PRF# 56972-ND2

Investigating the fossil record of early eukaryotes using sterane-specific antibodies

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Overview: *Where, when, and how did the eukaryotic cell originate? What sorts of ecologies and physiologies did early eukaryotes have? What selective processes led to the origin and diversification of eukaryotes?* These are some of the questions we hope to address through the developing, testing and application of sterane-specific antibodies. We have made some progress in several of our specific aims, including developing new antibodies and applying antibodies to biomarker detection in natural samples.

Developing new antibodies: Our initial goal was to evaluate and use our previously produced antibodies in collaboration to Dr. Alan Epstein at the University of Southern California. Unfortunately, the cell lines were inadvertently destroyed in his laboratory. As such, we worked with the USC antibody production lab to generate new anti-sterane monoclonal antibodies. We produced liposomes containing cholestane, which was used as the antigenic substrate for the antibody production by applying a previously described method for anti-lipid antibody production (Matyas et al., 2000). We tested a total of 675 antibody-secreting clones (hybridomas) resulted from the liposomal immunization strategy by analyzing the reactivity of antibodies using enzyme-linked immunosorbent assay (ELISA) against cholestane and compared to squalane, similarly to the method described by Matyas et al. (2000). A total of 27 clones selected from the ELISA results according to their reactivity to cholestane compared to squalane were further expanded and the culture supernatants were subsequently evaluated by immunoblot using the method we described to evaluate the binding of antibodies to geolipids (Medina Ferrer et al., 2018; **Figure 1**). Unfortunately, none of the antibodies evaluated showed reproducible results and we concluded that either the immunization strategy was not effective for cholestane or the screening method was ineffective.

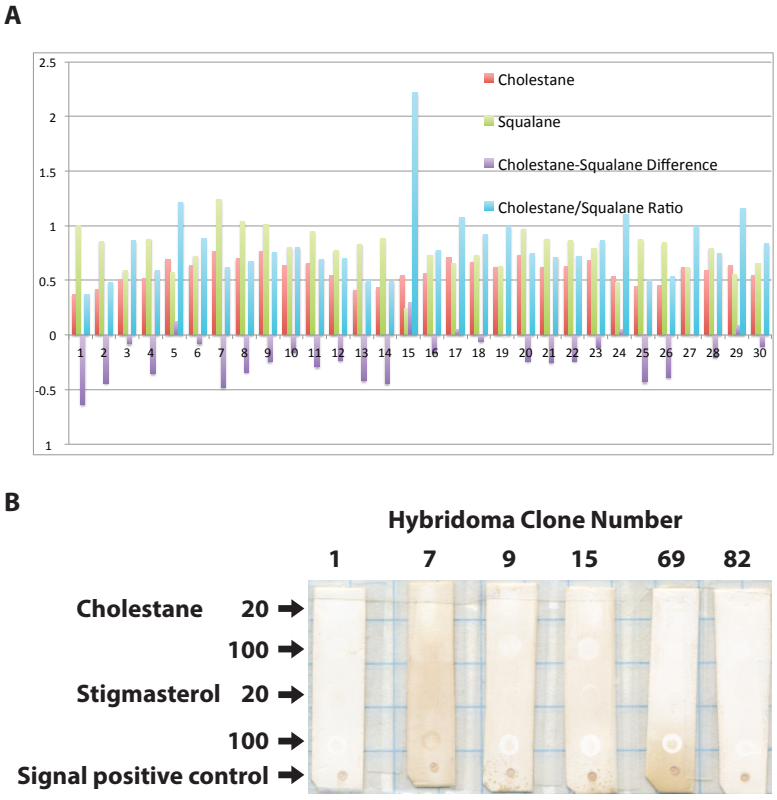

Figure 1*. Anti-cholestane antibody screening. (A) Signal quantification of the first 30 antibody clones evaluated by ELISA (numbered from 1 to 30) against cholestane and squalane. Clones showing either high overall signal or high cholestane-to-squalane signal ratio (such as clone #15) were selected for immunoblot evaluation. (B) Representative immunoblot on PVDF membranes of some selected clones against cholestane and stigmasterol standards (20 and 100 nmoles). The antibody binding signal is visualized by the precipitation of a purple-brownish insoluble product on the PVDF surface. In this figure, the clone #7 shows some reactivity against 100 nmoles of stigmasterol, however the signal was not reproducible in further experiments.*

To further evaluate the hybridoma lines produced by liposomal immunization, we plan to develop a method of anti-cholestane screening by immobilizing cholestane on protein surfaces, similar to what has been described for the production of antibodies to target small molecules (Li et al., 2016). Cholestane will be conjugated to albumin in the form of 3β-carboxy-5α-cholestane and carbodiimide coupling using hydroxysulfosuccinimide. The protein crosslinked to cholestane will be used for ELISA screening of the clones we already have and to test a different method of antibody production by injecting protein-immobilized cholestane as described previously for the production of antibodies against other small molecules (Pravetoni et al., 2014; Li et al., 2016). We plan to carry on this alternative immunization strategy in collaboration with the Immunology lab led by Dr. Marco Pravetoni at the University of Minnesota.

