深圳大学 2012 年硕士研究生入学考试初试试题

（答题必须写在答题纸上，写在本试题纸上无效）

考试科目代码：722 考试科目名称：医学细胞生物学

专业：生物医学工程

一、单项选择题（每题 2.5 分，共 100 分）：

1、Prokaryotic cells, but not eukaryotic cells, have:

A. endoplasmic reticulum.

B. histones.

C. nucleoid.

D. a nucleus.

E. a plasma membrane.

2、Mitochondria are associated with all of the following except.

A. ATP synthesis.

B. DNA synthesis.

C. protein synthesis.

D. hydrolysis of various macromolecules at low pH .

E. apoptosis.

3、Lysosomal enzymes:

A. are hydrolases .

B. usually operate at acidic pH.

C. are normally isolated from their substrates by the lysosomal membrane .

D. can lead to cellular digestion if the lysosomal membrane is disrupted.

E. all of the above are correct.

4、Cyclin-dependent kinases(Cdks):

A. occur only in the M phase.

B. are always inactivated by phosphorylation .

C. typically phosphorylate proteins on tyrosine residues.

D. in addition to binding cyclin , require other modifications for activity.

E. that phosphorylate Rb (retinoblastoma sensitivity) protein inhibit the synthesis of S-phase proteins.

三、解释下列专业名词（每题 5 分，共 50 分）：

1、胚胎干细胞（Embryonic stem cell）

2、操纵基因（Operator gene）

3、细胞表面(Cell surface)

4、蛋白质组(Proteome)

5、微粒体（microsomes）

6、实时荧光定量 PCR（Real-time fluorescent quantitative PCR）

7、化学渗透假说（Chemiosmotic coupling hypothesis）

8、反式作用因子（Tans-acting factor）

9、细胞周期检验点（Cell cycle checkpoint）

10、细胞去分化（Cell dedifferentiation）

四、简答题（每题 10 分，共 70 分）：

1、细胞质中合成的蛋白质如何转运至线粒体的功能部位并进行更新和组装？

2、细胞死亡有哪两种主要形式？分别给予解释。

3、说明 DNA 双螺旋模型的主要内容及其生物学意义。

4、pBR322 是一个经典的克隆载体，早期大量用于 DNA 片段的克隆等实验。该载体的酶切图谱见图 1。DL2000 为 DNA 分子量 marker，经 3%琼脂糖凝胶电泳分析结果见图 2。请用 BamHI 和 HindIII 双酶切 pBR322 载体后进行琼脂糖凝胶电泳分析，并用 DL2000 DNA 分子量 marker 做对照，请你绘制预期的核酸电泳结果示意图。

