2011

Identification of holocarboxylase synthetase chromatin binding sites in human mammary cell lines using the DamID technology

Dipika Singh
University of Nebraska-Lincoln

Angela K. Pannier
University of Nebraska-Lincoln, apannier2@unl.edu

Janos Zempleni
University of Nebraska-Lincoln, jzempleni2@unl.edu

Published in final edited form as:
Anal Biochem. 2011 June 1; 413(1): 55–59. doi:10.1016/j.ab.2011.02.001.

Identification of holocarboxylase synthetase chromatin binding sites in human mammary cell lines using the DamID technology

Dipika Singh[a], **Angela K. Pannier**[a,c], and **Janos Zempleni**[b,c,*]

[a] Department of Biological Systems Engineering, University of Nebraska at Lincoln

[b] Department of Nutrition and Health Sciences, University of Nebraska at Lincoln

Abstract

Holocarboxylase synthetase (HCS) is a chromatin protein that is essential for mediating the covalent binding of biotin to histones. Biotinylation of histones plays crucial roles in the repression of genes and repeats in the human genome. We tested the feasibility of DNA adenine methyltransferase identification (DamID) technology to map HCS binding sites in human mammary cell lines. Full-length HCS was fused to Dam for subsequent transfection into breast cancer (MCF-7) and normal breast (MCF-10A) cells. HCS docking sites in chromatin were identified by using the unique adenine methylation sites established by Dam in the fusion construct; docking sites were unambiguously identified using methylation sensitive digestion, cloning, and sequencing. Fifteen novel HCS binding sites were identified in the two cell lines and the following four out of the 15 overlapped between MCF-7 and MCF-10A cells: inositol polyphosphate-5-phosphatase A, corticotropin hormone precursor, ribosome biogenesis regulatory protein, and leptin precursor. We conclude that DamID is a useful technology to map HCS binding sites in human chromatin and propose that the entire set of HCS binding sites could be mapped by combining DamID with microarray technology.

Keywords

chromatin; DNA adenine methyl transferase; holocarboxylase synthetase; mammary cells

1. Introduction

Holocarboxylase synthetase (HCS) is a chromatin protein [1] that plays a pivotal role in catalyzing histone biotinylation [1,2]. Histone biotinylation is a recently discovered epigenetic mark [3]. Biotin is unique because biotin serves as an essential covalently bound cofactor for several mammalian carboxylases and also functions as a covalent histone modification. Initially, biotinylation of histones was thought to be catalyzed by biotinidase [4]. However, subsequent studies revealed that HCS is much more important than biotinidase for biotinylation of histones *in vivo* [1,5,6,7], despite biotinidase demonstrating histone biotinyl transferase activity *in vitro* [8,9,10]. Several histone biotinylation sites have been identified using recombinant histone biotin ligases, including HCS, and synthetic

*Corresponding Author: Dr. Janos Zempleni, 316C Ruth Leverton Hall, University of Nebraska at Lincoln, Lincoln, NE 68583, jzempleni2@unl.edu, Telephone Number: (402) 472-3270.
[c]Contributed equally to this project

histone tail peptides. The identified biotinylation sites are lysine (K)-9, K13, K125, K127, and K129 in histone H2A [9]; K4, K9, K18, and perhaps K23 in histone H3 [7,10]; and K8 and K12, and perhaps K5 and K16 in histone H4 [7,8,11]. *In vitro* biotinylation of K8, K12, and K16 in histone H4 has been confirmed by mass spectrometric analysis of mammalian histones [11]. The existence of biotinylated histones was recently questioned [12], but findings in three independent laboratories [13,14,15], in addition to ours [2,3,8,7,10,16], suggest that biotinylation is a natural histone modification. One might consider the evidence in two of these publications circumstantial [13,14]. However, the third study used LC/MS/MS to unambiguously demonstrate that about 50% of the histones in *Candida albicans* are biotinylated [15].

