Supporting Information

Modification of the 4Fe-4S Cluster Charge Transport Pathway

Alters RNA Synthesis by Yeast DNA Primase

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Figure S1. Anaerobic electrochemical analysis of the yeast 5YF p58C mutants. Bulk oxidation (left) and CV (right) of 5YF412 and 5YF431 after bulk oxidation. All scans were performed on 38 μM [4Fe4S] p58C in a buffer containing 20 mM Tris-HCl and 75 mM NaCl at pH 7.2, with a 100-mV/s scan rate for CV.
Figure S2. Anaerobic electrochemical analysis of the yeast p58C mutants. Bulk reduction (left) and CV (right) of p58C WT, 5YF412, 5YF431 and 6YF after bulk reduction. All scans were performed on 38 μM [4Fe4S] p58C in a buffer containing 20 mM Tris-HCl and 75 mM NaCl at pH 7.2, with a 100-mV/s scan rate for CV.
Figure S3. EPR analysis of WT and the 6YF mutant yeast p58C. EPR spectra were collected for oxidized wild type p58C, oxidized degraded 6YF, and oxidized 6YF. The wild type protein (blue) exhibits a trace signal at $g = 2.026$ (inset), within the range reported for $[4\text{Fe}-4\text{S}]^{3+}$ clusters in HiPiP proteins. In contrast, oxidation of 6YF (orange) gives rise to no signal, consistent with our proposal that $[4\text{Fe}-4\text{S}]$ cluster oxidation in this mutant will be inefficient because the Tyr-mediated electron hopping pathways have been mutated. Oxidization of aged, degraded 6YF (red) gives rise to a signal at $g = 3.12$, consistent with degradation of the cluster and demonstrating the lack of signal in a fresh sample is not due to inefficient incorporation of Fe. All spectra were acquired at 10 K using 12.85 mW microwave power, 2 G modulation amplitude, and a receiver gain of 30 x10³.
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Forward (5’ to 3’)</th>
<th>Reverse (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y352/353F</td>
<td>CCATCATTTGAGTTTTTTTGGGAGACAACAAC</td>
<td>GTTGTTGTCTCCCCAAAACCTCAAATGATGG</td>
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<tr>
<td>Y395F</td>
<td>GAGAAGTTCAATAAAGAATTCGTTACAGCTTCAGGC</td>
<td>CATTTGTGTTCCCATTCTTGTAATGC</td>
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<tr>
<td>Y397F</td>
<td>GAGAAGTTCAATAAGAAATACCGTTTCAGCTTCAGGC</td>
<td>CATTTGTGTTCCCATTCTTGTAATGC</td>
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<tr>
<td>Y412F</td>
<td>AACAGAATCAACTTCAAAACCATGGGAC</td>
<td>ACCTTCAAGACCGTAATTATGC</td>
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<tr>
<td>Y431F</td>
<td>TGGCGCGGAGATTTTTCATGGATGC</td>
<td>GGTCTGGGCTTGGAAGGATAGTGTG</td>
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<tr>
<td>Table S2: DNA oligonucleotides used in electrochemistry experiments</td>
<td></td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>Thiolated DNA</td>
<td>5’-SH-GTCGTGCAACGTGTCTGCGC-3’</td>
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<tr>
<td>Complementary DNA</td>
<td>3’-CAGCACGTTGCACAGACGCGTAC-5’</td>
<td></td>
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<tr>
<td>Abasic Site DNA</td>
<td>3’-CAG_ACGTTGCACAGACGCGTAC-5’</td>
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</table>
### Table S3: Distances between residues of interest in yeast p58C

<table>
<thead>
<tr>
<th>Residue pair</th>
<th>Distance (Å)</th>
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<tbody>
<tr>
<td>Fe-S to Y431</td>
<td>14.1</td>
</tr>
<tr>
<td>Fe-S to Y353</td>
<td>14.2</td>
</tr>
<tr>
<td>Y353 to Y352</td>
<td>5.8</td>
</tr>
<tr>
<td>Y352 to W376</td>
<td>12.1</td>
</tr>
<tr>
<td>Y352 to Y412</td>
<td>9.4</td>
</tr>
<tr>
<td>Y431 to W376</td>
<td>13.0</td>
</tr>
<tr>
<td>Y431 to Y395</td>
<td>15.1</td>
</tr>
<tr>
<td>W376 to Y395</td>
<td>9.3</td>
</tr>
<tr>
<td>Y395 to Y397</td>
<td>11.6</td>
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<tr>
<td>Y352 to Y412</td>
<td>9.4</td>
</tr>
<tr>
<td>W376 to Y397</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Measurements were recorded as distances between centroids of the aromatic moiety of Tyr/Trp residues in WT yeast p58C (PDB 6DI6) using Chimera protein visualization software.
**Table S4**: Crystallographic data collection and refinement statistics

