MITOCHONDRIAL GENES

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Edited by

Piotr Slonimski
Centre de Génétique Moléculaire du CNRS

Piet Borst
University of Amsterdam

Giuseppe Attardi
California Institute of Technology

Cold Spring Harbor Laboratory
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Preface

In the last few years, powerful new DNA, RNA, and protein technologies have been introduced. Combined with a refined genetic analysis, these technologies have promoted rapid developments in the investigation of the mitochondrial genome in a variety of organisms and have placed this system at the forefront of modern biological research. The Mitochondrial Genes meeting held at Cold Spring Harbor Laboratory in May 1981 testified to the extraordinary progress made in the understanding of the mitochondrial genetic system since the discovery, about 30 years ago, of the yeast "petite" mutation by Boris Ephrussi, to whom this book is dedicated, and his collaborators.

The 47 papers included in this volume cover a wide range of topics, including the mitochondrial genetic code, mitochondrial DNA replication, gene organization and expression, nuclear-mitochondrial interactions, and evolution of mitochondrial DNA. It thus provides a comprehensive account of the current knowledge concerning mitochondrial genes and a perspective of future directions of research in the field.

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P. Slonimski
P. Borst
G. Attardi

Meeting Participants

Agsteribbe, E., *State University, Groningen, The Netherlands*
Ainley, Mike, *University of Texas, Dallas*
Anderson, Stephen, *Medical Research Council, Cambridge, England*
Arnberg, A.C., *State University at Groningen, The Netherlands*
Attardi, Giuseppe, *California Institute of Technology, Pasadena*
Bandlow, Wolfhard, *University of Munich, Federal Republic of Germany*
Belcour, Leon, *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Bendich, Arnold, *University of Washington, Seattle*
Berlani, Roberta, *Columbia University, New York, New York*
Bernardi, Giorgio, *Institut de Recherche en Biologie Moléculaire, Paris, France*
Bertrand, H., *University of Regina, Canada*
Birky, William C., *Ohio State University, Columbus*
Blanc, Hughes, *Stanford University School of Medicine, California*
Boer, P.H., *Genetic Institute, Amsterdam, The Netherlands*
Bogenhagen, Daniel, *Carnegie Institution of Washington, Baltimore, Maryland*
Bonen, Linda, *University of Amsterdam, The Netherlands*
Bonitz, Susan, *Columbia University, New York, New York*
Borst, Piet, *University of Amsterdam, The Netherlands*
Breitenbach, Michael, *Institut für Allgemein Biochemie, Wien, Austria*
Brown, Gregory, *State University of New York, Stony Brook*
Burke, John, *Massachusetts Institute of Technology, Cambridge*
Butow, R.A., *University of Texas, Dallas*
Cann, Rebecca, *University of California, Berkeley*
Carignani, Giovanna, *Istituto di Chimica Biologica, Padova, Italy*
Castora, Frank, *State University of New York, Stony Brook*
Ching, Edwin P., *Rockefeller University, New York, New York*
Claise, M.L., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Clark-Walker, G.D., *Australian National University, Canberra*
Clayton, David A., *Stanford University, California*

