The molecular biology of the mammalian genetic apparatus

Volume 2

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PREFACE

With enthusiasm and optimism, we introduce Volume II of 'The Molecular Biology of the Mammalian Genetic Apparatus'. As indicated in the Preface to Volume I of this series, the chapters in this volume are also from the international symposium bearing the same name as this series which was held in December of 1975 at the California Institute of Technology. This symposium not only provided an opportunity to examine the recent achievements and future developments in the research on the mammalian genetic apparatus, but also recognized the contributions made to this field by the Caltech people (faculty, students, alumni, associates and friends) and, particularly, those of Professor James Bonner's laboratory on the occasion of his 65th birthyear. This volume is divided into two parts: Part A. DNA Organization and Gene Expression, and Part B. Relationship to Somatic Genetics, Cancer and Aging. Thus, this volume extends the contents of Volume I on Structure to the organizational and functional aspects of the genetic apparatus, and describes the relationship and the relevance of the research on the genetic apparatus to the challenging problems in cell biology and biomedical science. This volume closes with a chapter by Professor James Bonner entitled 'My Life as a Chromosomologist'. In this final chapter, James describes the events, the strategy, the human aspects and his personal evaluation and feelings on the histone/chromatin research over the past two decades.

With sadness and remembrance, we pay a special tribute in this volume to Professor Jerome Vinograd, a member of the Organizing Committee of this Symposium, a close colleague and a very dear friend. His passing away on July 3, 1976 was a great loss and sorrow for all of us who knew and loved him. To me in particular, he was a great teacher; from him personally I have learned English, physical chemistry, science, and most of all, how to be a man of understanding, compassion and quality. I vividly remember our first meeting in the Crelin Chemistry Building in 1953, when I had just entered Caltech as a graduate student, and he had just returned to an academic institution. It was a turning point

in my life when such a bond of learning and friendship was welded between us which has lasted nearly a quarter of a century. During the past 23 years, I have often asked myself, particularly when writing a challenging scientific paper, 'Would Jerry regard this as a paper of excellent quality, both in terms of science and presentation?' From him, I have learned to understand, appreciate, and strive for excellence.

With these two volumes, the Symposium has now become history. We have honored and benefitted from the pioneers who diligently tilled the soil of this virgin field of research on the genetic apparatus. More than 70% of the participants in this symposium of 400 people are under the age of 30. The best is yet to come, in terms of future symposia, future publications and future researchers. To paraphrase Newton, we can indeed build a greater structure of science, since we build on the foundation laid by scientists such as J. Bonner, J. Vinograd, and N. Davidson, to name just a few.

Again, we wish to thank those agencies and individuals responsible for the support and assistance given to the Symposium and to these two volumes as described in the Preface to Volume I.

Paul O.P. Ts'o

JEROME VINOGRAD (1913–1976)

Jerome Vinograd was born February 9, 1913 in Milwaukee, Wisconsin, and died July 3, 1976. After undergraduate work at the University of Minnesota, he studied colloid chemistry with H. Freundlich from 1931 to 1935, first in Berlin, and then in London. He completed his doctoral work with J.W. McBain at Stanford from 1937 to 1939. He was associated with the Shell Development Co. from 1941–1951 where he worked mainly on problems of surface films and colloids. At that time, he gave up a secure industrial career and came to the California Institute of Technology because of his intense desire to work in the exciting new area of molecular biology. His early work at Caltech dealt mainly with the physical chemistry of proteins.

His first outstanding contribution to molecular biology was the invention, in collaboration with Meselson and Stahl, of the method of equilibrium density gradient centrifugation of nucleic acids in cesium chloride solutions. This method has been elaborated in Vinograd's laboratory and other laboratories to include the effects of base composition, denaturation, alkaline titration, binding of metal ions and of dyes, and hydration on buoyant density. All told, equilibrium buoyant banding has been one of the key techniques in the explosive development of the nucleic acid side of molecular biology since the mid-1950's.

