Symbiosis becoming permanent: Survival of the luckiest

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Some might think we already know enough about endosymbiosis and organelle origins, and that speakers at the colloquium *Symbioses becoming permanent: The origins and evolutionary trajectories of organelles* would have little new to say. Indeed, since the 1980s, the idea that the mitochondria and plastids (chloroplasts) of eukaryotic cells arose through endosymbiosis has been about as universally accepted as such things ever are (1). However, enormous gaps remain in our knowledge about just how this happened. These gaps may, in part, be bridged by comparing organelles to other diverse endosymbiotic systems. Decades of rapid progress in both endosymbiont and organelle biology has revealed much about their respective natures, but has also allowed the fields to drift apart. It therefore seemed to us that a conceptual reunification was in order. Here we review some of the similarities and differences between endosymbiotic systems that have been uncovered in recent years, and reflect on how these advances change our view of the evolution of endosymbiotic partnerships.

We start, because we must, with the semantic problem that has hampered the field for decades: What is the definition of an organelle? In our view, the lack of a uniform and nonarbitrary definition reflects an oversimplification of the process, exemplified by a seemingly pedantic debate over words (2–5). The distinguishing characteristic most commonly used is genomic integration, or the movement of genes from the endosymbiont to the host genome, and the targeting of gene-products back to the endosymbiotic compartment (6). This targeting system is appealingly complex, and therefore rare. It is also relatively unambiguous to observe where it exists, providing a workable distinction, until recently. Now we know of endosymbiotic systems without gene transfer or targeting that are so highly integrated in other ways (e.g., into the cell cycle or metabolism) that they are also regarded as not just living in the cell, but part of it (7). Conversely, evidence is now mounting on several fronts for gene transfer and targeting in systems not traditionally regarded as organelles (8, 9). Because none of the prevailing definitions are adequate, and because it seems difficult to provide one today, we suggest that effort should be focused instead on considering more specifically what the similarities and differences between diverse endosymbiotic systems might tell us about the process of integration.

The shift from a transient inhabitant of another cell to a permanent and essential organelle was likely a protracted series of events, moving through levels of integration with no particular endpoint. Somewhere along this spectrum there may have been a turning point, or a no-going-back moment, perhaps marked by a sudden change in the tempo or mode of evolution. Perhaps this key change happened so long ago that any unambiguous evidence has been too obscured by time. Or, perhaps there was no such moment. To answer these questions, we need to bring together information from many sources and from many levels. We need detailed comparisons between "endosymbiotic" and "organelle" systems, and to consider these systems at different scales and from different angles: cell biology and the processes of communication, transport, gene transfer, and targeting; metabolism and energetics linking host and symbiont; genome evolution at the macro scale, leading to major changes in form and content; and genome evolution at the micro scale, to detect changes in mutational forces and population structure that may underpin other more easily observable changes.

There are two obvious directions from which to approach this problem. One can study organelles and their hosts to understand the outcomes of long-term integration. Or one can look at more recent endosymbiotic associations that we don't generally consider to be organelles to seek general principles, or counterexamples, to organelles. In the last century, comparing the two was very much in the foreground of research on symbiosis evolution, but these once-united fields have largely moved apart and do not often interact. Similarities that might reveal general principles may be sitting right under our noses, but going unnoticed because we lack connections between solitary fields focusing on "endosymbionts" or "organelles."

One purpose of this colloquium, therefore, was a family reunion of sorts: bringing ourselves back up to date on context and searching for characteristics that unite or distinguish what we vaguely call organelles or endosymbionts. Another was to examine both kinds of systems at several levels, from nucleotides to cells, and at the same time examine how we think about these systems and whether this impacts what we think about them. Still another purpose was to bring together philosophers and philosophically inclined biologists who have long been contemplating symbiosis as a challenge to Darwinian theory. For them, endosymbiosis holds important lessons for thinking about evolution as an inevitable ratcheting of complexity, what it is to be an "organism" or a "Darwinian individual" (not necessarily the same thing), and whether by focusing so much on explanations at the levels of genes and genomes we have not put the explanatory cart before the horse. Conversations begun between professional philosophers and empirically minded biologists will, we predict, continue and deepen.

