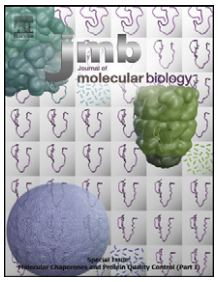

Lysine Deacetylases Regulate the Heat Shock Response Including the Age-Associated Impairment of HSF1

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Abstract

Heat shock factor 1 (HSF1) is critical for defending cells from both acute and chronic stresses. In aging cells, the DNA binding activity of HSF1 deteriorates correlating with the onset of pathological events including neurodegeneration and heart disease. We find that DNA binding by HSF1 is controlled by lysine deacetylases with HDAC7, HDAC9, and SIRT1 distinctly increasing the magnitude and length of a heat shock response (HSR). In contrast, HDAC1 inhibits HSF1 in a deacetylase-independent manner. In aging cells, the levels of HDAC1 are elevated and the HSR is impaired, yet reduction of HDAC1 in aged cells restores the HSR. Our results provide a mechanistic basis for the age-associated regulation of the HSR. Besides HSF1, the deacetylases differentially modulate the activities of unrelated DNA binding proteins. Taken together, our data further support the model that lysine deacetylases are selective regulators of DNA binding proteins.

Introduction

Homeostasis relies on a dynamic cellular environment to aptly react to fluctuating physiological demands [1]. Inducible pathways culminating in gene expression programs exemplify the need for prompt action. For example, physiological stressors such as elevated temperatures or steroid hormones trigger signaling systems that cascade into transcriptional responses. In general, these multistep paths are driven forward by cooperative interactions between select protein partners including the activated transcription factors, histone acetyltransferases, chromatin remodelers, and preinitiation factors [2]. As stress-signaling pathways require quick transduction of information, the systems are reliant on efficient transitions between the cooperatively assembled structures, as well as guidance to the next step [3]. Recent studies highlight the utility of molecular chaperones in mediating the rapid disassembly of transcription complexes and the prominent use of post-translational modifiers for guiding transcription pathways [4,5]. Here, we investigated how lysine deacetylases control stress-signaling pathways by directly modulating the DNA binding activities of transcription factors. As a molecular model, we focus on the mammalian heat shock response (HSR).

The HSR is an evolutionarily conserved stress-signaling process adapting cells to pathological challenges and is a paradigm for investigating systems directly reactive to physiological cues [6]. Significantly, HSR dysfunction has dire consequences. The capacity of the HSR declines when organisms age, impairing protein homeostasis and correlating with the onset of numerous ailments including neurodegeneration, type II diabetes, and heart disease [7]. Heat shock factor 1 (HSF1) is the principal mediator of the eukaryotic HSR as it coordinates cell's counteractions to both acute and chronic stressors [6]. Notably, HSF1 also is a key modulator of lifespan and a potent driver of oncogenesis [8,9]. Central to these events is HSF1 DNA occupancy, which is requisite to govern the gene programs driving the different cell fates. Therefore, it is imperative to understand how the DNA binding activity of HSF1 is controlled.

In transitioning from a quiescent monomer to an activated trimer, the post-translational modification façade of HSF1 is revamped [10]. For instance, correlations between serine/threonine phosphorylation levels and acquisition of HSF1 trimerization, nuclear

localization, and transcriptional potency have been found [6]. Besides phosphorylation, HSF1 is acetylated, sumoylated, and ubiquitinated [11]. While the significances of these modifications are not completely understood, lysine acetylation within the DNA binding domain (DBD) can inhibit DNA interactions whereas acetylation of the central regulatory domain (RD) deters degradation of HSF1 [12–14]. Hence, modification of select HSF1 lysines is a potent means to control the HSR and support cell vitality.

Lysine acetylation within the binding cleft of HSF1 is sufficient to block DNA interactions. Prior studies have shown that the GCN5 acetyltransferase selectively targets HSF1 lysine 80 (K80), which stabilizes HSF1 to DNA, whereas K80 is one of many residues modified by the P300 acetyltransferase [13,14]. In either case, acetylation of K80 impedes HSF1 DNA binding activity and limits the HSR. Minimally, the Sirtuin 1 (SIRT1) deacetylase relieves the inhibitory mark thereby enabling stress-induced HSF1 DNA binding and extending an HSR [12]. The role of SIRT1 is not universal since blockage of SIRT1 does not affect neuroprotection yet inhibiting type I/II histone deacetylases (HDACs) hinders neuronal heat defenses [15]. While the responsible HDAC has not been identified, the work illustrates that the reprieve of the constraining HSF1 modifications will involve lysine deacetylases (KDACs) specific to cell and disease.

