RECOLLECTIONS

Pride and prejudice: The discovery of the primer for glycogen synthesis

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The study of glycogen and its metabolism has been important for the progress of biochemistry as a whole because of the number of general concepts that have emerged therefrom. It was the first macromolecule to be synthesized *in vitro* by Carl and Gerty Cori. Its synthesis from UDPglucose by glycogen synthase was one of the crowning achievements of Luis Leloir, who discovered the nucleoside diphosphate sugars. Cyclic AMP and the second-messenger concept arose out of a study of the breakdown of liver glycogen. Protein phosphorylation and dephosphorylation, as a means of regulating enzyme activity, were discovered out of the realization that active muscle phosphorylase is a phosphorylated form of the inactive, unphosphorylated enzyme.

Arising from the discovery of how glycogen can be synthesized with phosphorylase and branching enzyme came the realization that a primer is necessary for this synthesis to take place. The primer can be glycogen itself. Originally it was thought that the

primer had some catalytic role, but later it was realized that the molecule acted as an acceptor of α -glucose residues, which became covalently attached by 1,4-bonds to this primer, already containing such residues. The same proved to be the case for starch synthesis (Whelan, 1976, 1992).

My entry into this field came in 1950, when we achieved the first preparative scale separation of maltosaccharides and showed with potato phosphorylase that a molecule as small as maltotetraose could prime amylose synthesis from glucose 1-phosphate, and that no improvement in rate of synthesis was achieved by using maltopentaose or the hexaose. The search for the means by which the primer for glycogen and starch synthesis is brought about was, therefore, defined as a mechanism for the synthesis of maltosaccharides. Even when it was discovered that the enzyme responsible for glycogen synthesis *in vivo* was synthase, and not phosphorylase, it was still observed that the minimum efficient primer was maltotetraose.

More than a decade after our 1950 report, there had been no progress in identifying the primer synthesizing mechanism, but in 1962, Cori and his colleagues reported that no special primer synthesizing mechanism was necessary. They claimed that if muscle phosphorylase was incubated with glucose 1-phosphate, the enzyme would somehow assemble a primer and synthesis would ensue. This was referred to as a *de novo* synthesis (Whelan, 1976).

I was intrigued by this observation and resolved to repeat it. I had the opportunity one year later, coincident with my having persuaded the CIBA Foundation in London to stage a symposium on the control of glycogen metabolism in honor of Luis Leloir, their corresponding member for Argentina (Whelan & Cameron, 1964) (see Fig. 1). I was aided in the selection of participants by the late Carl Cori and, looking back, the selection was prescient because by 1992, five of the 25 attendees had won the Nobel Prize. In 1963, Cori already had the Prize (1947), but Leloir (1970), Sutherland (1971), and Edmond H. Fischer, at the time of the 1963 Symposium, were to follow. Fischer, at the time of the 1963 Symposium, was about to spend part of a sabbatical year with me at the Lister Institute in London, and we took the opportunity to repeat the Cori *de novo* synthesis. We denied the claim because it was our conclusion that Cori's glucose 1-phosphate must have contained a trace of primer. A few years later, Lee, Schiefer, and I also refuted a claim of another kind of *de novo* synthesis, with a bacterial glycogen synthase acting on ADPglucose. Here it was not

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William J. Whelan was trained in organic chemistry at the University of Birmingham England where he obtained his Ph.D. in 1948. He held successive faculty appointments in Birmingham (1947–1948), at the University College of North Wales, Bangor (1948–1955), and the Lister Institute of Preventive Medicine, London (1956–1964). He became head of the new Department of Biochemistry at the Royal Free Hospital School of Medicine, University of London, in 1964. In 1967 he transferred to the corresponding position at the University of Miami where he remained chair of the Department until 1991, and continues as Professor of Biochemistry and Molecular Biology. His life-long researches have been into the structures and metabolism of starch and glycogen, most recently on the autocatalytic self-glucosylating proteins that initiate glycogen and starch synthesis, the subject of this article. In parallel, Dr. Whelan has held appointments with national, regional, and international biochemical organizations, for example, as the first Secretary General of FEBS (1965–1967) and PAABS (1970–1972). He was the General Secretary of the International Union of Biochemistry from 1973–1983 and is now its President (1997–). He took part in establishing several biochemical journals: the *European Journal of Biochemistry*, *FEBS Letters*, *Trends in Biochemical Sciences*, *Biochemistry and Molecular Biology International*, *BioEssays*, *BioFactors*, and most recently helped establish the *FASEB Journal*, of which, as also for *Trends in Biochemical Sciences* and *BioEssays*, he was the first Editor-in-Chief (1986–1996). His honors include the CIBA Medal of The Biochemical Society 1969, the FEBS Diplome d'Honneur 1974, Honorary Membership of the Royal College of Physicians of London 1985, of The Biochemical Society 1993, Honorary D.Sc. of LaTrobe University, 1997, and the Fellowship of The Royal Society of London, 1992.

