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FINAL TECHNICAL REPORT

A Molecular Genetic Approach to Understanding Eukaryotic Cellulose Synthesis

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Publications Attributable to DOE Funding

Blanton, R.L. In Press. Cellulose biogenesis in *Dictyostelium discoideum*. In *Proceedings of the 36th Yamada Conference: International Dictyostelium Conference* (Y, Maeda, I. Takeuchi, and K. Inouye, eds.), Tokyo: Universal Academy Press.

Grimson, M.J., C.H. Haigler, and R.L. Blanton. 1996. Cellulose microfibrils, cell motility, and plasma membrane protein organization change in parallel during culmination in *Dictyostelium discoideum*. *Journal of Cell Science* **109**: 3079-3087.

Blanton, R.L. and C.H. Haigler. 1996. Cellulose Biogenesis. In *Membranes: Specialized Functions in Plants* (M. Smallwood, J.P. Knox, and D.J. Bowles, eds.), Oxford, U.K.: BIOS Scientific Publishers. pp. 57-75.

Abstracts Reporting Work Attributable to DOE Funding

Blanton, R.L., M.J. Grimson, M.E. Bonner, and C.H. Haigler. 1996. Changes in patterns of cellulose deposion, cell motility, and membreane protein organization during stalk cell differentiation in *Dictyostelium discoideum*. Dicty'96: International *Dictyostelium* Conference, Sendai, Japan.

Blanton, R.L. 1996. Patterns of cellulose deposition by *Dictyostelium discoideum* during normal and abnormal development and in stalk cell monolayer cultures. Keystone Symposium on the Extracellular Matrix in Plants: Molecular, Cellular, and Developmental Biology. (Featured as an invited "short talk.")

Blanton, R.L., M.J. Grimson, and T.A. Kerr. 1995. Patterns of cellulose deposition by *Dictyostelium discoideum* during normal and abnormal development and in stalk cell monolayer cultures. Dicty 95: International *Dictyostelium* Conference, Dourdan, France.

Summary

The long history of frustrations, false leads, and set-backs that has characterized the study of cellulose biosynthesis may be entering a new and more satisfying era with the recent discovery of putative plant cellulose synthase genes [for a discussion of the history of the search for cellulose synthase genes and of the steps leading to the identification of the plant genes, see (Haigler and Blanton, 1996)] The long-term goal of my laboratory has been to bring to bear on the problem of cellulose biosynthesis the experimental versatility of *Dictyostelium discoideum* and its amenability to molecular biological manipulations. We have endeavored to apply every advance in the study of bacterial and plant cellulose biosynthesis to *Dictyostelium*, and have been fortunate to have relationships with nearly all of the major laboratories to make this possible. Unfortunately, we have yet to identify any genes from *Dictyostelium* that are involved in cellulose synthesis.

However, we have made a number of other interesting observations regarding the biochemistry, cell biology, and developmental biology of cellulose synthesis. We continue our

efforts to look for genes and are confident that one of our strategies will eventually bear fruit. The background of information on cellulose synthesis we have established, together with the experimental advantages of *Dictyostelium* will make it a key organism in unravelling the eukaryotic cellulose biosynthetic pathway and its overall place in cellular regulation.

Status of Individual Goals Stated in Original Project Abstract

It should be noted that the original project abstract was for a three-year project. Only one year of funding was provided; work continued on the project for a second unfunded year.

1. Use a newly developed method of tagged insertional mutagenesis to generate mutants in cellulose biosynthesis.

REMI (Restriction Enzyme Mediated Insertional Mutagenesis) is a method of tagged insertional mutagenesis adapted to *Dictyostelium* (Kuspa and Loomis, 1992) that has resulted in the identification of a number of novel genes important in development (Dynes et al., 1994; Harwood et al., 1995; Segall et al., 1995; Shaulsky et al., 1995). The major advantages of REMI are the high rates of insertion (as high as 4×10^{-5}) and the ability to recover the disrupted gene (Kuspa and Loomis, 1992; Kuspa et al., 1995). Cellulose biosynthesis is developmentally regulated (Blanton and Northcote, 1990; Blanton, 1993), so the method seemed an ideal one for identifying genes involved in cellulose biosynthesis.

I first screened a group of 110 mutants selected from 500 REMI-disrupted strains available in the laboratories of Drs. J.G. Williams (Imperial Cancer Research Fund, Clare Hall Laboratories, U.K.) and R.R. Kay (MRC Laboratory for Molecular Biology, Cambridge). None of the strains tested were lacking in cellulose, as indicated by fluorescence in the presence of the cellulose-indicating fluorescent brightening agent Tinopal LPW. Late developmental studies in my laboratory suggested that our assumptions regarding the possible phenotype of a cellulose-deficient mutant (deduced from the literature) were incorrect, which meant that the selected strains were likely arrested too late in development to be cellulose synthesis mutants. This emphasized to us again the import of a basic understanding of cellulose synthesis in *Dicytostelium*.

