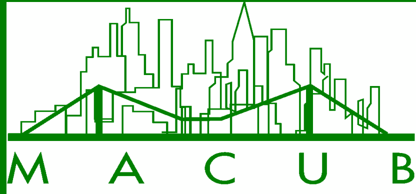

IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Fall 2010 Volume 32, Issue 1

43nd ANNUAL MACUB CONFERENCE

Saturday, October 23, 2010
at
Molloy College
Rockville Centre, New York

CONFERENCE THEME

" IT'S NOT NICE TO FOOL MOTHER NATURE: HUMAN IMPACT ON OUR BIOSPHERE "

KEYNOTE SPEAKERS

Susan S. Kilham is a Professor of Biology and Environmental Science at Drexel University, Philadelphia, Pennsylvania. Her research interests are aquatic ecology and climate change. Dr. Kilham supervises the Aquatic Ecology lab at Drexel where some of her many research projects include quantifying the ecological effects of catastrophic amphibian declines in neotropical streams, understanding tropical stream food weds, and restoration efforts in the Delaware Bay Estuary. Dr. Kilham will co-address this year's keynote and present the stark evidence on "Global Climate Change."

Anne and Jack Rudloe are naturalists and the directors of the Gulf Specimen Marine Laboratory (GSML) in Panacea Florida, a non-profit organization that supports marine research and education. Recently, they launched "Operation Noah's Ark," an effort by the GSML to rescue and preserve the marine animals threatened with extinction because of the Gulf of Mexico oil spill disaster. They are the authors of 9 published books, 35 magazine articles and numerous newspaper columns on marine life. Their keynote address will be on "Operation Noah's Ark, a Paradigm Shift of Restoring the Environment and Putting Fish Back into the Sea."

IN VIVO

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Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

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Affiliate Members — **inside back cover**

You are invited to participate in the 43nd Annual Fall MACUB Conference. Proposals are being accepted for member paper presentations and poster presentations

Member Paper Presentations

If you wish to make a paper presentation (20 min.) which will discuss the results of research or share ideas, please register on-line at the MACUB web site www.macub.org. If you have any questions contact Dr. Jodi Evans, at 516-678-5000 ext. 6179 (jevans@molloy.edu). Deadline for submission is October 6, 2010

Poster Presentations

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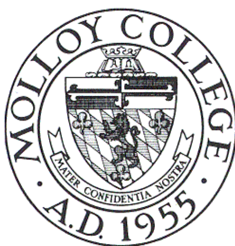

Molloy College is honored to host the 43rd Fall MACUB Conference! Members of the Molloy Community have a long established relationship with MACUB, and we look forward to involving our students and faculty your activities. Molloy is in the middle of a "renaissance" on a number of levels. Architecturally, MACUB guests will witness the construction of our "Public Square" building, housing a new communal center for students and a new theatre with increased capacity and advanced multimedia capabilities. Also, the first of three residence halls is under construction, allowing us to attract students from beyond our region. On an academic level, Molloy faculty are industrious in creating novel advances in our undergraduate and graduate programs. With 200 graduate and undergraduate programs to choose from, and recognition by US News and World Report as an outstanding comprehensive university, Molloy College has achieved recognition as a place where students have a choice, a voice, and can be effective competitors in their future goals. This Fall semester, we reached another milestone – the inauguration of our Ph.D. program in Nursing.

The Department of Biology, Chemistry, and Environmental Studies also experienced renewal. Starting in 2000, our Department underwent an extensive renovation involving all of our dedicated laboratory and lecture space. We now have two state-of-the-art laboratories for our students and faculty to conduct research with contemporary equipment. Our student research initiative will allow students to conduct research on campus or with our affiliations with Winthrop Medical Center, Weil Medical College of Cornell University, Downstate Medical Center and the Population Council at Rockefeller University, providing excellent opportunities for internships for our Biology majors.

We anticipate, with excitement, the MACUB Conference on October 23rd. This great organization, which has done so much for the advancement science for students and faculty , now becomes part of our "renaissance, " both College-wide and in our own Department of Biology, Chemistry and Environmental Studies.

Welcome, MACUB!

Tony Tolvo

Associate Professor of Biology
Dean, Division of Natural Sciences, Mathematics and Computer Studies,
Allied Health Sciences and Speech and Language Pathology

Age of Red Blood Cells In Muscovy X Pekin Hybrids

by

Donald Dorfman
Monmouth University, West Long Branch, NJ

The purpose of this study was to determine the age of red blood cells in Muscovy (♂) X Pekin (♀) hybrids employing the die-away of fetal hemoglobin. The technique used to follow the disappearance of fetal hemoglobin was described for Muscovy ducks (*Cairina moschata*)[(1)] and for Pekin ducks (*Anas platyrhynchus*) [(2)]. The hybrids, called moulards, or mules, are the sterile offspring of male Muscovy ducks and female Pekin ducks. They are raised commercially for foie gras production.

The hybrid ducks used for this study were raised as ducklings. They were donated by Hudson Valley Foie Gras, Ferndale, New York. The y were fed a commercial pelleted feed. The ducks were bled once each week initially, then more frequently as the percent of the fetal hemoglobin declined to less than five percent of the total hemoglobin.

The hybrids had three hemoglobins after hatching, two anodic adult hemoglobins , and a single cathodic hemoglobin. This is the same three peak hemoglobin pattern observed for each parent species.

Die-away of the fetal hemoglobin in the hybrid occurred on the 77th day after hatching, similar in the time period to that for Muscovy ducks of 80 days[(1)] as compared to that of 47 days for Pekin ducks[(2)].

Some fowl hybrids show a mixture of hemoglobins from the parental stock[(3)] . There may be mixing in the hemoglobins of the hybrids used in this study but, as shown, the die-away of the fetal hemoglobin is not an average of the different parental species, whereas the hatching time is. For example, hatching time for these hybrids is 32 days compared with 35 days for Muscovy ducks and 28 days for Pekin ducks. It would be interesting to determine the age of red blood cells of Pekin (♂) X Muscovy (♀) hybrids, called hinnies. These hybrids are also sterile. However, these are not readily available since they are not raised commercially.

References

[1]Dorfman, D., 2010. Age of red blood cells in the Muscovy duck (*Cairina moschata*). In Vivo **31(3):** 86.

[2]Dorfman, D., 2010. Fetal hemoglobin and age of red blood cells in fowl. In Vivo **31 (3):** 82-85.

[3]Prosser, C.L. 1973. *Comparative Animal Physiology* 3rd ed. W.B. Saunders, Phila. 966.

Correlation of β Subunit mRNA Amounts with ATP Synthase Enzymatic Levels in Yeast *Saccharomyces cerevisciae*

by

Georgia J. Lind
Kingsborough Community College, CUNY, Brooklyn, NY

Abstract

The adenosine triphosphate synthase (ATP synthase, also known as F1FO ATP synthase) of yeast (*Saccharomyces cerevisciae)* mitochondria is a 20 protein complex that uses a proton gradient generated by an electron transport chain to synthesize ATP. The proteins in the complex are synthesized both in the cytoplasm, from genes present on the cellular genome, and in the mitochondria, from genes that are part of the mitochondrial genome. As part of a large question, what regulates the production and assembly of the enzyme complex, I here ask the question, does the level of mRNA for the β subunit play a part in the regulation of the production of the enzyme complex? Results show that amounts of active β subunit mRNA increase during increases in ATP synthase activity through exponential phase growth in glucose, ethanol and raffinose. During stationary phase in cultures grown in raffinose, a decrease in ATP synthase enzyme activity accompanies a decrease in active β subunit mRNA, while in stationary phase cultures grown in glucose or ethanol, ATP synthase enzymatic activity remains high, while levels of active β subunit mRNA drop. This data supports a model where enzymatic activity and synthesis can be both coupled and uncoupled, with the proteins more stable than the mRNA.

Introduction

The adenosine triphosphate (ATP) synthase (F1FO ATP synthase) of mitochondria is one of the most important enzymes in cells. It synthesizes ATP, the energy currency of the cell, from ADP and phosphate groups. The driving force for the synthesis comes from a proton gradient across the inner mitochondrial membrane of the organelle that is established by the mitochondrial electron transport chain. As protons flow down the gradient, through the enzyme, the ATP is produced. A complex of similar function is also present in bacteria.

It is also a structurally fascinating enzyme. The structure has been extensively investigated in bacteria, bovine heart, and humans as well as in the yeast *Saccharomyces cerevisciae*. The complex is made up of two sections, a head (F1) and a tail (FO), and in yeast contains 20 different protein subunits[1]. Some of the subunits (mostly those of the F1 head) are designated with Greek letters (α,β,γ,δ,ε), others are lettered (a,b,c, etc). Figure 1 diagrams the arrangement of some of these parts.

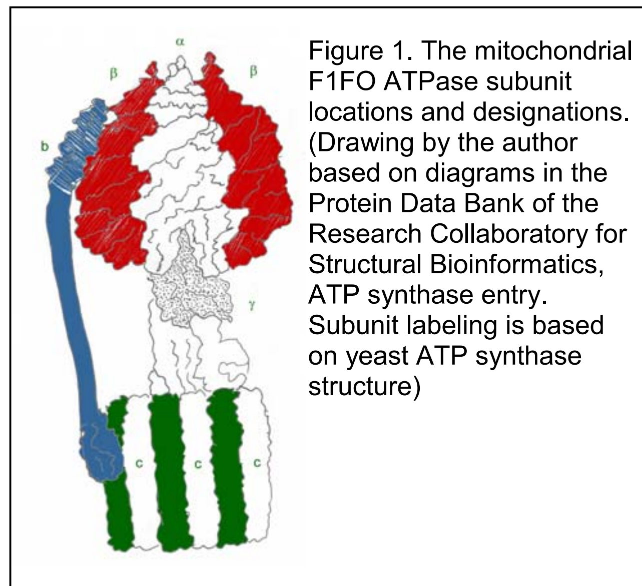

Figure 1. The mitochondrial F1FO ATPase subunit locations and designations. (Drawing by the author based on diagrams in the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics, ATP synthase entry. Subunit labeling is based on yeast ATP synthase structure)

The F1 head group is an assembly of three copies each of the α and β subunits. They alternate to make a collar around a central channel that contains the γ protein. As the center turns, the β subunits change configuration in a three-step process. First a phosphate group and an ADP molecule come together in a binding site on the β subunit. Second, the site changes shape and the precursors bind to each other. Finally after another shape change, the newly-formed ATP is released. The α and β proteins do not turn, because they are attached to the so-called stator

subunit (subunit b). The stator is a kind of fixed post that extends from the FO base around the outside of one side of the α, β, collar and keeps it from rotating.