Colson, Anne M., *University of Louvain, Louvain-La-Neuve, Belgium*
Coruzzi, Gloria, *Rockefeller University, New York, New York*
Cottrell, Stephen, *Brooklyn College, New York*
Cummings, Donald, *University of Colorado, Denver*
Dale, Roderick, *Yale University, New Haven, Connecticut*
Davies, R. Wayne, *University of Essex, Colchester, England*
Dayan, Jean, *Hartsdale, New York*
De Francesco, Laura, *City of Hope Research Center, Duarte, Colorado*
De Vries, H., *University of Groningen, The Netherlands*
De Zamaroczy, Miklos, *Institute of Molecular Biology, Paris, France*
Del Giudice, Luigi, *International Institute of Genetics/Biophysics, Napoli, Italy*
Deters, Donald, *University of Texas, Austin*
Dieckmann, Carol, *Columbia University, New York, New York*
Doersen, Claus J., *California Institute of Technology, Pasadena*
Dubin, Donald T., *Rutgers Medical School, Piscataway, New Jersey*
Dujardin, G., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Dujon, Bernard, *Harvard University, Cambridge, Massachusetts*
Englund, Paul, *Johns Hopkins University School of Medicine, Baltimore, Maryland*
Esser, Karl, *Ruhr Universität Bochum, Germany*
Farrelly, Frances, *University of Texas, Dallas*
Faugeron-Fonty, Godeleine, *Institute of Molecular Biology, Paris, France*
Fauron, C.M.R., *University of Utah, Salt Lake City*
Ferris, Stephen, *University of California, Berkeley*
Finzi, Eric, *Mt. Sinai School of Medicine, New York, New York*
Fox, Thomas, *University of Basel, Switzerland*
Freeman, K.B., *McMaster University, Hamilton, Canada*
Frontali, Laura, *University of Rome, Italy*
Fukuhara, M. Bolotin, *Laboratory of General Biology, Orsay, France*
Fukuhara, Hiroshi, *Institut Curie, Orsay, France*
Gelfand, Robert, *Purdue University, West Lafayette, Indiana*
George, Matthew, *San Diego Zoo, California*
Gillham, Nicholas, *Duke University, Durham, North Carolina*
Gollub, Edith, *Barnard College, New York, New York*
Goltz, Sherry, *Rockefeller University, New York, New York*
Goursot, Regina, *Institute of Research/Molecular Biology, Paris, France*
Grant, David, *St. Louis University, Missouri*
Gray, M.W., *Dalhousie University, Halifax, Nova Scotia, Canada*
Greenberg, Barry, *University of North Carolina, Chapel Hill*
Grivell, L.A., *University of Amsterdam, The Netherlands*
Grossman, Lawrence, *University of Michigan, Ann Arbor*
Hajduk, Stephen, *Johns Hopkins University School of Medicine, Baltimore, Maryland*
Halbreich, A., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Hanson, Deborah, *Indiana University, Bloomington*
Hare, James, *Portland, Oregon*
Hauswirth, William W., *University of Florida, Gainesville*
Heckman, Joyce, *Indiana University, Bloomington*

Hensgens, L.A.M., *University of Amsterdam, The Netherlands*
Howell, Neil, *Sidney Farber Cancer Institute, Boston, Massachusetts*
Hudson, Alan, *University of Texas, Dallas*
Hyman, Bradley, *University of Wisconsin, Madison*
Jacq, C., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Jayaraman, J., *Madurai Kamaraj University, Madurai, India*
Jubier, M.F., *Rockefeller University, New York, New York*
Kaudewitz, F., *Genetic Institute, University of Munich, Germany*
Kearsey, S.E., *Genetics Laboratory, Oxford, England*
Keyhani, E., *University of Pennsylvania Medical School, Philadelphia*
Kahn, Nasim, *Brooklyn College, New York*
Kidane, Gretachew, *University of California, Los Angeles*
Knight, Jeffery, *Mount Holyoke College, South Hadley, Massachusetts*
Koike, Katsuro, *Japanese Foundation for Cancer Research, Tokyo*
Kruszeweska, Anna, *Polish Academy of Sciences, Warsaw*
Kumar, C.C., *Massachusetts Institute of Technology, Cambridge*
Kuntzel, Hans, *Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany*
Laipis, Philip, *University of Florida, Gainesville*
Lambowitz, Alan, *St. Louis University Medical School, Missouri*
Lansman, Robert, *University of Georgia, Athens*
Lazowska, J., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Leaver, C.J., *University of Edinburgh, Scotland*
Li, May K., *Columbia University, New York*
Liebhard, Aurora B., *Center for Cellular Physics, Universidad Nacional Autonoma de Mexico, Mexico*
Linnane, A.W., *Monash University, Clayton, Australia*
Macino, Giuseppe, *University of Rome, Italy*
Mahler, Henry R., *Indiana University, Bloomington*
Mangin, M., *Institute of Research in Molecular Biology, Paris, France*
Marban, S. Lee, *Yale University School of Medicine, New Haven, Connecticut*
Marini, Joan, *Johns Hopkins School of Medicine, Baltimore, Maryland*
Marotta, Renzo, *Institute of Research in Molecular Biology, Paris, France*
Martin, Nancy, *University of Texas, Dallas*
Martin, Robert, *Institute of Molecular and Cell Biology, Strasbourg, France*
McKee, Edward, *University of Connecticut, Farmington*
Michaelis, Georg, *University of Biolefeld, Federal Republic of Germany*
Montoya, Julio, *California Institute of Technology, Pasadena*
Mounolou, Jean, *University of Texas, Dallas*
Nass, Margit, *University of Pennsylvania Medical School, Philadelphia*
Netter, P., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Newmark, Peter, *Nature Publishing Co., New York, New York*
Osinga, K.A., *University of Amsterdam, The Netherlands*
Pajot, P., *Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France*
Pepe, Gabriella, *Istituto Chimica Biolgica, Bari, Italy*
Perlman, Philip S., *Ohio State University, Columbus*
Pollack, Y., *Hebrew University Hadassah Medical School, Jerusalem, Israel*
Power, Scott D., *University of Connecticut, Farmington*