<table>
<thead>
<tr>
<th>Mutant</th>
<th>5YF412</th>
<th>5YF431</th>
<th>6YF</th>
</tr>
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<tbody>
<tr>
<td>PDB ID</td>
<td>7TL2</td>
<td>7TL3</td>
<td>7TL4</td>
</tr>
</tbody>
</table>

**Data collection**

| Wavelength | 0.97872 | 0.97857 | 0.97857 |
| Space group | P 21 21 21 | P 21 21 21 | P 21 21 21 |
| Resolution range | 26.02 – 1.53 | 30.36 – 2.07 | 29.61 – 1.80 |
| (1.58 – 1.53) | (2.12 – 2.05) | 1.86 – 1.80 |

**Cell dimensions**

| a, b, c (Å) | 40.62, 51.87, 89.48 | 41.04, 51.10, 90.22 | 39.52, 51.49, 90.03 |
| α, β, γ (°) | 90, 90, 90 | 90, 90, 90 | 90, 90, 90 |

**Reflections**

| Total | 29195 (13278) | 11684 (2866) | 16950 (7180) |
| Unique | 3387 | 1751 | 2173 |
| Completeness (%) | 99.56 (99.2) | 96.22 (89.3) | 96.72 (94.7) |

**Refinement**

| R_{work} | 0.04 | 0.15 | 0.09 |
| R_{free} | 0.043 (0.392) | 0.166 (0.549) | 0.095 (0.381) |
| R_{free} | 0.018 (0.169) | 0.078 (0.272) | 0.041 (0.172) |
| CC_{1/2} | 0.997 (0.945) | 0.969 (0.887) | 0.982 (0.965) |
| CC* | 0.999 (0.986) | 0.992 (0.970) | 0.995 (0.991) |
| Multiplicity | 3.1 (2.7) | 2.7 (2.0) | 2.9 (2.4) |
| Mean I/σ(I) | 39.03 (4.29) | 8.59 (2.69) | 12.02 (4.49) |

<p>| No. of non-H atoms | 1686 | 1546 | 1535 |
| Macromolecules | 1555 | 1503 | 1482 |
| Ligands | 8 | 8 | 8 |
| Solvent | 123 | 35 | 45 |
| R_{work}/R_{free} | 0.1516/0.1680 | 0.2356/0.2767 | 0.1962/0.2140 |
| (0.1810/0.2011) | (0.2919/0.3792) | (0.2332/0.2508) |
| RMSD (bonds, Å) | 0.014 | 0.0034 | 0.016 |
| RMSD (angles, °) | 1.207 | 0.494 | 1.253 |
| Ramachandran Favored (%) | 97.78 | 98.89 | 96.67 |
| Allowed (%) | 2.22 | 1.11 | 3.33 |
| Outliers (%) | 0 | 0 | 0 |
| Rotamer outliers (%) | 1.18 | 1.9 | 0 |
| Clashscore | 1.89 | 4.7 | 3.09 |
| Wilson B-factor (Å²) | 17.06 | 30.63 | 22.63 |
| Average B-factor (Å) | 26.99 | 42.77 | 40.53 |
| Macromolecules | 26.25 | 42.68 | 40.62 |
| Ligands | 37.33 | 49.48 | 32.69 |
| Solvent | 35.06 | 41.9 | 40.24 |
| RMSD vs WT (6DI6) | 0.42 | 0.24 | 0.47 |</p>
<table>
<thead>
<tr>
<th>Protein</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>4 ± 0.6</td>
</tr>
<tr>
<td>5YF412</td>
<td>13 ± 1.3</td>
</tr>
<tr>
<td>5YF431</td>
<td>8.9 ± 0.76</td>
</tr>
<tr>
<td>6YF</td>
<td>8.9 ± 1.6</td>
</tr>
</tbody>
</table>

Fluorescence anisotropy was performed with a 6FAM-labeled DNA substrate and various concentrations of yeast p58C (WT and mutants). The data were plotted and final apparent K<sub>D</sub> values were calculated using the one-site total binding equation in GraphPad Prism 8. The reported values represent the mean ± one standard deviation.