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Editor: Margaret Fox

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Proceedings of the 12th International Cancer Congress,
Buenos Aires, 1978

Volume IV

BIOLOGICAL BASIS FOR CANCER DIAGNOSIS

Editor:

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(Each volume is available separately.)

Foreword

This book contains papers from the main meetings of the Scientific Programme presented during the 12th International Cancer Congress, which took place in Buenos Aires, Argentina, from 5 to 11 October 1978, and was sponsored by the International Union against Cancer (UICC).

This organisation, with headquarters in Geneva, gathers together from more than a hundred countries 250 medical associations which fight against Cancer and organizes every four years an International Congress which gives maximum coverage to oncological activity throughout the world.

The 11th Congress was held in Florence in 1974, where the General Assembly unanimously decided that Argentina would be the site of the 12th Congress. Argentina was chosen not only because of the beauty of its landscapes and the cordiality of its inhabitants, but also because of the high scientific level of its researchers and practitioners in the field of oncology.

From this Assembly a distinguished International Committee was appointed which undertook the preparation and execution of the Scientific Programme of the Congress.

The Programme was designed to be profitable for those professionals who wished to have a general view of the problem of Cancer, as well as those who were specifically orientated to an oncological subspeciality. It was also conceived as trying to cover the different subjects related to this discipline, emphasizing those with an actual and future gravitation on cancerology.

The scientific activity began every morning with a Special Lecture (5 in all), summarizing some of the subjects of prevailing interest in Oncology, such as Environmental Cancer, Immunology, Sub-clinical Cancer, Modern Cancer Therapy Concepts and Viral Oncogenesis. Within the 26 Symposia, new acquisitions in the technological area were incorporated; such acquisitions had not been exposed in previous Congresses.

15 Multidisciplinary Panels were held studying the more frequent sites in Cancer, with an approach to the problem that included biological and clinical aspects, and concentrating on the following areas: aetiology, epidemiology, pathology, prevention, early detection, education, treatment and results. Proferred Papers were presented as Workshops instead of the classical reading, as in this way they could be discussed fully by the participants. 66 Workshops were held, this being the first time that free communications were presented in this way in a UICC Congress.

The Programme also included 22 "Meet the Experts", 7 Informal Meetings and more than a hundred films.

METHODOLOGY

The methodology used for the development of the Meeting and to make the scientific works profitable, had some original features that we would like to mention.

The methodology used in Lectures, Panels and Symposia was the usual one utilized in previous Congresses and functions satisfactorily. Lectures lasted one hour each. Panels were seven hours long divided into two sessions, one in the morning and one in the afternoon. They had a Chairman and two Vice-chairmen (one for each session). Symposia were three hours long. They had a Chairman, a Vice-chairman and a Secretary.

Of the 8164 registered members, many sent proferred papers of which over 2000 were presented. They were grouped in numbers of 20 or 25, according to the subject, and discussed in Workshops. The International Scientific Committee studied the abstracts of all the papers, and those which were finally approved were sent to the Chairman of the corresponding Workshop who, during the Workshop gave an introduction and commented on the more outstanding works. This was the first time such a method had been used in an UICC Cancer Congress.

"Meet the Experts" were two hours long, and facilitated the approach of young professionals to the most outstanding specialists. The congress was also the ideal place for an exchange of information between the specialists of different countries during the Informal Meetings. Also more than a hundred scientific films were shown.

The size of the task carried out in organising this Congress is reflected in some statistical data: More than 18,000 letters were sent to participants throughout the world; more than 2000 abstracts were published in the Proceedings of the Congress; more than 800 scientists were active participants of the various meetings.

There were 2246 papers presented at the Congress by 4620 authors from 80 countries.

The Programme lasted a total of 450 hours, and was divided into 170 scientific meetings where nearly all the subjects related to Oncology were discussed.

All the material gathered for the publication of these Proceedings has been taken from the original papers submitted by each author. The material has been arranged in 12 volumes, in various homogenous sections, which facilitates the reading of the most interesting individual chapters. Volume XII deals only with the abstracts of proffered papers submitted for Workshops and Special Meetings. The titles of each volume offer a clear view of the extended and multidisciplinary contents of this collection which we are sure will be frequently consulted in the scientific libraries.

We are grateful to the individual authors for their valuable collaboration as they have enabled the publication of these Proceedings, and we are sure Pergamon Press was a perfect choice as the Publisher due to its responsibility and efficiency.

Argentina
March 1979

Dr Abel Canónico
Dr Roberto Estevez
Dr Reinaldo Chacon
Dr Solomon Barg

General Editors

Introduction

The multidisciplinary attack on the problem of malignant disease is well illustrated in this volume. Many of the contributions are aimed at understanding the changes which occur in malignant cells at a molecular level and their biological significance. Such changes are thought to be the result of altered gene expression resulting in changes in the numbers and identity of proteins found in malignant cells both within the nucleus and cytoplasm and expressed on the cell surface.

At a clinical level alterations in gene expression in malignant cells result in paraneoplastic syndromes; this topic, the possible usefulness of "tumour markers" in monitoring the response of neoplastic disease to therapy, and the problems of screening for sub-clinical cancer, are also well covered.

