Workshop V

Primary Metabolism and Transporters
Chair: Daniel Ramon

Vo-1

Title to be announced

Daniel Ramon

Abstract not available at time of print.

Vo-2

CARBON CATABOLITE REPRESSION (CCR) IN ASPERGILLUS NIDULANS : THE EFFECT OF creB AND creC MUTATIONS ON THE UPTAKE OF REPRESSING SUGARS.

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CCR is a wide-domain regulatory circuit responsible for the repression of the catabolism of alternative growth substrates when carbon sources of higher nutritional value are present. In the filamentous fungus A. nidulans this is effected by the action of the DNA-binding transcriptional repressor CreA. To date, not much is known about the means by which CreA becomes functional in response to repressing carbon sources or how CCR adapts upon depletion of a preferred substrate. Along with creA derepressed mutants, creB/C mutants have been selected as suppressors of areA loss-of-function allowing growth on acetamide as the nitrogen source in the presence of glucose. creB/C mutants are glucose-derepressed for the utilization of certain but by no means of all alternative growth substrates. Actually, these mutants show a large number of phenotypes not related to CCR (i.e. not evident in creA mutants) all of which can be ascribed to uptake or excretion deficiencies. Since CCR is dependent on the concentration of the repressing substrate, a change in its uptake efficiency could well lead to changed repression characteristics. In this presentation we show that creB/C mutants exhibit abnormal glucose uptake characterized by loss of a low-affinity transport component predominantly responsible for glucose uptake in wild type. In addition, we show that the expression of glucose-inducible genes is altered in these mutants. Finally, we show that mutations in creB/C are additive to the sorA3 mutation which itself leads to altered high-affinity sugar transport. These data do not support the hypothesis of Lockington and Kelly (Mol. Microbiol. (2002) 43, pp. 1173) that CreB/C would interact directly with CreA, stabilizing the repressor under repressing growth conditions. Moreover, from our current transcript analyses it is clear that CreA still binds to repressible promoters under derepressing growth conditions as well as in creB/C mutants in the presence of repressing sugars. All our results suggest that the CreB and CreC proteins play some role on the process of glucose uptake, explaining why glucose derepression is observed in creB and creC mutants.

Vo-3

THE ALTERNATIVE D-GALACTOSE CATABOLIC PATHWAY IN HYPOCREA JECORINA: A STOWAWAY OF D-XYLOSE AND L-ARABINOSE CATABOLISM

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D-galactose metabolism via the Leloir pathway is a ubiquitous trait in pro- and eukaryotic cells. It involves the formation of galactose-1-phosphate by galactokinase and its subsequent stepwise conversion up to glucose-6-phosphate which is then channelled into glycolysis. In contrast to the situation in yeasts, a loss of function of H. jecorina (anamorph Trichoderma reesei) galactokinase Gal1 only partially impairs growth on D-galactose, suggesting the operation of an alternative pathway. A clue to the identification of this pathway was the detection of increased transient galactitol accumulation in a gal1 (1) mutant, suggesting that this pathway operates via reduction of D-galactose.
Here we will present genetic and biochemical evidence for the first two steps of this pathway, i.e. : NADPH-aldose reductase dependent reduction of D-galactose to galactitol, and subsequent NAD-dependent oxidation of galactitol to L-xylo-3-hexulose by either L-arabinitol dehydrogenase or xylitol dehydrogenase. The latter two enzymes act mutually exclusive, as either a gal1/lad1 or gal1/xdh1 mutant is unable to grow on D-galactose. Details on the genetic evidence and the properties of the respective purified enzymes will be presented. Investigations towards an identification of the enzyme catalyzing the breakdown of L-xylo-3-hexulose, which has so far not been identified as a metabolite in fungi, are in progress.
The alternative pathway of D-galactose metabolism is apparently not only a rescue bypass, but apparently operates also in parallel to a functioning Leloir pathway, as lad1-delta mutants alone already display reduced growth on D-galactose. This pathway must therefore have an as yet unknown physiological function. Its possible involvement in the regulation of cellulase induction by lactose is currently investigated.

(1) Seiboth, B., Hartl, L., Pail, M., Fekete, E., Levente, K., Kubicek, C.P. The galactokinase of Hypocrea jecorina is essential for cellulase induction by lactose but dispensable for growth on D-galactose. Mol Microbiol in press.