Histone biotinylation is a relatively rare event; less than 0.1% of histones are biotinylated in humans [3,13]. However, the abundance of an epigenetic mark is not the sole determinant its biologic importance. For example, only ~3% of cytosines are methylated, but the role of DNA methylation in gene regulation is substantial and undisputed [17]. Likewise, serine-14 phosphorylation in histone H2B and histone poly(ADP-ribosylation) are detectable only after induction of apoptosis and major DNA damage, respectively; yet the role of these epigenetic marks in cell death is unambiguous [18–20]. Despite the low global abundance of biotinylated histones, the epigenetic mark appears to be highly enriched in specific loci, consistent with its regulatory function. For example, evidence suggests that about one out of three histone H4 molecules are biotinylated at K12 in telomeric repeats [21]. Moreover, K12-biotinylated histone H4 (H4K12bio), K9-biotinylated H3 (H3K9bio), and K18-biotinylated H3 (H3K18bio) are enriched at transcriptionally repressed loci in mammalian genomes, suggesting a role for histone biotinylation in gene regulation [5,6,16,22]. The important role of HCS and subsequent biotinylation of histones has been confirmed in various studies. For example, phenotypes of HCS knockdown include decreased life span and heat survival in *Drosophila melanogaster* [1], and increased transcriptional activity of retrotransposons in metazoans, predisposing test organisms to chromosomal instability [6].

HCS has a dispersed and distinct distribution in chromatin; however its chromatin binding sites are poorly defined [1]. Therefore, the objective of this study was to employ DNA adenine methyl transferase identification (DamID) technology [23] to generate the first insight into HCS chromatin binding sites and to pave the way for more comprehensive mapping efforts in future studies. Briefly, DamID technology is based on fusing DNA adenine methyltransferase (Dam) from *Escherichia coli* to a chromatin protein or transcription factor of interest [23], e.g., HCS. Upon expression of the fusion protein in cultured cells or in an intact organism such as *Drosophila*, HCS guides Dam to HCS binding sites, where Dam creates a unique adenine methylation tag, which is absent in most eukaryotes [24]. Adenine methylation sites can be identified by methylation-sensitive sequencing procedures.

In this proof-of-concept study, DamID technology was used in mammary epithelial cell lines to map genomic HCS-binding sites in human chromatin for the first time, thereby identifying potential aberrant epigenetic marks in breast cancer and setting the ground for future breast cancer studies.

2. Materials and Methods

2.1 Cloning HCS-Dam fusion plasmid

The pIND (V5) EcoDam [23,25] vector was obtained from Dr. Bas van Steensel's laboratory at the Netherlands Cancer Institute. pIND (V5) EcoDam codes for *E.coli* Dam, and contains a multiple cloning site upstream of the Dam open reading frame. An HCS-Dam fusion construct was generated as follows. Full-length human HCS was PCR-amplified

using HCS-pET41a as a template [2], AccuPrime™ Pfx DNA polymerase SuperMix (Invitrogen, Carlsbad, CA) and the following primers: 5′-GTTCGAATTCATGGAAGATAGACTCCACATGG-3′ (forward, EcoRI site underlined) and 5′-GTTTCTCGAGCCGCCGTTTGGGGAGGATGAGG-3′ (reverse, XhoI site underlined). Following amplification, HCS and pIND (V5) EcoDam vector were digested with EcoRI and XhoI (Fermentas, Glen Burnie, MD), ligated using Fast-Link™ DNA Ligation Kit (Epicenter Biotechnologies, Madison, WI), and transformed into MAX Efficiency® DH5α™ Competent Cells (Invitrogen). The HCS-Dam fusion plasmid was sequenced (Eurofin MWG Operon, Huntsville, AL) to confirm its identity and was denoted "HCS-Dam." Two control plasmids were used in HCS mapping studies. (i) Plasmid pIND V5 EcoDam [11], codes for Dam only and was used to identify artifactual binding of Dam to chromatin that was not mediated by HCS. (ii) Plasmid "HCS" codes for full-length human HCS and was used to identify artifactual adenine methylation in the absence of Dam. Plasmid HCS was generated as described above for HCS-Dam, but the following reverse primer was substituted for the original reverse primer: 5′GTTTTCTAGATTACCGCCGTTTGGGGAGGATGAGG-3′ (reverse; XbaI site underlined). The stop codon in this reverse primer terminates translation after the HCS open reading frame.

2.2 Cell culture

MCF-7 and MCF-10A cells (ATCC, Manassas, VA) were used for all studies. MCF-7 cells were cultured in DMEM/F12 media (ATCC) with the following supplements (v/v): 1% L-glutamine (Invitrogen), 10% fetal bovine serum (Invitrogen), 1% antibiotic/antimycotic (Invitrogen), 1% nonessential amino acids (Invitrogen), and 0.06% bovine insulin (Sigma-Aldrich, St. Louis, MO). MCF-10A cells were cultured in mammary epithelial growth media (MEGM) + bullet kit (Lonza, Basel, Switzerland), 1% (v/v) fetal bovine serum, and 0.05% (v/v) cholera toxin (Sigma-Aldrich).

