# Chapter 6

## Gene Expression Changes in *Saccharomyces cerevisiae* in Response to Sequence-Specific DNA-Binding Polyamides

This chapter is from a collaborative project with Mazen Karaman and Joseph Hacia (USC)

**Abstract**

Eight-ring hairpin polyamides composed of *N*-methylimidazole and *N*-methylpyrrole amino acids bind a broad repertoire of DNA sequences with the affinity and specificity of natural transcription factors. The cell uptake and nuclear trafficking of hairpin polyamides can be altered through modification of the C terminal tail. Nuclear uptake in yeast is enhanced by a dication tail at the C terminus. In order to profile how these DNA binding molecules possibly interact with the gene regulatory pathways of a eukaryotic cell, gene expression patterns in the budding yeast *Saccharomyces cerevisiae* were examined in the presence of eight-ring hairpin polyamides that code for two different DNA sequences. An eight-ring hairpin that formally codes for a non-degenerate six base pair DNA sequence would be expected, on average, to bind once every 2,080 base pairs, or approximately 5,770 times in the 12 million base pair yeast genome. However, despite the large number of potential binding sites in the yeast genome, our results suggest a surprisingly limited transcriptional response to subtoxic doses of eight-ring polyamides. Two eight-ring hairpin polyamides of different DNA binding specificities with dication tails each effected the levels of less than 1% of the interrogated transcripts. Very few genes were similarly affected by both polyamides, even though the two polyamides differ by a single atom. Interestingly, the polyamides with dication tails increased the mRNA levels of many genes normally repressed by environmental stresses while decreasing the mRNA levels of many genes normally induced by these stresses. However, polyamides with similar DNA binding specificity but with monocation tails which do not enhance nuclear uptake showed gene expression changes consistent with those found in response to environmental stresses.

## 6.1 Introduction

Oligonucleotide and cDNA microarray-based gene expression assays are a comprehensive and unbiased means to access genome-wide transcriptional responses to xenobiotics or environmental stresses [1]. They have provided valuable insights into the specificity of siRNA-based mediated mRNA degradation in cultured mammalian cells [2-4]. Furthermore, they have been used to uncover genes that are regulated in response to multiple or distinct environmental stresses in *Saccharomyces cerevisiae* [5-8]. This information is especially valuable for studies aimed at discriminating between transcriptional responses to xenobiotics that are due to their designed function or are simply unintended side-effects of cellular stress.

Polyamides are synthetic molecules composed of N-methylpyrrole (Py) and N-methylimidazole (Im) amino acids that can be designed to bind to a large repertoire of DNA sequences with affinities and specificities comparable to those of naturally occurring transcription factors [9]. Properly targeted polyamides have demonstrated competition with numerous transcription factors for DNA binding *in vitro* [10], inhibition and activation of transcription in cell free systems [11-13], and inhibition of viral replication in cell culture [14]. What is less well understood, are the global effect of polyamides on the transcriptome of eukaryotic cells, and whether polyamide target sequence, as determined by Py-Im content, is a factor governing the transcriptional response to this class of molecules [15].

It was recently discovered that eight-ring hairpin polyamides with two positive charges at the C terminus demonstrate potent activity against several fungal strains *in vitro*, and activity against candidiasis in mouse model systems [16]. Transcriptional

interference has been proposed as the likely mechanism of action. Interestingly, polyamides with a beta-alanine dimethylaminopropylamine (β-Dp) group at the C terminus do not have antifungal properties, possibly due to reduced cellular uptake of these compounds.

In order to study the effects of polyamide target sequence on the mRNA expression profile of the model organism *Saccharomyces cerevisiae*, we have used cDNA microarrays to monitor the early transcriptional responses in *S. cerevisiae* grown in sub-toxic doses of eight-ring hairpin polyamides with a C-terminus dication tail. We demonstrate that a Bodipy-labeled hairpin polyamide-dication localizes in the nuclei of *S. cerevisiae*, whereas the Bodipy-labeled polyamide with the corresponding β-Dp tail is excluded. The gene expression profiles for two polyamide-dications that differ by a single atom, and code for DNA sequences that differ by one base pair according to the pairing rules, do not significantly overlap. Though not a proof, this would be consistent with the possibility that regulation of gene expression by cell permeable polyamides may be dependent on sequence specific DNA binding. Remarkably, dication polyamides increase the mRNA levels of many genes normally repressed by environmental stresses while decreasing the mRNA levels of many genes normally induced by these stresses. In contrast, the gene expression profile for the cell impermeable β-Dp polyamide appears to elicit a transcriptional response consistent with other environmental stresses.

## 6.2 Materials and Methods

**Synthesis of DNA-binding Polyamides.** Polyamides **1-5** were synthesized by solid-phase methods as previously described [17-19]**.**

**Cellular Localization Analysis.** Cellular localization analysis of conjugates **4** and **5** were examined using *S. cerevisiae* strain wt 4147 (kindly provided by Judith Campbell). Briefly, yeast were grown in normal YPD medium to a density of 1 x $10^4$ cells per milliliter and added to a 96 well plate with 5 μM **4** or **5** and incubated at 37 °C for 10 hours prior to confocal microscopy. Contents of each well were transferred to glass bottom culture dishes for imaging. Imaging was performed with a Zeiss LSM 5 Pascal inverted confocal microscope, equipped with a 40× oil immersion objective lens [19].

