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Optimization of the expression, purification and polymerase activity reaction conditions of recombinant human PrimPol

Boldinova E., Stojkovič G., Khairullin R., Wanrooij S., Makarova A.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

 Human PrimPol is a DNA primase/polymerase involved in DNA damage tolerance and prevents nuclear genome instability. PrimPol is also localized to the mitochondria, but its precise function in mitochondrial DNA maintenance has remained elusive. PrimPol works both as a translesion (TLS) polymerase and as the primase that restarts DNA replication after a lesion. However, the observed biochemical activities of PrimPol vary considerably between studies as a result of different reaction conditions used. To reveal the effects of reaction composition on PrimPol DNA polymerase activity, we tested the polymerase activity in the presence of various buffer agents, salt concentrations, pH values and metal cofactors. Additionally, the enzyme stability was analyzed under various conditions. We demonstrate that the reaction buffer with pH 6–6.5, low salt concentrations and 3 mM Mg 2+ or 0.3–3 mM Mn 2+ cofactor ions supports the highest DNA polymerase activity of human PrimPol in vitro. The DNA polymerase activity of PrimPol was found to be stable after multiple freeze-thaw cycles and prolonged protein incubation on ice. However, rapid heat-inactivation of the enzyme was observed at 37ºC. We also for the first time describe the purification of human PrimPol from a human cell line and compare the benefits of this approach to the expression in Escherichia coli and in Saccharomyces cerevisiae cells. Our results show that active PrimPol can be purified from E. coli and human suspension cell line in high quantities and that the activity of the purified enzyme is similar in both expression systems. Conversely, the yield of full-length protein expressed in S. cerevisiae was considerably lower and this system is therefore not recommended for expression of full-length recombinant human PrimPol.

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