2.3 Transfection of cells with HCS-Dam and control plasmids

For transfection with HCS-Dam, EcoDam and HCS plasmids, 3.5×10^6 cells were seeded in T-75 flasks and allowed to adhere for 18 h, after which time cells were transfected with one of the three plasmids as follows. DNA was complexed with Lipofectamine (LF) 2000 (Invitrogen) or FugeneHD (Roche, Madison, WI) in serum-free opti-MEM media (Invitrogen). For MCF-7 cells, 6.75 μg of each plasmid was complexed with 10.2 μl of LF 2000 in a 1:1.5 DNA to lipid ratio (μg of DNA to μl of LF2000). These complexes were allowed to incubate for 20 min and were then added to each designated flask. For MCF-10A cells, 8.25 μg of each plasmid was complexed with 20.625 μl of FugeneHD in a 1:2.5 DNA to Fugene ratio (μg of DNA to μl of FugeneHD). These complexes were incubated for 15 min and were then delivered to each designated flask. Forty-eight hours after transfection, cells were collected and lysed using protocols specific for isolation of RNA or genomic DNA.

2.4 Quantitative Real-time PCR (qRT-PCR)

Total RNA was collected using the RNeasy mini kit (Qiagen, Valencia, CA) and reverse transcribed using the ImProm-II Reverse Transcription System (Promega, Madison, WI). The cDNA was used to confirm successful transfection of cells with plasmids HCS-Dam, EcoDam, and HCS, using the following primers and SYBR green (Qiagen) in qRT-PCR expression analysis: (i) HCS-Dam = 5′-GCAACTCCTTCGACATGCTGAGAAA-3′ (forward, positioned in HCS region) and 5′-TCTTCATGCCGGTACGCGTAGAAT-3′ (reverse, positioned in Dam region); (ii) Dam = 5′-TCTGGTTGAGCGCCATATTCCAGT-3′ (forward) and 5′-TGTACAAAGCCAGCAGTTCGTCCA-3′ (reverse); and (iii) HCS = 5′-

ATGGAAGATAGACTCCACAT-3′ (forward) and 5′-TGAGACCTGATCCTTAACTTCC-3′ (reverse). Glyceraldehyde 3-phosphate dehydrogenase was used as the reference gene for qRT-PCR normalization, using primers 5′-TCCACTGGCGTCTTCACC-3′ (forward) and 5′-GGCAGAGATGATGACCCTTT-3′ (reverse) [6]. The cycle threshold values were used to calculate amplicon abundance [26].

2.5 Mapping HCS-binding sites of adenine-methylated sequences

Genomic DNA was purified by using the DNeasy mini kit (Qiagen) 48 h post transfection. DNA was digested with *DpnI* (Fermentas) and purified using a PCR purification kit (Qiagen). The digested DNA was ligated into the pBlueScript II sk(+) vector after vector linearization with *BamHI* (Fermentas); the Fast-Link™ DNA Ligation Kit was used for ligation. Note that digestion with *DpnI* and *BamHI* produces compatible overlaps. The ligation mixture was transformed into MAX Efficiency® DH5α™ Competent Cells and the cells were plated on LB-ampicillin plates. White colonies were randomly selected for sequencing by using the T7 promoter primer (Eurofins MWG Operon, Huntsville, AL). HCS-binding loci were identified by matching sequences against human genome using NCBI's BLAST tool, and were defined as sequences within the genes and sequences up to 100,000 basepairs upstream or downstream of the genes.

2.6 Statistics

The GraphPad Prism 5.0 program (La Jolla, CA) was used to plot qRT-PCR graphs. Data are presented as the mean ± SEM. Multiple comparisons were evaluated by one-way ANOVA, followed by post hoc Tukey test. Values were considered statistically significant, if $P < 0.05$.