**Strain and Growth Conditions.** *S. cerevisiae* strain BY4743 *MAT***a**/α *his3*Δ*1*/*his3*Δ*1 leu2*Δ*0*/*leu2*Δ*0 met15*Δ*0*/*MET15 lys2*Δ*0*/*LYS2 ura3*Δ*0*/*ura3*Δ*0* (Invitrogen, Carlsbad, CA) has been used for gene expression profiling of responses to diverse chemical treatments in different genetic backgrounds [1,5,20]. Colonies were grown on YAPG agar plates, inoculated into Synthetic Complete (SC) medium with 2% glucose, and grown overnight at 30 ºC. The cultures were diluted to 0.4 - 1.0 X $10^6$ cells/ml and grown an additional five to seven hours until reaching 0.4 - 1.0 X $10^7$ cells per ml [1]. When indicated, polyamides **1** - **3** were added at the beginning of the final five to seven hours of growth [1]. Total RNA was prepared using established protocols [21] using the RNeasy Mini RNA Purification Kit (Qiagen, Valencia, CA) as a final purification step.

**Oligonucleotide Microarray Experiments.** Total RNA (twenty micrograms per sample) were converted into biotin-labeled cRNA using standard protocols recommended by Affymetrix (Santa Clara, CA). For each sample, fifteen micrograms of fragmented cRNA was applied to GeneChip® Yeast Genome S98 microarrays and hybridized twelve to sixteen hours at 45 °C and processed as previously described [22].

**Oligonucleotide Microarray Data Analysis**. GENECHIP version 5.0 software

(Affymetrix) was used to calculate raw gene expression scores. Multiplicative scaling factors based on the median intensity of the 60th to 95th percentile of gene expression scores [22] were used to normalize the hybridization signals from each experiment. All gene expression scores below one hundred were set to one hundred in order to minimize noise associated with weak hybridization signals [22]. The GeneChip® Yeast Genome S98 microarrays contain 9,275 probe tilings that interrogate the abundance of over 6,400 *S. cerevisiae* (S288C strain) transcripts identified in the *Saccharomyces* Genome Database [23] as well as putative open reading frames identified by SAGE analysis, and ORFs from plasmids and other strains. All probe tilings, except those that interrogate tRNAs, rRNAs, snRNAs and transposable elements, were considered for the Venn diagrams in Figure 4. Since there is still debate about the precise number of protein-coding genes in the *S. cerevisiae* genome [24], only those with annotated functions or similarities to confirmed yeast genes were listed in Tables **1 - 3** in order to decrease noise and uninformative results from spurious genes. All raw and processed microarray data is available on our web site: http://lichad.usc.edu.

Eight-ring hairpin polyamide-treated samples and corresponding controls were analyzed using significance analysis of microarrays (SAM) [25,26]. This permutation-based analysis estimates the false discovery rate (FDR) of genes determined to be differentially expressed in response to treatment with eight-ring hairpin polyamides. We report those genes that show a low FDR (1 and 10%, respectively) based on seven hundred twenty permutations as well as a significant (≥1.8-fold) change in gene expression in response to eight-ring hairpin polyamides.

## 6.3 **Results**

It has been demonstrated that polyamides with two positive charges at the C terminus exhibit potent antifungal activity, and that this activity is abolished in structurally related singly charged analogs [16]. We synthesized polyamides **1** and **2** (Figure 6.1) each with two C terminal positive charges. According to the established polyamide pairing rules for these compounds, polyamide **1** binds to the DNA sequence 5'-WGGWCW, and polyamide **2** binds to 5'-WGGCCW, where W is either a T or A pair. Polyamide **3** was synthesized to target the same sequence as **1**, however it has a singly charged β-Dp moiety at the C terminus.

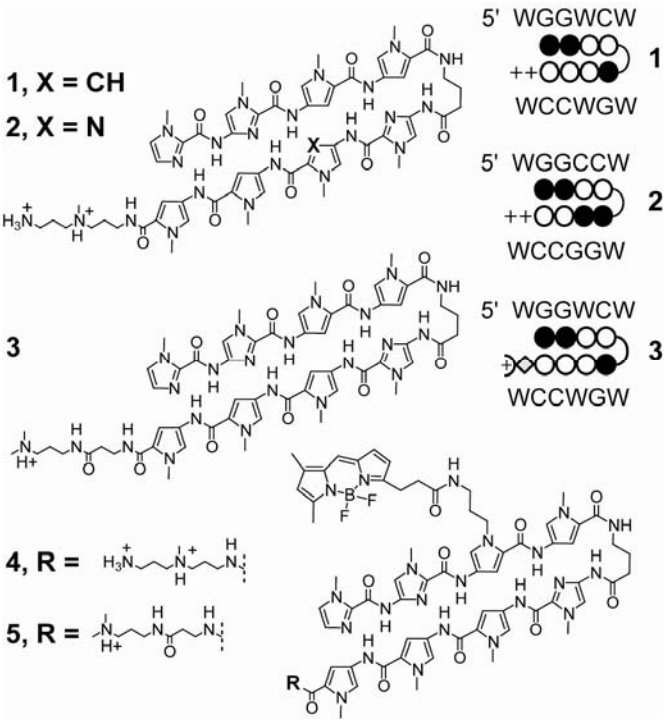


**Figure 6.1** Structures and schematic models of eight-ring hairpin polyamides. Polyamides **1** - **3** were used for gene expression studies. Polyamides **1** (ImImPyPy-γ-ImPyPyPy++) and **2** (ImImPyPy- γ -ImImPyPy++) have two positively charged groups at the C terminus. Polyamide **3** (ImImPyPy- γ -ImPyPyPy-β-Dp ) has a singly charged β-Dp at the C terminus. Im = N-methyl-imidazole, Py = N-methylpyrrole, and γ = γ -aminobutyric acid. Polyamide-Bodipy conjugates **4** and **5** were used for cell uptake and localization experiments. Polyamide conjugate **4** has a doubly charged and conjugate **5** a singly charged C terminus.

We measured the growth inhibitory effects on *S. cerevisiae* of polyamides **1, 2**, and **3** (Figure7.2). Doubly charged polyamides **1** and **2** inhibit growth by 30-50% at concentrations as low as 300 nM (0.36 μg/mL), while singly charged polyamide **3** has no measurable effect up to 1 μM (Figure 2), and even 10 μM (data not shown), consistent with previous work.

