DNA: from Damage to Mutation *via* Translesion Synthesis

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Abstract:
Ribonucleotides in DNA can promote genomic instability by causing mutations/strand breaks and are also linked to several diseases. The current studies investigate the impact of the DNA containing ribonucleotide rATP and its damaged analog 1,N^6-ethenoadenosine (1,N^6-εrA) on hpol η-mediated translesion synthesis (TLS) and RNase H2-mediated repair. The rATP and 1,N^6-εrA-bearing modified DNAs were synthesized employing solid phase synthesis. Bypass studies showed that the replication across from rATP was error-prone. TLS studies of 1,N^6-εrA indicated that the hpol η makes predominant deoxypurine insertion (*i.e.,* dATP and dGTP). Mass spectral analysis showed that 1,N^6-εrA in DNA generates extensive frameshifts, probably utilizing an adduct "skipping" mechanism during TLS, which can lead to genomic instability. The repair studies showed that RNase H2 recognizes 1,N^6-εrA but exhibits partial incision activity; this can lead to the persistence of 1,N^6-εrA adduct in DNA. The hpol η also acts as reverse transcriptase in the presence of damaged ribonucleotide 1,N^6-εrA but has poor RNA primer extension activities. Steady-state kinetic analysis of reverse transcription and RNA primer extension showed that hpol η favors the addition of dATP and dGTP opposite 1,N^6-εrA. These findings provided detailed insights on the mutagenic potential of 1,N^6-εrA adduct during replication, especially in humans.

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