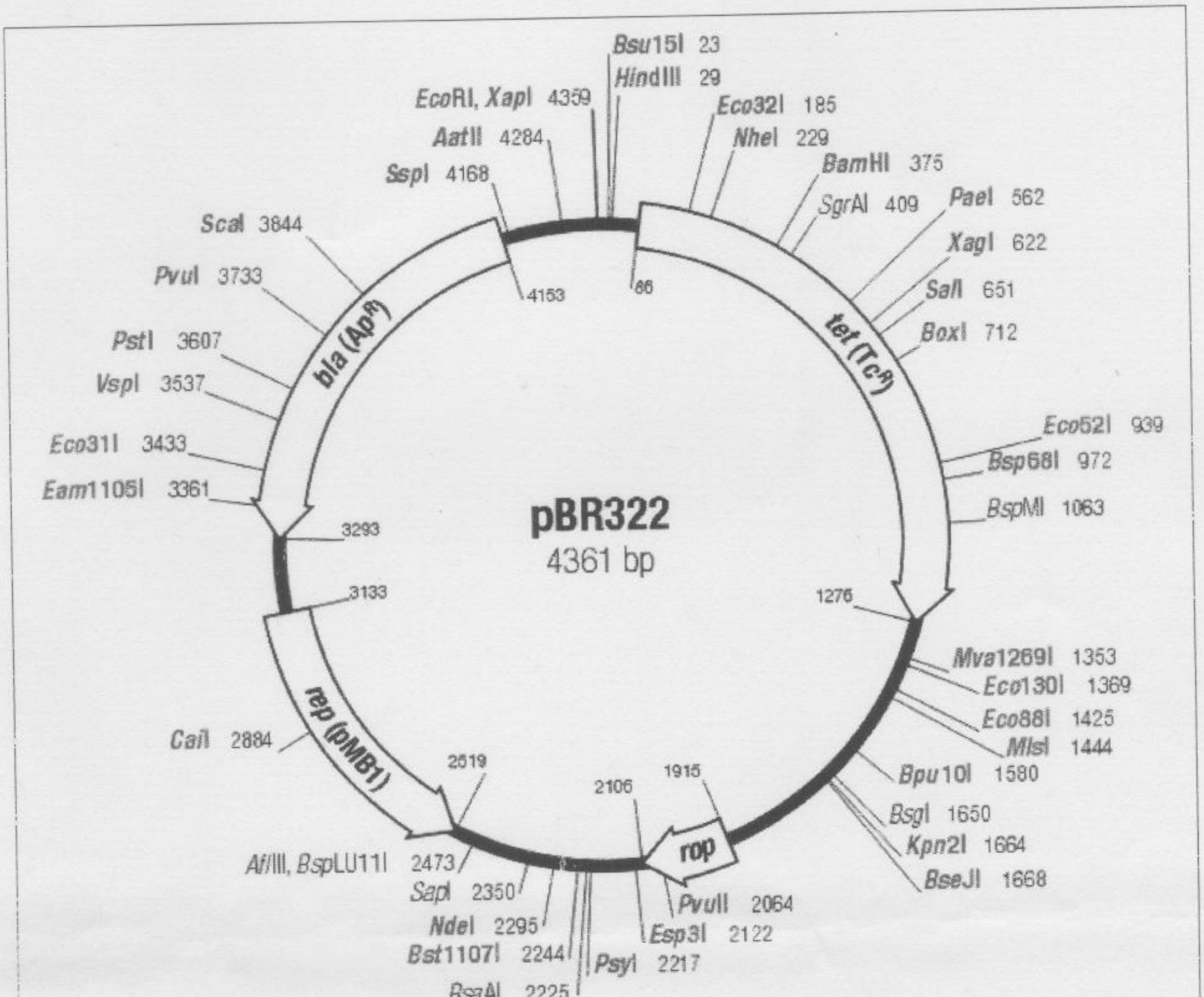

图 **1.pBR322** 载体的酶切图谱

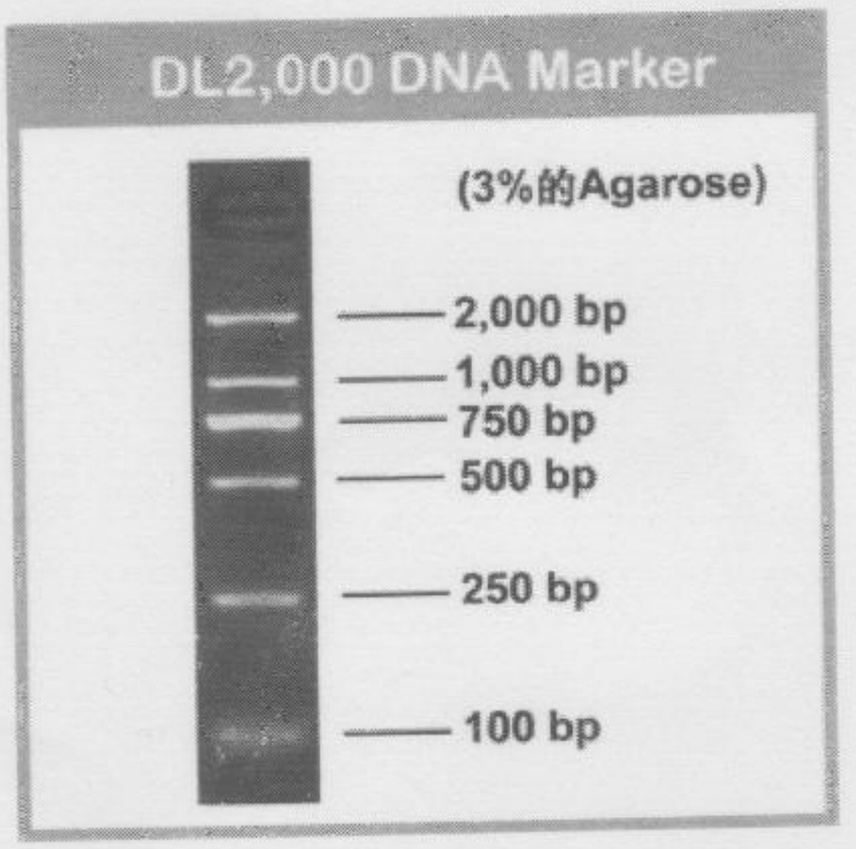

图 2 .DL2000 DNA Marker 电泳分析结果

5、实验室购买的细胞株以冻存管的形式运到以后，你应如何操作来复苏细胞（本题需要回答主要实验步骤）？

6、在一张细胞爬片上要同时检测 **Tubulin** 和 **Actin** 两种骨架蛋白，一抗和二抗应该如何选择（本题需要回答主要实验步骤）？

7、采用大肠杆菌表达外源基因时为何经常在目的基因 **5'**端或 **3'**端融合表达 **6** 个组氨酸（**6His-tag**）？

五、要求将下列文字翻译成中文（本题 20 分）：

Living cells are classified as either prokaryotes, which do not have a nucleus or internal membrane structures, or eukaryotes, which have a defined nucleus and intracellular organelles surrounded by membranes .Prokaryotes, which include bacteria and archaea(原生质体) , are usually unicellular but in some cases form colonies or filaments. They have a variety of shapes and sizes and can live under a variety of conditions, some very extreme. The plasma membrane is often invaginated(内陷) . Deoxyribonucleic acid, DNA, of prokaryotes is single-stranded and often segregated into a discrete mass, the nucleoid region that is not surrounded by a membrane or envelope. Even without defined membrane compartments, the intracellular milieu of prokaryotes are organized into functional compartments. Eukaryotes, which include yeasts, fungi, plants, and animals, have a volume 1000 to 10,000 times larger than that of prokaryotes. They have a well-defined membrane surrounding a central nucleus containing the bulk of the cell's DNA, along with a variety of intracellular structures and organelles. Intracellular membrane systems establish distinct cellular compartments, as described in Section 1.3, permitting a unique degree of subcellular organization. By compartmentalization, different chemical that require different environments can occur simultaneously. In addition, many reactions occur in or on specific membranes that create additional environments for diverse cellular functions.

六、请仔细阅读所附文献一，撰写该论文的中文摘要，总字数不得超过 400 个。（本题 **20** 分）

七、朊病毒的发现在科学上和医学上有何重要意义？通过阅读所附文献二阐明我国科学家在这一领域所做出的学术贡献（本题 20 分）。

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网络出版地址：http://www.cnki.net/kcms/detail/12.1329.R.20110726.1125.004.html

·基础研究·

miR-146a 调控骨髓间充质干细胞成骨分化的机制研究

随着生物材料和组织工程的兴起，骨髓间充质干细胞（bone marrow mesenchymal stem cells，BMSC）作为骨组织工程中重要的种子细胞越来越受到重视。虽然 BMSC 有多向分化的特点，但骨组织工程学要求其向单一方向分化，因此体外扩增和定向分化一直是制约其临床应用的重要因素[1]。笔者研究炎症和发育过程中重要的转录后调控分子——miRNAs 在 BMSC 成骨分化过程中的变化情况，探明其可能的分子机制，以期为寻找可能的 miRNA 药物提供理论依据，为提高干细胞修复组织缺损临床应用价值提供研究基础[2]。Runx 2 是成骨分化过程中重要的转录因子[3]，启动多个成骨相关基因的表达，可以作为成骨分化的重要标志，笔者研究中采用 Runx 2 的表达水平作为成骨分化的重要指征。

1 材料与方法

1.1 实验动物与试剂

Balb/c 小鼠 6 只，4 周龄；由广州军区广州总医院

作者单位：1.广州军区广州总医院 口腔科，广东 广州 510010；2.第四军医大学 口腔医学院 正畸科，陕西 西安 710032；3.美国加州大学 旧金山分校 牙医学院，美国 旧金山 94143；4.第四军医大学 唐都医院 口腔科，陕西 西安 710038

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动物中心提供,动物许可证号为 SCXK(军)2002-010。

达氏修正依氏培养液/F-12 (Dulbecco's modified Eagle's medium/ nutrient F-12 ham's, DMEM/F-12)(1∶1)(Gibco); 胎牛血清 (fetal bovine serum, FBS)(HyClone)(杭州四季青公司);0.25 %胰酶(Sigma); Lipofectamine 2000 试剂(Invitrogen);定量逆转录聚合酶链式反应 (quantitative reverse transcription-polymerase chain reaction, qRT-PCR)试剂盒(Omega),胰岛素(insulin)、消炎痛(indomethacin)、3-异丁基-1-甲基黄嘌呤(3-isobutyl-1-methylxanthine, IBMX)、地塞米松、维生素 C (vitamin C, VitC)、β-甘油磷酸钠(β-sodium glycerophosphate, β-GP)、骨形态发生蛋白-2(bone morphogenetic protein-2, BMP-2)(Sigma)。