Poyton, Robert, *University of Connecticut, Farmington*
Rabinowitz, Murray, *University of Chicago, Illinois*
RajBhandary, Uttam, *Massachusetts Institute of Technology, Cambridge*
Rickwood, David, *University of Essex, Colchester, England*
Roe, Bruce, *Kent State University, Ohio*
Saccone, Cecilia, *Istituto Chimica Biologica, Bari, Italy*
Sager, Ruth, *Sidney Farber Cancer Institute, Boston, Massachusetts*
Scheyen, R.J., *Genetisches Institut, Munich, Federal Republic of Germany*
Sena, Elissa, *Case Western Reserve University, Cleveland, Ohio*
Shade, Rosemary, *University of Georgia, Athens*
Simpson, Melvin, *State University of New York, Stony Brook*
Simpson, Larry, *University of California, Los Angeles*
Simpson, Agda, *University of California, Los Angeles*
Slonimski, Piotr, *Centre de Génétique du CNRS, Gif-sur-Yvette, France*
Solus, Joseph, *Yale University School of Medicine, New Haven, Connecticut*
Stanbridge, Eric, *University of California, Irvine*
Stepien, Piotr, *Warsaw University, Poland*
Strausberg, Robert, *Southern Methodist University, Dallas, Texas*
Suyama, Yoshitaka, *University of Pennsylvania, Philadelphia*
Tabak, H.F., *University of Amsterdam, The Netherlands*
Tait, Andrew, *University of Edinburgh, Scotland*
Thalenfeld, Barbara, *Columbia University, New York, New York*
Turner, Geoffrey, *University of Bristol, England*
Tzagoloff, Alex, *Columbia University, New York, New York*
Van den Boogaart, P., *State University Groningen, The Netherlands*
Van Etten, Richard, *Stanford University, California*
Wakabayashi, Kazuhiko, *University of Yamanashi, Japan*
Walberg, Mark, *Stanford University, California*
Wallace, Douglas, *Stanford University, California*
Werner, Sigurd, *Institut Physiologische Chemie, Munich, Federal Republic of Germany*
Wilkie, David, *University College London, England*
Wolf, Klaus, *Genetisches Institut, Munich, Federal Republic of Germany*
Wolstenholme, David R., *University of Utah, Salt Lake City*
Yin, Samuel, *Massachusetts Institute of Technology, Cambridge*
Zassenhaus, H. Peter, *University of Texas, Dallas*

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Boris Ephrussi and the Early Days of Cytoplasmic Inheritance in *Saccharomyces*

Herschel Roman
Department of Genetics, SK-50
University of Washington
Seattle, Washington 98195

This volume on mitochondrial genes is dedicated to the memory of Boris Ephrussi,[1] whose pioneering investigations of cytoplasmic inheritance in yeast set the stage for what is now a rapidly expanding area of molecular biology. I was fortunate to spend 1952–1953 in his laboratory, when he and his students were preoccupied with understanding the petite mutation, so called because its small-colony phenotype was expressed when mutant cells were grown on solid medium. The small size was due to a respiratory deficiency of the mutant cells. One of the students, Piotr Slonimski, concerned himself with the biochemistry of the petite mutation while Ephrussi was working out the genetics and physiological aspects of petiteness—the same Piotr Slonimski who is an editor of this volume. He and his collaborators have been the principal contributors to the theory of the structure and function of yeast mitochondrial DNA (mtDNA) and of its relation to petiteness.

INHERITANCE OF THE PETITE PHENOTYPE

The first evidence that the original petite mutation was cytoplasmic in its inheritance was reported in 1949 (Ephrussi et al. 1949b). Mutant cells, when mated with normal cells, gave a normal diploid; upon sporulation, this diploid produced asci in which all four spores were generally normal. Repeated backcrosses to the mutant gave the same result. Thus, since a 2:2 segregation would be expected if the mutation were nuclear in origin, it was concluded that the mutation was in fact cytoplasmic.

The petite story is an example of the unexpected discovery and the logical analysis of its meaning. When he began working with yeast, Ephrussi chose the bactericidal agent acriflavine as a possible specific mutagen because of its known interaction with nucleic acid. His expectation was realized when the preliminary experiment revealed that acriflavine induced in growing cultures a massive transformation to the petite

[1]Ephrussi made significant contributions in three quite separate areas of research. For a summary of his principal achievements, see Roman (1980).

state; i.e., most of the cells, when plated on solid medium, exhibited the small-colony phenotype. The high rate of mutation was surprising, as was the stability of the mutant: It did not revert to the normal phenotype. These were unusual characteristics for a gene mutation; so too was the fact that the small-colony phenotype occurred spontaneously with an unusually high frequency compared with those of known gene mutations in other organisms. It was fortunate that the yeast was capable of growing on either fermentable or nonfermentable substrates. Otherwise, of course, the petite mutations would have been lethal. Thus, the first impression was either that acriflavine was an unusual mutagen or that the petite mutation was exceptional (Ephrussi et al. 1949a). The petite mutation was thought to result from a loss of autoreproducing cytoplasmic particles (Ephrussi et al. 1949b). It was further hypothesized that these particles may be identical with the large cytoplasmic granules carrying cytochrome oxidase and corresponding to mitochondria (Slonimski and Ephrussi 1949).

