

中国科学院遗传研究所

研究工作年報

Annual Report
of
the Institute of Genetics
Academia Sinica

1979

《研究工作年報》編輯小組

北京大學出版社

中国科学院遗传研究所

研究工作年报

(1979)

《研究工作年报》编辑小组

北京大学出版社
1980

内 容 简 介

本《年报》共收集了研究论文 70 篇,反映了遗传研究所 1979 年所取得的主要成果,此外还简要报道了有关开展国内外协作和国际交流等方面的学术活动情况。可供从事细胞学、遗传学、分子生物学、生物化学、医学和农学工作者、大专院校生物系师生及中学生物教师参考。

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北京大学出版社出版

中国科学院印刷厂印刷

新华书店北京发行所发行

★

787 × 1092 16 开 14 印张

1980 年 8 月第一版

统一书号: 13209 · 9 定价: 1.80 元

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前 言

1979年我所的科学研究工作,在贯彻执行党中央关于“调整、改革、整顿、提高”八字方针和中国科学院提出的“两个侧重”(侧重基础,侧重提高)“两个服务”(为国民经济建设服务,为国防服务)的过程中,初步建立了正常的工作秩序,明确了研究方向,制定了发展规划,提出了当前的研究任务侧重在四个方面、五个中心课题和两个重点。这四个方面是:分子遗传学,细胞遗传学(Cell Genetics),进化遗传学与应用遗传学;五个中心课题是:基因的结构、表达与遗传工程,动物与人类医学遗传学,植物体细胞遗传学与细胞工程,细胞质遗传学与核质关系,远缘杂交与有性过程的遗传控制;两个重点是:基因的结构、表达与遗传工程,以及植物体细胞遗传学与细胞工程。

通过调整,加强了遗传学基础理论研究。抗原识别中的基因活动与枯草杆菌孢子形成的调节等分子遗传学工作有了新发现和初步形成了新见解。以细胞培养为手段,禾谷类植物为重点材料的体细胞遗传学研究,正在深入开展,这是我国当前植物遗传学研究的特色。在支农方面,通过育种新方法、新途径的研究培育的多穗玉米、孤雌生殖产生的小麦品系、杂交高粱以及夏播大豆等新品种和新品系已开始在生产上示范和推广应用。此外,各项目研究组已广泛应用同位素闪烁计数、自显影、电泳分析、染色体分类、组织培养以及原生质体融合等手段,初步建立了实验体系,使研究工作向纵深发展,加速了科学研究的进程。

一年来,我们还开展了国内、外协作和国际交流。在国内,已有17个项目组分别与上海生化研究所、吉林师范大学、湖南师范学院、首都医院等十多个单位进行协作;在国外,与美籍科学家丁玉澄、戴维廉、付维宁以及国际水稻研究中心等建立了对口协作关系,并已取得了一批阶段性成果和新的发展。全年有十五个国家和地区的科学家来访,我所有11人先后参加了六次国际学术讨论会,提出了有关分子遗传学、细胞杂交、花药培养、遗传工程、植物远缘杂交等七篇学术论文。此外,1979年我所已有7人分别派往西德、美、英、法国从事短期工作或学习。

为促进学科发展,检阅我所一年来的科研成果与工作进展,特将一年来主要研究工作和学术活动汇编成册,以资交流。

胡 舍 邵启全

1980年3月

Preface

In the duration of carrying out the policy of the Central Committee of the Party in 1979, the research work of our Institute has been got in good order, made appropriate research objectives and long-range programs. Current research tasks were emphasized studies on four spheres (i.e. 1. molecular genetics, 2. cell genetics, 3. evolutionary genetics and 4. applied genetics); five main subjects (i.e. 1. gene structure, gene expression and genetic engineering, 2. animal and human medical genetics, 3. plant somatic cell genetics and cell engineering, 4. cytoplasmic genetics and the relationship of nucleus and cytoplasm, and 5. distant hybridization and genetic control of sexual process); and two important aspects (i.e. 1. gene structure, gene expression and genetic engineering, and 2. plant somatic cell genetics and cell engineering).