Fig. 1. The participants in the CIBA Foundation Symposium on the Control of Glycogen Metabolism held in London in July 1963 (Whelan & Cameron, 1964). The five then and future Nobelists were: Carl F. Cori, standing, seventh from left; Luis F. Leloir, seated, second from left; Earl W. Sutherland, Jr., standing, fourth from left; Edmond H. Fischer, standing, eighth from right; Edwin G. Krebs, seated, third from right. The author is standing, third from right.

the substrate that was contaminated but the enzyme preparation (Whelan, 1986).

These negative findings generated a certain amount of heat but no light. That light was to come, however, in 1975 from Leloir's laboratory via his colleague, Clara Krisman, who reported that an extract of rat liver incubated with ^{14}C -labeled UDPglucose brought about the synthesis of glycogen-like material that was precipitable with trichloroacetic acid (TCA), from which she concluded that the glycogen was attached to a protein, and that the true primer was suggested to be a protein and not, as we had supposed, a free maltosaccharide. Krisman hypothesized that the primer protein acted as a recipient of α -1,4-linked glucose units added from UDPglucose by a hypothetical "glycogen initiator synthase." When four units long (maltotetraose), the attached saccharide could prime glycogen synthesis by synthase and branching enzyme.

I seized on this idea and wrote it up as a review in the first issue of *Trends in Biochemical Sciences* (Whelan, 1976). I invited Krisman to Miami, and together we repeated and extended her observations to other glycogen-storing tissues. I decided to search for the protein primer by examining glycogen for the possible presence of covalently bound protein. It did not follow that the protein primer was necessarily a permanent part of the molecule, but that was a chance we had to take.

The idea of protein in glycogen was hardly new. It had been reported as early as 1886, and formed the basis of a hypothesis formulated by Willstaetter in the 1930s that there were two kinds of glycogen. One was protein bound (desmoglycogen), the other

protein free (lyoglycogen). The two forms could be separated by taking advantage of the insolubility of desmoglycogen in dilute TCA. In the 1950s the possible physiological significance of the two fractions was quite extensively investigated, as recorded by Stetten and Stetten (1961). But a 1961 report by my former graduate student J.M. Bailey, a co-author on the papers reporting priming by maltotetraose, in which he claimed that the two glycogen components are artifacts, effectively brought to a halt further work on the Willstaetter hypothesis, which was not revived, until in 1990 we rediscovered desmoglycogen as a low molecular weight (mass 400 kDa) stable intermediate in the synthesis and degradation of glycogen. It contains one molecular proportion of the protein primer that I am about to describe and, therefore, is about 10% by weight of protein and as such is precipitated by dilute TCA. Mature glycogen (M_r 10^7 Da) also contains one molecular proportion of protein, but at 0.35% by weight, the protein content is insufficient to confer insolubility in TCA. Willstaetter's hypothesis was, therefore, modified. What we call proglycogen was his desmoglycogen; mature macroglycogen was his lyoglycogen. Both contain one molecular proportion of protein (Alonso et al., 1995).

Whether or not glycogen contains protein was a point that was often debated, for example, at the aforesaid 1963 CIBA Symposium (Whelan & Cameron, 1964), but there was a blind spot in the eyes of those who claimed that glycogen is free from protein. This was the fact that they were used to dealing with preparations obtained by the classical method of boiling tissue in 30% potassium hydroxide. Glycogen is the only macromolecule stable to

alkali, and can be cleanly recovered after tissue has been treated in this way, but it is hardly likely to contain any protein.

We obtained evidence that liver glycogen contains protein, but turned our attention to muscle glycogen because the protein component seemed more abundant. Joseph Lomako, a visiting Polish scientist, made the decisive breakthrough in 1979–1980 by isolating a protein from muscle glycogen that had mass 37 kDa and still contained covalently bound glucose residues. Obviously, the next step was to determine the nature of the covalent linkage between glycogen and the protein, which we named glycogenin. This proved to be the most difficult task of all. Progress was slow for three reasons. First of all, the bond proved to be an entirely new mammalian carbohydrate-to-protein linkage. The first glucose unit of glycogen is linked to a tyrosine hydroxyl group in glycogenin. Secondly, there was the technical difficulty that although we were enriching tyrosine in glycopeptides carved enzymically out of glycogen in the attempt to identify the linkage, tyrosine is notoriously unstable when hydrolyzed in acid in the presence of carbohydrate, so there were large losses, which lowered the tyrosine profile. The third reason was a financial one. When I came to attempt to renew my NIH grant that was paying for these researches, we had reached the point of being able to report that we had found that rabbit-muscle glycogen contained one molecular proportion of a covalently bound protein (glycogenin), and that we could not detect any protein-free glycogen molecules. We wished to pursue the identification of the linkage between glycogen and glycogenin. The NIH Study Section that reviewed our application was skeptical of this observation, and gave the proposal a priority score too low for it to be funded. With two devoted graduate students, Ignacio Rodriguez and Brent Kirkman, we worked on glycogenin for six years without any federal funding, obtaining small grants for other purposes, and pursuing the glycogenin story in our spare time. By 1985 we were able to return to the NIH, informing them that we had discovered the new glucose to tyrosine bond as the linkage between glycogen and glycogenin (Whelan, 1986). We proposed, having proven that glycogen contained covalently bound protein, now to test the enzymology implicit in Krisman's proposal by trying to obtain glycogenin in a pure form, as well as the hypothetical glycogen initiator synthase, so that we could test whether glycogenin is indeed the primer for glycogen synthesis. We felt rather confident of being able to make progress because we had provided the answer that the Study Section seemed to be looking for, namely, identifying and proving the covalent nature of the covalent glycogen–glycogenin bond. To our dismay, the proposal was again unfunded and the “pink sheet” that formed the report from the Study Section contained the following statement:

