生命学院朱斌教授团队研发新型RNA合成工具酶

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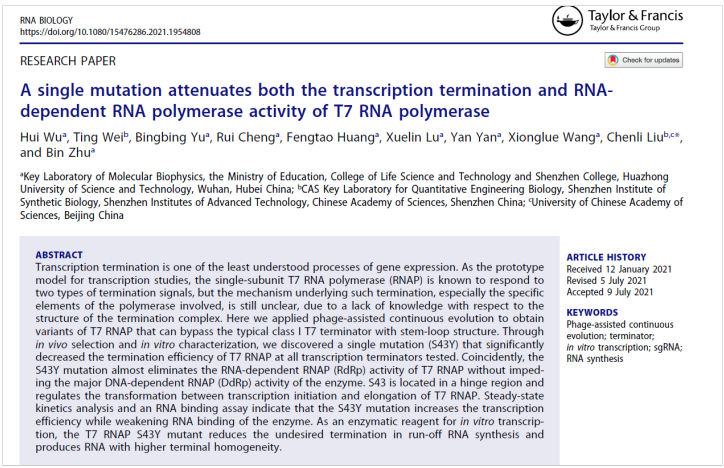
新闻网讯（通讯员 余兵兵）7月27日，生命学院朱斌教授团队与中科院深圳先进院刘陈立研究员团队合作，在国际RNA领域著名期刊《核糖核酸生物学》(RNABiology)发表题为"A single mutation attenuates both the transcription termination and RNA-dependent RNA polymerase activityof T7 RNA polymerase"的研究论文。



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RESEARCH PAPER

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A single mutation attenuates both the transcription termination and RNA-dependent RNA polymerase activity of T7 RNA polymerase

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ABSTRACT

Transcription termination is one of the least understood processes of gene expression. As the prototype model for transcription studies, the single-subunit T7 RNA polymerase (RNAP) is known to respond to two types of termination signals, but the mechanism underlying such termination, especially the specific elements of the polymerase involved, is still unclear, due to a lack of knowledge with respect to the structure of the termination complex. Here we applied phage-assisted continuous evolution to obtain variants of T7 RNAP that can bypass the typical class I T7 terminator with stem-loop structure. Through *in vivo* selection and *in vitro* characterization, we discovered a single mutation (S43Y) that significantly decreased the termination efficiency of T7 RNAP at all transcription terminators tested. Coincidently, the S43Y mutation almost eliminates the RNA-dependent RNAP (RdRp) activity of T7 RNAP without impeding the major DNA-dependent RNAP (DdRp) activity of the enzyme. S43 is located in a hinge region and regulates the transformation between transcription initiation and elongation of T7 RNAP. Steady-state kinetics analysis and an RNA binding assay indicate that the S43Y mutation increases the transcription efficiency while weakening RNA binding of the enzyme. As an enzymatic reagent for *in vitro* transcription, the T7 RNAP S43Y mutant reduces the undesired termination in run-off RNA synthesis and produces RNA with higher terminal homogeneity.

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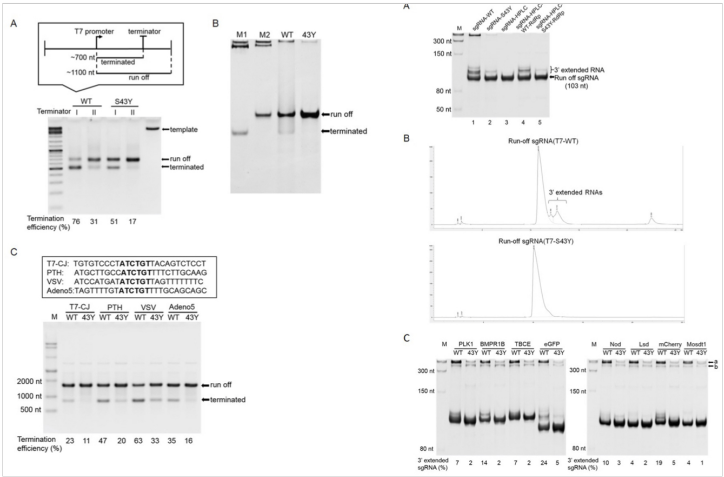
Phage-assisted continuous evolution; terminator; *in vitro* transcription; sgRNA; RNA synthesis

该研究工作充分发挥刘陈立团队蛋白质定向进化方法学优势与朱斌课题组长期以来在单亚基RNA聚合酶方向的研究特色，利用噬菌体辅助的连续定向进化手段筛选RNA领域的核心工具酶—T7RNA聚合酶能够克服转录终止信号的突变体，最终获得了一个单氨基酸突变体S43Y，相比野生酶对两类典型转录终止信号显著降低。其机理是改变了酶的构象使其转录延伸复合物更加稳定。该突变同时使得T7RNA聚合酶对RNA产物的重新结合能力大大降低，因此突变体几乎失去RNA依赖的RNA聚合酶活性。在应用方面，该单点突变有效减少了体外RNA合成产物中的两种主要杂质：由转录终止引起的中断副产物和由RNA依赖的RNA聚合酶活性引起的末端延伸副产物，为当前飞速发展的RNA研究和制药领域对体外合成RNA日益提高的质量要求提供了高效简便的解决方案。



华中科技大学生命学院博士研究生吴慧和中科院深圳先进院助理研究员魏婷为论文共同第一作者，华中科技大学为论文第一单位，华中科技大学生命学院朱斌教授与中科院深圳先进院刘陈立研究员为共同通讯作者。该研究受到国家自然科学基金及深圳市基础研究基金支持。

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