Basic research in genetics were strengthened this year, and thenceforward discoveries were made in molecular genetics, namely gene activation during antigen recognition and genetic regulation of sporulation of *Bacillus subtilis*, and some new information was obtained. Research projects were carried out in depth by means of cell and tissue culture and with cereal crops as experimental materials. This represents a special aspect of current plant genetic research in China. In aid-agriculture field, multi-ear maize, parthenogenetic wheat, hybrid sorghum and summer sowing soybean lines have been initially put on a demonstration or released in agricultural production. In addition, new techniques, such as isotope scintillation counting, radioautography, electrophoretic analysis, chromosome banding, tissue culture, and protoplast fusion were successively established for further development and progress of genetic research.

Cooperation and collaboration with research establishments domestically and abroad and scientific exchange were active in 1979. A total of 17 research groups of our Institute cooperated with research members in Shanghai Biochemistry Institute, Jilin Normal University, Hunan Normal College, and the hospital attached to Peking Medical Union. Cooperation was also established between American scientists of Chinese origin, Y. C. Ting, W. N. Fu and William Tai, and Chinese workers; and between foreign Institutes (for example IRRI) and our's wherein preliminary results have been obtained. During the year foreign scientists from 15 countries and regions visited our Institute, while eleven researchers of our Institute attended six international meetings and gave seven reports in molecular biology, cell hybridization, anther culture, genetic engineering, and plant distant hybridization. Furthermore, seven researchers went abroad for engaging short-term project of advance study and training.

This report was thus compiled for the exchange of experience with concerned establishment, the development of genetics and the evaluation of the research results of our Institute.

March, 1980

Hu Han Shao Qiquan

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615 小鼠和白血病 615 小鼠 (L615) 肝细胞 RNA 聚合酶 B 的比较研究

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真核生物基因表达是目前人们所关心的问题。原则上讲,转录调节起主要作用,它包括模板特征和 RNA 聚合酶的特异功能两个方面。高等生物的转录模板是染色质,染色质的组成成分蛋白质和 DNA 都起着重要的调节作用。不同类型的 RNA 用不同类型的 RNA 聚合酶转录。RNA 聚合酶 B 主要转录 Hn RNA。因此,在无细胞系统中研究真核生物 RNA 聚合酶 B 的结构、功能和它的作用,对了解细胞内基因转录调节是十分必要的。

我们选用了 615 小鼠和白血病 615 小鼠作为研究材料,从这两种小鼠的肝细胞中分离提取了 RNA 聚合酶 B,进行比较研究。这两种 B 酶在 DEAE-纤维素 DE52 层析柱上洗脱,分别是单一的峰区,酶活性峰和 A_{280} 吸收峰基本重合。

不变性条件下的聚丙烯酰胺凝胶电泳指出 615 和 L615 RNA 聚合酶 B 基本为一条电泳带,在此带之上还可以见到一条很微弱的电泳带。说明细胞内至少有两种形式的 B 酶存在,即正处于与染色质结合状态的酶,和与染色质分离的游离状态的酶。弱带为游离状态的酶。在变性条件下的聚丙烯酰胺凝胶电泳中 615 小鼠 RNA 聚合酶 B 有 11 条电泳带,其中两条大亚基分子量分别为 220,000 和 125,000 道尔顿。L615 B 酶在 615B 酶的第一、二条带之间有一条电泳带,在 615B 酶第九条带之上还有一条电泳带,这二条带可能是 L615 B 酶特异性亚基。Nakanishi 曾发现 Ehrlich 腹水癌细胞有两种蛋白质可以激活 RNA 聚合酶 B,使 B 酶与同源的 DNA 模板形成稳定的复合物。我们认为 L615 B 酶组成的变化是与 L615 白血病基因表达有关的变化。

