

## Extra View

# HDAC6 a new cellular stress surveillance factor

Patrick Matthias,<sup>3</sup> Minoru Yoshida<sup>4,6</sup> and Saadi Khochbin<sup>1, 2,\*</sup>

<sup>1</sup>INSERM, U823; Grenoble France; <sup>2</sup>Université Joseph Fourier; Institut Albert Bonniot; Grenoble France; <sup>3</sup>Friedrich Miescher Institute for Biomedical Research; Novartis Research Foundation; Basel Switzerland; <sup>4</sup>Chemical Genetics Laboratory; RIKEN; Wako, Saitama Japan; <sup>5</sup>Japan Science and Technology Corporation (JST); CREST Research Project; Kawaguchi, Saitama Japan; <sup>6</sup>Department of Biotechnology; The University of Tokyo; Tokyo Japan

**Abbreviations:** CFTR, cystic fibrosis transmembrane conductance regulator; MTOC, microtubule-organizing centre; HSF1, heat-shock factor 1; HDAC, histone deacetylase; AAA ATPase, ATPase associated with various cellular activities

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Less than a decade has passed since HDAC6 was first identified and regarded as an unusual histone deacetylase harbouring two catalytic domains. Early demonstration of its cytoplasmic localisation, its ubiquitin-binding and its tubulin-deacetylase activities took HDAC6 far away from everything known to involve other histone-deacetylases. Recent discoveries confirmed the very unique functions of HDAC6 among deacetylases and pointed to this protein as a master regulator of cell response to cytotoxic assaults. HDAC6 appears both as a sensor of stressful stimuli and as an effector, which, thanks to its wide range of activities, mediates and coordinates appropriate cell responses.

## HDAC6 controls a wide range of cellular activities through its impact on tubulin and actin cytoskeleton

The first clues on the involvement of HDAC6 in a specific biological pathway came after the observation of its ubiquitin binding activity<sup>1-3</sup> and its dramatic re-location in ubiquitinated aggresomes upon proteasome inhibition.<sup>4</sup> Aggresomes are specific structures juxtaposing the nucleus at the proximity of the microtubule-organizing centre (MTOC). They correspond to the endpoint of a microtubule-dependent transport of misfolded ubiquitinated protein aggregates and are believed to play a cytoprotective role by reducing the toxicity of scattered aggregates.<sup>5</sup>

The involvement of HDAC6 in this process became a first choice hypothesis since HDAC6 had previously been shown to also interact with the microtubule motor complex containing p150<sup>glued</sup>.<sup>6</sup> HDAC6 stimulated the formation of aggresomes containing the misfolded CFTR mutant and this function of the protein depended on both its ubiquitin-binding domain<sup>3</sup> and its deacetylase activity.<sup>4</sup>

Although aggresome formation could be a way to minimize the toxicity of scattered micro-aggregates, it does not overcome all the cellular defects triggered by the accumulation of protein aggregates in a cell, including the impairment of proteasome activity.<sup>7</sup>

Autophagy is one of the major degradation mechanisms conserved among eukaryotic cells, mediating the turnover and recycling of long-lived cytosolic proteins, excess or damaged organelles and aberrant protein aggregates.<sup>8</sup> The activation of autophagy therefore appears as an excellent solution to face the aggregates/aggresome-mediated troubles. Not long after the demonstration of the involvement of HDAC6 in aggresome formation, the Kopito laboratory showed an HDAC6-dependent autophagic clearance of mutant huntingtin aggregates.<sup>9</sup> The involvement of HDAC6 in the activation of autophagy received further support after the demonstration of its role in rescuing the cytotoxicity resulting from proteasome dysfunction or from expression of a mutant poly-glutamine expanded androgen receptor, in a transgenic drosophila model.<sup>10</sup>

Proteasome dysfunction and accumulation of protein aggregates also induce a third type of cellular response, leading to the activation of the major heat shock transcription factor, HSF1, which in turn induces the accumulation of the cellular heat shock proteins.<sup>11</sup> These chaperones possess well-known activities mediating protein folding and degradation of misfolded proteins and have a demonstrated role in safeguarding stressed cells.<sup>12</sup>

Very recent investigations showed that HDAC6 is also a critical player in this third type of cellular response to the accumulation of misfolded protein aggregates. This activity of HDAC6 does not depend on its catalytic activity but mostly relies on its ubiquitin-binding domain, which senses the accumulation of ubiquitinated proteins and transmits this information to HSP90, as will be discussed below.

