HHS Public Access

Author manuscript

FASEB J. Author manuscript; available in PMC 2021 November 08.

Published in final edited form as:

FASEB J. 2021 June ; 35(6): e21631. doi:10.1096/fj.202100566.

The 2021 FASEB Virtual Catalyst Conference on Extracellular and Organismal Proteostasis in Health and Disease, February 3–4, 2021

Chen Lior[1,†], **Francesca Hodge**[2,†], **Evandro A. De-Souza**[3,†], **Dimitra Bourboulia**[4], **Stuart Calderwood**[5], **Della David**[6], **D. Allan Drummond**[7], **Adrienne Edkins**[8], **Richard I. Morimoto**[9], **Veena Prahlad**[10], **Oded Rechavi**[11], **Lea Sistonen**[12], **Mark Wilson**[13], **Luke Wiseman**[14], **Maurizio Zanetti**[15], **Rebecca Taylor**[3,#], **Ruth Scherz-Shouval**[1,#], **Patricija van Oosten-Hawle**[2,#]

[1:]Department of Biomolecular Sciences, The Weizmann Institute of Science, Rehovot, Israel.

[2:]School of Molecular and Cell Biology and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.

[3:]Neurobiology Division, MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK.

[4:]Department of Urology, SUNY Upstate Medical University, Syracuse, NY, USA; Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY, USA; Upstate Cancer Center, SUNY Upstate Medical University, Syracuse, NY, USA.

[5:]Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02115, USA.

[6:]German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany.

[7:]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA.

[8:]Biomedical Biotechnology Research Unit, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa.

[9:]Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA.

[10:]Department of Biology, Aging Mind and Brain Initiative, 143 Biology Building East, 338 BBE, University of Iowa, Iowa City, IA 52242, USA.

[11:]Department of Neurobiology, Wise Faculty of Life Sciences & Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 69978, Israel.

[12:]Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, Tykistökatu 6, 20520 Turku, Finland; Turku Bioscience Centre, University of Turku and Åbo Akademi University, Tykistökatu 6, 20520 Turku, Finland.

[13:]Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW, 2522, Australia.

[#]**Correspondence:** p.vanoosten-hawle@leeds.ac.uk; ruth.shouval@weizmann.ac.il; rtaylor@mrc-lmb.cam.ac.uk.
[†]These authors have contributed equally to this work

[14:]Department of Molecular Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

[15:]Laboratory of Immunology, Department of Medicine and Moores Cancer Center, University of California at San Diego, La Jolla, CA 92093, USA.

A new addition to the FASEB Science Research Conference series is the Catalyst conference series. Catalyst conferences are two-day virtual meetings geared to establish a scientific community around an emerging scientific topic in the field. This was the intention of the first "Extracellular & Organismal Proteostasis in Health and Disease" conference that was held virtually on February 3-4, 2021, organized by Dr. Patricija van Oosten-Hawle (University of Leeds, UK), Dr. Ruth Scherz-Shouval (Weizmann Institute, Israel) and Dr. Rebecca Taylor (MRC LMB, UK). The meeting was attended by 372 people and brought together 15 speakers who are experts in different aspects of proteostasis. The main goal of this conference was to unite pioneers in the field of extracellular proteostasis who are exploring new horizons beyond the boundaries of the cell.

The proteostasis network is essential for the maintenance of cellular health and viability. To date, research in the field has largely focused on the intracellular functions of this network in health and disease. Recent advances have highlighted the equally important role of an extracellular proteostasis network that protects protein folding in the extracellular space and maintains inter-tissue signaling in physiology and pathology. This conference expanded the proteostasis community and provided a platform for this emerging research area. Over two days, the conference explored topics of extracellular proteostasis in 4 sessions, which included inter-tissue communication in organismal proteostasis, extracellular chaperones, and the role of extracellular proteostasis in immune responses, cancer, and neurodegenerative disease.

Prof. Richard Morimoto (Northwestern University, USA), commenced the meeting as keynote speaker with a broad overview of the proteostasis field on "Enhancing Proteostasis for Cellular Healthspan in Aging and Disease". Dr. Morimoto highlighted the vast complexity of autonomous and non-autonomous regulation of proteostasis and its relation with pathophysiology of age-related diseases. He led us through recent discoveries made in his research group, including results showing the beginning of the reproduction period in *C. elegans* being associated with a failure of the somatic transcriptional response to heat shock stress. Using a genetic screen, it was identified that inhibition of *cbd-1* – a gene involved in the eggshell's vitelline layer – improves the somatic proteostasis of the worm. The data suggests that there is an embryo-to-mother signaling pathway that regulates the balance between somatic proteostasis and quality of the progeny[1].