用 L615 RNA 聚合酶 B 制备的免疫抗血清对 615B 酶有免疫沉淀作用,而对 L615 RNA 聚合酶 B 则没有明显的免疫沉淀反应。

适当的硫酸铵浓度对 RNA 聚合酶 B 的转录活性有激活作用,在我们的实验条件下,90mM 硫酸铵对 615 B 酶和 L615 B 酶的转录达到最大的激活程度。在二价金属离子的激活实验中,可以看出锰离子比镁离子更适于激活 RNA 聚合酶 B。两种 B 酶要求的锰离子最适浓度都是 2 mM,但是,615 小鼠 B 酶在镁离子也是 2 mM 时,它的激活能力只有锰离子的三分之一,而对 L615 小鼠 B 酶则是二分之一。所以我们认为 L615 B 酶比 615B 酶更偏向要求镁离子。

α -鹅膏蕈碱是鉴别真核生物 RNA 聚合酶的一种重要试剂,它特异性地选择抑制 RNA 聚合酶 B。615 小鼠 B 酶在 0.2 微克/毫升的 α -鹅膏蕈碱作用下,RNA 合成受到 50% 的抑制。L615 小鼠 B 酶在 0.1 微克/毫升的 α -鹅膏蕈碱作用下,活性受到 50% 以上的抑制。当 α -鹅膏蕈碱浓度为 1 微克/毫升时,抑制 615 小鼠 B 酶活性的 80%,抑制 L615 小鼠 B 酶活性的 90%。 α -鹅膏蕈碱主要抑制 RNA 合成的第一个磷酸二酯键形成,所以

它是抑制转录的启动阶段。根据 L615 B 酶构成情况,我们认为 L615 小鼠 RNA 聚合酶 B 有一些蛋白质因子对 α -鹅膏蕈碱亲和力较强,一旦这些因子结合了 α -鹅膏蕈碱,转录的启动就受到抑制。

RNA 聚合酶 B 在变性 DNA 上转录更为有效。以 20 微克蛋白质的 B 酶为酶的用量,分别加入不同浓度的变性 DNA,在 DNA 为 30 微克时,转录能力达到饱和,再增加 DNA 的量,转录水平没有显著增加。这时 615 小鼠 RNA 聚合酶 B 利用天然 DNA 模板与变性 DNA 模板的比值为 0.18,而 L615 小鼠 RNA 聚合酶 B 利用两种模板的比值则是 0.37。因此,L615 B 酶比 615B 酶利用天然 DNA 的能力高一倍。

A COMPARATIVE STUDY ON RNA POLYMERASE B IN LIVER CELL OF MOUSE 615 AND L615

Liu Lianrui Wang Bin Zhang Dada
Huang Chongxi Wang Jinxia

ABSTRACT

The main function of RNA polymerase B is responsible for the synthesis of Hn RNA. Therefore, it is necessary to study the structure and function of RNA polymerase B for investigating the regulation of transcription. RNA polymerase B of normal and Leukemic cells from mouse 615 were studied. It was found that there existed in liver cells two forms of RNA polymerase B, one of them was in an engaged form, another, in free form. In comparing RNA polymerase B in leukemic mouse (L615) with normal mouse (615), it was found that there were two proteins bands closely associated with L615 enzyme B, but not with 615 enzyme B. An antiserum directed against the 615 mouse RNA polymerase B was prepared. In immunodiffusion tests, the antiserum gave a precipitation reaction with 615 mouse RNA polymerase B, but failed to precipitate with L615 mouse RNA polymerase B. For these two forms of enzyme, the optimum ionic strength of ammonium sulphate was about 90 mM; for stimulating activities of the two enzymes, manganese ion was stronger than magnesium ion. The two kinds of RNA polymerase B were very sensitive to the inhibition of α -amanitin, but the L615 mouse RNA polymerase B was far more sensitive. The denature DNA was a suitable template for transcription of the two RNA polymerase B.