3. Results and Discussion

3.1 Plasmid expression

Transfection of mammary cells with plasmids Dam-HCS, EcoDam, or HCS produced the expected expression patterns compared with non-transfected control cells. When cDNA was analyzed using PCR primers for HCS, the transcript abundance was 170-fold and 62-fold greater in MCF-7 cells transfected with HCS and HCS-Dam, respectively, than in non-transfected control cells (Fig. 1A); the abundance of HCS transcript was not altered by transfection with EcoDam (negative control) as it did not contain HCS. When cDNA was analyzed using PCR primers for the HCS-Dam fusion protein, the transcript abundance was 2000-fold greater in MCF-7 cells transfected with HCS-Dam than in non-transfected control cells (Fig. 1B); the abundance of HCS-Dam fusion transcript was not altered by transfection with EcoDam and HCS (negative controls) as these controls lacked HCS-Dam fusion. When cDNA was analyzed using PCR primers for Dam, the transcript abundance was 660-fold and 800-fold greater in MCF-7 cells transfected with EcoDam and HCS-Dam, respectively, than in non-transfected control cells (Fig. 1C); the abundance of Dam transcript was not altered by transfection with HCS (negative control) as it did not contain Dam. Results were similar in MCF-10A cells (Fig. 2).

3.2 HCS-docking sites

Following the validation of expression of fusion and control plasmids, these plasmids were expressed in MCF-7 and MCF-10A cell lines, genomic sequences methylated by Dam were cloned into the pBlueScript II sk(+) cloning vector, and then positive clones were obtained and sequenced. When five clones from HCS-Dam positive MCF-7 cells were annotated by NCBI's basic local alignment search tool (BLAST), eight unique HCS-binding sequences, within specific genes, were detected (Table 1). Each clone returned more than one sequence,

because of the complimentary ends created during digestion with *DpnI* and the random ligation of these fragments during ligation and cloning. Transfection with plasmid HCS (negative control) did not produce any clones in MCF-7 cells. We conclude that HCS greatly enhances the binding of Dam at distinct genomic loci. Results in MCF-10A cells were similar to those in MCF-7, including five clones identified from HCS-Dam positive MCF-10A cells that contained eight unique sequences when annoted by BLAST, (Table 2).

Within these data, some important observations were noted. First, when MCF-10A cells were transfected with plasmid HCS, one clone with one single sequence was identified compared with the seven clones in cells transfected with HCS-Dam, indicating that the false positive rate due to these artifacts might be up to 14% in Dam-based assays. Also, for the MCF-10A cell line, a locus within X-linked neuroligin4 gene was disregarded as a potential HCS docking site, as it appeared due to artifactual methylation by pIND (V5) EcoDam control. No such artifactual methylation by Dam was observed for MCF-7 cells. Finally, three HCS-docking sites were found to be common between MCF-7 and MCF-10A cells, indicating a 26% overlap in the HCS binding regions identified in the two epithelial cell lines, increasing confidence that the results produced by HCS-Dam technology are real. Note that clones were selected randomly in this proof-of-concept study and, therefore, one must not expect a 100% overlap in clones. Comprehensive maps can only be generated by using microarray-based technologies or high-throughput sequencing and may show an expected overlap of 100% between the identified genes, as both cell lines used in this study are of epithelial origin or alternatively could allow for determination of non-overlapping loci specific for either cell type. Also note that the following gene sets share a common HCS-docking locus: *CRH* and *RRS1* (Tables 1 and 2), cadherin 8 and cadherin 11 (Table 1) and, SRY-box4 and prolactin (Table 2).

This study proves feasibility of the DamID technology to identify, for the first time, potential HCS binding sites in human mammary epithelial cell lines. DamID technology eliminates the need for large cell numbers and antibodies and thus represents a viable alternative method to the chromatin immunoprecipitation (ChIP) assay, a frequently used tool to investigate DNA-chromatin interactions. With the establishment of the ability of DamID technology to identify chromatin protein binding sites, the future goal of this project is to use the technology along with cDNA microarray sequencing to monitor chromatin proteins in small cell samples, such as oocytes. While the combination of techniques employed in this study only provided a snapshot of events occurring at the genomic level, it proves the DamID technology's utility and when combined with cDNA microarray sequencing, has the potential for full genome analysis.

Acknowledgments

A contribution of the University of Nebraska Agricultural Research Division, supported in part by funds provided through the Hatch Act (NEB-21-146). Additional support was provided by NIH grants DK063945, DK077816, DK082476 and ES015206, USDA CSREES grant 2006-35200-17138, Nebraska Research Initiative, American Heart Association, and by NSF grant EPS 0701892.

