During replication, Okazaki fragment maturation is a fundamental process that joins discontinuously synthesized DNA fragments into a contiguous lagging strand. Efficient maturation prevents repeat sequence expansions, small duplications and generation of double-stranded DNA breaks. To address the components required for the process, Okazaki fragment maturation was reconstituted in vitro using purified proteins from Thermococcus species 9°N. The similarities to both bacterial and eukaryotic systems and evolutionary implications of archaean Okazaki fragment maturation are discussed.

DNA replication is a conserved process throughout all domains of life. Due to the antiparallel nature of double-stranded DNA and the unidirectionality of DNA polymerases, the leading strand replicates continuously while the lagging strand is synthesized discontinuously from a series of Okazaki fragments. The Okazaki fragment on the lagging strand sits as a short RNA primer synthesized by the primase. Then the processivity factor (the sliding clamp) assembles around the primer and binds DNA polymerase. The DNA polymerase-sliding clamp extends the RNA primer to synthesize the complementary strand. To form an uninterrupted lagging strand, RNA primers are removed, the gap in the DNA is filled and the Okazaki fragments are joined together. Each domain of life accomplishes this objective using different mechanisms and components. In bacteria, the lagging strand is synthesized by DNA polymerase III holoenzyme (pol III) while DNA polymerase I (pol I) is the major polymerase that carries out Okazaki fragment maturation. Pol I uses its polymerase activity to extend nicks or gaps left by pol III and its 5'-3' exonuclease activity to degrade the downstream RNA primer. The nick is sealed by DNA ligase. In eukaryotes, the same requirements are fulfilled using a different repertoire of enzymes. The lagging strand polymerase, pol δ, synthesizes the lagging strand and displaces the RNA primers into a flap structure. Flap endonuclease (Fen1) removes the flap and the nick is sealed by DNA ligase to generate a continuous double-stranded DNA.

Lagging strand synthesis and Okazaki fragment maturation are not as well understood in eukarya and archaea. The majority of characterized archaean species (excluding the known Crenarchaeae) encode both members of Family B DNA polymerase (polB) as well as the archaean specific Family D DNA polymerase (polD). Several lines of evidence suggest that polD is the main replicative polymerase for both the leading and lagging strand synthesis. In some species, polD is the only essential DNA polymerase for cell viability while in others, both polD and polB are required. PolD forms complexes with several key replisome proteins in vivo while polB does not. In addition, the ability of polD to efficiently extend an RNA primer fulfills a requirement for both a leading and lagging strand DNA polymerase. Thus, polD was suggested to replicate at least the lagging strand and likely the leading strand as well.

In this study, the roles of polD and polB during Okazaki fragment maturation were evaluated using in vitro assays with proteins purified from Thermococcus species 9°N or cell extracts. The data suggest that Okazaki fragment maturation is a hybrid of the bacterial and eukaryal systems.

**RESULTS**

**ABSTRACT**

During replication, Okazaki fragment maturation is a fundamental process that joins discontinuously synthesized DNA fragments into a contiguous lagging strand. Efficient maturation prevents repeat sequence expansions, small duplications and generation of double-stranded DNA breaks. To address the components required for the process, Okazaki fragment maturation was reconstituted in vitro using purified proteins from Thermococcus species 9°N. The similarities to both bacterial and eukaryotic systems and evolutionary implications of archaean Okazaki fragment maturation are discussed.

**INTRODUCTION**

**CONCLUSIONS**

- PolD is essential for viability in Thermococcus and may synthesize both the leading and lagging strands.
- PolD synthesis stops before downstream Okazaki fragments and requires polB for strand displacement and subsequent processing.
- In Thermococcus, efficient Okazaki fragment processing requires polB, flap endonuclease and DNA ligase.
- Okazaki fragment maturation in Thermococcus shares similarities to both bacterial and eukaryal systems.