The colloquium was a collaboration between the US National Academy of Sciences and the Canadian Institute for Advanced Research, and spanned a wide range of overlapping themes in symbiosis: major

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evolutionary transitions and the nature of individuality (10–13); the mosaic phylogenetic nature of organelle proteomes (14–17); the risks and costs associated with long-term endosymbiosis (18, 19); the structure and dynamics of symbiont and organelle genomes (20–22); the metabolic complexities that develop during the establishment and integration of endosymbionts (23–31); and how endosymbiosis has been understood in the history of biology and how it might be thought of in the future (32, 33).

Many meetings convene with goals beyond the sum of the talks and fail, but in this colloquium characteristics unifying organelle and endosymbiont were repeatedly brought to light. For example, details about how fundamental processes, such as mutation rate and shifts in selective regimes can drive dramatic changes in genome structure, emerged frequently. The similarity in outcomes of these processes in endosymbionts and organelles suggest that there may be a key shift in the mode of evolution that occurs well before we previously imagined the endosymbiont-to-organelle transition. Another theme was the inherent risks and negative outcomes that can come with a stable endosymbiotic relationship. We tend to think of endosymbiotic events as major adaptive transitions, but the evolutionary destinies of host and symbiont do not always stay perfectly aligned. This is manifest in the extravagant and sometimes ridiculous genomic complexity seen in some symbionts and organelles: when deleterious endosymbiont genotypes cannot be purged by the host they can be fixed in the population, even in highly integrated mutualistic endosymbioses. Following this logic, it seems that the "specialness" of organelles comes not from any complex mechanism, or singularity of events, it is just that they are old and have managed not to go extinct. They are the lucky ones.

The model of endosymbiosis that one might find in a text book description of organelle origins as an organism "choking on its food" is clearly overly simple. Prevailing models focus instead on more drawn-out processes, often involving multiple, prolonged endosymbiotic associations leading up to a final seemingly permanent association. The more extreme cases of genomic change now being discovered in diverse symbiotic systems reflect the potential perils of sticking to a single symbiont, and suggest that long-term host–endosymbiont codependency may provide fertile ground for nonadaptive complexity to arise (34–36). The oddities in form and content that we associate with reduced endosymbiont and organelle genomes eventually become so extreme that they are untenable. But if the endosymbiont dies, so too will the host. One solution, for the host at least, is the constant renewal of genetically degenerating endosymbionts with new ones. This has profound implications for how we view genome evolution in these symbiotic systems, and also converges with organellogenesis as a series of endosymbiotic partnerships, and not just a single, sudden event.

Why genes are retained in organelle genomes has been examined in many ways, including at this colloquium, but we must also ask why genes move to the nucleus. Adaptationist ideas about control or protection from mutagenic metabolic processes in the organelles are often proposed, and doubtless hold for many genes, but it is also likely that random chance plays a role in a process as widespread as this. The development of a specific targeting system and the movement of genes that initiated the cascade of genetic integration may have been linked to the population dynamics and related mutational forces now being observed in endosymbiotic systems in an intriguing way. If highly functionally integrated endosymbionts tend to die off in mutational oblivion, then replacing the endosymbionts maybe good for the host but offers little to the endosymbionts. If, alternatively, the endosymbiont stumbles on a means to shelter its genes from the increasingly strange mutational circus we know organelle (and now some endosymbiont) genomes to be, then this might offer a way for both to survive.

The mitochondria and plastids we see today may, accordingly, have only been the luckiest of a longstanding series of doomed endosymbionts who were saved by transfer of genes to the nucleus. Gene transfer might also have begun long before the terminal symbiont was even taken up and protein targeting may have originated not to return their gene products to their source, but to target host or even previously acquired foreign genes to an endosymbiont (9, 37, 38). Initially, therefore, genomic integration might not have served any purpose to do with organelle function, but because it did take place fortuitously, the symbiont did not die off in a blaze of genetic weirdness resulting from its host being too good at looking after its many needs. Instead, it survived as part of the host cell. It is possible that the mode of evolution in the endosymbiont genome changed once more, limiting potentially terminal change. However, if one looks at the range of diversity of organelle genomes, it is also possible that gene transfer simply rendered the extremes of genome reduction nonlethal because of the availability of previously transferred genes, and by allowing for the endosymbiont to retain an exceedingly low number of genes. In the most extreme cases of all, we now know that number is zero.

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