Carcino-Embryonic Proteins

Chemistry, Biology, Clinical Application

Volume I

Frank-Günter Lehmann Editor

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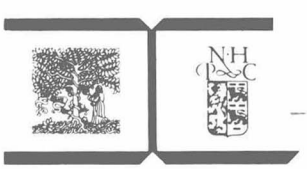
FRANK-GÜNTER LEHMANN



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PREFACE

The organization of an international congress and the publication of the proceedings should be especially justified at a time when international conferences and the number of publications in life sciences and medicine increase enormously from year to year. During the last decade, the concept of a common gene expression in development and oncology became increasingly important for biology and medicine. The discovery of onco-developmental gene products, e.g. carcino-embryonic proteins, in a qualitative and quantitative similar amount during development and carcinogenesis stimulated the hypothesis of a common, gene related, basic process as well in development as in carcinogenesis. The occurrence of carcino-embryonic proteins during intra-uterine fetal development, in extra-embryonic tissues like in the placenta and in vitelline structures, during cell differentiation in inflammatory or regenerating states, and in carcinogenesis favoured the concept of malignant transformation of cells as a special phase of development. Cancer cells and normal cells have the same genomic content, but express different particular portions of their genomic information; during several phases of development (and of cancer) these parts of the gene can be re-expressed as carcino-embryonic proteins due to a de-repression. In fact, the discovery of carcino-embryonic proteins stimulated enormously the research in basic sciences like molecular biology, immunochemistry, immunology, and developmental sciences as in clinical disciplines like clinical oncology, gastroenterology or gynecology and obstetrics. This independent, interdisciplinary interest and approach raised the need for the foundation of an international, multidisciplinary organization. In 1972, the "International Research Group for Carcino-Embryonic Proteins" was established in order to facilitate interdisciplinary communication, to induce multidisciplinary research and to favour exchange and standardization of onco-developmental gene products and reagents. The scientific meetings and publications (1–5) became increasingly important during the last 5 years (Fig. 1). The continuous publication of the proceedings (1–5) was necessary due to the lack of one or more common international journals suitable for the publication of these interdisciplinary approach to the problem of cancer. Original publications are distributed over more than 30 different journals in different languages ranging from basic sciences oriented publications in chemistry up to clinically oriented publications in obstetrics. Therefore, the proceedings of the previous meetings provided an unique collection of unpublished original work in different disciplines.

The main reason for the organization of the 6th Meeting of the "International Re-

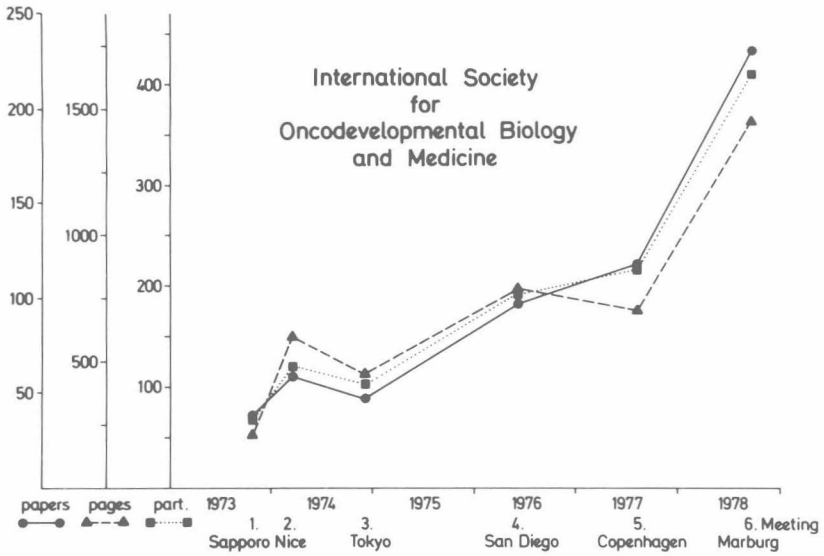

Fig. 1. Development of the "International Research Group for Carcino-Embryonic Proteins" (now changed into "The International Society for Oncodevelopmental Biology and Medicine"): Number of papers presented at the first six meetings, volume of the conference proceedings and number of participants.

