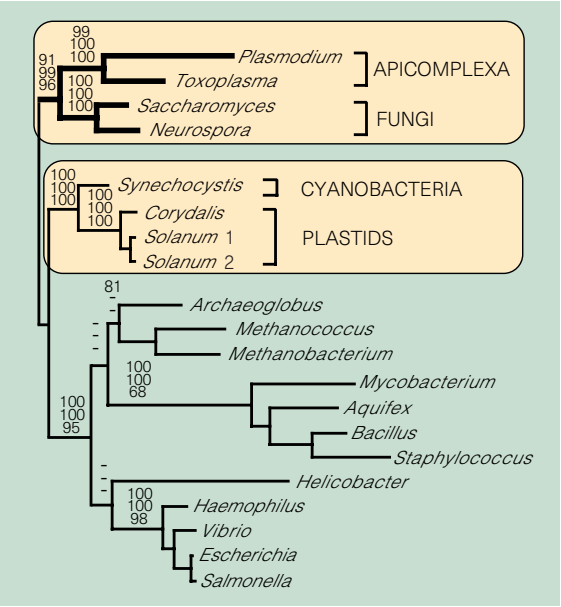

Figure 1 Phylogeny of chorismate synthase. The figure shows a Fitch–Margoliash tree, based on maximum-likelihood distances corrected by the Jones, Taylor and Thornton substitution matrix. Other methods (neighbour-joining, maximum parsimony, protein maximum likelihood, and quartet puzzling) all also support the grouping of fungi and Apicomplexa, and of cyanobacteria and plastids. Numbers at the nodes correspond to support for the major groups from (top to bottom): Fitch–Margoliash bootstrap, protein maximum-likelihood resampling estimated log-likelihood bootstrap, and per cent occurrence in quartet-puzzling steps. Dashes indicate support of less than 50 per cent.

with cyanobacteria, as expected. The grouping of the fungal/apicomplexan lineage with the cyanobacterial/plastid lineage, although curious, should not be mistaken for plastid origin because this tree is unrooted, the cytosolic genes do not branch specifically with plastids, and fungi lack plastids and indeed probably never had them.

The evolutionary origin of cytosolic shikimate-pathway enzymes in eukaryotes is unclear, as the distribution of the cytosolic pathway in eukaryotes is poorly understood and the sampling of sequences is limited to fungi and Apicomplexa. Nevertheless, in view of the phylogeny of chorismate synthase and the absence of leader peptides, there is no reason to believe that the shikimate pathway of Apicomplexa is either derived from or functions in the plastid.

Even if the shikimate pathway does take place in the cytosol in Apicomplexa, this does not detract from the importance of the sensitivity of these parasites to glyphosate. Vertebrate hosts of Apicomplexa lack this pathway, so it remains a specific chemo-therapeutic target. However, before glyphosate sensitivity can be exploited, we need to define the role, if any, of the plastid in the apicomplexan shikimate pathway.

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Roberts et al. *reply* — Although our study[1] was stimulated by the discovery of the apicomplexan plastid, we did not claim to have provided proof that the shikimate pathway occurred in the plastid in apicomplexans. We believe that the site of this pathway remains an open question, despite the similar data emerging from our own[2] and Keeling *et al.*'s phylogenetic analyses of our chorismate synthase gene sequences.

The premise for the phylogenetic analyses was that if the shikimate pathway occurs in the plastid, then these gene sequences are likely to show evolutionary relatedness to those in green algae, like the genes present on apicomplexan plastid DNA. However, phylogenetic analysis of chorismate synthases in the apicomplexans does not support this hypothesis; rather, it points to a closer similarity with fungal enzymes. From this, Keeling *et al.* conclude that the shikimate pathway is cytosolic (as it is in fungi).

This situation might have been expected if apicomplexans had evolved directly from a common fungal lineage, but this is not generally believed to be the case. At present, the best evidence available aligns apicomplexans more closely with ciliates and plants, with fungi as distant relatives.

Caution is needed in drawing conclusions about the subcellular locations of proteins from phylogenetic analysis. Such studies are fraught with difficulty because of the frequency with which horizontal gene transfer occurs during evolution, especially when the data are from a relatively small number of organisms. We believe that phylogenetic analysis is an inadequate tool for determining the location(s) of the shikimate pathway in apicomplexans: direct experimental evidence of the location of key proteins is required.

The two apicomplexan chorismate synthases do not have an obvious amino-terminal leader sequence like that found on plastid chorismate synthases of higher plants. However, apicomplexan protein sequences differ from all other reported chorismate synthases in that they have a number of large insertions. The function(s) of these insertions remain(s) unknown, but one of them might act as a targeting sequence, as proteins can be targeted to plastids even without an amino-terminal leader sequence[3].

The location of the shikimate pathway in apicomplexan parasites remains an open question. A rigorous, practical approach should reveal that all, some or even none of the shikimate enzymes function within the plastid, or the pathway may turn out to operate both outside and inside the plastid in apicomplexans, as in *Euglena*[4]. Irrespective of where the pathway is located, however, it presents a prime target for antiparasitic agents. A cytosolic location would be more accessible to a target enzyme as fewer membranes would need to be crossed.

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