Cell Stress and Chaperones (2017) 22:307–315
DOI 10.1007/s12192-016-0754-9

SHORT COMMUNICATION

Loss of malin, but not laforin, results in compromised autophagic flux and proteasomal dysfunction in cells exposed to heat shock

Navodita Jain[1] · Anupama Rai[1] · Rohit Mishra[1] · Subramaniam Ganesh[1]

Received: 13 June 2016 / Revised: 4 December 2016 / Accepted: 5 December 2016 / Published online: 14 December 2016

Abstract Heat stress to a cell leads to the activation of heat shock response, which is required for the management of misfolded and unfolded proteins. Macroautophagy and proteasome-mediated degradation are the two cellular processes that degrade polyubiquitinated, misfolded proteins. Contrasting pieces of evidence exist on the effect of heat stress on the activation of the above-mentioned degradative pathways. Laforin phosphatase and malin E3 ubiquitin ligase, the two proteins defective in Lafora neurodegenerative disorder, are involved in cellular stress response pathways and are required for the activation of heat shock transcription factor - the heat shock factor 1 (HSF1) - and, consequently, for cellular protection under heat shock. While the role of laforin and malin in the proteolytic pathways is well established, their role in cellular recovery from heat shock was not explored. To address this, we investigated autophagic flux, proteasomal activity, and the level of polyubiquitinated proteins in Neuro2a cells partially silenced for laforin or malin protein and exposed to heat shock. We found that heat shock was able to induce autophagic flux, proteasomal activity and reduce the polyubiquitinated proteins load in the laforin-silenced cells but not in the malin-deficient cells. Loss of malin leads to reduced proteasomal activity in the heat-shocked cells. Taken together, our results suggest a distinct mode of action for laforin and malin in the heat shock-induced proteolytic processes.

Electronic supplementary material The online version of this article (doi:10.1007/s12192-016-0754-9) contains supplementary material, which is available to authorized users.

✉ Subramaniam Ganesh
sganesh@iitk.ac.in

[1] Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208016, India

Keywords Heat shock response · Ubiquitin-proteasome system · Autophagy · Epilepsy · Post-translational modifications

Introduction

Heat shock response is characterized by the induction of heat shock proteins (HSPs) that protect cellular proteins from misfolding and aggregation. HSPs also induce the E3 ubiquitin ligases that assist in the proteasome-mediated clearance of the misfolded proteins (McDonough and Patterson 2003). This conserved cellular heat shock response pathway is triggered and regulated by a master regulator, the heat shock factor 1 (HSF1), a transcription factor activated by thermal stress (Cotto and Morimoto 1999). HSF1 directly regulates the transcription of genes coding for the HSPs and indirectly regulates the expression of a number of genes by transactivating other transcription factors (Anckar and Sistonen 2011). Thermal stress is known to denature proteins and promote their aggregation. To clear the increased load of abnormal proteins, the heat shock response is thought to be followed by the activation of proteolytic processes such as autophagy and ubiquitin proteasomal system, suggesting a cross talk between the heat shock response and the proteolytic processes (Dokladny et al. 2015). Indeed, there is evidence suggesting that HSF1 induces autophagy through the direct transcriptional regulation of an autophagy gene. For example, HSF1 was shown to induce the transcription of *Atg7*—gene critical for inducing autophagy and cell survival (Desai et al. 2013). Similarly, the overexpression of HSF1 in mouse models of Alzheimer's disease led to autophagy induction (Pierce et al. 2013). A study also suggests heat shock to be a protective mechanism against neurodegeneration as it induces autophagy (Liu et al. 2010). Few reports also demonstrate the

negative effect of HSF1 on proteolytic processes. For example, loss of HSF1 led to the activation of basal autophagy (Dokladny et al. 2013), and, conversely, active HSF1 was also shown to suppress the expression of LC3 under heat stress (Zhao et al. 2009). While these studies suggest a functional link between the heat shock response and the proteolytic processes, these contradictory findings also call for further studies in this direction.

Lafora disease (LD) is a fatal form of a teenage onset, progressive myoclonus epilepsy, characterized by other symptoms such as ataxia, dementia, psychosis, and muscle wasting (Delgado-Escueta et al. 2001; Ganesh et al. 2006). LD patients die within about 10 years of the first epileptic episode (Serratosa et al. 2012). LD is caused due to the mutations in the *EPM2A* or the *NHLRC1* gene, coding for the laforin protein phosphatase or the malin E3 ubiquitin ligase, respectively (Singh and Ganesh 2009). One of the characteristic features of LD is the formation of polyglucosan bodies, called the Lafora bodies, in the affected tissues including the neurons (Delgado-Escueta et al. 2001; Ganesh et al. 2006). LD mouse models developed polyglucosan bodies in neurons and other tissues and displayed widespread neurodegeneration (Ganesh et al. 2002). Laforin and malin are thought to function as a complex and have been shown to play a role in proteolytic processes. These include ubiquitin-proteasome system (Mittal et al. 2007; Garyali et al. 2009) and autophagy; cells deficient for laforin or malin showed autophagy blockade (Aguado et al. 2010; Puri et al. 2012; Criado et al. 2012; Singh et al. 2013; Garyali et al. 2014). A direct role for these proteins was also shown in heat shock response; laforin and malin were shown to be required for the activation and nuclear translocation of HSF1 (Sengupta et al. 2011). Since the heat shock response and proteolytic processes have functional links and since the LD protein functions in both pathways, in the current submission, we assessed the role of laforin and malin on the cellular proteolytic machinery, namely the autophagy and proteasome-mediated degradation, during recovery from heat shock.