The FO base anchors the enzyme in the inner mitochondrial membrane, and contains multiple copies of the c protein. Protons travel through a central section of the group of c proteins from the interchristae space to the intermembrane space which causes rotation of the central γ protein in the center of the α, β F1 channel. This rotation induces the above-described changes in configuration of the β subunits[2]. In 1997, the structure and function of the ATP synthase were recognized as important enough to warrant part of the Nobel Prize in Chemistry[3].

Current evidence strongly supports a model for the origin of mitochondria in eukaryotes in which mitochondria are former prokaryotes that joined larger cells in a symbiotic relationship. The synthetic origin of the F1FO ATP synthase demonstrates how intimately related these two systems have become since they merged. Three genes found in the mitochondrial genome (ATP6, ATP9 and ATP8) produce subunits of the FO section of the enzyme, the a, c and an unknown protein, all of which are synthesized by mitochondrial ribosomes using mitochondrial tRNAs. Synthesis of many others, including the α, β, γ, δ and ε proteins of the F1 head group, and the b protein stator, takes place in the cell cytoplasm on cytoplasmic ribosomes, using mRNA transcribed from nuclear DNA[4]. In order to enter the mitochondria, the α, β and γ proteins that are synthesized in the cytoplasm include a short (20 amino acid) end sequence that directs them to the intra-mitochondrial space after synthesis[5]. These small sequences are removed before the proteins are assembled into the functional enzyme.

Production of a mitochondrial enzyme protein that is imported from the cytoplasm could be regulated at any of a number of steps from DNA to final product. These include mRNA synthesis rate, mRNA processing rate, translational rate, rate of importation and assembly. I approached the problem at two of these steps. First, mRNA synthesis and processing. Does the amount of mRNA that can actively direct subunit synthesis vary with the amount of ATP synthase activity? This was assayed using a reticulocyte lysate protein synthesis system programmed by yeast mRNA. Secondly, we investigated the translation output. How much α and β protein is present? This was assayed by immunoprecipitating protein molecules from radioactively-labeled yeast cultures, and will be presented in a separate manuscript.

Making possible this work is the fact that yeast are facultative anaerobes. The state of their mitochondria responds to their metabolic activity. Cells alternate between highly functional mitochondria, present when yeast are in the presence of oxygen and a non-fermentable carbon source such as ethanol or lactate, and what are called repressed mitochondria, found in yeast growing on glucose or at low oxygen levels. It has been found that repressed mitochondria are light, have few proteins, have inactive electron transport systems, low ATP synthase activity, and in some cases seem to be present in the cells in low numbers. When the yeast are derepressed, the cell has highly functional mitochondrial electron transport systems, and very active levels of ATP synthase[6]. By growing yeast in various carbon sources, we can cause them to have either repressed or active mitochondria. It is also possible to monitor the yeast transition from repressed to derepressed. As a fermentable carbon source such as glucose is exhausted, yeast switch to non-fermentable sources, and their mitochondrial activity levels increase. As these changes take place, we can study how ATP synthase enzyme production is regulated.

I isolated F1 complexes and designed a protocol to prepare pure mitochondrial ATP synthase α and β subunit proteins, which I will describe briefly here. I used the proteins to raise antibodies in goats (F1 head group) or rabbits (α and β subunits).

I then grew yeast in several metabolic environments. I confirmed the mitochondrial activity levels in each of these environments, and then investigated the amount of β mRNA present. The four different metabolic states were: repressed (exponential growth in glucose), derepressed (growth in lactate or ethanol), and transitioning from inactive to most active state, that is, when we believed they would be most vigorously producing and assembling ATP synthase (extended growth in glucose). In addition, growth on the carbon source raffinose had been reported to produce a state called "intermediate" repression[7]; since it was not clear what level of β mRNA would be present in this metabolic environment, we investigated it as well. We sampled yeast, determined mitochondrial activity levels, and quantitatively determined the amounts of active β subunit mRNA found in these metabolic environments.

Materials and Methods

In order to minimize space in the Methods section, the protocols used are only summarized here. The additional details of these procedures were part of a Ph.D. thesis, and can be obtained from Prof. Lind at glind@KBCC.CUNY.edu.

Protein isolation and antibody preparation

Subunits α and β were purified from commercial (Red Star) yeast. The isolation of F1 is based on Douglas *et al*[8]. Samples were mechanically broken, and mitochondria were isolated using centrifugal cellular fractionation. Mitochondria were sonicated to produce submitochondrial particles, and treated with a chloroform- phenylmethyl sulfonyl fluoride (PMSF) solution to release F1 head groups. The F1 head group was separated from contaminants by charge affinity on a DE 52 column eluted with a NaCl gradient, then by size using a Sephadex G 150 sizing column. Fractions were selected for rutamycin-sensitive ATP synthase activity (see below). These combined samples were lyophilized and are termed the F1 fractions.

The subunit proteins present in the F1 fractions were separated using acrylamide gels that were 10-15% 1/3 exponential under 2/3 linear gradients. Samples were loaded into one large well per gel. Edges of the gels were stained, and slices of gel containing isolated α or β subunit proteins were homogenized and injected into rabbits to induce antibodies.

All antibodies present in collected serum from the rabbits were separated by ammonium sulfate precipitation, and antibodies specifically against α or β were selected using a cyanogen bromide-activated Sepharose 4B affinity column prepared from submitochondrial particles. This step was particularly important because the presence of non-specific heavy chain antibody proteins interfered with proper electrophoresis of alpha and beta subunits.

Yeast cell growth

Yeast *Saccharomyces cerevisiae* strain D273-10B (ATCC 25657) was grown under sterile conditions at 28°C with aeration by shaking in flasks filled to 20% volume. Basic medium contained 1% yeast extract, 2% Bacto-Peptone (both from Difco) and 0.1% D-glucose which were autoclaved together. For glucose medium, glucose concentration was raised to 5%. For lactate medium, basic medium was made up to 3% with DL lactic acid, and pH was adjusted to 5.4 with KH_2PO_4. Raffinose medium was prepared by adding D+ raffinose pentahydrate to 2%. Ethanol growth medium was prepared from basic medium after autoclaving and cooling. Just before inoculation, the flasks were made up to 2% (final concentration) ethanol with 95% ethanol which was filter sterilized using a 0.22 µm Millipore filter. A sterile 1:10 dilution of Sumycin (Squibb, 125 mg/5 ml) tetracycline syrup in H_2O was added to all growth cultures at 0.8 ml per 100 ml media just before inoculation. Cell numbers and growth in culture were monitored either by counting cells using a hemocytometer, or by taking the optical density of cultures grown in flasks with side-arm tubes using a Klett-Sommerson Photoelectric Colorimeter (red filter).

Mitochondrial Enzyme Assays

Respiratory activity (cellular oxygen consumption)

Whole yeast cells were sedimented out of growth media by centrifugation for 2 min at room temperature, resuspended in aerated 0.5% glucose H_2O solution, and immediately tested using a Gilson oxygraph with a platinum electrode (1.5 ml chamber with magnetic stirring) coupled to a chart recorder. The conversion factor used was 10.4 nmol O_2 cm^{-1}. This is based on an initial baseline oxygen content of 260 nmol oxygen, and a 25 cm chart distance per minute tested. After the test, the number of cells suspended per ml of glucose solution was counted. Oxygen consumption is expressed in units of nmol O_2 min^{-1} $[10^8 \text{ cells}]^{-1}$.

ATP synthase activity

The technique of Taussky and Shore[9] was used to assay inorganic phosphate (Pi) released by digestion of ATP by cellular ATPases (ATP synthase run in reverse). Mitochondrial ATP synthase activity was the amount of total activity that was inhibited by Rutamycin (Eli Lilly & Co., 6 µg/ml). Reagents for the enzyme assay are from Tzagoloff[10]. Each assay included Pi standards, and results are expressed quantitatively by comparison with a standard curve. Activity is expressed as rutamycin-inhibited µmoles phosphate liberated per min per mg protein.

Amounts of Active β Subunit mRNA

Summary

Total mRNA was extracted from cells at various times during growth using standard methods. mRNA for the β subunit was translated using an optimized reticulocyte lysate protein translation system in the presence of radioactive ($^{35}SO_4$) amino acids. Tests showed that reticulocyte lysate synthesized yeast protein more effectively than wheat germ. 7.5 µg mRNA per assay was found to produce maximum protein output (data not shown). The β subunit protein produced was captured using anti-subunit antibodies coupled to protein A-Sepharose beads. The captured material was separated using PAGE and the bands exposed to x-ray film. The bands on the films were scanned and used to quantify the amount of β subunit synthesized.

Isolation of RNA

Isolation of RNA was done at all times under sterile conditions, using sterile plastic or autoclaved equipment and solutions containing RNAase inhibitors, to control for RNAase activity. Briefly, at various time points during growth, 1-2 g of cells were broken with glass beads, and supernatant cellular contents were extracted three times with buffered phenol and chloroform. The resultant RNA was precipitated with ethanol, then dried, resuspended, and used to program lysates.

