THE CLONING AND ORGANISATION OF GENES FOR BACTERIOCHLOROPHYLL AND CAROTENOID BIOSYNTHESIS IN RHODOBACTER SPHAEROIDES

Shirley A. Coomber*, Maliha Chaudri and C. Neil Hunter

Department of Molecular Biology and Biotechnology
University of Sheffield, Sheffield S10 2TN
*Present address: Dupont Experimental Station,
P.O.Box 80402 Wilmington, Delaware 19880-0402, U.S.A.

INTRODUCTION

Photosynthetic bacteria such as Rhodobacter sphaeroides and Rb. capsulatus are capable of chemoheterotrophic growth in the dark, and under conditions where oxygen is not limiting can repress the synthesis of the photosynthetic apparatus almost completely. The removal of oxygen initiates the coordinated synthesis of the pigments, proteins and lipids of the photosynthetic membrane which grows as invaginations of the cytoplasmic membrane (Niederman et al., 1976; Chory et al., 1984). By the time this process is complete, light harvesting (LH) domains of several thousand bacteriochlorophyll (bchl) and carotenoid (crt) molecules have been assembled, consisting of LH2 units which surround and interconnect cores containing LH1 and the photochemical reaction centre (Hunter et al., 1985. Vos et al., 1988). Nevertheless, such cells still contain small amounts of non-pigmented cytoplasmic membrane (Parks and Niederman, 1978) and membrane regions enriched in newly synthesised pigment protein complexes. (Niederman et al., 1979)

The onset of photosynthetic membrane assembly relies upon the coordinated expression of a large number of genes. Starting with the work of Yen and Marrs in 1976, it was increasingly clear that many of these genes are physically linked on a small stretch of the chromosome. In 1981, Marrs isolated an R-prime plasmid which was able to complement an array of lesions in the biosynthesis of photosynthetic pigments and proteins. A genetic and physical map of the 46 kb cluster borne on this R-prime plasmid emerged two years later. (Taylor et al., 1983). Further characterisation by localised mutagenesis with transposon Tn 5.7 identified several new putative genes for bacteriochlorophyll and carotenoid synthesis (Zsebo & Hearst 1984). Interposon mapping and DNA sequencing has defined the crt cluster in detail (Giuliano et al., 1988; Armstrong et al., 1989); the regions around and including puh and put genes for reaction centre and light harvesting complexes have also been sequenced (Youvan et al., 1984).
In *Rb. sphaeroides* there were a few indications that genes for photosynthetic membrane assembly may be linked in a cluster similar to that found in *Rb. capsulatus*. Sistrom and colleagues (1984) suggested that genes for light-harvesting and reaction centre polypeptides and for pigment biosynthesis may be linked on an unspecified plasmid, pWS2. *Rb. sphaeroides* *pgh*, *pgf* and *pg£* genes have been cloned and sequenced (Williams et al., 1983; Williams et al., 1986; Ashby et al., 1987; Kiley and Kaplan, 1987; Donohue et al., 1986; Williams et al., 1986; Kiley et al., 1987).

Furthermore, Pemberton and Harding (1986) isolated two cosmids which between them encompass a 60 kb region of the *Rb. sphaeroides* chromosome, and which bear a 15 kb cluster of carotenoid biosynthesis genes. Subsequently, three clones pSCN5-1, pSCN6-1 and pSCN22-1 were isolated from a *Rb. sphaeroides* gene library cloned in E.coli using the mobilisable vector pSUP202 (Hunter and Coomber, 1988). Transfer of these clones restores wild type phenotype to mutants N5, N6 and N22 respectively, each of which is blocked at a different stage of bacteriochlorophyll synthesis. These clones were characterised by restriction endonuclease mapping using EcoRI, HindIII, PstI and BamHI. pSCN6-1 and pSCN5-1 were found to overlap when their restriction maps were compared.

More recently, several more overlapping clones were isolated from this *Rb. sphaeroides* gene bank. Following conjugative gene transfer from *Escherichia coli* these clones restored a wild type phenotype to several mutants unable to synthesise bacteriochlorophyll (Coomber and Hunter, 1989). The insert DNA was analysed by restriction mapping, and taken together these clones form the basis of the first restriction map, 45 kb in length, of the photosynthetic gene cluster of *Rb. sphaeroides*. This cluster is defined on one side by *pgh* A encoding the reaction centre H polypeptide and on the other by the *pgf* operon encoding reaction centre L and M apoproteins and light harvesting LH1 α and β polypeptides.

The availability of these clones in the vector pSUP202 facilitates the examination of cloned inserts by localised transposon Tn5 mutagenesis (Hunter, 1988). In this paper we report a physical map of this cluster obtained using this technique.

The position of each Tn5 insertion has been mapped and the mutant phenotype identified in each case. Analysis of these insertions provides a physical map of the photosynthesis cluster in *Rb. sphaeroides*.

**MATERIALS AND METHODS**

The isolation and restriction mapping of plasmids pSCN5H-1, pSCN5-1, pSCN6-1, pSCN6-20, pSCN22-1 and pSCN22-15 are described in Hunter and Coomber (1989). They are recombinants of the vector pSUP202 (Simon et al., 1983). The conditions for growth of *Rb. sphaeroides* on plates and in liquid culture are described by Hunter and Turner (1988). Localised Tn5 mutagenesis was carried out as in Hunter (1988) with one modification, described below.

Six clones in vector pSUP202, pSCN5H-1, pSCN5-1, pSCN6-1, pSCN6-20, pSCN22-1 and pSCN22-15 (Coomber and Hunter, 1989) were subjected to Tn5 mutagenesis as described in Hunter (1988). *E. coli* colonies which showed resistance to neomycin (Tn5), chloramphenicol and ampicillin (pSUP202) and which were assumed to bear different Tn5 insertions were chosen for each parental clone. Although Hunter (1988) used a triparental system to transfer pSUP202::Tn5 derivatives from *E. coli* to *Rb. sphaeroides* this was found to be unreliable.