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General discussion

The discussion presented below results from papers presented at The Royal Society Discussion Meeting.

J. F. Allen (*Plant Biochemistry, Lund University, Lund, Sweden*). Session 1 was called 'Symbiosis and genome function'. There were talks by John Raven, Angela Douglas, Tom Cavalier-Smith and myself. The chairman was Chris Leaver. Do you feel, Professor Raven, your case was adequately addressed in subsequent discussions in the meeting? Your talk on the roles of cyanobacteria and proteobacteria in symbioses with eukaryotes?

J. A. Raven (*School of Biological Sciences, University of Dundee, Dundee, UK*). Yes.

J. F. Allen. Good. I thought so, too, actually. There might be a general issue about what extant symbioses have to tell us about the dark and mysterious goings on two billion years ago, between organisms of which we can guess and argue and speculate on; clearly symbiosis is a very popular way of doing things and is not the wild and improbable and unlikely way of introducing genetic innovation in evolution. But what about the specifics? I mean, is the cyanobacterium actually a good model for the proto-chloroplast?

J. A. Raven. It seems to be the best we have. Do you have any others?

J. F. Allen. Is it possible the cyanobacterium brought into the deal the possibility to fix nitrogen, which the host cell could not? We all think about photosynthesis and ATP synthesis.

J. A. Raven (*Department of Biological Sciences, University of Dundee, Dundee, UK*). This was something I hedged on; obviously, I have thought about this before. I have got a couple of papers coming out soon that go into this in perhaps a little more detail, without coming to any particular conclusion, really, as to whether the plastid ancestor or ancestors were diazotrophs, and if they were, why the diazotrophy was lost. As far as today goes, of course we have no extent examples of any, shall we say for the purposes of argument, diazoplasts, that is to say organelles that specifically fixed nitrogen, from either the cyanobacteria or the *Frankia* line, the Posibacteria, or from the rhizobial line, but I think, possibly Angela Douglas....

A. E. Douglas (*Department of Biology, University of York, York, UK*). Well, I was wondering if you could possibly help us, or perhaps other people can, on the reason why there are not any nitrogen-fixing organelles, since we have perfectly good nitrogen-fixing symbioses in protists, I believe.

J. A. Raven. Yes, I think yes. Well, Tom [Cavalier-Smith]?

T. Cavalier-Smith (*Department of Zoology, University of Oxford, Oxford, UK*). It may not be a complete reason but if the symbiotic origin of mitochondria and chloroplasts came about in a phagotroph, I think a phagotroph does not need to fix nitrogen because it eats it.

J. A. Raven. That is a very good point. Certainly organisms go to very significant lengths to scavenge nitrogen from combined sources, rather than to fix nitrogen in many cases. There are a few exceptions like as *Azolla* and *Gunnera*, which you cannot really persuade to stop fixing nitrogen, except by removing the symbionts where that is possible. Many legumes, for example, will scavenge rather than fix, under really quite severe nitrogen-deficient conditions.

A. E. Douglas. And, perhaps consistent with that, nitrogen-fixing symbioses are really rather rare in animals, being restricted largely to wood-feeding termites, which are not your typical phagotrophs.

J. A. Raven. Not really, no.

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A. E. Douglas. Not in terms of their diet.

J. A. Raven. There are, of course, technical difficulties with diazotrophy in oxygen-evolving organisms, which clearly heterocystous cyanobacteria have got around, and *Richia* and *Hemiaulus* do nocturnal nitrogen fixation, and *Trichodesmium* switches between photosynthesis and diazotrophy during the day. So there are complications there; it may not have been that easy to combine photosynthesis with diazotrophy. But that is not the only option; multi-cellular organisms, either non-photosynthetic organisms or those that have photosynthesis in one part and diazotrophy in another, certainly could combine the two, but as I say we have no evidence of organelles that actually do that.

R. E. Blankenship (*Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona, USA*). I just want bring up this question of the possibility of the prochlorophytes being the ancestors of the higher plant chloroplast. Of course, when the prochlorophytes were first discovered 30 some years ago, they were hailed as the likely predecessor of the higher plant chloroplast, which went through a decline as it appeared as if the different prochlorophytes were polyphyletic. But now people have gone in and sequenced the chlorophyll *b* synthase genes and they all seem to be homologous, and that seems to swing the pendulum back a bit, towards that possibility. Do you have some thoughts on that? It could obviously be lateral gene transfer of that particular gene, that one gene you need to make chlorophyll *b*.