Translation

Rabbit reticulocyte lysate was prepared from rabbits as outlined in Maniatis, Fritsch and Sambrook[11]. On the day of use, the lysate was treated with micrococcal nuclease. Translation was done using a 20 amino acid mixture containing $^{35}SO_4$ methionine, and mRNA isolated from various yeast culture time points. The translated material was precipitated with TCA, washed with ethanol and resuspended. Controls contained no mRNA.

Capture of subunit protein

Anti-β antibodies were attached to protein A sepharose beads. These were tumbled with the radioactively-labeled products of the reticulocyte lysate system. The beads were pelleted with their attached proteins using a mini-fuge. The adherent proteins were released using PAGE loading buffer containing sodium dodecyl sulfate (SDS), and separated by electrophoresis. Equal amounts (cpm) of TCA-insoluble translated material were used for comparative gels, that is, each gel sample well contained the captured subunit proteins from a fixed amount of trichloroacetic acid (TCA)-insoluble lysate. Gels were dried, exposed to x-ray film for 30-35 days, and the developed films were photographed or scanned. It was found that a brief rotation of the lysate products before immune capture with protein-A sepharose beads to which non-immune serum proteins were attached removed contaminant material, so this was routinely done.

Detection

Bands on x-ray film were scanned using a laser scanning densitometer (LKB Instruments, Inc.) whose output was analyzed using a Hewlett Packard 3390 A reporting integrator. A baseline was set by scanning an area of the film clear of bands. Each band was scanned 4-10 times (top to bottom) at various locations left to right, and the peak volume averaged. The width of the band was hand-measured using a ruler. An amount of pre-beta produced (arbitrary units) was determined by multiplying the width by the average vertical peak. The same technique was used for the S protein. If various experiments were compared, each amount was normalized to TCA-insoluble material loaded by division of the scan information by the load information. All results are expressed in arbitrary units.

Results

Antibody specificity

The antibodies were tested using Ouchterlony plates and found to bind to α and β subunits (Figure 2a). Antibodies did not cross-react (i.e., anti-α did not bind to β nor vice-versa), see also Fig 2b. Both antibodies inhibited ATP synthase activity (data not shown).

In a second test, yeast mitochondrial proteins were separated by electrophoresis, transferred to nitrocellulose paper, and antibodies to α or β subunits were applied, decorated with horseradish-peroxidase-coupled anti-Fab fragments, and reacted for color. The anti-α protein and anti-β protein antibody each bound to only one alpha- or beta-sized band, even when the yeast proteins were a complex mixture (F1) (Figure 2 b).

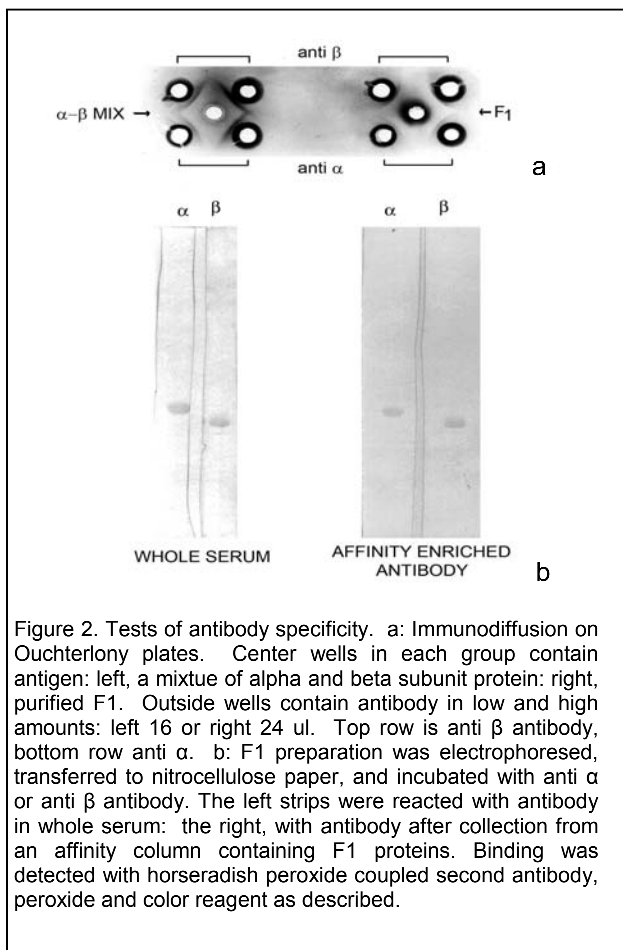

Figure 2. Tests of antibody specificity. a: Immunodiffusion on Ouchterlony plates. Center wells in each group contain antigen: left, a mixtue of alpha and beta subunit protein: right, purified F1. Outside wells contain antibody in low and high amounts: left 16 or right 24 ul. Top row is anti β antibody, bottom row anti α. b: F1 preparation was electrophoresed, transferred to nitrocellulose paper, and incubated with anti α or anti β antibody. The left strips were reacted with antibody in whole serum: the right, with antibody after collection from an affinity column containing F1 proteins. Binding was detected with horseradish peroxide coupled second antibody, peroxide and color reagent as described.

The anti-β antibody was used to immunoprecipitate materials produced by total yeast mRNA in cell-free lysates. When the captured material was examined, it was found that the antibody preparation captured two proteins, labeled R and S in Figure 3. Neither was the size of the β subunit found in our standard ATP synthase F1 preparation. Yet it was clear that the antibody preparation bound to the β subunit in F1.

To investigate the identity of these bands, samples of $^{35}SO_4$-labeled R and S were subjected to protease digestion, as were samples of β subunit labeled with either $^{35}SO_4$ or ^{125}I. Results are shown in Figure 4. The R band produced degradation products very similar to beta, and was considered to be a beta precursor, with a molecular weight approximately 2,000 MW larger than beta. Band S seemed to be unrelated to mitochondrial ATP synthase β subunit.

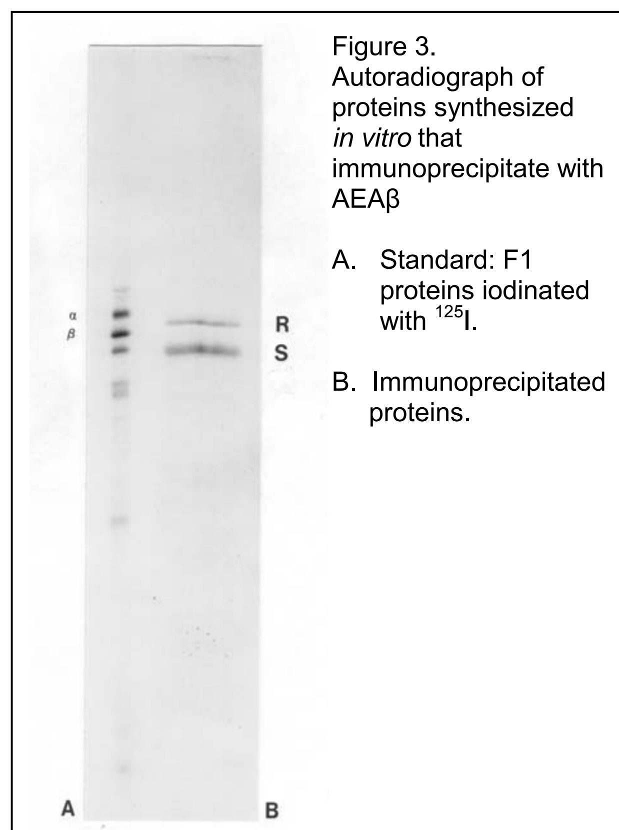

Figure 3. Autoradiograph of proteins synthesized *in vitro* that immunoprecipitate with AEAβ

A. Standard: F1 proteins iodinated with ^{125}I.

B. Immunoprecipitated proteins.

Mitochondrial activity in various metabolic environments

The first goal was to verify that our results agreed with previous authors regarding mitochondrial activity in various carbon sources, and to determine the specific effects of these environments on ATP synthase activity.

First we tested the oxygen consumption of cultures grown in four carbon sources. Oxygen is the final electron receiver of the mitochondrial electron transport chain; thus oxygen consumption is a measure of cellular respiration (mitochondrial activity). Each culture was sampled at two time points during mid-exponential growth. Figure 5 shows that our cultures reflected the reported results: glucose-grown (repressed) cells show low cellular respiration rates, and lactate and ethanol produce high cellular respiration rates (derepressed cells). Also as reported, mid-exponential cells grown in raffinose show intermediate cellular respiration levels.

Next we expanded our sampling times. We were interested in whether we could observe changes in cellular respiration rate as cells in glucose continued to grow in culture, and made a transition from the repressed to a derepressed

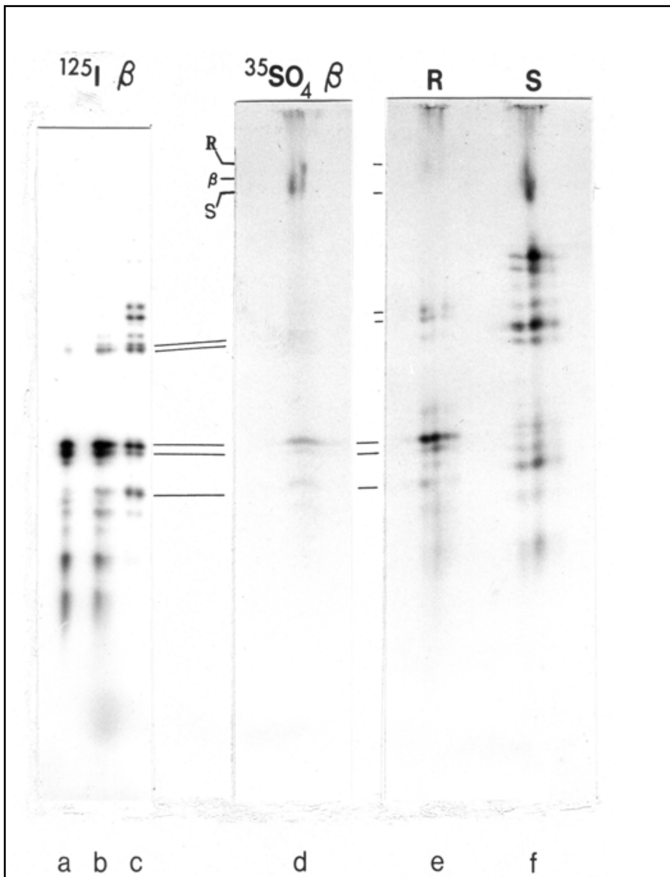

Figure 4. Protease digestion of bands immunoprecipitated by AEAβ antibody. Tracks a, b and c, iodinated beta subunit. d, beta subunit from yeast grown in $S^{35}O_4$. R and S were cut from a gel like the one in Fig. 3. One µl of protease solution (0.5 mg/ml) was added to samples c, d, e, and f; samples a and b were treated with 10 and 2 µl respectively. Positions of intact proteins are marked on track d. Lower lines show aligned digestion products.

