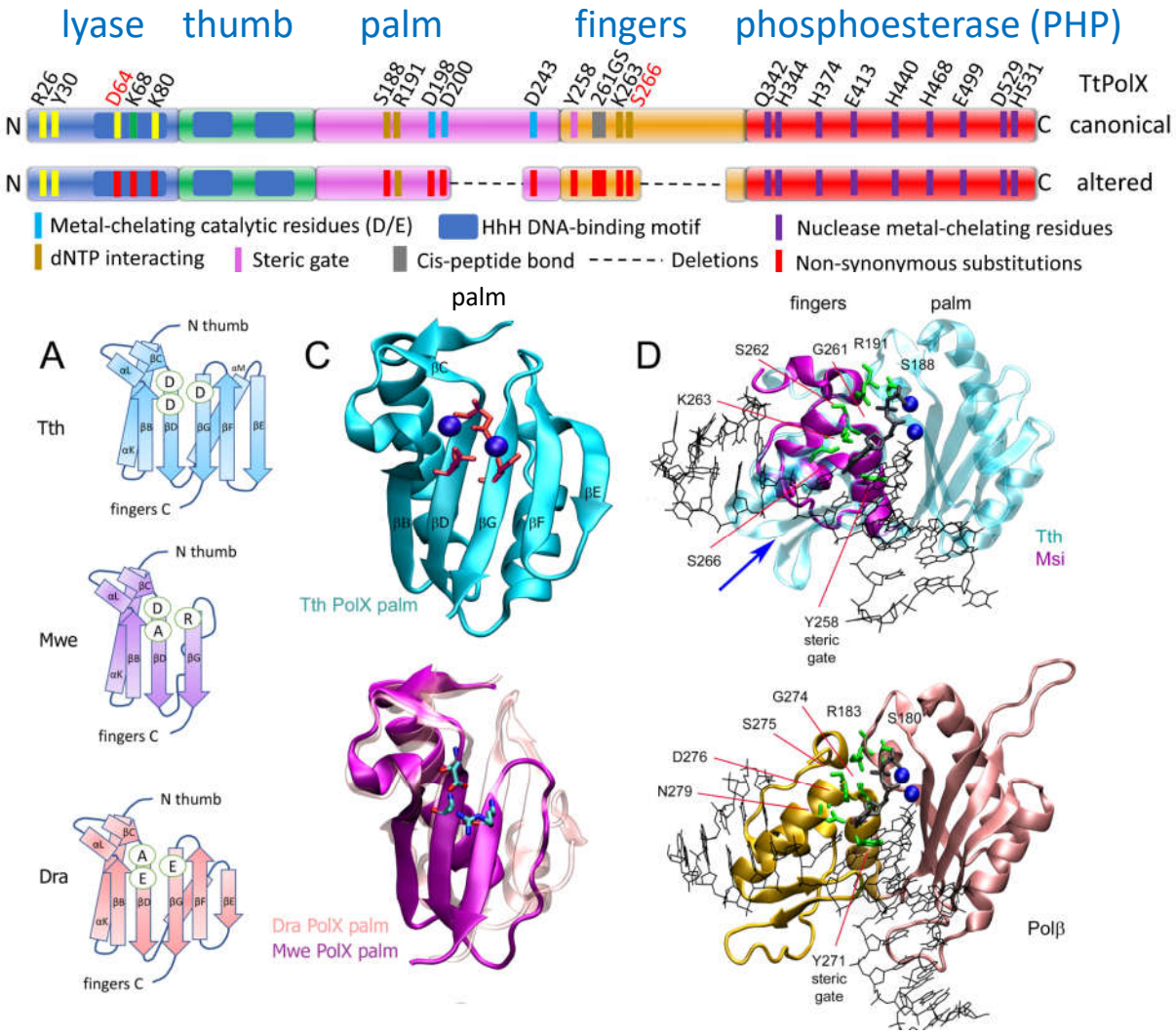
In the non-redundant collection of prokaryotic proteins we found noncanonical X-family polymerases, which differ from the canonical ones by deletions and mutations in palm and fingers domains, but not in phosphoesterase (PHP) domain.