March 1979 MARGARET FOX

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Cell Biology and Cancer

Control of DNA Synthesis in Normal and Cancer Cells

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ABSTRACT

DNA replication in eukaryotic cells is a complex process involving many different steps, the nature of which is still poorly understood. The present knowledge on eukaryotic DNA polymerases is briefly reviewed, and evidence is presented suggesting that in stimulated lymphocytes there are two distinct DNA polymerases-α. The utility of cancer cells as natural mutants to elucidate some aspects of DNA biosynthesis is illustrated, and the presence of an altered nuclear DNA polymerase in a case of acute lymphoblastic leukemia is reported. The presence of genetic diseases and *in vitro* conditional mutants with alterations in DNA replication is discussed. The role of the different factors involved in DNA synthesis may be investigated through the analysis of *in vitro* systems: permeabilized cells, isolated nuclei, subcellular extracts. Experiments on permeabilized lymphocytes are reported demonstrating that deoxyribonucleoside triphosphates may enter these cells and that dCTP is a precursor *in vitro* both to DNA and to phosphatidyl-dCMP.

INTRODUCTION

DNA replication in eukaryotic cells is a highly complex process involving many different metabolic reactions, highly coordinated between them. In spite of the effort dedicated to their study the nature of many of these reactions and how they are regulated is far from understood. The knowledge in this field, which has been recently reviewed by Sheinin, Humbert and Pearlman (1978), lays behind that of DNA replication in prokaryotes, where the last 10 years have witnessed a remarkable progress in our understanding of DNA replication. An essential factor which contributed to the achievements in the latter field was the extensive use of genetics which through the obtention of conditional mutants of DNA synthesis and its manipulation permitted the analysis of the intervening factors in DNA replication (Hirota and others, 1972).

Conditional mutants in eukaryotic cells are more difficult to obtain and characterize, and the role of the different factors involved in DNA synthesis has been studied mainly through the analysis of a great variety of cellular systems ranging from normal cells to tumor cells either growing *in vivo* or which have been transformed *in vitro*. The emerging picture from these studies suggest that the concept of units of

DNA replication or replicons initially proposed for prokaryotes (Jacob, Brenner and Cuzin, 1963) may also apply to eukaryotes. There is already genetic, biochemical and biophysical evidence that indicates that the simple genomes of the viruses of the eukaryotic cells (e.g. papovaviruses, adenoviruses and herpes viruses) can be classified as replicons. Although the replicons of the eukaryotic genome cannot still be identified, we are able to define replication units. They consist of a segment of DNA double helix varying from 10-100 μm lenght and operationally defined from origin to terminus. The replication units appear to be tandemly arranged within the chromosomal DNA in clusters composed of 2 to 250 units. The DNA synthesis is initiated at an origin within the replication unit and proceeds bidirectionally by the movement of two replication forks toward the two distant termini.

With respect to the role of the different factors involved in eukaryotic DNA replication, the diversity of systems utilized for its study obviously makes it difficult to interpret the information obtained since many observed characteristics may be peculiar to one type of cell or be determined by its growth conditions and may not be applicable to other cells. This inconvenient appears specially when DNA replication is analyzed in cells which have undergone a cancerous transformation and where, almost by definition, a mutation(s) has occurred somewhere in the chain of events controlling cell division. However, since cancer cells are "natural mutants" their analysis could help to understand some features of DNA synthesis in their normal counterparts, and it would be preferable that experimental systems be used where this correlation is possible. Also, since many chemotherapic agents act through the inhibition of DNA synthesis at some step, knowledge gained on any existing difference between normal and cancer cells could be useful to design more effective and selective chemotherapic agents or to obtain better results with the existing ones. The work which will be presented here will deal with two aspects of DNA synthesis. Our present knowledge on DNA polymerases will be briefly reviewed, and data from our laboratory will be presented demonstrating how the use of cancer cells as "spontaneous mutants" may help to clarify some obscure points. The second aspect to be treated refers to the use of *in vitro* systems for the analysis of DNA synthesis.

RESULTS

DNA Polymerases in Eukaryotic Cells

During the past few years considerable information has accumulated concerning the type and number of DNA polymerases in eukaryotic cells, and the field has been recently reviewed (Weissbach, 1977). Due to the diversity of eukaryotic tissues studied there was a great variety of nomenclatures for DNA polymerases, and in 1975 it was decided to establish a uniform system of nomenclature (Weissbach and others, 1975). Only the enzyme classes representing distinct entities were included, and were named with Greek letters according to the order of discovery. In this system were not included the reverse transcriptases or RNA tumor virus associated DNA polymerases, neither the virus induced DNA polymerases. Four polymerase classes were identified:

1) DNA polymerase-α. It is the high molecular weight (> 100.000) DNA polymerase, first identified in calf thymus extracts almost 20 years ago (Bollum, 1960). This enzyme is mainly detected in the cytoplasmic extracts of growing cells, but

when non aqueous solvents are used to break the cells, most of the activity is found in the cell nucleus. The α -polymerase is particularly active in copying "activated" double stranded DNA, and it is inhibited when sulfhydryl groups are blocked. It has recently been purified to near homogeneity and was shown to be a dimer composed of two dissimilar subunits of 76.000 and 66.000 daltons present in equimolar ratio (Fisher and Korn, 1977).