Materials and methods

Cell culture and transfection

Neuro2a cell line was obtained from the National Centre for Cell Science, Pune, and was grown in DMEM (Sigma-Aldrich), supplemented with 10% fetal bovine serum (Gibco International) and 1% Pen/Strep (Invitrogen). For the heat shock experiments, the cells were incubated at 42 °C in a water bath for 1 h and harvested at the end of the heat shock or were allowed to recover at 37 °C and then were harvested at 2, 4 8, or 16 h post-heat shock (see Fig. 1a for the study design). This post-heat shock time window tested will hereafter be referred to as the "recovery period." Cells were treated with 4 μM Bafilomycin A1 (Sigma-Aldrich) for 2 h preceding the harvest.

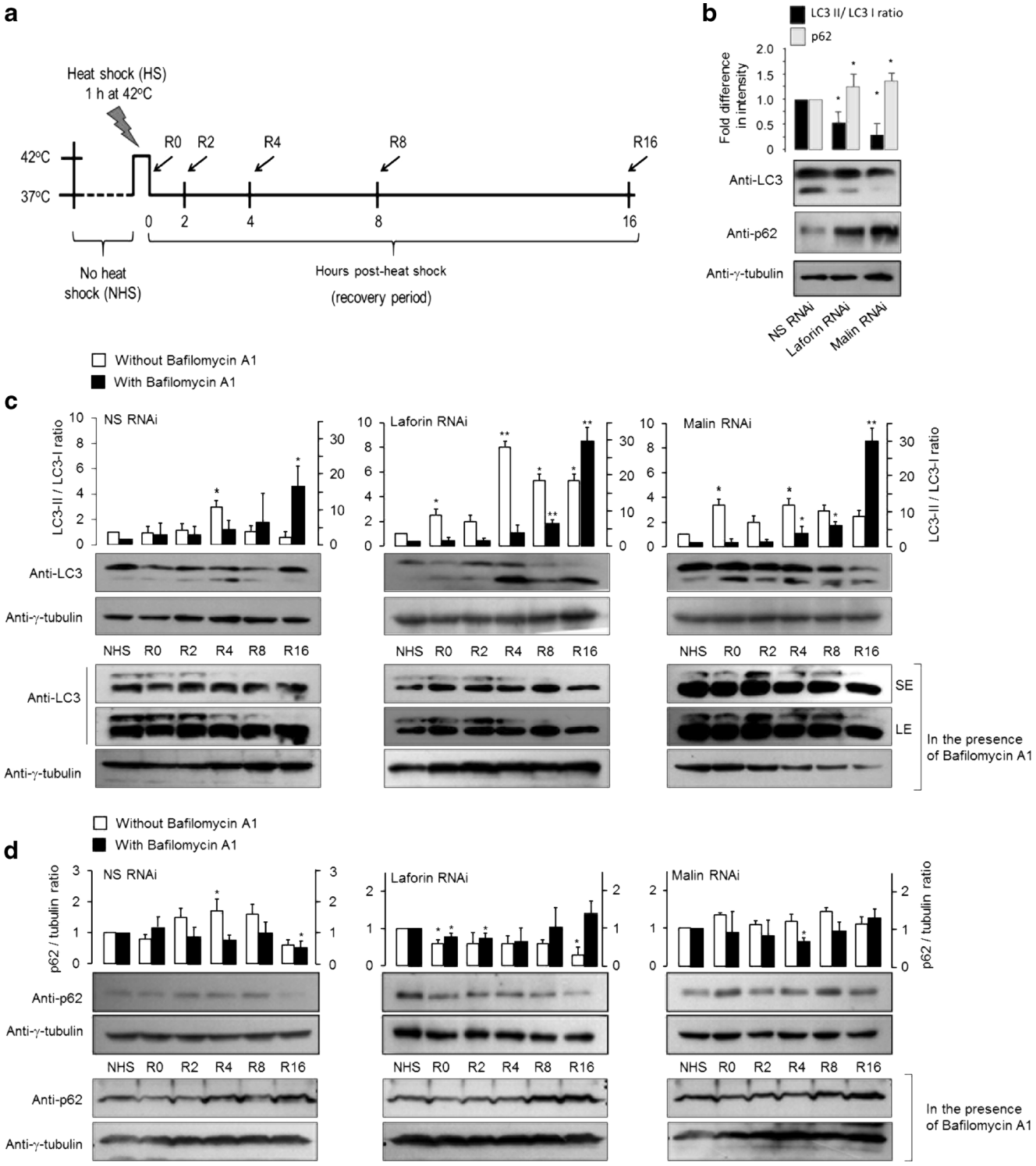
Expression constructs

The RNAi constructs (shRNAmir) for laforin and malin were originally purchased from Open Biosystems, USA (Expression Arrest microRNA-adapted shRNA libraries). In these constructs, with the pLKO.1 backbone vector, the double stranded RNA against the target genes is expressed using the U6 pol III promoter; thus, the knockdown efficiency is much higher than conventional siRNA duplex as the pol III construct is expected to express up to 72 h post-transfection. The efficiency of knockdown constructs was confirmed by semi-quantitative PCR and also by using overexpression constructs for laforin or malin (Supplementary Fig. S1). These knockdown constructs have been reported in our previous studies as well (Garyali et al. 2009; Jain et al. 2016).