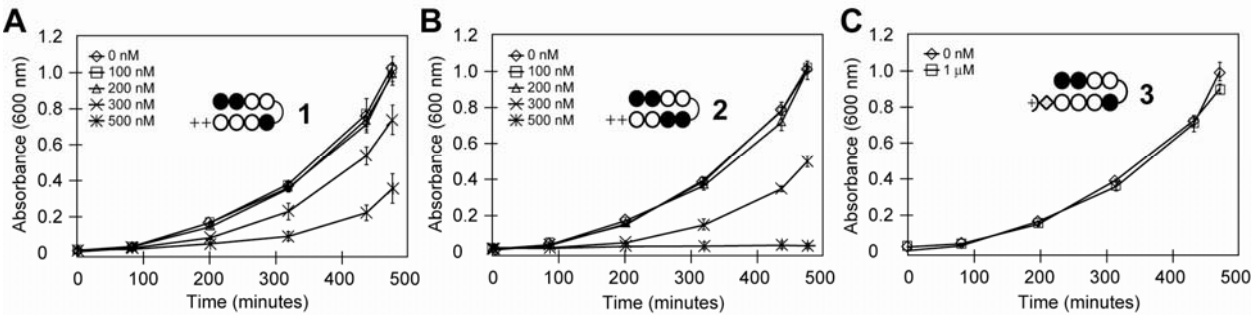


**Figure 6.2** Effects of eight-ring hairpin polyamides on growth rates. *S. cerevisiae* were grown in the presence of 100, 200, 300, and 500 nM of (*A*) polyamide 1 or (*B*) polyamide 2. (*C*) *S. cerevisiae* were grown in the presence of 1.0 μM polyamide 3. All experiments were performed in triplicate with the average $A_{600}$ reading provided along with error bars representing one standard deviation.

In order to determine whether differences in cellular uptake were consistent with growth inhibition, polyamide-Bodipy conjugates **4** and **5** were synthesized (Figure 6.1) and their cellular localization analyzed by confocal microscopy (Figure 3). Conjugates **4** and **5** have the same DNA binding functionalities and C terminal structures as polyamides **1** and **3**, respectively. Conjugate **4** appears to localize in the nuclei of live *S. cerevisiae*, while conjugate **5** is excluded from the nucleus (Figure 7.3), consistent with the antifungal activities of polyamide **1** and inactivity of **3**.

In order to determine transcriptional responses towards polyamides **1** and **2** in the absence of cellular toxicity, we measured gene expression profiles of these polyamides at the highest concentrations (100 and 200 nM) that had minimal effect on growth rate.

Similarly, we measured gene expression responses to 1 μM polyamide **3**, also a concentration without measurable growth inhibition. We used significance analysis of microarrays (SAM) [25] to determine the number of genes that were differentially expressed at least 1.8-fold with a less than 10% false discovery rate (FDR) in response to each compound relative to untreated control (methods).

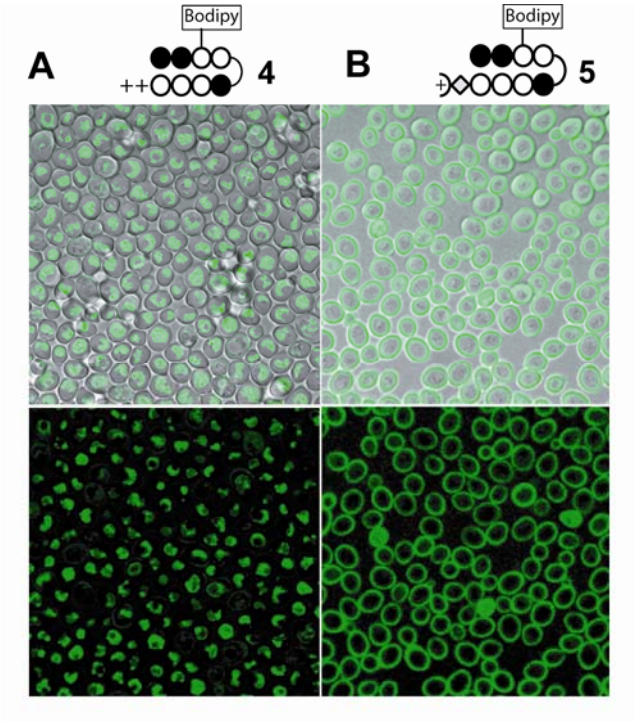


**Figure 6.3** Localization of polyamide-Bodipy conjugates. (*A*) Localization of doubly charged conjugate 4 in live yeast, (*bottom*) fluorescent image, (*upper*) bright field/fluorescent merged image. (*B*) Localization of singly charged conjugate 5 in live yeast, (*bottom*) fluorescent image, (*upper*) bright field/fluorescent merged image.

We first considered data from all 9,275 probe tilings in the Yeast Genome S98 microarray in order to obtain the broadest overview of differential gene expression (Figure 6.4). These tilings interrogate annotated genes with experimental proof of existence as well as those with weak and often conflicting evidence based on computational analysis (Methods). Thirty-six and ninety one probe tilings indicated

differential gene expression in response to 100 nM polyamide **1** and **2**, respectively (Figure 6.4*A*). Only five tilings (YAL046C, *FMC1*, *XBP1*, YPL222W, and a non-annotated genomic sequence) are in common between these groups. Growth in 200 nM polyamide **1** and **2** resulted in eight and two probe tilings indicating differential gene expression, respectively (Figure 6.4*B*). None of the above described probe tilings were held in common between 200 nM polyamides **1** and **2** (Figure 6.4*B*). Twenty eight tilings indicated differential gene expression in response to 1 μM polyamide **3** (Figure 6.4). Cultures treated with 1 μM polyamide **3** had two tilings (*NCE103* and YDR336W) in common with 100 nM polyamide **1**, one tiling (*CPD1*) in common with 100 nM polyamide **2**, and one tiling (*RPS26A*) in common with 200 nM polyamide **1**.