1.2 骨髓间充质干细胞培养

取 4 周龄大鼠的股骨和胫骨,直接用培养液冲出骨髓,尽量把干垢端的骨髓冲洗干净。冲洗后不离心直接接种在培养瓶里,接种后 60 ~ 80 min,换液去除悬浮细胞;原代培养,24、48 h 各换液 1 次;观察细胞情况,在原代培养 7 d 左右时,如观察到成片的典型形态的细胞,在瓶底标记,用 0.25 %胰酶消化;传代到新瓶中, 加入少量培养液, 孵箱静置 20 ~ 30 min 后,BMSC 大多牢固贴壁。原代或传代的细胞如观察到少量成片的杂细胞,在超净台里用长吸管尖端机械刮除,吸出去掉。

1.3 成骨和成脂分化

取体外培养扩增的第 2 ~ 4 代 BMSC,在 12 孔板上细胞密度 1×10^4/孔 BMSC 铺板培养,共分 3 组,每组 3 个培养孔。对照组:用增殖培养液(growth medium, GM)培养;成脂诱导组:加入成脂肪培养液;成骨诱导组:加入成骨培养液(defined medium, DM),隔天换液。成骨分化诱导液成分如下:地塞米松浓度 1×10^{-8} mol/L, VitC 质量浓度 0.05 g/L, β-GP 浓度 0.01 mol/L, BMP-2 质量浓度 100 μg/L。隔天换液。成脂分化诱导液成分为:IBMX 浓度 0.5 mmol/L, 地塞米松浓度 1.0 μmol/L, 胰岛素质量浓度 2 mg/L。于成骨、成脂培养 14 d 后分别进行茜素红和油红 O 染色,确定分化效果。

1.4 miRNA 的定量逆转录聚合酶链式反应

收集对照组及成骨诱导组细胞 (作为实验组), 用 Trizol 提取 RNA, 采用 miScript Reverse Transcription Kit 试剂盒, 按照说明书进行反转录。设计 miR-146a 的聚合酶链式反应(polymerase chain reaction, PCR) 引物, 即 5′-TGAGAACTGAATTCCATGGGTT-3′, 下游引物由 Qiagen 试剂盒提供,采用的方法为 Sybr Green 荧光 qRT-PCR。同时,以 U6B 作为内参照,具体引物由 Qiagen 试剂盒提供。实时荧光定量 PCR 扩增目的基因, 测得相应的 Ct 值。miR-146a 表达丰度 $=2^{-(Ct_{miR-146a}-Ct_{U6B})}$,实验组 miR-146a 表达相对丰度 $=2^{-(Ct_{miR-146a}-Ct_{U6B})_{实验组}-(Ct_{miR-146a}-Ct_{U6B})_{对照组}}$。

1.5 茜素红染色和油红 O 染色

细胞成骨诱导分化结束后, 用磷酸盐缓冲溶液(phosphate buffered solution, PBS) 洗涤 3 次,75 %乙醇溶液固定 30 min。将固定后的细胞置于 0.1 %茜素红-Tris-HCl 液(pH 8.2)中染色 10 min;染色结束后用 1 %醋酸溶液快速冲洗, 不同浓度的乙醇依次脱水;用数码相机照相后分析成骨分化程度[4]。

油红 O 染色步骤如下: 称取油红 O 0.5 g, 溶于 100 mL 异丙醇(含量 98 %),制备成油红 O 饱和液。染色时,用蒸馏水充分洗涤,取油红 O 饱和液 6 mL, 加蒸馏水 4 mL,静置 5 ~ 10 min 后过滤制成稀释液,用油红 O 稀释液染色 10 ~ 15 min,避光,再用 60 %乙醇在光学显微镜下洗涤至间质清晰。

1.6 细胞瞬时转染

细胞按每个孔 5×10^5 个密度铺板,培养 12 h 后转染化学合成的阴性对照 RNA 双链或 miR-146a mimic(miR-146a antagonism)。转染操作如下:首先按待转染双链 RNA 以终浓度为 20 nmol/L 和 Lipofectamine 2000 试剂每孔 6 μL 的量将它们分别与 250 μL 无血清的 DMEM 混匀,5 min 后混合,在 37 ℃条件下孵育 25 min。吸尽 6 孔板中的培养液,每孔各加入无血清的 DMEM 800 μL, 将上述孵育好的 DNA/Lipofectamine 2000 混合物加入各孔, 轻轻振动培养板,混匀;转染细胞 37 ℃孵箱培养 4 ~ 6 h 后,将含有未转染入细胞的脂质体和质粒的无血清的培养液更换为含有常规培养液,继续培养。

1.7 miR-146a 对成骨分化相关基因 Runx 2 表达

首先将转染有阴性对照 RNA 序列和 miR-146a mimic(或 antagonism)的 BMSC 以 5×10^5 密度接种于 6 孔培养板上培养。在成骨分化培养液条件下培养 7 d。收集细胞,提取 RNA,反转录成 cDNA。采用 PCR 检测分化相关基因 Runx 2 的表达水平,同时以 β-actin 为内参照,观察细胞分化状态的改变。PCR 反应条件:95 ℃预变性 4 min,95 ℃,30 s,55 ℃,20 s,72 ℃,40 s,30 ~ 35 cycles。Runx2 的引物序列为:上游 5′-ATTCCTGTAGATCCGAGCAC-3′,下游 5′-ACGGTAACCACAGTCCCATC-3′;β-actin 的引物序列为:上游 5′-AGATCATGTTTGAGACCTTC-3′, 下游 5′-CATCTCCTGCTCGAAGTCTA-3′。PCR 产物经琼脂糖凝胶电泳(1.5 %的琼脂

糖凝胶，5 V/cm 的电压电泳 30 min 左右，使溴酚蓝电泳至琼脂糖胶的底端，终止电泳），紫外分析仪下观察 Runx 2 和 β-actin 的扩增条带的亮度，并照相。

1.8 统计学方法

采用 Graphpad 统计作图软件进行统计学分析，ANOVA 方差分析，比较组间差异，$P<0.05$ 为差异有统计学意义。

2 结果

2.1 骨髓间充质干细胞体外细胞培养建立

通过贴壁培养分离获取了 BMSC，光学显微镜下细胞成梭形（封三插图图 1A）。为了进一步确立 BMSC 的干细胞性质，将 2～4 代 BMSC 在体外进行成脂、成骨诱导。如封三插图图 1B 所示，在成脂分化培养液条件下，BMSC 能分化为脂肪细胞，油红 O 染色可见脂滴的形成。在成骨分化条件下，茜素红染色发现 BMSC 细胞可以分化为茜素红阳性的成骨细胞（封三插图图 1C）。

