THE MOLECULAR BIOLOGY OF BACTERIAL GROWTH

Schaechter
Neidhardt
Ingraham
Kjeldgaard

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A Symposium held in honor of Ole Maaløe, at the University of Alabama, Tuscaloosa

Edited by

Moselio Schaechter
Tufts University School of Medicine

Frederick C. Neidhardt
University of Michigan Medical School

John L. Ingraham
University of California, Davis

Niels Ole Kjeldgaard
University of Aarhus, Denmark

With the Help of David Freifelder, University of California, San Diego

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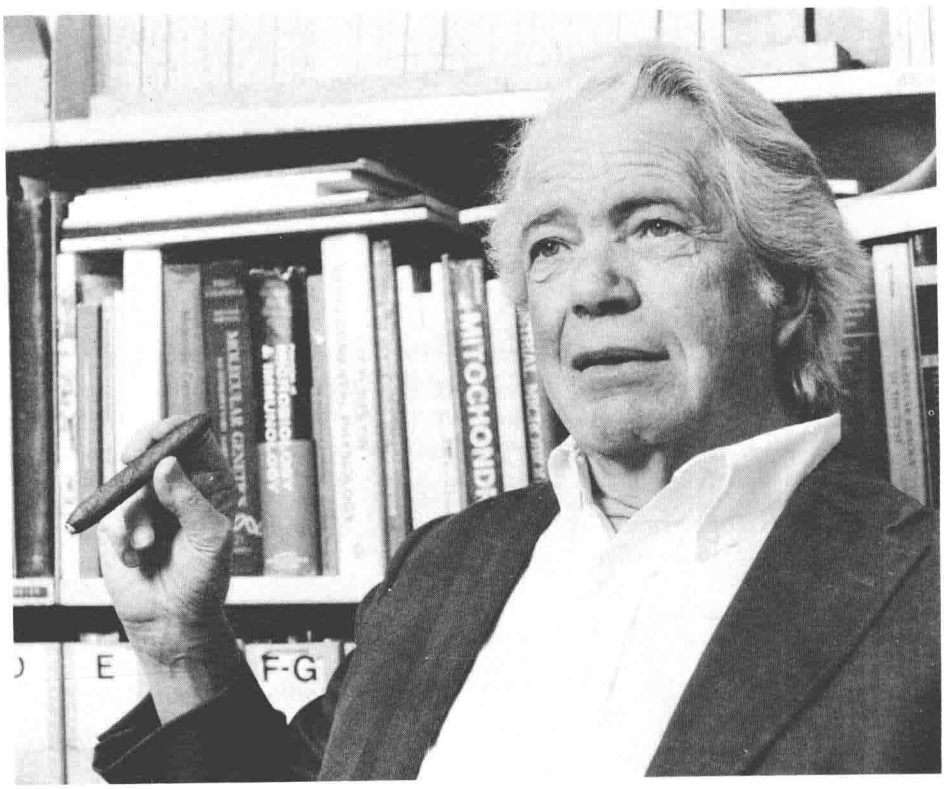


Ole Maaløe

INTRODUCTION

Several years ago, the University of Alabama, Tuscaloosa, Department of Microbiology and the Interdisciplinary Biochemistry Program initiated a new lecture series called Frontiers in Modern Biology. Well-known scientists were brought to Tuscaloosa nearly weekly for about a year, providing an exciting and educational experience for faculty, staff, and students. With unusual wisdom the administration of the University funded this program amply, recognizing the value that it would have for the scientific community of the university. Even though the program does not exist any more, the University continues to hold short courses on specific topics given by respected scientists. I have been given the responsibility to select the speakers for these special programs. In 1983 Professor Robert Haynes (York University, Toronto, Canada) gave a mini-course on biological repair processes.