Antibodies and reagents

The antibodies used for the immunoblotting experiments and their sources are as follows: anti-LC3 (Cell Signaling Technology), anti-p62 and anti-polyubiquitin (Enzo Life Sciences), and anti-γ-Tubulin (Sigma-Aldrich Pvt. Ltd.). Secondary antibody conjugated to horseradish peroxidase (HRP) was procured from Jackson ImmunoResearch Inc. The 7-amino-4-methylcoumarin (AMC) conjugated fluorescent proteasome substrates, with peptides cleaved by the enzymatic activities of cellular trypsin-like (Substrate VI), chymotrypsin-like (Suc-LLVY-AMC, Substrate III), and caspase-like (Substrate II) were obtained from Merck.

Fig. 1 Autophagy flux during the heat shock recovery in cells silenced for laforin or malin. **a** Schematic diagram depicting the experimental design to test the role of laforin and malin in heat shock response. Neuro2a cells grown at physiological temperature (37 °C) were exposed to a heat shock at 42 °C for 1 h (identified by the *thunder sign*), allowed to recover at 37 °C, and were harvested at 0, 2, 4, 8, or 16 h post-heat shock exposure. The post-heat shock recovery period is referred to as "recovery period," and the time points of harvest are identified as R0 to R16. **b** Representative immunoblots showing the increased cellular levels of endogenous p62 and reduced LC3-II/LC3-I ratio in cells partially silenced for laforin or malin. The *bar diagram* shown above indicates the fold change in the p62 level and LC3-II/LC3-I ratio in the laforin- or malin-silenced cells as compared with the cells transfected with the non-silencing RNAi construct. **c**, **d** Immunoblots showing the relative levels of endogenous LC3 (**c**) or p62 (**d**) in cells exposed to the heat shock and harvested at indicated time points during the recovery (*upper panel* in each figure). Immunoblots representing endogenous p62 and LC3 levels in cells exposed to Bafilomycin A1 for 2 h prior to the harvest served as controls for autophagy flux (*lower panel* in each figure). Since bafilomycin treatment results in increased levels of target proteins, *images* representing the short exposure (*SE*) and the long exposure (*LE*) for the same set of blots are shown. Tubulin served as a loading control (**$p < 0.001$; *$p < 0.05$; two-tailed *t* test; *p* value calculated against *NHS*)



Bafilomycin A1 was obtained from Sigma-Aldrich India Pvt. Ltd. (B1793).

Immunoblotting

Protein samples were resolved on SDS-polyacrylamide gel, transferred to nitrocellulose membrane, and were processed for immunoblotting essentially as described earlier (Singh et al. 2013; Jain et al. 2016) The blot was incubated with the primary and the secondary antibodies according to the manufacturer's instructions, visualized on an X-ray film or a chemiluminescence detector (Bio-Rad). The chemiluminescent substrate (SuperSignal West Pico) was procured from Thermo

Life Sciences. Quantitation of bands and further analysis were done by using the ImageJ software. For the analysis of polyubiquitin proteins, band intensity of the entire lane was considered and normalized with the intensity of tubulin bands.

Proteasome activity assay

Cells were lysed in a buffer with the following composition: 50 mM HEPES (pH 7.5), 5 mM EDTA, 150 mM NaCl, and 1% Triton X-100 on ice. The lysates were centrifuged at 8600×*g* for 15 min at 4 °C. The supernatant was estimated for protein concentration. Ten micrograms (10 μg) of protein was used for proteasome assay, using the fluorogenic proteasome substrate and the assay buffer (250 mM HEPES, 5 mM EDTA, 0.5% NP40) at 60 °C for 1 h. MG132 (Sigma-Aldrich India Pvt. Ltd.) was used as controls at a final concentration of 10 μM. Readings were taken at excitation maxima of 380 nm and emission maxima of 440 nm.

Statistical analyses

Each experiment was repeated at least thrice. Intensity estimation of western blots was carried out through ImageJ (NIH). The standard deviation was estimated using Microsoft Excel; GraphPad Software was used for two-tailed *t* test and *p* value calculations.

Results

Neuro2a cells lacking malin, but not laforin, exhibited compromised autophagic flux upon exposure to heat shock in Neuro2a cells

Cells lacking either laforin or malin are known to display lower levels of LC3-II/LC3-I ratio and higher p62 levels, even when they are not exposed to a heat shock. Malin and laforin are necessary for autophagic induction and loss of either result in autophagy blockade (Aguado et al. 2010; Puri et al. 2012, Criado et al. 2012; Singh et al. 2013; Garyali et al. 2014). The Neuro2a cells used in the present study also showed a similar effect upon a transient loss of laforin or malin (Fig. 1b). To test the effect of heat shock on the autophagic flux, Neuro2a cells were transiently transfected with the shRNAi construct (for the knockdown of laforin or malin) and were subjected to a heat shock at 42 °C for 1 h, followed by recovery at 37 °C for indicated time points, with concurrent assessment of LC3-II and p62 levels (Fig. 1a, b). Interestingly, as shown in Fig. 1c, the LC3-II/LC3-I ratio was higher during the recovery periods for the cells silenced for laforin, especially during the 4- to 16-h period post-heat shock. This correlated well with a decrease in the p62 levels, a substrate of autophagic clearance (Bjorkoy et al. 2005), during the same period (Fig. 1d), suggesting that loss of laforin does not compromise the heat shock-induced autophagy. Intriguingly, the LC3-II/LC3-I ratio was much higher in the laforin-silenced cells during the 4- to 16-h period post-heat shock. However, p62 showed a significant difference in the level only at 16 h post-heat shock (Fig. 1d). This shift in the LC3-II/LC3-I ratio and the change in the level of p62 was fully inhibited when the cells were treated with Bafilomycin A1, a lysosomal proton pump inhibitor, for 2 h preceding the harvest of cells (Fig. 1c, d), suggesting that the observed changes in the laforin silent cells are indeed due to the heat shock-induced autophagy flux. In cells silenced for malin, the LC3-II/LC3-I ratio did increase during the post-heat shock recovery period (Fig. 1c). However, p62 levels remained constant throughout the recovery period, suggesting sub-optimal clearance of autophagic targets (Fig. 1d).