2.2 成骨分化过程中 miR-146a 表达

在成骨分化中，实验组随着细胞的分化，miR-146a 表达丰度下降，14 d 时为 $2^{-4.5}$，而对照组为 $2^{-3.4}$；两组比较，差异有统计学意义（$P<0.05$）（表 1）。见图 2。

表 1 成骨分化过程中 miR-146a 的表达变化

Table 1 Change of expression of miR-146a during the osteogenic differentiation

项目	Ct_{18S}	$Ct_{miR-146a}$	ΔCt	ΔΔCt	P
对照组	21.2 ± 0.12	24.6 ± 0.15	3.4	0 ± 0.15	< 0.05
实验组	21.3 ± 0.15	25.8 ± 0.11	4.5	1.1 ± 0.11	

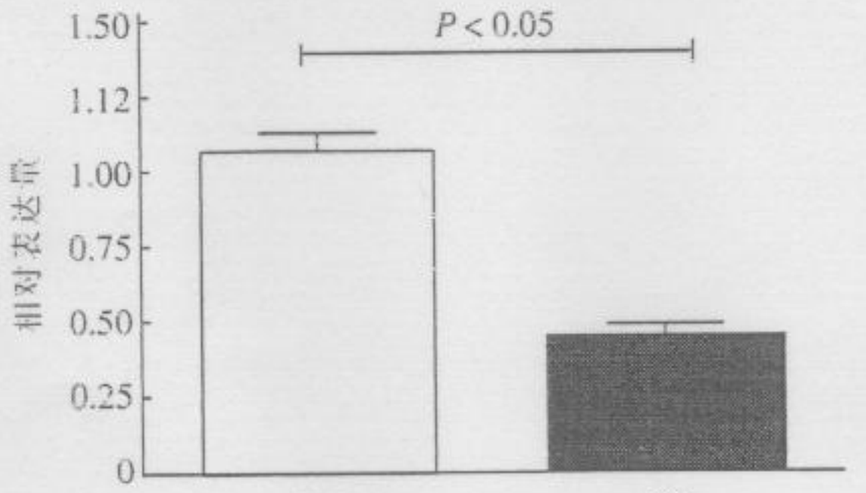

图 2 成骨诱导条件下 BMSC 中 miR-146a 表达下降

Figure 2 Decreased miR-146a expression under osteogenic differentiation

2.3 miR-146a 抑制成骨分化

上述结果提示 miR-146a 可能参与 BMSC 成骨分化的调控。为此，首先在 BMSC 过表达 miR-146a 或对照 RNA 序列，同时给予成骨分化诱导剂。结果发现，miR-146a 显著抑制了细胞的成骨分化标志 Runx 2 的表达，表明 miR-146a 抑制成骨细胞分化。相反，转染化学合成的 miR-146a 拮抗体 antago-miR-146a，发现早期分化标志 Runx 2 的表达水平增加；进一步说明 miR-146a 可以抑制成骨分化。见图 3。

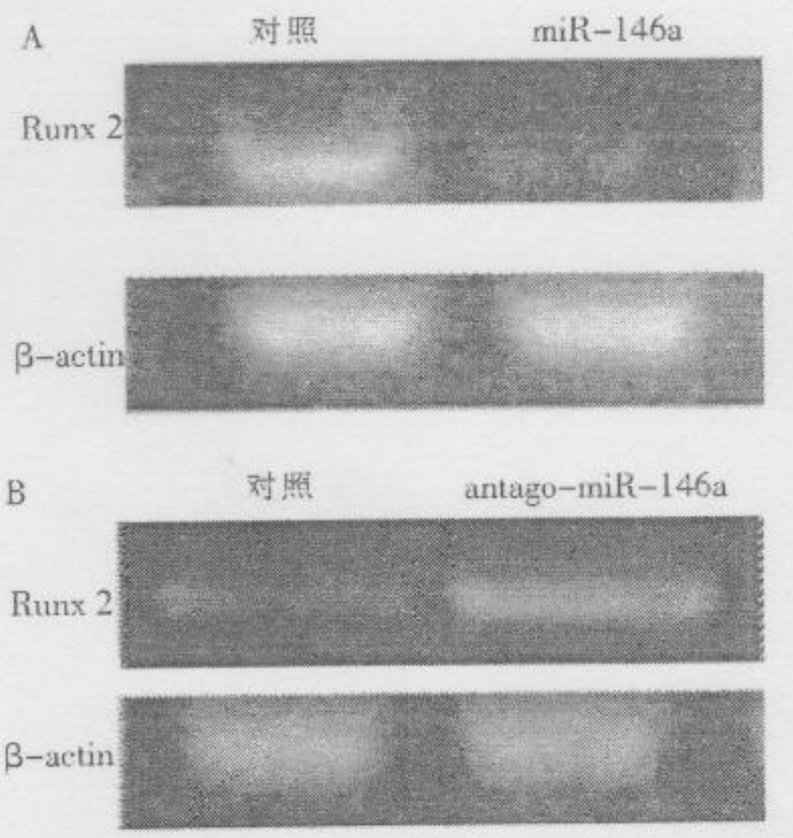

图 3 miR-146a 对成骨分化的影响（A.细胞过表达 miR-146a 可以抑制早期分化标志 Runx 2 的表达；B. 细胞转染 antago-miR-146a 可以增加早期分化标志 Runx 2 的表达）

Figure 3 Effect of miR-146a in osteogenic differentiation(A.over-expression of miR-146a inhibits expression of Runx 2; B. Cell transfected antago-miR-146a could increased Runx 2 expression)

3 讨论

在骨组织工程中，骨组织再生要求有 3 个基本的生物学因素参与[5]，即种子细胞、生长和分化因子、细胞外基质材料。从促进骨再生的治疗角度而言，如何增强 BMSC 体外扩增效率，同时保证移植入患者的细胞能够定向分化成目的细胞是该领域的关键课题[6,7]。而阐明 BMSC 增殖和分化的分子机制对于实现增强体外扩增和体内分化至关重要。随着基因技术的发展，可以在种子细胞体外扩增时对其进行基因修饰，使种子细胞更适于组织工程的需要。

就间充质干细胞的成骨分化而言，多个细胞因子多条信号通路参与分化调控，影响 BMSC 向成骨细胞分化的因素很多，其中生长因子的影响尤为重要[8]。越来越多的研究表明，BMP 是 BMSC 成骨分化中最为重要的一类调节分子，是 Runx 2 诱导成骨分化必须的信号刺激之一[9]。笔者研究发现 miRNA 是 BMSC 成骨分化的重要调控分子。

miRNA 是一类含有 18～24 bp 的非编码 RNA，装载入 RISC 复合体的单链 miRNA 通过识别与自身部分互补（主要是与 miRNA 第 2～8 的 seed sequence 互补）的靶分子 mRNA，调节靶分子的稳定性和翻译