Following the successful series by Dr. Haynes, I was asked to conceive of and organize a symposium, for the spring of 1984, that would honor someone whose is reknowned in both microbiology and biochemistry. It did not take more than a few minutes to call to mind Professor Ole Maaløe, University Institute of Microbiology, Copenhagen, Denmark. Ole was my mentor during a postdoctoral year in 1962 and has influenced my thinking about cell growth, and the thinking of many others, since that time. Ole Maaløe was the founder of what has become known as the "Copenhagen School" and was instrumental in making cell growth an analytical science. His influence on many young scientists and on the field of microbiology has been profound and felt worldwide. Furthermore, 1984 was Ole's 70th year, and for some time I had been thinking of some way to honor his accomplishments. I made the proposal to various faculty and administrators at the University of Alabama, and they were quickly infected with the notion of a "Maaløe Symposium". The plan was to bring together most of the people who had worked in Ole's lab since the 1950s, to summarize the work of the past thirty years, and to plan for future understanding of cell growth. Our model for the Symposium was to be the highly successful Cold Spring Harbor meetings. I contacted Ole, asking him if he would agreed to be so honored, if he would be available at a time convenient for the University of Alabama, and if he had suggestions for participants. Ole agreed and suggested that the Symposium be planned by four former colleagues, Niels Ole Kjeldgaard (with whom he had worked in Copenhagen for more than a decade), Moselio Schaechter (one of the earliest postdocs in the Copenhagen Institute), and Fred Neidhardt and John Ingraham, with whom he had just authored a book on bacterial growth. These four friends, feeling as I did that honoring Ole was long overdue, contacted Ole's former students and the Symposium began to materialize; it was held in April, 1984.

The University of Alabama was especially generous in providing airfare for many Europeans and Americans, for the general funding

of the meeting, and for a fantastic banquet. Professor Harry Heath of the Department of Microbiology, his wife Lucie, and his graduate students, were responsible for local arrangements and cannot be thanked enough. It took very little talking to convince Donald Jones and Arthur Bartlett of Jones and Bartlett, Publishers, Inc., that the Symposium should be published, not only to honor Ole Maaløe, but because it is an up-do-date and valuable collection of information about cell growth. Authors, editors, and the members of the University of Alabama hope that the readers of the volume can appreciate the unique contribution of Ole Maaloe to this field and can sense some of the excitement that took place at the Symposium.

January, 1985
San Diego, California

David Freifelder

IN RETROSPECT

By October 1983 our institute in Copenhagen celebrated its 25th anniversary, and the occasion was marked by a great party. Sadly enough, not one of our many foreign friends and collaborators was in Denmark at that time. The symposium presented in this volume has made up for this in a splendid manner, and the silver anniversary in Copenhagen and the banquet in Tuscaloosa complement each other to give a true picture of the life and spirit in our laboratory, as I like to think of it.

In 1950 while I still worked in three small rooms at the State Serum Institute, fortune brought the first foreign scientists to the lab: Jim Watson, Günther Stent, Niels Jerne and I worked together in Copenhagen for a year at the end of which the group disintegrated. Jim went to Cambridge to meet Francis Crick, Günther went to André Lwoff's lab in Paris, and I joined the phage group in Max Delbrück's lab in Pasadena. Jerne stayed in Copenhagen, but not for long.

Back from Caltech it worried me that the exciting collaboration with scientists from other parts of the world might have come to an end. Nothing of the kind, our lab had been put on the map, and throughout the years of plenty, when fellowships to study abroad were easy to obtain, a large number of foreign scientists came to work with us (mostly post-docs and senior scientists on sabbatical leave from the US, but also quite a few from other countries in Europe.)

I like to think that our guests were attracted by the particular approach to the study of bacterial growth that grew out of work done in the small rooms in the Serum Institute by Gordon Lark, Victor Bruce, Elio Schaechter, Niels Ole Kjeldgaard and myself. Gordon and I first worked out a scheme involving shifts between 25 and 37 C that induced division synchrony in broth cultures of *S. typhimurium;* however, we went on to find that the temperature treatment distorted the normal pattern of DNA replication. At that point we abandoned the system, both being interested primarily in *normal growth* (it was a sad decision, for the synchrony curves looked quite nice).

