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Hypothesis

A different path: Revealing the function of staphylococcal proteins in biofilm formation

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ABSTRACT

***Staphylococcus aureus* and *Staphylococcus epidermidis* cause dangerous and difficult to treat medical device-related infections through their ability to form biofilms. Extracellular poly-*N*-acetylglucosamine (PNAG) facilitates biofilm formation and is a vaccination target, yet details of its biosynthesis by the *icaADBC* gene products is limited. IcaC is the proposed transporter for PNAG export, however a comparison of the Ica proteins to homologous exo-polysaccharide synthases suggests that the common IcaAD protein components both synthesise and transport the PNAG. The limited distribution of *icaC* to the Staphylococcaceae and its membership of a family of membrane-bound acyltransferases, leads us to suggest that IcaC is responsible for the known *O*-succinylation of PNAG that occurs in staphylococci, identifying a potentially new therapeutic target specific for these bacteria.**

1. Introduction

Staphylococcus aureus and *Staphylococcus epidermidis* infections associated with the insertion of medical devices can arise due to their ability to form biofilms on surfaces [1]. A biofilm is a multicellular, three-dimensional bacterial community anchored to a surface; bacteria within a biofilm are surrounded by an exopolymeric matrix, reducing bacterial exposure to antibiotics and the host immune system [2]. As a result, infections involving biofilms are very difficult to eradicate often leading to prolonged antibiotic therapy and/or surgical removal of the device and the surrounding infected tissue, placing a significant financial burden on health services [3]. The ability to form a biofilm is recognised as a major virulence factor of *S. epidermidis* in particular [4], and dissecting the molecular mechanisms underlying staphylococcal biofilm formation will aid the development of novel therapeutics. The first-discovered and most studied mechanism of intercellular adhesion in staphylococcal biofilms is mediated by the polysaccharide intercellular adhesin (PIA), which is a β-1,6-linked poly-*N*-acetylglucosamine (PNAG) molecule that is partially *N*-deacetylated [5–7]. More recently, both PNAG and *N*-deacetylated (dPNAG) have been studied as potential vaccine targets in therapeutic approaches against a range of human microbial pathogens [8,9].

In both *S. aureus* and *S. epidermidis*, biosynthesis of PNAG, and biofilm formation, requires the IcaADBC proteins, encoded in the *icaADBC* operon (Fig. 1); the sequences of the four proteins being highly conserved (⩾79% similarity) between the two staphylococci [10,11]. We have compared the *ica* encoded PNAG biosynthesis system to analogous systems in Gram-negative bacteria and also to Ica systems in other Gram-positive bacteria. Our analysis of published data suggests that PNAG is likely to be synthesised *and* translocated across the inner membrane by IcaA/D and that the integral membrane protein IcaC, rather than acting as the PNAG transporter (as proposed since its discovery in 1996) [11], is likely to be an enzyme that adds *O*-linked succinyl groups to PNAG residues. Thus we present a revised model for the function of the Ica proteins in PNAG synthesis in the Staphylococci and in Gram positive bacteria more generally.

2. Current models of PNAG synthesis in the Staphylococci

A role for the *ica* gene products in the synthesis of *S. epidermidis* PNAG was discovered in 1996 by Götz and colleagues [11]. Inactivation of genes in a three gene operon, *icaABC*, led to loss of detectable PNAG and cell aggregation. Later a fourth small gene, *icaD*, was identified between *icaA* and *icaB* [12], giving the final gene order of *icaADBC* (Fig. 1). The small gene *icaD* is unusual in that it

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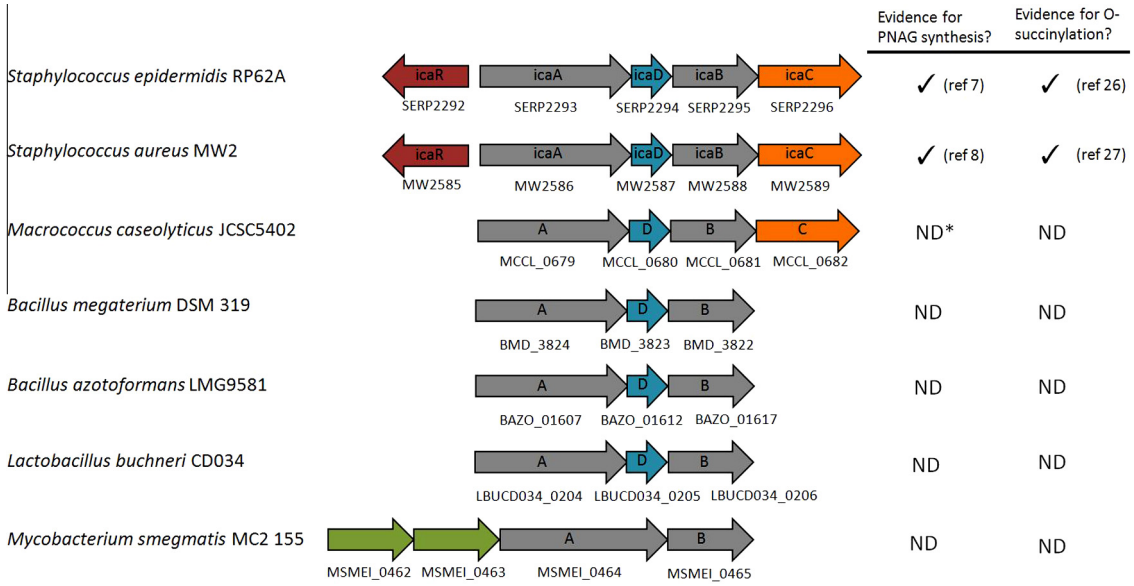