效率，有效地抑制基因的表达[5]。近年来，研究表明 miRNA 参与的转录后调控在胚胎发育、系统发生、组织分化及疾病演变中扮演非常重要的功能；在间充质干细胞成骨分化过程中，miRNA 发挥重要功能。首先在间充质干细胞中存在大量 miRNA 的表达，它们在干细胞的功能维持和定向分化中扮演重要作用[10]。研究表明，miR-196a 可以通过抑制 HOXC8 基因的表达，促进脂肪细胞来源的间充质干细胞增殖而抑制其成骨分化[11]。而 miR-26 可以通过抑制 Smad1 的表达抑制成骨分化，相反其拮抗体可以增强 Smad1 的表达，促进分化[12]。笔者研究发现，在 BMSC 诱导分化的过程中，miR-146a 的表达下降，当过表达 miR-146a 时，分化培养条件下 BMSC 的分化早期相关基因 Runx 2 下调，说明过表达 miR-146a 对成骨分化有抑制作用。而利用 antago-miR-146a 可以补救 Runx 2 的下降，说明拮抗 miR-146a 对骨 BMSC 成骨分化有促进作用。因此，miR-146a 在 BMSC 成骨分化过程中扮演重要的作用。既往研究表明，炎症等刺激可以改变 miR-146a 的表达，进一步探讨炎症是否通过改变 miR-146a 的表达影响成骨细胞分化具有重要意义[13]。

笔者研究所用的转染为瞬时转染，在体内有效时间一般为 3 d，因此只能观察到分化早期的标志分子 Runx 2，因此笔者所在课题组目前正在尝试建立长效载体系统，已取得初步成功，进一步的研究期望通过转染后细胞体内移植，观察过表达和拮抗 miR-146a 对分化过程中的 BMSC 的成骨能力的影响，进一步确定该分子在 BMSC 体内体外成骨能力调控方面的作用，为临床合理利用小分子 RNA 干预提供一定的理论依据。

BMSC 作为骨组织工程的重要种子细胞之一，其移植为解决骨折不愈合、骨囊肿、骨缺损、股骨头缺血性坏死等提供了重要的手段。但 BMSC 移植中还有很多问题有待解决。笔者研究重点关注 miRNAs 在炎症和发育过程中重要的转录后调控分子的研究，阐明其在 BMSC 分化过程中的调控作用，以期为控制 BMSC 成骨分化向着临床需要的方向进行提供理论依据。

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miR-146a 调控骨髓间充质干细胞成骨分化的机制研究(正文第 415 页插图)

图 1　BMSC 体外培养及诱导分化(A.BMSC 的光学显微镜下形态；B.细胞成功分化为脂肪细胞；C.细胞成功分化为成骨细胞)

Figure 1　Culture and differentiation of BMSC *in vitro*(A. morphology of BMSC by optical microscope; B. adipogenic differentiation of BMSC; C. osteogenic differentiation of BMSC)

Generating a Prion with Bacterially Expressed Recombinant Prion Protein

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The prion hypothesis posits that a misfolded form of prion protein (PrP) is responsible for the infectivity of prion disease. Using recombinant murine PrP purified from *Escherichia coli*, we created a recombinant prion with the attributes of the pathogenic PrP isoform: aggregated, protease-resistant, and self-perpetuating. After intracerebral injection of the recombinant prion, wild-type mice developed neurological signs in ~130 days and reached the terminal stage of disease in ~150 days. Characterization of diseased mice revealed classic neuropathology of prion disease, the presence of protease-resistant PrP, and the capability of serially transmitting the disease; these findings confirmed that the mice succumbed to prion disease. Thus, as postulated by the prion hypothesis, the infectivity in mammalian prion disease results from an altered conformation of PrP.

Transmissible spongiform encephalopathies (TSEs or prion disease) are infectious neurodegenerative disorders. The prion hypothesis (*1*) proposes that the infectious agent is an aberrant conformational isoform of the normal PrP (PrP^C), a glycosylphosphatidylinositol (GPI)–anchored glycoprotein. By virtue of its self-perpetuating characteristic, the aberrant isoform (PrP^{Sc}) converts host PrP^C into the PrP^{Sc} conformation and leads to neurodegeneration (*2–4*). Despite strong supporting evidence (*5–11*), a crucial prediction derived from the prion hypothesis—that an infectious prion can be generated with bacterially expressed recombinant PrP (recPrP)—remains unfulfilled (*2*, *12*), leaving lingering doubts about the prion hypothesis (*13*).

Recombinant PrP has been folded into various forms similar to PrP^{Sc}, but none of them fully recapitulates the characteristics of the infectious agent (*2*, *12*). The amyloid fiber of a recPrP fragment (recPrP89-230) causes prion disease in transgenic mice overexpressing PrP89-231 (*10*), but a prolonged incubation time in mice overexpressing PrP has led to uncertainty about whether the infectivity is indeed derived from recPrP89-230 amyloid fibers (*2*, *12*). The difficulty in creating a recombinant prion is likely due to the lack of proper facilitating factors (*14*). Polyanions, particularly RNA, have been found to facilitate PrP conversion and promote de novo prion formation (*9*, *15–17*). We investigated lipid as a potential facilitating factor because GPI-anchored PrP^C is in the vicinity of lipid membranes and the interfacial lipid bilayer region strongly influences protein structure (*18*). Encouraged by the findings that lipid interaction converts recPrP to a PrP^{Sc}-like form (*19*), we applied protein misfolding cyclic amplification (PMCA) (*8*) to study recPrP

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conversion in the presence of both lipid and RNA.

Using a serial PMCA protocol (*20*), we tested 16 different conditions in which recPrP was mixed with various combinations of lipids and/or total RNA isolated from normal mouse liver. In the presence of the synthetic anionic phospholipid POPG (1-palmitoyl-2-oleoylphosphatidylglycerol) and RNA, a 15-kD proteinase K (PK)–resistant band was detected after 17 rounds of PMCA (Fig. 1A). Once formed, the PK-resistant recPrP (rPrP-res) was able to serially propagate (Fig. 1A and fig. S1). The same procedure was repeated several times, and the overall efficiency of de novo rPrP-res formation was found to be ~20% (fig. S2).

Serial PK-digestion of rPrP-res revealed that the PK-resistant band was detectable after digestion with high concentrations of PK (200 μg/ml; PK:recPrP molar ratio > 50:1) (Fig. 1B). After centrifugation, the rPrP-res was detected only in the pellet fraction (Fig. 1C); moreover, the 15-kD PK-resistant band was not recognized by the 8B4 antibody, which detects an N-terminal epitope of PrP (*21*) (Fig. 1C). These findings show that, similar to PrP^{Sc}, rPrP-res is aggregated, is PK-resistant, and contains a C-terminal PK-resistant core.