HDAC6 therefore appears to mediate all the three major cellular response pathways to the cytotoxic accumulation of misfolded and aggregated proteins. First, it favours the formation of aggresomes, second, it activates autophagy and third, it mediates the activation of heat shock proteins accumulation (Fig. 1).

Proteasome dysfunction, which may result as a consequence of the accumulation of protein aggregates and aggresome formation, is somehow sensed by HDAC6 to activate the two other types of responses. This sensing mechanism was clearly established in the "heat-shock" pathway, where ubiquitin-binding by HDAC6 was shown to mediate the dissociation of the repressive HSP90-HSF1 complex.<sup>13</sup> In the case of autophagy, however, the molecular basis of the events linking its activation to the impairment of the proteasome activity remains an open issue.

\*Correspondence to: Saadi Khochbin; Institut Albert Bonniot; Domaine de La Merci; La Tronche Cedex 38706 France; Tel.: 33.4.76.54.95.83; Fax: 33.4.76.54.95.95; Email: khochbin@ujf-grenoble.fr

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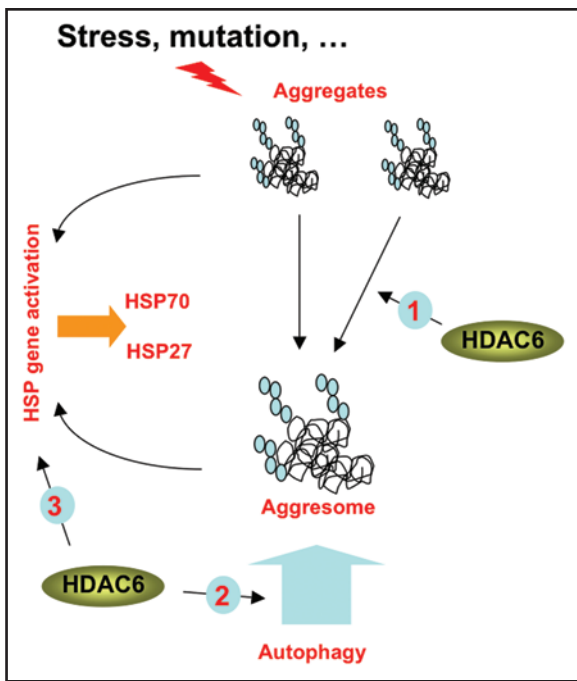


Figure 1. HDAC6 mediates and coordinates three major cell response pathways to the cytotoxic accumulation of protein aggregates. (1) Simultaneous binding of HDAC6 to polyubiquitin chains in the aggregates and to the dynein motors mediates the transport of these aggregates towards MTOC and the formation of an aggresome. (2) The accumulation of aggregates impairs the proteasome activity and enhances the accumulation of ubiquitinated cellular proteins. HDAC6 senses proteasome dysfunction through an unknown mechanism and activates the autophagic clearance of protein aggregates. (3) Ubiquitin-binding by HDAC6 signals proteasome dysfunction to the HSP90-HSF1 complex and induces the release and activation of HSF1 transcription factors, which in turn activates responsive HSP genes, leading to the accumulation of the major chaperones, HSP70 and HSP27.