Fig. 1. Arrangement of the *ica* operon in *S. epidermidis*, *S. aureus* and other Gram-positive bacteria. The genes are centred on *icaB* and coloured based on similarity to the *ica* genes in *S. epidermidis*. Systematic gene names are indicated for each species and evidence for the presence of PNAG and *O*-succinylation of PNAG is indicated. No IcaR homologues were found outside staphylococcal species. *ND = Not Determined.

overlaps with both the 3′-end of *icaA* by 37 nucleotides and the 5′-end of *icaB* by four nucleotides [12]. Finally, a divergently transcribed transcriptional regulator *icaR* was identified in this region, which encodes a TetR family protein and is involved in environmental regulation of *icaADBC* operon expression [13]. The presence of the *ica* operon correlates with increased antibiotic resistance and biofilm formation in clinical isolates of *S. epidermidis*, where 45% of nosocomial infection isolates were found to be *ica* positive [14]. Several studies have also demonstrated that synthesis of PNAG is critical for staphylococcal biofilm formation in animal infection models [15,16], highlighting the key role for PNAG in persistence.

Since the discovery of the *ica* genes, a variety of models for Ica function have been proposed (reviewed in [17]) (Fig. 2A). Based on sequence homology, IcaA was identified as a probable *N*-acetylglucosamine transferase, with a likely function in the synthesis of the β-1,6-linked *N*-acetylglucosamine polymer [11]. IcaA is predicted to contain four transmembrane (TM) helices [11] with the glycosyltransferase 2 domain (GT-2, CAZY classification, EC 2.4.1) located between TM1 and TM2 in a cytoplasmic location. IcaD is a much smaller integral membrane protein, comprising two predicted TM helice, that is required for full activity of IcaA [12] but its precise role remains unclear. IcaB has been demonstrated experimentally to be a specific PNAG *N*-deacetylase, and a proportion of the PNAG (around 15–20% in *S. epidermidis*) is known to be *N*-deacetylated [18,19]. IcaC was originally (in 1996) described as an integral membrane protein with six TM helices and was suggested to play a role in PNAG export [11]; later work suggested that it must be coexpressed with IcaA/IcaD and was needed to produce ‘mature’ PNAG that could be recognised

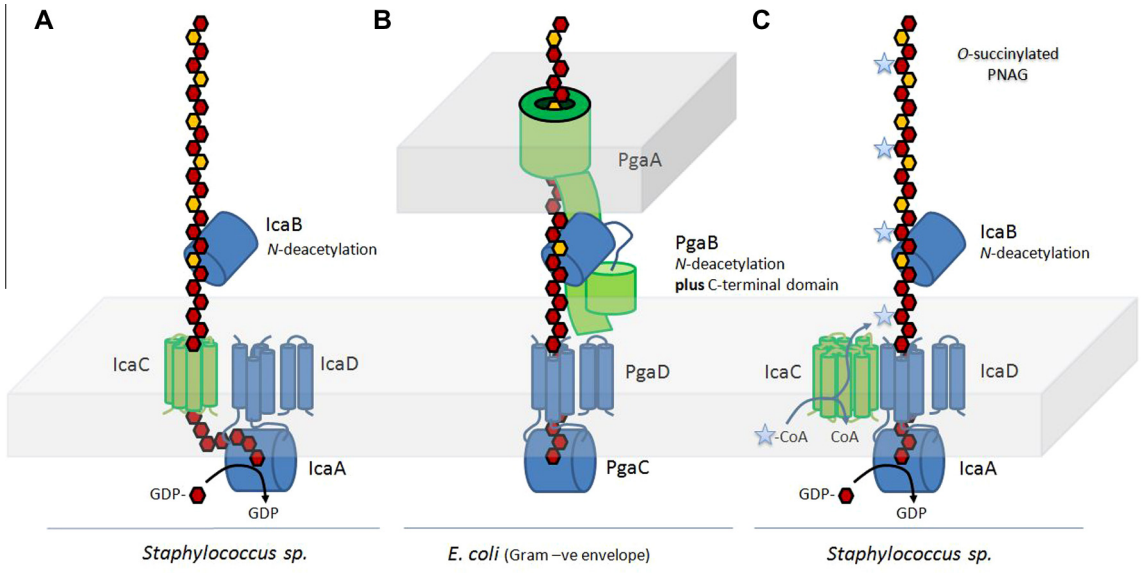

Fig. 2. Schematic comparison of the models for Ica and Pga gene functions in the biosynthesis of PNAG. (A) The current model of Ica gene function in PNAG biosynthesis in Gram-positive bacteria. (B) The Pga system from the Gram-negative bacterium *E. coli*. (C) Our refined model that has a different route for PNAG export through IcaA/D and the added *O*-succinylation of the PNAG which we propose is catalysed by IcaC. In each system shared components are coloured blue and unique components are coloured green. The indicated degree of deacetylation and *O*-succinylation are not stoichiometrically accurate and are for illustrative purposes only.