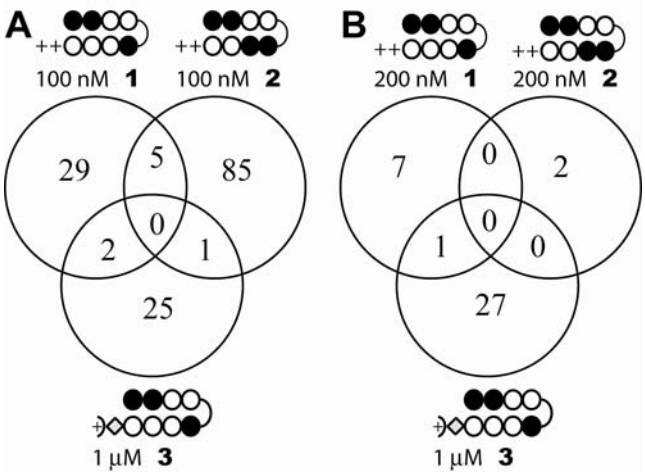


**Figure 6.4** Transcriptional responses to hairpin eight-ring polyamides. Venn diagrams of all genes at least 1.8-fold differentially expressed (less than 10% false discovery rate) in response to (*A*) 100 nM polyamides **1** and **2** and 1.0 μM polyamide **3** or (*B*) 200 nM polyamides **1** and **2** and 1 μM polyamide **3** are shown.

A comparison of the differentially expressed genes by polyamides **1** and **2** reveals the importance of polyamide target sequence on the transcriptional response. A single atom change between **1** and **2**, which changes specific DNA binding from 5'-WGGWCW

to 5'-WGGCCW for **1** and **2**, respectively, drastically alters the observed gene regulation pattern.

The identities of probe tilings indicating differentially expressed genes is also strongly influenced by polyamide concentration in the growth media. Interestingly, considerably more genes were differentially expressed in response to polyamides **1** and **2** at low (100 nM) relative to high (200 nM) concentrations. Only two probe tilings indicating differentially expressed genes (*RPL4A* and YAL046C) were held in common between 100 and 200 nM **1**. Similarly, no probe tilings were held in common for treatment with 100 and 200 nM **2**.

Next, we focused our attention on more closely examining the functions of differentially expressed genes for which there is strong experimental or computational proof of existence (Tables 6.1 – 6.3) (Methods). We compared the expression patterns of the differentially expressed genes in response to polyamides **1** - **3** (Tables 6.1 – 6.3) to what is found in response to environmental stresses such as heat shock, acid, base, and salt treatment, osmotic shock, and diauxic shifts [6,7]. Interestingly, genes induced by treatment with 100 nM polyamides **1** or **2** were frequently repressed in response to common environmental stresses (e.g. *RPO31*, *ILV5*, *CYB5*, *UTP10*, *PHO3*, *RNR1*, *HAM1*, *BUD19*, and *NEW1*) (Tables 1 and 2). Conversely, genes repressed by treatment with 100 nM polyamides **1** or **2** were frequently induced in response to common environmental stresses (e.g. *XBP1*, *NCE103*, *FIS1*, YGR043C, *GUT2*, *HXT5*, and *DCS2*) (Tables 6.1 and 6.2). Interestingly, the *XBP1* gene, a stress induced transcriptional repressor, is held in common between 100 nM polyamides **1** and **2**.

Several genes involved in glycolytic and glyoxylate pathways [6] such as *GDB1*,

*JEN1*, *FBP1*, and *CIT2*, are differentially expressed in response to polyamide **3**. In contrast to 100 nM polyamides **1** and **2**, the differentially expressed genes show more similar patterns of expression relative to environmental stress responses (Table 6.3). For example, *RPS26A*, *GDB1*, and *FBP1* are up-regulated both in response to polyamide **3** as well as other environmental stresses. Likewise, *RPL7B*, *NOG1*, *NOG2*, *CIC1*, and *ERB1* are down-regulated in response to polyamide **3** as well as other environmental stresses. Nevertheless, there were exceptions to these observations (i.e. *HSP30*, *TPO2*, and *NCE103*).

We next examined whether any of these differentially expressed genes contained an over-abundance of the preferred binding sites for polyamides **1**, **2**, or **3** in their upstream regulatory regions. In only one case (*FMC1*) were there a statistically significant increased number of polyamide **1** binding sites (nine sites present) within one kilobase upstream of the predicted start codon ($p<0.05$). However, *FMC1* was also differentially expressed in response to 100 nM polyamide **2**, but does not have polyamide **2** binding sites in this same region. However, such an analysis is expected to yield at best limited information, given that specific interactions of transcription factors for every gene are not thoroughly characterized, and, moreover, since the regulation of a particular gene by a polyamide may be indirect, acting through a protein whose expression itself was effected upstream.

**Discussion**

Polyamides **1** and **2**, like those characterized by Marini et al, are eight-ring polyamides with two C terminal positive charges and exhibit marked activity against *S.*

*cerevisiae* [16]. However, the structure of the doubly charged C terminus of polyamides **1** and **2** differs. Previously characterized dication polyamides possessed a branched C terminus consisting of aspartic acid joined with two Dp residues, -(Dp)-Asp-Dp. Polyamides **1** and **2** obtain their positive charges from a linear group consisting of a methyl substituted tertiary amine and a terminal primary amine separated by three methylene carbons. It is interesting to note that the antifungal properties of polyamides **1** and **2** are conserved even though the structure of the C terminal groups of these two sets of polyamides are significantly different.

