Identifying the Function of GGGAATC Repeat Sequences in Xanthomonas Campestris

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G-Quadruplex structures can be adopted by G-rich DNA and RNA sequences. Proof of the existence of quadruplexes *in vivo* is still scarce, as is evidence for their suggested functions. We are focusing on a potential quadruplex forming sequence in the plant-pathogenic bacteria *Xanthomonas campestris pv. campestris* and its role in gene regulation. We identified 97 GGGAATC repeats in the *X. campestris* genome. The repeats are primarily found in intergenic regions. *In vitro* analysis of the DNA oligo $(GGGAATC)_3GGG$ indicated formation of a (3+1) hybrid G-quadruplex in the presence of K^+. Analysis of the neighboring genes revealed various genes coding for proteins involved in cell wall synthesis, motility, energy metabolism, adaption and in particular osmoadaption. This is of special interest as a massive uptake of K^+ ions from the environment occurs in many non-halophilic bacteria after an osmotic upshift. *X. campestris* also faces high K^+ concentration when infecting host plants. We hypothesize that GGGAATC repeats could be involved in osmoregulation. There the motif may act as potassium sensor affecting gene expression upon increase of intracellular potassium.

Identification of Negative Regulators of Integrin-Mediated Cell Adhesion

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Cells recognize and respond to their microenvironment through a multitude of transmembrane proteins such as the well characterized integrin superfamily. These tightly regulated receptors mediate transmembrane signaling by connecting the actin cytoskeleton via cytoplasmic adapter proteins to the extracellular matrix. Regulation of integrin activity is a fundamental process involved in a lot of physiological events like angiogenesis, tissue formation, migration or survival. The regulation of their ligand affinity based on conformational changes has been intensively studied. In the recent years a lot of proteins have been identified that positively affect integrin activity like talin and kindlins. In contrast, only few factors that negatively regulate integrins are currently known.

We identified the serine/threonine phosphatase Popx2 as a negative regulator of integrin activation in a shRNA-based screening approach. The knockdown of Popx2 results in an increased cell adhesion potential coinciding with enhanced ?1 integrin activation. These findings establish Popx2 as a novel regulator of integrin-mediated cell adhesion.

A detailed understanding of the mechanism of integrin affinity modulation will give us new starting points to manipulate integrin-mediated cell adhesion and the turnover of focal adhesion sites, both physiological events involved in a lot of pathological processes like cancer, asthma or multiple sclerosis.

Synthesis of Strained and Electron-Rich Dienophiles for Diels-Alder Reactions with Inverse Electron Demand and their Application in Metabolic Oligosaccharide Engineering

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Within the last years, metabolic oligosaccharide engineering (MOE) (Figure 1) has been established as an important tool to monitor carbohydrates *in vivo* and *in vitro.*[1] Different ligation methods can be used to detect the unnatural sugars that have been metabolically incorporated. In addition to the well-established azide-alkyne cycloaddition, our lab also uses Diels-Alder reactions with inverse electron demand (DAR_{inv}) as ligation reaction. One of the advantages of the DAR_{inv} is that it does not require catalysis by toxic metals and that the release of nitrogen during the reaction makes it irreversible. Moreover, the DARinv can be carried out in the presence of azides, and it is, thus, possible to label two different metabolically incorporated sugars in the same experiment.[2] This DAR_{inv} with an electron-poor tetrazine and an alkene and following retro-Diels-Alder reaction may be further improved by using dienophiles with a higher electron density or strained alkenes. Thus, new sugars (Figure 2) fulfilling these criteria were synthesized to make the reaction more efficient.

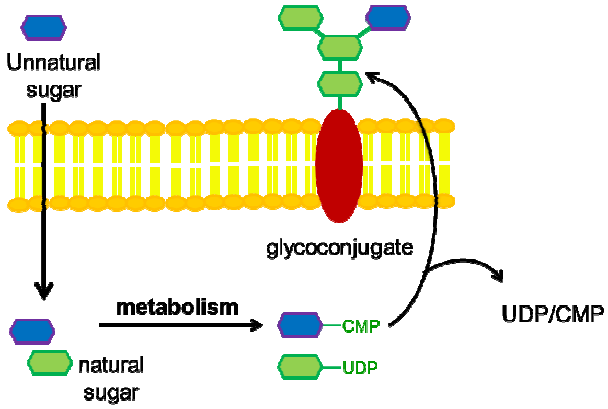

Figure 1: MOE principle.

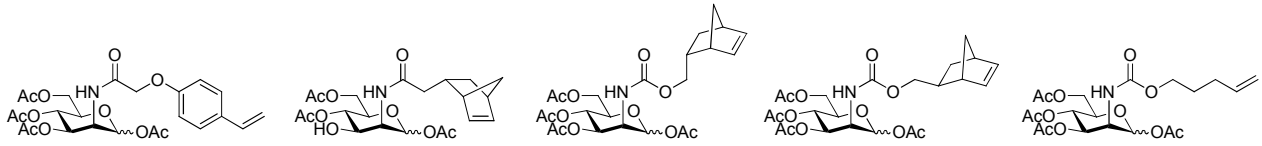

Figure 2: Novel dienophiles for DAR_{inv}.

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