J. A. Raven. Yes, I certainly would not rule out the possibility that Tomitani and coworkers put forward in 1999, that the ancestor of the plastids, assuming a monophyletic origin of plastids, had both phycobilisomes and chlorophyll *b*. That certainly seems plausible, and a number of the prochlorophytes seem to have residual phycobilisomes as well as chlorophyll *b*, so there is certainly a precedent for that. Certainly, when one starts to look at the glaucocystophytes, they are rather different from the reds (red algae) in terms of their RUBISCO—they have a type 1B RUBISCO rather than the 1D of the reds—so clearly there was significant, presumably horizontal, gene transfer then into the reds to retain the phycobilins in the reds, and the glaucocystophytes kept the 1B RUBISCO as the reds got 1D from some proteobacteria.

J. F. Allen. Session 2 was called 'Bacterial homologues of compartments and organelles'. The session was chaired by Bob Whatley, and talks were given by Ford Doolittle, Bill Martin, Siv Andersson and Carl Bauer. Are there any comments or questions to any of those speakers?

E. Lopez-Juez (*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK*). Earlier, we heard about the RegA–RegB redox control of very diverse phenomena in *Rhodobacter*. Now the electron sensor we had in protein X was widespread, among all kinds of organisms. What is protein X? Could it be related to redox control in mitochondria and plastids or would it be specific for certain type of organelle?

J. F. Allen. This is a question directed to Carl Bauer.

C. E. Bauer (*Department of Biology, Indiana University, Bloomington, Indiana, USA*). Yes, it is a mitochondrial protein associated with cytochrome oxidase. It has been found in all the mitochondria, and in everything from bacteria all the way to higher organisms.

J. F. Allen. This is what I think is called a tease, Carl. It is not Oxa1, is it?

C. E. Bauer. No, no.

J. F. Allen. Is it an unknown component that we know by another name?

C. E. Bauer. Yes, it is one of these peripheral proteins that are present. You have a core of Cox I, Cox II, Cox III, but then there is a series of peripheral proteins associated with cytochrome oxidase; this is one of them that is associated with it.

J. F. Allen. In mitochondria, is it nuclearly encoded?

C. E. Bauer. Yes.

J. F. Allen. Always?

C. E. Bauer. Yes.

J. F. Allen. Let us narrow this down. Let us play 20 questions.

J. A. Raven. How many more questions do we have?

C. E. Bauer. Let us have this discussion of meeting in two years and I shall be able to tell you everything about it.

J. F. Allen. Could I comment? Bill Martin made a presentation on a grand unified theory of everything, I think, and there is nothing wrong with that. It was enormously thought provoking, but it was on behalf of Mike Russell also, who is here, who will be a co-author on the paper, and that title was the 'On the origin of cells' (Martin & Russell 2003). Although this is not a meeting on the origin of life, one has to consider where all these things came from in the first place, and I found it really quite startling, the idea that the fundamental division between bacteria and Archaea was in the iron–sulphide bubbles in the Makinite.

M. J. Russell (*Isotopes Geology Unit, Scottish Universities Environmental Research Centre, East Kilbride, Glasgow, UK*). Mackinawite.

J. F. Allen. I mean that seems like quite a wild idea, actually. Mike [Russell], you agree with all this?

M. J. Russell. Yes, I do agree with all that.

J. F. Allen. The Archaea popped out of a different inorganic iron–sulphide bubble from the ones that the bacteria popped out of?

M. J. Russell. The Earth, 4.4 billion years ago, was like a giant photoelectrochemical cell, the output was about 500 mV, and that really needed to get spent. Metabolism is how it got spent. And what happens often when you get a far-from-equilibrium structure, kinetic structure, whatever that might be, whether it is a volcano or life, is that often you get differentiation. So we had the idea that microbes differentiated right from scratch because of the different lipids in the membrane and so forth. So, yes, right at the beginning. I should say you could have two parallel origins of life, emergences of life.

