Literature Report

Reporter: Chunyu Yan

Date: 2020-08-13

Literature Source

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

Cell- and polymerase-selective metabolic labeling of cellular RNA with 2'-azidocytidine

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J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.0c04566 • Publication Date (Web): 07 Aug 2020

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Literature Source

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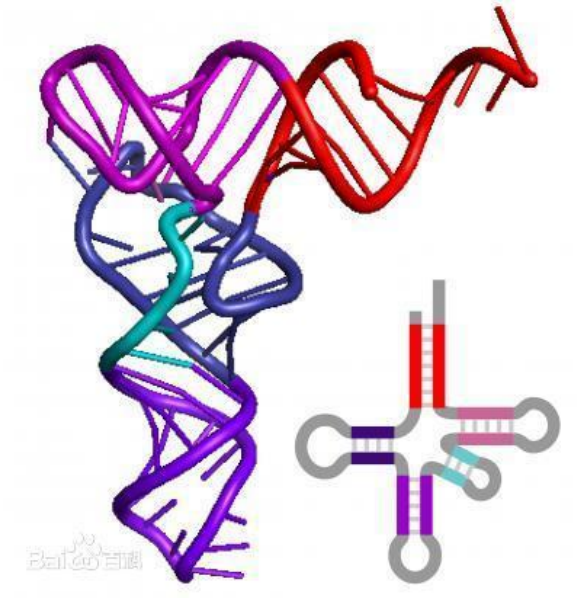
WORKS IN CHEMICAL BIOLOGY

To date, over 100 structurally distinct chemical modifications have been found, including both enzymatic and non-enzymatic modifications of the canonical ribonucleotides; however, there is a major gap in our understanding of how these chemical modifications impact RNA function.

Our goal is to decipher the chemical complexity of cellular RNA. Towards this end, we are developing and employing novel approaches integrating chemistry and biology to investigate the functional significance of RNA modifications and the interplay of RNA chemistry with cellular mechanisms regulating RNA function and integrity. Our studies will rely heavily upon synthetic and chemoenzymatic strategies for generating modified nucleic acids, chemical proteomics, and quantitative cellular imaging, and aim to reveal fundamental biological mechanisms maintaining cellular homeostasis.

Introduction

RNA is a unique polymer. It carries out a broad range of functions, from ①translating genetic information into the molecular machines and structures of the cell to ②regulating the activity of genes. It can also ③bind specific proteins or small molecules, and, remarkably, RNA can ④catalyze chemical reactions, including joining amino acids to make proteins. *https://www.umassmed.edu/rti/biology/role-of-rna-in-biology*



RNA由核糖核苷酸经磷酸二酯键缩合而成长链状分子。一个核糖核苷酸分子由磷酸，核糖和碱基构成。RNA的碱基主要有4种，即A（腺嘌呤）、G（鸟嘌呤）、C（胞嘧啶)、U(尿嘧啶)，核糖核酸在体内的作用主要是引导蛋白质的合成。

Introduction

生物正交反应：也称活细胞化学修饰，指那些能够在活体细胞或组织中能够在不干扰生物自身生化反应条件下可以进行的化学反应，这个概念最早由Carolyn R. Bertozzi在2003年提出。

需求：RNA的有效标记及探针在活细胞内特定位置的结合

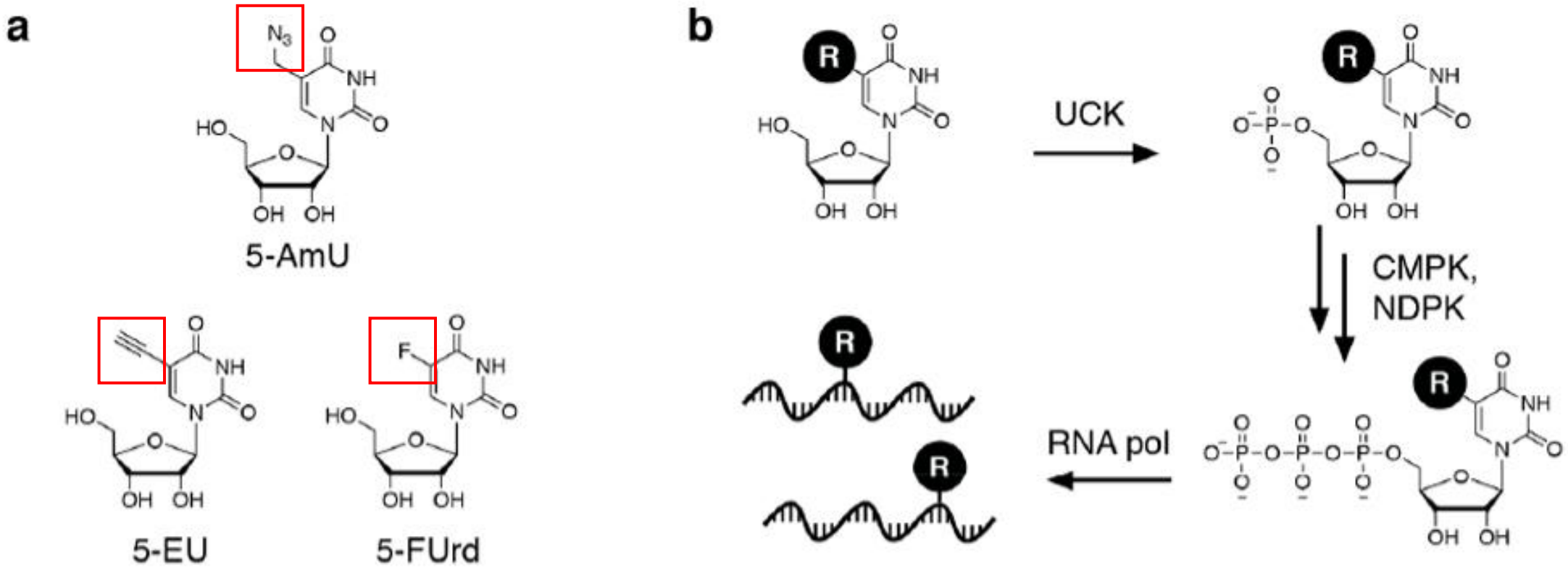
对单核苷酸进行修饰之后整合到细胞的RNA中，是目前一种研究RNA生物学的有效修饰方法。核苷酸具有双正交、光亲和的特性，可作为化学探针用于研究活细胞的转录动力学和蛋白质-RNA的相互作用。