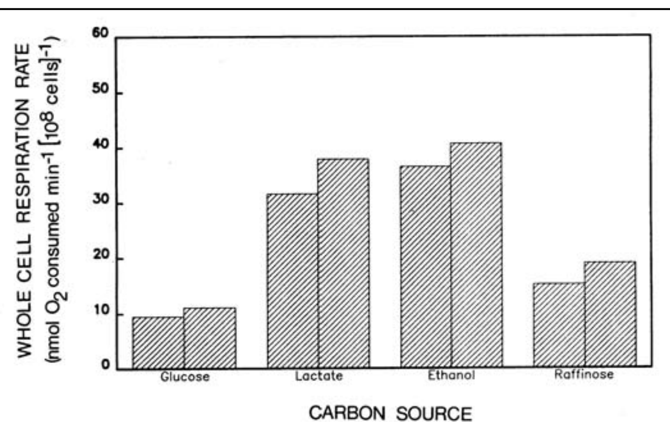

Figure 5. Respiratory activity of mid-exponential growth cultures that used four carbon sources. Each bar is one time point; samples were taken 1.5 hrs apart.

state. When did this happen? Was it gradual, or abrupt? We also investigated changes in cellular respiration during growth of cells in ethanol (derepressed) and in raffinose.

Each culture showed a very different pattern (Figure 6). Glucose was as expected: it had low levels of respiratory activity during exponential growth, then increased activity through stationary phase. (The midpoint between exponential and stationary phase growth, marked S, was used to align data among cultures.) In raffinose, cellular respiration level is intermediate during exponential growth, and increases with time in culture. The ethanol-grown cells start with a high respiratory rate; however, somewhat surprisingly, oxygen consumption levels continue to increase substantially during exponential and early stationary phase growth. A slight late-culture decrease was observed, but oxygen consumption activity only declines after 2-3 hrs of stationary phase growth. Note that activity of cells in glucose culture at maximum is only slightly higher than the lowest activity found in cells grown in ethanol.

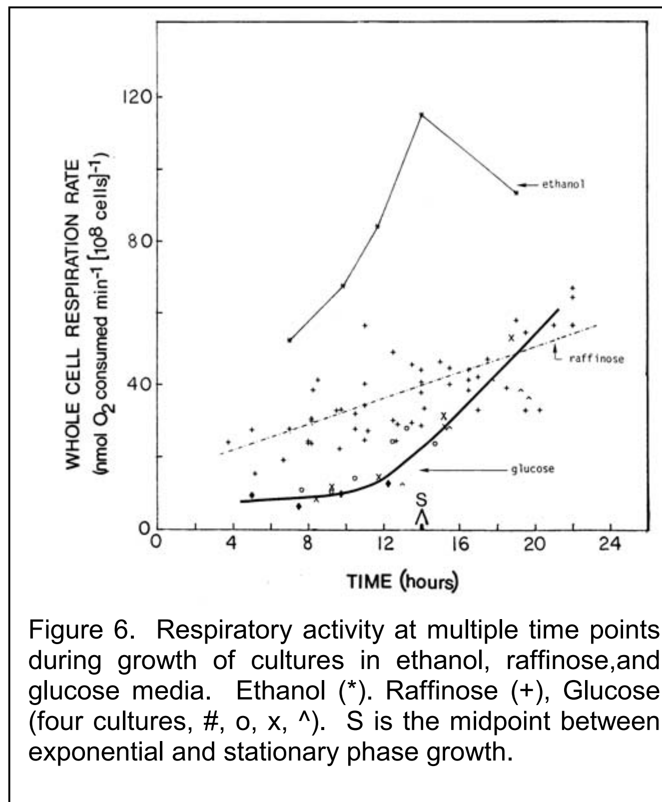

Figure 6. Respiratory activity at multiple time points during growth of cultures in ethanol, raffinose,and glucose media. Ethanol (*). Raffinose (+), Glucose (four cultures, #, o, x, ^). S is the midpoint between exponential and stationary phase growth.

ATP synthase activity in various metabolic environments

Figure 7 shows the results from a similar set of studies assaying mitochondrial ATP synthase specific activity during growth in the same three carbon sources. In glucose, changes in the ATP synthase activity parallel those of cellular respiration. In ethanol culture, like respiratory activity, an increase occurs during exponential and early stationary phase growth, but there is no late-culture decrease in ATP synthase activity. The activity of the mitochondrial respiratory chain and ATP synthase are the most different in cells during late culture growth in raffinose. As the culture transitions toward stationary phase (times after 12 hrs in culture), cellular respiratory chain activity increases steadily, in contrast to which ATP synthase activity decreases substantially.

Possible Regulation of ATP Synthase at the mRNA level

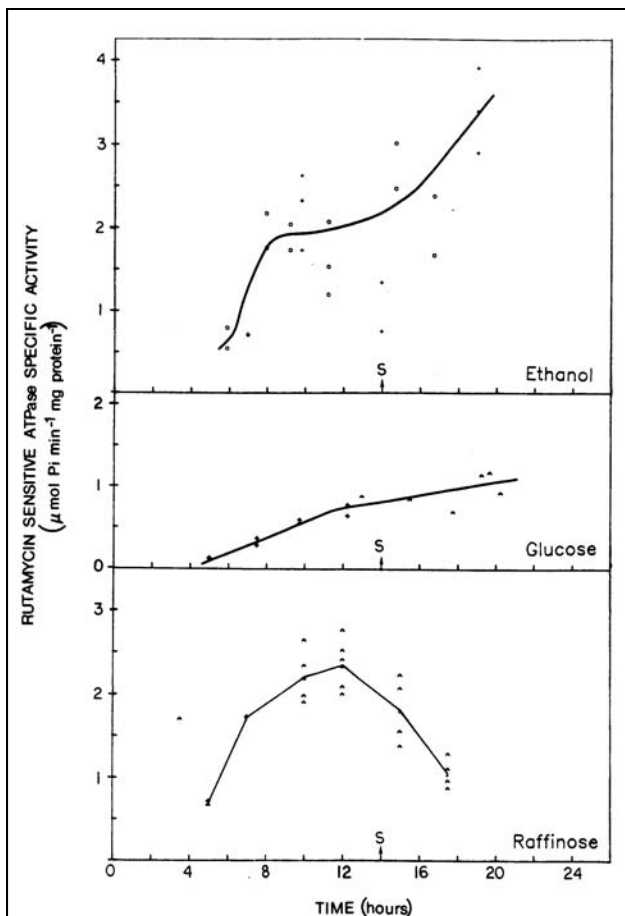

Figure 7. Rutamycin-sensitive ATPase specific activity of cultures grown in ethanol, glucose and raffinose media. In ethanol and glucose data, different symbols represent data from different cultures.

The final investigation was of the amount of active β subunit mRNA present during growth in these three metabolic environments. Since the enzyme activity patterns varied considerably, we were interested in investigating one of the regulatory steps that might produce these patterns.

Glucose

The observed amounts of active β subunit mRNA during growth in glucose culture are shown in Figure 8a. Figure 8b plots ATP synthase activity and cell number over the same interval. During hours 4-13.5 (almost to S), as the amount of ATP synthase activity increases, the amount of active β subunit mRNA also increases. Between hours 13.5 and 16, ATP synthase activity slows its increase, and so does the amount of active β subunit mRNA. From hour 17 to the end of observation (about hour 22), although enzymatic activity continues to increase slightly, the amount of active β subunit mRNA decreases precipitously.

Ethanol

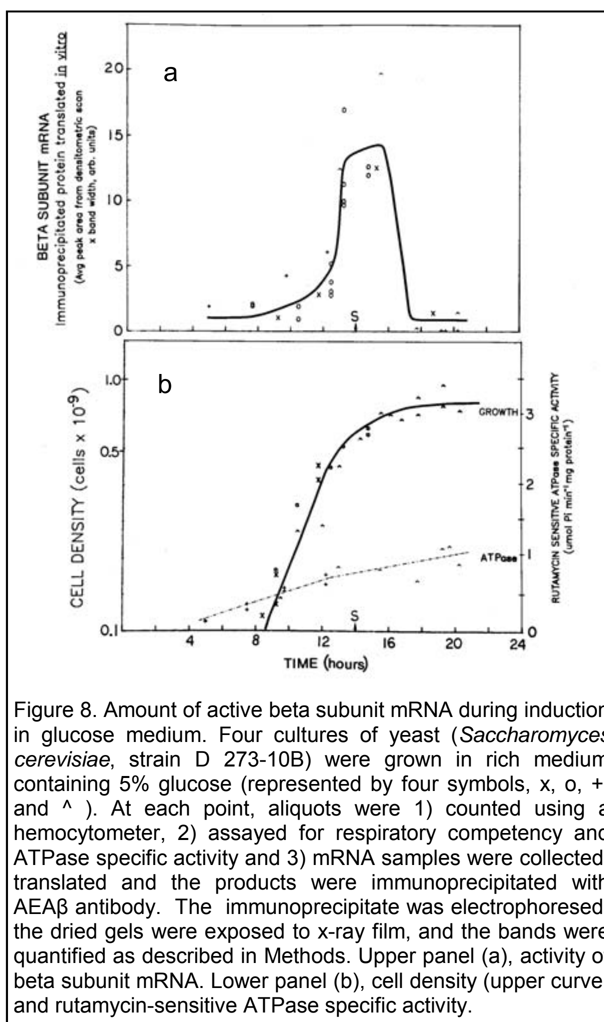

Figure 8. Amount of active beta subunit mRNA during induction in glucose medium. Four cultures of yeast (*Saccharomyces cerevisiae*, strain D 273-10B) were grown in rich medium containing 5% glucose (represented by four symbols, x, o, +, and ^). At each point, aliquots were 1) counted using a hemocytometer, 2) assayed for respiratory competency and ATPase specific activity and 3) mRNA samples were collected, translated and the products were immunoprecipitated with AEAβ antibody. The immunoprecipitate was electrophoresed, the dried gels were exposed to x-ray film, and the bands were quantified as described in Methods. Upper panel (a), activity of beta subunit mRNA. Lower panel (b), cell density (upper curve) and rutamycin-sensitive ATPase specific activity.