T. Cavalier-Smith. It seems to me a great problem with them being that far back. Virtually everything we know about cell biology—molecular biology—suggests that their common ancestor had over a thousand different proteins; it had membranes with complex targeting, essentially all of the basic metabolic processes, development, and so on. And furthermore, some features shared by eukaryotes and archaebacteria are also present in a highly derived subgroup of the Gram-positive bacteria, the actinobacteria, and I cite specifically the proteosome. I think it just does not make any phylogenetic sense to suggest that archaebacteria were that old, and there is not a scrap of geological evidence that they were, either.

W. Martin (*Institute of Botany III, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany*). Well, Dr Nisbet responded to that comment earlier, and he is welcome to respond again. His comment was that, up to 2.7 billion years ago, the carbon isotope data indicate that marine ecosystems were very much the same as they are now, meaning that there is good isotopic evidence for the ultralight carbon going back 2.7 billion years, and that is an indication for the presence of methanogenesis. Your prime objection to this model is the independent origins of the membrane biochemistry that surrounds free living cells, the two kinds of prokaryotes, archaebacteria and eubacteria, but it is not only the lipid biochemistry, but also the cell wall biochemistry that matters. Let us not forget that Otto Kandler and Carl Woese discovered archaebacteria differently, independently. Otto Kandler discovered them by their lack of murein, and we know that their cell wall biochemistry, in addition to the lipids themselves, is completely different, and shares no common components. So theories such as yours are fully articulated, and you are very explicit; however, you do not differ fundamentally from van Valen (van Valen & Maiorana 1980) on this issue. To explain this difference, you start with a single origin of free-living cells, from what I do not know. The step to cellular compartmentation is an extremely difficult one. There is the *obcell theory*, which posits that somehow genetic systems became compartmentalized in membranes that they synthesized *de novo* from a non-compartmentalized cytosol, which I think is completely untenable; this, at least, is at odds with all the conserved attributes of cells, which are compartmentalized redox compartments, and this organic soup that would have contained an ancestor is not compartmentalized, and so it would have had no possibility of an energy or redox chemistry and could not have proliferated itself. So that is the obcell. So we solved that problem differently, and by using pre-formed structures.

The second criticism that you voice is when you said that it is possible that a bacterium—and in your last paper it was 800 million years ago, or 850 million years ago, in other papers it has been earlier, but it does not make any difference when that occurred—that a bacterium, with eubacterial lipids, actually the common ancestor of eubacteria and eukaryotes, possessed eubacterial lipids, and then the common ancestor of all archaebacteria, a single cell, reinvented its lipid and its cell wall biochemistry, so this was inherited by all of those common descendents. Now if you have problems with our model, we have problems with that.

T. Cavalier-Smith. I do not see any problem with that. But listen, you said 'in the absence of peptidoglycan in the

archaebacteria', but it is known that it has been lost on other occasions, for instance in the origin of mitochondria, the origin of chloroplasts, and in the origin of mycoplasmas, but nothing in what you said answered the actual objections I raised, which were about the large number of things in common. Not a single thing answered that. And, secondly, nothing you said gave any evidence whatsoever for an equal age for archaebacteria and eubacteria. A time of 2.7 billion years is much less than 3.5 billion years and even if that 2.7 date were correct, my point would stand, that there is no evidence of an equal age.

W. Martin. OK, to counter that...

J. F. Allen. Dr Nisbet was going to make a comment.

E. Nisbet (*Department of Geology, Royal Holloway, University of London, Egham, Surrey, UK*). I was simply going to say that the evidence for methanogenesis at 2.7 Ga is very strong indeed. When you go older than 2.7 Ga you obviously get into a sea of fog, but there is moderately good evidence from Barberton in South Africa at 3.5 Ga that there is quite a diverse community. I think that is as far as I will go. There is evidence for eukaryotes at 2.7 Ga from relict biochemistry: work by Brocks, Logan, Buick and Summons. Certainly the –40 per mil fractionation, or lighter, seen in 2.7 Ga rocks, is strong evidence for methanogenesis—I think you just cannot argue around that.

T. Cavalier-Smith. Well, I can.

E. Nisbet. How? I agree with you that at 3.5 and 3.7 billion years ago the arguments are quite complex. For example, I would imagine Minik Rosing might defend oxygenic photosynthesis at 3.7 Ga in Greenland (Rosing 1999): he has a good case for plankton. There are arguments against as well. But at 2.7 Ga I think the isotopic diversity recorded in carbon and sulphur minerals and kerogen is comparable with the same sort of environment today. The spreads are not quite as wide as today, but they are wide. That we can say from the geological record in the Late Archaean: that life is very diverse.

