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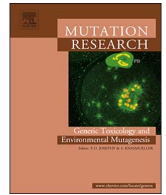

Short communication

Genome-wide bisulfite sensitivity profiling of yeast suggests bisulfite inhibits transcription

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ABSTRACT

Bisulfite, in the form of sodium bisulfite or metabisulfite, is used commercially as a food preservative. Bisulfite is used in the laboratory as a single-stranded DNA mutagen in epigenomic analyses of DNA methylation. Recently it has also been used on whole yeast cells to induce mutations in exposed single-stranded regions *in vivo*. To understand the effects of bisulfite on live cells we conducted a genome-wide screen for bisulfite sensitive mutants in yeast. Screening the deletion mutant array, and collections of essential gene mutants we define a genetic network of bisulfite sensitive mutants. Validation of screen hits revealed hyper-sensitivity of transcription and RNA processing mutants, rather than DNA repair pathways and follow-up analyses support a role in perturbation of RNA transactions. We propose a model in which bisulfite-modified nucleotides may interfere with transcription or RNA metabolism when used *in vivo*.

1. Introduction

Sodium bisulfite is a common food preservative used in wines and dehydrated produce, and is also a widely used industrial chemical. Moreover it is an important reagent in the laboratory where it is used in bisulfite sequencing applications to study DNA methylation patterns [1]. Bisulfite sequencing exploits the ability of bisulfite to react with cytosine in single-stranded DNA (ssDNA), but not with 5-methylcytosine, and convert it to uracil. This phenomenon has been exploited for studies beyond bisulfite sequencing, including footprinting single-stranded regions of interest (e.g. for DNA:RNA hybrid prone loci [2]). Indeed, bisulfite footprinting was among key experiments that established the single-stranded character of the immunoglobulin class-switch recombination region as a target of the Activation Induced Cytidine Deaminase (AID; [3]). More recent studies in yeast showed that bisulfite acted as a potent ssDNA mutagen in live cells that were engineered to expose long tracts of ssDNA around a mutation reporter locus [4]. This is consistent with its known bias to ssDNA during *in vitro* cytosine deamination reactions [1].

Despite its importance across sectors and in biological research, surprisingly little is known about genetic modifiers of bisulfite sensitivity. One of the most powerful ways to profile genes whose disruption leads to chemical sensitivity is to screen the yeast knockout collection (YKO) [5]. This approach has allowed profiling of homozygous deletions in non-essential genes and heterozygous deletions in essential genes for thousands of compounds [6]. Other efforts to profile essential genes in haploid cells have created additional collections of tetracycline repressible [7], decreased abundance by mRNA perturbation (DAmP; [8]) or temperature sensitive [9–11] mutant collections. Combining the YKO with resources to study essential genes in haploids enables complete and parallel assessment of both non-essential and essential gene mutants that are sensitized to a particular set of conditions.

Here we apply yeast functional genomics resources to screen the mutants covering nearly the entire genome, and identify genes that regulate the cellular response to bisulfite. Given previous literature and the biochemistry of bisulfite *in vitro*, we expected to identify genes that regulate the exposure of ssDNA. Instead, we identify a set of genes that regulate transcription and/or the cellular response to elongation inhibitors such as 6-azauracil. We go on to show that bisulfite reduces expression from a reporter plasmid, total RNA yields, and reduces the formation of RNA processing bodies (P-bodies), whose assembly is RNA dependent, in otherwise unperturbed cells. Together our data support a model in which bisulfite modified nucleotides interfere with RNA metabolism. Potential mechanisms for this effect are discussed.

