Acetylation of MyoD by p300 Requires More Than Its Histone Acetyltransferase Domain*

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MyoD, an essential transcription factor involved in muscle cell terminal differentiation, is regulated by acetylation, as are a number of other transcription factors, but the histone acetyltransferase enzyme responsible for this acetylation is a matter of controversy. In particular, contradictory findings have been reported concerning the ability of CBP/p300 to acetylate MyoD in vitro. Here we provide an explanation for this discrepancy: although full-length p300 does indeed acetylate MyoD, a fragment of p300 corresponding to its histone acetyltransferase domain does not. In addition to clearly demonstrating that p300 acetylates MyoD in vitro, these results underscore the necessity of using full-length histone acetyltransferase enzymes to draw valid conclusions from acetylation experiments.

Acetylation has recently emerged as a central mode of regulation for proteins. Histone acetyltransferases (HATs), which are able to acetylate histone and non-histone proteins, are involved in a variety of essential cellular processes such as muscle-cell terminal differentiation. Muscle-cell terminal differentiation involves several families of transcription factors, including myogenic basic helix-loop-helices (MyoD, Myf-5, myogenin, and MRF-4) (1), and transcriptional co-regulators with histone acetyltransferase activity, the PCAF/GCN5 family (2–4) and the CBP/p300 family (5, 6). PCAF (7) and CBP/p300 (8, 9) are found in the same complexes (7), although they acetylate distinct targets (10).

HATs are involved at different steps of the differentiation program; differentiation triggers the acetylation of histones on muscle-specific promoters (11). In addition, the myogenic basic helix-loop-helix protein MyoD is also acetylated in myogenic cells (12). MyoD acetylation increases its transcriptional activity by influencing its ability to bind DNA (13) and to interact with other proteins (11). In vitro, MyoD is acetylated by PCAF (13). Acetylation of MyoD by CBP/p300, on the other hand, has been somewhat controversial. We reported that MyoD is acetylated by CBP or p300 with an efficiency similar to that observed in p300 in vitro (14). The latter study relied on a truncated version of the p300 protein (from amino acid 965 to amino acid 1810) that is often employed in this type of study. It is of note that the p3001065–1810 fragment has lost the main protein-protein interaction domains of p300. In particular, the regions of p300 previously shown to interact with MyoD, the CH3 domain (amino acids 1620–1891) (15) and the N-terminal KIX domain (amino acids 379–654) (16), are truncated or altogether deleted in p3001065–1810. Thus a possible explanation for the discrepancy between these two series of experiments might be that a physical contact between the HAT and its substrate is required for acetylation and that the interaction domains are critical for the reaction to take place. Here, we present the results of a direct test of this hypothesis, which show that acetylation of MyoD by p300 is linked to the ability of the two proteins to physically interact.

EXPERIMENTAL PROCEDURES

HAT Assay and Protein Acetylation—Recombinant GST-PCAF and FLAG-tagged p300 and p3001065–1810 were purified from bacteria and insect cells as described previously (12). HAT activity was measured as described previously (17) using either nucleosomes purified from HeLa cells or a peptide corresponding to the first 24 amino acids of histone H3. Bacterially produced recombinant MyoD was produced and acetylated as described previously (12).

Co-immunoprecipitation—20 ng of recombinant Escherichia coli-produced MyoD protein was incubated with 50–100 ng of baculovirus-produced FLAG-p300 wild type or FLAG-p3001065–1810 in 25 mM HEPES, pH 7.2, 150 mM potassium acetate, 2 mM EDTA, and 0.1% Nonidet P-40 followed by immunoprecipitation with anti-FLAG antibody (M-2, Sigma). Proteins were resolved on SDS-polyacrylamide gel electrophoresis, and the presence of MyoD in the complexes was revealed by Western blot with anti-MyoD antibody (C-20, Santa Cruz Biotechnology).

RESULTS AND DISCUSSION

To determine whether p300 integrity is required for MyoD acetylation, we directly compared the two forms of p300 for their ability to acetylate MyoD. The histone acetyltransferase activities of the two proteins, as well as of PCAF, were first standardized based on their ability to acetylate histones, as measured by incorporation of 14C from radiolabeled acetyl-CoA. We used as substrates either a synthetic peptide corresponding to the first 24 amino acids of histone H3 (17) (Fig. 1A) or nucleosomes prepared from HeLa cells (Fig. 1B). Note that we purposely chose to use a concentration of the p3001065–1810 fragment that acetylates histone substrates with a significantly higher efficiency than the full-length p300 and PCAF proteins (about 3-fold higher). We next assayed the three HATs using MyoD as a substrate. In contrast to the results obtained with the histones, the p3001065–1810 fragment did not induce detectable incorporation of 14C into MyoD (Fig. 1C). As published previously (12), PCAF and p300, when full-length, catalyzed the incorporation of 14C into MyoD to similar levels.

These data demonstrate that MyoD acetylation by p300 requires more than just its HAT domain. The most likely explanation is that physical interaction between the enzyme and its structures.
substrate is required for efficient acetylation of the substrate. Indeed, the results of a co-immunoprecipitation assay indicated that although full-length p300 strongly interacts with MyoD in vitro, p300\textsuperscript{965–1810} fragment does not (Fig. 2). These results show a correlation between MyoD acetylation and physical interaction with MyoD. They demonstrate without ambiguity that p300 \textit{is} in fact able to acetylate MyoD \textit{in vitro}, and most importantly, they show that, in order to arrive at valid conclusions, full-length histone acetyltransferases must be used in \textit{in vitro} assays. In light of this finding, and given that the widely used p300\textsuperscript{965–1810} fragment has lost the main protein-protein interaction domains of p300, some previously published data may need to be re-evaluated.

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\textbf{FIG. 1.} Full-length p300, but not p300\textsuperscript{965–1810}, acetylates MyoD \textit{in vitro}. A, recombinant GST-PCAF or baculovirus-produced p300 or p300\textsuperscript{965–1810} incubated with a peptide corresponding to the first 24 amino acids of histone H3 and C14 acetyl-CoA. The radioactivity was measured in a \textit{B} counter (mean of three determinations). B and C, nucleosomes purified from HeLa cells (B) or recombinant MyoD (C) acetylated \textit{in vitro} as above. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis. Coomassie stains and autoradiograms of the gels are shown as indicated.

\textbf{FIG. 2.} p300\textsuperscript{965–1810} does not physically interact with MyoD \textit{in vitro}. FLAG-tagged p300 or p300\textsuperscript{965–1810} was incubated with recombinant MyoD and immunoprecipitated using anti-FLAG antibodies. A, immunoprecipitates analyzed by Western blot using anti-MyoD antibodies. B, a Coomassie stain of the FLAG tagged proteins used in panel A.
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