The localization of polyamide-Bodipy conjugate **4** (Figure 7.3) in the nuclei of live yeast, and the apparent failure of yeast nuclei to take up conjugate **5**, sheds light on the antifungal activity of **1** and **2,** and lack of activity of **3**. Given the similar structures of conjugates **4** and **5**, it may be surprising that the cellular localization would be different. However, subtle differences in polyamide structure have been observed to affect significant differences in cellular localization in other studies [27].

Previously, it has been shown that the antifungal effects of doubly charged polyamides are dependent upon the number of binding sites in the *S. cerevisiae* genome as well as the ploidy of the strain [16]. Additionally, yeast deletion experiments were more consistent with discrete sites of action, rather than a global effect, for these polyamides. This suggests that the mechanistic basis for growth inhibition involves interference with DNA-dependent processes, such as transcription or replication.

We found that there is a limited transcriptional response to eight-ring hairpin polyamides **1** and **2** at 100 nM and 200 nM (the highest concentrations that do not affect growth). This data is summarized in Figure 6.4, and Tables 6.1 and 6.2. The relatively

limited effect on global gene expression by polyamides is consistent with studies in mammalian systems [15,28]. Polyamides **1** and **2** target the DNA sequences 5'-WGGWCW and 5'-WGGCCW, respectively. This difference in binding site arises from a single atom change from a carbon to a nitrogen at the third residue from the C terminus, allowing acceptance of a hydrogen bond from the exocyclic amine of guanine in the minor groove of DNA at a GC base pair [29]. Binding of polyamide **1** to the match site for **2** is expected to be 50-100 fold less than for its own match site. Although polyamides **1** and **2** differ only by a single atom, they displayed distinct sets of differentially expressed genes (Figures 6.1 and 6.4). The lack of overlap between differentially expressed genes by **1** and **2** at 100 nM suggests that gene regulation by polyamides could be dependent upon DNA target sequence and that the gene regulation may be the result of specific polyamide-DNA interactions. Furthermore, the localization of polyamide-Bodipy conjugate **4** in the nucleus of yeast lends further supports to a mechanism taking place in the nucleus, such as interactions with DNA and the transcriptional machinery. However, we must emphasize that interactions with other cellular components cannot be ruled out as an alternative explanation.

At 100 nM dosage, cell permeable polyamides **1** and **2** show differential gene expression of stress response genes in the opposite direction than what has been observed in response to heat, osmotic, and oxidative stresses (Tables 6.1 – 6.2) [6]. This also suggests that the expression of environmental stress response genes in *S. cerevisiae* can be repressed as well as induced relative to basal levels. Interestingly, others have found that eight-ring polyamides down-regulate the DNA damage-inducible transcript 3 (*GADD153*) in cultured human colon cancer cell lines [28]. This suggests that subclasses of

eight-ring hairpin polyamides can invert the normal regulation of certain environmental stress-response genes in different biological systems. It also suggests that these polyamides do not cause substantial DNA damage in our experiments. This would be in agreement with studies showing that *S. cerevisiae* strains with increased sensitivity to agents that disrupt chromosomal integrity are not hyper-sensitive to eight-ring hairpin polyamides [16].

Due to their similar concentration dependent inhibitory effects, it appears likely that polyamides **1** and **2** share an important mechanism for growth inhibition. This could be due to the occupancy of a critical number of sequence tracts that lead to an inhibition of important DNA-dependent processes influencing the nuclear and/or the mitochondrial genome. The match sites for polyamides **1** and **2** appear 38,822 and 8,659 times, respectively, in the *S. cerevisiae* genome (*Saccharomyces* Genome Database webserver, PatMatch search engine, http://www.yeastgenome.org/). However, it is currently unknown how many of these optimal binding sites are accessible to polyamide binding, or are critical for gene expression.

One possible explanation for the antifungal properties of polyamides **1** and **2** is the down-regulation of stress response pathway genes, thereby leaving the cells more susceptible to toxicity. Interestingly, a similar phenomenon has been observed in mammalian cells in response to treatment with genestein, a potent inhibitor of tyrosine kinase activity [30]. It has been proposed that, in part, the anti-cancer effects of genestein could be related to the repression of stress-response genes. If so, then eight-ring hairpin polyamides could be of use in further dissecting and modulating stress response pathways that have therapeutic relevance.

The transcriptional response to polyamide **3** at 1.0 μM (Figure 6.4 and Table 6.3) is consistent with a mechanism not involving direct binding to DNA and is supported visually by confocal microscopy. The preponderance of genes up-regulated in response to this polyamide are also up-regulated in response to other environmental stresses, and those down-regulated are similarly down-regulated in other environmental stresses. In this case, several of the differentially expressed genes appear to be involved in carbohydrate metabolism and are regulated in a similar fashion in responses to diauxic shifts [6]. These observations are consistent with the localization of polyamide-Bodipy conjugate **5** outside of yeast nuclei. Therefore, it appears that the previously observed reversal of environmental stress-related responses by 100 nM polyamides **1** and **2** are likely due to interactions between the eight-ring polyamides and intercellular components, such as genomic DNA. Unexpectedly, there was a strong concentration dependence on gene expression responses to polyamides **1** and **2** with lower dosages (100 nM) providing considerably more differentially expressed genes than higher dosages (200 nM) (Figure 6.4). This suggests complex interactions between polyamides with multiple DNA binding sites and other cellular components that affect stress responses and other biochemical pathways. Because of this ambiguity, we are currently running experiments at the 50 nM concentration.