Intertwined within the acetylation process that controls transcription factor DNA binding activities is the p23 molecular chaperone [13]. Before the lysines coordinating DNA interactions can be acetylated, p23 must dissociate the DNA-bound complex, as DNA binding conceals the target lysine [13]. The involvement of p23 in transcription factor acetylation events was founded in a synthetic genetic array linking the chaperone to both acetylases and deacetylases along with the discovery that the loss of p23 triggers increased expression of GCN5 and HDAC1 [13,16]. In our early model, we suggested that the increase in HDAC1 balanced the elevation in GCN5—extra HDAC1 might remove the superfluous GCN5 acetylation modifications thereby reestablishing the DNA binding activities of target proteins [13]. Here, we tested this basic hypothesis and discovered that regulation through lysine deacetylase action is complicated as KDACs both positively and negatively modulate DNA binding activities using deacetylase-dependent and deacetylase-independent mechanisms.

Results

Select lysine deacetylases remove the inhibitory GCN5 mark on HSF1

To test the impact of HDAC1 on GCN5-mediated inhibition of the HSR, we exploited the heat-induced DNA binding activity of HSF1 in the human embryonic kidney cell line 293T. *A priori*, we had anticipated that most, if not all, lysine deacetylases (KDACs) would suffice in removing the inhibitory K80 acetyl group. Given our previous genetic data linking p23/GCN5/HDAC1, we believed that HDAC1 would certainly counter the GCN5 mark on HSF1 [13]. While overexpression of GCN5 by transient transfection effectively diminished the binding of HSF1 to the proximal heat shock element (HSE) of the *HSP70* promoter following exposure to heat stress, coexpression of HDAC1 did not rescue binding activity (Fig. 1a). However, joint expression of SIRT1 was adequate to recover stress-induced HSF1 DNA binding (Fig. 1a). Hence, the HDAC1 and SIRT1 deacetylase display discriminate regulation of HSF1 in conjunction with GCN5.

To further explore the selectivity of KDACs with a non-histone protein target, we screened a panel of 12 deacetylases with representatives from the three main KDAC groups. Based on protein structure and catalytic activity, there are 18 known mammalian KDACs divided into four clusters: class I (HDAC1–HDAC3 and HDAC8); class II (HDAC4–HDAC7, HDAC9, and HDAC10); class III (SIRT1–SIRT7); and class IV (HDAC11) [17,18]. Only three KDACs (HDAC7, HDAC9, and SIRT1) countered the GCN5 inhibitory effect on heat-induced HSF1 DNA binding (Fig. 1b).

Lysine deacetylases discriminately regulate HSF1 activities

To determine if the specificity of the deacetylases was particular to the GCN5 mark, we checked the intrinsic heat-triggered HSF1 pathway. Acetylation of HSF1 increases under heat shock conditions thereby dampening the response [12,13]. As heat shock promoters are maintained in nucleosome-depleted states *in vivo*, we continued to exploit electrophoretic mobility shift assays (EMSAs) as a means to monitor HSF1 DNA interactions. Similar to the relief of GCN5 inhibition, overexpression of HDAC7, HDAC9, and SIRT1 by transient transfection stimulated heat-triggered HSF1 DNA binding (Fig. 2a). Hence, these three KDACs can counter acetylation marks that are repressive to HSF1 DNA binding activity independent of the modifying source.

Unexpectedly, HDAC1 overexpression in 293T cells was sufficient to impair HSF1 DNA interactions (Fig. 2a). The impact of the KDACs is independent of cell context, as heat-activated HSF1 was differentially modulated by the deacetylases in both rabbit reticulocyte lysate and mouse embryonic fibroblasts (MEFs) (Supplementary Fig. 1). Since only the individual KDACs were expressed with HSF1, the effects likely do not involve large deacetylase complexes (e.g., NuRD or SIN3). Rather, the individual KDACs appear sufficient to control HSF1 DNA binding activity.