Minireview

Functional genomics of the yeast DNA-damage response
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Abstract

High-throughput approaches are beginning to have an impact on many areas of yeast biology. Two recent studies, using different experimental platforms, provide insight into new pathways involved in the response of yeast to DNA damage.

Large-scale sequencing projects in the 1990s ushered in a series of genome-wide studies aimed at addressing gene function. Termed ‘functional genomics’ or in some contexts ‘systems biology’ [1], a decade of work on the budding yeast Saccharomyces cerevisiae has resulted in a body of knowledge describing gene-expression patterns, gene-disruption phenotypes, and protein–protein and protein–DNA interactions. While certain levels of experimental error are associated with these data, analyses have shown that combinations of the individual datasets result in gene function predictors of considerable power [2]. Two recent studies [3,4] have taken these observations into account and describe work aimed at further characterizing the yeast response to DNA damage by using different and complementary experimental platforms.

The DNA-damage response has been a target of high-throughput studies because of its complexity as well as its relevance to human cancer. Many kinds of damage occur to DNA during growth, whether in the presence or absence of DNA-damaging agents (Figure 1). Invariably, damaged DNA that is processed to single-stranded DNA elicits a checkpoint response that stalls the cell cycle, allowing time for repair. Distinct types of DNA damage, such as mismatched bases and double-strand breaks, are detected by proteins or protein complexes (for example, MutS proteins and the Ku heterodimer), and are processed to expose single-stranded DNA. The presence of damage is signaled through specific phosphorylation pathways, such as those involving the yeast protein kinases Mec1 and Dun1, that eventually alter the activity of transcription factors (for example, Crt1) that effect the expression of a large number of proteins that rebuild and repair the damaged DNA (for example, Rad51). No single current technology can interrogate these different organizational levels and so several approaches have been used.

Parallel approaches to studying DNA damage
Early studies using DNA microarrays indicated that transcriptional responses often reflect underlying biology: for instance, the expression of cell-cycle genes that cycle in tandem with fluctuations in the respective proteins [5]. Several groups have investigated gene expression in response to DNA damage in yeast. Jelinsky et al. [6] treated cells with different alkylating agents, ionizing radiation (IR), and peroxide, and found a variety of upregulated genes that had not previously been implicated in DNA repair. Brown and co-workers [7] found a set of genes whose expression increased following methylmethanesulfonate (MMS) and IR treatment: it included RAD51, RAD54, RNR2 and RNR4. Other groups have investigated the sensitivity of homozygous deletion strains to various
DNA-damaging treatments. Chang et al. [8] described a set of 103 genes for which homozygous deletion mutants are significantly sensitive to MMS, and Bennett et al. [9] investigated γ-ray sensitivity. A more recent study generated quantitative drug-sensitivity profiles using 51 different cytotoxic or cytostatic agents [10]. Finally, a number of groups have studied the interactions of proteins following addition of DNA-damaging agents, including direct physical interactions [11] and, in many cases, genetic interactions [12]. In general, these studies have provided valuable insights into the biology of the DNA-damage response, but they fail to give an overall perspective.

In general, there is very little overlap in the genes identified in the different studies, even in those that used the same agent, such as MMS. There are probably several reasons. First, no two studies exactly reproduce the same conditions. Second, the inherent 'biological noise' that is now known to underlie many cellular responses may influence the findings [13,14]. Whatever the basic reasons, cellular responses involving hundreds of genes are very complex, and complete understanding would require not only an exact description of the responses of the genes at a single point in time, but the complete dynamics of such a response. For instance, scores
of gene products are involved in DNA replication during the normal cell cycle, and the state of these proteins at the particular time of DNA damage may influence their subsequent behavior (see Figure 1). Calls for experimental design that focuses on understanding the cell as a system are motivated by these factors, but efforts to do this are limited by techniques, by resource availability and perhaps by our conceptualization of the nature of the biology. For instance, what is the appropriate unit for studying DNA damage - the protein, the pathway or the cell? Perhaps the answer is all three.

A physical DNA-protein binding approach

In a recent study of DNA damage in response to MMS in yeast, Workman and colleagues [3] focused initially on detecting the physical interactions of transcription factors with their DNA targets using the chromatin immunoprecipitation-DNA microarray assay (ChIP-chip) [15], but have extended this significantly by examining the genetic and physical context of the interactions. In other words, they attempt to place the transcriptional response to DNA damage in yeast within the context of the cell as a system. Previous work has suggested that this response involves not only the induction of repair enzymes, but also less obvious aspects of cell biology such as lipid metabolism, cytoskeleton remodeling and cell-cycle checkpoints [16]. Workman et al. [3] mapped the binding sites of 30 transcription factors implicated in the DNA-damage response following addition of MMS and compared the results with an earlier study carried out under normal growth conditions [17]. They found that six transcription factors bound many more genes under DNA-damage conditions than during normal growth, whereas eight bound significantly fewer genes. The authors [3] noted upstream DNA elements enriched in gene sets bound by particular transcription factors, and searched for sets of target genes common to different transcription factors. Some of these relationships are intriguing: for example, the transcription factor Cad1 shares downstream target genes with Hsf1 under DNA-damage conditions but with Yap1 under normal conditions. Also, the number of genes bound by each transcription factor varied widely, from 13 (each) for Dig1 and Adr1 to 1,078 for Ino4.