Having seen how easy it is to introduce artifacts by submitting a growing culture to such "mild" treatments as shifts between two temperatures that both permit exponential growth, we went for the simplest possible experimental designs, banning all interference with the process of growth. Elio, Niels Ole and I began to analyze cultures in balanced growth in media supporting different rates of growth. The rigor with which we defined the state of balanced growth, and stressed the importance of the growth rate as a basic variable are spelled out at some length in the monograph Niels Ole and I wrote several years later (*Control of Macromolecular Synthesis,* Benjamin 1966). Our rule has therefore been, first, to measure at different growth rates the parameters that can be measured without disturbing

the culture, and, second, only to interfere with growth if we know the *primary effect* of the agent or procedure used. Examples are starvation for a required amino acid, or addition of reagents that interfere with growth in a clearly defined way such as chloramphenicol, rifampicin or alpha-methyl-glucoside. Probably, our main achievement was to introduce experimental designs that emphasized simplicity and thereby facilitated the interpretation of the data obtained. This attitude in a sense defined the Copenhagen School of bacterial growth physiology, a recent product of which is the textbook by Ingraham, Maaløe and Neidhardt (*Growth of the Bacterial Cell,* 1983.).

These rules of conduct should, I think, be applied to studies of bacteria in general; we concentrated on *Escherichia coli* to permit us to use genetic analysis. I don't intend to present a chronology of people nor of their individual contributions over the last 25 years. However, looking back, a few key experiments stand out—most of them fruits of collaborative efforts. These experiments are referred to or implied in many of the contributions to this book.

Our work developed along two lines. One was based on the first data obtained with cultures in balanced growths at different rates (Schaechter, Maaløe and Kjeldgaard, 1958). The strong hint of a constant rate of protein synthesis per "nucleoprotein particle" (not yet known as a ribosome, let alone identified as the site of polypeptide synthesis) made us focus on the protein-synthesizing system (PSS) as a whole. Secondly, we were concerned with DNA replication *in vivo*. Shortly after the lab had moved to the present location in the Botanical Garden, a "run-out experiment" was done, which showed that in the absence of protein synthesis a round of DNA replication, once initiated, would run to completion, but without initiation of a new round (Maaløe and Hanawalt, 1961). The obvious is not always easy to spot, and only many years later was it realized by K. V. Rasmussen in our lab that the run-out technique offers direct estimates of the number of origins in a population of replicating genomes.

For at least 15 years after we moved we worked in the broad area of growth physiology. On the PSS project techniques were invented or adapted to permit quantitative measurements of parameters such as the elongation rates of RNA and polypeptide chains, the rate of synthesis of ribosomal proteins and other factors involved in protein synthesis, and the amounts and stability of mRNA. Extensive use was made of rifampicin to carry out run-out experiments on RNA synthesis by Pato, von Meyenburg and Molin, and 2-D gels to measure mRNA half-life and to estimate amounts of nonribosomal PSS proteins by Pedersen and Neidhardt. A project run exclusively by in-house people should be mentioned: The instantaneous down-shift caused by adding alpha-methyl-glucoside to a culture in glucose minimal medium was analyzed by Molin, von Meyenburg, Karlstrøm and a graduate student, Knud Johnsen (see Chapter 8 in Ingraham et al., 1983). Knud taught me a lesson; he declined an offer to continue a very promising scientific career. He was quite sure he wanted to teach biology in high school, and he was right, for he is a great teacher.

Together these measurements establish the high and nearly constant efficiency of ribosomes engaged in protein synthesis, and the more-or-less constant average protein yield per polysome (see my paper in this volume). Embedded in these studies was a thorough examination of ppGpp synthesis and levels at different growth rates in stringent as well as relaxed strains by Fiil, Friesen, and von

Meyenburg. They showed conclusively that ppGpp is of minor importance for the adjustment of the size of PSS to growth rate. This work was important in another way: it marked the beginning of genetic studies in our lab and involved isolation of mutants requiring elaborate selection techniques.

On the DNA project a new turn was marked by the visit to Copenhagen of Cooper and Helmstetter (1963-64), who taught us to use the "Baby-machine" to obtain division synchrony by selection. Their technique should introduce few, if any, artifacts, so this time we did not hesitate to use synchronized cultures. The experiments and thoughts of the following years were focussed on the initiation of replication, and the ideas of Pritchard and of Donachie played important roles. During this time the concept of autoregulation was introduced by Sompayrac and Maaløe (1973). It was suggested as a possible means of "timing" initiation.