Next, we performed PMCA and cell culture analyses to determine whether rPrP-res could seed glycosylated and GPI-anchored endogenous PrP^{C}. With normal mouse brain homogenate as substrate, PMCA was carried out with or without rPrP-res seed. The PK-resistant endogenous PrP, as demonstrated by higher molecular weights of glycosylated PrP, was detected in samples seeded with rPrP-res (Fig. 1D). In reactions without rPrP-res seed, no PK-resistant PrP was detected, allowing us to rule out de novo PrP-res formation or insufficient PK digestion. The cell infection assay was performed on SN56 cells, a murine neuronal cell line susceptible to prion infection (*22*). Endogenous PrP^{C} in SN56 cells was glycosylated and sensitive to PK digestion (Fig. 1E). After rPrP-res infection, the PK-resistant endogenous PrP was detected in cells after 2 passages and remained detectable after 17 passages (Fig. 1E). A similar experiment revealed that the rPrP-res–converted normal mouse brain homogenate (Fig. 1D) could infect SN56 cells as well (fig. S3). Thus, rPrP-res is able to propagate its PK-resistant conformation to endogenous PrP^{C}.

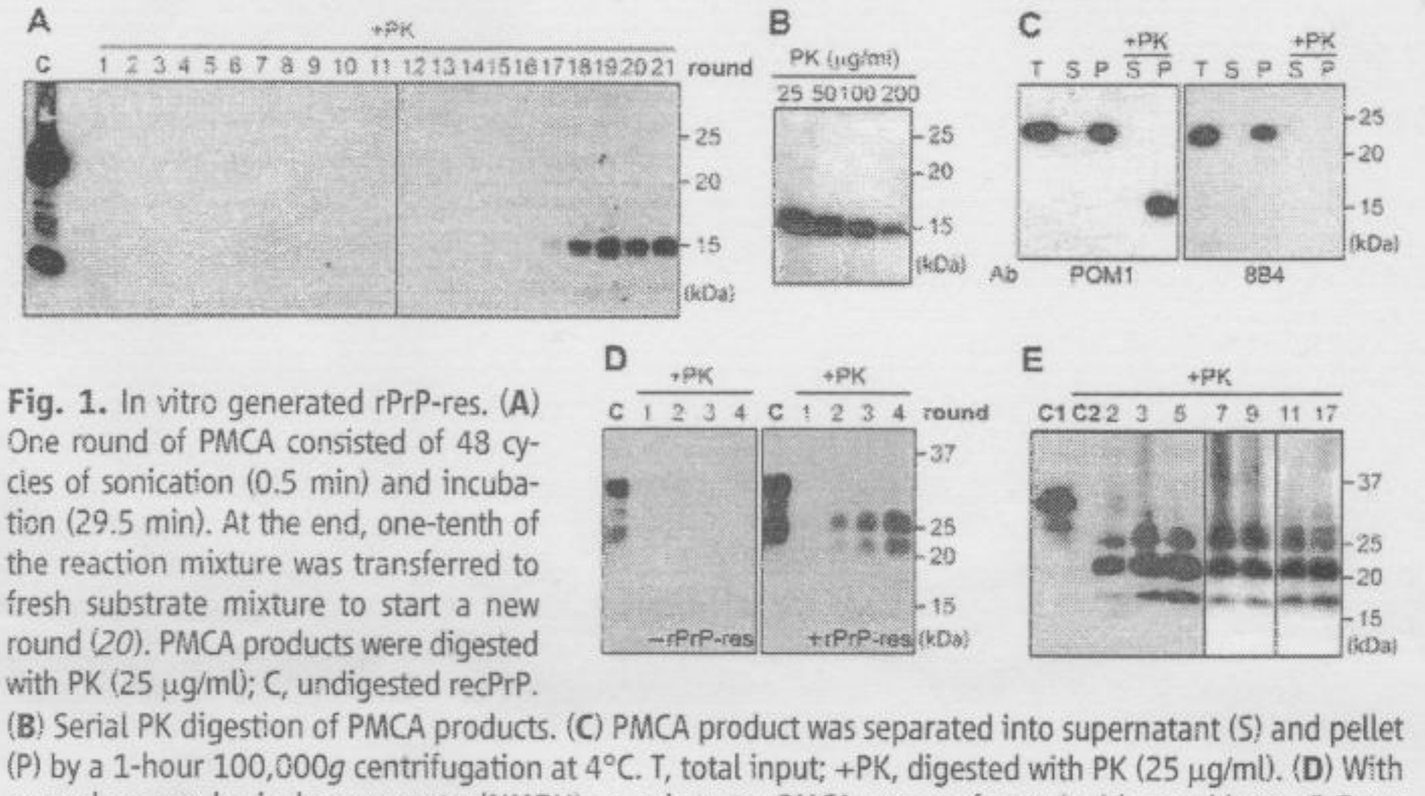

Fig. 1. In vitro generated rPrP-res. (**A**) One round of PMCA consisted of 48 cycles of sonication (0.5 min) and incubation (29.5 min). At the end, one-tenth of the reaction mixture was transferred to fresh substrate mixture to start a new round (*20*). PMCA products were digested with PK (25 μg/ml); C, undigested recPrP. (**B**) Serial PK digestion of PMCA products. (**C**) PMCA product was separated into supernatant (S) and pellet (P) by a 1-hour 100,000*g* centrifugation at 4°C. T, total input; +PK, digested with PK (25 μg/ml). (**D**) With normal mouse brain homogenate (NMBH) as substrate, PMCA was performed with or without rPrP-res seed. Product was digested with PK (100 μg/ml); C, undigested NMBH. (**E**) After rPrP-res infection, SN56 cells were lysed, digested with PK (25 μg/ml), and centrifuged. The PK-resistant PrP in the pellet was detected by immunoblot analysis. Numbers indicate cell passages. C1, undigested SN56 cell lysate; C2, pellet of PK-digested, uninfected SN56 cell lysates. In all panels, PrP was detected by immunoblot analysis with POM1 antibody to PrP except for the right panel of (C), where 8B4 antibody was used. PK digestion was carried out at 37°C for 30 min [(A), (B), and (C)] or 1 hour [(D) and (E)].

To determine whether rPrP-res was capable of causing bona fide prion disease, we infected 8-week-old female CD-1 mice by intracerebral injection. The rPrP-res (inoculum 4) was prepared by propagating rPrP-res through 24 rounds of PMCA. All PMCA products were pooled together and centrifuged through a sucrose cushion. The pellet was washed, resuspended, and used for inoculation. Three control inocula were used for animal study (Table 1). Inoculum 1, consisting of all the components used for rPrP-res propagation except for recPrP and rPrP-res seed, was subjected to the same treatments as inoculum 4. Inoculum 2, consisting of all the components of rPrP-res propagation except for rPrP-res seed, was incubated at 37°C for 24 days without sonication and subjected to the same pelleting and washing treatments. Omitting the sonication step prevented the de novo rPrP-res formation in this control sample, as confirmed by the PK digestion analysis described below (Fig. 2A). Inoculum 3 was prepared by directly mixing recPrP, POPG, and RNA in the inocu-

Table 1. Intracerebral inoculation of rPrP-res.

