# 論　文　概　要

論 文 題 目 ： Natural genetic transformation mediates MRSA emergence（自然形質転換により MRSA は出現する）

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目　　的:

*Staphylococcus aureus* is a Gram-positive bacterium that inhabits the nasal cavity in about ~30% of the population. As an opportunistic pathogen, it can cause a wide range of infections ranging from minor to potentially fatal. Minor infections include superficial skin abscesses and food poisoning, while life threatening diseases include endocarditis, pneumoniae, sepsis, and toxic shock syndrome. Staphylococcal infections are largely associated with biofilm formation, providing protection against treatment and causing a huge burden on the healthcare system. *S. aureus* is also notorious for its rapid ability to acquire resistance against antibiotics. The emergence and dissemination of drug resistant strains, especially MRSA (methicillin-resistant *S. aureus*), is a global health concern. Resistance to methicillin and β-lactam antibiotics in MRSA is conferred by *mecA* gene, which is carried by the mobile genetic element termed Staphylococcal Cassette Chromosome *mec* (SCC*mec*: 21-60 kb). SCC*mec* can be transferred horizontally from methicillin-resistant coagulase negative staphylococci (MR-CoNS) to *S. aureus* and probably among *Staphylococcus* species according to epidemiological observations, but the exact mechanism of SCC*mec* transfer has been debated for over half a century. Among the major horizontal gene transfer (HGT) mechanisms in *S. aureus*, bacteriophage-mediated transduction is considered the primary, however, transduction cannot mediate transfer of large SCC*mec* elements (>45 kb), and conjugation is not common among staphylococci. Natural genetic transformation has been recently identified in *S. aureus* and could demonstrate the transfer of the large SCC*mec* element type II (~52 kb) to genetically modified *S. aureus* competent cells. However, the role of natural transformation has been regarded to be extremely limited, since the transformation frequencies were under detection limit in unmodified cells.

Development of natural genetic transformation in bacteria is generally tightly regulated. The environmental cues governing this regulation differ among species and is unknown in *S. aureus*. Two-component systems (TCSs) are major sensory means for mediating the extracellular signals and modifying the cellular response. TCSs are composed of a histidine kinase sensor embedded in the membrane and a cognate response regulator, which upon phosphorylation regulates gene expression.

This study aims to clarify the unknown condition(s)/signal(s) required for competence development and for efficient natural transformation in *S. aureus*, to help gain better insights into its role and relevance in staphylococcal evolution.

対象と方法:

To achieve this, the involvement of *S. aureus* TCSs in the activation of the competence operon *comG* was investigated by employing the reporter $P_{comG}$-gfp and testing its activity in a series of TCS deletion strains.

結　　果:

The GFP reporter assay indicated that TCS13 (AgrCA) and TCS17 (BraSR) are necessary for the full $P_{comG}$ activity, while TCS12 (VraSR) plays an inhibitory role. These results suggest that cell-surface stress and quorum sensing signaling are involved in the regulation of competence development. TCS13 and TCS17 were also found to be important for biofilm formation, and the transformation frequencies of the cells were increased (up to ca. $10^{-6}$) in statically grown biofilm conditions in a dependent manner on TCS13 and TCS17. Based on this finding, a new transformation protocol was established, and was used it to test the transformability of several clinical isolates of *S. aureus*. A series of experiments demonstrated for the first time intercellular transfer of various SCC*mec* elements (type I to IV) from MRSA and methicillin-resistant coagulase negative staphylococci to methicillin-sensitive *S. aureus*, which has long been predicted epidemiologically. SCC*mec* transformation was dependent on the site-specific insertion/excision system mediated by cassette chromosome recombinases (Ccr), while the stability of SCC*mec* in the transformants varied depending on SCC types and recipients.

考　　察:

This is the first study demonstrating efficient natural transformation development and showing its importance in mediating SCC*mec* transfer in *S. aureus*. The fact that any SCC*mec* type (21-60 kb) could be transferred and the fact that Ccr was important for the transfer were not demonstrated in the other known HGT mechanisms, suggesting that natural transformation is the major intercellular transmission mechanism of SCC*mec*.

結　　論:

Natural transformation is the key process in the emergence of MRSA.