found in the Antibodypedia. The web portal is publicly available, and through user contribution of experimental validation data we anticipate the Antibodypedia to become a valuable resource for everyone working with antibodies

doi:10.1016/j.nbt.2009.06.962

Poster 4.6.02

Genome-wide analysis of the Zur regulon of *Corynebacterium glutamicum* ATCC 13032

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Zinc is an essential nutrient for all living cells. Accordingly, the maintenance of zinc homeostasis and its genetic regulation have been characterized in various bacteria with increasing interest. The protein Zur (Cg2502) of *Corynebacterium glutamicum* is a metalloregulator and belongs to the ferric uptake repressor (Fur) family of DNA-binding transcriptional regulators. The regulatory role of Zur was investigated in detail by transcriptomics and molecular genetics approaches.

Whole-genome DNA microarray hybridizations comparing a Zur-deficient mutant and the *C. glutamicum* wild-type strain revealed 22 genes with enhanced expression in the mutant, six of them implicated in the uptake of zinc. The transcript levels of eight genes were verified by real-time RT PCR, and complementation of the mutant reversed the effects of differential gene expression. The data demonstrated that Zur provides the repression of two operons coding for putative manganese/zinc ABC transporters and, additionally, for genes encoding a putative oxidoreductase and a putative GTPase. DNA band-shift assays illustrated that purified Zur protein specifically binds in a zinc-dependent manner to 19-bp operator sites that are similar to those of Zur from *Mycobacterium tuberculosis*. Our data provided clear evidence that Zur (Cg2502) is the key transcriptional regulator for genes of zinc transport systems in *C. glutamicum*.

doi:10.1016/j.nbt.2009.06.963

4.6.03

Colorimetric screening of recombinant RNAi vectors without using PCR

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RNAi is widely used to knock down gene expression in plants, animals and many fungi. RNAi provides a powerful technique for the derivation and analysis of loss of function of a gene. The procedure of gene knock down (silencing) includes a series of step to design a expression vector to produce hpRNA/double stranded RNA/siRNA. The vectors used for the introduction of gene fragment undergoes series of recombination and transformation. After transformation of these vector to the host bacterial cells/cell lines requires screening of desired colonies. The screening of the positive clones are generally performed with PCR. Although PCR screening is most accurate there are chances to get false positive results mostly due to cross contamination. The abstract describes about the colorimetric screening using gold nano particle (GNP). This method is robust and do not utilize PCR to screen the positive clones. In theory ssDNA (Probe) present in colloidal gold prevent the induced aggregation and confers red color. The induction is caused by addition of NaCl which screen the repulsive force among the GNP. The GNP in colloidal state is bright red whereas the fully aggregated GNP shows purple color. The separate hybridization step was conducted to scavenge complementary ssDNA probe added to the dsDNA before hybridization. The RNAi vectors containing the desired sequence binds to the probes and the solution turns blue after addition of NaCl. The Gateway Technology (Invitrogen) was used to construct the silencing vector. Briefly the target sequence for the induction of RNAi in Arabidopsis was amplified by adapter PCR and introduced into the hairpin RNA-expressing pWatergate vector (CSIRO Plant industry). A fragment of GGT2 (gamma-glutamyl-transferase) gene of 363 bp from nucleotides 104 to 467 was amplified using the primer containing attB1 and attB2 recombination sites. The amplified PCR product was first cloned into the donor vector pDONR221 (Invitrogen) to create an entry clone and after sequence verification, the gene specific sequence was transferred to the binary T-DNA destination vector pWatergate in reactions mediated by BP and LR Clonase reaction. For the screening of the recombinant plasmids, GGT2-pDONR221 and GGT2-pWATERGATE the DNA probe was designed which bind to the GGT region of the plasmid. The plasmid were isolated using the miniprep method. 50 ng of plasmid was found sufficient for the screening. This method is rapid and performed in few minutes. This method is useful in high throughput screening of the recombinant plasmid.

doi:10.1016/j.nbt.2009.06.964

4.6.04

A spatiotemporal analysis of sucrose synthase during seed development in wild-type and EMS mutant embryos of common bean (*Phaseolus vulgaris* L.) using *in situ* hybridization

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Sucrose is one of the principal end products of photosynthesis in most plants and also the major carbohydrate transported to sink tissues. Sucrose synthase (Sus), one of the key enzymes in the control of sucrose synthesis, has been studied extensively in various plant species. Sus showed mainly involvement in starch and protein synthesis at the storage phase of embryo development. The function of Sus during seed development of common bean (*Phase-*

olus vulgaris L.) is not known. Using immunolocalization, in some species such as *Zea mays*, *Hordeum vulgar*e and *Arabidopsis thaliana* Sus showed expression in the basal endosperm transfer cells and in developing embryos. In order to better define the role of Sus in *Phaseolus*, we have compared the pattern expression of Sus during developmental progression of wild-type and an ethyl methanesulfonate (EMS) mutant embryos. Homozygous embryos isolated from mutant aborted pods were arrested at the globular stage; they present a developmental delay during embryogenesis and lethality in seed growth.

