Supplemental Methods

Auto-acetylation of picNuA4—Acetylation of picNuA4 was monitored by phosphorimaging. 3 μM enzyme was incubated with 52 μM 1'-^{14}[C]-acetyl-CoA in 50 mM Tris, pH 7.5. Reactions were quenched at various times in SDS-PAGE loading dye without reducing agent. Proteins were separated by 12% SDS PAGE, gel dried, and exposed to phosphorimage screen for 2 days. A time course of acetylation was produced using the filter-binding assay. Reaction conditions were 8 μM picNuA4, 75 μM ^{3}[H]-acetyl-CoA, and 50 mM Tris, pH 7.5.

Pulse-chase of acetylated enzyme with CoA—5 μM picNuA4 was incubated with 3 μM ^{14}C-acetyl-CoA for 1 hour at 25 ºC. Enzyme was then diluted into buffered solutions of 0, 100 or 500 μM CoA. Reaction was allowed to proceed for 5 minutes before quenching into non-reducing SDS-PAGE dye. Electrophoresis and phosphorimaging were performed as above.

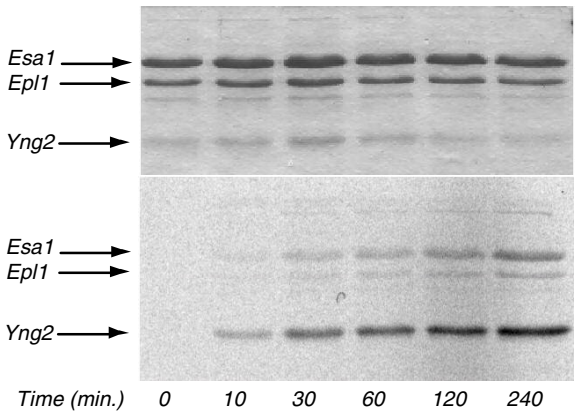
Determination of CoA formed—Assays to determine the rate of CoA formation were performed as described previously with 13-20 μM picNuA4 at 0 or sub-saturating levels (30 μM) of $H4_{1-20}$. Time points were quenched in 0.05% TFA. Samples were centrifuged to remove precipitate and then injected onto a C18 column (Grace-Vydac, Hesperia, CA) in 0.05% TFA in H_2O. CoA and acetyl-CoA were then eluted by a linear gradient of 0-22% 0.02% TFA in acetonitrile for 27 minutes. CoA eluted at 26 minutes and acetyl-CoA at 28 minutes. A ratio of the CoA peak area to the total peak area of CoA and acetyl-CoA was then calculated for each time point to determine CoA formed.

Further analysis of the amount CoA formed was performed using the DTNB assay as described in previous sections for propionyl-CoA.

Supplemental Figure Legend

Figure 1—Characterization of the auto-acetylation of picNuA4 (*A*) 0.5 μM picNuA4 was incubated in 50 mM Tris and 52 μM ^{14}C-labeled acetyl-CoA for times shown and then quenched in non-reducing SDS-PAGE loading dye. The Coomassie stained gel of picNuA4 time points is shown in the top panel with the phosphorimage showing the levels of acetyl-incorporation shown in the bottom panel. **(*B*)** 5 μM picNuA4 was incubated with 3 μM ^{14}C-labeled acetyl-CoA for one hour and then diluted into buffered solutions containing 0, 100, and 500 μM CoA to determine if Esa1 acetylation was reversible. All reactions were in 50 mM Tris, pH 7.5. **(*C*)** and **(*D*)** CoA formation by picNuA4 as monitored by HPLC (C) or DTNB assay (D). HPLC reactions were performed in 50 mM Tris, pH 7.5 with 13-20 μM picNuA4, and 75 μM acetyl-CoA. Reaction without peptide denoted with triangles and with 30 μM peptide with boxes. CoA formation and acetyl-CoA loss was monitored by reverse-phase HPLC. The rate of CoA formation was determined to be 0.0021 ± 0.001 s^{-1}. Experiment was repeated in duplicate with representative data shown. Reactions for the DTNB assay contained 50 mM Tris, pH 7.5 at 25 ºC and 1 mM EDTA with 0.2 μM picNuA4, 180 μM acetyl-CoA, and 0 or 840 μM $H4_{1-20}$. Reactions were quenched in 6% SDS with 1 mg/mL DTNB. Reaction with peptide denoted with circles and without peptide with boxes. **(*E*)** Rate of picNuA4 autoacetylation was determined by filter binding assay. Reaction contained 50 mM Tris, pH 7.5, 8 μM picNuA4, and 75 μM ^{3}H-labeled acetyl-CoA. Data were fitted to linear equations in Kaleidagraph to determine rate. Rate was determined to be 7 ± 0.2 x 10^{-4} s^{-1}. Experiment was repeated in triplicate with representative data shown.

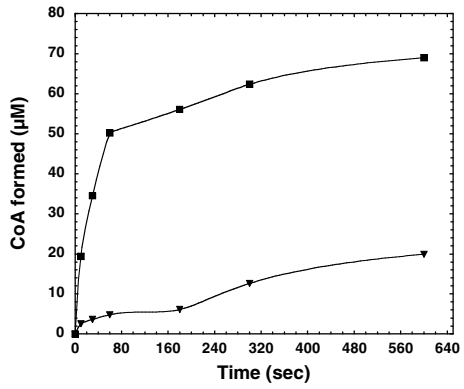
Supplemental Figure 1A



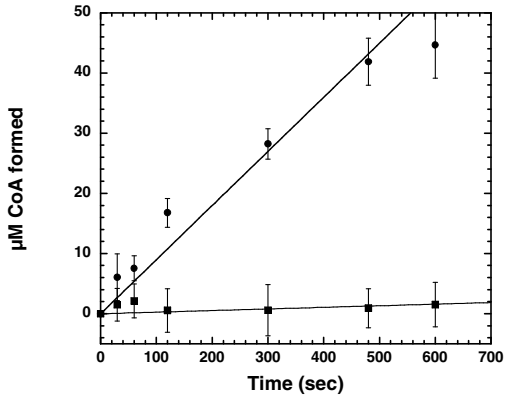
B

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

C



D



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