Loss of laforin and malin resulted in contrasting effects on proteasome activity during the post-heat shock recovery period

Ubiquitinated proteins serve as important nodes for cellular signaling, and, depending upon the ubiquitin linkages, the proteins might be targeted for autophagic clearance or for the proteasomal-mediated degradation (Li and Ye 2008). For evaluating the cellular proteasome activity, we assayed the caspase-, chymotrypsin-, and trypsin-like protease activities of the proteasomal proteases. For this, the Neuro2a cells were silenced for either malin or laforin, subjected to heat stress, and harvested at various time points. Cellular lysates were prepared at 4 °C and in a buffer lacking SDS. An equal amount of protein lysates were incubated with fluorogenic peptides, and a reaction with the proteasomal inhibitor MG132 was used as control (lysate of unstressed cell incubated with MG132). As shown in Fig. 2, we found no significant change in any of the three protease activities of the proteasome upon exposure to a heat shock and during the post-heat shock recovery period for cells transfected with the non-silencing vector. For the cells silenced for laforin or malin, there was no difference in activities under unstressed condition (non-heat shocked (NHS)). However, partial loss of laforin or malin did affect the protease activities at the end of the heat shock or during the post-heat shock recovery periods, although to different extents (Fig. 2). Malin deficiency led to a significantly reduced activity of all three proteases upon exposure to heat shock, and during the post-heat shock recovery periods, while loss of laforin for the same time points resulted in a significant increase for the protease activities (Fig. 2), indicating a differential effect of laforin and malin on the proteasomal function.

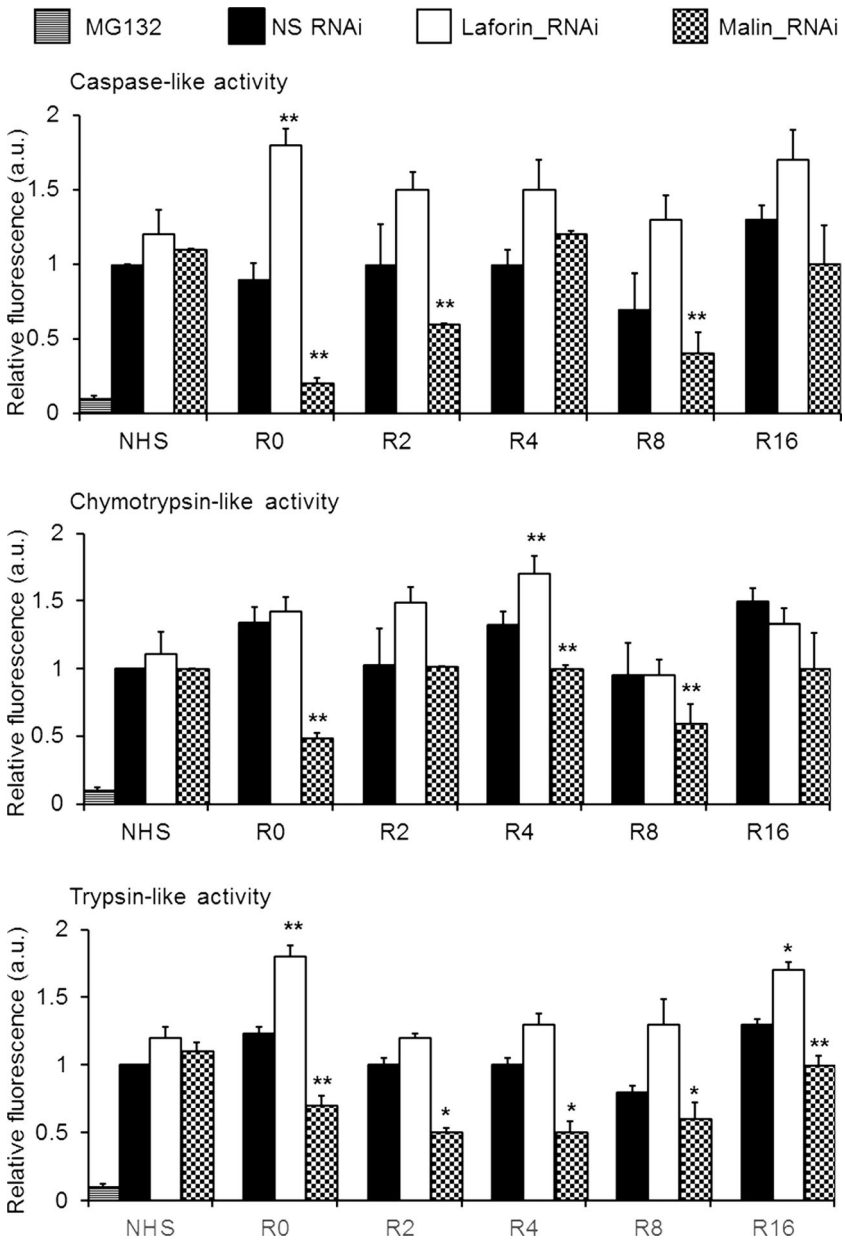


Fig. 2 Proteasomal activity during heat stress and recovery in cells silenced for laforin or malin: Equal amount of protein lysate from the Neuro2a cells, obtained at the indicated time points, was incubated with the fluorogenic substrate for measuring the proteasomal activity. Unstressed cells treated with the proteasomal inhibitor, MG132, served as control. The experiments were conducted with cells transiently transfected with the control or the knockdown constructs for laforin or malin. *Bar* represents average value (+/− SD). ($n = 6$; $^{**}p < 0.001$; $^{*}p < 0.05$; two-tailed *t* test, relative to the value obtained for the unstressed cells transfected with the non-silencing RNAi construct [*NS RNAi*])

Overall, loss of malin resulted in lowering the protease activity during the post-heat shock recovery period.