To validate their findings, Workman et al. [3] determined the gene-expression profiles of 27 viable transcription factor deletion strains and focused on transcription factor-gene pairings that showed differential expression under normal versus DNA-damage conditions, but which lost this difference in the transcription factor knockout strains. They call this phenomenon ‘deletion buffering’. Such a relationship would appear to offer strong evidence that the transcription factor regulated the corresponding gene following DNA damage, and this was indeed the case for the transcriptional repressor Crt1 and components of the ribonucleotide reductase complex (Rnr2, Rnr3 and Rnr4), which is induced in response to DNA damage [18]. In total, 341 such pairings were discovered, and Workman et al. [3] noted a positive relationship between the number of genes buffered by a transcription factor and the sensitivity of the deletion strain to MMS. This might have been expected, but they also found 16 examples of genes that only became MMS-responsive in transcription factor deletion strains. It is more difficult to envisage how this relationship occurs - perhaps the transcription factor serves as a repressor or has some general function in limiting the damage response. Furthermore, of the 341 transcription factor-gene pairings with a ‘deletion buffering’ relationship, only 37 are connected by ChIP-chip experiments. How does one find meaning in this hall of mirrors?

These results suggest that the architecture of the transcriptional response to DNA damage is complex, if not baroque, and requires modeling that extends beyond simple binary transcription factor-gene pairings to higher-order motifs and pathways. In fact, transcription factors compete for binding to particular DNA elements; they function as either activators or repressors depending on context, and their expression and function may also vary temporally and spatially [19]. Workman et al. [3] constructed such a model using Bayesian statistics on a set of over 10,000 transcription factor-gene pairings and over 14,000 physical protein-protein interactions from their own work and from the literature. The result is an admirable overview of the protein-protein and protein-DNA interaction network of the DNA-damage response based on current knowledge, and includes over 80 indirect regulatory loops that are newly proposed. The model is also valuable in linking the central enzymatic machinery of DNA repair (Rnr1, Rnr2, Rnr4, Rfa1, Mag1, Crt1, Din7, Dun1) with proteins of the cell cycle, the stress response, and lipid and nucleotide metabolism.

A genetic mapping approach

Another recent study of the yeast DNA-damage response, by Boeke and colleagues [4], focused on genetic interactions in the regulatory and effector pathways rather than the transcriptional response. Parallel screens for buffering, or epistatic, interactions between genes (pairs of genes where disruption of both gives a different phenotype than disruption of either gene alone) have been very successful at mapping functional pathways within yeast cells [20-22]. The diploid-based synthetic lethality analysis on microarrays (dSLAM) method measures differential growth of disrupted strains in competitive cultures [20]. Diploid strains are used because they show robust genetic properties and because essential genes can be used in the assay. Pan et al. [4] take a wide view of the DNA-damage response, and include DNA replication, cell-cycle checkpoints and other contributors to DNA integrity. Beginning with 74 genes involved in these pathways, they generated a network of 4,956 genetic interactions comprising 875 genes, less than 10% of which had previously been described. Although the network is rich in protein complexes and pathways determined from previous
work, one weakness of using high-throughput methods is that it is difficult to determine when the resulting data represents a single functional unit or a multi-step pathway.

Several workers have noticed that genetic interactions are frequently observed among groups of genes involved in the same biological process but are rare among genes involved in the same linear pathway or protein complex [21,23,24]. This makes sense: when two proteins mediate sequential steps in a pathway, one expects that the net effect of disrupting both proteins would be the same as disrupting just one. However, when two proteins contribute to related functions in branched or distinct biochemical pathways, removing them both is likely to prove disruptive. Pan et al. [4] defined 16 such functional modules by grouping sets of genes with similar dSLAM genetic-interaction profiles or sensitivities to DNA-damaging agents, but excluding those with internal genetic interactions. These dSLAM gene sets included the homologous recombination module (Rad50, Rad51, Rad54, Rad55, Rad57, Mre11 and Xrs2) and a Mec1 kinase module (Mec1, Lcd1 and Rad53). Significantly, these modules are consistent with many earlier studies reported in the literature. Another module identified, the Bre1 module (Rad6, Bre1 and Lge1), illustrates the power of the approach, as it accurately defines a complex that ubiquitinates histone H2B [25,26]. Bre1 and Lge1 shared very similar genetic-interaction profiles when measured by dSLAM (123 of 129 Bre1 and 142 Lge1 interactions were overlapping), suggesting very similar roles for these proteins, but Rad6 had a slightly different profile. Rad6 is also a component of the post-replication repair module along with Rad5 and Rad8, but only Rad6 shared dSLAM profiles with other chromatin-remodeling proteins. Therefore, these types of behavior can illuminate subtle aspects of the roles of these proteins in DNA-damage responses and related activities. In the future, more quantitative genetic analyses will undoubtedly provide further insight into these and other biological processes.

Taken together, the two studies by Workman et al. [3] and Pan et al. [4] show that creative technological approaches continue to be applied in yeast, and that they can provide new insights into complex cellular responses, that are relevant to all organisms.

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