

canonical Wnt pathway was revealed using an additional transgenic zebrafish line, one expressing a GFP-tagged, dominant negative form of the downstream transcription factor T-cell factor (TCF) from a heat shock promoter to inhibit the Wnt/ β -catenin pathway. Furthermore, the time-frame of liver specification determined by inhibiting β -catenin coincides with the temporal bilateral expression profile of *wnt2bb* in the lateral plate mesoderm, and is independent of any endodermal signals. Grafting of wild-type labelled cells into the lateral plate mesoderm rescued the prt phenotype and showed that the prt gene is essential to mediate mesodermal–endodermal crosstalk during liver specification. Combined, these data provide strong evidence for a precise and essential role for Wnt signalling in specification of the zebrafish liver.

Although a role for Wnt signalling in determining cell fate and differentiation of many tissue types during development has been well documented [11], there is little evidence in the literature for a role in liver specification. In fact, expression of the *secreted frizzled-related protein 5 gene*, which encodes a Wnt inhibitor, in the foregut endoderm in mouse [15] suggests that inhibition of the Wnt pathway may be required during hepatic specification. Therefore, the finding that Wnt2bb can mediate liver specification in zebrafish in such a spatiotemporal manner is intriguing. Furthermore, the expression pattern of Wnt2bb in zebrafish closely resembles that reported for the mouse orthologue [16], Wnt13, perhaps advocating a yet unknown role for Wnt signalling in mammalian species.

It is possible that precisely executed waves of inhibition and activation of Wnt signalling may mediate the various stages of endoderm patterning, such as liver development, or that convergence of other pathways known to operate during this time, such as BMP signalling, may act to enhance the Wnt pathway, as BMP2 enhances Wnt2b expression in keratinocytes [17]. Interestingly, FGF8 and BMP4 are essential for

hepatic induction and differentiation and both are known targets of Wnt signalling [18,19]. Whether these factors act downstream of, or in a feedback loop with, an earlier temporal specifying Wnt signal in the mouse liver remain to be elucidated.

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Endosymbiosis: Double-Take on Plastid Origins

Plastids — the light-harvesting machines of plant and algal cells — evolved from cyanobacteria inside a eukaryotic host more than a billion years ago. New data reveal that a mysterious unicellular alga acquired its photosynthetic apparatus much more recently than other eukaryotes, affording a second look at the primary endosymbiotic origin of plastids.

John M. Archibald

“I call this experiment ‘replaying life’s tape.’ You press the rewind button and, making sure you thoroughly erase everything that actually happened, let the tape

run again and see if the repetition looks at all like the original.”

Stephen J. Gould (*Wonderful Life*)

In his famous treatise on the Cambrian fossils of British Columbia’s Burgess Shale,

Stephen J. Gould [1] considered the role of chance in evolution and posed the ‘thought experiment’ quoted above. Would life ever evolve the same way twice? The largely historical nature of evolutionary biology makes this an extremely difficult question, yet on rare occasions evolution provides us with the means to glimpse an answer. Such is the case with the origin of eukaryotic photosynthesis. In this issue, Yoon *et al.* [2] report evidence that the unicellular alga *Paulinella chromatophora* acquired its light-harvesting abilities through the uptake of a *Synechococcus*-type cyanobacterium completely independent of — and much more recently than — the endosymbiosis that gave rise to the plastids of all other eukaryotes. Detailed genomic studies of *P. chromatophora* promise to elucidate the molecular processes underlying the transition from free-living bacterium to fully integrated eukaryotic organelle.

Oxidative photosynthesis first evolved in cyanobacteria [3] and its subsequent spread to eukaryotes via ‘primary’ endosymbiosis ranks as one of the most important events in the history of life. Three eukaryotic lineages — green algae (and their multi-cellular cousins, land plants), red algae, and glaucophytes — harbor plastids whose ancestry can be traced directly back to the cyanobacterial endosymbiont [4,5]. While green and red algal plastids are known to have diffused across the eukaryotic tree by ‘secondary’ (eukaryote–eukaryote) endosymbiosis [6], it is widely believed that primary plastids evolved from cyanobacteria only once in life’s history (for example [7]). Or did they?

Mounting evidence suggests that the photosynthetic organelles of the enigmatic fresh-water amoeba *P. chromatophora* (Figure 1) represent a second primary endosymbiosis in its early stages. First isolated by the German biologist Robert Lauterborn in 1894 [8,9], this organism has a long but sporadic

history in the scientific literature, having been discovered at a time when the evolutionary connections between cyanobacteria and plastids were far from clear. Remarkably, in his initial description of *P. chromatophora*, Lauterborn [8] is said to have touched “on the possible endosymbiotic origin of the chromatophores (plastids) without explicitly advancing this hypothesis (as did Mereschkovsky 10 years later)” [9]. The rarity of *P. chromatophora* in nature and its resistance to stable culturing has meant that progress towards understanding the significance of its endosymbiont has been slow.