本文的研究背景：

1. 使用4-thiouridine (4-SU)或5-ethynyluridine (5-EU)两种修饰过的核苷酸进行RNA标记的效果比较好，但是标记量有限。*Proc Natl Acad Sci USA 2008, 105 (41), 15779-84*，*Cell 2010, 141 (1), 129-41*
2. 缺乏在细胞中毒性小能在体内应用的细胞特异性标记方法。*Nat Methods 2009, 6 (6), 439-41*
3. 缺乏基于RNA聚合酶的特异性标记方法。*RNA Biol 2013, 10 (10), 1623-30*

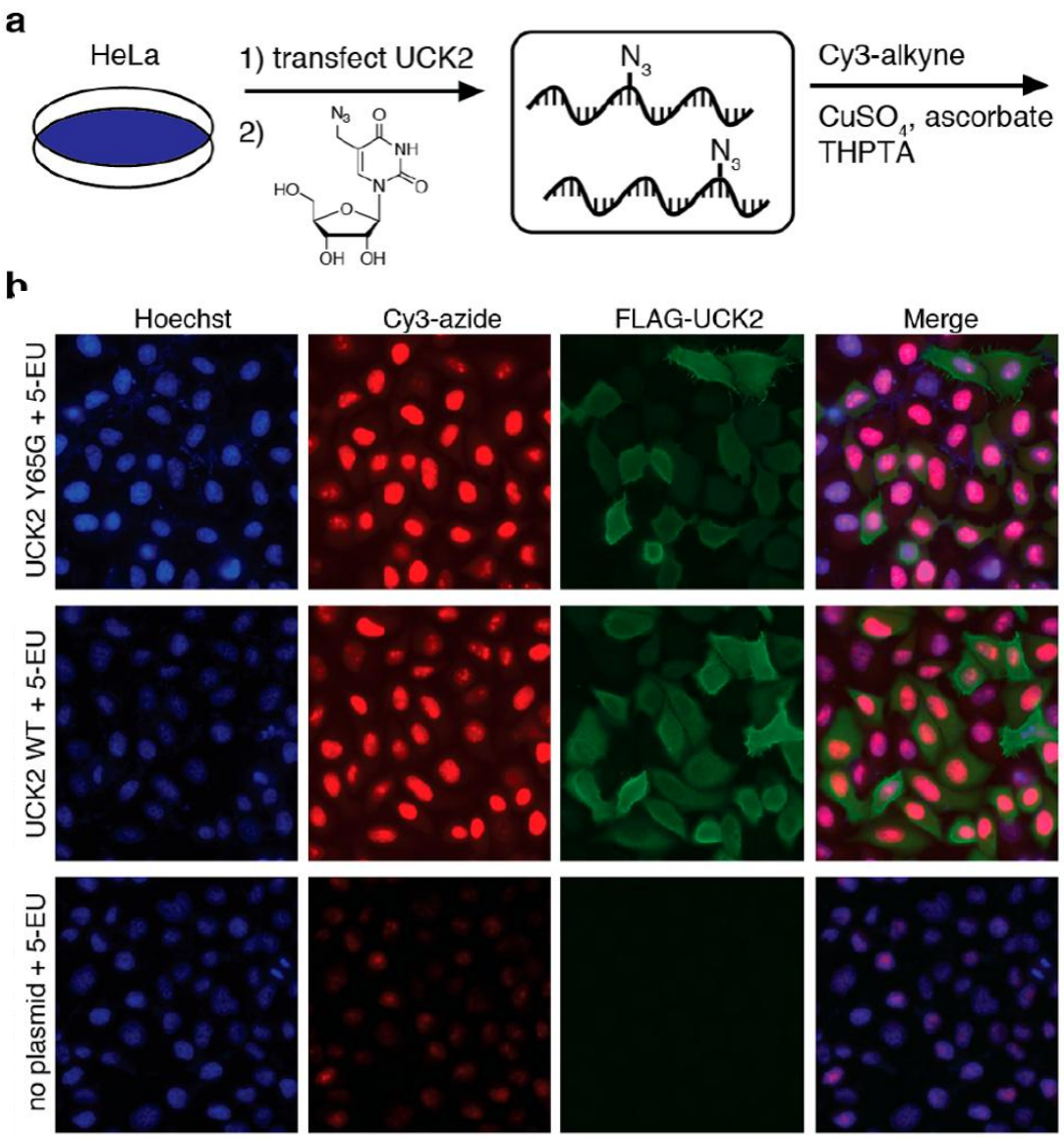
Introduction

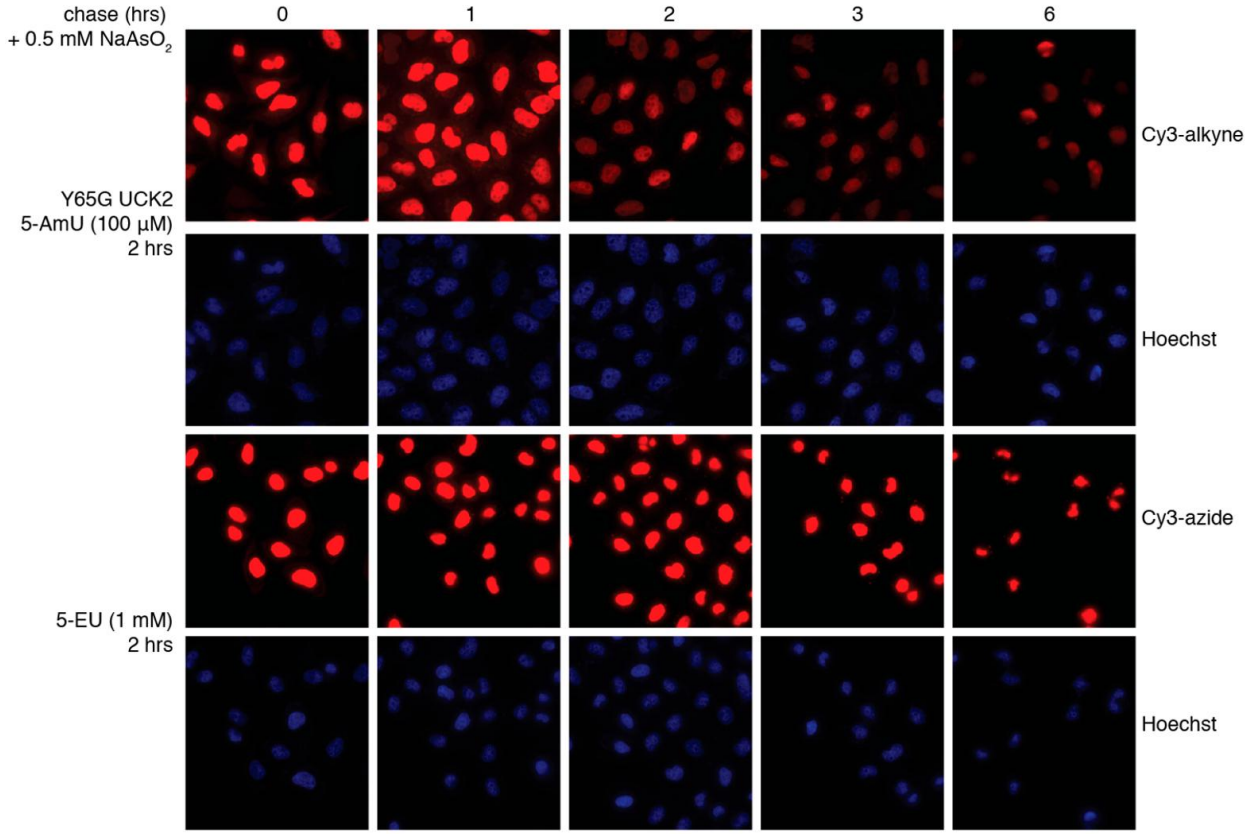
本文的研究基础：



J Am Chem Soc 2019, 141 (8), 3347-3351

Introduction





1. 5-FUrd具有细胞毒性，无法满足活细胞修饰要求
2. 5-AmU的成像效果比5-EU差，但能够在外部条件改、变的情况下对RNA的转移进行时间尺度观察

Introduction

- 嘧啶核苷酸代谢
  - 分解代谢
  - 合成代谢
    - 从头合成
    - 补救合成
      - 嘧啶+PRPP ⟶ 嘧啶核苷酸
      - 尿嘧啶核苷+ATP ⟶ 嘧啶核苷酸

FEBS Lett. 1985 Dec 2;193(2):203-7. doi: 10.1016/0014-5793(85)80151-4.

Azidocytidine is incorporated into RNA of 3T6 mouse fibroblasts

Abstract

