研究简报

小鼠腺苷脱氨酶 mRNA 特异 Ribozyme 计算机设计*

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摘要 小鼠腺苷脱氨酶 mRNA 经计算机分析，包括：ribozyme 切点位置选择，二级结构预测，基因生物学功能和基因同源性分析，筛选出四个锤头结构 ribozyme. 结果表明上述 ribozyme 底物切点两翼碱基形成发夹结构，切点位于单链环区，切点所在基因片段位于该基因生物功能区内，并同已知小鼠其它基因不同源.

关键词 腺苷脱氨酶 mRNA，ribozyme，计算机设计

1986 年 Symons 等比较了类病毒、卫星病毒和拟病毒的 RNA 自我剪切（self-cleavage）规律，通过对这些 RNA 二级结构分析，提出“锤头结构”（hammerhead structure）模型 ribozyme. 它由 13 个保守核苷酸残基和三个螺旋区构成[1]，锤头 ribozyme 自我剪切活性依赖于结构和构象的完整性. 此后又相继发现了“发夹结构”（hairpine）[2]，丁型肝炎病毒（HDV）[3]和链孢霉线粒体 VSRNA[4]等 ribozyme 二级结构特征. 在上述 ribozyme 中，锤头结构 ribozyme 是目前已知最小的 ribozyme，并易于设计，因而得到广泛的应用[5—7].

本文旨在选择小鼠腺苷脱氨酶（adenosine deaminase，ADA）mRNA 作为靶 RNA 分子，以锤头结构为模型，采用计算机人工设计能特异性切割 ADA mRNA 的 ribozyme 分子，为 ribozyme 的计算机辅助设计提供参数.

1 材料与方法

1.1 选择 Yeung 等[8]构建的 ADA cDNA 克隆 pADA5-29 基因序列做为分析序列.

1.2 采用 ribozyme 设计软件寻找小鼠 ADA mRNA 上 ribozyme 的切点位置（含 GUH 结构，其中 H 为 A，C 和 U）.

1.3 采用 pcFOLD 软件（加拿大国家科学院 Zuker 编写）对切点两翼 RNA 碱基序列进行二级结构计算机预测，确定 ribozyme 切点位置.

1.4 根据 Symons 的锤头结构模型[1]设计 ribozyme 切割“催化中心”碱基序列（22nt）5′ CUGAUGAGUCCGUGAGGACGAA3′. 根据 ribozyme 切点两翼碱基序列（7—13nt）设计 ribozyme 切割“结合部位”碱基序列，它们同相应的底物碱基序列互补，形成氢键对，对上述 ribozyme 进行二级结构计算机预测，确定能形成最佳二级结构的 ribozyme 分子.

1.5 采用 RBSA DNA 序列分析软件（美国国立卫生研究院 Lipman 和 Wilbur 编写）对上述 ribozyme 底物切点所在基因片段的功能进行分析，选择具有重要生物学功能的基因片段，做为 ribozyme 靶基因片段.

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1.6 采用 NCBI GenBank（美国国立医学图书馆和美国国立卫生研究院编写）对底物切点两翼基因片段同源分析，参与形成核苷酸对的碱基序列同小鼠已知其它基因片段进行同源性分析，排除 ribozyme 非特异切割其它基因片段的可能性.

2 结果与讨论

ADA mRNA 全长 1379 碱基，其中翻译编码区为 71—1129 碱基，该基因 ATG 5′（71 碱基）上游存在多个 SP_1 因子结合位点. 在 ADA mRNA 上共有 38 个 GUH 的 ribozyme 切点，对这些切点两翼（60nt）碱基二级结构分析，其中在 262，455，583 和 862 四个切点的二级结构较为理想，分别命名为 ADARz262，ADARz455，ADARz583 和 ADARz862，上述 ribozyme 底物切点所在基因生物学特性如表 1.

