遗传 2005 27 (2): 279-283 ISSN: 0379-4172 CN: 11-5450/R

技术与方法

DNA聚合酶高保真机理的新发现及其在SNP分析中的应用

陈琳玲1, 2, 张 佳, 彭翠英2, 廖端芳2, 李弘剑3, 高汉林4, 李 凯1~4

1.津脉公司，天津300192；2.南华大学SNP研究所，衡阳421001；3.暨南大学生命科学与技术学院，广州510632；4.希望城国家医学中心，加利福尼亚，美国

收稿日期 2004-2-9 修回日期 2004-11-17 网络版发布日期 接受日期

摘要 高保真DNA聚合酶在遗传与进化等生命活动中具有十分重要的生理与病理意义。高保真聚合酶除具有广为人知的校正功能外，最近的实验进一步表明，由不能及时校正或难于纠正的错配碱基引发的“关”闭DNA聚合反应的效应，同样保证了DNA聚合反应终产物的纯度。高保真聚合酶这一“关”闭DNA聚合反应的能力，促成了其与耐外切酶消化的3′末端碱基特异性引物共同构成一个SNP敏感性纳米级复合分子“开/关”，高保真聚合酶分子中相距三纳米的聚合中心和3′→5′外切酶酶解中心则既合作又独立地起到了复合分子开关中“开”和“关”的效能:对于配对的引物，则直接在该酶的聚合中心进行聚合反应，即“开”的效应；而对于3′末端错配的引物，则从该酶的聚合中心转移至3′→5′外切酶的酶解中心，由于引物修饰了的3′末端耐外切酶的特点，继而出现了一种长时间无酶解产物的酶解过程，最后因酶的聚合中心空转而“关”闭DNA聚合反应，即“关”的效应。这一新的复合分子“开/关”在很大程度上满足了后基因时代对SNP分析的要求。该SNP分子开关的应用，使基因诊断提高到单碱基水平。同时，利用该方法通过SNP对基因组扫描，在单基因遗传病病因研究及法医学鉴定上具有很强的理论和实用价值。

关键词 DNA聚合酶 校正 分子开关 单碱基多态性

分类号

Novel Mechanism of 3′ Exonuclease of Polymerase in Maintenance of DNA Replication Fidelity and Its Application in SNP Assay

CHEN Lin-Ling1,2,ZHANG Jia1,PENG Cui-Ying2,LIAO Duan-Fang2,LI Hong-Jian3,GAO Han-Ling4,LI Kai1~4

1.Genomapping Inc, Tianjin 300192,China;2. Department of SNP, Nanhua University, Hengyang 421001, China;3. The Institute of Life Science and Biotechnology, Jinan University, Guangzhou 510632,China; 4.City of Hope National Medical Center，CA，USA

Abstract

Polymerase with 3′ to 5′exonulcease plays an important role in the maintenance of in vivo DNA replication fidelity. In order to develop more reliable SNP assays, we revisit the underlying molecular mechanisms by which DNA polymerases with 3′ exonucleases maintain high fidelity of DNA replication. In addition to mismatch removal by proofreading, we recently discovered a premature termination of polymerization by a new mechanism of OFF-switch. This novel ON/OFF switch turns off DNA polymerization from mismatched primers and turns on DNA polymerization from matched primers. Two SNP assays were developed based on the proofreading and the newly identified OFF-switch respectively: terminal labeled primer extension and the ON/OFF switch operated SNP assay. These two new methods are well adapted to conventional techniques such as electrophoresis, real time PCR, microplates, and microarray. Application of these reliable SNP assays will greatly facilitate genetic and biomedical studies in the post-genome era.

Key words polymerase proofreading ON/OFF switch SNP

DOI:

通讯作者 李 凯 kaili34@yahoo.com