Why is *P. chromatophora* so interesting? Each cell has one or two cytoplasmic bodies, historically known as cyanelles, which resemble free-living cyanobacteria much more so than they resemble canonical plastids. The bodies themselves cannot be cultured in isolation, do not appear to reside within a food vacuole, and divide synchronously with their host [10–13], suggesting at least a certain level of host–endosymbiont integration. Most intriguingly, a close relative of *P. chromatophora*, *P. ovalis*, is not photosynthetic but actively feeds on cyanobacteria that are similar to members of the genus *Synechococcus* [11].

Molecular data brought to bear on the question of the origin of *P. chromatophora* have confirmed its unusual evolutionary history. Like the plastids of glaucophytes, the *P. chromatophora* endosymbiont possesses a cyanobacterial-like peptidoglycan wall, and it initially seemed possible that the endosymbionts of these two groups shared a common origin. However, phylogenetic analyses of the nucleus-encoded 18S ribosomal DNA (rDNA) gene revealed that the host component of *P. chromatophora* is not related to glaucophytes, but is instead allied with testate amoebae, chlorarachniophytes, and other members of the super-assembly of eukaryotes collectively known as Cercozoa [14,15]. More recently, Marin *et al.* [16] sequenced the complete



Figure 1. Light micrograph of *Paulinella chromatophora*.

Image by D. Patterson, provided with permission by <http://microscope.mbl.edu>.

rDNA operon of the *P. chromatophora* endosymbiont and convincingly showed, as has long been suspected, a robust phylogenetic connection between it and modern-day cyanobacteria, more specifically with members of the *Synechococcus/Prochlorococcus* clade [16].

While it is now clear that *P. chromatophora* acquired its photosynthetic apparatus independent of the endosymbiotic origin of all other plastids [16,17], an important question remains: to what extent can the *P. chromatophora* endosymbiont be considered a bona fide organelle? More specifically, what is the extent of the genetic integration between the host and endosymbiont components of *P. chromatophora*? One of the hallmarks of canonical plastid genomes is their diminutive size relative to those of free-living cyanobacteria. Sequenced cyanobacterial genomes range from ~1.7 to >7 megabase-pairs (Mbp) in size and possess thousands of genes, whereas plastid genomes rarely have more than 200 genes (<http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi>). During the early stages of the association between endosymbiont and host, many of the cyanobacterial genes no longer essential for intracellular life were presumably lost, and many more were transferred to the

host's nuclear genome where they acquired the primary sequence information necessary to target their protein products back to the endosymbiont.

The nuts-and-bolts of the targeting process are reasonably well understood in a wide range of plants and algae [18,19]. Concomitant with the evolution of the protein import system itself, the initial surrender of essential genes to the host nucleus is thought to represent the 'click of the ratchet' beyond which the autonomy of the endosymbiont is lost. Unfortunately, the evolutionary gulf between modern-day plastids and cyanobacteria is so vast that most of the evolutionary information about the early stages of primary endosymbiosis has been erased. Where does the *P. chromatophora* endosymbiont lie on the continuum between food particle and plastid, and what can it tell us about this evolutionary transition?

To address this issue, Yoon *et al.* [2] isolated and sequenced two fragments of the *P. chromatophora* endosymbiont genome (9.4 and 4.3 kilobase pairs in size) and compared them to homologous regions of available cyanobacterial genomes. Their results indicate that the endosymbiont is essentially cyanobacterial in nature: the highest degree of gene order conservation is shared with *Synechococcus* sp. WH5701, and several of the genes identified in the *P. chromatophora* cyanelle genome, for example *psbO*, are always (or most often) located in the nuclear genomes of photosynthetic eukaryotes — they are the result of plastid-to-nucleus gene transfers. The gene order data are consistent with rDNA [2,16] and protein phylogenies [2] in showing a specific association between the *P. chromatophora* endosymbiont and members of the *Synechococcus/Prochlorococcus* clade. While the actual size of the genome cannot be inferred from the data in hand, Yoon *et al.* speculate that it could well be similar in size to that of free-living *Synechococcus/Prochlorococcus*-type

cyanobacteria, to which it appears most closely allied.

The stage is now set to explore the molecular and cell biology of *P. chromatophora* in much more detail. Although its photosynthetic organelle is demonstrably cyanobacterial, the degree of biochemical and cellular integration between the *P. chromatophora* endosymbiont and host [10–13] leads Yoon *et al.* [2] to speculate that at least some endosymbiont-to-host-nucleus gene transfers have occurred, such as those involved in organelle division (for example *ftsZ*) and metabolite transport. In this sense, the sequence of the *P. chromatophora* nuclear genome should be as informative as that of its endosymbiont.

Exploration of nucleus-encoded proteins involved in organelle protein import will be particularly interesting. In plant and algal plastids, the import apparatus comprises more than a dozen proteins [19], some of which are cyanobacterial and others that appear to be eukaryote-specific proteins 'invented' in the common ancestor of red and green algae (data are currently unavailable for glaucophytes) [20]. When it comes to predicting the composition and complexity of the protein import apparatus in *P. chromatophora*, all bets are off. The immediate goal will be to glean as much information about the early stages of primary endosymbiosis as possible by comparing and contrasting *P. chromatophora* genomic sequences with those of all available algae and cyanobacteria. Such analyses will provide a rare opportunity to assess the relative contributions of chance and necessity in the evolution of life.

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