Organization of Ansamycin Biosynthetic Gene Clusters in *Streptomyces hygroscopicus* subsp. *duamyceticus* JCM4427

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The geldanamycin, benzoquinone ansamycin antibiotic produced by *Streptomyces hygroscopicus* JCM4427, is assembled using 3-amino-5-hydroxybenzoic acid (AHBA) as a starter unit (Figure 1). Geldanamycin binds to the N-terminal ATP binding site of heat-shock protein (Hsp) 90, inhibiting the chaperone activity of the protein (1). Thus, geldanamycin and its analogs may enhance the activity of a variety of approved anticancer drugs and agents in clinical development.

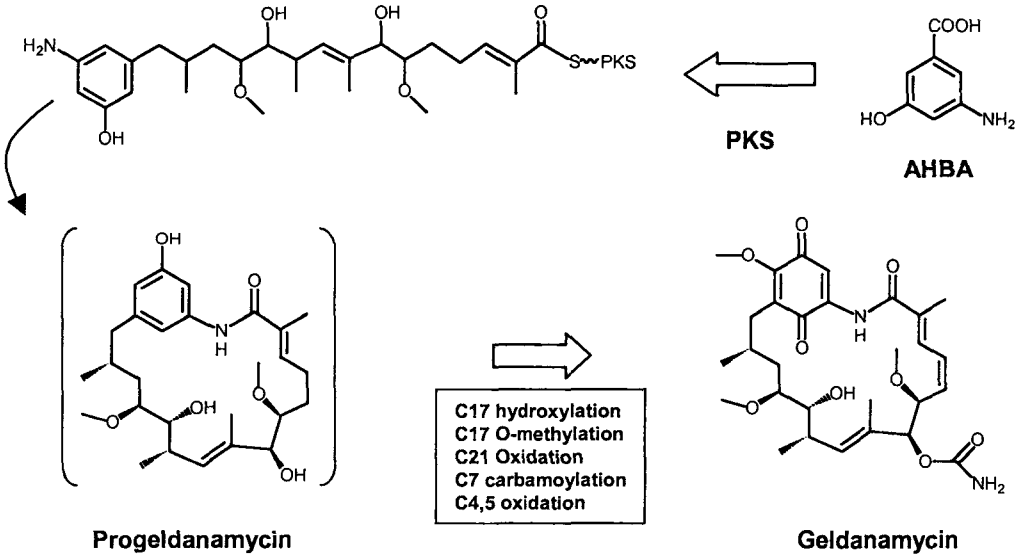

Figure 1. Proposed biosynthetic pathway from AHBA precursor to geldanamycin.

In an attempt to understand the biosynthesis of this compound in more detail, we have cloned two-separated gene clusters required for the biosynthesis of the geldanamycin (2). A set of genes identified, *nap12-18* was unique for the biosynthesis of starter unit in whole genome. Five type I polyketide synthase genes, *napA-E*, followed by the downloading *napF*, encode eight homologous sets of modules together, each catalyzing a specific round of chain initiation, elongation, or termination step. Remarkably, there are polyketide synthase gene cluster that may have a functions in putative naphthoquinone formation. Another set of genes, *gelA-D*, encodes the polyketide formation of the benzoquinone ansamycin, geldanamycin, which are the same genes that already reported from

other streptomycetes (3) (Figure 2).

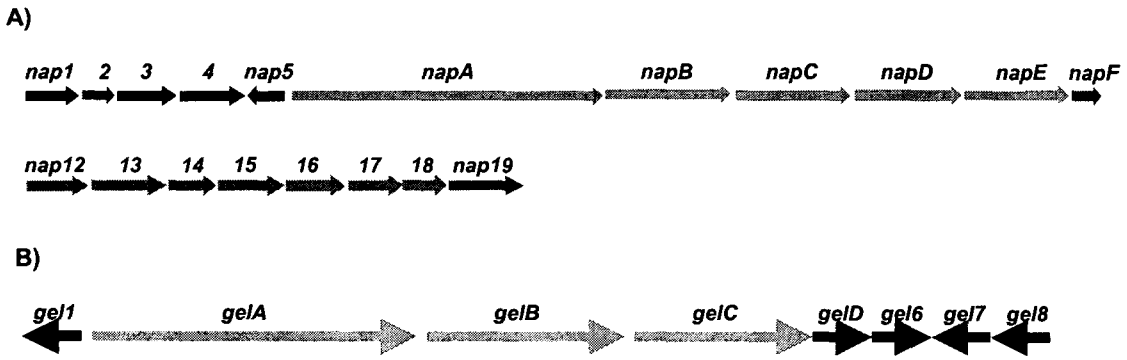

Figure 2. Organization of the AHBA biosynthetic genes and putative naphthoquinone-type PKS gene cluster (A) and geldanamycin PKS gene cluster (B) in *Streptomyces hygroscopicus* JCM4427.

There is considerable precedent that the genes involved in an assembly of the antibiotics from simple carbon precursors, are clustered with the genes providing self-resistance and the regulatory genes that coordinate expression of the biosynthetic genes. In particular, genes involved in pathways that convert primary metabolites to precursors like AHBA, often appear to be located within these gene clusters (4). Recently, the genes involved in the formation of an AHBA are dispersed in separate regions in the ansamitocin producing strain, *Actinosynnema pretiosum* (5). Also, the genes encoding AHBA synthase were identified in two separate antibiotic biosynthetic gene cluster in ansatrienin and naphthomycin producer, *S. collinus* (6). Thus, despite commonality in biosynthesis, the ansatrienin and naphthomycin biosynthetic gene clusters showed clear organizational differences and carried separate sets of genes for AHBA biosynthesis. Those results suggest that the biosynthetic gene cluster of geldanamycin also include a set of genes for AHBA biosynthesis in the neighboring region. But, the observation about cloning of the geldanamycin genes showed that all but one of the remaining genes for AHBA biosynthesis in the geldanamycin pathway are clustered elsewhere in the genome, more than 20kb from the end of the cosmid that contains the polyketide gene cluster (Figure 2).

In the present article, we report the cloning, sequencing, and characterization of the AHBA biosynthetic genes, which provides AHBA for the assembling processes of the geldanamycin polyketide and the neighbor PKS gene cluster. Thus, this neighbor PKS gene cluster encodes putative naphthoquinone ansamycin polyketide, which have no effective function of the geldanamycin production proven by gene disruption experiment. Its genetic organization is significantly different from those of previously reported ansamycin PKS gene cluster.

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