W. F. Doolittle (*Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada*). I think Tom [Cavalier-Smith] made two points that have gotten conflated, and I do not want to get into the issue of how old the Achaea are, but the first point he made was that Bill's [W. Martin's] hypothesis, drawn on those beautiful slides, sort of implies that life got to the point of having much of the basic biochemistry—most of the first few chapters of any basic biochemistry textbook—in place before they got around to having envelopes around themselves. I guess I find that a bit implausible, too.

M. J. Russell. I did not say that.

W. F. Doolittle. Well, I think that that is what you are implying, because bacteria and Archaea have many shared features. No, Bill implies that. Bill's scheme implies that we had an organism surrounded by iron sulphide, a mineral-bound organism, with ribosomes and almost everything that Archaea and bacteria now share, before it invented an envelope, and I find that difficult to believe myself.

W. Martin. To address that directly, before we get into this, Ford [W. F. Doolittle], would you please tell me where the precursors came from to derive any other sort of organism? You are doing the same thing the RNA world does—you just take your precursors for life and pull them out from behind your back. Ford, I do not think that is the same question at all.

At this point, the General Discussion gave way to a live broadcast, of about 15 minutes, of the BBC Radio 4 programme 'The material world'. This broadcast was essentially a radio report of the Discussion Meeting. The programme contained a conversation between its presenter, Quentin Cooper, J. F. Allen, and W. F. Doolittle, and had been recorded on the previous evening. The BBC's production title for the feature was 'Mighty mitochondria'.

J. F. Allen. Just before we got distracted, Mike [Russell] in fact had his hand politely raised for some 15 or 20 minutes.

M. J. Russell. Yes, I think that there is some unfinished business, and I think that geologists really have to talk to biochemists, biologists and microbiologists and vice versa. What we have got to realize is that you are backtracking, and fair enough, you have got to go back in time, but we have got to consider a bottom–up approach, in other words, we take initial conditions seriously. So we have got to see why life would have started and when it did and also to remind people that geological time is not something you can just use willy-nilly. In fact, in order to get a living system going would probably take days or weeks. If you just think of the incubator where life started it would self-organise very quickly, otherwise it would not get off the ground, so to speak. So it is much shorter; the span of life originating is short, and the molecules that we use are much shorter than people have assumed. You are not using them as energy, you are using basic molecules like carbon monoxide, methane, formaldehyde, and so

forth, but the scale of the system from which life generates is solar, of course, because we have to have light coming to split charge. That does not mean to say it is photosynthesis, just photolysis to give you redox. So that has all happened, and then, even when you get down to the Earth and the system, then you have got to measure it in thousands of cubic kilometres. Because of the importance of convection, metabolisms to this day are coupled to convective processes. Those are the two major, far-from-equilibrium, dissipative structures on a planet—convection and life—and they must be coupled. These are coupled today, and they must have been coupled from the beginning. So that is why we had this idea of starting right from scratch from an alkaline spring, and a lot happens early on, including differentiation.

W. Martin. One last point, and then I promise not to say anything else, unless asked. The point is, both Tom and Ford have made a statement that is to a certain degree correct, and that is that the last common ancestor of all cells must have had, say, a thousand genes. But when you say a thousand genes, what you actually mean is a thousand biochemical functions. Now we know very well—I am not going to go into class one and class two enzymes—there is a broad diversity of enzymes, particularly across the archaebacteria and the eubacteria, that catalyse exactly the same reaction but they do it completely differently. Yes, they are completely different enzymes; they may have a different mechanism, but the product is the same. So what is generally conserved? I know for a fact that what is conserved in the evolution of biochemical pathways is the set of reactions, the set of intermediates, the chemical conversions, for example the Calvin cycle—but the individual enzymes that catalyse those reactions are completely different. A specific prediction of our model is, for example, that such fundamental processes as amino acid biosynthesis or purine biosynthesis should be fundamentally different in the archaebacteria and the eubacteria.

