降解，但仍适于用 PCR 进行基因重排分析. 在用于 PCR 的模板 DNA 提取中，我们对常规提取方法，与简化 NP40-蛋白酶 K 法，煮沸法进行了比较（未发表资料）. 结果发现，NP40-PK 法（不经酚抽提）亦可得到满意的扩增；但煮沸法提取 DNA 虽较简便，但煮沸既可释放 DNA，亦可破坏 DNA，故不适于基因重排分析的模板制备[7,8]. 在本组 14 例 B 细胞性淋巴瘤中，11 例检测出 Ig 重链基因重排，其敏感性和特异性均不亚于采用新鲜组织 DNA 提取物所进行的 DNA 印迹杂交分析. 而且，PCR 简便快速，不需要同位素、模板 DNA 质量要求不高，在临床上有着更广泛的用途.

参 考 文 献

1 Goelz S E, Hamilton S R, Vogelstein B. Biochem Biophys Res Commun, 1985; **130**: 118

2 Trainor K J, Brisco M J, Wan J H *et al*. Blood, 1991; **78**: 192

3 Wu A M, Winberg C D, Sheibani K *et al*. Hum Pathol, 1990; **21**: 1132

4 Miles C, Houldsworth L, Chaganti R S K. Am J Surg Pathol, 1991; **15**: 169

5 Dubeau L, Chandler L A, Glow J R *et al*. Cancer Res, 1986; **46**: 2964

6 Dubeau L, Weinberg H, Jones P A *et al*. Am J Pathol, 1988; **130**: 588

7 Greer C E, Peterson S L, Nancy H T *et al*. Am J Pathol, 1991; **95**: 117

8 Jackson D P, Lewis F A, Taylor G R *et al*. J Clin Pathol, 1990; **43**: 499

人脑髓鞘碱性蛋白 cDNA 体外扩增、克隆和鉴定*

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摘要 采用聚合酶链反应（PCR）从人脑 cDNA 文库中扩增出 600bp 的髓鞘碱性蛋白（MBP）cDNA 片段，与载体 pGEM-3Zf（+）平端连接. 重组质粒 DNA 转化宿主菌 JM109，在含 X-gal 和 IPTG 的平板上直接筛选阳性克隆. 限制性内切酶分析和成套引物扩增鉴定证明，该克隆含有 7 个外显子的 21.5kD 人脑 MBP 全长编码序列.

关键词 聚合酶链反应（PCR），人脑 cDNA 文库，髓鞘碱性蛋白编码序列，载体 pGEM-3Zf（+）

髓鞘碱性蛋白（MBP）是脊椎动物中枢神经系统少突细胞和周围神经系统雪旺细胞合成的一种强碱性（pI>12）膜蛋白，它可能与细胞磷脂阴离子基团相互作用，促使胞浆面融合而成髓鞘多片层结构的主要致密线，对有鞘神经的绝缘和冲动快速传导起重要作用[1].

人和鼠脑 MBP 基因编码区和部分非编码区序列基本上已确定，普遍认为两个种属这一基因均含 7 个外显子（Ⅰ—Ⅶ）而且其同源性高达 90%以上，但两者原始转录物的剪接方式不同，已确定鼠脑有 5 种即 21.5，18.5，17_a 和 17_b 和 14kD MBP，人脑有 4 种即 21.5，20，18.5 和 17kD MBP[2]. 但近年有人采用 MBP 不同外显子探针和聚合酶链反应(PCR)又从鼠

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脑 cDNA 文库中发现系列新的外显子（O. I. 和 V.），表明该基因结构及其表达比原预料的更复杂[3,4].

作者设计并合成系列特异性引物，采用 PCR 技术对 21.5kD 人 MBP 全长编码序列进行扩增，克隆和鉴定. 现报道如下：

1 材料和方法

1.1 材料

λZAP Ⅱ /EcoR Ⅰ 人脑 cDNA 文库 (Stratagene)；pGEM-3Zf（+）载体，小牛肠碱性磷酸酶 (CIAP)，T4 DNA 连接酶，DNA 聚合酶 Klenow 片段，Sma Ⅰ (Promega)；5-溴-4-氯-3-吲哚-β-半乳糖苷 (X-gal)，异丙基-β-D-硫代半乳糖苷 (IPTG) (Sigma)；DNA 合成试剂盒 (Applied Biosystems)；DNA 扩增试剂盒（上海复旦大学遗传所）；其他试剂为国产分析纯.

