Identification of the starter units for the biosynthesis of blepharismins of the heterotrich ciliate *Blepharisma japonicum*

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**SUMMARY**

The heterotrich ciliate *Blepharisma japonicum* has red pigment blepharismins (BPs). These pigments have been known to have three functions: light perception, chemical defense against predators, and protection against UV radiation. The chemical structures of BPs were shown to be polycyclic (p-hydroxybenzylidene) and benzodianthrone derivatives, respectively. Recently, we reported that the dibenzoperylene quinone moiety of BPs was biosynthesized via the polyketide pathway. This was discovered during our studies of $^{13}$C NMR assignment for BP-C by analysing 2D spectra of $^{13}$C-enriched samples, which were obtained by feeding experiments using $^{13}$C-labeled sodium acetates. As a result of these studies, we hypothesized that the starter unit of BP-C was derived from L-leucine or isovaleryl-CoA, based on its incorporation of $^{13}$C-labeled sodium acetate. Polyketide synthases utilize a wide assortment of starter units, such as branched-chain fatty acids and amino acids. In many cases, the nature of a starter unit provides important structural and biological features to the molecule. In this study, we identify that the starter units of biosynthesis of BPs are isovaleryl-CoA and butyryl-CoA, using HPLC and LC/ESI-MS analyses of pigments that were obtained from feeding experiments with L-leucine, sodium butyrate (in excess), and deuterium-labeled L-leucine.