THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 285, No. 36, Issue of September 3, p. e11, 2010

Printed in U.S.A.

Classics

A PAPER IN A SERIES REPRINTED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial
1905–2005
100 Years of Biochemistry and Molecular Biology

Isolation and Characterization of Cytochrome *c* Mutants: the Work of Fred Sherman

The Mutational Alteration of the Primary Structure of Yeast Iso-1-cytochrome *c* (Sherman, F., Stewart, J. W., Parker, J. H., Inhaber, E., Shipman, N. A., Putterman, G. J., Gardisky, R. L., and Margoliash, E. (1968) *J. Biol. Chem.* 243, 5446–5456)

Identification of Missense Mutants by Amino Acid Replacements in Iso-l-cytochrome *c* from Yeast (Putterman, G. J., Margoliash, E., and Sherman, F. (1974) *J. Biol. Chem.* 249, 4006–4015)

Fred Sherman

Fred Sherman was born in Minneapolis, Minnesota in 1932. He attended the University of Minnesota where he earned a bachelor's degree in chemistry in 1954. Sherman then enrolled in the biophysics graduate program at the University of California, Berkeley and began working with Robert K. Mortimer on the induction of ρ^- mitochondrial mutants by elevated temperature in *Saccharomyces cerevisiae*.

Sherman received his doctorate in biophysics in 1958 and moved to Seattle where he did a postdoctoral fellowship at the University of Washington, studying recombination in yeast with Herschell Roman. In 1960, Sherman went abroad to the Laboratorie de Génétique Physiologique at the Centre National de la Recherche Scientifique (National Center for Scientific Research (CNRS)) in Gif-sur-Yvette, France. There he worked with Boris Ephrussi and initiated a research program that led to the discovery of the cytochrome c_1 (*CYC1*) gene and its mutant, *cyc1-1* (1).

At the time the *cyc1-1* mutant was discovered, scientists were inferring all information on gene structure from mutationally altered proteins because DNA sequencing had yet to be invented. Genetic codes that were deciphered were done by analysis of amino acid replacements and mutagenic specificity. There were only three proteins that were readily amenable to mutational analysis, tryptophan synthetase from *Escherichia coli*, lysozyme from bacteriophage T4, and the tobacco mosaic virus (TMV) coat protein. Thus, the discovery of an additional mutant on a protein that could easily be purified was of considerable importance.

Sherman returned to the United States in 1961 and joined the faculty of the University of Rochester where he continued his cytochrome *c* investigations and discovered two forms of

cytochrome *c* in yeast: iso-1-cytochrome *c* and iso-2-cytochrome *c* (2). He also began to devise methods to detect cytochrome *c*-deficient mutants, and using low temperature (−196 °C) spectroscopic examination of large numbers of strains on the surface of nutrient agar plates, he discovered a second mutation at the *CYC1* locus, *cyc1-2*, as well as mutations in the *CYC2* and *CYC3* loci (3). (The *CYC* gene was later shown to encode heme lyase (4), and *CYC2* was sequenced and shown to encode a mitochondrial protein required for normal mitochondrial import of cytochrome *c* (5).)

A few years later, Sherman developed a better mutant isolation procedure that relied on the staining of colonies with benzidine reagents. As reported in the first *Journal of Biological Chemistry* (JBC) Classic reprinted here, the yeast colonies were exposed to an H_2O_2 solution and then a benzidine solution. Because the colonies were often disrupted when they were transferred to the solutions, Sherman and his colleagues searched for a method that would fix the colonies but still allow effective contact with the solutions. After testing numerous agents, they discovered that a gentle spraying of the Petri dish surfaces with ordinary hair spray was effective. Using this method, Sherman was able to detect six cytochrome *c*-deficient mutants (cy_1, cy_2, cy_3, cy_4, cy_5, and cy_6) that could be divided into two major classes. The first class of revertants had normal or altered iso-1-cytochromes *c* and the low amounts of iso-2-cytochrome *c* found in normal strains. The second type contained only iso-2-cytochrome *c*, usually in amounts higher than normal.