Heat shock promotes the clearance of the polyubiquitinated proteins in laforin-deficient cells

Polyubiquitinated proteins are targets of autophagic and proteasomal-mediated clearance (Olzmann and Chin 2008). Heat stress leads to the accumulation of detergent-resistant polyubiquitinated proteins; silencing of autophagic proteins in *Arabidopsis* prior to heat stress has been shown to enhance this accumulation (Zhou et al. 2013). Indeed, loss of laforin or malin resulted in increased levels of long-lived, ubiquitinated proteins in the mouse models of LD (Aguado et al. 2010; Puri et al. 2012; Criado et al. 2012). Therefore, we wanted to test if the heat shock-induced clearance of polyubiquitinated proteins is effective in the absence of laforin or malin. Corroborating earlier reports (Aguado et al. 2010; Puri et al. 2012, Criado et al. 2012; Singh et al. 2013; Garyali et al. 2014), we found that cells partially silenced for laforin and malin have higher level of polyubiquitinated proteins, compared to the control (Fig. 3a). We assessed the clearance of polyubiquitinated proteins during the post-heat shock recovery period (Fig. 3b). For each such comparison, the level of the total polyubiquitinated protein in the non-stressed cells (NHS) was considered as one, and the fold difference in the level at subsequent time points of investigations was plotted (Fig. 3b). The level of polyubiquitinated proteins attained its

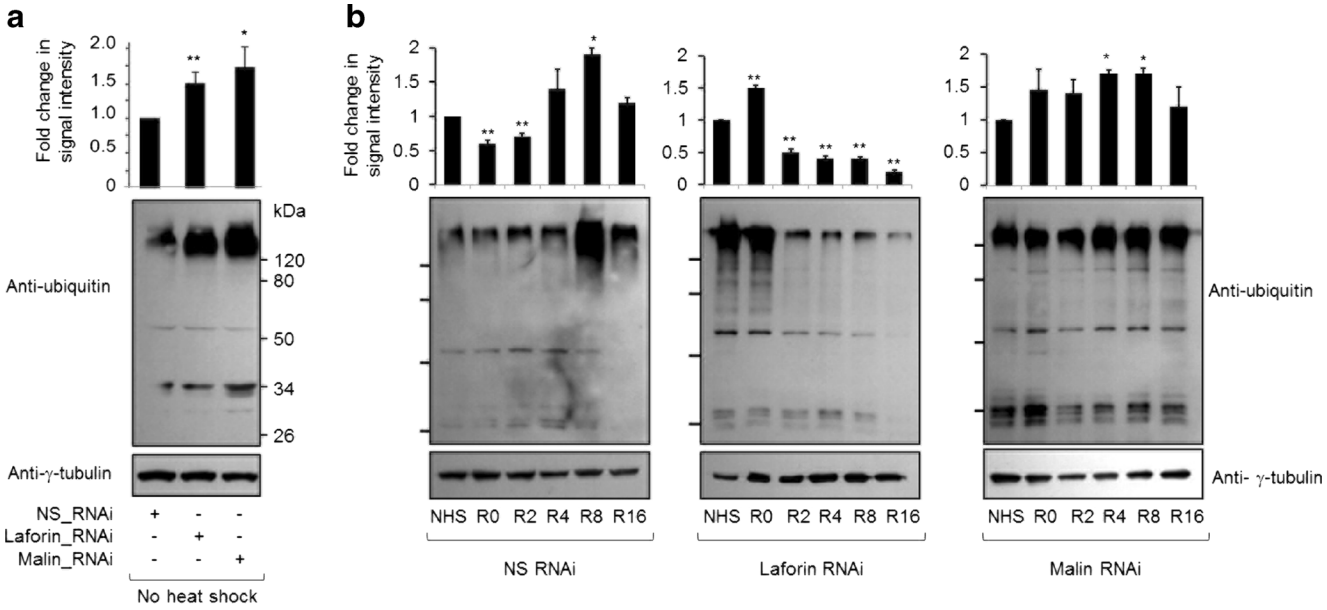


Fig. 3 Clearance of polyubiquitinated proteins during heat shock recovery. **a** Images of representative immunoblots showing levels of total cellular polyubiquitinated proteins in lysates of Neuro2a cells partially silenced for laforin or malin. Note the increased level of polyubiquitinated species in the laforin- or malin-silenced cells. **b** Immunoblot showing the relative levels of polyubiquitinated proteins in cells transiently transfected with the non-silencing construct (*NS RNAi*) or the knockdown construct for laforin or malin, exposed or not exposed to heat shock, and harvested at indicated time points. Tubulin has been used as loading control. The *bar diagram above* represents the fold change in the signal intensities for the polyubiquitinated protein as compared to cells that were not exposed to the heat shock (**$p < 0.001$; *$p < 0.05$; two-tailed *t* test)

maximum at 8 h post-heat shock in the control cells (NS_RNAi), suggesting a surge in the polyubiquitination activity during the post-heat shock recovery period (Fig. 3b), and this parallels with the autophagy induction and the increased levels of p62 during the post-heat shock recovery period (Fig. 1c, d). On the contrary, the level of polyubiquitinated proteins in the laforin-silenced cells was higher in cells unexposed to heat shock (Fig. 3a) but dramatically reduced during the post-heat shock recovery period (Fig. 3b). This reduction in the polyubiquitinated species also mirrored the induction of the autophagy and reduction in the level of p62 during the same time points (Fig. 1c, d), suggesting that the heat shock-induced autophagy and the proteasomal activity could possibly be the reasons behind the clearance of the polyubiquitinated proteins. The cells silenced for malin, however, did not show such a change; the level of ubiquitinated proteins did not decrease during the post-heat shock recovery period as compared to the unstressed (NHS) cells (Fig. 3b), corroborating the observations with the autophagy markers that the loss of malin attenuates the heat shock-induced autophagy.