Vo-4

FORMATION OF PROPIONYL-COA AND ITS EFFECT ON CELLULAR METABOLISM IN FILAMENTOUS FUNGI

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Propionate is one of the most abundant carbon sources in soil. Therefore, many aerobic microorganisms, including fungi and bacteria, are able to degrade this monocarboxylic acid. Activation of propionate to propionyl-CoA is the first and essential step in all known degradation pathways of propionate (1). However, accumulation of propionyl-CoA has been shown to disturb normal cellular metabolism, as well in bacteria (2) as also in eucaryotes (3).
In this study we used the filamentous fungi Aspergillus nidulans and Aspergillus fumigatus, which both metabolise propionate via the methylcitrate cycle (4). Disruption of one of the key enzymes, methylcitrate synthase, leads to a strong retardation of growth on media containing glucose and propionate as carbon sources. This phenomenon is accompanied with the accumulation of propionyl-CoA in the mutant strain, which is not visible to that extend in the wild type. Interestingly, addition of acetate releases growth inhibition followed by decreasing propionyl-CoA levels. In order to examine this observation, we specifically searched for enzymes of glucose metabolism, which are strongly inhibited by propionyl-CoA and furthermore, investigated the ability of cell extracts to activate acetate and propionate, respectively, to the corresponding acyl-CoA-esters.
Furthermore, we were able to show that accumulation of propionyl-CoA correlates with a disturbed polyketide formation, which is easily visualised by altered spore colour formation of both, A. nidulans and A. fumigatus, which carry a disrupted gene coding for methylcitrate synthase. The addition of propionate or amino acids like isoleucine, valine or methionine, which are degraded to propionyl-CoA, lead to the formation of white conidia. Therefore, we can conclude that accumulation of propionyl-CoA is not only toxic but also directly influences secondary metabolism.

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NITROGEN METABOLITE REPRESSION OF ARGININE CATABOLISM GENES IN ASPERGILLUS NIDULANS.

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The arginine catabolism genes agaA and otaA, coding for arginase and ornithine transaminase (OTAse) respectively, are specifically induced by arginine and repressed by ammonia (Dzikowska et al. 1999 ; Borsuk et al. 1999 ; Dzikowska et al. 2003). In A. nidulans nitrogen metabolite repression is mediated by transcriptional activator AREA. AreA 600 loss of function mutant does not grow on arginine as a nitrogen source but we have shown that arginase and OTAse are fully inducible in this mutant. The same was shown for agaA and otaA mRNA. This suggests that an arginine permease gene can be a target for AREA activator. It also seems that some negatively acting factor participates in the ammonia repression of agaA and otaA. We have already shown that areB participates directly or indirectly in otaA repression by ammonia since there is no repression of OTAse activity in areB loss of function mutant. We try to find additional evidences for regulation of agaA and otaA by AREB.

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NITROGEN STARVATION RESPONSES IN A SYMBIOTIC FUNGUS: A TRANSCRIPTOMIC ANALYSIS IN THE ECTOMYCORRIZAL ASCOMYCETE Tuber borchii

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In fungi, N deprivation is one of the most general and conserved stimuli of compensatory metabolic as well as morphogenetic modifications aimed to induce an increased nutrient acquisition capacity. Most of these morphogenetic transitions are accompanied (and perhaps determined) by the overexpression of cell wall-associated or secreted surface proteins. N deprivation has additional, eco-physiological implications in ectomycorrhizal fungi. These organisms, in fact, have a superior capacity to mobilize and take up soil N compared to their host plants and the regulated release of N-derived compounds to the phytobiont in exchange with carbohydrates plays a key role in the economy and ontogenesis of the ectomycorrhizal symbiosis. We are addressing N starvation responses in the symbiotic ascomycete Tuber borchii by using both a targeted approach, aimed at isolating and functionally characterizing key assimilation components such as various N-assimilation enzymes and permeases, as well as an “untargeted” approach relying on hybridization to a dedicated macroarray containing 2100 ESTs.
Data produced by the latter analysis clearly indicate that the earliest and most intense responses to N starvation rely on the upregulation of mRNAs coding for surface-associated or secreted proteins involved in cell wall and/or membrane modification, rather than on the upregulation of true N assimilation components. The only “early upregulated” mRNA that has been functionally identified so far codes for a Ca^{2+}-activated, secreted phospholipase A2 (TbSP1) belonging to a new group of enzymes (group XIII) that are unique of filamentous microorganisms. It thus appears that two radically different responses take place upon N deprivation in T. borchii: an early response, that is most likely involved in an as yet unidentified morphogenetic change and cell surface modification; and a delayed response aimed to improve the N assimilation capacity of free-living mycelia. Particularly interesting in this regard is the observation that the upregulation of the NH4+ and NO3- transporters under N-limiting conditions is accompanied by a switch from a GDH (glutamate dehydrogenase)-based ammonium assimilation pathway as in most fungi, to a GS/GOGAT (glutamine synthetase/glutamate synthase) pathway that is typical of plants and of certain filamentous fungi. Since N deprivation is one of the most important environmental factors that favour mycorrhization, data obtained from these studies may also provide initial insights into pre-infection events that may predispose the fungus towards a productive interaction with its plant symbiotic partner.