1.2 方法

1.2.1 PCR 引物合成和纯化

根据人脑 MBP 基因已知序列[5]，作者设计并采用 381A DNA 合成仪合成系列引物和 20%聚丙烯酰胺凝胶电泳（PAGE）纯化. P_1——TTCGAATTCATGGCGTCACAGAAGAGAC，P_2——TCAGGATCCTTAGCGTCTAGCCATGGGT，P_3——GGTGAATTCATGGACTCACACCACCCGGCAA，P_4——GGCGGATCCCTAAATGTTCTTGAAGAAGTGG，P_6——GGATCCAGCTAAATCTGCTCAGGGACAG.

1.2.2 人脑 MBP cDNA 体外扩增

按本室报道的程序[6]. 采用引物 P_1 和 P_2 从 λ ZAP Ⅱ /EcoR Ⅰ 人脑 cDNA 文库中扩增出 600bp 的特异性区带.

1.2.3 重组体 pGEMP 的构建和转化[7]

载体 pGEM-3Zf（+）经 Sma Ⅰ 酶切和 CIAP 去 5′-磷酸；上述 PCR 产物经 DNA 聚合酶 Klenow 片段切平. 预处理的载体和靶 DNA 按等摩尔数混合，进行平端连接反应. 按常规 $CaCl_2$ 法转化宿主菌 JM109 后，铺于含 100μg/ml 氨苄青霉素. 1mgX-gal 和 2.4mg IPTG 的 LB 平板上，在 37℃孵育过夜.

1.2.4 限制性酶谱分析和成套引物（nested primers）扩增

挑多个白色单菌落，按碱裂解法提取质粒 DNA 并经 EcoR Ⅰ 酶切，确定插入 DNA 的有无及其方向. 重组质粒 DNA 经 EcoR Ⅰ 和 Pst Ⅰ 酶切，回收其插入片段，用 Kpn Ⅰ，Taq Ⅰ 单酶和双酶消化和 12%PAGE 分析. 按上述 PCR 程序，以重组质粒 DNA 为模板，分别用引物 P_1-P_2，P_1-P_4，P_3-P_2 和 P_1-P_6 对 MBP cDNA 不同外显子区进行扩增鉴定.

2 结果与讨论

作者根据人脑 MBP 基因已知序列设计并合成特异性引物 P_1 含外显子 Ⅰ 的第 1—16 位核苷酸，P_2 含外显子 Ⅵ 的第 576—591 位核苷酸互补序列，在两者 5′端分别还加有起始密码 ATG 和终止密码 TAA 以及不同的限制性内切酶位点. 采用这对引物从人脑 cDNA 文库中扩增出 600bp 区带，其长度与阳性对照完全一致（图 1）. 在人脑 cDNA 文库中还有另一条短而弱的 PCR 产物，其性质有待鉴定.

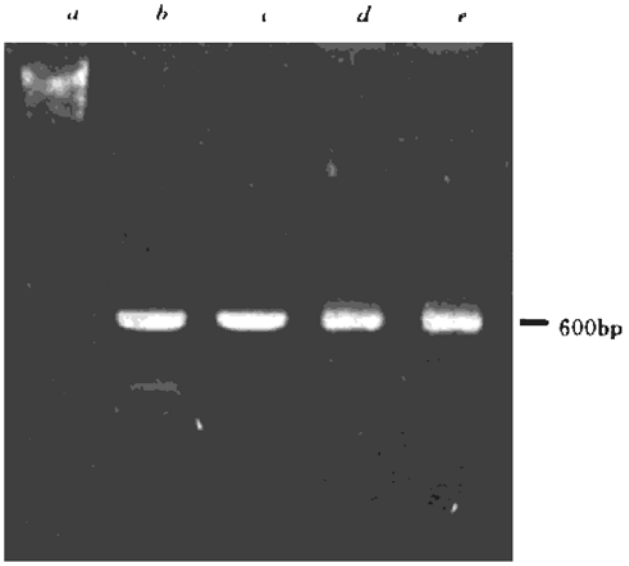

图 1 人脑 MBP 全长编码序列的 PCR 扩增

a：λ-Hind Ⅲ 片段；*b* 和 *c*：人脑 cDNA 文库的 PCR 产物；*d* 和 *e*：阳性对照（模板为 C. Campagnoni 所赠的重组质粒 pRK41-1.2，含人 MBP 编码区和 3′-非翻译区）.