During the last 10 years molecular genetics has been an increasingly important part of our work, but the link to growth physiology has been maintained. Most significantly, some of the autoregulations that seem to balance the synthesis of the different ribosomal components have been analyzed by Fiil, Pedersen, and Johnsen with strong inputs from a number of graduate students, and in collaboration with scientists in the U.S. (Nomura's group in Madison; Lindahl's lab in Rochester; Cathy and Craig Squires' lab in New York), in Canada (Friesen's group in Toronto; Dennis' lab. in Vancouver), and in the Wittmann group in West Berlin. Several papers in this volume illustrate these activities, the results of which have been so important for my own attempts to draw an integrated picture of a growing *E. coli* cell (see my paper in this volume).

On the DNA front, genetics came to be equally important. In the beginning there were a few mutants with altered DNA/mass ratios, some of them isolated the hard way by Knud Rasmussen and Flemming Hansen (they scanned agar plates with lots of colonies with only 50-100 cells, looking for abnormally large or small cells). The full switch to molecular genetics came with Kaspar von Meyenburg, who in 1975 turned his attention from ribosomes to DNA replication. As you know, we had already focussed on the act of initiation as the key to understanding the control of replication, and Kaspar's success in identifying and sequencing the site of initiation (*oriC*) was most encouraging. However, this turned out to be another case in which the sequence itself did not suggest a molecular mechanism. Scanning the region around *oriC*, the *dnaA* gene and its product were analyzed with two interesting results: (1) the DnaA protein was shown to be autoregulated, and (2) certain suppressors of *dnaA* mutants, isolated and studied by Tove Atlung, show that the DnaA protein interacts directly with the RNA polymerase, presumably in the act of synthesizing the RNA primer at the site of initiation of replication. This and more is presented by Kaspar, Tove, Flemming, Knud Rasmussen and others in this volume.

I have now brought the history of our activities up to the present, and I have included recent work in the two segregated groups: Kjeldgaard's group at the University of Aarhus (established in 1968), and von Meyenburg's group at the Technical University in Lyngby (1978). A third group has just been created by Søren Molin, next door to Kaspar's unit. It is time therefore to tell what I feel has been my own role during all these years. The two papers I wrote

with Kjeldgaard and Schaechter and published in 1958 clearly shaped the course we have followed up to the present. Apart from this initial input, and a few later ones already mentioned, my main contribution has been to keep us on course. What this means is perhaps best seen by comparing my latest attempt to describe the properties of a growing *E. coli* cell (this volume) to a quote from the contribution I made to the 1960 Symposium of the Society for General Microbiology. The final paragraphs read:

The sketch of the growing bacterium presented here is based essentially on the idea of exchange of information between different molecular levels of organization in the cell. A flow of information is assumed to descend from a linear, genetic specification on a DNA strand, via RNA and protein and to give to a small molecule, such as an amino acid, its three-dimensional individuality. Equally specific information is believed to pass from the level of the small molecules back in the direction of the nucleus. This feedback of information, which produces the phenomenon of repression, is thought to be responsible for one of the remarkable properties of the cell: its ability to adjust the size and activity of different synthetic systems to the set of nutrients present in the medium; an adjustment that results in the establishment of a definite partitioning of energy and matter among the synthetic systems, to which corresponds a definite growth rate and cell composition.

In a paper like this, interpretations and generalizations certainly play an important role. It should therefore be made clear that we have adopted the view that RNA templates exist and are formed by direct contact with the DNA of the nucleus, and that repression involves the function but not the formation of the protein-synthesizing systems, because we find that alternative mechanisms, even if they cannot be excluded, appear less plausible."

These paragraphs at once show where we aimed and that we started out when molecular biology was in its infancy. They also give an idea of the great amount of work and thought contributed by my friends at home and abroad to put substance into the primitive 1960 sketch.

Sydney Brenner once offered a very brief description of our work. At a small symposium in Cambridge (England) he asked me what I wanted to talk about, and when I had explained the main idea, he said: "Oh, you are going to talk about the effects of banging on a network."