HDAC6 catalytic activity was also shown to be required for the HDAC6-dependent aggresome formation and autophagic clearance of protein aggregates. Here also, the exact function of HDAC6 catalytic activity is far from clear. HDAC6-controlled tubulin acetylation may have an important indirect role in the intracellular microtubule-dependent trafficking. Indeed, recent investigations showed that microtubule acetylation causes the recruitment of molecular motors dynein and kinesin-1 that ferry cargoes along microtubule tracks.<sup>14</sup> There are clear evidences that microtubule acetylation in neuronal cells, upon HDAC6 inhibition or knock-down, stimulates polarized protein trafficking to subcellular domains.<sup>14,15</sup> In contrast, the catalytic activity of HDAC6 was found to be necessary for aggresome formation and autophagy, two processes which depend on intracellular trafficking. The current model for HDAC6-mediated transport of ubiquitinated protein aggregates is indeed based on the capacity of HDAC6 to simultaneously bind the polyubiquitin of the aggregates and the dynein/dynactin minus end directed motors, transporting the cargo toward the MTOC.<sup>4</sup>

At present the literature is somewhat confusing: while the results of Kawaguchi and colleagues,<sup>4</sup> Iwata and colleagues<sup>9</sup> and Pandey and colleagues,<sup>10</sup> clearly demonstrated the necessary role of HDAC6 catalytic activity for aggresome formation and autophagy promotion, the data presented by Reed et al.<sup>15</sup> and Dompierre et al.<sup>14</sup> rather suggest that HDAC6-dependent tubulin deacetylation should reduce the interaction of molecular motors with microtubules and therefore

interfere with these processes.

It is possible that another factor, also involved in the control of intracellular trafficking, could be the direct target of HDAC6 catalytic activity. Cortactin, recently identified as a new substrate of HDAC6,<sup>16</sup> appears as a possible candidate. Cortactin interacts with F-actin to promote polymerisation and branching and, interestingly, its acetylation prevents actin binding and therefore carries strong functional consequences.<sup>16</sup> This observation might provide an explanation for the role of HDAC6 catalytic activity, at least in autophagy, through the modulation of actin filament-based trafficking,<sup>17</sup> which might affect autophagy.<sup>18,19</sup>

The second emerging general function of HDAC6 is its role in the control of cell motility. Here again, the first clue on HDAC6-regulated cell motility came after the discovery of its tubulin-deacetylase activity by Yao's group who showed that HDAC6-overexpressing NIH3T3 cells move significantly faster than control cells in response to serum.<sup>6</sup> In agreement with this observation, the treatment of cells with a specific inhibitor of HDAC6, tubacin, slightly decreased their motility.<sup>20</sup>

The mechanism requiring HDAC6 catalytic activity in the control of cell motility is also undefined. Early investigations suggested that a decreased tubulin acetylation reduces microtubule stability, accounting for the HDAC6-mediated increased cell motility.<sup>6</sup> This mechanism was also suggested to be responsible for the HDAC6-dependent control of the stability of cilia.<sup>21</sup> Acetylation-controlled microtubule stability seems however to be a complex phenomenon, which is difficult to precisely control, as reflected by the fact that different laboratories have reported conflicting results on the impact of tubulin acetylation on microtubule stability.<sup>20,22-24</sup> Moreover, at least in the case of lymphocyte motility and transmigration, although a modulatory effect of HDAC6 was evidenced, no role for its tubulin-deacetylase activity was found.<sup>25</sup>

Very recent investigations may however help to better understand how HDAC6 functions in this process: Specific inhibition of HDAC6 catalytic activity was found to increase the total adhesion area of the cell with a reduced adhesion turnover, directly affecting cell migration capacity.<sup>24</sup> It is probable that the role of HDAC6 catalytic activity in cell adhesion, and subsequently cell motility, is due to a target other than tubulin. Indeed, the ability of HDAC6 to control actin cytoskeleton may provide an alternative explanation for its control of cell motility. HDAC6 may control formin-mediated actin polymerization.<sup>26,27</sup> Furthermore, the ability of HDAC6 to modulate the cortactin-actin interaction, through the modulation of cortactin acetylation, may also highly impact actin-dependent cell motility and establish a new molecular basis for the involvement of HDAC6 in this process.<sup>16</sup>

Related to these issues, emerging data indicate the involvement of HDAC6 in a third type of general cellular activity, also based on its regulatory actions on the cellular cytoskeleton, which is the control of the formation of specific structures such as cilia. Indeed, HDAC6 catalytic activity was found to promote ciliary disassembly, hence controlling the stability of cilia. In growth-arrested cells, one of the centrioles differentiates into the "basal body", a structure that organizes the microtubule bundles of the ciliary axonema. The tubulin deacetylase activity of HDAC6 is proposed to destabilize the axonema leading to cilium destabilization and resorption.<sup>21</sup> The investigation of the molecular basis of HDAC6 function in ciliary

disassembly revealed the involvement of Aurora A kinase and HEF1 in the phosphorylation and activation of HDAC6 catalytic activity.<sup>21</sup> This Aurora A-mediated HDAC6 activation should also have a profound impact on other HDAC6 functions based on tubulin and actin cytoskeleton, i. e., intracellular trafficking and cell motility, all of which are modulated by HDAC6 catalytic activity.