Inoculum	Component	Processing	Preparation for injection	Diseased/ inoculated	Survival time (dpi)*
1	Buffer + POPG + RNA (the amount of each component equaled that in the rPrP-res propagation reaction)	Serial PMCA	Pelleting through a sucrose cushion and washing twice with PBS	0/15	>360
2	Buffer + POPG + RNA + recPrP (the amount of each component equaled that in the rPrP-res propagation reaction)	Incubated at 37°C without sonication	Pelleting through a sucrose cushion and washing twice with PBS	1†/14	>360 (286†)
3	POPG + RNA + recPrP (the amount of each component equaled that in the final pool of inoculum 4)	No processing	No preparation	0/5	>360
4 (rPrP-res)	Buffer + POPG + RNA + recPrP + rPrP-res seed	Serial PMCA	Pelleting through a sucrose cushion and washing twice with PBS	15/15	150 ± 2.2 (mean ± SEM)

*One mouse from each group was euthanized at 275 dpi to serve as controls. †One mouse died from an unrelated disease at 286 dpi. It had no neurological signs or weight loss.

5、利用放射性同位素标记物质能使照相乳胶感光的原理来检测细胞内某种物质的含量与分布的方法是：

A. 免疫荧光显微镜技术

B. 放射自显影技术

C. 单克隆抗体技术

D. 离心技术

E. 核酸分子杂交技术

6、蛋白质分子的β折叠是：

A. 基本结构

B. 一级结构

C. 二级结构

D. 三级结构

E.四级结构

7、分离出单个细胞在适当的条件下使之增殖成均一的细胞群体称为：

A. 细胞培养

B. 原代培养

C. 传代培养

D. 细胞融合

E. 细胞克隆

8、催化生物体代谢的酶的化学本质是：

A. 核蛋白

B. 糖苷

C. 脂类

D. 蛋白质

E. 维生素

9、DNA 和 RNA 水解后的产物比较是：

A. 碱基相同，核糖不同

B. 碱基不同，核糖相同

C. 核糖不同，部分碱基不同

D. 碱基不同，核糖不同

time was 166 ± 1.5 days (fig. S11). The marginal increase in the survival time of second-round transmission could be due to the reported variation among inoculation experiments (*23*) or to the influence of other components in the brain homogenate used in second-round transmission. Nonetheless, PrP^{Sc} was detected in all groups of mice inoculated with diseased mouse brain homogenates, but not in control mice (Fig. 3H). The spongiosis pattern remained similar to that of rPrP-res–inoculated mice (fig. S12). Thus, similar to natural prion disease, the rPrP-res–caused disease can be serially transmitted.

Inadvertent contamination is always a concern for PMCA. The only naturally occurring prion used in our lab was the RML strain, which was used only three times in our failed attempts to convert recPrP. During the past 2 years while we were working with rPrP-res, absolutely no naturally occurring prion was used. Our latest de novo rPrP-res formation (fig. S2) was achieved in a new sonicator, and the substrate was prepared in a lab that has never been exposed to prion. Furthermore, both the behavioral and pathological phenotypes of rPrP-res–inoculated mice were clearly different from those reported for RML-infected mice (*24*). Thus, it is highly unlikely that rPrP-res formation was due to an inadvertent contamination. Note also that before the inoculum was prepared, the rPrP-res had been propagated for more than 35 rounds of PMCA. Thus, even if the initial rPrP-res formation were due to contamination, the $>10^{35}$ dilution had ensured that recPrP was the only PrP in the inoculum (Fig. 2A). We therefore conclude that the disease-causing agent was rPrP-res.

The three main components in our system were recPrP, POPG, and RNA. The purity of recPrP was verified by silver staining, and recPrP was the only protein detected (fig. S13). The mouse liver RNA was chosen because PrP is not normally expressed in liver and because ectopic PrP expression in the liver of PrP-null mice does not support prion propagation (*25*). Because synthetic polyanions that do not encode protein can replace RNA in cell-free prion formation and propagation (*9*, *16*, *17*), the likely role of RNA in generating infectious prions is to facilitate PrP conversion rather than to encode an infectious protein. Indeed, we were able to propagate rPrP-res with the use of synthetic polyadenylated RNA (fig. S14), which shows that rPrP-res can be generated with virtually completely defined components. The requirement of lipid is in accordance with previous reports of higher prion infectivity in lipid membrane–associated PrP^{Sc} (*22*, *26*). Notably, the purified GPI-anchored PrP^{C}, which was used to produce infectious prion de novo (*9*), contained stoichiometric amounts of copurified lipids, supporting a general role of lipid in PrP conversion. Of note, the POPG and RNA used here may simply mimic one or more unknown in vivo facilitating factors. Further studies are required to identify these factors.

Our results provide direct evidence in support of the prion hypothesis. We found that rPrP-res is in a conformational state similar to the pathogenic PrP^{Sc} isoform, that rPrP-res possesses the self-perpetuating characteristic of a prion, and that rPrP-res causes bona fide prion disease in wild-type mice. The fact that only rPrP-res–inoculated mice developed prion disease establishes that prion disease is caused by the altered conformational form of PrP.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1183748/DC1
Materials and Methods
Figs. S1 to S14
Movies S1 to S3
References

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E. 核糖相同，部分碱基不同

10、核酸分子的空间结构呈三叶草形是：

A. DNA

B. mtDAN

C. tRNA

D. rRNA

E. mRNA

11、原核细胞和真核细胞都具有的细胞器的是：

A. 线粒体

B. 高尔基体

C. 中心体

D. 核糖体

E. 细胞骨架

12、密码子的简并性指：

A. 一些密码子可缺少一个嘌呤碱或嘧啶碱

B. 密码子有许多碱基

C. 大多数氨基酸有一组以上的密码子

D. 一些密码子适用于一种以上的氨基酸

E. 以上都不是

13、蛋白质分子中能表现蛋白质特异性的部位是：

A. 氨基

B. 羧基

C. 肽键

D. 氢键

E. 侧链

14、不同细胞的细胞周期时间的差别主要取决于什么期的长短：

A. G_0

B. G_1

C. S

D. G_2

E. M

15、线粒体的嵴是由:

A. 线粒体内膜凹陷形成的

B. 线粒体外膜凹陷形成的

C. 内膜和外膜凹陷形成的

D. 内膜或外膜凹陷形成的

E.基粒聚合成的

16、细胞膜中含量最丰富的脂质是:

