SUPPORTING INFORMATION

Radical-translocation Intermediates and Hurdling of Pathway Defects in "Super-oxidized" (Mn^{IV}/Fe^{IV}) *Chlamydia trachomatis* Ribonucleotide Reductase

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Simulation of Tyrosyl Radicals. Simulation of the X-band EPR spectra of the radicals shown in Figure 3 was carried out by using *EasySpin* (*www.easyspin.org*).[1] A total electronic spin (S_{total}) of ½ was assumed. Hyperfine interactions with six nonequivalent nuclei, two C_β methylene protons and the four protons on the phenyl ring, were considered. The principal *g*-values of tyrosyl radicals have a polynomial dependence on the spin density of the C1 carbon, with the g_x component being the most sensitive one.[2] The g_y and g_z principal values vary less among the different radical signals studied.[2] For both radicals, the line widths were assumed to be isotropic and pseudo-Voigtian, with an additional anisotropic line broadening contribution being introduced for the radical in the wt α•β complex. The hyperfine coupling constants of the ring protons are quite conserved among different tyrosyl radicals.[2] Therefore, their values in the simulations were allowed to vary within a narrow range of 2 MHz of the reported, typical values.[2] The Euler angles between the A_x tensor component of the ring protons and the g_x direction were assumed to be similar to those previously reported.[2] Analysis of various tyrosyl radicals by ENDOR spectroscopy has revealed that the hyperfine tensors for the methylene protons are nearly axial, with the largest component being A_x ($A_{parallel}$) and the other two components being nearly equal [$A_y \approx A_z$ ($A_{perpendicular}$)].[2] The axial symmetry was an additional constraint for the determination of the hyperfine couplings of the methylene proton by simulations. Overall, the difference between the g_x values obtained for the β-wt•α-wt and the β-Y_{338}F•α-wt complexes are indicative of different spin densities on the C1 carbon, whereas the different hyperfine couplings and their degree of anisotropy ($A_{parallel}/A_{perpendicular}$) reflect a different dihedral angle, θ, between the C_β-H

bond and the axis normal to the plane of the aromatic ring. The simulation parameters are provided in Table S1.

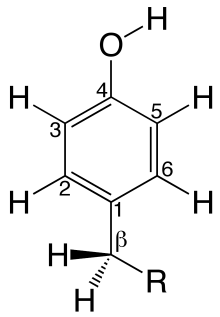
Spin Quantification of EPR Signals. The quantification of duplicate samples of *Ct* β Mn^{IV}/Fe^{IV} and the RT pathway Y•(s) (see Materials and Methods for preparation of samples) were carried out as previously described.[3] The results are presented in **Table S2**. Samples were prepared as described in the legend of **Figure S2**.

Table S1: Simulation parameters of RT pathway radicals in *Ct* RNR

Species	**β-wt•α-wt**	**β-Y_{338}F•α-wt**
$\mathbf{A_{Hβ1}}$ (MHz)	31.3, 27.3, 24.0	56.0, 49.0, 49.0
$\mathbf{A_{Hβ2}}$ (MHz)	16.0, 15.0, 15.0	2.0, 1.0, 1.0
$\mathbf{A_{H3}}$ (MHz)[a]	-24.7, -8.0, -22	-24.7, -8.0, -20.0
$\mathbf{A_{H5}}$ (MHz)[b]	-26.7, -8.0, -22.4	-25.5, -8.0, -20.4
$\mathbf{A_{H2}}$ (MHz)[c]	5.0, 7.5, 1.5	5.0, 7.5, 1.5
$\mathbf{A_{H6}}$ (MHz)[d]	5.0, 7.5, 1.5	5.0, 7.5, 1.5
Line width (mT)	0.24, 0.25, 0.26	0.15
g	2.0104, 2.0045, 2.0016	2.0074, 2.0046, 2.0018

[a] **A** was rotated into the frame of **g** around A_z using Euler angles of α =22°, [b] α =-22°, [c] α =10°, and [d] α =-10°.

Sample	Total Area (arbitrary units)	Relative amount of Mn^{IV}/Fe^{IV} (%)	Relative amount of % Y• (%)
− CDP, ATP (trial 1)	30	100	0
+ CDP, ATP (trial 1)	37	54	46
− CDP, ATP (trial 2)	28	100	0
+ CDP, ATP (trial 2)	29	56	44



Scheme S1: Schematic drawing of the phenol ring of a tyrosine residue with numerical assignments used for the simulation of the radicals.

