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Introducing human-like mutations in yeast iso-1-cytochrome *c* to decrease peroxidase activity in apoptosis

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Introduction

- Cytochrome *c* functions as a signalling agent in the intrinsic pathway of apoptosis, known as programmed cell death
- Yeast does not contain all components of the human apoptotic pathway, resulting in a 20-fold higher intrinsic peroxidase activity in yeast. This suggests an evolved "off" switch to limit peroxidase activity in the human intrinsic pathway

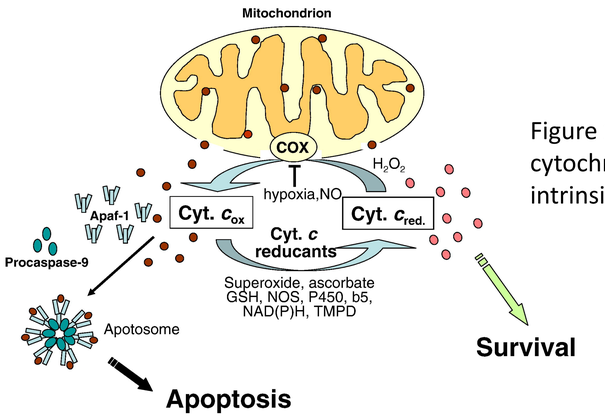


Figure 1: Diagram of cytochrome *c*'s role in the intrinsic apoptosis pathway[1]

- During peroxidase activity, conformational changes cause dissociation of Met 80 ligation from the heme
- Ω-loop C (residues 40-57) and Ω-loop D (70-85) are implicated in providing access to the peroxidase conformers due to the ability of their hydrogen bonding network to alter the conformational dynamics of cytochrome *c*

- We introduced all possible single, double, and triple humanizing mutations at sites known to co-evolve[4]: S40, V57, and N63 (S40T, V57I, N63T) in yeast iso-1-cytochrome *c* and hypothesize that relative to wild type the variants will have:
  - A stabilizing effect on global stability and overall secondary structure
  - Decreased peroxidase activity

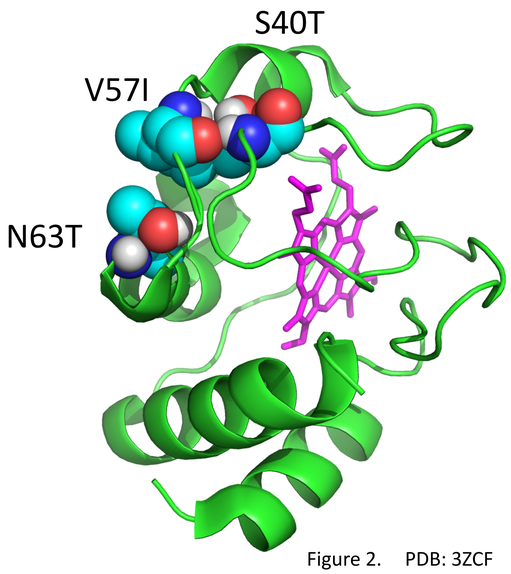


Figure 2. PDB: 3ZCF

Data

- Yeast iso-1-cytochrome *c* mainly consists of α-helical secondary structure
- Global stability was measured using CD spectroscopy
- Ellipticity of the variants was monitored at 222 nm using 250 nm as background correction ($\theta_{222corr} = \theta_{222}-\theta_{250}$)

Figure 3

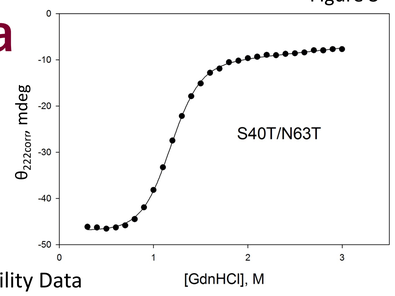


Table 1: Global Stability Data

Variant	ΔG (k_{cal}/mol)	M ($k_{cal} * mol^{-1} * M^{-1}$)	C_m (M)
WT	5.05 ± 0.30	4.24 ± 0.13	1.19 ± 0.04
S40T	4.99 ± 0.09	3.94 ± 0.16	1.27 ± 0.03
N63T	5.38 ± 0.12	4.01 ± 0.10	1.34 ± 0.01
V57I	4.22 ± 0.13	3.54 ± 0.12	1.19 ± 0.01
S40T/ N63T	4.50 ± 0.12	3.85 ± 0.07	1.17 ± 0.01
N63T/ V57I	5.40 ± 0.16	3.87 ± 0.12	1.40 ± 0.03
S40T/ V57I	4.23 ± 0.07	3.66 ± 0.22	1.16 ± 0.06