### Control of HSP90 cellular circuits by HDAC6

After tubulin, HSP90 was identified as the second substrate of HDAC6.<sup>28</sup> This observation suggested for the first time that, besides cytoskeleton-related functions, HDAC6 may also act on completely different cellular circuits.

Early investigations showed that HDAC6-dependent HSP90 acetylation affects the binding of p23, an essential HSP90 co-chaperone and, consequently, the maturation of the glucocorticoid receptor.<sup>28</sup> Later studies showed the effect of HDAC6-regulated acetylation of HSP90 on several other client proteins (for review see Boyault et al.<sup>29</sup>). However, importantly, HSP90 harbours multiple acetylation sites and its acetylation status is a strong determinant in the binding of client proteins and co-chaperones but HDAC6 does not deacetylate all these sites.<sup>30</sup>

Recent investigations also revealed a new role of HDAC6 on HSP90 activity, which depends on ubiquitin-binding by HDAC6. Indeed, a large fraction of HDAC6 was found to be present in a complex with HSP90 in resting cells.<sup>13,28</sup> The interaction of HDAC6 with ubiquitin induces the complex dissociation, leading to the release and activation of one of HSP90 client proteins, the Heat Shock transcription Factor 1, HSF1. The interaction of HSF1 with HSP90 does not seem to be regulated by an HDAC6-controlled acetylation of HSP90, since in cells lacking HDAC6, although HSP90 is hyperacetylated, the HSP90-HSF1 complex remains stable.<sup>13</sup> In this case, p97/VCP a chaperon of the AAA ATPase family with well-known segregase activities, was demonstrated to be responsible for dissociation of the HSP90-HSF1 complex. Early studies had previously identified p97/VCP as a partner of HDAC6 and also showed that ubiquitin-binding by HDAC6 leads to the dissociation of the HDAC6-p97/VCP complex.<sup>1</sup> In the case of excessive accumulation of misfolded ubiquitinated proteins after proteasome dysfunction, ubiquitin-binding by HDAC6 releases p97/VCP, which then uses its segregase activity to dissociate the repressive HSP90-HSF1 complex.<sup>13</sup> Interestingly, these investigations also led to the demonstration of a role for p97/VCP in the recovery of active HDAC6 and the reformation of a basal dormant HSP90 complex.<sup>13</sup> By extension, it is therefore possible to propose that p97/VCP plays a critical role in recycling of HDAC6 after various types of stressful stimuli.

### Discrete functions for HDAC6 in non-stressed cells

The data discussed above point to HDAC6 as a key regulatory molecule involved in the control of vital cellular functions. However, HDAC6 deficient mice have been recently generated and found, perhaps surprisingly, to be viable. Under standard laboratory conditions they look superficially normal, but also exhibit specific phenotypes, such as limited bone defects or a moderately impaired immune response. Remarkably, in these mice tubulin acetylation is greatly elevated; depending on the organ examined, the increase in tubulin acetylation ranges from a few fold (in the spleen) to more than twenty fold (in the testis). These observations definitively identify

HDAC6 as the major tubulin deacetylase and also indicate that there is a remarkable flexibility in how much acetylated tubulin is tolerated by the cell in an in vivo setting (Matthias P, unpublished data).

Altogether, these data support the idea that HDAC6 functions become essential mostly in response to various stimuli and that in non-stressed cells, despite all the functions potentially involving HDAC6, no essential role for this enzyme could be evidenced. It is therefore very likely that HDAC6 deficient mice will exhibit increased susceptibility to various forms of stress when challenged, and this will be examined in the future.

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