Overall, this study suggests that the transcriptional states of surprisingly few genes appear to be effected by eight-ring hairpin polyamides, despite the large number of potential binding sites throughout the yeast genome. A tightly regulated transcriptional apparatus that is not easily perturbed by minor groove binding ligands, or inaccessibility of much of the genetic material could account for this limited effect. However, despite

the striking structural similarity of polyamides **1** and **2**, which are two nearly identically shaped molecules that differ in only one atom out of a molecular weight of almost 1200 daltons, it is still possible that the observed changes in gene expression are due to mechanisms other than direct polyamide-DNA binding. Clearly, further genomic studies of a more diverse library of polyamides are needed to more adequately characterize the genetic targets, mechanisms of action, and limitations of these chemical DNA binding probes.

**Table 6.1** Expression changes of annotated genes in response to polyamide **1**.

| | | | Expression Level* | | | Stress† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Symbol | Biological Process | Control | Test | FC | H | A | B | Ox | S | Os | D |
| 100 nM | RPO31 | RNA polymerase III large subunit | 100 | 202 | 2.0‡ | | | | - | - | - | - |
| | NRD1 | Control of pre-mRNA accumulation | 161 | 317 | 2.0 | | | - | - | | + | |
| | BIO5 | Vitamin/cofactor transport | 1316 | 2676 | 2.0 | | | | + | | | |
| | YML023C | Required for viability / similar to Nmd2p | 109 | 201 | 1.9‡ | | | - | - | - | - | |
| | MNT4 | Carbohydrate metabolism | 123 | 225 | 1.8 | | | + | | | | |
| | ILV5 | Amino acid biosynthesis / mtDNA stability | 1304 | 2482 | 1.9 | | | - | - | - | - | - |
| | XBP1 | Stress induced transcription repressor | 499 | 230 | -2.2 | + | | + | + | | + | + |
| | YFL052W | Similar to Mal63p, YPR196w and Mal13p | 238 | 116 | -2.1‡ | | | | | | | |
| | RPL4A | Ribosomal protein | 1054 | 501 | -2.1 | | | | - | | | - |
| | SRB6 | Part of Srb/Mediator transcription complex | 223 | 111 | -2.0 | | | + | | + | | |
| | YLR327C | Similar to Stf2p | 3075 | 1661 | -1.9‡ | + | + | + | + | + | + | + |
| | CBP6 | Translational activator of cytochrome b | 277 | 143 | -1.9 | | | | | + | m | |
| | FMC1 | Involved in assembly of ATP synthase | 291 | 152 | -1.9 | | | | | + | + | + |
| | SMP2 | Plasmid maintenance / aerobic respiration | 425 | 218 | -1.9 | | | - | - | | | |
| | HSE1 | Mediates endosomal protein sorting | 282 | 150 | -1.9 | - | + | + | | + | + | |
| | INO4 | Transcription factor | 235 | 125 | -1.9 | + | | - | + | | | |
| | 4001 at§ | Cytochrome-c oxidase subunit I | 674 | 362 | -1.9 | | | | | | | |
| | YRB2 | Part of the nuclear pore complex | 269 | 144 | -1.9 | + | | | | + | + | |
| | HUL5 | Ubiquitin-protein ligase | 352 | 187 | -1.9 | + | | | | + | m | |
| | NCE103 | Non-classical protein export | 1979 | 1021 | -1.9 | + | | + | + | + | + | - |
| | SCO2 | Copper transport | 566 | 295 | -1.9 | + | | + | | + | + | |
| | FIS1 | Mitochondrial division | 415 | 230 | -1.8 | + | | + | | + | + | - |
| | PHO89 | $Na^+/P_i$ symporter | 433 | 237 | -1.8 | - | - | + | - | | | |
| 200 nM | RPS26A | Ribosomal protein | 10885 | 25109 | 2.3 | + | + | + | - | + | + | - |
| | GPI2 | GPI anchor biosynthesis | 109 | 205 | 1.9‡ | | | | | | | |
| | RPL4A | Ribosomal protein | 1054 | 485 | -2.2‡ | | | | - | | | - |
| | BSD2 | Copper transporter | 264 | 138 | -1.9‡ | | | + | | + | + | |

False discovery rate (FDR) <10%. ORF, open reading frame; FC, fold change.

*Average expression level of three independent experiments.

†Stress, H: heat, A: acid, B: base, Ox: $H_2O_2$, S: salt, Os: sorbitol (ref. 21). D: diauxic shift (ref. 20). +, indicates an induction of >3 in at least one time point. -, indicates a repression of >3 in at least one time point. m, indicates a mixed response across time points.

‡Significant at FDR <1%.

§Affymetrix probe ID.