2. Materials and methods

2.1. Yeast strains and sensitivity screening

Yeast were grown according to standard procedures on either Yeast

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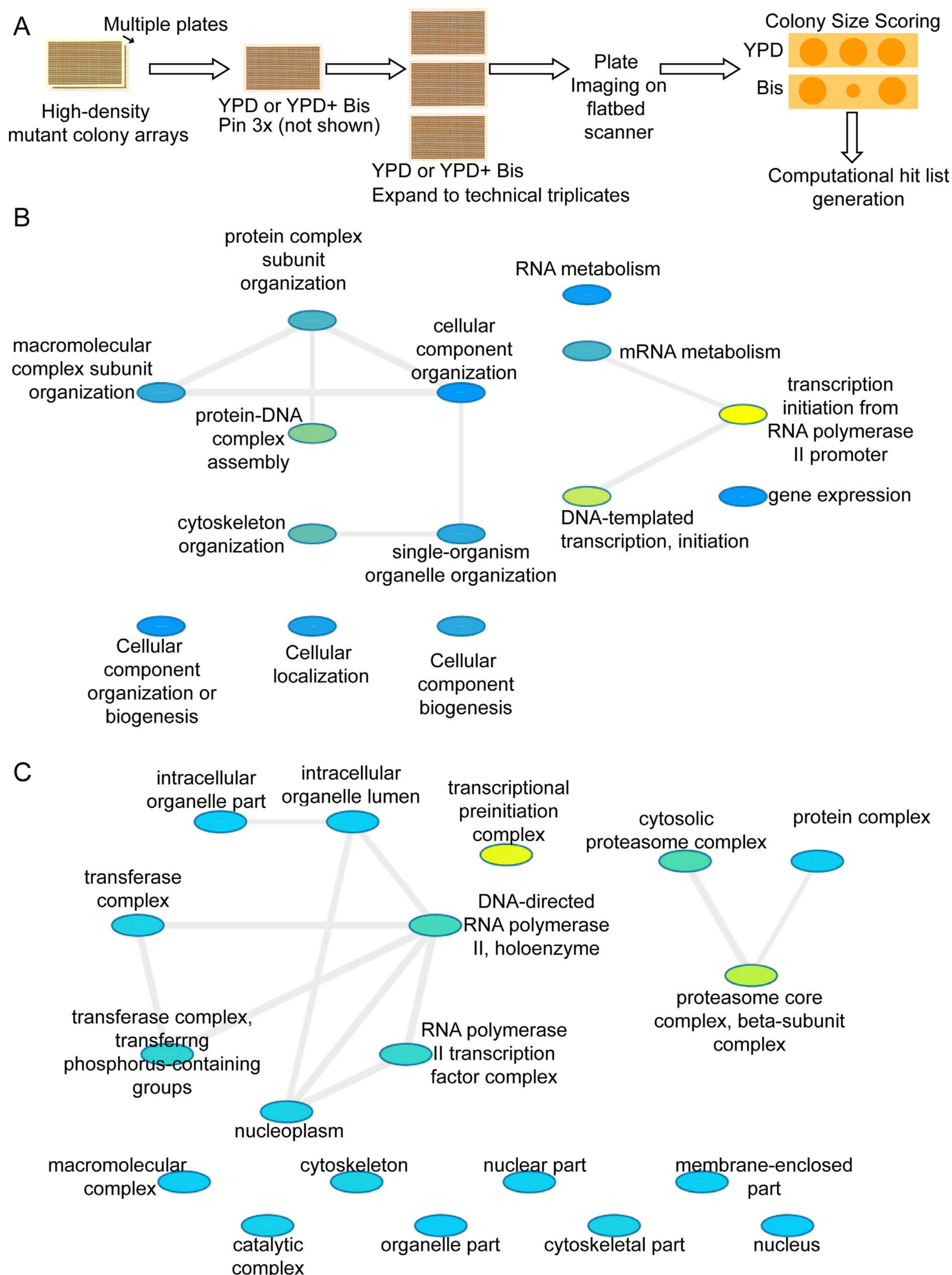

Fig. 1. Gene ontology of bisulfite sensitive yeast genes. (A) Flow chart of bisulfite sensitivity screen. See Materials and Methods for more details. Briefly, high-density colony arrays (left) were pinned in triplicate onto YPD or bisulfite plates. These were expanded into technical replicates and plate images were taken after a growth period. An example of three idealized colonies on each media is shown on right, two grow normally, but one is bisulfite sensitive and therefore smaller. Colony scoring compares the size of colonies on the control to the size on bisulfite and generates a measure of colony size differences and reproducibility for each individual strain. Large, reproducible differences in growth indicate the strains which populate the hit list. (B) GO Biological Processes enriched and (C) GO Cellular Components enriched in the hit list. (B and C) Significant ($p < 0.01$) negative hits with a magnitude < -0.5 difference between bisulfite and control were analyzed by the generic GO termfinder (WWW) using the gene list screened as a background set for GO enrichment calculations (Table S2). A network of non-redundant GO terms was created by ReviGO (WWW) and colored by term fold enrichment in cytoscape. Nodes as colored by fold enrichment from low enrichment (blue) to high enrichment (yellow).

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