The results for β subunit mRNA amounts in ethanol culture are shown in Figure 9a. Figure 9b shows that ATP synthase activity is high (compare to glucose culture, for example), rises steeply between 6 and 8 hours, and either rises slowly or stays reasonably stable from 14 hours to end of observation (18.5 hrs). The rise to hour 8 is paralleled by a rise in active β subunit mRNA. However, from hours 8 to S (at hour 14), active mRNA level drops, and stays low from S to end of observation. As in glucose, the end time period does not show a drop in enzyme activity, only a drop in amount of active β subunit mRNA.

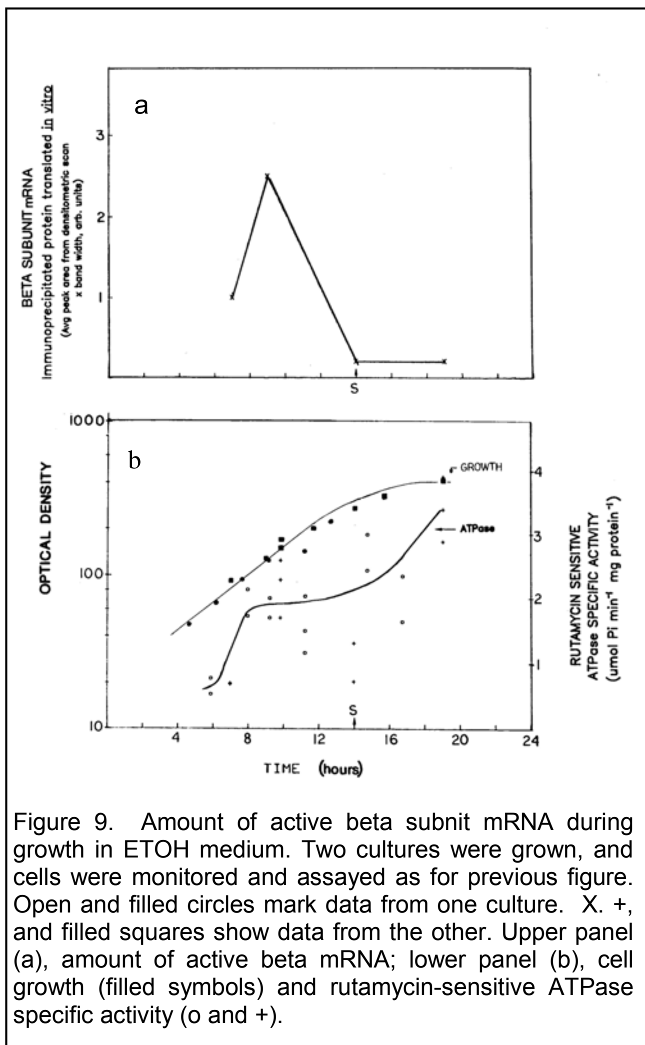

Figure 9. Amount of active beta subnit mRNA during growth in ETOH medium. Two cultures were grown, and cells were monitored and assayed as for previous figure. Open and filled circles mark data from one culture. X. +, and filled squares show data from the other. Upper panel (a), amount of active beta mRNA; lower panel (b), cell growth (filled symbols) and rutamycin-sensitive ATPase specific activity (o and +).

Raffinose

Finally, the results for β subunit mRNA amounts in raffinose culture are shown in Figure 10. In the lowest panel, ATP synthase activity in these two cultures shows the same inverted U shape that was seen previously, with the maximum occurring between 3.5 and 4 hours before S. As found in both glucose and ethanol cultures, there is a rise in active β subunit mRNA at early time points (between hours 4 and 10 (upper curve) or 12.5 (lower curve) hours). This maximum β subunit mRNA activity roughly parallels the maximum ATP synthase enzymatic activity (also between hours 10 and 12.5). Unlike either of the previously investigated cultures, note that as the amount of active β subunit mRNA decreases, the ATP synthase enzymatic activity also goes down.

Discussion and Conclusions

The antibodies prepared to the α and β subunits appeared specific when tested using Oucterlony plates and Western blots. However, when anti-β subunit antibodies were used to collect proteins produced from total mRNA in cell-free lysates, a protease digest was required to identify the proteins collected. The R protein showed digest products that aligned in several places with β subunit digest products. The increased size agrees with previously reported data[12-14] about small extensions that function as signal sections to guide mitochondrial proteins synthesized in the cytoplasm to their proper locations. The data suggests that for the β subunit, the signal peptide is of about 2,000 mw.

Our results support the idea that yeast mitochondria show various levels of cellular respiratory activity in response to their metabolic environment. Figure 5 shows that oxygen consumption can be low, high, and intermediate. The studies of cultures over time reveal some other changes. As previously reported, in continuous glucose culture oxygen consumption transitions between low and high[13-17]. Cells in both raffinose and ethanol show changing levels of respiratory chain activity, including a gradual increase in both cultures, followed by an obvious decline during stationary phase in ethanol culture.

To a reasonable extent, the mitochondrial ATP synthase enzymatic activity aligns with the cellular respiratory activity. There are also notable differences. During early growth in glucose, ATP synthase activity seems to increase earlier (between hours 4 and 12) than cellular respiration, which begins to increase just before hour 12. Also, cellular

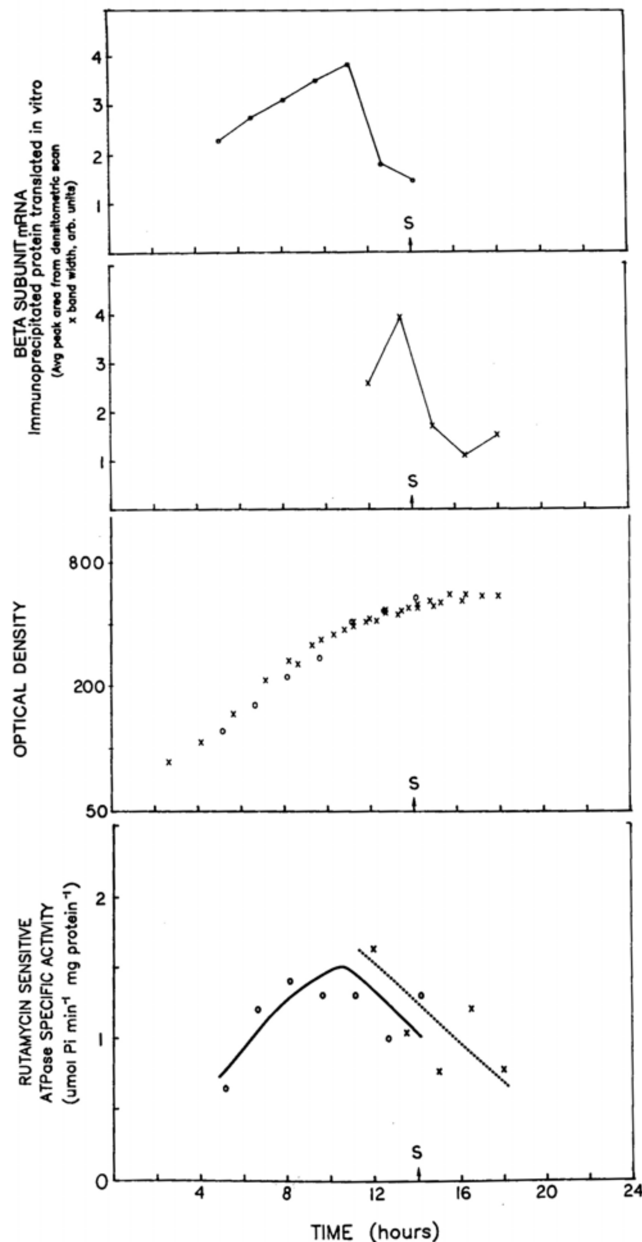

Figure 10. Amount of active beta subunit mRNA during growth in raffinose culture. Two cultures (o and x) of Saccharomyces cerevisiae strain D273-1OB were grown in 2% raffinose medium. Cells were harvested, broken, RNA was extracted, and mitochondria were isolated and assayed for protein and rutamycin-sensitive ATPase activity as in Methods. The RNA was used to direct synthesis in a reticulocyte cell-free translation system, and the beta mRNA was quantified by immunoprecipitation and analysis as for previous figure. Upper two panels, activity of beta subunit mRNA. Upper panel: 9-day film exposure, immunoprecipitation of 4.8 x 10^5 cpm TCA-precipitable translate per point. Second panel: 30-day film data; immunoprecipition of 1.1 to 1.2 x 10^5 cpm TCA-precipitable translate. Third panel: cell density. Fourth panel, rutamycin-sensitive ATPase specific activity.

respiration increases steadily, while ATP synthase activity slows its rate of increase just after hour 12. During growth in ethanol, the most striking difference is the drop in respiration rate during stationary phase
that is not seen in enzyme activity. Finally, during growth in raffinose, as the culture transitions toward stationary phase after 12 hrs, while cellular respiration increases steadily, ATP synthase activity decreases substantially.