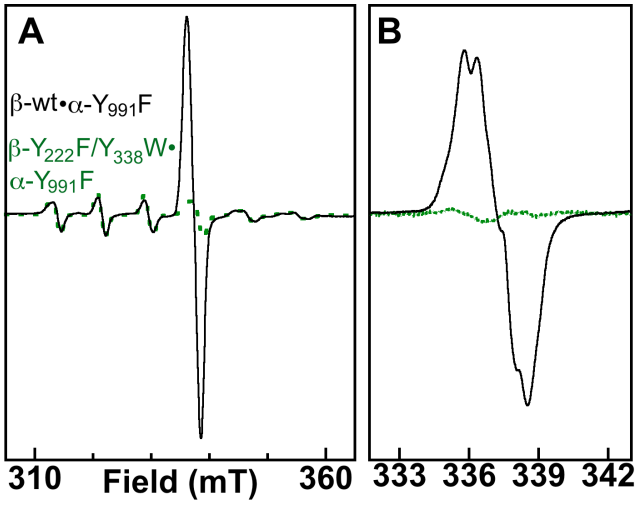


Figure S1. X-band EPR spectra of samples prepared with the *Ct* α-$Y_{991}F$ variant demonstrating the generation of an organic radical when complexed with *Ct* β-wt (black spectra), but failure to form any significant organic radical when in complex with *Ct* β-$Y_{222}F/Y_{338}W$ (green spectra). The "raw" spectra are shown in panel **A**, whereas spectra in panel **B** have had the contribution of the Mn^{IV}/Fe^{IV} states removed. Sample preparation and spectrometer conditions are identical to those described in the legend of **Figure 1**.

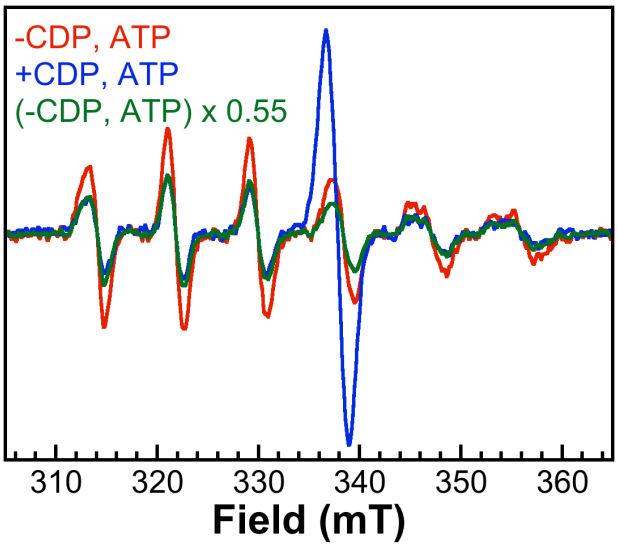


Figure S2. Representative X-band EPR spectra demonstrating loss of signal intensity attributable to the Mn^{IV}/Fe^{IV} intermediate upon formation of the Y•. Mn^{II}/Fe^{II}-β was first mixed with O_2-saturated buffer to form the Mn^{IV}/Fe^{IV} intermediate, and after 2 s, the resultant solution was mixed either with α, $MgSO_4$, and DTT (**red spectrum**) or with one α, CDP, ATP, $MgSO_4$, and DTT (**blue spectrum**). After mixing with the α-containing solution, the reaction was quenched after 1 s by spraying the reaction mixture into cold (~ 120 K) 2-methylbutane. The intensity attributable to the Mn^{IV}/Fe^{IV} intermediate is ~ 45% less in the spectrum of the sample in which the Y• is formed than in the sample in which no Y• accumulates (**green spectrum**). This loss of intensity correlates well to a ~ 45% increase in signal intensity in the fourth line of the six packets of resonances observed. This increased intensity is attributable to the Y•. Final concentrations after mixing were: 0.2 mM β, 0.15 mM Mn, 0.15 mM Fe, 0.3 mM α, 1 mM CDP (when present), 0.5 mM ATP (when present), 10 mM $MgSO_4$, 10 mM DTT. The spectrometer conditions were: T = 14 ± 0.2 K, microwave frequency = 9.47 GHz, microwave power = 20 μW, modulation

frequency = 100 KHz, modulation amplitude = 10 G, time constant = 167 ms, scan time = 167 s.

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