“This reviewer is troubled by the repeated inferences that glycogenin is the primer for glycogen synthesis.”

“Troubled”? The reviewer could not see his or her way to voting a fundable priority to support what was for us the entirely reasonable proposal to try to determine the significance of the presence in glycogen of this covalently bound protein.

It was at this point that the research might have stopped. Lacking any funds, there was little prospect of continuing. I had just reached the age of 60, a point at which superannuation to faculty in the British university system became payable. When I had moved to Miami from London in 1967, I had continued to pay into the British system to add value to the retirement benefits I had already accumulated there. My wife, Margaret, who had always taken an

intense interest in our researches and had shared the highs and lows of the work on glycogenin, proposed to me, after the latest failure with NIH, that I should use some of these retirement funds to continue the research. I agreed to try this for one year, and made my first act the expenditure involved in inviting to Miami, from Buenos Aires, another member of the Leloir Institute, Juana S. Tandecarz. At a primer protein symposium in Buenos Aires in November, 1984, that I had arranged with Clara Krisman, Juana Tandecarz had described her researches into the question whether starch is synthesized on a protein primer. She reported that she could radioglucosylate a protein in a potato membrane preparation using 5 μ M radiolabeled UDPglucose and 5 Mn^{2+} . (The reaction was totally dependent on manganese.) When the labeled preparation was subjected to SDS-PAGE, and the gel radioautographed there was seen to be a radioactive band corresponding to mass 38 kDa. I was impressed by the discovery of experimental conditions that could detect the kind of protein that formed the basis of Krisman's hypothesis. Dr. Tandecarz agreed to visit Miami, and together with Ignacio Rodriguez performed one of those very rare experiments that work the first time and give the hoped-for result. With a rabbit-muscle extract, a protein having the same M_r as glycogenin was found to be radioglucosylated. Furthermore, it gave a positive Western blot with antibody to the glycogenin we had isolated from rabbit-muscle glycogen. Armed with this information, I once again applied to the NIH for funding, and in 1987 was successful. We have been funded ever since.

The rest of the story has been related by us in a recent review (Alonso et al., 1995). In this we describe how Joseph and Wieslawa Lomako in Miami and others elsewhere (Smythe & Cohen, 1991) learned that glycogenin and glycogen initiator synthase are one and the same protein. Glycogenin is self-glucosylating. I was pleased to see our review described in a new textbook of biochemistry and molecular biology as:

“A beautiful article describing the role of glycogenin. Summarizes new concepts in a field many thought was a closed book” (Elliott & Elliott, 1997).

So the believability in glycogenin is far removed from the dark days of 1985, and I take pride in the fact that the prejudice toward a primer role for glycogenin that was displayed at that time has disappeared, particularly in the citation that accompanied the announcement of my election, in 1992, as a Fellow of The Royal Society of London, which reads in part:

“Distinguished for the elucidation of the role of the primer protein in the synthesis of glycogen.”

It was especially gratifying that my wife was alive to share this moment of vindication. She died the following year. Not among the skeptics, and indeed perhaps the first onlooker publicly to acknowledge the significance of glycogenin, was Lubert Stryer (1988), who, in the third edition of his textbook of biochemistry, wrote that:

“Glycogen synthesis requires a primer. This function is carried out by a protein containing an oligosaccharide of α -1,4-glucose units attached to the phenolic oxygen atom of a tyrosine residue.”

In almost all textbooks published since then glycogenin is acknowledged as the primer. No longer do textbook authors beg the question of what is the nature of the primer.

Acknowledgments

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In order to provide a compact bibliography, the original literature has not been cited. Instead, the following reviews and a monograph may be used to pursue the topic in depth.

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