Earlier work has shown that azidocytidine inhibits the growth and DNA synthesis of 3T6 mouse fibroblasts by inactivation of the enzyme ribonucleotide reductase. RNA synthesis, as measured by incorporation of [3H]cytidine was not affected. Here I show that azidocytidine is incorporated into RNA, but not into DNA. Incorporation of the analogue into RNA may under special circumstances contribute to the biological effect of the nucleoside.

14. Akerblom, L.; Reichard, P., Azidocytidine is a specific inhibitor of deoxyribonucleotide synthesis in 3T6 cells. *J Biol Chem* **1985,** *260* (16), 9197-202.

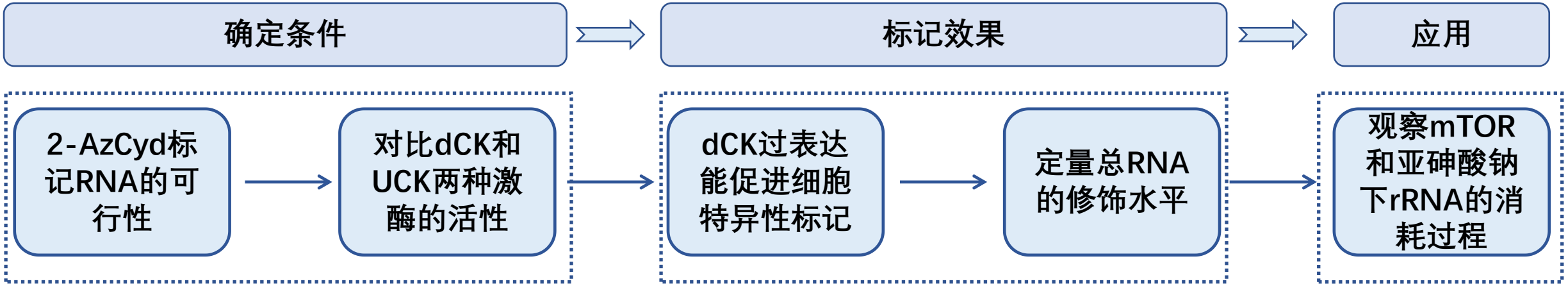
15. Akerblom, L., Azidocytidine is incorporated into RNA of 3T6 mouse fibroblasts. *FEBS Lett* **1985,** *193* (2), 203-7.

16. Kruger, K.; Grabowski, P. J.; Zaug, A. J.; Sands, J.; Gottschling, D. E.; Cech, T. R., Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. *Cell* **1982,** *31* (1), 147-57.