Ole Maaløe

SYMPOSIUM PARTICIPANTS

Tove Atlung, *University Institute of Microbiology; Copenhagen, Denmark.*

Michael L. Berman, *Litton Institute of Applied Biotechnology; Rockville, Maryland.*

Patrick P. Dennis, *Department of Biochemistry, The University of British Columbia; Vancouver, B.C.*

William D. Donachie, *Department of Molecular Biology, Edinburgh University; Edinburgh, Scotland.*

Abraham Eisenstark, *University of Missouri; Columbia, Missouri.*

David Freifelder, *University of California, San Diego.*

James D. Friesen, *Department of Medical Genetics, University of Toronto; Toronto, Ontario.*

Jonathan Gallant, *Genetics Department, University of Washington; Seattle, Washington.*

Kirsten Gausing, *Department of Molecular Biology and Plant Physiology, University of Aarhus; Aarhus, Denmark.*

Larry Gold, *Department of Molecular, Cellular and Developmental Biology, University of Colorado; Boulder, Colorado.*

Flemming G. Hansen, *Department of Microbiology, The Technical University of Denmark; Lyngby-Copenhagen, Denmark.*

Harry E. Heath, *University of Alabama, Tuscaloosa.*

Lucie S. Heath, *University of Alabama, Tuscaloosa.*

Charles. E. Helmstetter, *Department of Experimental Biology, Roswell Park Memorial Institute; Buffalo, New York.*

John L. Ingraham, *University of California, Davis.*

Morten Johnsen, *Institute of Microbiology, University of Copenhagen Copenhagen, Denmark.*

Herman M. Kalckar, *Chemistry Department, Boston University; Boston, Massachusetts.*

Niels Ole Kjeldgaard, *Institute of Molecular Biology, University of Aarhus; Aarhus, Denmark.*

Tokio Kogoma, *Department of Biology, University of New Mexico; Albuquerque, New Mexico.*

Peter Kuempel, *Department of Molecular, Cellular and Developmental Biology, University of Colorado; Boulder, Colorado.*

Charles G. Kurland, *Department of Molecular Biology, The Biomedical Center; Uppsala, Sweden.*

Karl G. Lark, *University of Utah; Salt Lake City, Utah.*

Lasse Lindahl, *Department of Biology, The University of Rochester; Rochester, New York.*

Cyrus Levinthal, *Columbia University; New York, New York.*

Ole Maaløe, *University Institute of Microbiology; Copenhagen, Denmark.*

Millicent Masters, *Department of Molecular Biology, Edinburgh University; Edinburgh, Scotland.*

Agnete Munch-Petersen, *University Institute of Biological Chemistry B; Copenhagen, Denmark.*

Frederick C. Neidhardt, *Department of Microbiology and Immunology, University of Michigan Medical School; Ann Arbor, Michigan.*

Jan Neuhard, *Enzyme Division, University Institute of Biological Chemistry B; Copenhagen, Denmark.*

Donald P. Nierlich, *Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles.*

Masayasu Nomura, *Institute for Enzyme Research and Departments of Genetics and Biochemistry, University of Wisconsin; Madison, Wisconsin.*

Martin Pato, *National Jewish Hospital; Denver, Colorado.*

Steen Pedersen, *Institute of Microbiology, University of Copenhagen, Copenhagen, Denmark.*

Robert H. Pritchard, *Department of Genetics, University of Leicester; Leicester, England.*

Moselio Schaechter, *Department of Molecular Biology and Microbiology, Tufts University; Boston, Massachusetts.*

Robert Schleif, *Biochemistry Department, Brandeis University; Waltham, Massachusetts.*

Catherine L. Squires, *Department of Biological Sciences, Columbia University; New York, New York.*

Gunther Stent, *Department of Molecular Biology, University of California, Berkeley.*

Annamaria Torriani, *Department of Biology, Massachusetts Institute of Technology; Cambridge, Massachusetts.*

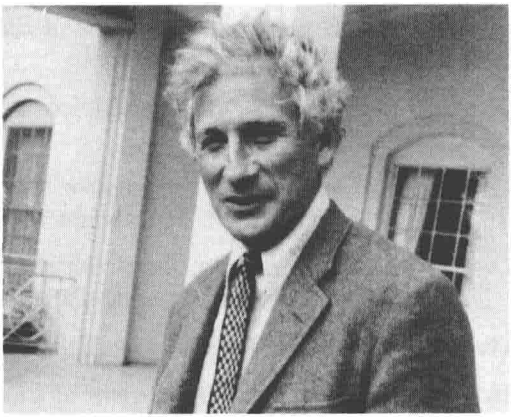
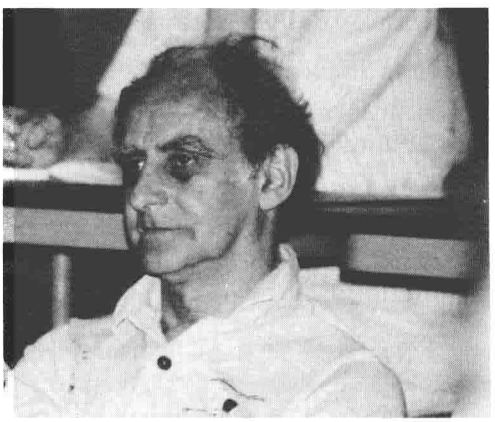
Kaspar von Meyenburg, *Department of Microbiology, The Technical University of Denmark; Lyngby-Copenhagen, Denmark.*