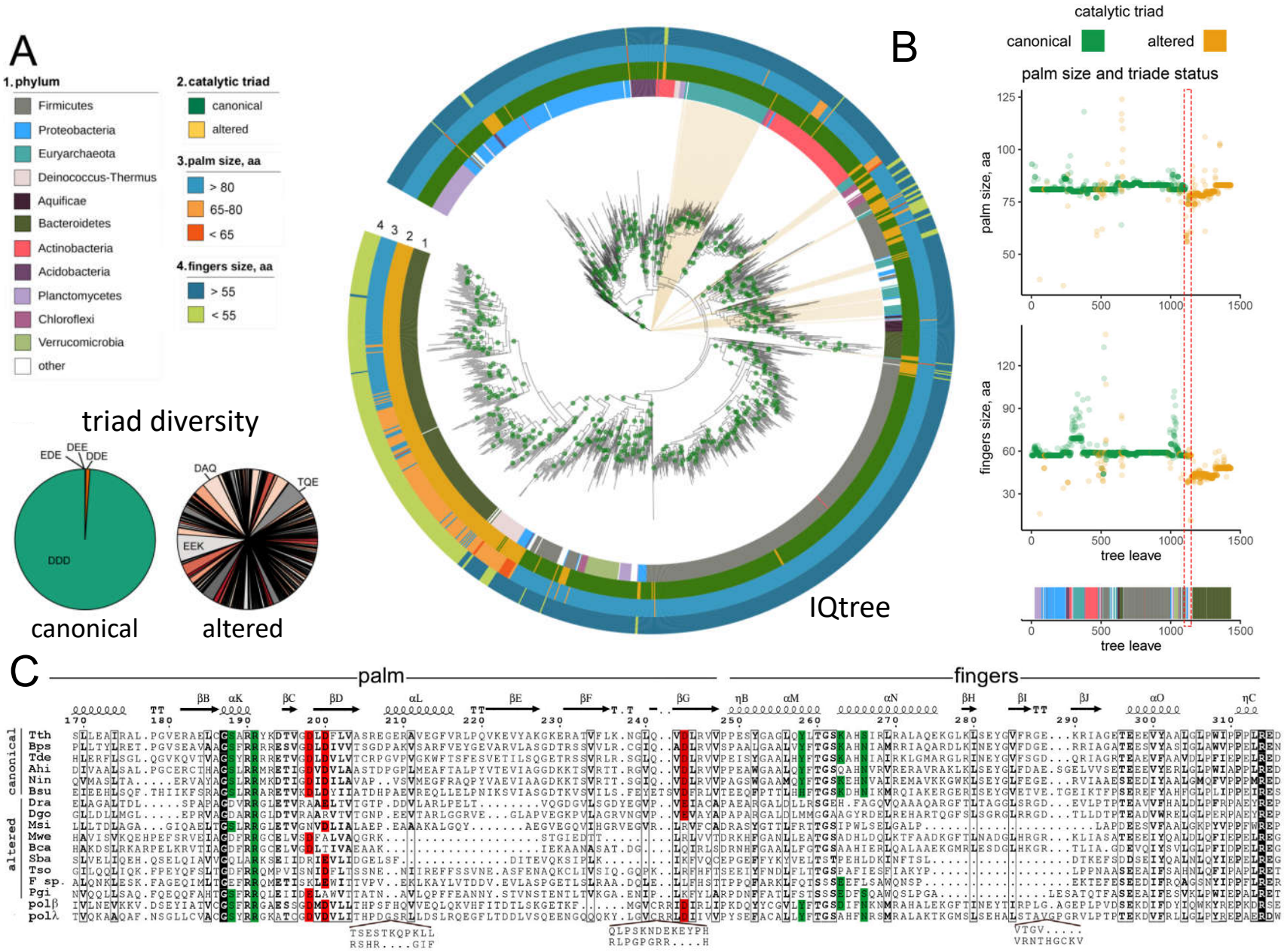
Most remarkable mutations are alterations of aspartate triade of polymerase active site located in palm domain. These mutations should eliminate polymerase activity of these proteins. We called such polymerases “altered”.

For example, palm domains of *Mesorhizobium wenxiniae* (Mwe, AlphaFold model) *and Deinococcus radiodurans* (Dra, PDB: 2W9M) PolXs contain mutations of catalytic aspartate triade to DAR and AEE respectively (C) and deletion of β-strand E (Dra) or even two β-strands E and F (Mwe) in contrast to palm domain of canonical *Thermus thermophilus* (Tth, PDB: 3AU0) PolX. While fingers domain of *Meiothermus silvanus* (Msi, AlphaFold model) PolX contain deletion of entire β-sheet (D, blue arrow).



Most of altered polymerases are united in one branch on phylogenetic tree (bootstrap value 98) and belong to *Bacteroidetes*, *Deinococcus-Thermus* or *Proteobacteria* phyla (A).

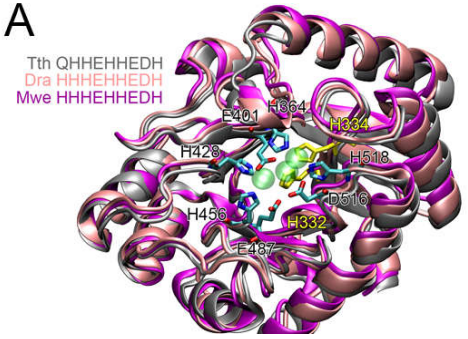
In this branch alterations in catalytic triade of palm domain often accompanied with shorter palm domain and/or shorter fingers domain (B). Shortening of domains revealed to be due deletions in them (C, catalytic triad is shown in red, NTP binding motif is shown in green).



Phosphoesterase (or PHP domain) of prokaryotic polXs, in contrast to palm and fingers domains, is very conservative and retain all residues of active site almost invariant (A).