Now, people are working on purine biosynthesis in archaebacteria, and one of the steps that is catalysed by tetrahydrofolate in eubacteria and eukaryotes is catalysed by a formate-dependent reaction in archaebacteria by a fundamentally different chemistry and a fundamentally different enzyme. That is highly compatible with the type of organism that we are addressing. My warning here is not to make the assumption; you are just assuming—and this is a criticism, a direct criticism—that because it has the same functions, it also has the same genes and the same enzymes, and I think if you look you will find fundamental differences at certain steps, and that would provide evidence in favour of our case. So that is it.

J. F. Allen. Thank you for that. As a neutral, I do not have any investment in this at all, but Mike Russell is absolutely right. I mean, biologists are trying to think further and further backwards, and Earth scientists are trying to think how things could have developed, and sometime or other you have to meet and have a consistent explanation as to how cells originated and why there would be differences between Archaea and bacteria. It might be here or it might be there, but actually saying 'look, it's here' is a start; you can begin to negotiate and discuss and test hypotheses. So, go for it. And Tom and Ford will say why it is wrong if it demonstrably is, and that is what we are here for, is it not?

Session 3 was called 'Chloroplasts' and was chaired by myself, and had Chris Howe, Reinhold Herrmann, John Gray and Iain Wilson. Has anyone anything to say arising from this session? Well, that wraps it up for chloroplasts. Session 4 was called 'Mitochondria' and was chaired by Angela Douglas. The speakers were Axel Brennicke, Martin Embley and Louis Tielens. I withdraw my remarks about hydrogenosomes, unreservedly. But one question is, do hydrogenosomes qualify? Martin was quite keen, or someone was quite keen, that we should not just call the session 'Mitochondria', but call it 'Mitochondria and hydrogenosomes', but we thought leaving it at just 'Mitochondria' was a bit more provocative. Are hydrogenosomes mitochondria? I mean, is it useful to think of them in this way?

T. M. Embley (*Department of Zoology, The Natural History Museum, London, UK*). You define them by a phenotype. Hydrogenosomes produce hydrogen and mitochondria carry out oxidative phosphorylation, unless they are anaerobic, such as Louis talked about. So, I do not see what the problem is. I think that as sophisticated human beings we can appreciate that there are many similarities, but also differences.

J. F. Allen. While you are there, you put up the biochemistry of hydrogenosomes and I thought 'great, now I shall understand', but you sort of zipped on. What they do is produce hydrogen, but what for? They are reducing protons with electrons to make hydrogen, but where do they get the electrons?

T. M. Embley. From pyruvate through ferredoxin. As Ford said, we seldom know why, but I think many people have suggested that it helps to maintain the redox balance.

J. F. Allen. So they are just blowing off hydrogen, like an oilrig that has surplus hydrocarbons to get rid of? It is as simple as that. Just like purple bacteria; they do something like that, I believe.

T. M. Embley. It could be wrong but it seems plausible.

F. R. Whatley (*Department of Plant Sciences, University of Oxford, Oxford, UK*). Then they make ATP. The point about going back again and throwing away hydrogen is how to get back to the pyridine nucleotide so you can oxidize it. It is the same balance that everybody else has been talking about. It is a fermentation reaction, really. There is an oxidative fermentation, and a reductive fermentation.

J. Tovar (*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK*). I would just like to make a comment on an aspect of the genome of mitochondria, relating to this redox poise and its involvement in the retention of an organellar genome. We have some evidence, working with *Entamoeba histolytica*. We have identified a mitosome, which is related to the mitochondrion. We have looked very hard for a genome in this organelle and we are unable to find one. Now the interesting point here, as many of you are aware, is that *Entamoeba* lacks any oxidative phosphorylation and any haem biosynthesis, so there is no redox potential or redox balance to be maintained. And it is this organelle that we find has lost completely its genome. So I think we have here an interesting example of a mitochondrially derived organelle that has lost its genome, and I think it ties in very neatly with the hypothesis of the redox poise. I do not know if other people have any comments on it.

J. F. Allen. My comment is, wonderful, thank you; it cheers me up no end.

J. A. Raven. Finally, we thank all of those who acceded to our request to come and talk, and chair sessions. The quality of the presentations was very high, and they were extremely interesting. The discussion was particularly stimulating: this alone distinguishes the Meeting and these proceedings from simply a collection of a lot of chapters for a thematic volume.

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