Discussion

The heat shock response and the autophagic process are two distinct pro-survival cellular pathways that are induced by a wide variety of physiological stresses. These include, but are not limited to, thermal stress, hypoxia, oxidative stress, and starvation. Although a direct functional link between these two very well-conserved pathways is yet to be established, emerging reports suggest cooperation between these two processes (Dokladny et al. 2015). While the heat shock response is considered a preventive measure limiting the protein damage due to unfolding, autophagy is essentially a catabolic process involved in the degradation of damaged and unwanted proteins (Sisti et al. 2015). It is therefore not surprising that autophagy is induced upon exposure to a thermal stress in mammalian cells, since elevated temperature is known to increase the cellular load of misfolded proteins, and the cell induces autophagy to clear the abnormal proteins and to provide nutrients during the post-heat shock recovery phase. Unlike the heat shock response wherein the HSF1 is considered to be the master regulator, the autophagy process is known to be regulated by multiple signaling pathways (Ravikumar et al. 2010), suggesting multiple players in this catabolic process. Similarly, cross talks between the autophagy and the ubiquitin-proteasome system have also been established (Ravikumar et al. 2010). In the current study, we attempt to address the conflict using neuroblastoma cell line as our model, as cells of neuronal origin are considered to be the most vulnerable to proteotoxic stress (Morimoto 2008). Consistent with these views, we find an increased load of polyubiquitinated proteins in the control Neuro2a cells during the post-heat shock recovery period and the concomitant induction of autophagy. We, however, did not observe a

significant change in the proteolytic activity of the proteasome at the end of the heat shock period or during the tested post-heat shock recovery period, suggesting that the autophagic process could be the major player in the clearance of the damaged proteins. Another study that followed the cellular autophagic status during heat shock recovery elucidated the role of transcription factor NFκB in autophagic induction (Nivon et al. 2009). Similar to our findings, this study documents the accumulation of insoluble, polyubiquitinated proteins peaking at 6 h post-heat shock recovery and the lowering to basal levels at 24 h of the recovery period (Nivon et al. 2009). The authors showed that the loss of NFκB increased the cellular polyubiquitinated levels and decreased the LC3 levels for recovery periods, suggesting the role of a transcription factor during the recovery period after the heat shock.

The loss of either laforin or malin is known to result in the autophagy blockade (Aguado et al. 2010; Puri et al. 2012; Criado et al. 2012; Singh et al. 2013; Garyali et al. 2014; Jain et al. 2016). One of the intriguing observations of the present study is the contrasting effect on heat shock-induced autophagy induction in the Neuro2a cells deficient for laforin as compared with cells that were deficient for malin. Heat shock was able to induce autophagy in the laforin-deficient cells but not in the malin-deficient cells. While we do not know the exact reason behind the difference, it is of interest to note that the pathways involved in the autophagy blockade appear to be different in the two LD models. While the autophagy defects in the laforin-deficient state appear to be mTOR dependent (Aguado et al. 2010; Singh et al. 2013), it is likely to be mTOR independent in the case of malin deficiency (Criado et al. 2012) and this could possibly contribute to the observed difference. Indeed, a recent study demonstrates that overexpressed HSF1 "phenocopies" mTOR inhibition via rapamycin (Pierce et al. 2013), suggesting a similar function might operate in laforin-deficient cells. Similarly, treatment of cells with okadaic acid (an inhibitor of protein phosphatases) increases autophagy flux (Yoon et al. 2008) and induces heat shock protein expression (Arias et al. 1998). Thus, it is likely that the loss of the laforin's phosphatase activity did not affect the heat shock-induced autophagy as compared to the cells that were partially deficient for malin. This argument brings in an important question as to why heat shock is unable to induce autophagy in the absence of malin. Apart from a direct regulation of autophagic genes, heat shock induces small chaperones (HspB8/Bag3/Hsc70/CHIP) that are known to have a mechanistic role in macroautophagy (Crippa et al. 2010). These chaperones are induced in an HSF1-independent manner and are regulated by post-translational modifications. The role of small chaperone BAG3-HspB8 was shown upon heat stress-induced autophagy (Nivon et al. 2012). Oxidative stress also induces small heat shock protein beta-1 (HSPB1) which induces autophagy in renal cells (Matsumoto et al. 2015). These examples suggest that malin might regulate autophagy during stress recovery in an HSF1- and mTOR-independent manner, possibly through the post-translational modulation of some critical factors, which could be very different from the way laforin regulates autophagy. Indeed, laforin appears to regulate basal autophagy via FOXO3a—a transcription factor that regulates the expression of autophagy genes *Map1LC3b* and *Atg12* (Jain et al. 2016). A similar, but a distinct, mode of action could possibly exist for malin, and thus further studies are required to understand the role of malin in autophagy regulation. Nonetheless, our findings are clear enough to suggest that the autophagy blockade resulting from the loss malin could not be induced by heat shock and is, therefore, independent of HSF1. Our findings also suggest that activation of heat shock response pathway might restore autophagy in laforin-deficient cells.