References

1. Camporeale G, Giordano E, Rendina R, Zempleni J, Eissenberg JC. *Drosophila melanogaster* holocarboxylase synthetase is a chromosomal protein required for normal histone biotinylation, gene transcription patterns, lifespan, and heat tolerance. J Nutr. 2006; 136:273542.
2. Bao B, Pestinger V, Hassan YI, Borgstahl GE, Kolar C, Zempleni J. Holocarboxylase synthetase is a chromatin protein and interacts directly with histone H3 to mediate biotinylation of K9 and K18. J Nutr Biochem.

3. Stanley JS, Griffin JB, Zempleni J. Biotinylation of histones in human cells. Effects of cell proliferation. Eur J Biochem. 2001; 268:5424–9. [PubMed: 11606205]
4. Hymes J, Fleischhauer K, Wolf B. Biotinylation of histones by human serum biotinidase: assessment of biotinyl-transferase activity in sera from normal individuals and children with biotinidase deficiency. Biochem Mol Med. 1995; 56:76–83. [PubMed: 8593541]
5. Gralla M, Camporeale G, Zempleni J. Holocarboxylase synthetase regulates expression of biotin transporters by chromatin remodeling events at the SMVT locus. J Nutr Biochem. 2008; 19:400–8. [PubMed: 17904341]
6. Chew YC, West JT, Kratzer SJ, Ilvarsonn AM, Eissenberg JC, Dave BJ, Klinkebiel D, Christman JK, Zempleni J. Biotinylation of histones represses transposable elements in human and mouse cells and cell lines and in *Drosophila melanogaster*. J Nutr. 2008; 138:231622.
7. Kobza KA, Chaiseeda K, Sarath G, Takacs JM, Zempleni J. Biotinyl-methyl 4 (amidomethyl) benzoate is a competitive inhibitor of human biotinidase. J Nutr Biochem. 2008; 19:826–32. [PubMed: 18479898]
8. Camporeale G, Shubert EE, Sarath G, Cerny R, Zempleni J. K8 and K12 are biotinylated in human histone H4. Eur J Biochem. 2004; 271:2257–63. [PubMed: 15153116]
9. Chew YC, Camporeale G, Kothapalli N, Sarath G, Zempleni J. Lysine residues in N terminal and C-terminal regions of human histone H2A are targets for biotinylation by biotinidase. J Nutr Biochem. 2006; 17:225–33. [PubMed: 16109483]
10. Kobza K, Camporeale G, Rueckert B, Kueh A, Griffin JB, Sarath G, Zempleni J. K4, K9 and K18 in human histone H3 are targets for biotinylation by biotinidase. FEBS J. 2005; 272:4249–59. [PubMed: 16098205]
11. Chew YC, Raza AS, Sarath G, Zempleni J. Biotinylation of K8 and K12 co-occurs with acetylation and mono-methylation in human histone H4. J FASEB. 2006; 20:A610.
12. Healy S, Perez-Cadahia B, Jia D, McDonald MK, Davie JR, Gravel RA. Biotin is not a natural histone modification. Biochim Biophys Acta. 2009; 1789:719–33. [PubMed: 19770080]
13. Bailey LM, Ivanov RA, Wallace JC, Polyak SW. Artifactual detection of biotin on histones by streptavidin. Anal Biochem. 2008; 373:71–7. [PubMed: 17920026]
14. Takechi R, Taniguchi A, Ebara S, Fukui T, Watanabe T. Biotin deficiency affects the proliferation of human embryonic palatal mesenchymal cells in culture. J Nutr. 2008; 138:680–4. [PubMed: 18356320]
15. Ghosh, S. PhD thesis. School of Biological Sciences, University of Nebraska-Lincoln; 2009. Physiology, regulation, and pathogenesis of nitrogen metabolism in opportunistic fungal pathogen *Candida albicans*. Ph.D. advisor: Ken W. Nickerson
16. Pestinger V, Wijeratne SS, Rodriguez-Melendez R, Zempleni J. Novel histone biotinylation marks are enriched in repeat regions and participate in repression of transcriptionally competent genes. J Nutr Biochem. 2011 in press.
17. Li D, Da L, Tang H, Li T, Zhao M. CpG methylation plays a vital role in determining tissue- and cell-specific expression of the human cell-death-inducing DFF45-like effector A gene through the regulation of Sp1/Sp3 binding. Nucleic Acids Res. 2008; 36:330–41. [PubMed: 18033804]
18. Cheung WL, Ajiro K, Samejima K, Kloc M, Cheung P, Mizzen CA, Beeser A, Etkin LD, Chernoff J, Earnshaw WC, Allis CD. Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. Cell. 2003; 113:507–17. [PubMed: 12757711]
19. Boulikas T. At least 60 ADP-ribosylated variant histones are present in nuclei from dimethylsulfate-treated and untreated cells. EMBO J. 1988; 7:57–67. [PubMed: 3359995]
20. Boulikas T. DNA strand breaks alter histone ADP-ribosylation. Proc Natl Acad Sci USA. 1989; 86:3499–503. [PubMed: 2726732]
21. Wijeratne SSK, Camporeale G, Zempleni J. K12-biotinylated histone H4 is enriched in telomeric repeats from human lung IMR-90 fibroblasts. J Nutr Biochem. 2010; 21:310–6. [PubMed: 19369050]
22. Camporeale G, Oommen AM, Griffin JB, Sarath G, Zempleni J. K12 biotinylation histone H4 marks heterochromatin in human lymphoblastoma cells. J Nutr Biochem. 2007; 18:760–8. [PubMed: 17434721]

