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Functional expression of carotenoid biosysthesis genes from marine bacterium, paracoccus haeundaensis

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The astaxanthin biosynthesis gene cluster in Paracoccus haeundaensis consists of six genes: crtW, crtZ, crtY, crtI, crtB, and crtE contain 726, 486, 1158, 1503, 912, and 879 base pairs, respectively. Individual carotenoid biosynthesis genes of P. haeundaensis have now been expressed in E. coli and each gene product has been purified to homogeneity.

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Genetic analysis of a conserved pseudoknot structure in 530 stem-loop of *Escherichia coli* 16S rRNA

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The 530 stem-loop is a 46 nucleotide stem-loop structure found in all small-subunit ribosomal RNAs. Phylogenetic and mutational studies by others suggest the requirement for Watson-Crick interactions between the nucleotides 505-507 and 524-526 (530 pseudoknot), which are highly conserved. To examine the nature and functional significance of these interactions, a random mutagenesis experiment was conducted in which the nucleotides in the proposed pseudoknot were simultaneously mutated and functional mutants were selected and analyzed. Genetic analysis revealed that the particular nucleotide present at each position except 524 was not exclusively critical to the selection of functional mutants. It also indicated that base-pairing interactions between the positions 505-507 and 524-526 were required for ribosomal function, while much weaker base-pairing interactions than those of the wild-type also allowed high ribosomal function. Our results support the hypothesis that the 530 pseudoknot structure may undergo a "conformational switch" between folded and unfolded states during certain stages of the protein synthesis process by interacting with other ligands present in its environment.

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Genetic characterization of a large mosaic bacteriocin, glycinecin R from *Xanthomonas axonopodis* pv. glycines 8ra

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X. a. pv. glycines 8ra is known to strongly inhibit the growth of closely related xanthomonas species by producing bacteriocin named glycinecin. In this study a clone containing a novel glycinecin of glyR gene product, glycinecin R. A series of deletion and transposon-insertion mutagenesis revealed that the 5 kb genomic region is responsible for the bacteriocin activity. Southern hybridization analysis revealed that the glyR homologs exist in only the xanthomonads up to three copies. Thus, the glyR may be useful as the genus-specific DNA marker for the identification of xanthomonads. The glyR gene is 4,422 bp in length capable of producing the high molecular weight glycinecin R. The glycinecin R protein consists of two domains, core and core extension. The core is made up of a motif, GXXXXYXYDXXGRLT, repeated 31 times. Homologs of the glyR core domain at the amino acid level were also identified in a wide spectrum of unrelated bacterial genomes by BLAST search and the 60-80 amino acids in the C-terminal end of their cores were aligned using ClUSTAL algorithm. Extensive sequence analysis revealed that the core domain of the glycinecin R shows homology to the cores of Rhs element of unknown function in E. coli and toxin complex (tc) gene of Photorhabdus luminesc

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Human lactoferrin enhances angiogenesis via upregulating expression of KDR/Flk-1, a receptor for vascular endothelial growth factor in human endothelial cells

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Lactoferrin (LF) is a multifunctional iron-binding glycoprotein with potent antimicrobial and immunomoduatory activities. Recent studies indicated that LF contributes to angiogenic modulation. In this study, we demonstrated that human LF promotes the VEGF-A-induced migration of HUVECs at an optimal concentration of 5 μg/ml, which is within physiological concentration. Treatment of HUVECs with LF time- and dose-dependently upregulated KDR/Flk-1 mRNA, but not Flt-1 mRNA. Western blot and confocal microscopic analyses demonstrated that LF stimulated KDR protein levels in HUVECs. LF exposure of HUVECs potentiated the cells to the mitogenic activity of VEGF-A, and significantly increased VEGF-induced ERK MAP kinase phosphorylation. LF stimulated VEGF-mediated angiogenesis in vivo in CAM assay. The optimal LF concentration for maximal KDR expression is 5 μg/ml, which is correlated with LF-induced increase in cell migration and proliferation. These findings demonstrate that LF stimulates expression of KDR in endothelial cells, and suggest roles of LF in angiogenesis.