search Group for Carcino-Embryonic Proteins" (now turned into the "International Society for Onco-developmental Biology and Medicine"), held in Marburg/Germany from September 17th to 21st, 1978, and for the integral publication of the papers presented at this conference is the continued need for a forum for international multidisciplinary research and collaboration from the viewpoint of onco-developmental gene expression. Normally, a new unpublished discovery in basic science needs approximately 5 to 8 years until clinical application is possible; one may calculate that a new finding in immunochemistry, biochemistry or molecular biology needs 1 to $1\frac{1}{2}$ years for the publication in a basic science oriented journal. Then, this result may come to the attention of a clinically oriented research worker who will repeat the experiment and publish the results in a more medicine related journal; this needs another year for the experiments and another 1 to $1\frac{1}{2}$ years for the publication. Then, a clinician may pick up this information and perform a pilot study under clinical conditions necessitating again the same time for performing the experiments and publication. Thus, 6 years may easily accumulate between the discovery in a basic laboratory and the publication of the first clinical pilot study provided that every new finding is immediately recognized and re-evaluated in the next "generation" of research workers. In any case, the shift of information is considerably retarded and a considerable amount of substantial findings will be lost and never reach the end of this "cascade" of research workers. Therefore, this type of interdisciplinary international conference and publication is necessary not only to enable and to shorten the shift of information from the basic scientist to the clinician but also to avoid useless parallel research, to

save financial investments and at least to win time for the introduction of new methods in diagnosis and therapy control of cancer patients as well as in fetal monitoring. Furthermore, no international forum (conference, publication, or journal) exists to provide the "reversed type" of information, e.g. the shift of information from the clinician to the basic scientist. Again it is the aim of this conference as well as of this publication to favour the understanding of the basic scientists for clinical problems and to direct basic research into the teleological direction of a possible clinical application. Thus, efforts have been made during the conference to enforce this interdisciplinary collaboration, and this publication has been arranged to provide comprehensive information by understandable reviews of leading experts as well as to accumulate unpublished original research data from basic and clinical disciplines. A pre-conference workshop had been organized two years before the meeting [6] in order to stimulate research in oncodevelopmental sciences in Germany.

The selection and composition of these two books was achieved by four sequential steps: first, the best internationally recognized specialists were invited by the editor to contribute to the conference and to the publication either by reviews or by original results of their laboratory. Special attention had been paid to the fact that the complete field of onco-developmental gene products ranging from established carcino-embryonic proteins, placental proteins, isoferritins, isoenzymes up to newly recognized, not yet definitively characterized carcino-embryonic proteins, and of the related methodology were represented in appropriate parts with particular emphasis on marginal fields like hormones, cell-mediated immunity, and the use of antibodies to carcino-embryonic proteins. Second, research workers, without any limitation, were invited to submit original scientific papers for the conference and the publication. Anonymous selection, i.e. without knowlege of authors and institutions, was performed by an international, interdisciplinary program committee (Table I). After the grading procedure, the results of the individual members of the program committee were evaluated and all "borderline papers" were discussed in detail by the European membranes of the program committee before a paper was rejected or accepted. In addition, several abstracts could be accepted only after substantial revision. Then, the editor attributed an appropriate number of pages for the publication to every author taking into account the final results of the grading procedure. Third, all manuscripts were reviewed during the conference by the chairmen of the correspond-

TABLE I

PROGRAM COMMITTEE OF THE 6TH MEETING OF THE I.R.G.C.P., SEPTEMBER 1978, MARBURG

E. Alpert, Boston, U.S.A.
P. Burtin, Villejuif, France
W.H. Fishman, La Jolla, U.S.A.
H. Hirai, Sapporo, Japan
F.-G. Lehmann, Marburg, Germany
R. Masseyeff, Nice, France
M. Seppälä, Helsinki, Finland

TABLE II

CHAIRMEN OF THE 6TH MEETING OF THE I.R.G.C.P., SEPTEMBER 1978, MARBURG, WHO REVISED THE SUBMITTED MANUSCRIPTS (Other chairmen not listed in this table)