Purified recombinant altered polymerases of *Deinococcus radiodurans* (Dra) and *Deinococcus gobiensis* (Dgo), as it was predicted, did not possess polymerase activity (C, E, F), but retain Mn^{2+} -dependent 3'-5' exonuclease activity (C, E, F), which was proved to be located in PHP domain (F). While recombinant canonical polymerase from *Bacillus subtilis* had both polymerase and 3'-5' exonuclease activities (G).

A



PHP domain has conservative structure

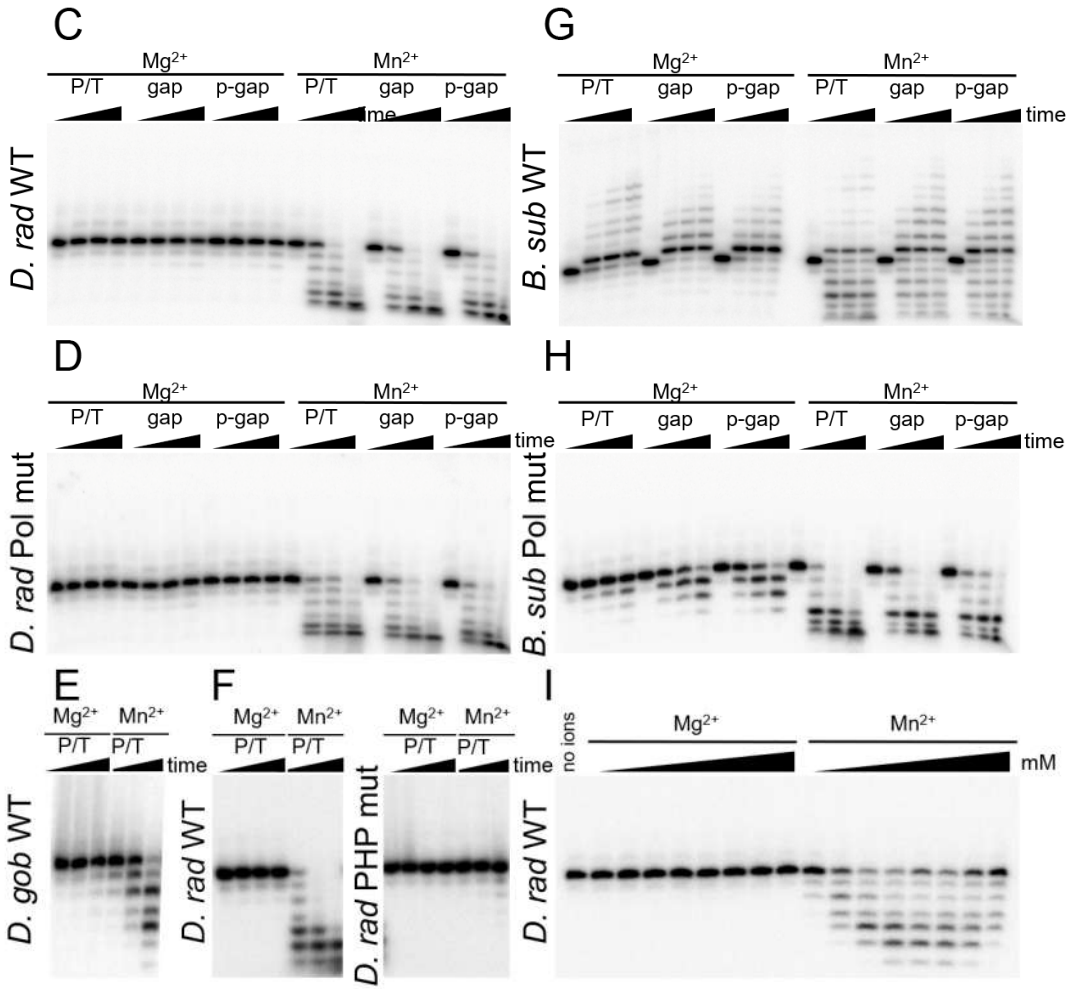
B

P/T
```
5′-P32-GTCACAGAGATACTAC
      CAGTGTCTCTATGATGTACACGCTGACAAGTC - 5′
```

gap
```
5′-P32-GTCACAGAGATACTAC TGTGCGACTGTTCAG - 3′
      CAGTGTCTCTATGATGTACACGCTGACAAGTC - 5′
```

p-gap
```
                        P
5′-P32-GTCACAGAGATACTAC TGTGCGACTGTTCAG - 3′
      CAGTGTCTCTATGATGTACACGCTGACAAGTC - 5′
```

Primer extension experiments



Based on the observation, that DNA repair and recombination proteins are often adjoin PolX gene in genomes, we assume, that exonuclease activity of PolX can be utilized by the cell in DNA reparation and recombination events (C).

We noticed that in genome of *Alphaproteobacteria* PolX is encoded in proximity to NHEJ (non-homologues end joining) protein ligase D (C).

We investigated non-redundant collection of complete prokaryotic genomes and showed, that PolX indeed significantly (χ^2-test) co-occurred with NHEJ proteins – Ku and LigD (with or without nuclease domain) in different phyla (A, B) independently from genome size (D).

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