Taq DNA 聚合酶具有非模板依赖性末端转移酶活性，使其合成的 PCR 产物 3′ 端突出一个脱氧腺苷酸 (dA)，该产物平端连接效率甚低. 本文采用 Klenow 片段处理 600bp 的 PCR 产物[8]，与 Sma I 和 CIAP 处理的载体 pGEM-3Zf (+) 重组后转化宿主菌 JM109，获得预期的阳性结果 (图 2). 转化率为

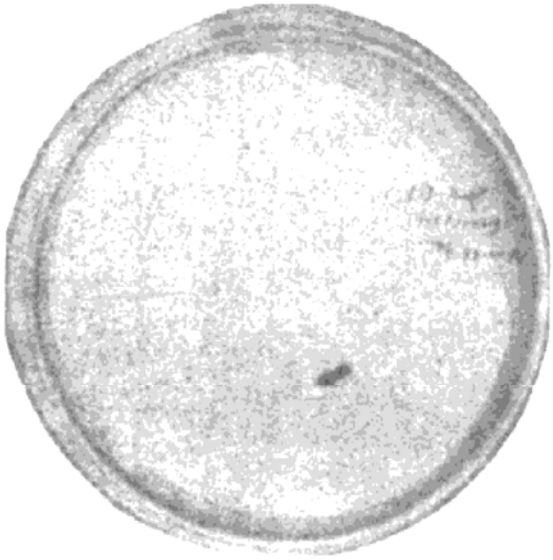

图 2 含重组体 pGEMP 菌落直接筛选

箭头指示白色的阳性克隆.

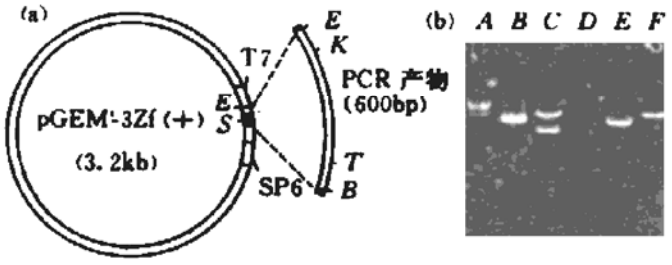

图 3 重组体 pGEMP 的构建和电泳鉴定

(a) pGEMP 的构建：*E*=EçoR I，*S*=Sma I，*B*=BamH I，*K*=Kpn I，*T*=Taq I. T7 代表 T7 启动子，SP6 代表 SP6 启动子. (b) 电泳鉴定：*A*：λ-Hind Ⅲ 片段；*B*：pGEMP-7 DNA；*C*：pGEMP-7 DNA+EcoR I (3.2 和 0.6kb)；*D*：纯化 PCR 产物 (0.6kb)；*E*：pGEMP-10 DNA；*F*：pGEMP-10 DNA+EcoR I (3.8kb).

$1.2\times10^5/\mu g$. 直接从含指示剂的平板选 4 个白色单菌落. 其质粒 DNA 经 EcoR I 酶切结果表明：含 3.8kb 片段的克隆 pGEMD-2 和-10 为正向插入，含 3.2 和 0.6kb 片段的克隆 pGEM-7 和-5 为反向插入 (图 3).