表 1 ADA mRNA 特异 ribozyme 底物切点所在基因片段生物学特性

ribozyme	切点	GUH	编码氨基酸	外显子	配对碱基	同源性分析[1]	
						核苷酸	氨基酸
Rz262	262	GUU	Lys-Phe	Ⅲ	8-8	100%	100%
Rz455	455	GUU	Val	Ⅴ	7-8	75%	64%
Rz583	583	GUA	Lys-Tyr	Ⅵ	8-8	88%	60%
Rz862	862	GUC	Trp-Ser	Ⅸ	8-8	100%	100%

[1] 小鼠 ADA 基因和人 ADA 基因比较.

上述切点的共同特点是切点两侧 7 至 8 个碱基形成环状突出结构，而其它碱基则形成茎状结构，使整个二级结构形成发夹结构，从而使 ribozyme 切点充分暴露出来，同时切点远端的二级结构对其影响较少，这种结构有利于 ribozyme 和底物分子结合，加速 ribozyme 切割反应. 我们同时对上述四个切点 ribozyme 自身的二级结构进行分析，发现它们均能形成 ribozyme 切割活性所必需的催化活性中心结构. 上述 ribozyme 的结构如图 1.

上述四个切点分别位于 ADA mRNA 的前部，中部和后部位置，均处在 ADA 编码翻译区内，切点两翼 mRNA 片段同人 ADA mRNA 同源性较高（表 1），其中切点 262 和 862 在基因片段同人的基因完全相同，表明这些基因片段处在 ADA 基因的功能保守区，是 ADA 的重要功能结构区域.

基因库检索，在已知的小鼠的基它基因中未发现同上述基因片段同源的序列.

由上述结果可以看到 ribozyme 底物切点的选择主要根据二个方面：a. 基因功能：ribozyme 切点所在基因片段必需在该基因的重要功能区，如 HIVgagRNA，而使该基因在切点处被切断后，相应蛋白质的功能即丧失，同时切点两翼配对碱基顺序应较为保守，使 ri-

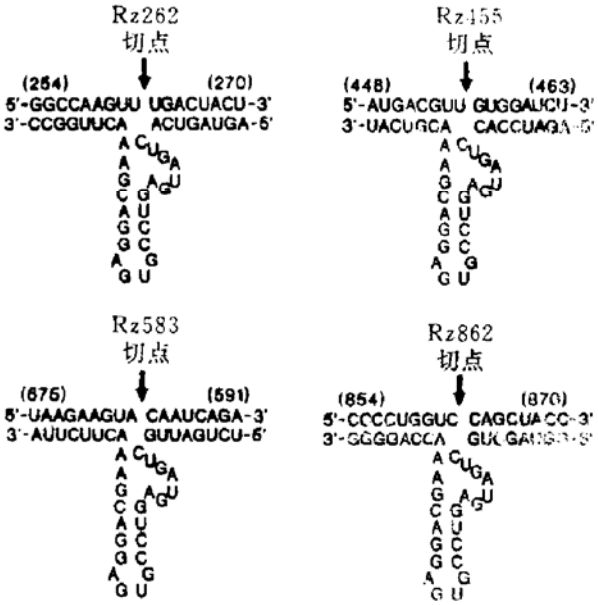

图 1 ADA mRNA 特异 ribozyme 结构和底物各切点处的碱基序列

bozyme 切割谱更广，并且不因某些碱基的突变丧失切割作用，特别是一些病毒 RNA，如 HIV 病毒分子. b. 底物二级结构：切点所在基因在全长 mRNA 中应处于单链环区内，而非发生碱基配对的茎状结构内，在计算机模拟二级结构时，由于 RNA 过长后不易得到正确的二级结构，我们采用切点两翼各 30 个碱基进行二级结构预测，理想的 ribozyme 切点应形成发夹结构. 此外还应考虑底物同细胞内蛋白质因子的相互作用：细胞内蛋白质因子同 RNA 结合后将封闭 ribozyme 的切点，因此 ribozyme 切点所在 mRNA 片段应为非蛋白质因子结合区，因此 RNA 结合蛋白质的发现及 RNA 结合蛋白质功能的研究对 ribozyme 的设计将起一定的推动作用.