23. van Steensel B, Henikoff S. Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. Nat Biotechnol. 2000; 18:424–8. [PubMed: 10748524]
24. van Steensel, B. DamID, Frequently Asked Questions. [accessed 1/29/2011]. Available from: http://research.nki.nl/vansteensellab/DamID%20info/FAQ.htm
25. van Steensel, B. DamID Plasmid. [accesses 1/26/2011]. http://research.nki.nl/vansteensellab/damid.htm
26. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25:402–8. [PubMed: 11846609]

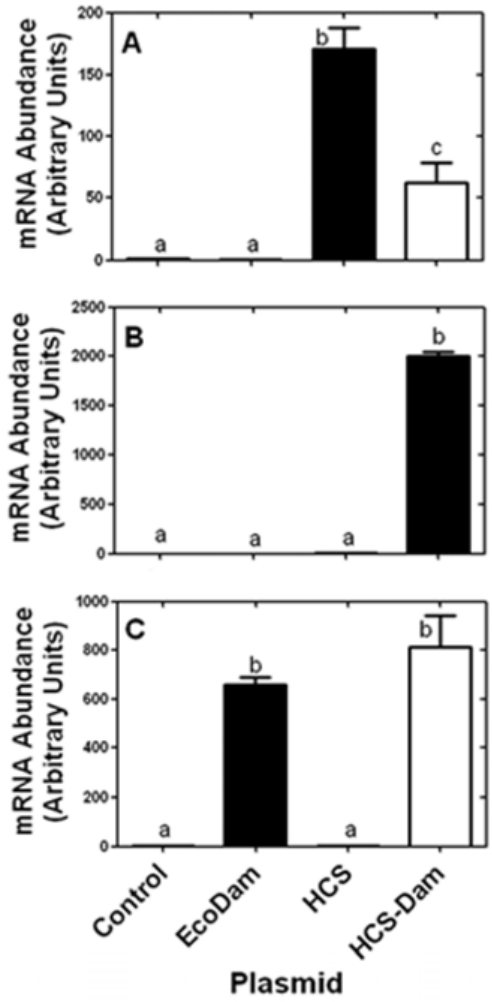

Figure 1.

Transcript abundance in MCF-7 cells transfected with plasmids HCS-Dam, EcoDam and HCS. Transcripts were quantified by qRT-PCR using gene-specific primers for HCS (A), HCS-Dam fusion (B), and EcoDam (C). Each bar represents the mean ± SEM, n = 3. [a,b,c]Bars with different letters indicate significant differences ($P < 0.05$) between treatments.