L. Belanger, Québec, Canada	B. Nørgaard-Pedersen, Copenhagen, Denmark
T.M. Chu, Buffalo, U.S.A.	W. Rapp, Heidelberg, Germany
J.W. Drysdale, Boston, U.S.A.	E. Ruoslahti, Duarte, U.S.A.
Y. Endo, Tokyo, Japan	V. Ryatsep, Tallinn, U.S.S.R.
W.H. Fishman, La Jolla, U.S.A.	F. Schapira, Paris, France
D.M. Goldenberg, Lexington, U.S.A.	M. Seppälä, Helsinki, Finland
R.B. Herberman, Bethesda, U.S.A.	A. Talerman, Rotterdam, The Netherlands
C.H.W. Horne, Aberdeen, U.K.	Y.S. Tatarinov, Moscow, U.S.S.R.
J. Kohn, London, U.K.	C.W. Todd, Duarte, U.S.A.
J.-P. Mach, Lausanne, Switzerland	S. Weinhouse, Philadelphia, U.S.A.
A. Munro Neville, Sutton, U.K.	H. Weitzel, Hannover, Germany

ing sessions (Table II). Fourth, the editor additionally reviewed all manuscripts after the conference in order to decide whether or not the manuscripts could be accepted. In addition to the rejected papers, several manuscripts had to be revised completely before publication. The editor acknowledges the substantial support of the program committee for the selection procedure and of the chairmen of the scientific sessions for the revision of the manuscripts.

The intention of the publication is to provide a complete overview of the field of carcino-embryonic proteins and to collect all new original work available at the present time. Furthermore, the speed of publication is of special interest. Therefore, the publication is performed in two volumes, both arranged and grouped into the same chapters: Volume I contains all reviews (27%), review papers which contain new unpublished original work (20%), and selected original papers (53%) characterized by their outstanding findings or their multidiciplinary importance. This volume is edited uniformally, type setted and arranged in a way that every topic is covered by one contribution. Volume II contains all original scientific contributions arranged in the same chapters like Volume I and representing the actual interest of different fields within the onco-developmental sciences. This volume is produced by camera ready copy procedure in order to enhance considerably the speed of publication. Both volumes provide substantial information on chemistry, biology and clinical application of "established" carcino-embryonic proteins like carcino-embryonic antigen, alpha-fetoprotein, pregnancy associated β_1-glycoprotein, newly recognized carcino-embryonic proteins, isoferritins, isoenzymes, hormones, cell-mediated immunity, methodology and standardization and antibodies to carcino-embryonic proteins.

The organization of the Marburg conference and the publication of the scientific contributions would have been impossible without the academic and financial support of the patrons, sponsors and contributors from 9 different countries, listed in Table III. Especially the generous support of the Deutsche Forschungsgemeinschaft in gratefully acknowledged. Furthermore, the editor is greatly indebted to Drs. C. Gropp and T. Wegener, Department of Medicine, University of Marburg, to the staff and the research fellows

TABLE III

PATRONS, SPONSORS AND CONTRIBUTORS OF THE 6TH MEETING OF THE I.R.G.C.P., SEPTEMBER 1978, MARBURG

Patrons
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In collaboration with
World Health Organization/International Agency for Research on Cancer (WHO/IARC), Geneva/Lyon

Co-sponsors
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Foundation for Basic Research in Oncology, Tokyo

Contributors

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Asta	Farmitalia
Behringwerke	Hoffmann-La Roche
Bioscientia	Immuno
Biotest	Isotopen Dienst West
Boehringer Mannheim	Merck
Ciba-Geigy	Nordmark
Commissariat à l'Energie Atomique	Pharmacia
Dako immunoglobulins	Serono
Delalande	

of his laboratory and last not least to his wife Dorothee; their continuous help and encouragement substantially contributed to the success of the Marburg conference and the preparation of this publication. Finally, continuous suggestions and advice from several members of the International Society for Oncodevelopmental Biology and Medicine, e.g. the members of the program committee (Table I), equiped the editor with additional information and knowledge for the composition of the program and this publication.