Vinograd's excellent work in the last 12 years has been in the study of closed circular DNA. The distinction between supercoiled closed circles and open circles was recognized in 1965. The ethidium bromide method of buoyant banding for isolation of closed circular DNA was invented in 1967. This method, which makes it possible to analyze for, and to isolate, closed circular DNA in the presence of massive quantities of linear and open circular DNA is widely used and is one of the main reasons why there has been such exciting progress in the study of closed circular DNAs in different kinds of cells.

Later work has dealt with the properties of mitochondrial DNA from malignant cells and with the study of the replication of mitochondrial DNA.

This latter topic was initiated by the discovery of D-loops in Vinograd's laboratory. More recently, his students have made several important contributions to the studies of the properties of the nicking and closing enzyme which relaxes supercoiled DNA while leaving it covalently closed. The Vinograd group, as well as the groups of Keller at Cold Spring Harbor and Wang at Berkeley observed that one can use gel electrophoresis and relaxation by an enzyme to count the number of turns in a superturn in molecules which are closed under an equilibrium condition.

Vinograd was by early training and in his approach to molecular biology a physical chemist. He had a flair for recognizing when an anomalous and unexplained observation, if subjected to fundamental physical chemical analysis, would lead to an unexpected and important result in molecular biology. The 'special Vinograd touch' we called it.

A simple example is the development of the velocity band centrifugation method by Vinograd, Bruner, Kent, and Weigle in 1963. Shortly before that Jean Weigle discovered that he could layer a dilute solution of viruses in aqueous buffer onto a sucrose solution (not a gradient) in a centrifuge tube, centrifuge and get a good sharp virus band. Vinograd was puzzled as to why the band was sharp and reasoned that there must be some effect providing convective stability. He recognized that there had to be a self-generating (by diffusion) density gradient to provide convective stability; a careful study then led to the development of this general and widely used method.

At a Caltech seminar in late 1966 or early 1967 J.B. LePecq described his studies on the binding of ethidium bromide (Etd Br) to DNA. An important point was that, unlike the common acridine dyes, there was a reasonable amount of binding at high salt concentration. Vinograd pointed out at a discussion immediately following that if the dye ion bound in 6 M CsCl it would cause a large shift in the buoyant density of the DNA because it would displace a Cs^+ ion. Experiments by W.R. Bauer (then a graduate student) showed that Etd Br would indeed decrease the buoyant density of DNA, but that the shift was much larger for a relaxed than for a closed circular DNA. On learning of this result, Vinograd immediately perceived the correct explanation in terms of the topological constraint on unwinding of closed circular DNA, thus leading to the development of an extremely useful method for isolating closed circular DNA, as well as for studying the free energy of supercoiling.

One of the many distinguished honors which Vinograd received throughout his scientific career was the election to membership in the National Academy of Sciences in 1968. He was a recipient of the Kendall Award in Colloid and Surface Chemistry in 1970 from the American Chemical Society, as well as the recipient of the Helen Hay Whitney Foundation T. Duckett Jones Award in 1972. He was also a national lecturer at the 1972 Biophysical Society Annual Meeting. Vinograd also served on the editorial boards of BBA, JCCP, BP, ABMP, and

Intervirology. At the time of his death, Vinograd was the Ethel Wilson Bowles and Robert Bowles Professor of Chemical Biology at Caltech.

He suffered a major heart attack in 1954 and a second one in 1969. He accepted the resulting restrictions on his overall activities philosophically. But his condition did not affect the intensity with which he devoted himself to his scientific work. We remember him through all these years being in the laboratory regularly on evenings and weekends discussing and analyzing experimental data in painstaking detail with his students, seeking fundamental explanations for unexpected and unexplained results. His students and colleagues will miss his penetrating analyses of technical problems and his wise counsel on general policy issues.

Norman Davidson
James Bonner

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Chapter 1

GENE MAPPING BY ELECTRON MICROSCOPY

NORMAN DAVIDSON

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The present status of several techniques that are under development and/or in use in our laboratory for gene mapping by electron microscopy is presented in this communication.

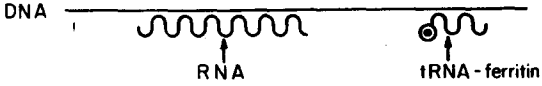

Fig. 1. Schematic diagram of RNA : DNA hybrids. The reasonably long RNA : DNA duplex on the left (> 500 nucleotides) can be recognized and measured in electron micrographs. In order to recognize a very short RNA : DNA hybrid as for tRNA, a ferritin label is attached to the tRNA, as shown on the right.