In my own laboratory, I recruited a graduate student, Stephen Fuhrmann, to work on the REMI project. Mr. Fuhrmann established the procedure in my laboratory, initially using the system as described and provided by Dr. Kuspa (Kuspa and Loomis, 1992), and later adopting the more efficient method of Adachi *et al.* (Adachi et al., 1994). Our initial screening continued to be absence of fluorescence by colonies grown on agar containing Tinopal LPW. Mr. Fuhrmann performed the REMI procedure approximately 25 times, each time generating 50 culture plates with between 25-50 colonies per plate. Therefore, 30,000-50,000 colonies were screened. Very few of these tested negative with the Tinopal test. Of these, most turned out to be colonies that had not developed to the multicellular stage (and hence were not producing cellulose). The remainder proved to have cellulose upon closer examination.

The REMI project was hindered by the absence of an efficient screening system that would allow testing hundreds of thousands of colonies. We attempted to adapt a monolayer culture system without success. We attempted to screen for absence of cellulose in spores without success. We consulted with a wide variety of *Dictyostelium* biologists, but no one was able to suggest a screening strategy that we had not already tried.

2. Exploit the monolayer culture system to identify differences in mRNA populations between DIF-induced (and cellulose-synthesizing) versus non-induced cells.

One of the most interesting aspects of cellulose synthesis in *Dictyostelium* is its inducibility; cells that never made cellulose will be induced to synthesize cellulose. This induction can be studied in the absence of other morphogenetic processes in monolayer culture systems, in which amoebae are plated as a cell monolayer submerged in a liquid culture medium. If cells of HM44

[the mutant strain that is incapable of synthesizing the chemical that induces stalk cell formation (DIF, differentiation inducing factor)] are plated in monolayers, then formation of stalk cells (and cellulose synthesis) will depend upon addition of DIF. The induced and non-induced monolayer cultures would be useful sources of mRNA for cDNA-based techniques for identifying differentially expressed genes. We could not pursue this part of the project as intensively as we would have liked given the short-term funding. However, Dr. Vas Achar, a research associate hired to work part-time on the project and I made several attempts to generate a subtracted cDNA library. We prepared mRNA from induced and non-induced HM44 cultures and used the Invitrogen Subtractor kit to prepare subtracted cDNA. Although we obtained high quality mRNA from both cell types, we were unable to obtain subtracted cDNA of sufficient quality/quantity to prepare a library.

Other laboratories have attempted to use methods such as differential display and subtracted libraries to obtain novel DIF-induced genes, but none have succeeeded.

Additional Projects Pursued during the Project Period

1. Membrane specializations that accompany the onset of cellulose synthesis

The experience my laboratory has developed with monolayer cultures has led to several interesting insights concerning the developmental regulation of cellulose synthesis in *Dictyostelium*. Mark Grimson, departmental EM technician in our department and my Ph.D. student, used freeze-fracture electron microscopy to examine changes in membranes of stalk cells during development. The only aspect of the work receiving DOE support was in the establishment of the monolayer culture. The results are well-summarized in the published paper, a copy of which is attached to this report.

2. Continued efforts in the biochemical characterization of the *Dictyostelium* cellulose synthase

Several interesting observations of the biochemistry of the *Dictyostelium* cellulose synthase remained from our first DOE grant. Dr. Achar worked on some of these, specifically the effects of various cations, inhibitors, and other reagents and some of the supernatant and washing effects that we had observed. I hope to be able to publish the final results of our biochemical efforts in the coming year.

3. A PCR-based strategy to identify the *Dicytostelium* cellulose synthase

As each cellulose synthase gene sequence has been published, we have done multiple-sequence alignments, identified potential regions of homology, designed PCR primers, and attempted to isolate the *Dictyostelium* cellulose synthase by PCR. Our lack of success in the past was not surprising, since there were a limited number of genes from which homologous regions could be induced, and all of these genes were from bacteria. Only with the publication of the first plant cellulose synthase sequences were the important regions of limited homology across kingdoms made clear. We designed PCR primers to these regions, both using standard degeneracy and *Dictyostelium* codon bias (Warrick and Spudich, 1988). To date, we have yet to identify a convincing fragment from *Dictyostelium*, although our efforts continue.

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