On the occasion of the Marburg conference, the "International Research Group for Carcino-Embryonic Proteins" turned into the "International Society for Oncodevelopmental Biology and Medicine". Hopefully, this more formal organization, the next meetings in London, Great Britain (1979), Tallinn/Estonia, USSR (1980), Calgary/Alberta, Canada (1981) and Sapporo/Hokkaido, Japan (1982), and the complete information on our present knowledge in onco-developmental sciences provided by this publication may further favour and stimulate the international and multidisciplinary approach to our common enemy, the cancer.

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CURRENT CONCEPTS IN ONCO-DEVELOPMENTAL GENE EXPRESSION

COLE MANES

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SUMMARY

This paper is a brief review of recent research results in the area of eukaryotic gene expression and of their relevance to a consideration of cancer as a problem in developmental biology. Such fundamental concepts as the constancy of the genome and variable gene expression are undergoing important revisions. It is also appearing more likely that all of the potentially coding sequences in the genome are expressed as protein during the lifetime of the organism. If the generation of the malignant phenotype does not require exogenous genetic information, then it seems probable that "oncogenes" are expressed normally at some point in development.

RESULTS AND DISCUSSION

The synthesis of carcino-embryonic proteins by malignant cells has not only proven useful to the clinician but has also suggested to some investigators that cancer may ultimately be viewed as a problem in developmental biology. Such a view implies that cancer cells do not differ from normal cells by virtue of any differences in their genomic content, but rather by virtue of the particular portions of their genomic information which they choose to express as proteins, just as all other phenotypic differences are generated during the course of development. Even if this view of cancer turns out to be true, the problem of cancer will not be significantly simplified, since the mechanisms which regulate gene expression in normal cells are for the most part still obscure. The inclusion of cancer within the scope of developmental biology will most likely benefit both oncologists and developmental biologists, however. The aberrant or "ectopic" protein syntheses occurring in the cancer cell may well provide important insights into the mechanisms of gene regulation in normal cells. On the other hand, the demonstration that malignancy is ultimately a matter of the abnormal regulation of normal genes will provide the oncologist with a unifying concept in the face of a multi-faceted disease problem.

During the past three decades, two tenets in developmental theory have become elevated almost to the level of Dogma: the Constancy of the Genome, and the concept of Variable (or Differential) Gene Expression [1]. In the light of recent experimental results, both of these tenets are undergoing interesting revisions–revisions, furthermore, which have important implications as to the points in the flow of genetic information from

DNA to protein where perturbations may occur in abnormal cells.

The idea that all differentiated cells of an organism contain equivalent genetic information has gained support from direct studies of the DNA of various cell types by molecular hybridization, and from nuclear transplantation. The hybridization studies were initially performed under conditions which would detect only repetitive DNA sequences [2]; more recently, comparisons of single copy DNA from various organs have been carried out [3]. Both types of study, however, agree that there are no major gains or losses in genetic information to accompany phenotypic differentiation. Minor alterations in the genetic content, below the level of 5% difference, cannot be detected in these experiments. If we consider that the total mammalian genome contains some $3-5 \times 10^9$ base pairs, it becomes evident that even 1–2% of this DNA could carry with it a significant amount of genetic information. Thus, on the basis of the molecular hybridization studies, the absolute constancy of the genome throughout development must remain an open issue.

The transplantation of nuclei from intestinal epithelial cells of Xenopus tadpoles [4] or of nuclei from cultured Xenopus epithelial cells [5] into enucleated eggs, and the subsequent development of these eggs into apparently normal adult frogs, have been cited as proof that no genetic information is lost from nuclear DNA during the course of development. It is also evident in this work, however, that the later in development one obtains nuclei for transplantation, the lower the success rate in supporting normal development of the enucleated egg. This result suggests that the DNA is undergoing modification, albeit potentially reversible modification, with advancing development. If that is true, then although the total amount of genetic information in various nuclei may remain constant, it cannot strictly be termed "equivalent".