对克隆 pGEMP-10 插入片段的限制性酶分析，Kpn I (449/184bp)，Taq I (515/118bp)，Kpn I 和 Taq I (331/184/118bp) 片

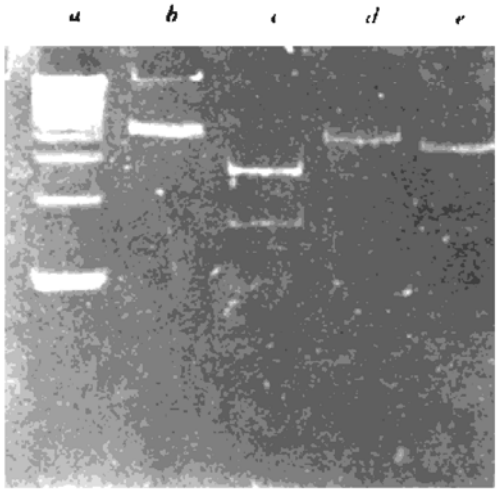

图 4 克隆 pGEMP-10 插入 DNA 的限制性分析

a：123 bp ladder 标准；*b*：插入 DNA；*c*：插入 DNA + Kpn I 和 Taq I；*d*：插入 DNA+Taq I；*e*：插入 DNA+Kpn I.

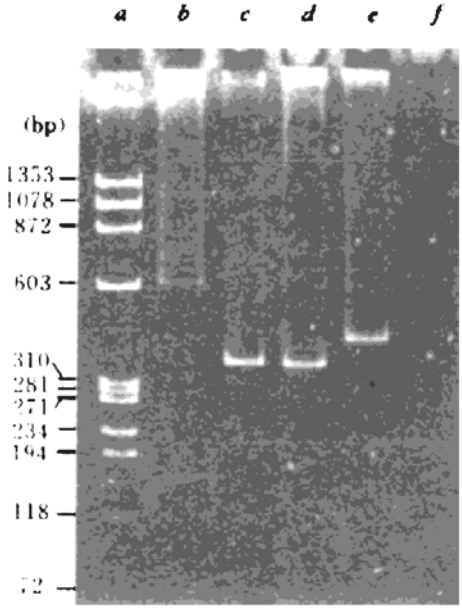

图 5 人脑 MBP 外显子区的成套引物扩增

a：ΦX-Hae Ⅲ 片段；*b*：引物 P_1-P_2 扩增外显子 I—Ⅶ；*c*：引物 P_1-P_4 扩增外显子 I—Ⅲ；*d*：引物 P_3-P_2 扩增外显子 Ⅲ—Ⅶ；*e*：引物 P_1-P_6 扩增外显子 I—Ⅴ；*f*：阴性对照 (λDNA 为模板，加成套引物扩增).

段长度与21.5kD人MBP编码序列相应的酶切位点相符(图4). 以该克隆的重组质粒DNA为模板，分别用P_1-P_2，P_1-P_4，P_3-P_2和P_1-P_6扩增出600，350，330和420bp的特异性区带，与人MBP外显子Ⅰ—Ⅶ，Ⅰ—Ⅲ，Ⅲ—Ⅶ和Ⅰ—Ⅴ等区段长度一致（图5）. 这表明成套引物扩增能对MBP家族中不同cDNA分子进行快速鉴定.

有关人脑MBP基因表达的研究报道甚少. Kamholz等[9]首次用鼠MBP cDNA克隆片段作探针，从人脑干/小脑cDNA文库中发现18.5kD（缺外显子Ⅱ），17.2kD（缺外显子Ⅱ和Ⅴ）和21.5kD等三种cDNA. Roth等[10]从人胎脊髓cDNA文库中发现20.5kD（缺外显子Ⅴ）cDNA，未从人脑中检出17和14kD（均缺外显子Ⅵ）的表达产物. 本文构建含人脑MBP全长编码序列的pGEMP克隆，为该基因体外转录和翻译的研究奠定了基础.

参 考 文 献

1 Mikoshiba K, Okano H, Tamura T *et al*. Annu Rev Neurosci, 1991; **14**: 201

2 Campagnoni A T. J Neurochem, 1988; **51**: 1

3 Kitamura K, Newman S L, Campagnoni C W *et al*. J Neurochem, 1990; **54**: 2052

4 Aruga J, Okano H, Mikoshiba K. J Neurochem, 1991; **56**: 1222

5 Streicher R, Stoffel W. Biol Chem Hoppe-Seyler, 1989; **370**: 503

6 陈俊杰，程汉华，李昌隆等. 科学通报，1993；**38**：1151

7 Sambrook J, Fritsch E F, Maniatis T. Molecular cloning, A laboratory manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989