**Table 6.2** Expression changes of annotated genes in response to polyamide **2**.

| | | | Expression Level* | | | Stress† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Symbol | Biological Process | Control | Test | FC | H | A | B | Ox | S | Os | D |
| 100 nM | CYB5 | Cytochrome b5 | 287 | 731 | 2.5 | | | - | - | - | - | - |
| | UTP10 | Pre-rRNA processing | 204 | 503 | 2.5 | | | - | - | - | - | - |
| | ICY2 | Chromatin structure / nuclear transport | 875 | 2171 | 2.5 | | | | + | | - | |
| | YFL067W | Similar to mouse period clock protein | 101 | 244 | 2.4 | | | | | | | |
| | POL30 | DNA polymerase processivity factor activity | 230 | 495 | 2.2 | | | | - | - | | |
| | PHO3 | Acid phosphatase | 501 | 978 | 2.0 | - | | - | - | - | - | - |
| | RNR1 | Ribonucleotide reductase | 534 | 1084 | 2.0 | - | | - | - | - | - | - |
| | BUD28 | Bud site selection | 100 | 202 | 2.0 | + | | + | | - | - | - |
| | HAM1 | Controls 6-N-hydroxylaminopurine sensitivity | 129 | 248 | 1.9 | | | | - | - | - | |
| | YER130C | Similar to Msn2p and Msn4p | 135 | 255 | 1.9 | - | | | | + | + | |
| | BUD19 | Bud site selection | 140 | 269 | 1.9 | | | | | - | - | - |
| | NEW1 | Possible ABC transporter | 1363 | 2520 | 1.8 | | | | - | - | - | - |
| | RPL22B | Ribosomal protein | 173 | 313 | 1.8 | | | | | | | |
| | YGR043C | Similar to transaldolase | 354 | 160 | -2.2 | + | + | + | + | + | + | + |
| | SPG1 | Cell division | 240 | 108 | -2.2 | | | | | + | + | + |
| | HSL7 | Bud formation | 325 | 151 | -2.2 | | | - | - | | | |
| | GUT2 | Mitochondrial GPD | 604 | 285 | -2.1 | + | | + | + | + | + | |
| | XBP1 | Stress induced transcriptional repressor | 499 | 236 | -2.1 | + | | + | | + | + | + |
| | YNL194C | Similar to YDL222c and Sur7p | 3279 | 1667 | -2.0 | + | | | + | + | + | + |
| | DDR2 | DNA damage/stress response | 4502 | 2265 | -2.0 | | | | | | | + |
| | SUC4 | Sucrose catabolism | 949 | 468 | -2.0 | | | | | | | |
| | CPD1 | Chromatin structure | 274 | 139 | -2.0 | - | | | | - | - | |
| | YDL222C | Cell wall organization | 651 | 326 | -2.0 | + | + | + | + | m | + | |
| | BEM3 | Bud growth | 498 | 245 | -2.0 | | | | - | - | - | |
| | FMC1 | Involved in assembly of ATP synthase | 291 | 152 | -1.9 | | | | | + | + | + |
| | YPT10 | Golgi organization and biogenesis | 414 | 221 | -1.9 | | | - | | | | |
| | PIG2 | Regulator of protein phosphatase | 403 | 216 | -1.9 | + | | | + | + | + | |
| | HXT5 | Hexose transport | 685 | 374 | -1.8 | + | | + | + | + | + | + |
| | DCS2 | Regulated by Msn2/4p | 1453 | 807 | -1.8 | + | + | + | + | + | + | |
| | PHR1 | Photolyase | 206 | 113 | -1.8 | + | | | | | m | + |
| | RPL7B§ | Ribosomal protein | 645 | 1192 | 1.8‡ | | | - | - | - | - | - |

False discovery rate (FDR) <10%. ORF, open reading frame; FC, fold change.

*Average expression level of three independent experiments.

†Stress, H: heat, A: acid, B: base, Ox: $H_2O_2$, S: salt, Os: sorbitol (ref. 21). D: diauxic shift (ref. 20). +, indicates an induction of >3 in at least one time point. -, indicates a repression of >3 in at least one time point. m, indicates a mixed response across time points.

‡Significant at FDR <1%.

§Differentially expressed in response to 200 nM of polyamide **2**.

**Table 6.3** Expression changes of annotated genes in response to polyamide **3**.

| | | Expression Level* | | | Stress† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Symbol | Biological Process | Control | Test | FC | H | A | B | Ox | S | Os | D |
| ADH2 | Alcohol dehydrogenase II | 163 | 397 | 2.4 | | | | | - | | |
| MUP3 | Methionine uptake | 193 | 446 | 2.3 | | | + | - | + | + | |
| RPS26A | Ribosomal protein | 10885 | 22595 | 2.1 | + | + | + | - | + | + | - |
| JEN1 | Lactate transporter | 585 | 1123 | 1.9 | + | - | | - | | | + |
| GDB1 | Glycogen debranching enzyme | 1674 | 3105 | 1.9 | + | + | + | + | - | + | + |
| GAP1 | General amino acid permease | 362 | 658 | 1.8 | | | + | | | | |
| FBP1 | Fructose-1,6-bisphosphatase | 153 | 279 | 1.8 | + | - | + | | + | | + |
| HSP30 | Heat shock protein | 4796 | 1886 | -2.5 | - | + | | + | + | + | + |
| ICY2 | Chromatin structure / nuclear transport | 874 | 370 | -2.4 | | | | + | | - | |
| TPO2 | Polyamine transport | 2650 | 1224 | -2.2‡ | + | + | + | + | + | + | + |
| NCE103 | Non-classical protein export | 1979 | 961 | -2.1‡ | + | | + | + | + | + | - |
| MAE1 | Mitochondrial malic enzyme | 553 | 260 | -2.1 | | | | - | - | - | |
| CPD1 | Chromatin structure | 274 | 130 | -2.1 | - | | | | - | - | |
| RIM8 | Regulator of sporulation | 223 | 110 | -2.0‡ | + | + | | | + | + | |
| RPL7B | Ribosomal protein | 645 | 330 | -2.0‡ | | | - | - | - | - | - |
| UPC2 | Sterol uptake | 288 | 143 | -2.0‡ | | | | | | | |
| FUI1 | Uridine transport | 464 | 231 | -2.0 | + | | + | - | - | - | |
| NOG1 | Nucleolar GTP-binding protein 1 | 396 | 201 | -2.0 | | | - | - | - | - | - |
| NOG2 | Nucleolar GTP-binding protein 2 | 257 | 131 | -2.0 | | | | - | - | - | - |
| FCY2§ | Purine transport | 1289 | 661 | -1.9‡ | | | | - | - | - | - |
| CIC1 | Ribosome biogenesis protein | 198 | 106 | -1.9‡ | | | | - | - | - | - |
| ERB1 | rRNA processing | 479 | 264 | -1.8‡ | | | - | - | - | - | - |
| RPR1 | tRNA processing | 800 | 435 | -1.8‡ | | | | | | | |
| CIT2 | Citrate synthase | 1692 | 936 | -1.8 | - | m | + | - | + | | + |

False discovery rate (FDR) <10%. ORF, open reading frame; FC, fold change.