There is now abundant evidence from work with prokaryotes and plants that regulatory DNA sequences, termed "insertion elements", are regularly excised from the chromosomal DNA and re-integrated at other sites, thereby altering gene expression. Although there is as yet no published evidence that such "insertion elements" exist in the higher animals or that they function to regulate genetic expression during development, a search for these elements is on and an answer should not be long in coming. If they are found, it will again be necessary to ask whether two genomes, containing the same DNA sequences but in different ordered arrangements, should be considered "equivalent". Another property of prokaryotes which is being widely exploited in molecular biology is their possession of modification and restriction enzymes. Only recently higher organisms have been examined for the presence of these enzymes, and a sequence-specific restriction endonuclease in the African green monkey is reported [6]. The presence of this restriction endonuclease implies the presence of its companion "modification" enzyme, as in prokaryotes, to protect the local DNA from endonuclease attack. The modification enzymes generally protect cleavage sites by methylating adjacent bases, so that cell type-specific DNA methylation sites in higher organisms appear to be increasingly probable, as predicted by Holliday and Pugh [7]. Such a process would be consistent with the reversible modifications of DNA suggested by the nuclear transplantation work described above.

Finally, it is now documented that some dividing mammalian cells, under extreme

selective pressure, can amplify certain genetic sequences which have survival value [8]. Thus, while two genomes might be informationally "equivalent", portions of that information may be over-represented in one of them, rendering the genome something less than "constant". It is too early to know how general this phenomenon may be, or whether it ever occurs outside the artificial conditions imposed by the laboratory. The experimental results, as a minimum, demonstrate that it is at least a possibility.

The concept of Variable Gene Expression, as an explanation for the generation of widely differing phenotypes from a constant genotype during development, has somewhat imprecisely been taken to imply that coding sequences within the genome are either "on" or "off", and that the major site of gene regulation was at the level of transcription. If a coding sequence were once transcribed, it appeared highly probable that it would eventually be translated into protein. It is becoming increasingly clear that there are a number of control points in gene expression beyond the level of transcription, and that any of these are potentially vulnerable to disturbance by carcinogens or viruses. The specificity at the level of transcription, as implied by some versions of the Variable Gene Expression concept, has been challenged by recent investigations of the complexities of heterogeneous nuclear RNA (hnRNA) populations in various cell types within a given organism. When this hnRNA is hybridized in excess with single copy DNA in the sea urchin, it is found to be virtually identical in all embryonic and adult cell types tested [9]. In a similar fashion, hnRNAs from brain, liver, and kidney in the adult rat were demonstrated to constitute a "nested set" of transcripts, such that all transcripts found in liver were a subset of those in brain, and those in kidney a subset of those in liver [10]. It is evident that a much more complex messenger RNA (mRNA) class exists in the nucleus of the cell than in its cytoplasm, and that many potentially coding transcripts are formed in the nucleus which are never, or very infrequently, translated at the polysome level.

If selectivity is not a prominent feature of transcription, then surely it must be demonstrable at the level of the polysome where the proteins, the determiners of cell phenotype, are actually synthesized. Comparisons of polysomal mRNAs between different phenotypes within the same organism have yielded some surprising results, depending upon one's prejudice and upon the method used for the comparison. If complementary DNA (cDNA) is prepared to total polysomal mRNA and used for the comparison, then only minor differences (3–5%) are detected [11]. However, polysomal mRNAs are represented in vastly different proportions. Some 20% of the mRNA species comprise as much as 95% of the total mRNA mass, indicating that there are between 100 and 1000 copies of each of these messages per cell [12]. It is these mRNAs which are predominantly represented when cDNA is prepared to total polysomal mRNA, and they may over-represent common structural or housekeeping genes. The remaining 75–80% of the mRNA species are present at 1–10 copies per cell, and large differences in this "complex" mRNA class have been observed in different phenotypes [13]. It is argued that the synthesis of a number of significant cell proteins can be adequately supported by mRNAs present in less than 5 copies per cell, and thus that differences in this complex mRNA class are of biological importance [13]. More investigations of this type, using several differentiating systems, will be required in order to determine the generality of