The conclusion to be drawn here seems to be that neither during induction, nor most evidently during stationary phase, is the amount of ATP synthase enzymatic activity tightly coupled to the activity of the cellular respiration system. The emphasis here should be on the word “tightly”. Obviously highly active cells grown in ethanol have high levels of both cellular respiration and ATP synthase enzymatic activity. But in continuous glucose culture, the ATP synthase activity increases before the activity of the cellular respiration complex. And during stationary phase, both in raffinose and ethanol cultures, the amounts of ATP synthase enzymatic activity and cellular respiration are uncoupled. It is not clear how the ATP synthase would be active *in situ*, in the absence of an electron transport chain-generated proton gradient.

Finally, what does our evidence allow us to conclude about the possible regulatory role of subunit mRNA? Our first observation was “Aha! In glucose-grown cells there is no active β subunit mRNA, and in ethanol-grown cells there is!! mRNA amounts must control enzyme amounts!!” Of course, the picture got more complicated as we studied more time points, but to a great extent, particularly in exponentially growing cultures, our results are consistent with this model. In glucose culture, as the enzyme activity rises, so does the amount of β subunit mRNA. This is also true in raffinose culture (before S), and in ethanol culture (before hour 9).

Some of the data also supports the idea that, in the absence of active β subunit mRNA, ATP synthase enzyme activity declines. This is seen in raffinose culture after hours 10-12.

But it can also be seen that, particularly late in continuous culture, the level of ATP synthase can be uncoupled from the amount of active β subunit mRNA. This is apparent in both late glucose and late ethanol cultures, where the enzyme is active, but the amount of active β subunit mRNA is almost undetectable. Consistent with these results would be a model in which, during these times, the enzyme complex proteins are more resistant to degradation than the subunit mRNA. This suggests that for proteins, stationary phase is a

conservative metabolic environment, where turnover is restrained, while mRNAs are degraded. The model also predicts that ATP synthase proteins would cease to be synthesized during late times in cultures; this could be examined in further studies. Raffinose continues to be an interesting carbon source, since in culture in this carbon source, the ATP synthase is not stable during stationary phase.

Acknowledgments

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The Effects of Copper and Copper Blocking Agents on Gill Mitochondrial O_2 Utilization in *Crassostrea virginica*

by

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Abstract

While essential in trace amounts, excess copper is toxic to cells and tissues. Copper is a major aquatic pollutant. Previously, our lab demonstrated that tissues of the bivalve mollusc *Crassostrea virginica* readily accumulated copper and other metals from their surrounding environment. In this study we showed that O_2 consumption in isolated gill mitochondria of *C. virginica* was impaired by *in vitro* copper additions and that copper's deleterious effects on mitochondria respiration could be blocked by the presence of the membrane channel blocker diltiazam.

Introduction

Copper is an essential nutrient. In addition to its role in activation or repression of transcription of various genes, copper is required as an integral component of at least 12 major proteins involved in such processes as cellular respiration, catecholamine production, connective tissue biosynthesis, superoxide dismutation, iron metabolism and blood coagulation[1,2]. While essential in trace amounts, excess tissue copper can cause both structural and functional impairment. Excess cellular copper can disrupt normal cell metabolism by displacing other ions at their metal binding sites or through non-specific binding to enzymes, DNA, and other biomolecules[3]. Alternatively, free copper ions can cause oxidative damage by catalyzing reactions that generate hydroxyl and other oxyradicals[4]. The 2 common oxidation states for copper are Cu (I) and Cu (II) and the easy exchange between these two oxidation states endows copper with redox properties that may be of an essential or deleterious nature in biological systems. It is also noteworthy that the generation of hydroxyl radicals from hydrogen peroxide and superoxide via the Haber-Weiss reaction can only take place when catalytic concentrations of transition metals like iron or copper are present[5]. Indeed, the oxidative damage caused by hydroxyl radicals and other reactive oxygen species are thought to be major contributing factors to the development of cancer, diseases of the nervous system and aging[6]. Mitochondria are particularly sensitive to oxidative damage and depend upon various antioxidants and anti-oxidizing systems to defend against oxidative stress. As the major site of O_2 utilization, mitochondria are not only a source of reactive oxygen species[7] but are important targets for oxidative damage. The presence of excess copper and resulting oxyradicals can overwhelm cellular defensive mechanisms, especially in mitochondrial, compromising respiratory function and further impairing cellular health and survival. Brain tissue is especially susceptible to oxidative damage because of its extensive supply of polyunsaturated fatty acids and the fact that it consumes about 20% of the oxygen utilized by the body and many neurodegenerative and neuropsychiatric diseases, including depression, have been linked to oxidative stress.

Copper is a major aquatic pollutant. Bivalves are filter feeders that readily accumulate heavy metals[8,9,10] and other pollutants from their environment. Previously it was determined that oyster seed from *C. virginica*, transplanted from an oyster farm to a polluted site in Jamaica Bay NY, grew well[11] despite accumulating significant amounts of metal pollutants in their tissues[12]. Atomic absorption spectrometry revealed that the soft tissues accumulated copper in the ug/g dwt range, which was comparable to other published reports for *C. virginica* growing in other areas[9,13]. Copper accumulations were not homogeneously distributed throughout the oyster's soft tissues with greater amounts accumulating in the gill, heart and palps; shell tissues also accumulated copper, but to a lesser extent[12]. In other studies with isolated gill tissue, a three day copper (0,5 mM) treatment increased gill copper by more than 300%[14]. The purpose of this study was to determine the effect of acute copper additions on

O_2 uptake in isolated gill mitochondria of the bivalve *C. virginica* and to test potential metal blocking agents for their ability to reduce any deleterious effect that copper had on mitochondrial O_2 utilization. Lanthanum and diltiazem are well known calcium channel blockers. Lanthanum is an element that forms a trivalent cation that strongly reacts with calcium binding sites and affects most membrane transport processes involving Ca^{2+} ions[15-18]. Diltiazem is a benzothiazepine that acts selectively on the voltage-dependent L-type Ca^{2+} channels[19] and is used therapeutically in the treatment of angina pectoris, hypertension and supraventricular arrhythmias[20]. Recently, atomic absorption spectrometry and histochemical staining showed that copper accumulations were reduced when gills were treated with diliazem[14]. In this study we determined the effects of diltiazem and lanthanum in the presence of copper on O_2 consumption in gill mitochondria.

Materials and Methods

Adult oysters *C. virginica* of approximately 80 mm shell length were obtained from Frank M. Flower & Sons oyster farm in Oyster Bay, NY. Animals were transported on ice to Medgar Evers College within 2 hours of collection and placed in recirculating aquaria with artificial seawater (Instant Ocean®, Aquarium Systems Inc., Mentor, OH) at 16-18°C, specific gravity of 1.024 ± 0.001 and pH of 7.2 ± 0.2. Animals were used in the experiments within one week of arriving in the lab. Each animal was tested for health prior to experimentation by the resistance it offered to being opened. Animals that fully closed in response to tactile stimulation and required at least moderate hand pressure to being opened were used for the experiments. Lanthanum chloride, diltiazem, cupric sulfate, and all other chemicals were obtained from Fisher Scientific (Pittsburgh, PA).

Mitochondria were isolated from oyster gills using a method modified from Ballantyne and Moyes[21]. The isolation buffer consisted of 540 mM glycine, 250 mM sucrose, 2 mM EDTA, 20 mM Tris-HCl, pH 7.4, and 0.2% bovine serum albumin (BSA, essentially fatty acid free). For each experimental trial, the gills from one animal were removed, blotted dry, weighed and placed in 10 ml of ice cold isolation medium. The tissue was homogenized with three passes of a Potter-Elvehjem homogenizer equipped with a loosely fitting Teflon pestle. The homogenate was centrifuged at 2°C for 10 min at 600 X g in a Sorval RC5B centrifuge to remove the nuclear pellet. The supernatant was centrifuged at 2°C for 20 min at 10,000 X g to pellet the mitochondria. The resulting mitochondrial pellet was washed twice with ice-cold EDTA-free isolation buffer to minimize copper binding by the chelator and resuspended in 2 ml of ice cold EDTA-free isolation buffer.

Oxygen uptake by mitochondria was measured in a Micro-Biological Oxygen Monitor with a 600 µl micro-batch chamber (YSI, Yellow Springs, OH). Continuous data acquisition was made using a DI-700 Data Acquisition System (DATAQ Instruments, Inc., Akron, OH). Temperature in the mitochondrial respiration chamber was maintained constant at 20°C using a Haake water circulator.

An aliquot (100 µl) of isolation medium containing mitochondria was added to the micro-batch chamber containing respiratory buffer (0.25 succinate, 0.05 mM ADP, 5 mM $MgCl_2$, 10 mM K_2HPO_4, 540 mM glycine, 250 mM sucrose, 20 mM Tris-HCl, pH 7.4, 0.2% BSA) and mitochondrial respiratory rates (state 3) were measured in the presence or absence of copper with or without potential copper blockers. Basal respiration was measured for 10 minutes after which time 20 µl of copper or blocking agents were added to the chamber, respiration measured for 10 minutes and 20 µl of copper or blocking agents were added to the chambers again, according to the experimental protocols. For all experiments, N ranged from 5-24 and statistical analysis was determined by ANOVA with Tukey post test.