2) DNA polymerase-β: It is the low molecular weight enzyme (30.000-50.000) detected mainly in nuclear extracts (Weissbach and others, 1971). It is an ubiquitous enzyme in the animal kingdom but it is absent in bacteria, plants and protozoa (Chang, 1976). This enzyme is resistant to the blocking of sulfhydryl groups and in the presence of an activated DNA template has a significant ability to incorporate a single deoxynucleoside triphosphate in the absence of the other three depending on the DNA: enzyme ratio (Franze de Fernández, Mordoh and Fridlender, 1975).

3) DNA polymerase-γ. This is the most recently described DNA polymerase that copies preferently An:dT$_{15}$ rather than natural or synthetic DNA templates (Fridlender and others, 1972), and it is inhibited by sulfhydryl blocking compounds.

4) Mitochondrial (mt) DNA polymerase. This enzyme is separable from the others and it is so named for its subcellular localization (Meyer and Simpson, 1968). However, recent studies suggest that this enzyme may be a form of DNA polymerase-γ (Bolden, Noy and Weissbach, 1977).

The physiological role of each of the DNA polymerases is still unclear. Most of the studies performed to ascribe a role to the DNA polymerases have been correlative, consisting in the measure of DNA polymerase activities under varying conditions of growth or quiescence. In general, it has been found that in growing cells the predominant activity is the DNA polymerase-α while in resting cells the predominant activity belongs to DNA polymerase-β (Weissbach, 1977).

Our studies on the control of DNA replication have been mainly done using the system of human peripheral blood lymphocytes. While these cells are normally resting, they can be induced to proliferate by a variety of substances such as phytohemagglutinin (PHA) (Nowell, 1960). The DNA polymerase activities of non stimulated lymphocytes were analyzed by DE52 column chromatography. In the cytoplasmic fraction (Fig. 1A) the presence of two peaks of activity were observed, CI_n and CII_n, which eluted at 0.07 M NaCl and 0.13 M NaCl respectively. The soluble nuclear fraction showed a single peak of activity, designed as NI_n, which did not adsorb to DE52 (Fig. 1B).

When the DNA polymerases from stimulated lymphocytes were analyzed by DE52 column chromatography, the pattern shown in Fig. 2 was generally observed. In the cytoplasm there was a single peak of activity (CII_s) eluting at 0.12 M NaCl (Fig. 2A) while in the soluble nuclear fraction two peaks of activity appeared (Fig. 2B): NI_n which did not adsorb to DE52, while the second peak, NII_s, eluted at 0.07 M NaCl.

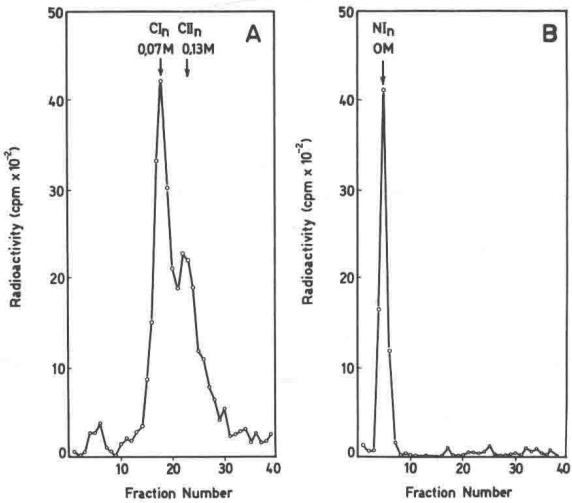

Fig. 1. DNA polymerase DNA dependent activities from non stimulated lymphocytes. The cytoplasmic (A) and nuclear soluble fractions (B) were chromatographed on DE52 cellulose (data from Fridlender and others, 1974).

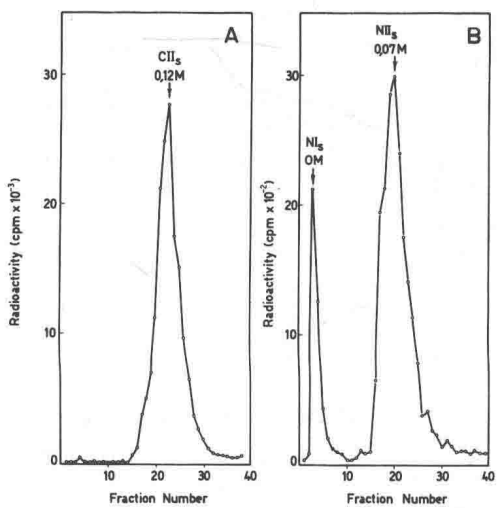

Fig. 2. DNA polymerase DNA dependent activities from stimulated lymphocytes. The cytoplasmic (A) and nuclear soluble fractions (B) were chromatographed on DE52 cellulose (data from Fridlender and others, 1974).