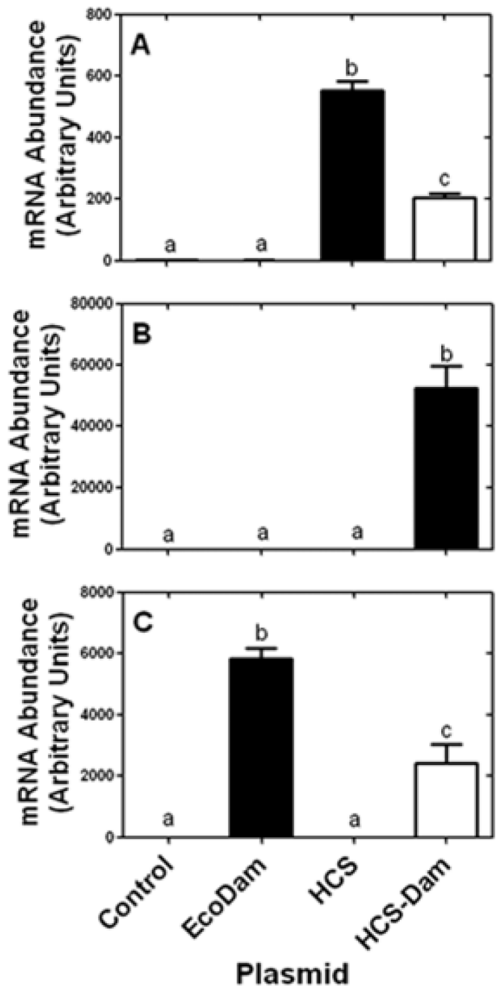

Figure 2.

Transcript abundance in MCF-10A cells transfected with plasmids HCS-Dam, EcoDam and HCS. Transcripts were quantified by qRT-PCR using gene-specific primers for HCS (A), HCS-Dam fusion (B), and EcoDam (C). Each bar represents the mean ± SEM, n = 3. [a,b,c]Bars with different letters indicate significant differences ($P < 0.05$) between treatments.

Table 1

Genomic HCS binding sites in MCF-7 cells.

MCF-7 HCS-Binding Loci	Genes	Chromosome	GenBank Acession Number
5′- CCACAGAATCAGGGGATAACGCAGGAAAGAACAT- 3′	Inositol polyphosphate-5-phosphatase A	10	NM_005539.3
5′- CAGGAAAGAACATGTGAGCAAAAG- 3′	Corticotropin releasing hormone precursor (CRH), And Homolog of yeast ribosome biogenesis regulatory protein (RRS1)	8	NM_000756.2 NM_015169.3
5′- ATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA- 3′	Cadherin 8 and, Cadherin 11 Type2 preproproteins	16	NM_001796.2 NM_001797.2
5′- ACCATGCTCTTTCCCCCTTGCAACCTCACTCATTCTCTCTCCTA TTC-3′	Schwannomin interacting protein 1 (SCHIP1)	3	NM_014575.2
5′- GGTTTTTATCTTTGCAAGCAGCAGAT-3′	Neuron navigator 3 (NAV3)	12	NM_014903.4
5′ ATTCCACACAACATACGAGC-3′	Leptin precursor	7	NM_000230.2
5′- AAAGGGATTTTGGGGCATGAGATCATC- 3′	Apoptosis inhibitor 5 isoform b	11	NM_006595.3
5′- AAAGGGATTTTGGGGCATGAGATCATC- 3′	Netrin-G1 ligand	11	NM_020929.1

Table 2

Genomic HCS binding sites in MCF-10A cells.

MCF-10A HCS-Binding Loci	Genes	Chromosome	GenBank Acession Number
5′- CCACAGAATCAGGGGATAACGCAGGAAAGAACAT- 3′	Inositol polyphosphate-5-phosphatase A	10	NM_005539.3
5′- TCGCCCTATAGTGAGTCGTAT-3′	Homo sapiens myosin, heavy chain9, non-muscle (MYH9)	22	NM_002473.4
5′- CAGGAAAGAACATGTGAGCAA AAG- 3′	Corticotropin releasing hormone precursor (CRH), and Homolog of yeast ribosome biogenesis regulatory protein (RRS1)	8	NM_000756.2 NM_015169.3
5′- TGAGCTAACTCACATTAATTGCGTTG- 3′	SRY (sex determining region Y)-box 4, and Prolactin	6	NM_003107.2 NM_001163558.1
5′- CCTGTTTTGCAGGGTGCTTT- 3′	Purinergic receptor P2X5 isoform A and B	17	NM_002561.2
5′- GAGCTCCAGCTTTTTTTCTCTTTAG- 3′	Tetratricopeptide repeat domain 7B	14	XM_510118.2
5′- CCTGGGGTGCCTAATGAGTG-3′	CUB and Sushi multiple domains 1	8	Q96PZ7-1
5′- ATTCCACACAACATACGAGC-3′	Leptin precursor	7	NM_000230.2