应用计算机模拟选择 ribozyme 切点是一种经济有效的实验手段，但它很大程度上依赖于计算机软件的功能，现有软件的设计是有效的，但并不是最优的，进一步优化软件设计，提高计算机软件的分析功能仍在进行之中，特别是应考虑到切点远端碱基序列对切割活性的影响，同时上述设计结果仍有待于在体外，细胞甚至整体动物体内得到证实.

除计算机模拟外，国外也采用其它多种方法选择 ribozyme 切点. 如 Draper 等[5]采用 RNase H 消化和移动足迹方法选择最佳 ribozyme 切点，挑选出不发生碱基配对的 ribozyme 切点. 在第三届 ribozyme 研究研讨会上[5]，有的科学家建议采用细胞抽提液做为 ribozyme 的切割反应体系，选择 ribozyme 切点，从而排除蛋白质因子同切点所在 mRNA 结合的可能性. 避免蛋白质因子封闭 ribozyme 切点. 如将计算机模拟和上述功能实验结合起来，就有可能得到更为理想的 ribozyme 设计.

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胎盘型谷胱甘肽 S-转移酶基因在胃癌中的表达*

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摘要 用 Dig-GST-π cDNA 探针分子杂交方法，检测了正常胃组织、胃癌及相应癌旁正常组织中 GST-π DNA 和 GST-π RNA 水平. 发现 GST-π DNA 水平没有明显变化，而 GST-π RNA 在 8 例胃癌组织中有 6 例高于正常胃组织，在 12 例低分化腺癌中有 7 例癌旁正常组织高于相应癌组织. 表明 GST-π 基因表达增加与胃癌有关，而且早于细胞形态的变化.

关键词 人胎盘型谷胱甘肽 S-转移酶，胃癌，基因表达

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yield. At the same time, AC, a by-product which was not contaminated by G_s, can be used for assay of G_s activity after reconstituting it into asolectin vesicls. This method of assaying G_s activity has been proved to be simple, reliable and sensitive.

Key words stimulatory GTP-binding protein, adenylate cyclase, bovine brain

FOS/JUN Mediates Endothelin-1 Gene Expression Induced by Phorbol Ester in Endothelial Cells. Wen Jinkun, Hu Jing, Qiao Yaming, Zhang Chenhui, Zhou Airu, Tang Jian. (*Institute of Basic Medicine, Hebei Medical College, Shijiazhuang* 050017). *Prog. Biochem. Biophys.* (China), 1994; **21** (5): 457—458

Gel shift of electrophoresis and Northern and Western blotting analysis were used to detect the effect of c-jun antibody on the interact between AP-1 site of ET-1 gene and nuclear proteins as well as the effect of TPA on c-fos/c-jun gene expression. The results showed that AP-1 binding activity in vascular endothelial cells was stimulated by c-fos/c-jun, whose expression was induced by TPA, and that the electrophoretic mobility of band of DNA-protein complexes was altered by the antibody against c-jun. These results suggest that ET-1 gene expression induced by TPA is mediated by c-fos/c-jun.

Key words ET-1 gene, endothelial cells, c-fos/c-jun, TPA

A Rapid and Reliable Method for Direct Sequencing of PCR Products. Wang Liang, Zhang Jinsan, Zhu Dan, Yin Luo, Wang Xiuqin, Wu Min. (*National Laboratory of Molecular Oncology, Cancer Institute, Chinese Academy of Medical Sciences and Peking Union Medical College, Bejing* 100021). *Prog. Biochem. Biophys.* (China), 1994; **21** (5): 458—459

A simple, rapid and reliable sequencing method for double stranded PCR products is described. This method presented utilizing the unique property of T7 DNA polymerase which remains active at low temperature to allow the sequencing reaction performed at low temperature. Excellent sequencing results have been obtained by this method for various PCR products.