In the second JBC Classic reprinted here, Sherman and his colleagues report on the isolation and partial determination of the structure of altered peptides from chymotryptic digests of iso-1-cytochromes *c* that were obtained from two revertants of the *cyc1-6* mutant, one revertant of the *cyc1-10* mutant, and one revertant of the *cyc1-15* mutant. They discovered that *cyc1-6* contains a missense mutation, and although they could not prove it definitively, *cyc1-10* and *cyc1-15* were probably also missense mutants.

Eventually, Sherman developed an even better method for isolating Cyc^- mutants that was based on the finding that mutants that still contain approximately 5% normal cytochrome *c* levels are defective in the utilization of lactate but are still able to utilize other nonfermentable substrates such as glycerol or ethanol. The mutants unable to utilize lactate are also resistant to the toxic action of the analogue chlorolactate, which Sherman used to enrich mutants partially defective in cytochrome *c* (6).

Since the first report of *CYC1* in 1964 (1), Sherman has published approximately 240 papers using the isocytochrome *c* system to investigate a variety of topics, including informational transcription, translation, co-translational and post-translational modification, mitochondrial import, heme attachment, enzymatic functions, and protein degradation.

Sherman remains at the University of Rochester as an emeritus professor in the department of biochemistry and biophysics. He served as an instructor at the Cold Spring Harbor Laboratory and was a coauthor of the Cold Spring Harbor manual on yeast genetics and molecular biology. He also was an associate editor for *Genetics* and *Molecular Cell Biology* and currently is the editor of *Yeast*. Sherman has received several awards and honors for his research, including the Wander Memorial Lectureship in 1975, the Arthur Kornberg Research Award in 1999, the George W. Beadle Award from the Genetic Society of America in 2006, and the Lifetime Achievement Award from the Genetic Society of America in 2006. He was elected to the National Academy of Sciences in 1985 and became a AAAS fellow in 2006.[1]

Sherman's coauthor on both papers, Emanuel Margoliash (1920–2008), also made several important contributions to science. He probably was best known for his work on molecular evolution with Walter Fitch; the pair devised a method for constructing evolutionary trees based on protein sequences, known as the Fitch-Margoliash method. Margoliash also was an expert in sequencing cytochromes *c*. He earned his M.D. from the American University in Beirut and served as a medical officer in the Israeli Army in the 1948 War of Independence. Margoliash held research positions at Hebrew University-Hadassah Medical School, the Nobel Institute, the University of Utah College of Medicine, the McGill-Montreal General Hospital Research Institute, the University of Illinois at Chicago, Abbott Laboratories, and Northwestern University. He was elected to the National Academy of Sciences in 1975 and passed away in 2008.

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

[1] Biographical information on Fred Sherman was taken from Ref. 7.

REFERENCES

1. Sherman, F., and Slonimski, P. P. (1964) Respiration-deficient mutants of yeast II. Biochemistry. *Biochim. Biophys. Acta* **90,** 1–15
2. Sherman, F., Taber, H., and Campbell, W. (1965) Genetic determination of isocytochromes *c* in yeast. *J. Mol. Biol.* **13,** 21–39
3. Sherman, F. (1964) Mutants of yeast deficient in cytochrome *c. Genetics* **49,** 39–48
4. Dumont, M. E., Ernst, J. F., and Sherman, F. (1987) Identification and sequence of the gene encoding cytochrome *c* heme lyase in the yeast *Saccharomyces cerevisiae*. *EMBO J.* **6,** 235–241
5. Dumont, M. E., Schlichter, J. B., Cardillo, T. S., Hayes, M. K., and Sherman, F. (1993) CYC2 encodes a factor involved in mitochondrial import of yeast cytochrome *c. Mol. Cell. Biol.* **13,** 6442–6451
6. Sherman, F., Stewart, J. W., Jackson, M., Gilmore, R. A., and Parker, J. H. (1974) Mutants of yeast defective in iso-1-cytochrome *c. Genetics* **77,** 255–284
7. Sherman, F. (2005) The importance of mutation, then and now. Studies with yeast cytochrome *c. Mutat. Res.* **589,** 1–16

DOI 10.1074/jbc.O110.000234