Identification of a novel signalling cascade from N-acetylglucosamine transport to siderophore biosynthesis in Streptomyces coelicolor.

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Introduction:
Iron is an essential element for all organisms as it is required for vital life processes such as respiration and DNA synthesis. To circumvent the low bioavailability of the Fe³⁺ ion, microorganisms produce and excrete high affinity iron chelators, known as siderophores, to scavenge iron by forming soluble Fe³⁺ complexes that can be imported into the cell. As an excess of iron is toxic, the maintenance of strict homeostasis is crucial, and siderophores are released only when intracellular iron concentrations are low. In S. coelicolor, iron utilisation is controlled by DmrR1 [1] and iron boxes have been found upstream of the des and cch clusters involved in the biosynthesis of the siderophores desferrioxamines [2] and coelicchelin, respectively. Interestingly, we found a DasR responsive element upstream of dmrtR. DasR is a global regulator which controls all genes devoted to N-acetylglucosamine (GlcNAc) transport and utilisation [3] as well as antibiotic pathway specific activators such as actII-4 and act3 (for actinorhodin and prodiginines biosynthesis, respectively) [4]. Our in silico predictions enabled us to propose a signaling cascade linking GlcNAc, and the transcriptional repressors DasR and DmrR1, to a regulatory pathway of siderophore biosynthesis. The rush for iron is thus of utmost importance in the environment colonisation battle. The highlighted signaling cascade from GlcNAc transport to siderophore production suggests that glycans blocks derived from Streptomyces' own peptidoglycan and/or from the cell wall of fungi (chitin) may define the status of the colonising bacteria and adapt iron supply or privation.

Conclusion:
The signaling cascade initiated by N-acetylglucosamine and leading to siderophore biosynthesis repression has been validated in vivo. EMSAs validated the binding of DasR to the predicted direct upstream site of dmrtR. RT-PCR confirmed the repression and inducibility effects of DasR and GlcNAc, respectively, on dmrtR expression. GlcNAc's subsequent repression on siderophore biosynthesis has been validated in vivo. Therefore, we can conclude that the siderophores desferrioxamine and coelicchelin are, after actinorhodin and prodiginines [4], two new secondary metabolites which regulatory pathways are N-acetylglucosamine and DasR dependent. Furthermore, synthesis of this group of secondary metabolites is usually regulated according to the intracellular concentration of the ion it chelates. To our knowledge, this is the only example linking biosynthetic pathways to a nutritional molecule.

Effect of iron deficiency and abundance on the development of S. coelicolor:
Iron, along with other oligoelements, is required for numerous essential physiological processes. As mentioned above, microorganisms need to maintain intracellular iron levels within a restricted range of concentration conducive to growth. In order to gain a better understanding of the effect of iron on the life cycle, we investigated the phenotype of S. coelicolor M145 in response to low (deprivation) and high extracellular iron concentrations.

Antibiotic(s) are required to bypass the development blockade induced by GlcNAc

S. coelicolor M145

<table>
<thead>
<tr>
<th>Antibiotic(s)</th>
<th>R2YE</th>
<th>R2YE + GlcNAc</th>
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<tr>
<td>M145</td>
<td></td>
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<tr>
<td>M510</td>
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<td>M510 (Rif+4)</td>
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Conclusion:
Addition of N-acetylglucosamine to rich media, such as R2YE, represses siderophore biosynthesis and blocks S. coelicolor's development at the vegetative mycelium stage. Our experiments suggest that these effects might not be independent one from another. Indeed, deprived of their iron chelators, streptomycetes are not able to scavenge iron from the environment resulting in low intracellular iron concentration. In this case, iron could be the limiting factor preventing aerial hyphae and spore formation. Furthermore, our postulate is supported by the fact that double mutants are unable to grow without addition of exogenous siderophores [5].

We demonstrated that addition of iron relieves the developmental repression exerted by GlcNAc (sufficient intracellular iron). Interestingly, the GlcNAc effect is not bypassed when the iron chelators are removed.

Desferrioxamines, coelicchelin and actinorhodin are defined as secondary metabolites. Our experiments suggest that these molecules possess primary functions as they are required for proper development of S. coelicolor. These compounds are new examples commanding a redefinition of the term “secondary metabolites.”

References

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