The next speaker was Dr. Della David (University of Tübingen, Germany), who presented exciting work detailing for the first time a mechanism by which *C. elegans* can coordinate extracellular proteostasis through the stress-activated map kinase, KGB1. Dr. David showed how the elimination of coelomocytes promotes age-dependent aggregation of lipid-binding protein 2 (LBP-2) in the *C. elegans* pseudocoelom. LBP-2 is a secreted protein with homology to Myelin P2 protein associated with Charcot-Marie-Tooth disease. Utilizing

a genetic screen, she identified several extracellular regulators that suppressed LBP2 aggregation in the extracellular space. Upon linking these with the innate immune response, a role for these regulators in host survival upon pathogenic attack was elucidated[2].

Dr. Veena Prahlad (University of Iowa, USA) took us through the narrative of discovering a novel mechanism expanding our understanding of HSF-1 in stress and heredity. It was found that exposing adult *C. elegans* to heat stress activates HSF-1 within the germline[11]. This subsequently modified the chromatin of the maternal germline creating an epigenetic memory of the stress in soon-to-be fertilized oocytes, increasing the stress resistance of these offspring. Along with her previous findings of serotonin-mediated HSF-1 control by the maternal nervous system, Dr. Prahlad is working to provide further insight into how stress is integrated between tissues within the mother to alter offspring physiology.

Transmission of UPR activation between tissues leads to increased lifespan and stress resistance. Dr. Rebecca Taylor (MRC LMB, UK) presented evidence that UPR (Unfolded Protein Response)-induced activation of intestinal lysosomes and alterations to lipid metabolism underlie this enhanced longevity and proteostasis. Furthermore, it was demonstrated that tyramine synthesis is required for neurons to trigger intestinal UPR activation, and that expression of XBP-1s in the two pairs of neurons that synthesize tyramine is sufficient to drive intestinal UPR activation. This biogenic amine also enables neuronal UPR activation to modify organismal behavior and reproduction, suggesting that activation of the UPR in neurons acts as a global coordinator of responses to stress[3].

Opening the second session, Dr. Luke Wiseman (Scripps Research, USA), discussed the importance of ER (Endoplasmic Reticulum) proteostasis in regulating extracellular protein aggregation in the context of systemic amyloid disease. Dr. Wiseman presented data showing how imbalances in ER proteostasis increase the secretion of amyloidogenic proteins in non-native, aggregation-prone conformations that accelerate their aggregation into soluble oligomers and amyloid fibrils. Pharmacologically targeting either the amyloidogenic protein within the ER or the ER proteostasis environment itself improved the quality of secreted proteins reducing the toxic extracellular aggregation of amyloidogenic proteins implicated in disease. These findings highlight the importance of the ER in the pathogenesis of systemic amyloid disease and define a therapeutic strategy that can be broadly applied to mitigate the pathologic extracellular aggregation of proteins implicated in diverse protein aggregation disorders[12].

Questioning one of the paradigms of proteotoxic stresses, Dr. Allan Drummond (University of Chicago, USA) started his talk showing that proteotoxic stresses have long been thought to trigger widespread misfolding of endogenous proteins. However, studies of the actual behavior of endogenous proteins during heat shock have revealed no toxic misfolding, but point to adaptive bio-molecular condensation of proteins. Dr. Drummond has reconstituted the chaperone dispersal of phase-separated condensates of the authentic endogenous heat-triggered substrate poly(A)-binding protein (Pab1) by its cognate chaperones. Remarkably, Pab1 dispersal is orders of magnitude faster than the common "model" misfolded substrate, firefly luciferase, and goes rapidly to completion. Dr. Drummond proposes that physiological heat shock is often productively viewed as an environmental signal of

changing conditions to which cells have exquisitely adapted, during which chaperones act as regulatory factors for adaptive protein condensation[10].

Next, Dr. Patricija van Oosten-Hawle (University of Leeds, UK) discussed the finer detailing of the transcellular chaperone signaling mechanism which is a nonautonomous stress response mediating chaperone expression across tissues. This talk highlighted how the balance of stress in one tissue is integrated through several mechanisms in the entire organism, suggesting that different types of stress may have different inter-tissue signals. Suppression of the heat shock response (HSR) in the intestine depended on secreted innate immune peptides such as the aspartic protease ASP-12 to signal the upregulation of HSP-90 in the muscle[4]. Conversely, a decrease in intestinal HSP-90 induced the HSR within the intestine and signaled through a novel mechanism by activating an alternate transcription factor to HSF-1 in the muscle. It was further detailed how HSF-1 activity was in fact a suppressor of this alternative stress response highlighting once again the multi-faceted nature of HSF-1 in its regulation of stress.