8 Hemsley A, Amheim N, Toney M D *et al*. Nucleic Acids Res, 1989; **17**: 6546

9 Kamholz J, Ferra F D, Puckett C *et al*. Proc Natl Acad Sci USA, 1986; **83**: 4962

10 Roth H J, Kronquist K E, Rosbo K D *et al*. J Neurosci Res, 1987; **17**: 321

脯氨酰顺-反异构酶的纯化及对重组蛋白质折叠反应的催化性能*

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摘要 脯氨酰异构是蛋白质折叠反应的限速步骤之一，体内被脯氨酰顺-反异构酶（PPI）所催化. 为了研究PPI在重组蛋白体外折叠复性中的作用，我们自猪肾脏中纯化了PPI，并对重组蛋白的酶促折叠过程进行了探讨. 结果表明，PPI催化的重组蛋白的折叠反应主要是提高了它们的折叠速率，而不增加正确折叠率和比活性，PPI在很低的浓度下即有很高的催化活性.

关键词 重组蛋白，折叠，脯氨酰顺-反异构酶

蛋白质中的多数肽键，由于共振和立体效应，通常采用反式构象. 但脯氨酰由于其五元环的空间限制，当反应处于平衡时，约有10%—20%的Pro残基处于顺式构象，室温下Pro的顺-反异构反应约需数分钟完成. 当肽链完全伸展时Pro的异构反应处于自由进行的状态，而当肽链处于折叠状态时其异构反应就受到一定的限制，当Pro处于错误构象时，蛋白质的折叠反应根本无法进行，因为Pro的异构反应较蛋白质折叠反应慢得多，因而Pro异构

*“863”项目的一部分.

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Preparation and Properties of Long Half-Life Recombinant Human Superoxide Dismutase. Luo Xunyi. Wang Jingyi. Xie Bangtie. Li Zhanqing. Liu Xiaolin. Chen Xiaosui. (*Laboratory of Molecular Biology, Naval General Hospital, Beijing* 100037). *Prog. Biochem. Biophys.* (China). 1994; **21** (3): 234

Recombinant human Cu/Zn SOD (rhCu/Zn SOD) obtained from *E. coli* was covalently linked with an amphipathic molecule poly-(styrene-co-maleic acid) butyl ester (SMA) via amide linkage. When 42% free amino groups of the enzyme were modified, 88% remained enzyme activity was obtained. The results of circular dichroism of rhCu/Zn SOD and SMA-rhCu/Zn SOD indicated that the structure of the modified rhCu/Zn SOD was scarcely changed. Its biological half-life in blood was prolonged 22 times, and its abilities to resist pepsin and trypsin were increased significantly.

Key words rhCu/Zn SOD, chemical modification of protein, SMA, genetic engineering

Purification and Properties of the Alkaline CMCase Derived from *Bacillus sp.* O74. Wang Dong, Song Guijing, Gao Peiji. (*Institute of Microbiolgy, Shandong University, Jinan* 250100). *Prog. Biochem. Biophys.* (China). 1994; **21** (3): 237

An alkaline CMCase was partially purified from the culture medium of *Bacillus sp.* O74. The enzyme was purified 27.9 fold by sephadex G-100 gel filtration, ion-exchange chromatography and hydrophobic interaction chromatography. The enzyme was characterized by demonstration of optimum activity at 50 C and pH 7.0, and its molecular weight of 52 500 determined by gel filtration. The pH range of the enzyme showing the activity is from pH 4 to 12, and at pH 9 and 10, it can keep 80% and 70% of the maximum activity respectively. The enzyme was stable in the presence of the most metal ions, surface active agents and auxiliaries.