615 小鼠肝细胞核 RNA 聚合酶 A、B、C 的提取、分离和鉴定

王 斌 刘连瑞 张大达 王金霞 黄崇喜 王恢鹏 宁益华 冯 尚

真核生物基因转录的调控机制目前还不十分清楚,但是,各种调控机制都直接或间接地与依赖于 DNA 的 RNA 聚合酶有关。因此,弄清 615 和 L615 小鼠各种 RNA 聚合酶的性质、结构及功能,将为研究 L615 白血病基因表达及在癌变过程中基因转录的调控提供一条有效的途径。

我们从 615 小鼠肝细胞核中提取、分离并鉴定出了 A、B、C 三种 RNA 聚合酶。整个提取过程除了特别指出外都在 0—4℃ 条件下进行。按 60 克肝(湿重)所得到的细胞核为标准,加入 200ml MS(5),充分摇匀后定容到 300ml。边搅拌边加入 67.5ml 饱和硫酸铵,这时溶液变得极为粘稠。用 MSE 高速匀浆器剧烈搅拌去粘,用双层纱布过滤,滤液按每 100 ml 加 13.3 克固体硫酸铵,使溶液的硫酸铵达到 40% 饱和度,电磁搅拌 45 分钟,酶蛋白就会沉淀下来。用 12,000rpm, 4℃, 离心 30 分钟,收集沉淀并溶于 100 ml MS (30) 中。此步的提取物称为 P40。

为了进一步除去核酸类物质,我们在 100 ml P40 溶液中加入 5ml 1% 的硫酸鱼精蛋白(pH5.5),继续搅拌 30 分钟,然后用 35,000rpm, 4℃, 离心 60 分钟,收集上清液,用 MS(30) 稀释到 3.5 倍体积,使硫酸铵浓度降为 70mM。这一步的酶提取物称为 PS。在 80mM 硫酸铵浓度下,使 B 酶选择性地吸附在 DEAE-纤维素 DE52 上,过滤(流出液中含有 A、C 酶),用含 500mM 硫酸铵的 MS(30) 进行洗脱,分部收集,测定在 280nm 的光吸收,并取样测定 RNA 聚合酶 B 的活性。结果表明, A_{280} 吸收峰和酶活性峰基本重合。在有 1 μ g/ml α -鹅膏蕈碱的情况下, B 酶活性受到明显抑制,峰型明显降低。从酶粗提物到这一步, B 酶纯化了 40.5 倍。

含有 A、C 酶的流出液用 MS (30) 稀释到硫酸铵浓度为 50mM,然后在 50mM 硫酸铵浓度下使 A 酶和 C 酶吸附在 DEAE-Sephadex A25 上。用 50-500mM 硫酸铵线性梯度进行洗脱, A_{280} 曲线和酶活曲线都呈现为两个独立的峰区,第一个峰区的酶活性在有 200 μ g/ml 的 α -鹅膏蕈碱时没有受到抑制,第二个峰区的酶活性在有 100 μ g/ml 的 α -鹅膏蕈碱时受到明显抑制,峰型降低,但 1 μ g/ml 的 α -鹅膏蕈碱对第二个峰区的酶活性没有影响。所以第一个峰区是 A 酶,第二个峰区是 C 酶。从粗提取物到这一步, A 酶纯化了 55.7 倍, C 酶纯化了 83.3 倍。

α -鹅膏蕈碱是鉴定真核生物 RNA 聚合酶的标准试剂。我们测定了所提取的三种 RNA 聚合酶对 α -鹅膏蕈碱的敏感性,结果表明: A 酶在 α -鹅膏蕈碱浓度为 200 μ g/ml 时,完全不受抑制; B 酶在 0.2 μ g/ml 的 α -鹅膏蕈碱浓度下,酶活受到 50% 以上的抑制; C 酶在 100 μ g/ml 的 α -鹅膏蕈碱时酶活受到 50% 以上的抑制。