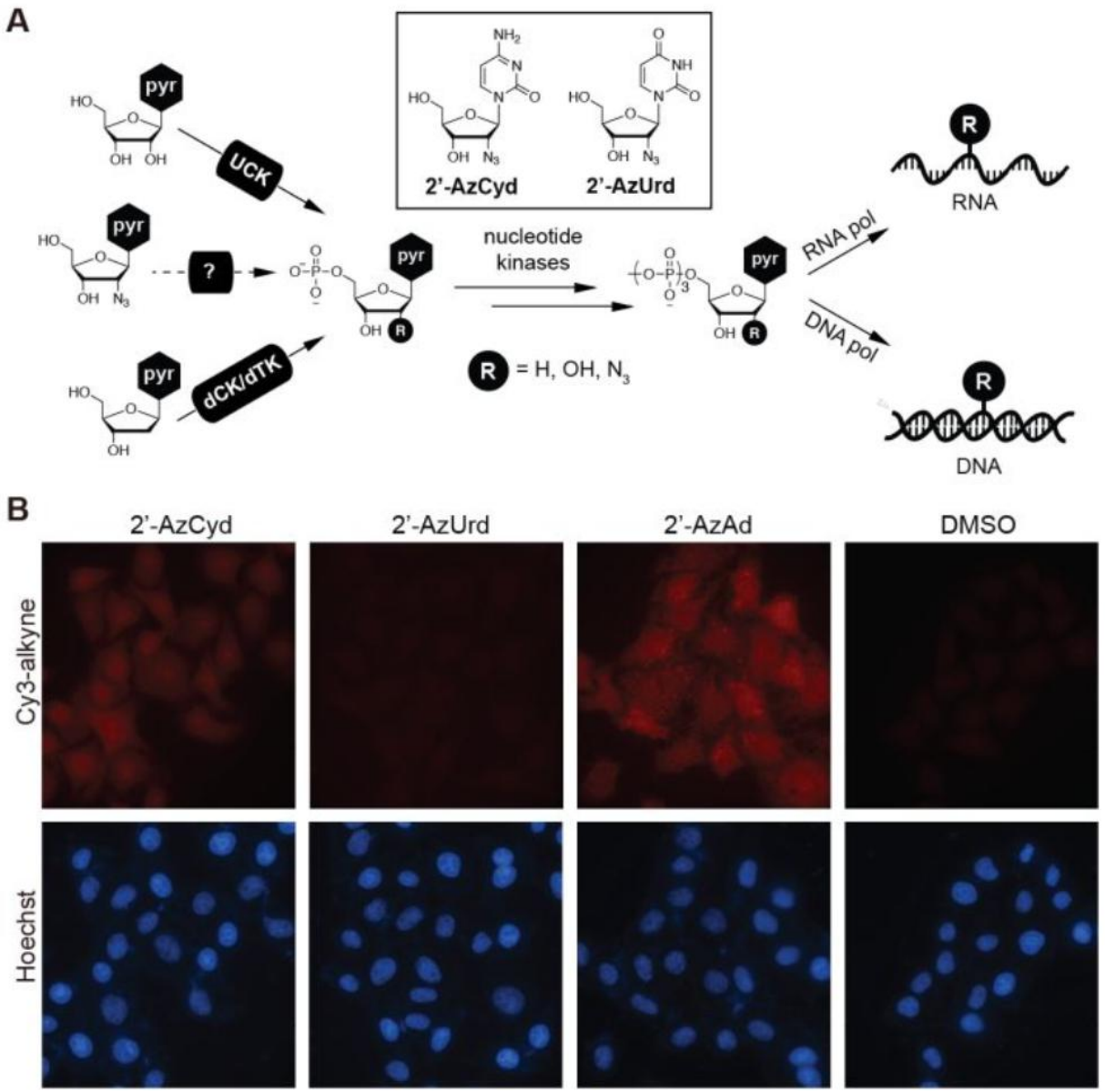
Problem and Solution

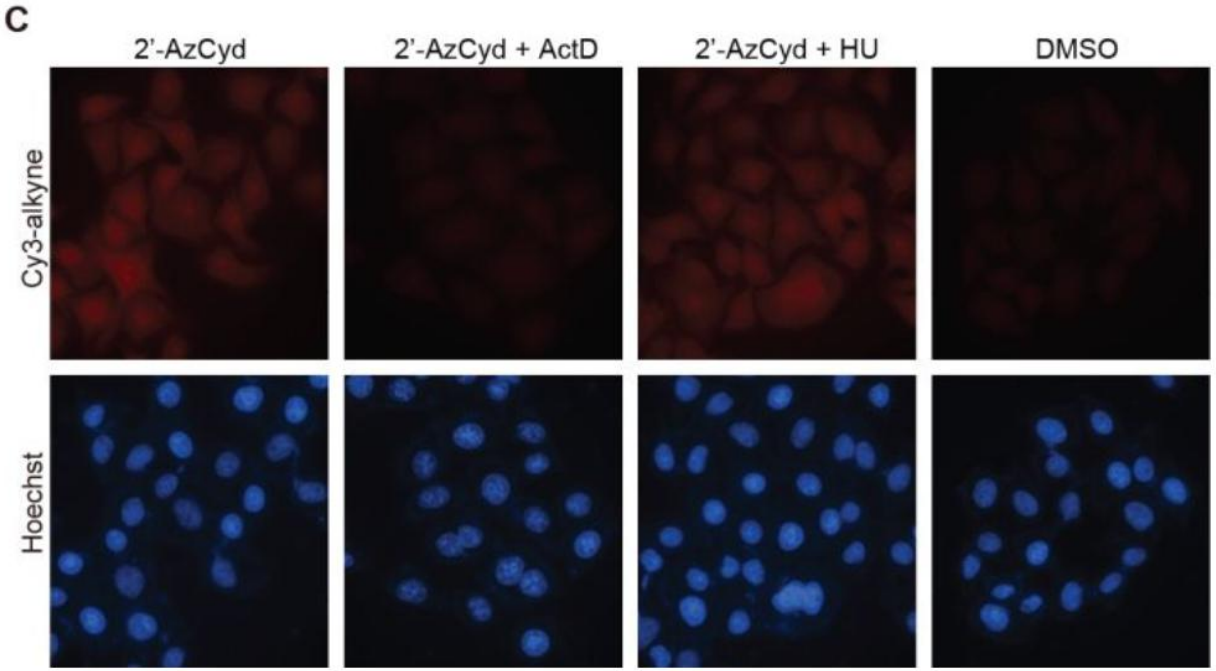
解决的科学问题： 采用非已知尿苷的胞苷完成了细胞的核苷酸代谢补救途径，对细胞无毒且标记信号强烈。分析了核糖体RNA在外部条件胁迫下的合成消耗运输过程，提供了一种新的活细胞RNA标记方法。

