BIOCHEMICAL LETTERS

Myxococcus xanthus C-factor, a morphogenetic paracrine signal, is similar to *Escherichia coli* 3-oxoacyl-[acyl-carrier-protein] reductase and human 17 β -hydroxysteroid dehydrogenase

Starvation induces cellular differentiation in the soil bacterium *Myxococcus xanthus* by initiating a series of temporally regulated events over 24 h that involve cell aggregation, formation of a fruiting body and sporulation [1,2]. Progression through this process is mediated by at least four different cell-cell signals synthesized by *M. xanthus*. One signal, C-factor, a peptide of 166 residues [3,4], acts during the interval between 12 and 18 h after

starvation-induced cell differentiation to mediate expression of genes necessary for completion of cell aggregation and initiation of sporulation. Here, we report a sequence similarity between C-factor and the catalytic domain of *Escherichia coli* 3-oxoacyl-[acyl-carrier-protein] reductase [5] and its homologues [6–8], such as human 17 β -hydroxysteroid dehydrogenase, enzymes that catalyse the interconversion of secondary alcohols and ketones [9]. The similarity indicates that C-factor and these proteins are probably descended from a common ancestor. A notable difference is that C-factor lacks the domain that binds the nucleotide cofactor, suggesting that the secondary-alcohol dehydrogenase family [9] contains a branch with non-enzyme proteins, one of which is a signal for bacterial cell-cell communication.

Figure 1 shows a multiple alignment of *M. xanthus* C-factor

Figure 1 Multiple sequence alignment of *M. xanthus* C-factor with *E. coli* 3-oxoacyl-[acyl-carrier-protein] reductase (3KetoReductase), human 17 β -hydroxysteroid dehydrogenase (17 β -OH-Steroid DH), *B. japonicum* FixR and mouse adipocyte p27 protein

The progressive alignment program of Feng and Doolittle [10] was used to construct the multiple alignment. Positions where C-factor has either identities, denoted by dark-shaded boxes, or conserved residues, denoted by light-shaded boxes, with at least three of the four other proteins are boxed. The α -helices and β -strands for the tertiary structure of 3 α ,20 β -hydroxysteroid dehydrogenase [11] as modified from the structure of dihydropteridine reductase [12] and aligned to 17 β -hydroxysteroid dehydrogenase [6] are shown to suggest the possible location of α -helices and β -strands for C-factor. The ALIGN scores of C-factor with FixR and adipocyte p27 protein are 8.6 S.D. units. Pairwise alignments of C-factor yield the following identities and conservative replacements, respectively: *E. coli* 3-oxoacyl-[acyl-carrier-protein] reductase 26.5% and 36%; human 17 β -hydroxysteroid dehydrogenase 30% and 21.5%; *B. japonicum* FixR 34% and 16%; mouse adipocyte p27 protein 25% and 33%.

with *E. coli* 3-oxoacyl-[acyl-carrier-protein] reductase, human 17β -hydroxysteroid dehydrogenase, *Bradyrhizobium japonicum* FixR and mouse adipocyte p27 protein. The similarity covers about 70% of the C-factor sequence. Pairwise comparisons between C-factor and the proteins in Figure 1 show 25–34% identical residues. This similarity is reflected in the comparison scores from an ALIGN analysis [13,14] of C-factor with 3-oxoacyl-[acyl-carrier-protein] reductase and 17β -hydroxysteroid dehydrogenase. The ALIGN scores are 9.1 and 8.75 S.D., respectively, higher than that of 1000 comparisons of randomized sequences of these proteins. The probability of getting a score of 9.1 S.D. by chance is 10^{-19} , which is why we propose that these sequences are derived from a common ancestor.

C-factor binds tightly to the membrane of *M. xanthus* and is active at nanomolar concentrations [4]. This affinity is 10^3 times higher than the μ M affinity of substrates for dehydrogenases. C-factor lacks the nucleotide-binding domain of dehydrogenases; the alignment of C-factor sequence is with the catalytically active domain in secondary-alcohol dehydrogenases [15]. Of special interest is the alignment of tyrosine-86 and lysine-92 of C-factor with a tyrosine and a lysine residue that have been shown by mutagenesis studies to be important in catalysis in *Drosophila* alcohol dehydrogenase [15,16], rat 11β -hydroxysteroid dehydrogenase [17] and human 15-hydroxyprostaglandin dehydrogenase [18]. In alcohol dehydrogenase, the equivalent tyrosine is proposed to abstract a proton from the substrate and the spatially close lysine to lower the pK_a of tyrosine from ~ 10 to ~ 7.6 [15]. Alternative pairwise alignments show C-factor's tyrosine-88 aligned with the catalytic tyrosine. The tyrosine's pK_a may be lowered by the positively charged arginine-89 and lysine-92. Either or both tyrosines may be important in C-factor activity, perhaps in binding to the membrane on *M. xanthus*.

The origins of signals, either peptides or small hydrophobic molecules, that mediate cell-cell communication are poorly

understood [19]. C-factor is the first reported paracrine signal in a dehydrogenase family that is notable because it contains enzymes that indirectly regulate hormone-mediated gene transcription [19–21] by catalysing the interconversion of active and inactive forms of steroids. In contrast, C-factor itself is a signal for the completion of cell aggregation and initiation of sporulation in *M. xanthus*.

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