A statement of the general problem is given in fig. 1. We are given a DNA strand containing a gene coding for a particular RNA molecule. If the RNA can be isolated in a fairly pure state, the gene can be physically mapped by hybridizing the RNA onto the DNA and observing in the electron microscope the position of the RNA : DNA hybrid region relative to the ends of the molecule or to other features. In the standard formamide, cytochrome *c* spreading method, duplex regions are thicker and smoother than single strand regions. If the duplex region is fairly long (say >500 nucleotides) it is usually easy to discriminate between DNA : DNA duplexes and single strands. The contrast between RNA : DNA hybrids and DNA single strands is not as good as for DNA : DNA hybrids but in cases where it is possible to prepare and observe many molecules sufficient data can be obtained to give reliable results [1,2,3].

However if the RNA : DNA hybrid region is quite short – for example the length of a tRNA gene or of a 5S RNA gene (approximately 80 or 120 nucleotides respectively) – the duplex region is much too short to be recognized in a cytochrome *c* spread. Some time ago a ferritin labeling method for mapping tRNA

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and other short RNA genes was introduced. In this procedure the electron opaque label ferritin was covalently attached to the 3′ end of the tRNA by suitable in vitro chemical reactions [4]. The tRNA-ferritin conjugate was hybridized to the DNA strand. The position of the ferritin along the DNA strand can be readily recognized in the electron microscope and defines the position where the tRNA has hybridized.

This ferritin labeling procedure was not entirely satisfactory because the preparation of the tRNA-ferritin conjugate in high yield required meticulous control of the reaction conditions and because the hybridization of the tRNA-ferritin molecule in vast excess to DNA containing tRNA genes never resulted in more than 50% saturation of the genes.

We have therefore investigated an alternative procedure. The philosophy of this new approach is to attach the small molecule, biotin, to the tRNA. The tRNA-biotin conjugate is then hybridized to the DNA. Because biotin is fairly small it is anticipated that the rate and yield of hybridization will be the same as for unlabeled tRNA. Heitzmann and Richards have independently conceived of and developed a method based on the biotin-avidin reaction for ferritin labeling of cell surface components [5].

The protein, avidin, is covalently attached to the electron opaque label ferritin. It is known that biotin reacts with avidin to form a very strong stable non-covalent complex with an estimated dissociation constant of 10^{-15} M [6]. The DNA : tRNA-biotin hybrid is then incubated with ferritin-avidin, and thus, the ferritin label is attached to the position of the tRNA genes.

The overall procedure is as follows:

1) Preparation of tRNA-biotin: a) periodate oxidize the 3′ terminus of the tRNA to give a dialdehyde. This is a good reaction with a yield of 90 to 100%; b) form a Schiff base by reaction of the tRNA with excess 1,5-pentanediamine; c) stabilize the Schiff base against dissociation and β elimination by BH_4-reduction. Reactions (b) and (c) together are somewhat unsatisfactory in our hands, in that the yield varies uncontrollably from 20 to 80%; d) attach biotin to the newly attached terminal amino group on the tRNA by acylation with the N-hydroxy succinimide ester of biotin; e) purify the tRNA-biotin by affinity chromatography on avidin-agarose. Because of the strength of the biotin-avidin bond, the elution of the tRNA-biotin requires extreme conditions, 6 M guanidium hydrochloride, pH 2.5. It is probable that the combination of step c and the acidic elution in step e results in chain breakage of some tRNA's at 7-methyl guanine and dihydrouridine positions.

2) Preparation of ferritin-avidin: a) acylate ferritin with the N-hydroxy succinimide ester of bromoacetic acid [7] to give about 10 active bromide groups per ferritin. Add SH groups to avidin by acylation with the N-hydroxy succinimide ester of dithiodiglycollic acid, followed by exposure of the SH groups by treatment with DTT. Bromo-ferritin is then treated with SH-avidin to give a ferritin-avidin