技术路线：



Result and Discussion



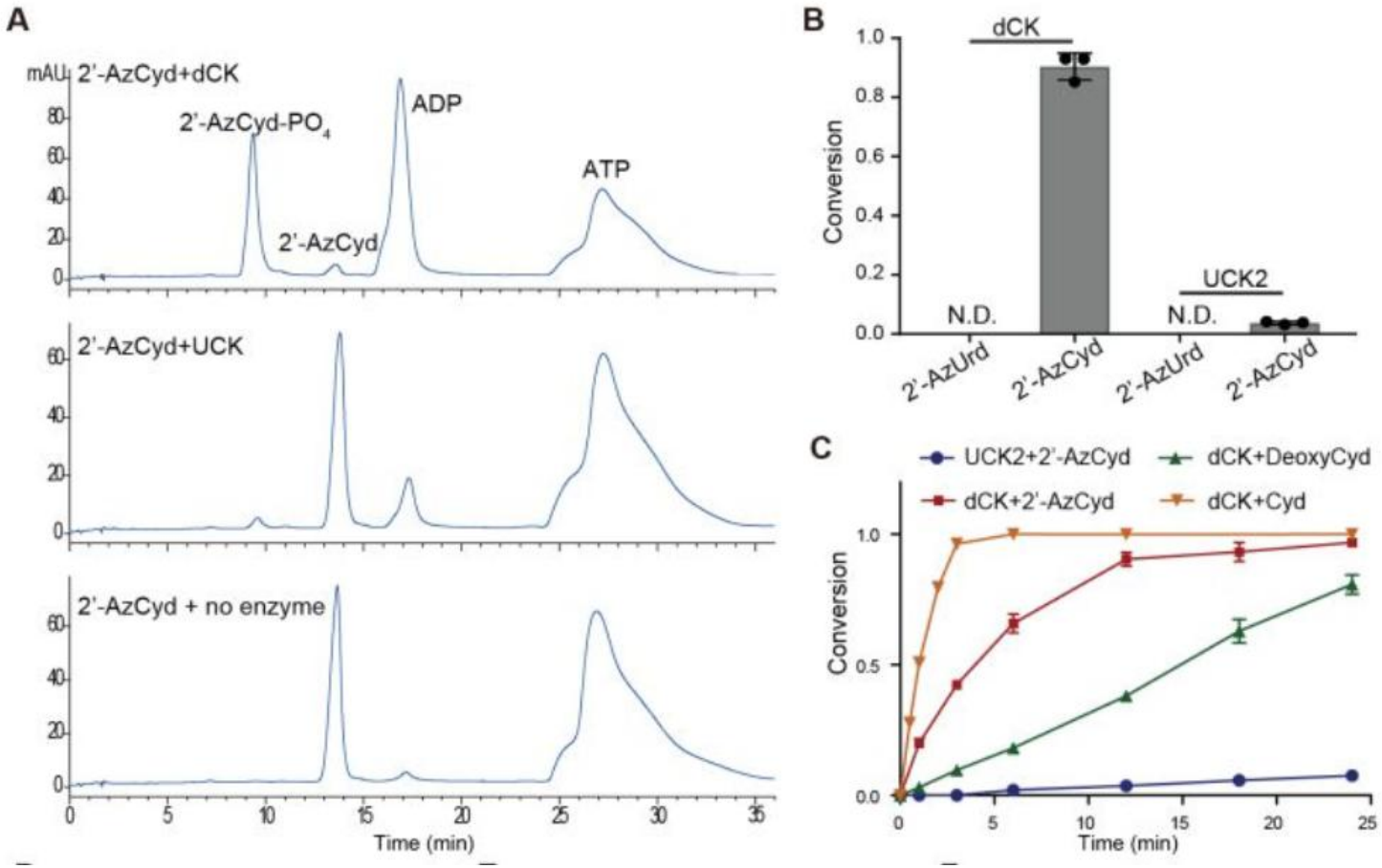


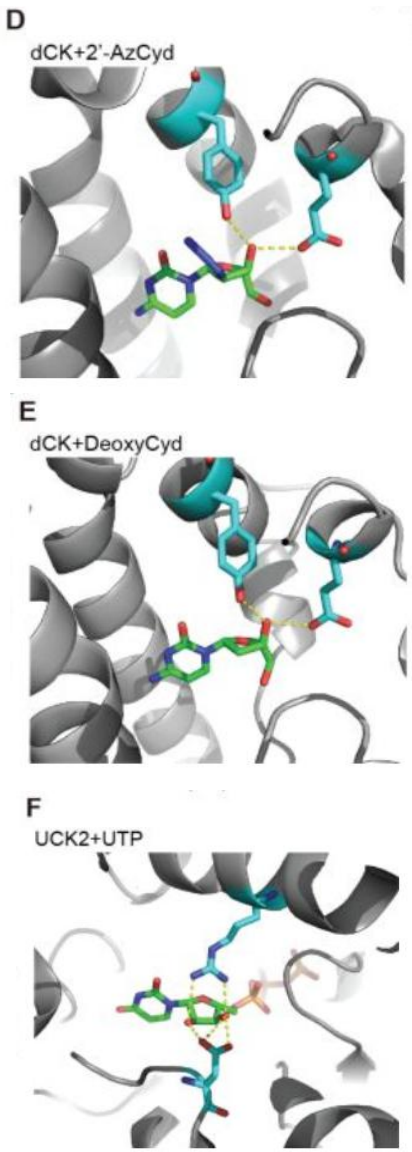
目的：证明叠氮胞苷用于RNA标记的可行性

方法：用赫斯特染料与其他已报告叠氮核苷作对比

证明：分别使用ActD（抑制RNA合成）、HU（抑制DNA合成）反向证明叠氮胞苷确实结合在RNA上

Result and Discussion



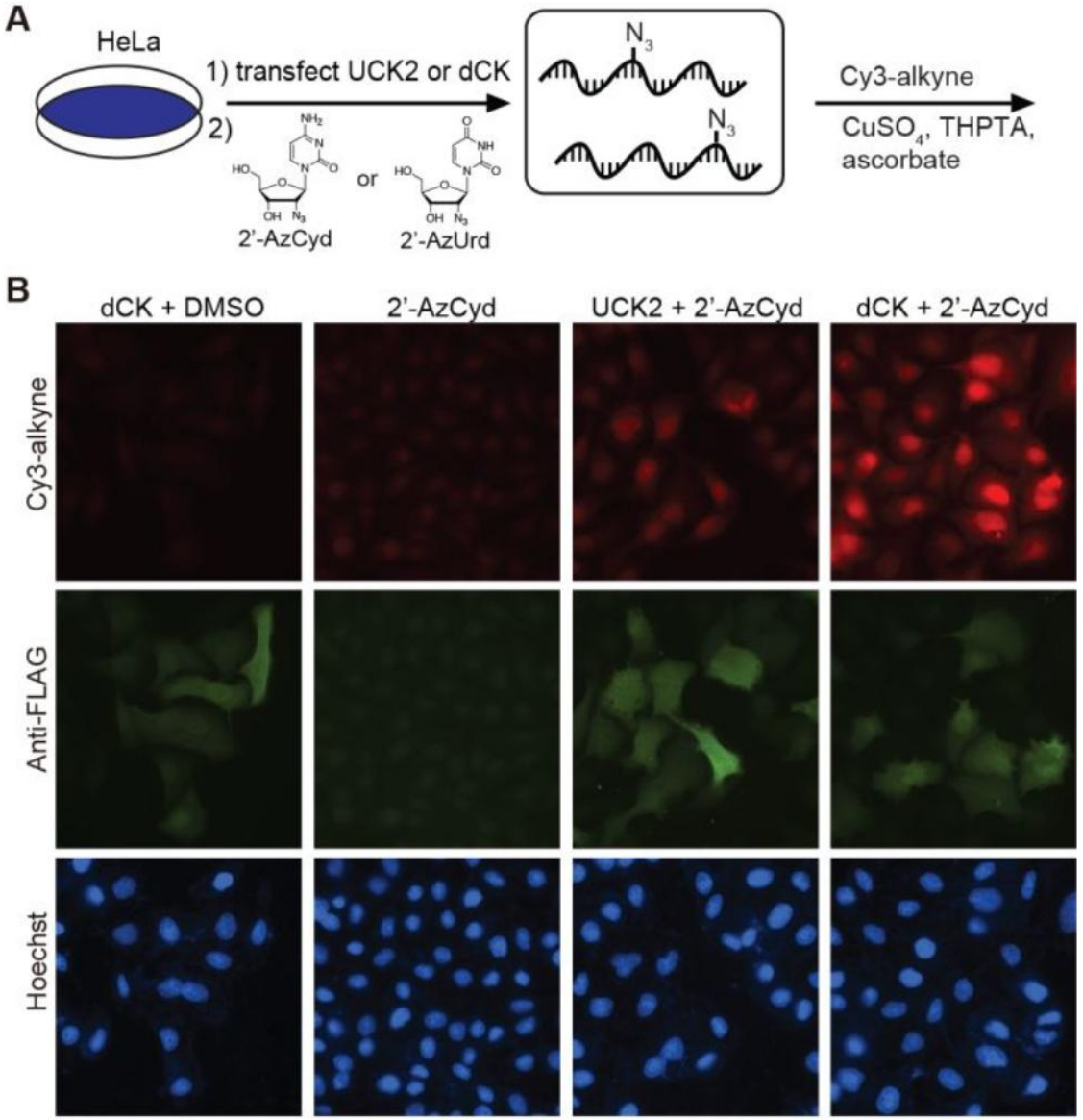


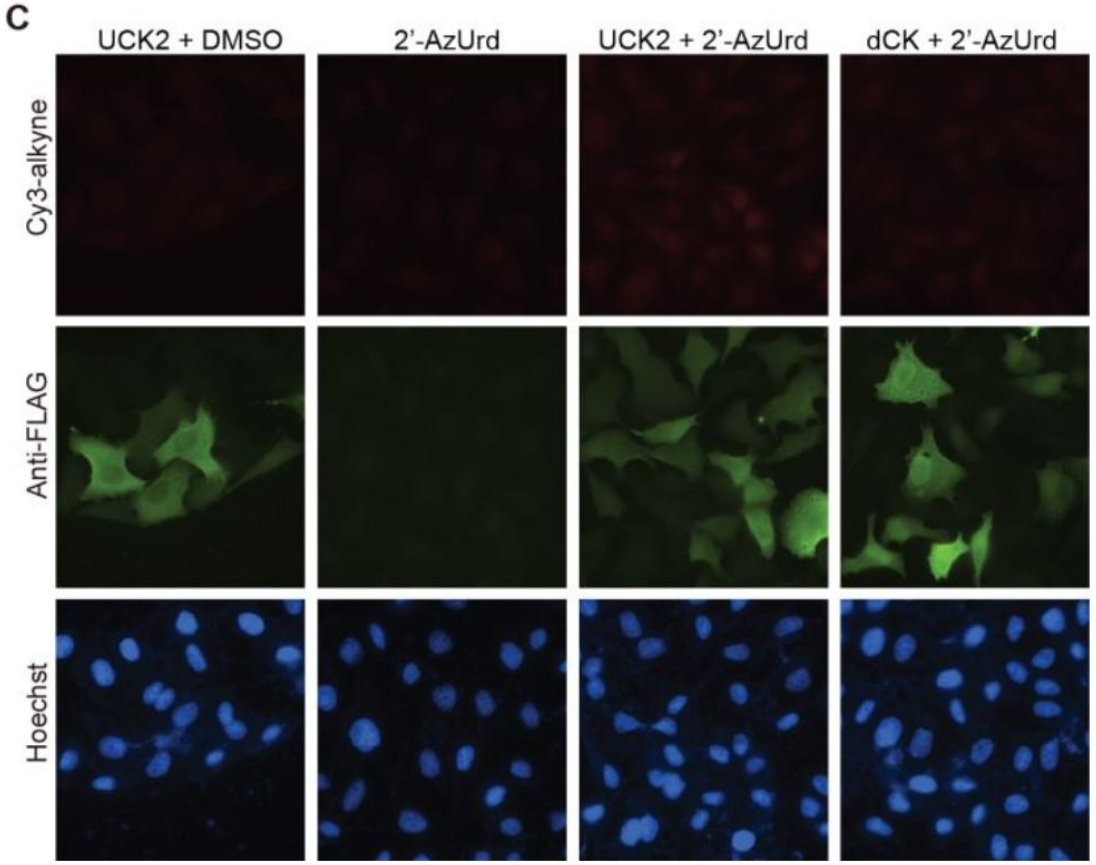
目的：通过胞苷磷酸化对比dCK和UCK两种激酶的活性

方法：用HPLC和时间测定底物的消耗和磷酸化产物的生成

证明：通过底物和产物的相关性变化正向证明了dCK激酶对底物胞苷具有更高的磷酸化活性

Result and Discussion