The fact that the petite phenotype disappeared in the diploid in matings with normals and did not reappear in the haploid progeny was itself quite fortuitious. Ephrussi et al. (1955) found another type of petite, indistinguishable in ordinary tests from the first, that in matings with normals gave diploids in which the frequency of petites, although more or less specific for a given parentage, varied over a wide range and sometimes approached 100%. This type of petite was called suppressive, since the normal phenotype was suppressed when the two were mated. The results obtained in crosses with a suppressive petite were quite different from those obtained with a neutral petite, as the first type came to be called. Thus, the interpretation of the results of crosses would not have been as straightforward if a suppressive petite had been encountered at the start.

The paper citing genetic evidence in favor of the cytoplasmic nature of the petite (Ephrussi et al. 1949b) was met with a certain reserve. There were several reasons for this lack of appreciation. First, yeast was unfamiliar to geneticists, and its rules of inheritance were suspect. Gene conversion was a controversial issue that added to the idea that yeast was not a reliable genetic organism. Second, cytoplasmic inheritance itself was regarded as an oddity, being confined to such examples as plastids in plants and the killer factor in *Paramecium* (see Ephrussi 1951). Third, it was thought by many that the cytoplasm was under the control of the nucleus and that all phenomena attributed to cytoplasmic inheritance could be explained if the actions of nuclear genes were fully understood.

The next important finding in yeast that bore on the nucleus/cytoplasm argument was again made in Ephrussi's laboratory (Chen et al. 1950) with the discovery of a spontaneously occurring petite that proved to be the result of a nuclear gene mutation. A mating between this petite and a normal cell gave a normal diploid which, upon sporulation, gave the two

phenotypes in the 2:2 ratio expected of Mendelian segregation. Moreover, when the segregational petite was mated with the neutral petite, the ensuing diploid was normal in phenotype and the asci again exhibited the 2:2 segregation. This was interpreted to mean that the neutral petite bore a wild-type nuclear gene and was defective in its cytoplasm, whereas the segregational petite was mutant for the wild-type gene but carried the normal cytoplasmic factor. The diploid from the mating was therefore heterozygous for the mutant gene and had a normal cytoplasm.

THE SUPPRESSIVE PETITE

The third and last petite phenotype that was found in Ephrussi's laboratory was the suppressive petite (Ephrussi et al. 1955) referred to earlier. When matings were made between the suppressive petite and a normal and the diploid cells were sporulated immediately, asci of two types were obtained: those for which the four spores gave rise to cultures that were all normal and those which produced cultures that were all petite. When the diploids were grown for several generations, only asci of the first type were obtained.

It was first considered that the suppressive petite consisted of two types of cells, those which were like the neutral petite and those which were 100% suppressive, i.e., totally efficient in replacing the normal cytoplasmic factor. This hypothesis could account for the sporulation results if it were assumed that the suppressive factor took some time in replacing the normal factor. Thus, some diploid cells still had the capacity for sporulation (petite diploids do not sporulate) but produced only petite spore cultures. A simple test showed that this hypothesis was wrong. On plating a petite that was 50% suppressive, one would expect to obtain 50% neutrals and 50% total suppressives. This proved not to be the case. Instead, subcloning a suppressive petite gave again the same average amount of suppressiveness but, in addition, gave an array of subclones with different degrees of suppressiveness (Ephrussi and Grandchamp 1965; Ephrussi et al. 1966). It became obvious that, although there were three general classes of petites—the nuclear, the neutral, and the suppressive—all with identical phenotypes as determined by the methods then available, there was in fact a much larger number, if the different degrees of suppressiveness are evidence of different molecular (DNA) explanations. One such explanation is given for a highly suppressive strain by Blanc and Dujon (1980). (It also became clear that a large number of nuclear genes can mutate to produce a respiratory-deficient phenotype and that several of these mimic the cytoplasmic petite.)

It is a curious fact that even in the later papers (cited above) by Ephrussi and his collaborators, no mention is made of mtDNA as a possible site of the petite mutation. Although he could not have been oblivious of the