Global analysis of RNA oxidation in *Saccharomyces cerevisiae*

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Oxidative RNA damage has been linked to loss of RNA function and to the development of many human age-related diseases. Consequently, a need exists for methods to identify and quantify the extent of RNA oxidation on a genome-wide basis. We developed such a method by combining affinity selection of mRNA containing 8-hydroxyguanine with high throughput DNA sequencing. We demonstrate that this assay is suitable for detecting differences in the extent of oxidation between RNA transcripts. We applied this method to the yeast *Saccharomyces cerevisiae* grown under physiological conditions and in response to hydrogen peroxide, and detected significantly oxidized RNA transcripts.

Table 1. Sequencing data acquisition and mapping statistics

<table>
<thead>
<tr>
<th>Library</th>
<th>Reads acquired</th>
<th>Reads mapped</th>
<th>Percent mapped</th>
<th>Unique, non-rRNA reads</th>
<th>Unique, non-rRNA reads (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized RNA</td>
<td>64,581,760</td>
<td>57,775,230</td>
<td>89.46%</td>
<td>19,008,947</td>
<td>32.90%</td>
</tr>
<tr>
<td>Total RNA</td>
<td>68,738,128</td>
<td>61,787,617</td>
<td>89.89%</td>
<td>19,678,020</td>
<td>31.84%</td>
</tr>
<tr>
<td>Oxidized RNA + H$_2$O$_2$</td>
<td>75,142,091</td>
<td>65,930,518</td>
<td>87.74%</td>
<td>17,592,399</td>
<td>26.68%</td>
</tr>
<tr>
<td>Total RNA + H$_2$O$_2$</td>
<td>83,896,685</td>
<td>72,330,257</td>
<td>86.21%</td>
<td>23,539,861</td>
<td>32.54%</td>
</tr>
</tbody>
</table>

To define mappable bases we identified all unique 36 base sequences from both strands of the reference assembly. We defined mappable bases as all positions covered by these sequences.
the immunoprecipitation procedure using beads alone. In contrast to elution from the antibody-coupled beads, which yielded ~400 ng of RNA, the elutions from the negative control beads yielded ~100 ng or less of lower quality RNA, suggesting that <5% of the RNA applied to control beads bound. Approximately 50 ng of oxidized RNA and mRNA-enriched total RNA were fragmented (Ambion Fragmentation kit, Ambion) and used for strand-specific Illumina sequencing library preparation (10).

Strand-specific Illumina sequencing libraries were constructed from RNA isolated from two independent cultures grown in yeast extract-peptone dextrose (YPD) medium at 30 °C to exponential growth phase (OD400 = 0.5–0.6). The cultures had either been left untreated, or were treated prior to harvest for 15 min with 0.5 mM hydrogen peroxide. After aligning reads to the genome, we filtered out low quality alignments and rRNA sequences. The reproducibility between the two biological replicates of immunoprecipitated oxidized RNA libraries, and between the replicates of the total RNA libraries, was robust (Pearson’s r2 > 0.99). Therefore, we merged reads from biological replicates and obtained four data sets: total RNA from the untreated and hydrogen peroxide-treated yeast, and oxidized RNA from the untreated and hydrogen peroxide-treated yeast (Table 1).

Of the 6,300 yeast transcript models, ~40%–55% were significantly expressed in either total or oxidized RNA libraries, as measured against the background from intergenic regions using a Poisson distribution (P < 0.01) (Table 2). For each significantly expressed yeast transcript in each data set, we calculated an RNA oxidation index as the ratio of normalized reads in the oxidized RNA to those in the total RNA. We defined significantly oxidized transcripts as transcripts with read density in an oxidized RNA sample significantly higher than in the corresponding total RNA sample using a Poisson exact test (Q-value cutoff of 0.001). This analysis revealed that 14% and 19% of the yeast transcripts were significantly oxidized in the untreated sample and hydrogen peroxide-treated sample, respectively (Table 2).

Approximately 91% (815 of 892) of the transcripts found to be oxidized in untreated yeast were also identified from yeast subjected to oxidative stress, suggesting that these transcripts might possess features that make them vulnerable to physiological levels of free radicals. Treatment with hydrogen peroxide resulted in an additional 407 transcripts classified as significantly oxidized. Approximately 9% (77 of 892) of the transcripts were detected as oxidized only under physiological growth conditions. Some of these transcripts may represent nonspecifically bound RNAs, or they may carry the 8-OHG modification to regulate a step in RNA metabolism, such as stability, splicing, transport, or translational efficiency.

### Table 2. Significantly expressed and significantly expressed oxidized transcripts

<table>
<thead>
<tr>
<th>Library</th>
<th>Significantly expressed</th>
<th>Significantly expressed &amp; oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized RNA</td>
<td>2,921 (46.40%)</td>
<td>892 (14.16%)</td>
</tr>
<tr>
<td>Total RNA</td>
<td>3,454 (54.80%)</td>
<td></td>
</tr>
<tr>
<td>Oxidized RNA + H₂O₂</td>
<td>2,444 (38.79%)</td>
<td>1,222 (19.40%)</td>
</tr>
<tr>
<td>Total RNA + H₂O₂</td>
<td>3,451 (54.78%)</td>
<td></td>
</tr>
</tbody>
</table>
We examined the effect of oxidative stress on the overlapped set of 815 yeast transcripts identified as oxidized. We predicted that these transcripts overall should be more oxidized in response to H_2O_2 treatment. For each transcript, we calculated a ratio of its oxidative index in the H_2O_2-treated sample to that in the untreated sample. This analysis revealed that 594 out of the 815 (73%) transcripts had a ratio >1 (with all but one transcript having a ratio below 2), suggesting that these are sensitive to oxidative stress, whereas for 221 (27%) of the transcripts, the ratio fell below 1.

We explored the correlation between the extent of RNA oxidation and features of the RNA. To examine the correlation with RNA folding, we determined RNA folding energies after normalizing the minimal free energy of RNA folding (ΔG) (11) by transcript length (ΔG/length) (12). This analysis revealed a modest correlation (Spearman ρ < -0.372) between RNA oxidation index and ΔG/length, indicating that highly structured RNAs are less oxidized by ROS. Susceptibility to oxidative damage may be affected by RNA subcellular localization, because mRNA targeted to mitochondria likely encounters higher levels of ROS. We compared the RNA oxidation index with the mitochondria location index (MLR) calculated for 3106 yeast transcripts based on their association with free and mitochondrion-bound polysomes (13). We observed a modest correlation between these parameters (Spearman ρ > 0.41). We observed no significant correlation between RNA oxidation and either RNA abundance, transcript length, or ribosomal density (14).

In summary, we developed a high-throughput method that is suitable to identify oxidized RNA species on a genome-wide scale, and applied this method to S. cerevisiae. Similar studies performed on RNA isolated from tissues of patients affected by neurodegenerative diseases might shed light on the role of RNA oxidation in these diseases.

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Competing interests
The authors declare no competing interests.

References

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