*Average expression level of 3 independent experiments.

†Stress, H: heat, A: acid, B: base, Ox: $H_2O_2$, S: salt, Os: sorbitol (ref. 21), D: diauxic shift (ref. 20). +, indicates an induction of >3 in at least one time point. -, indicates a repression of >3 in at least one time point. m, indicates a mixed response across time points.

‡Significant at FDR <1%.

§Another probe tiling a fold change of -2.1.

**References**


1. Hughes, T. R. et al. Functional discovery via a compendium of expression profiles. *Cell* 102, 109-126 (2000).
2. Chi, J. T. et al. Genomewide view of gene silencing by small interfering RNAs. *Proceedings of the National Academy of Sciences of the United States of America* 100, 6343-6346 (2003).
3. Jackson, A. L. et al. Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnology* 21, 635-637 (2003).
4. Semizarov, D. et al. Specificity of short interfering RNA determined through gene expression signatures. *Proceedings of the National Academy of Sciences of the United States of America* 100, 6347-6352 (2003).
5. Giaever, G. et al. Functional profiling of the Saccharomyces cerevisiae genome. *Nature* 418, 387-391 (2002).
6. DeRisi, J. L., Iyer, V. R. & Brown, P. O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278, 680-686 (1997).
7. Causton, H. C. et al. Remodeling of yeast genome expression in response to environmental changes. *Molecular Biology of the Cell* 12, 323-337 (2001).
8. Gasch, A. P. et al. Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell* 11, 4241-4257 (2000).
9. Dervan, P. B. & Edelson, B. S. Recognition of the DNA minor groove by pyrrole-imidazole polyamides. *Current Opinion in Structural Biology* 13, 284-299 (2003).
10. Gottesfeld, J. M., Turner, J. M. & Dervan, P. B. Chemical approaches to control gene expression. *Gene Expression* 9, 77-91 (2000).

11. Gottesfeld, J. M., Neely, L., Trauger, J. W., Baird, E. E. & Dervan, P. B. Regulation of gene expression by small molecules. *Nature* 387, 202-205 (1997).

12. Mapp, A. K., Ansari, A. Z., Ptashne, M. & Dervan, P. B. Activation of gene expression by small molecule transcription factors. *Proceedings of the National Academy of Sciences of the United States of America* 97, 3930-3935 (2000).

13. Arora, P. S., Ansari, A. Z., Best, T. P., Ptashne, M. & Dervan, P. B. Design of artificial transcriptional activators with rigid poly-L-proline linkers. *Journal of the American Chemical Society* 124, 13067-13071 (2002).

14. Dickinson, L. A. et al. Inhibition of RNA polymerase II transcription in human cells by synthetic DNA-binding ligands. *Proceedings of the National Academy of Sciences of the United States of America* 95, 12890-12895 (1998).

15. Dudouet, B. et al. Accessibility of nuclear chromatin by DNA binding polyamides. *Chemistry & Biology* 10, 859-867 (2003).

16. Marini, N. J. et al. DNA binding hairpin polyamides with antifungal activity. *Chemistry & Biology* 10, 635-644 (2003).

17. Belitsky, J. M., Nguyen, D. H., Wurtz, N. R. & Dervan, P. B. Solid-phase synthesis of DNA binding polyamides on oxime resin. *Bioorganic & Medicinal Chemistry* 10, 2767-2774 (2002).

18. Baird, E. E. & Dervan, P. B. Solid phase synthesis of polyamides containing imidazole and pyrrole amino acids. *Journal of the American Chemical Society* 118, 6141-6146 (1996).

19. Belitsky, J. M., Leslie, S. J., Arora, P. S., Beerman, T. A. & Dervan, P. B. Cellular uptake of N-methylpyrrole/N-methylimidazole polyamide- dye

conjugates. *Bioorganic & Medicinal Chemistry* 10, 3313-3318 (2002).

20. Winzeler, E. A. et al. Functional characterization of the S-cerevisiae genome by gene deletion and parallel analysis. *Science* 285, 901-906 (1999).

21. Ausubel, F. M. *Current protocols in molecular biology* (Greene Publishing Associates, Brooklyn, N. Y., 1987).

22. Karaman, M. W. et al. Comparative analysis of gene-expression patterns in human and African great ape cultured fibroblasts. *Genome Research* 13, 1619-1630 (2003).

23. Weng, S. et al. Saccharomyces Genome Database (SGD) provides biochemical and structural information for budding yeast proteins. *Nucleic Acids Research* 31, 216-218 (2003).

24. Mackiewicz, P. et al. How many protein-coding genes are there in the Saccharomyces cerevisiae genome? *Yeast* 19, 619-29 (2002).

25. Tusher, V. G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Sciences of the United States of America* 98, 5116-5121 (2001).

26. Piper, M. D. W. et al. Reproducibility of oligonucleotide microarray transcriptome analyses - An interlaboratory comparison using chemostat cultures of Saccharomyces cerevisiae. *Journal of Biological Chemistry* 277, 37001-37008 (2002).

27. Best, T. P., Edelson, B. S., Nickols, N. G. & Dervan, P. B. Nuclear localization of pyrrole-imidazole polyamide-fluorescein conjugates in cell culture. *Proceedings of the National Academy of Sciences of the United States of America* 100, 12063-

12068 (2003).

28. Supekova, L. et al. Genomic effects of polyamide/DNA interactions on mRNA expression. *Chemistry & Biology* 9, 821-827 (2002).

29. Kielkopf, C. L., Baird, E. E., Dervan, P. B. & Rees, D. C. Structural basis for G.C recognition in the DNA minor groove. *Nature Structural Biology* 5, 104-9 (1998).

30. Zhou, Y. H. & Lee, A. S. Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy. *Journal of the National Cancer Institute* 90, 381-388 (1998).