- During the alkaline transition, cytochrome *c* Met-80 ligation to the heme is displaced by deprotonated Lysine residues
- Loss of heme-Met 80 ligation was monitored by the disappearance of the 695 nm absorbance band at varying pH

Table 2: Alkaline Transition Data

Variant	pKa	n
WT	8.00 ± 0.05	0.980 ± 0.010
S40T	7.99 ± 0.06	0.983 ± 0.167
N63T	8.01 ± 0.05	0.946 ± 0.057
V57I	8.17 ± 0.06	1.13 ± 0.12
S40T/N63T	7.84 ± 0.11	0.984 ± 0.120
N63T/V57I	7.64 ± 0.04	0.961 ± 0.053
S40T/V57I	7.94 ± 0.16	1.05 ± 0.13
S40T/V57I/N63T	7.96 ± 0.10	0.890 ± 0.083

Figure 4

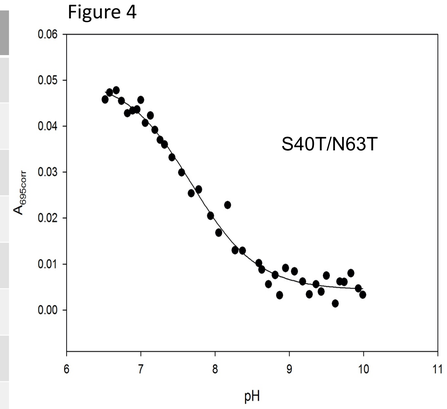
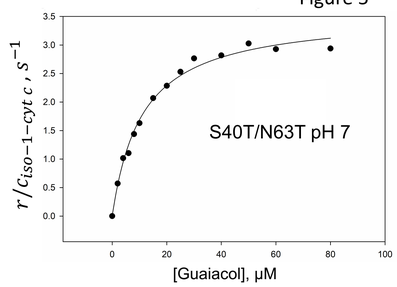


Figure 5



- Peroxidase assays monitor the formation of tetraguaiacol at 470 nm
- Data were fit to the Michaelis-Menten equation:

$$\frac{\nu}{[iso-1-cytochrome\ c]} = \frac{k_{cat} \bullet [Guaiacol]}{K_M + [Guaiacol]}$$

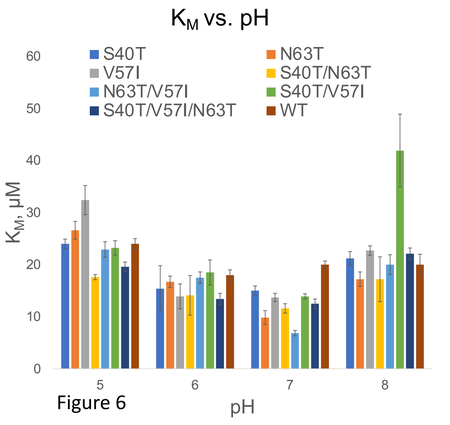


Figure 6

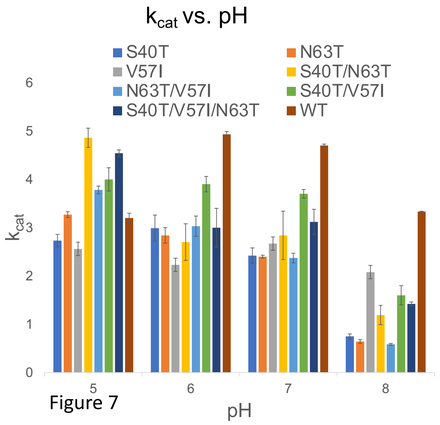


Figure 7

Discussion & Conclusions

- Each of the single and double mutation variants have moderate effects on global stability when compared to wild type yeast iso-1-cytochrome *c*
- The alkaline transition $pK_{apparent}$ values for the N63T/V57I and S40T/N63T are lower when compared to wild type, whereas the single mutation variants, S40T/V57I, and S40T/N63T/V57I are similar in value to WT
- Peroxidase assays indicate minimal effects of all variants on K_M compared to that of wild type yeast iso-1-cytochrome *c*. The k_{cat} for each variant is similar at pH 5 but decreases at increased pH values in relation to WT
- The effect of human-like mutations at these positions that co-evolve in cytochrome *c* are modest, indicating that they co-evolve to preserve rather than change function. Substitutions that control peroxidase activity must lie elsewhere in the protein.

References

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Acknowledgments

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