A Self-Replicating Linear DNA

- Pei Sheng Liew
  ,
- Tze Hao Tan
  ,
- Yin Cheng Wong
  ,
- Edmund Ui Hang Sim
  ,
- Choon Weng Lee
  , and
- Kumaran Narayanan*****

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SUBJECTS:

- Rodent models,
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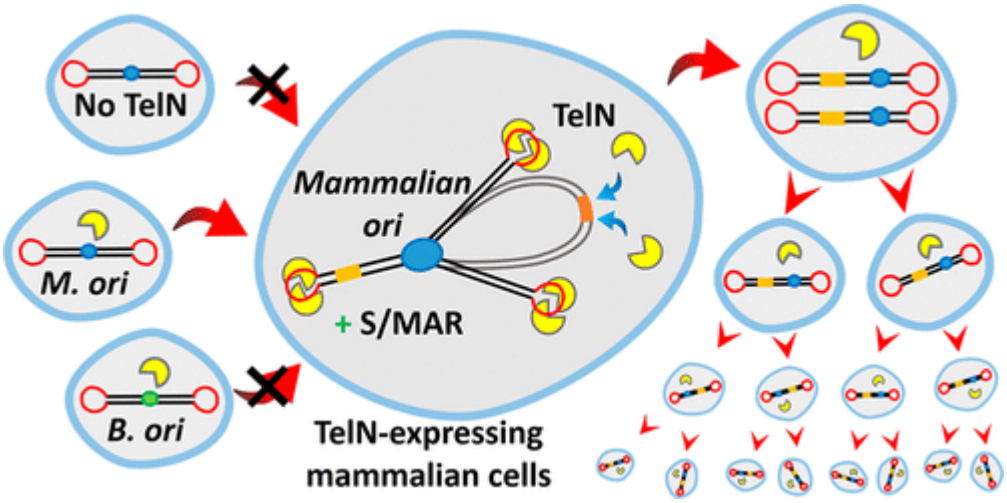
Abstract



TelN and *tos* are a unique DNA linearization unit isolated from bacteriophage N15. While being transferable, the TelN cleaving-rejoining activities remained stable to function on *tos* in both bacterial and mammalian environments. However, TelN contribution in linear plasmid replication in mammalian cells remains unknown. Herein, we investigated the association of TelN in linear *tos*-containing DNA (*tos*-DNA) replication in mammalian cells. Additionally, the mammalian origin of replication (*ori*)

that is well-known to initiate the replication event of plasmid vectors was also studied. In doing so, we identified that both TelN and mammalian initiation sites were essential for the replication of linear *tos*-DNA, determined by using methylation sensitive DpnI/MboI digestion and polymerase chain reaction (PCR) amplification approaches. Furthermore, we engineered the linear *tos*-DNA to be able to retain in mammalian cells using S/MAR technology. The resulting S/MAR containing *tos*-DNA was robust for at least 15 days, with (1) continuous *tos*-DNA replication, (2) correct splicing of gene transcripts, and (3) stable exogenous gene expression that was statistically comparable to the endogenous gene expression level. Understanding the activities of TelN and *tos* in mammalian cells can potentially provide insights for adapting this simple DNA linearization unit in developing novel genetic engineering tools, especially to the eukaryotic telomere/telomerase study.

KEYWORDS:

- N15 TelN protelomerase
- linear DNA replication
- mammalian initiation sites
- human S/MAR