A neuronal disorder that is associated with a mutation in the gene coding for an ubiquitin ligase E6AP is known to regulate proteasomal function. Interestingly, the mutation in this gene leads to defective proteasomal activity and is proposed to be a causative factor in disease development (Tomaic and Banks 2015). Loss of laforin or malin in the unstressed cells did not lead to a significant difference in proteasomal activity. However, loss of laforin and malin had contrasting effects on proteasomal activity during post-heat shock recovery period. While the loss of malin led to a significant reduction in the protease activity, loss of laforin resulted in enhanced activity at least at a few time points tested. Since ubiquitin ligases are known to promote proteasomal processivity (Aviram and Kornitzer 2010), and as malin is associated with proteasome (Mittal et al. 2007), it could be argued that the loss of malin might compromise proteasome function. An alternate explanation could be the increased level of p62 in the malin-deficient cells, as p62 accumulation is known to inhibit proteasomal function (Korolchuk et al. 2009). However, a reason for the increased activity of proteasome observed upon the partial loss of laforin during recovery from the heat shock is not obvious to us. One possible mechanism could be the post-translational modification of critical regulators of the proteasome complex. For example, the 26S proteasome phosphatase UBLCP1 is known to negatively regulate the proteasome activity (Guo et al. 2011). Given that laforin associated with proteasome in the stressed cells (Mittal et al. 2007), it is possible that laforin is a negative regulator of the proteasome, and its loss might enhance its activity. Clearly, further studies are required on the regulatory roles of laforin and malin in the proteasomal activity under cellular stress.

Acknowledgements This work was supported in part by a research grant [2010/21/02-BRNS/2115] from the Department of Atomic Energy, Govt. of India (to SG), and a research fellowship from the Council of Scientific and Industrial Research, Government of India (to NJ and AR). The authors wish to thank the anonymous referees for their suggestions on an earlier version of the manuscript.

References

Aguado C, Sarkar S, Korolchuk VI et al (2010) Laforin, the most common protein mutated in Lafora disease, regulates autophagy. Hum Mol Genet 19:2867–2876. doi:10.1093/hmg/ddq190

Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80: 1089–1115. doi:10.1146/annurev-biochem-060809-095203

Arias C, Becerra-García F, Arrieta I, Tapia R (1998) The protein phosphatase inhibitor okadaic acid induces heat shock protein expression and neurodegeneration in rat hippocampus in vivo. Exp Neurol 153: 242–254. doi:10.1006/exnr.1998.6900

Aviram S, Kornitzer D (2010) The ubiquitin ligase Hul5 promotes proteasomal processivity. Mol Cell Biol 30:985–994. doi:10.1128/MCB.00909-09

Bjorkoy G, Lamark T, Brech A et al (2005) p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171:603–614. doi:10.1083/jcb.200507002

Cotto JJ, Morimoto RI (1999) Stress-induced activation of the heat-shock response: cell and molecular biology of heat-shock factors. Biochem Soc Symp 64:105–118

Criado O, Aguado C, Gayarre J et al (2012) Lafora bodies and neurological defects in malin-deficient mice correlate with impaired autophagy. Hum Mole Genet 21:1521–1533. doi:10.1093/hmg/ddr590

Crippa V, Sau D, Rusmini P et al (2010) The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). Hum Mol Genet 19:3440–3456. doi:10.1093/hmg/ddq257

Delgado-Escueta AV, Ganesh S, Yamakawa K (2001) Advances in the genetics of progressive myoclonus epilepsy. Am J Med Genet 106: 129–138. doi:10.1002/ajmg.1575

Desai S, Liu Z, Yao J et al (2013) Heat shock factor 1 (HSF1) controls chemoresistance and autophagy through transcriptional regulation of autophagy-related protein 7 (ATG7). J Biol Chem 288:9165–9176. doi:10.1074/jbc.M112.422071

Dokladny K, Myers OB, Moseley PL (2015) Heat shock response and autophagy—cooperation and control. Autophagy 11:200–213. doi:10.1080/15548627.2015.1009776

Dokladny K, Zuhl MN, Mandell M, Bhattacharya D, Schneider S, Deretic V, Moseley PL (2013) Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. J Biol Chem 288:14959–14972. doi:10.1074/jbc.M113.462408

Ganesh S, Delgado-Escueta AV, Sakamoto T et al (2002) Targeted disruption of the Epm2a gene causes formation of Lafora inclusion bodies, neurodegeneration, ataxia, myoclonus epilepsy and impaired behavioral response in mice. Hum Mol Genet 11:1251–1262. doi:10.1093/hmg/11.11.1251

Ganesh S, Puri R, Singh S et al (2006) Recent advances in the molecularbasis of Lafora's progressive myoclonus epilepsy. J Hum Genet 51:1–8. doi:10.1007/s10038-005-0321-1

Garyali P, Segvich DM, DePaoli-Roach AA, Roach PJ (2014) Protein degradation and quality control in cells from laforin and malin knockout mice. J Biol Chem 289:20606–20614. doi:10.1074/jbc.M114.580167

Garyali P, Siwach P, Singh PK et al (2009) The malin-laforin complex suppresses the cellular toxicity of misfolded proteins by promoting their degradation through the ubiquitin-proteasome system. Hum Mol Genet 18:688–700. doi:10.1093/hmg/ddn398