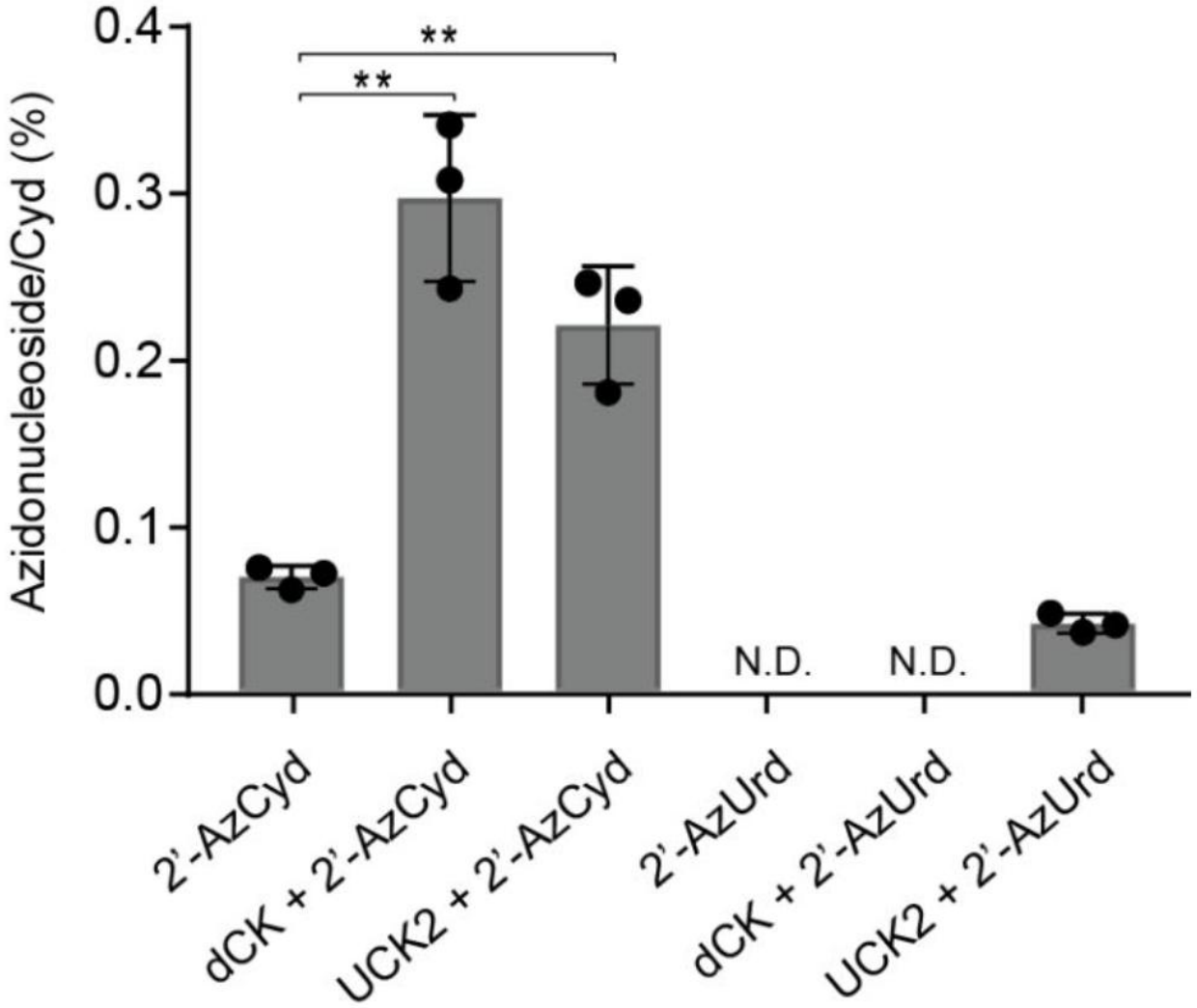


目的：根据dCK的能力推测其过表达能够促进细胞特异性标记

方法：不同核苷和不同激酶条件的对比细胞成像验证

证明：通过控制单因素变量证明只有两者同时存在时才有效

Result and Discussion

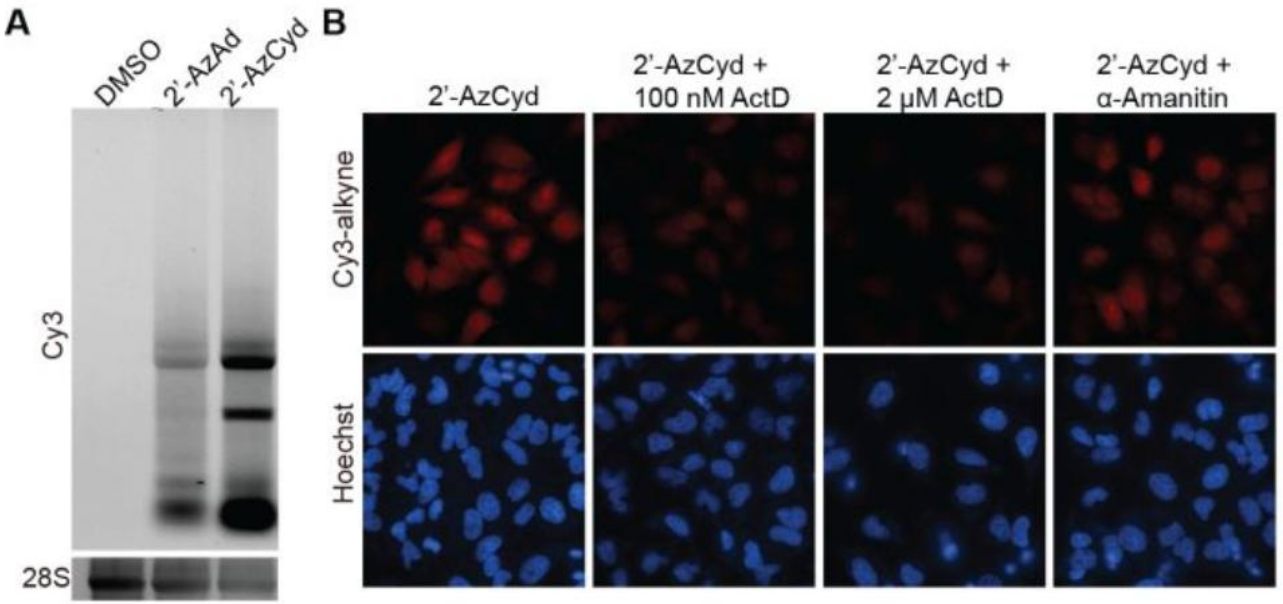


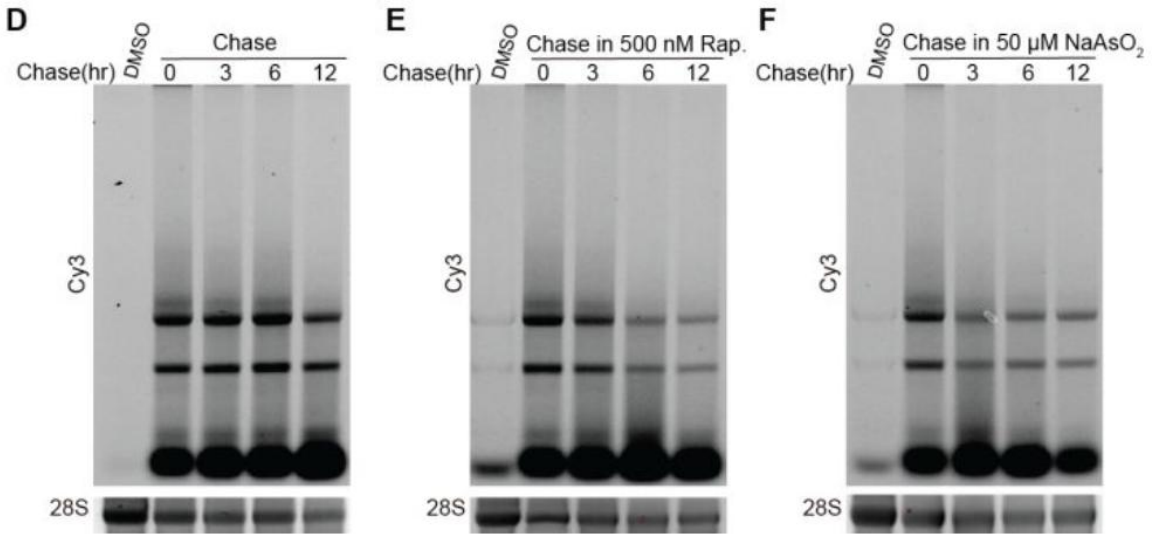
目的：定量测量总RNA的修饰水平

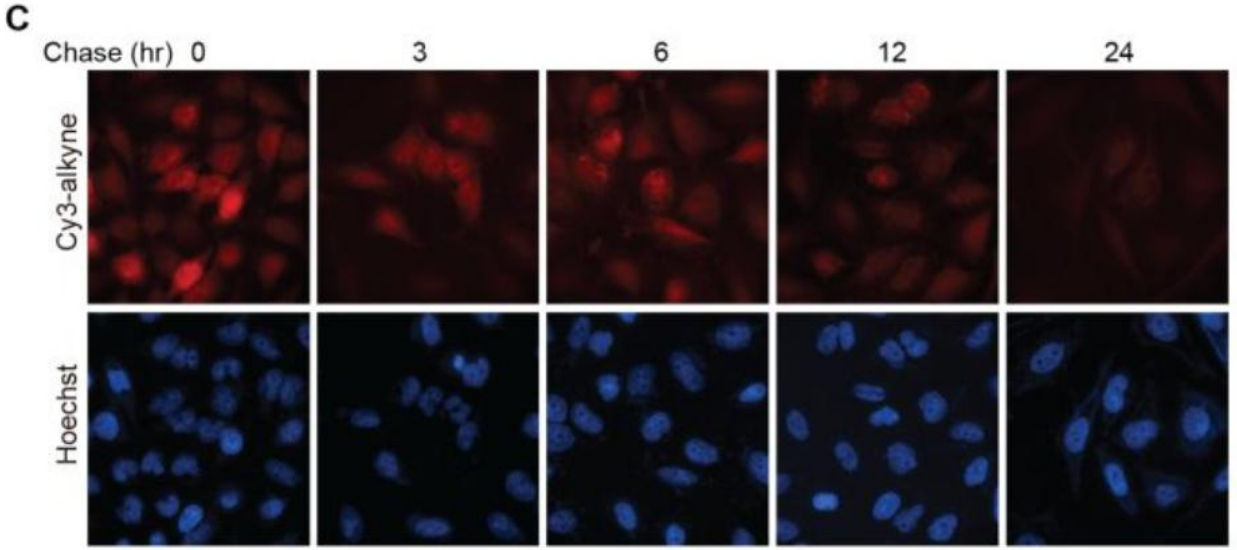
方法：提取总RNA，酶切成核苷酸LC-MS/MS绘制标准曲线定量

在有dCK酶存在的情况下，2-AzCyd整合到RNA上的标记量提高了4.3倍，与已经报告过的UCK2酶与2-AzUrd作用的代谢标记效果高10倍。

Result and Discussion







目的：观察mTOR和亚砷酸钠下rRNA的消耗过程

方法：通过不同时间下细胞荧光信号的变化

证明：加入抑制剂证明对RNA Pol I的特异结合，同时加以电泳辅助验证

Perspective

总结与展望：

① Azido-nucleotides does not result in RNA degradation.

②2'-AzCyd is less toxic than the commonly used ribonucleoside and can label RNA without enrichment.

③ 2'-AzCyd-dCK pair to be superior as it facilitates RNA labeling with 10-fold higher efficiency.

④ provides a tool for dissecting the mechanism of this process in mammalian systems.

⑤ It demonstrates a unique synergy between deoxynucleoside salvage and RNA polymerases that can be exploited to incorporate modifications in place of the ribose 2'-OH, presenting new opportunities for labeling cellular RNA with diverse chemical groups for probing biological processes.

Inspiration

对自己工作的启发

1. 文献查阅方面：

①重视所查文献的引用文献和被引用文献

②溯源文献中出现的重点内容

2. 研究思路方面：

①可以思考新的核酸标记方法

②可以研究在活细胞下核酸相关的生命过程或光学检测

请大家批评指正

THANK YOU