Results

Mitochondrial respiration was measured with a Yellow Springs 5300 Biological Oxygen Monitor with a Microbath chamber (Fig. 1). Basal O_2 consumption (QO_2) was typically 10 - 15 µl/g/hr over a 10 - 20 minute period. Adding copper to the microbath chamber (1 - 20 mM) after recording basal activity for 10 minutes decreased QO_2 up to 90% in a dose dependent manner (Fig. 2). Lanthanum was not effective in blocking the deleterious effects of copper (Fig. 3 and Fig. 4). When copper (5 - 10 mM) was tested after adding 5 or 10 mM of lanthanum, QO_2 was reduce up to 60%. Diltiazem was effective in blocking the deleterious effects of copper (Fig. 5 and Fig 6). When copper (5 - 10 mM) was tested after adding 5 or 10 mM of diltiazem QO_2 was not significantly reduced.

YSI Model 5300 Biological Oxygen Monitor with a Micro-Bath

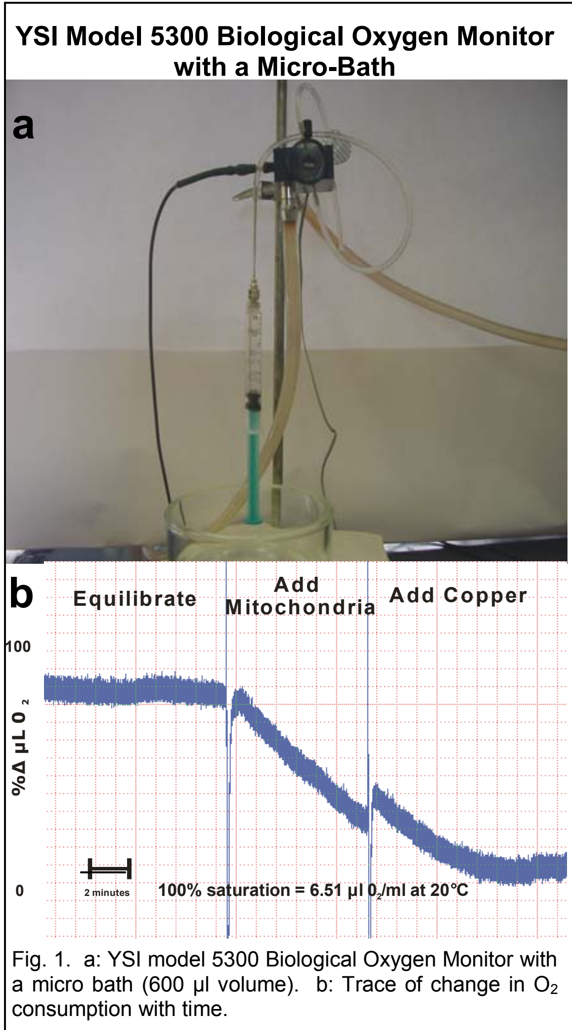

Fig. 1. a: YSI model 5300 Biological Oxygen Monitor with a micro bath (600 µl volume). b: Trace of change in O_2 consumption with time.

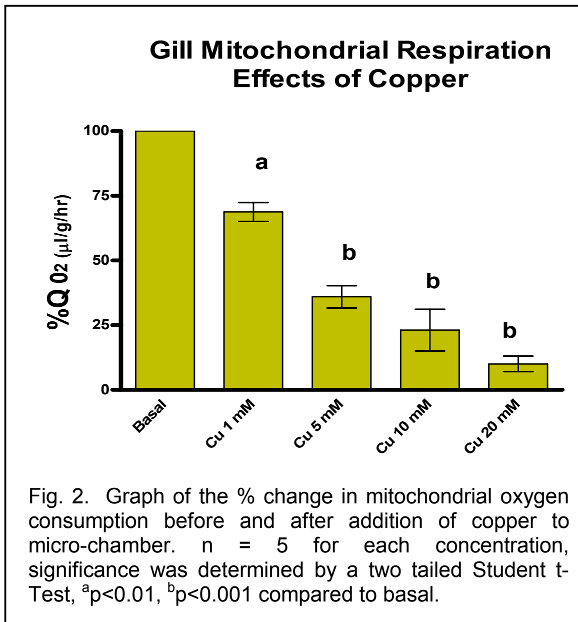

Fig. 2. Graph of the % change in mitochondrial oxygen consumption before and after addition of copper to micro-chamber. n = 5 for each concentration, significance was determined by a two tailed Student t-Test, [a]p<0.01, [b]p<0.001 compared to basal.

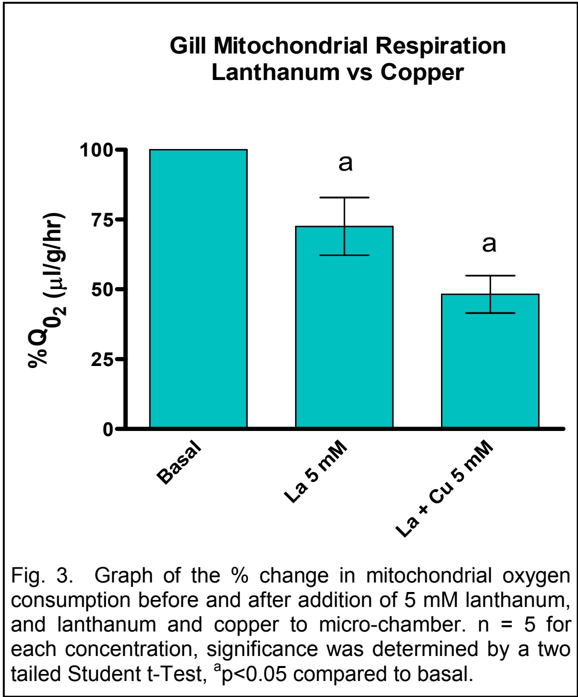

Fig. 3. Graph of the % change in mitochondrial oxygen consumption before and after addition of 5 mM lanthanum, and lanthanum and copper to micro-chamber. n = 5 for each concentration, significance was determined by a two tailed Student t-Test, [a]p<0.05 compared to basal.

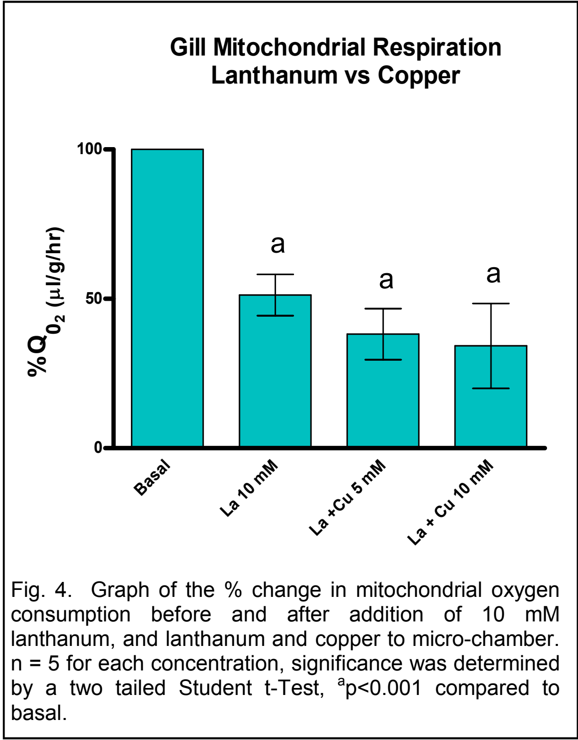

Fig. 4. Graph of the % change in mitochondrial oxygen consumption before and after addition of 10 mM lanthanum, and lanthanum and copper to micro-chamber. n = 5 for each concentration, significance was determined by a two tailed Student t-Test, [a]p<0.001 compared to basal.

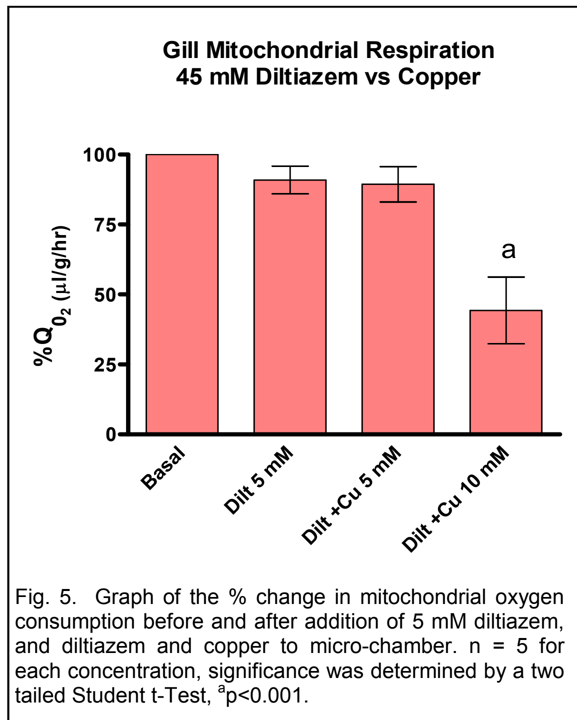

Fig. 5. Graph of the % change in mitochondrial oxygen consumption before and after addition of 5 mM diltiazem, and diltiazem and copper to micro-chamber. n = 5 for each concentration, significance was determined by a two tailed Student t-Test, [a]$p<0.001$.

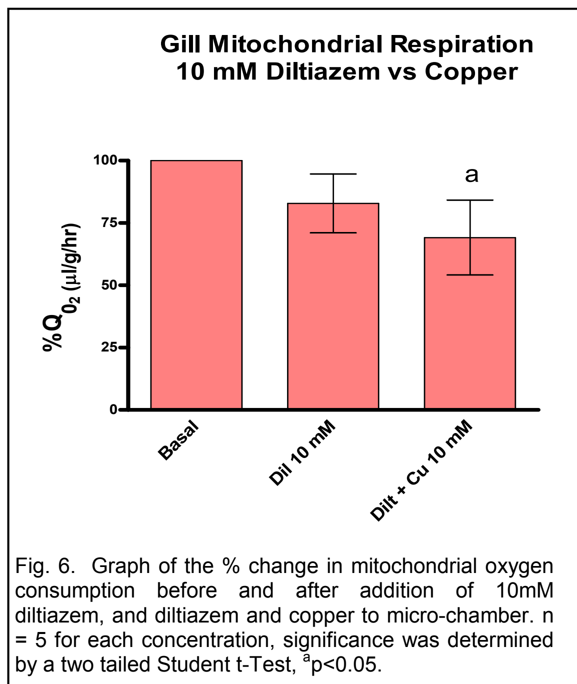

Fig. 6. Graph of the % change in mitochondrial oxygen consumption before and after addition of 10mM diltiazem, and diltiazem and copper to micro-chamber. n = 5 for each concentration, significance was determined by a two tailed Student t-Test, [a]$p<0.05$.