A. 磷脂

B. 胆固醇

C. 糖脂

D. 神经节苷脂

E. 唾液酸

17、分裂前期开始的标志:

A. 间期的染色质纤维开始螺旋化、折叠

B. 核仁解体

C. 核膜消失

D. 纺锤体形成

E. 染色体向赤道面运动

18、远距离细胞之间的分化调节作用是通过什么完成的:

A. 细胞诱导

B. 细胞抑制

C. 细胞识别

D. 激素

E. 细胞黏附因子

19、外显子是指:

A. 基因突变的表现

B. 断裂开的 DNA 片段

C. 不转录的 DNA，即反义链

D. 真核生物基因中为蛋白质编码的序列

E. 真核生物基因的非编码的序列

20、克隆羊的成功一方面说明了体细胞核保存着全部的遗传信息，另一方面也说明了下列哪项对细胞的决定和分化起着关键性的作用：

A. 体细胞质

B. 卵细胞核

C. 卵细胞质

D. 体细胞核

E. 细胞器

21、三羧酸循环发生于线粒体的：

A. 外膜

B. 内膜

C. 基质

D. 膜间腔

E. 基粒

22、染色体末端具有能维持染色体结构稳定性的结构是：

A. 着丝粒

B. 动粒

C. 端粒

D. 随体

E. 副缢痕

23、矽肺的形成主要是由于：

A. 线粒体呼吸链破坏

B. 溶酶体膜破坏

C. 溶酶体酶缺乏

D. 过氧化物酶体内氧化酶缺乏

E. 过氧化物酶体膜破坏

24、显微镜油镜常用的油为：

A. 汽油

B. 煤油

C. 液状石蜡

D. 香柏油

E. 松油

25、下述不属于反式作用因子结构域的是：

A. 锌指结构域

B. α 螺旋-转角-α 螺旋结构域

C. 亮氨酸拉链

D. 基质相关区域

E. 螺旋环螺旋结构域

26、对细胞癌基因叙述正确的是：

A. 只在肿瘤细胞中出现

B. 加入化学致癌物在正常细胞才会出现

C. 正常人细胞也检测到癌基因

D. 细胞经转化后才出现

E. 正常人感染了致癌病毒才会出现

27、细胞凋亡的最显著特征是：

A. 光镜下可见细胞肿胀

B. 细胞代谢障碍并有脂肪变性

C. 细胞成分自控性的自我降解

D. 细胞结构广泛酶分解

E. 蛋白质分子的合成

28、TATA 框的作用是：

A. 决定转录起始的频率

B. 决定转录起始点

C. 具有转录终止功能

D. 能增强转录能力

E. 位于基因的任何位置

29、在适当的条件下，两条互补的单链 DNA 可以配对形成双螺旋的过程称为：

A. 活化

B. 融合

C. 复制

D. 变性

E. 复性

30、真核细胞具有的特征：

A. 转录和翻译在同一场所同时进行

B. 有各种膜性结构的细胞器

C. 都含有细胞壁

D. 无细胞骨架

E. 以上都是

31、Rb 基因是：

A. 结构基因

B. 操纵基因

C. 调节基因

D. 抑癌基因

E. 癌基因

32、真核细胞的核外遗传物质存在于：

A. 核膜

B. 核蛋白体

C. 细胞质

D. 内质网

E. 线粒体

33、目前被广泛接受的生物膜分子结构模型是：

A. 片层结构模型

B. 单位膜模型

C. 液态镶嵌模型

D. 晶格镶嵌模型

E. “脂筏”模型

34、干细胞增殖的最主要特征是：

A. 干细胞增殖的缓慢性和不确定性

B. 干细胞增殖的缓慢性和干细胞增殖系统的自稳定性

C. 干细胞增殖的缓慢性和干细胞增殖系统的不稳定性

D. 干细胞增殖迅速，可大量获得分化细胞

E. 干细胞增殖系统的不稳定性导致其必须依靠程序性细胞死亡来保持干细胞数量的恒定

35、关于膜流，下面方向正确的是：

A. 质膜——大囊泡——高尔基复合体

B. 高尔基复合体——粗面内质网——质膜

C. 粗面内质网——高尔基复合体——滑面内质网

D. 内质网——高尔基复合体——质膜

E. 滑面内质网——大囊泡——高尔基体

36、糖酵解的场所是：

A. 核基质

B. 细胞质基质

C. 线粒体内膜

D. 线粒体基质

E. 线粒体的膜间腔

37、多聚核糖体是指：

A. 细胞中有两个以上的核糖体集中成一团

B. 一条 mRNA 串联多个核糖体的结构组合

C. 细胞中两个核糖体集成簇状或菊花状结构

D. rRNA 的聚合体

E. 附着在内质网上的核糖体

38、细胞骨架系统的主要化学成分是：

A. 多糖

B. 脂类

C. 蛋白质

D. 核酸

E. 糖脂

39、常染色质与异染色质的共同特点是：

A. 在核内的分布

B. 化学成分

C. 转录活性

D. 折叠和螺旋化程度

E. 染色深浅

40、在表达过程中不受时间限制的基因是：

A. 管家基因

B. 奢侈基因

C. 免疫球蛋白基因

D. 血红蛋白基因

E. 胰岛素基因

二、填空题（每题 2 分，共 20 分）：

1、_____是指细胞或生物体的一套完整的单倍体遗传物质，是所有染色体上全部基因和基因间的 DNA 的总和。

2、真核细胞结构复杂，具有精细的专门功能的结构单位，如：生物膜系统、遗传信息表达系统、_____、核糖体和细胞质溶胶等。

3、酵母双杂交主要用于研究_____ 。

4、与受体结合的生物活性物质统称为_____，包括激素、神经递质、生长因子、某些药物和毒物等。

5、 细胞内信使是指受体被激活后在细胞内产生的、能介导信号转导的活性物质，又称为第二信使。已经发现的细胞内信使有许多种，其中最重要的有：cAMP、cGMP、二酯酰甘油、三磷酸肌醇和_____离子等。

6、细胞连接是维系细胞间相对稳定的特化连接装置，也是相邻细胞之间协同作用的重要组织形式。细胞连接有多种类型，根据其结构和功能特点可分为三大类，即封闭连接，_____和通讯连接。

7、核骨架是细胞核中一个以_____（成分）为主构成的纤维网架结构，它不仅在维持细胞核的形态方面，而且在染色体组装、DNA 复制和基因转录调控等一系列活动中发挥重要作用。

8、_____是高尔基复合体中最具有特征性的标志酶。

9、目前一般将基因分为结构基因和_____。

10、对蛋白质的分离主要依靠柱层析法，其中_____层析效果最好。