Differences in Specificity and Selectivity Between CBP and p300 acetylation of histone H3 and H3/H4 *Ryan A. Henry, Yin-Ming Kuo, Andrew J. Andrews*

Supporting Information

SUPPLEMENTAL FIGURE 1. Determination of steady-state kinetic parameters of CBP- and p300mediated acetylation of histone H3 when titrating H3. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays contained from 1 to 150 nM p300 or 0.5 to 10 nM CBP, with varying concentrations of H3 (0.25 - 15 μ M), and constant (200 μ M) acetyl-CoA. Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-C): H3K14, H3K18, and H3K23. The acetylation sites for p300 are (from D-G): H3K14, H3K18, H3K23, and H3K9. The apparent kinetic parameters are summarized in Table 1.

SUPPLEMENTAL FIGURE 2. Determination of steady-state kinetic parameters of CBP- and p300mediated acetylation of histone H3 when titrating acetyl-CoA. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays for p300 contained 50 nM p300, 17.5 μ M H3, and varying concentrations of acetyl-CoA (1- 200 μ M). Assays for CBP contained 7 nM CBP, 7.5 μ M H3, and varying concentrations of acetyl-CoA (1- 200 μ M). Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-C): H3K14, H3K18, and H3K23. The acetylation sites for p300 are (from D-G): H3K14, H3K18, H3K23, and H3K9. The apparent kinetic parameters are summarized in Table 2.

SUPPLEMENTAL FIGURE 3. Determination of steady-state kinetic parameters of CBP and- p300mediated acetylation of H3 on H3/H4 when titrating H3/H4. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays contained from 1 to 22.5 nM CBP or 1 to 50 nM p300, with varying concentrations of H3/H4 (0.25 - 10 μ M), and constant (200 μ M) acetyl-CoA. Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-C): H3K14, H3K18, and H3K23. The acetylation sites for p300 are (from D-G): H3K14, H3K18, H3K23, and H3K9. The apparent kinetic parameters are summarized in Table 3.

SUPPLEMENTAL FIGURE 4. Determination of steady-state kinetic parameters of CBP- and p300mediated acetylation of H4 on H3/H4 when titrating H3/H4. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays contained from 1 to 22.5 nM CBP or 1 to 50 nM p300, with varying concentrations of H3/H4 (0.25 - 10 μ M), and constant (200 μ M) acetyl-CoA. Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-D): H4K5, H4K8, H4K12, and H4K16. The acetylation sites for p300 are (from E-H): H4K5, H4K8, H4K12, and H4K16.

SUPPLEMENTAL FIGURE 5. Determination of steady-state kinetic parameters of p300-mediated acetylation of H3 on H3/H4 when titrating acetyl-CoA. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays contained either 20 nM CBP or 50 nM p300, 7.5 μM H3/H4, and varying concentrations of acetyl-CoA (1- 200 μM). Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-C): H3K14, H3K18, and H3K23. The acetylation sites for p300 are (from D-G): H3K14, H3K23, and H3K9. The apparent kinetic parameters are summarized in Table 4.

SUPPLEMENTAL FIGURE 6. Determination of steady-state kinetic parameters of CBP-mediated acetylation of H4 on H3/H4 when titrating acetyl-CoA. Experiments were performed at 37 °C in 100 mM Ammonium bicarbonate and 50 mM HEPES buffer (pH 7.8) at 37°C. Assays contained either 20 nM CBP or 50 nM p300, 7.5 μ M H3/H4, and varying concentrations of acetyl-CoA (1- 200 μ M). Experiments were quenched with 4 volumes of TCA and boiled at 95 °C for 5 minutes. The acetylation sites for CBP are (from A-D): H4K5, H4K8, H4K12, and H4K16. The acetylation sites for p300 are (from E-H): H4K5, H4K8, H4K12, and H4K16. The apparent kinetic parameters are summarized in Table 4.

Supplemental Figure 1





0.000-5.0×10⁻⁰⁵ 1.0×10⁻⁰⁴ 1.5×10⁻⁰⁴ 2.0×10⁻⁰⁴ 0 Acetyl-CoA [M]

1.5×10⁻⁰⁵

4.5×10⁻⁰⁵

Supplemental Figure 3



H3/H4 [M]