Alvin L. Winters, *University of Alabama, Tuscaloosa*

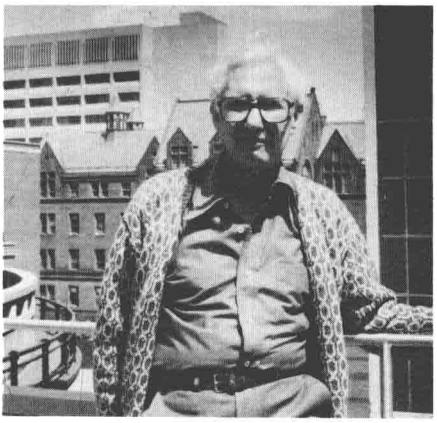
Richard E. Wolf, Jr., *Department of Biological Sciences, University of Maryland, Baltimore County; Catonsville, Maryland.*

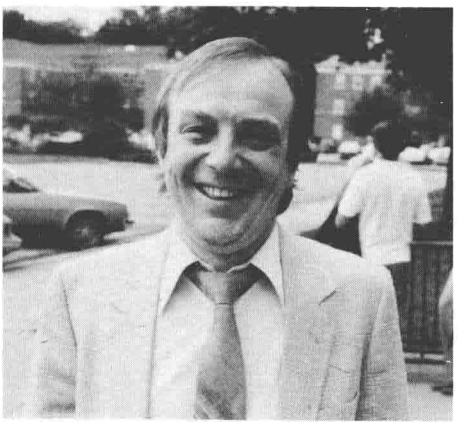
Andrew Wright, *Tufts University Medical School; Boston, Massachusetts.*

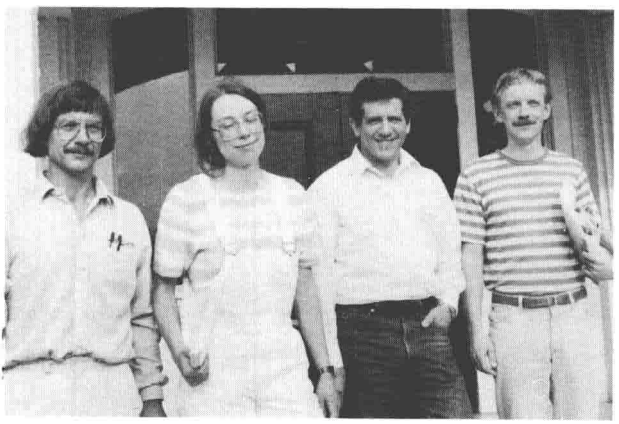
Charles Yanofsky, *Department of Biological Sciences, Stanford University; Stanford, California.*

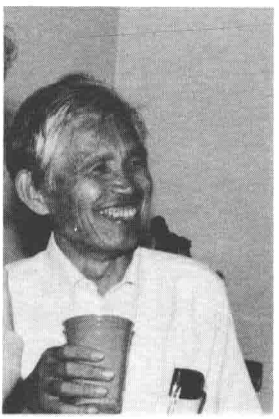





Top Row (left to right): O. Maaløe; K.G. Lark.
Middle: R. Pritchard; R. Schleif.
Bottom: C. Squires; O. Maaløe, A. Wright, A. Torriani, P. Kuempel, W. Donachie.









Top Row: C. Levinthal; L. Lindahl,
Middle: F. Hansen, T. Atlung, R. Wolf, M. Johnsen; M. Nomura.
Bottom: T. Kogoma; J.D. Friesen, L. Gold.