Discussion

Copper is an essential micronutrient needed as an integral component in a large number of enzymatic and structural proteins. However, the potential for copper toxicity exists. Soluble copper ions can catalyze Fenton/Haber-Weiss reactions generating the formation of highly reactive hydroxyl radicals, which result in membrane peroxidation and other signs of oxidative stress. As the major site of cellular O_2 utilization, mitochondria are particularly sensitive to pro-oxidizing agents. Superoxide and other reactive oxygen species are unavoidable consequences of the electron transport chain and mitochondrial cytochrome P450 activities and mitochondria utilize various antioxidants and anti-oxidizing systems to defend against resulting oxidative stress. The added oxidative stress caused by excess copper may overwhelm cellular defensive mechanisms, especially in the mitochondrial, compromising respiratory function and impairing cellular health and survival. In this study we found that oyster gill mitochondrial treated with high doses of copper had impaired O_2 utilization. Addition of $CuSO_4$ (1-20 mM) to the micro-batch chamber caused a dose dependent decrease in gill mitochondrial O_2 consumption.

The study also showed that under our experimental condition, diltiazem was effective in blocking copper's inhibitory effects on gill mitochondrial O_2 consumption. The presence of diltiazem (5-10 mM) did not alter mitochondrial O_2 utilization and its presence was able to significantly reduce by over 50% the inhibitory effect of copper treatments on respiration. The metal blocker lanthanum (5-10 mM) was less effective at protecting against the inhibitory effects of added copper on gill mitochondrial O_2 utilization. While both drugs are known calcium channel blockers it is unknown why diltiazem was more effective than lanthanum at blocking the deleterious effects of copper on mitochondrial respiration. However, our results collaborate with our previous findings that diltiazem, but not lanthanum, blocked the uptake of copper in oyster gill tissue[14] supporting the idea that the mechanism of action of diltiazem as a copper blocker may not be related to its role as a metal channel blocker.

A better understanding of the toxic effects of copper on gill mitochondria could be of physiological significance to the growth and long-term health of aquatic animals living in a copper polluted environment. Fish and other aquatic animals living in a copper polluted environments show damage to a variety of tissues especially gills, liver, kidneys and nervous system[22] and excess copper has been shown to interfere with fish sense of smell, thus preventing them from choosing good mates or finding their way to mating areas[23].

In humans, the potential for toxicity exists and copper homeostasis must be tightly regulated so that the concentration of free copper remains extremely low. A number of disorders in copper homoeostasis exist such as Wilson Disease [24,25,26], a condition leading to progressive accumulation of copper with resulting cirrhotic and neurological damage. More recently, many neurodegenerative and neuropsychiatric diseases have been linked to oxidative stress and copper toxicity has been cited as a potential contributive if not causative agent. Copper and zinc are known to bind to amyloid beta proteins[27], and this bound form is thought to mediate the production of reactive oxygen species in the brain of Alzheimer's patients[28]. In fact, free copper levels were found to be significantly elevated in Alzheimer's disease patients compared to age matched controls[29] and furthermore, that these levels could be predictive of the rate of decline in these patients over time[300]. Humans accumulate copper through food, and it has been recently discovered that varying degrees of copper intake originate from drinking water piped through copper plumbing[31] and a recent study suggests that copper toxicity is a cause of loss of cognition in the general population, as well as in Alzheimer disease[32]. Several authors have reported evidence of oxidative stress in depressed patients[33,24,35] and copper toxicity may also be associated with clinical depression. Women suffering from post-partum depression showed elevated serum copper[36] and in a recent study, free copper excess was found to affect cortical glutamatergic neurotransmission and the clinical state of depressed patients[37].

Studying the toxic effects of copper and other metals on mitochondrial physiology will provide insight into causes of mitochondrial dysfunction and associated pathologies. Identifying copper blocking agents and determining their mode of action will further the understanding of metal transport mechanisms and may be beneficial in the therapeutic treatment of copper toxicity especially in humans.

Acknowledgments

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Benjamin Cummings/MACUB
Student Research Grants

Purpose
To provide investigative research support for undergraduate students working under the supervision of faculty who are current members of MACUB.

Awards
Applications will be evaluated and awards granted based on the scientific merit and overall quality of the proposed research experience.

- 4 grants of $500 each will be awarded annually (provided by BC).
- Complimentary registration for the annual fall conference of MACUB and membership in MACUB for student research grant awardees (provided by MACUB)

Eligibility
- Only undergraduate students currently enrolled at the institution of a MACUB faculty mentor may apply.
- Undergraduates who are graduating seniors must plan to complete their research prior to graduation.
- A student is only eligible to receive one award.

Requirements
- Student research grants may be used to support scientific investigation in any field of biology.
- Funding may be used to purchase equipment or supplies required for the proposed project, and/or travel to and from a research location.
- Grant winners are required to present the results of research supported by this award at the MACUB annual fall conference following the year of the award.
- Institutional support is required. This may include research supplies, travel expenses, in-kind matches, and other forms of support.
- All application materials must be submitted on-line at http//www.macub.org by February 1, 2011 and all applicants will receive notification of award status by February 28, 2011.

Application
- On-line proposal requires:
  - Student contact information.
  - Faculty advisor contact information.
  - Faculty reference letter from the research advisor. This letter must include a statement of institutional support for the project.
  - Proposal title.
  - Proposal (maximum of 500 words). The proposal should provide a brief background on the project with reference, a statement of the proposed question or hypothesis to be tested, and a description of the experimental approach.
  - References
  - Basic budget justification.
  - Include discipline. For example, molecular biology, cell biology, genetics, etc.
  - Register on-line at: www.macub.org

CALL FOR NOMINATIONS

The terms of office for the following positions will be up for re-election to serve on the Year 2011 Executive Board:

President
Corresponding Secretary
Members-at-Large - 2 positions

The duties of these officers will involve attending all Executive Board meetings in addtion to specific duties as described below:

The President will serve as chairperson of the Executive Board, will appoint and charge, with the approval of the Executive Board, the chairperson and members of all committees, and will carry out other activities usually pertaining to the office.

The Corresponding Secretary of the Association shall receive and validate applications for membership, respond to all inquiries, assemble and update the directory, and any other duties that usually pertain to this office.

The Members-at Large shall chair committees (Articulation, Exhibition, etc.) and handle other assignments as directed by the Executive Board.

Normally, each candidate for President, and Corresponding Secretary should have been a Member-at-Large for at least one term and each candidate for Member-at-Large should have attended at least one Annual Conference.

DEADLINE FOR NOMINATIONS is October 10, 2010

If you are interested in running for office (or wish to nominate anyone else), please send a letter of nomination to

Dr. Margaret Carroll
Biology Department
Medgar Evers College
1150 Carroll Street
Brooklyn, NY 11225

Student Membership

We encourage your students to become Associate Members in MACUB. Many of them will go on to graduate and professional schools. Their membership, participation and attendance at conferences such as these can enhance the experiences they include on their applications and discuss during interviews.

2010 MACUB Conference Registration Form
43rd Annual MACUB Conference at Molloy College
Saturday, October 23, 2010

❑Dr.	❑Regular Member	❑Student Member[1]
❑Prof.	❑Full-Time Faculty	❑Member's Spouse/Guest
❑Mr.	❑Ms.	❑Adjunct Faculty[1]
❑______		

* Name: ______________________ * School Phone: ______________________
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____

*The above information may appear in a Directory of Members unless you indicate otherwise.

Home Address: ______________________
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I prefer MACUB mailings to be sent to my:
❑School ❑ Home[1]

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[1]Student and adjunct mailings will normally be sent to your home address.

	Early Bird by 9/21	In Advance by 10/13	On-Site 10/23	
❑Regular Member	$45	$50	$55	Includes 2011 Membership dues, conference registration, continental breakfast and luncheon.
❑Student Associate Membership	$35	$35	$40	Includes 2011 Associate Membership dues, conference registration, continental breakfast and luncheon.
❑Member's Spouse/Guest	$35	$35	$40	Includes conference registration, continental breakfast and luncheon.

❑I will not be attending the Conference but enclosed is my 2011 membership dues.
❑Regular Member $20 ❑Student Member $10

Return this registration form by October 13, 2010
Please make checks payable to: MACUB
Send registration form and check to:
Dr. Paul Russo
Division of Natural Sciences & Mathematics
Bloomfield College
467 Franklin Street
Bloomfield, NJ. 07003

Registration fees are refundable upon written notification by **October 13, 2010.** The membership fee ($20 for regular members and $10 for student members) will be deducted. *No refunds will be given postmarked after October 13, 2010.*

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The MACUB web site is now up and running. We now call for members to use the web site for registration information. Register for the 43rd Annual Fall Conference on-line. Submit your poster presentation abstract on-line. Submit your member paper presentation on-line. If you are a MACUB member in good standing and have a web site that you would like linked to our web site, submit the URL address to: gsarinsky@kbcc.cuny.edu

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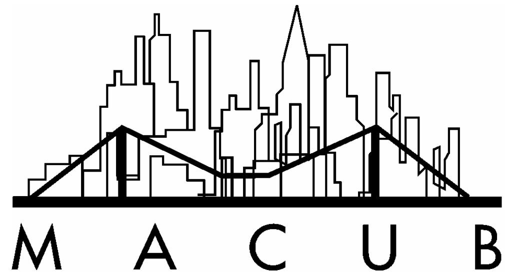

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