Guo X, Engel JL, Xiao J et al (2011) UBLCP1 is a 26S proteasome phosphatase that regulates nuclear proteasome activity. Proc Natl Acad Sci U S A 108:18649–18654. doi:10.1073/pnas.1113170108

Jain N, Mishra R, Ganesh S (2016) FoxO3a-mediated autophagy is down-regulated in the laforin deficient mice, an animal model for Lafora progressive myoclonus epilepsy. Biochem Biophys Res Commun 474:321–327. doi:10.1016/j.bbrc.2016.04.094

Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC (2009) Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol Cell 33(5):17–527. doi:10.1016/j.molcel.2009.01.021

Li W, Ye Y (2008) Polyubiquitin chains: functions, structures, and mechanisms. Cell Mol Life Sci 65:2397–2406. doi:10.1007/s00018-008-8090-6

Liu TT, CH H, Tsai CD, Li CW, Lin YF, Wang JY (2010) Heat stroke induces autophagy as a protection mechanism against neurodegeneration in the brain. Shock 34:643–648. doi:10.1097/SHK.0b013e3181e761c1

Matsumoto T, Urushido M, Ide H et al (2015) Small heat shock protein beta-1 (HSPB1) is upregulated and regulates autophagy and apoptosis of renal tubular cells in acute kidney injury. PLoS One 10: e0126229. doi:10.1371/journal.pone.0126229

McDonough H, Patterson C (2003) CHIP: a link between the chaperone and proteasome systems. Cell Stress Chaperones 8:303–308. doi:10.1379/1466-1268(2003)008

Mittal S, Dubey D, Yamakawa K, Ganesh S (2007) Lafora disease proteins malin and laforin are recruited to aggresomes in response to proteasomal impairment. Hum Mol Genet 16: 753–762. doi:10.1093/hmg/ddm006

Morimoto RI (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. Genes Dev 22:1427–1438. doi:10.1101/gad.1657108

Nivon M, Abou-Samra M, Richet E, Guyot B, Arrigo AP, Kretz-Remy C (2012) NF-kappaB regulates protein quality control after heat stress through modulation of the BAG3-HspB8 complex. J Cell Sci 125: 1141–1151. doi:10.1242/jcs.091041

Nivon M, Richet E, Codogno P, Arrigo AP, Kretz-Remy C (2009) Autophagy activation by NFkappaB is essential for cell survival after heat shock. Autophagy 5:766–783

Olzmann JA, Chin LS (2008) Parkin-mediated K63-linked polyubiquitination: a signal for targeting misfolded proteins to the aggresome-autophagy pathway. Autophagy 4:85–87

Pierce A, Podlutskaya N, Halloran JJ et al (2013) Over-expression of heat shock factor 1 phenocopies the effect of chronic inhibition of TOR by rapamycin and is sufficient to ameliorate Alzheimer's-like deficits in mice modeling the disease. J Neurochem 124:880–893. doi:10.1111/jnc.12080

Puri R, Suzuki T, Yamakawa K, Ganesh S (2012) Dysfunctions in endosomal-lysosomal and autophagy pathways underlie neuropathology in a mouse model for Lafora disease. Hum Mol Genet 21: 175–184. doi:10.1093/hmg/ddr452

Ravikumar B, Sarkar S, Davies JE et al (2010) Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev 90: 1383–1435. doi:10.1152/physrev.00030.2009

Sengupta S, Badhwar I, Upadhyay M, Singh S, Ganesh S (2011) Malin and laforin are essential components of a protein complex that protects cells from thermal stress. J Cell Sci 124:2277–2286. doi:10.1242/jcs.082800

Serratosa JM, Minassian BA, Ganesh S (2012) Progressive myoclonus epilepsy of Lafora. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds). Jasper's basic mechanisms of the epilepsies. National Center for Biotechnology Information (US)

Singh PK, Singh S, Ganesh S (2013) Activation of serum/glucocorticoid-induced kinase 1 (SGK1) underlies increased glycogen levels, mTOR activation, and autophagy defects in Lafora disease. Mol Biol Cell 24:3776–3786. doi:10.1091/mbc.E13-05-0261

Singh S, Ganesh S (2009) Lafora progressive myoclonus epilepsy: a meta-analysis of reported mutations in the first decade following the discovery of the EPM2A and NHLRC1 genes. Hum Mut 30: 715–723. doi:10.1002/humu.20954

Sisti G, Kanninen TT, Ramer I et al (2015) Interaction between the inducible 70-kDa heat shock protein and autophagy:

effects on fertility and pregnancy. Cell Stress Chaperones 20: 753–758. doi:10.1007/s12192-015-0609-9

Tomaic V, Banks L (2015) Angelman syndrome-associated ubiquitin ligase UBE3A/E6AP mutants interfere with the proteolytic activity of the proteasome. Cell Death Dis 6:e1625. doi:10.1038/cddis.2014.572

Yoon SY, Choi JE, Kweon HS, Choe H, Kim SW, Hwang O, Lee H, Lee JY, Kim DH (2008) Okadaic acid increases autophagosomes in rat neurons: implications for Alzheimer's disease. J Neurosci Res 86:3230–3239. doi:10.1002/jnr.21760

Zhao Y, Gong S, Shunmei E, Zou J (2009) Induction of macroautophagy by heat. Mol Biol Rep 36:2323–2327. doi:10.1007/s11033-009-9451-4

Zhou J, Wang J, Cheng Y, Chi YJ, Fan B, JQ Y, Chen Z (2013) NBR1-mediated selective autophagy targets insoluble ubiquitinated protein aggregates in plant stress responses. PLoS Genet 9:e1003196. doi:10.1371/journal.pgen.1003196