Rounding off the first day of the conference, Prof. Mark Wilson (University of Wollongong, Australia), spoke about the progress he has made in his pursuit of mapping the extracellular chaperome through pinpointing key actors of extracellular proteostasis[16]. Dr. Wilson presented a new streamlined method for identifying chaperones from bodily fluids and gave examples of preliminary results this approach had yielded in discovering previously unknown extracellular chaperones. Another exciting observation described for the first time a selective preference of certain chaperones for inhibition of either amorphous or amyloid aggregation providing another route in understanding how proteostasis machinery can be harnessed in developing new therapies for human diseases.

Opening the second day of the conference, the keynote speaker Prof. Lea Sistonen (Åbo Akademi University and Turku Bioscience Centre, Finland), started her talk introducing extracellular vesicles (EVs) and their role in cancer. In order to elucidate how the transition of a tumor to an invasive phenotype affects the secretion and characteristics of EVs Prof. Sistonen's group developed an *in vivo* model mimicking extracellular matrix-based 3D cultures for EV isolation and investigation from tumor organoids. They found a dramatic increase in EV secretion during the transition to invasive phenotype, and while the EVs from invasive and non-invasive organoids were similar in shape and size, they had different cargo, which was analyzed using LC-MS/MS analysis. This remarkable work sheds light on the mechanisms by which cancer cells communicate with their surroundings[5]. Additionally, Prof. Sistonen presented work on the mechanisms by which repeated exposure to heat stress improves the cellular ability to cope with additional stress. Promoter-proximal RNA Pol II pausing, pause-release and transcriptional termination are the rate-limiting steps involved in establishing a transcriptional memory over cell divisions.

The next speaker was Dr. Dimitra Bourboulia (SUNY Upstate Medical University, USA). Dr. Bourboulia presented the roles and mechanism of action of extracellular chaperone HSP90 (eHSP90). Despite reports that eHSP90 interacts with and stabilizes secreted proteolytic enzyme MMP2 in the extracellular space to promote tumor cell invasion, the mechanisms involved are not fully understood. Dr. Bourboulia presented studies that led

to the identification of the tissue inhibitor of metalloproteinase 2 (TIMP2) as a bona fide secretory co-chaperone of eHSP90[6]. More specifically, TIMP2 direct interaction with eHSP90 impacts not only on eHSP90 chaperone function, but also on client MMP2 activity, by interfering with the formation of the HSP90:MMP2 complex[6]. Thus, it is conceivable to think that TIMP2 would likely impact other eHSP90 client proteins. The Bourboulia lab are currently investigating the role of extracellular post-translational modifications, and specifically TIMP2 phosphorylation by secreted c-Src tyrosine kinase, in regulating the eHSP90 chaperone machinery[7].

Prof. Adrienne Edkins (Rhodes University, South Africa) presented studies on “HSP90 Regulation of Fibronectin ECM Dynamics *in vitro”*. Prof. Edkins introduced Fibronectin (FN) as an abundant extracellular matrix (ECM) protein that undergoes a transition from a secreted, cell-derived, soluble monomer to an insoluble fiber as part of its physiological functions in the ECM. Prof. Edkins then demonstrated that FN is also an extracellular HSP90 client protein, which interacts with the HSP90 M domain via the N terminal FN30 and FN45 domains. The binding affinity was determined by inherent stability of the FN fragments and the presence of the FN type I repeat motif. *HSP90* RNAi led to remodeling and turnover of the FN ECM. Remarkably, Prof. Edkins showed that exogenous extracellular HSP90 was sufficient to enhance FN ECM formation, an effect which was at least in part dependent on the ability of HSP90 to interact directly with FN[13].

The next speaker was Dr. Ruth Scherz-Shouval (The Weizmann Institute of Science, Israel). In her talk, Dr. Scherz-Shouval presented research investigating stress responses in the tumor microenvironment. Tumors are dynamic and stressful ecosystems comprised of malignant and non-malignant components. Dr. Scherz-Shouval’s research focuses on cancer-associated fibroblasts (CAFs), which, unlike cancer cells, are genomically stable. HSF1, a master regulator of the heat shock response, was shown to be activated in CAFs, and to regulate their reprogramming to a more pro-tumorigenic phenotype. How exactly does this occur? Dr. Scherz-Shouval presented two tumor-promoting activities of stromal HSF1 recently discovered in her lab. In one study, Dr. Scherz-Shouval’s team has found that HSF1 is activated in fibroblasts by inflammation, which subsequently leads to ECM remodeling, mediating the transition from chronic intestinal inflammation to colon cancer[8]. In an inflammation-induced cancer mouse model, depletion of HSF1 prevented the disruption and ablation of ECM, which resulted in disease suppression. Complementing this effect, a separate study focusing on gastric cancer was also presented, showing how HSF1 affects the content of extracellular vesicles (EVs) secreted from CAFs to the tumor microenvironment to promote cancer[9]. Using samples from human gastric patients as well as mouse models, they defined a CAF signature associated with poor disease outcome and identified two HSF1-regulated proteins – THBS2 and INHBA – secreted from CAFs via extracellular vesicles, which are then taken up by the cancer cells to promote tumorigenesis.