Evaluating antibody binding in standards and natural samples: As a proof-of-concept for the use of antibodies to detect hydrocarbons in environmental and geologically relevant samples, we have been used already produced anti-squalene antibodies from the study of Matyas et al. (2000). Anti-squalene antibodies were useful to develop our in situ staining procedures as well as showing the antibody potential as an organic geochemistry tool (Medina Ferrer et al., 2018). We are still using those anti-squalene antibodies to further advance our understanding of antibody binding to hydrocarbon standards and in natural samples as well as to develop new methods for the detection of lipids in organic geochemistry. We expect to reproduce the methods developed using the anti-squalene antibody once we have our anti-sterane specific antibodies. We have been working on purifying the anti-squalene antibodies and use the purified antibodies to detect lipids in natural samples. Preliminary data show that the sensitivity and signal-to-noise ratio are improved when purified antibodies are used instead of the culture supernatant (**Figure 2**). We anticipate that purified anti-squalene antibodies will help us visualize lipids preserved in fish fossils as well in bitumen and kerogen fractions of fossil organic matter.

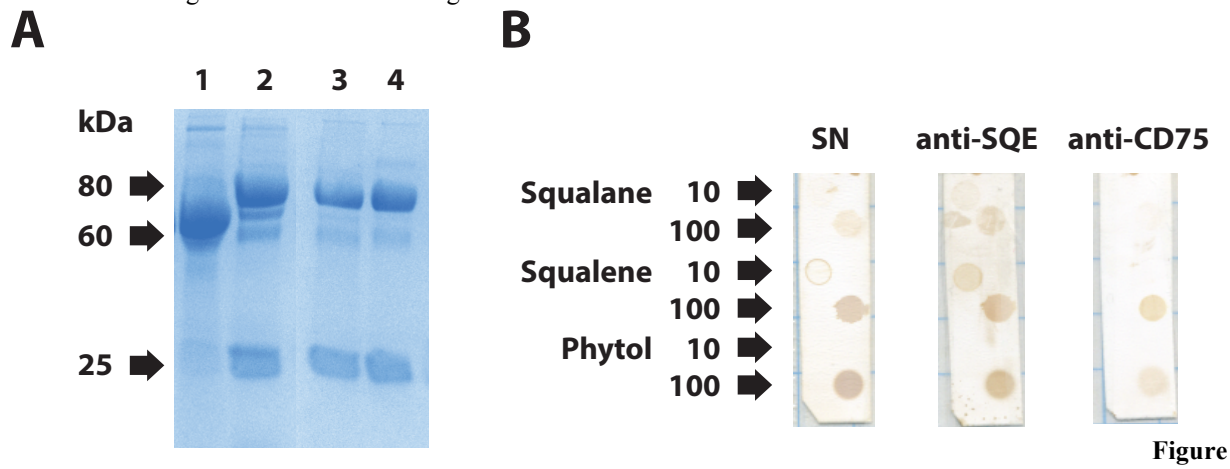

Figure 2. Purification of antibodies. (A) SDS-PAGE of purified anti-squalene antibodies (lane 4) and anti-CD75 (isotype control; lane 3) antibodies using protein L from culture supernatants (lane 1) and culture supernatant precipitated after dialysis against water (lane 2). (B) Immunoblot signal of using purified antibodies (anti-SQE and anti-CD75) compared to culture supernatants (SN) for incubation of membranes containing squalane, squalene and phytol (10 and 100 nmoles).

In addition, we developed a simple chromatographic method based on the separation of hydrocarbons using PVDF membranes as a stationary phase and applying a format similar to paper chromatography. The method was tested using lipid standards and SARA (saturate, aromatics, resin and asphaltic) fractions obtained from crude oil samples with the subsequent detection of anti-squalene-reactive hydrocarbons. To our knowledge, such chromatographic method has not been described thus far. We are currently writing a manuscript describing the chromatography of biological and geological relevant samples using PVDF membrane chromatography.

These results, while still preliminary, do suggest that antibodies raised against biomarker compounds can be produced and used to target sedimentary organic matter by using a variety of formats that we are currently developing.

Participants: The work was primarily performed by my Ph.D. student, Mr. Fernando Medina Ferrer who is using this project as the basis of his Ph.D. thesis.

Dissemination of research results: Mr. Medina Ferrer presented some of his research results from this project at the IV Annual ESCI Student Research Symposium, April 14 (2017), University of Minnesota, and at the Goldschmidt Conference, August 12-17 (2018) in Boston, MA, USA.

Publications derived from the project: Medina Ferrer F, Bailey J, Corsetti F, Moldowan JM, Barbanti B, Caron D, Bryant-Huppert J. (2018) Assessing biomarker syngeneity: an in situ approach using monoclonal antibodies. *Organic Geochemistry* 124:112–122.