Expression profiling of Sus by reverse transcriptase polymerase chain reaction (RT-PCR) showed that the gene was active at low levels in vegetative wild-type tissues such as leaves, flowers, cotyledons and roots. Highest level of expression was observed in seeds and nothing in stems.

In situ hybridization studies in wild-type, 3 days after pollination (DAP), show the gene activity in endosperm, suspensor, embryo, but also in a group of cells located in the outer and inner integumentary layer. At 7 and 8 DAP Sus gene product mRNA was detected specifically in suspensor and no signal detect in other tissue. However, at 9 and 12 DAP Sus expression was revealed in suspensor, endothelium layer, and cotyledons, but was absent from the endosperm, outer and inner integumentary layer. These temporal changes in cellular localization of Sus gene during common bean seed development are comparable with those reported in Arabidopsis seed. These data suggested a possible role of Sus in carbon portioning during early to mid stages of seed development, as a process to maintain sink strength. In later stage, Sus play a role within the cotyledon in sucrose utilization. In the EMS mutant plant, disruption in Sus pattern and expression highlight the roles of Sus gene during seed development in common bean.

doi:10.1016/j.nbt.2009.06.965

4.6.05

Functional mapping of MuA transposase by pentapeptide insertion mutagenesis

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Bacteriophage Mu propagates by the use of DNA transposition reaction catalyzed by MuA transposase that is member of the retroviral integrase superfamily (RISF). MuA functions in transposition as a tetramer, synapsing the two transposon ends. This protein-DNA complex or transpososome is the key molecular machine that executes the critical DNA cleavage and joining reactions. To analyze structure-function relationships in the MuA action, a large-scale pentapeptide insertion scanning mutagenesis was performed, i.e. five amino acid insertions were distributed in random positions along the primary sequence. A quantitative in vivo assay was used to monitor the activity of over 230 mutants. This assay is based on in vivo expression of MuA and transposition of a lacZ marker gene-containing mini-Mu transposon (without promoter) into expressed gene locus in the bacterial chromosome, yielding blue papillae in otherwise white colonies on indicator plates. The quantitative data were mapped onto primary sequence and the known MuA domain 3D structures. The results pinpoint those regions that withstand insertions without affecting the function and indicate positions that are critical for function. As expected, most of the insertions within Igamma to IIIalpha region totally destroyed the transposase activity, confirming a variety of predictions made earlier. However, three loop/linker regions were detected, in which insertions improved the MuA activity: the linker Ialpha to Ibeta, the loop N281 to G282, and the loop A473 to R478. Many insertion-tolerant regions were found in the Ialpha domain and in IIIbeta domain. This comprehensive data set will be useful for the design of transposase variants with altered properties. In addition, activities in RNase H fold region provide insight into the molecular details of action of other members of RISF.

doi:10.1016/j.nbt.2009.06.966

4.6.06

Gene expression, data analysis and modelling

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The traditional approach to research in Molecular Biology has been an inherently local one, examining and collecting data on a single gene, a single protein or a single reaction at a time. This is, of course, the classical reductionist stance: to understand the whole, one must first understand the parts. Over the years, this approach has led to remarkable achievements, allowing us to make highly accurate biochemical models of such favorites as bacteriophage Lambda.

However, with the advent of the "Age of Genomics" an entirely new class of data is emerging. To date, analysis of this large scale data has consisted of little more than descriptions of how many genes were previously unknown, which genes are over- or under-expressed under certain circumstances, etc. Of course, such data is a valuable resource for researchers who are focusing on individual genes. But can we really expect to construct a detailed biochemical model of, say, an entire yeast cell with some 6000 genes (only about 1000 of which were defined before sequencing started, and about 50% of which are clearly related to other known genes), by analyzing each gene and determining all the binding and reaction constants one by one? Likewise, from the perspective of drug target identification for human disease, we cannot realistically hope to characterize all the relevant molecular interactions one-by-one as a requirement for building a predictive disease model.

There is a need for methods that can handle this data in a global fashion, and that can analyze such large systems at some intermediate level, without going all the way down to the exact biochemical reactions.

This process may be conceptualized as a genetic feedback network, in which information flows from gene activity patterns through a cascade of inter- and intracellular signaling functions back to the regulation of gene expression. Gene sequence information in cis regions (regulatory inputs) and protein coding