Key words purification, cellulase, alkaline

Extraction of DNA From Formalin-Fixed, Paraffin-Embedded Tissues for the Analysis of Immunoglobulin Heavy Chain Gene Rearrangement. Zhang Jianzhong, Zhu Yuanxiao, Wang Jianan, Zheng Jianqiang. (*Department of Pathology, Kegongwei Hospital, Beijing* 100101). *Prog. Biochem. Biophys.* (China). 1994; **21** (3): 241

Gene rearrangement analysis plays an increasing important role in the diagnosis of lymphomas. The possibility of detecting immunoglobulin heavy chain gene rearrangement in formalin-fixed, paraffin-embedded tissues was examined. 30 cases of lymphoid lesions, only 11 have been extracted with high molecular DNA which could be used in southern blot, and others showed DNA degradation in some degree. The degraded DNA could also be used in antigen receptor gene analysis after amplified by polymerase chain reaction. DNA analysis using paraffin-embedded tissues has potential clinical and research applications in detecting gene abnormalities in rare and difficult cases of lymphomas in which fresh specimen was not available.

Key words paraffin-embedded tissues, extraction of DNA, gene rearrangement analysis, lymphoma

Amplification, Cloning and Identification of Full-Length Coding Sequence for Human Myelin Basic Protein. Chen Junjie, Wang Ruohan, Cheng Hanhua, Chen Pu, Li Changlong.

Yang Luchuan. (*Department of Biochemistry, West China University of Medical Sciences, Chengdu* 610041). *Prog. Biochem. Biophys.* (China), 1994: **21** (3): 244

A series of DNA primers specific for human brain myelin basic protein (MBP) gene was designed and synthesized. MBP cDNA fragment about 600bp in length was amplified from human brain cDNA library by using polymerase chain reaction (PCR) with the specific primers P_1 and P_2. The recovered PCR product was flushed by klenow fragment and inserted into pGEM-3Zf (+) vector pretreated with Sma I and calf intestinal alkaline phophatase. The recombinant plasmid was used to transform competent cell JM 109. The positive colonies were directly screened on indicator plates. The recombinant plasmid DNA and insert fragment isolated from four positive colonies were analyzed by digestion with EcoR I, Kpn I and Taq I. The different coding sequences including MBP exon I—Ⅶ, I—Ⅲ, Ⅲ—Ⅶ and I—Ⅴ were amplified from these clones with their corresponding nested sets of primers respectively. These results show that these cDNA clones contain full-length coding sequence for 21.5kD human MBP.

Key words polymerase chain reaction (PCR), human brain cDNA library, coding sequence for myelin basic protein, vector pGEM-3Zf(+)

Studies on the Purification of PPI and its Catalyzing Activity for Folding of Recombinant Proteins. Xu Mingbo, Meng Wenhua, Ma Xiankai. (*Institute of Basic Medical Sciences*, P. O. Box 130-3, *Beijing* 100850). *Prog. Biochem. Biophys.* (China), 1994: **21** (3): 247

Proline isomerization catalyzed by peptidyl-prolyl *cis-trans* isomerase (PPI) *in vivo* is a limited procedure in protein folding. In order to study the catalyzing activity of PPI on the refolding of recombinant proteins *in vitro*, PPI is purified from pig kidney, and is investigated the effects of the enzyme on catalyzing the refolding process. Results indicate that PPI increases the folding rate without increasing the correct folding ratio and specific activity, and PPI has a high catalyzing activity even at very low concentration.

Key words recombinant protein, refolding, PPI

The Research on Vasoactive Intestinal peptide Binding With Nucleotides. Zhu Ping, D. MANN, R. GREENBERG. (*Veterinary Institute, University of Agriculture and Animal Sciences of PLA, Changchun* 130012). *Prog. Biochem. Biophys.* (China), 1994: **21** (3): 251

The interaction between VIP and nucleotide was tested with advanced photo-affinity technique. It was found that VIP can bind radiolabled GTP specifically and this binding could be competitively inhibited by cold GTP (unlabled GTP). The experiment indicated that not only GTP could inhibit the binding between VIP and hot GTP, but also all other nucleoside triphophates such as ATP, TTP, UTP could competitively inhibit this binding, although their inhibitions were a little weaker than GTP. It means VIP binding nucleoside triphosphate was a typical reversible binding reaction. It was found also that GDP, GMP at low concentration did not inhibit VIP binding hot GTP but enhanced the binding. Connecting with other researchers' results of GTP influencing on VIP-receptor interaction, it was considered that VIP could reversibly bind one of nucleoside triphosphates and this binding