Key words polymerase chain reaction (PCR), DNA sequencing, T7 DNA polymerase

Determination of the Content of AchE in the Plasma of Patients With PNH. Xu Caimin, Lu Hong, Pan Huazhen, Zhang Zhinan. (*National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing* 100005). *Prog. Biochem. Biophys.* (China), 1994; **21** (5): 460

The contents of AchE in plasma and erythrocyte membrane of patients with PNH were determined by ELISA. The results show that the content of AchE is low in PNH erythrocyte membrane but high in plasma when it is compaired with that in normal.

Key words paroxysmal nocturnal hemoglobinuria (PNH), soluble acetylcholinesterase (soluble AchE), plasma, erythrocyte memberane

Computer Design of Murine Adenosine Deaminase mRNA Specific Ribozyme. Chen Hua, Chen Nongan, Lu Changde, Qi Guorong. (*Shanghai Institute of Biochemistry, Academia Sinica, Shanghai* 200031). *Prog. Biochem. Biophys.* (China), 1994; **21** (5): 461—463

With computer analysis of the adenosine

deaminase (ADA) mRNA, which included cleavage site selection, secondary structure analysis, biological function and homology analysis of the gene fragment around the cleavage site, four hammerhead ribozymes were designed. These ribozymes targeted sequence around the cleavage site have hairpine structure in which the cleavage site is in the ring part. This gene fragment is of biological importance in ADA gene. No homologies were found between these gene fragments and other murine genes. These characteristics of the gene fragment make them easy to be paired and cleaved by their respective ribozymes.

Key words adenosine deaminase, ribozyme, computer design

GST-π Gene Expression in Gastric Tumor. Qi Chunhui, Li Chunhai. *(Institute of Basic Medical Sciences, Beijing* 100850*). Prog. Biochem. Biophys.* (China), 1994; **21** (5): 463—466

Increased expression of the glutathione S-transferase (GST; E. C. 2. 5. 1. 18) π class isoenzyme is associated with both tumor and preneoplastic tissues. In order to further characterize the alteration of GST-π gene expression during progression of carcinoma, both the levels of GST-π DNA in one normal gastric tissue and 20 gastric tumor with perineoplastic normal tissue, and the GST-π RNA in the normal gastric tissue and 12 gastric poorly differiented adenoearcinomas with corresponding perineoplastic normal tissues were tested using Dig-GST-π cDNA probe by Dot blot hybridization. No significant change of GST-π DNA level, but the expression level of GST-π RNA in 6 of eight gastric tumors was higher than in normal gastric tissue, and in 7 perineoplastic normal tissue of twelve poorly differentiated adenocarcinoma was higher than that in its corresponding tumor. This suggests that the elevation of GST-π gene expression is related to gastric tumor, and earlier than the changes of cell morphology.

Key words human placental glutathione S-transferas, gastric tumor, gene expression

A Study of the Solution Behavior of Bovine Serum Albumin by Viscosimetry. Zuo Ju, Wang Zhigang, Liang Bo, Ouyang Di, Zhou Yongqia. *(Chemistry Department of Nankai University, Tianjin* 300071*). Prog. Biochem. Biophys.* (China), 1994; **21** (5): 466—469

The various differences of the hydrodynamic behavior of bovine serum albumin in NaCl, KSCN and KI solutions were evidenced by viscosimetry. Based on the empirical formula and viscosity data, the apparent axial ratios and apparent molecular volumes were calculated, which were also affected by the salt properties, correspondingly.

Key words bovine serum albumin, relative viscosity, intrinsic viscosity, apparent axial ratio, apparent molecular volume

The Ways to Enhance Cloning Efficiency of PCR Amplification Products. Shi Yanhong, Zhao Shimin, Sun Yongru. *(Institute of Genetics, Academia Sinica, Beijing* 100101*). Prog. Biochem. Biophys.* (China), 1994; **21** (5): 470—471

Some ways are introduced to enhance the cloning efficiency of PCR amplification produts. The purification of PCR products, the speciality of PCR amplification, the remainder of Tag polymerase, the 3′-end projection of PCR products and the blunt end ligation are the main factors to affect the cloning efficiency.

Key words PCR, cloning efficiency, ligation