Prof. Oded Rechavi (Tel Aviv University, Israel) opened a conversation into the role of inter-tissue stress in the context of evolution. In *C. elegans* mating is initiated by hermaphrodite pheromone secretion upon sperm depletion in aged adults. Interestingly, Prof. Rechavi described how stress induced in a *C. elegans* population over several generations at elevated temperatures led to premature initiation of mating. This premature "attractiveness

for mating" was found to be inherited through several generations once the population was returned to physiological temperatures. Utilizing an intricate assay tagging the small RNA regulator *hrde-1* with AID to allow inducible depletion of small RNAs, it was determined that the inheritance was indeed mediated via small RNAs. Importantly it was found that when equal numbers of these prematurely attractive *C. elegans* were mixed with those not descended from a stressed phenotype, and males were added, there was a significant overrepresentation of alleles from prematurely attractive worms in the subsequent population. This showed that stress survival of individuals can enhance their genotype within the population pool, steering the evolution of *C. elegans*.[14]

Next, Prof. Stuart Calderwood (Harvard Medical School, USA) showed that extracellular HSP90 (eHSP90) activates the NRF2 pathway in macrophages and CNS-resident microglia. Prof. Calderwood examined the role of eHSP90 in the toxic and neuroinflammatory effects of amyloid beta fibrils, causal agents in the morbidity of Alzheimer's disease. He found that eHSP90 protects neuronal viability and function when exposed to beta amyloid in the presence of mouse brain derived microglia. These effects were accompanied by changes in gene expression in the microglia, including induction of NRF2-dependent antioxidant genes, autophagy genes and genes encoding anti-inflammatory receptors. Prof. Calderwood's findings suggest a potential role for eHSP90 in CNS homeostasis and highlight a potential role for this chaperone in therapeutic interventions for beta-amyloid mediated diseases.

The final speaker of this conference was Prof. Maurizio Zanetti (University of San Diego, USA). Prof. Zanetti presented work on the unfolded protein response, aneuploidy and immune cells dysregulation in cancer. In a tumor, the immune system reacts to the emergence of cancer cells by deploying its adaptive and innate branches. Over the tumor evolution, perturbations in the tumor microenvironment can disrupt the balance between adaptive and innate immune cells, leading to progressive loss of the anti-tumor effect. Endoplasmic reticulum (ER) stress promotes the UPR that has emerged as a major contributor to local immune dysregulation. In his talk, Prof. Zanetti presented evidence that the transmission of ER stress signals from cancer cells to myeloid cells (macrophages and dendritic cells) imparting these cells with pro-inflammatory/immune suppressive characteristics[15]. This phenotype also occurs *in vivo* during induced or spontaneous tumorigenesis. *In vitro*, the effects of ER stress transmission are attenuated in *Ern1*-deficient macrophages, and in mice with conditional KO of Ern1 in macrophages, thus leading to increased tumor growth. Prof. Zanetti also showed that cancer cell aneuploidy, quantified through a novel single somatic copy-number alteration (SCNA) score, correlates with loss of intra-tumor cytotoxic T cells, and that the UPR is the likely link between aneuploidy and immune dysregulation. Additionally, Prof. Zanetti presented evidence that experimentally induced aneuploidy activates the UPR, and that the conditioned medium of these cells transmits signals that induce proinflammatory cytokines and immunosuppressive molecules in recipient bone marrow-derived macrophages. These studies confirm that the UPR plays an important role in dysregulating local anti-tumor immunity, and that aneuploidy is a potential cell-endogenous trigger of such events.

In summary, the first "Extracellular & Organismal Proteostasis in Health and Disease" conference was a great success and the organizers would like to thank all speakers

and participants for making this a highly interesting conference with fruitful discussions after each session. The findings presented in this conference highlighted the relevance of extracellular proteostasis to different aspects of physiology and pathology across organisms. The high interest in the research topic was reflected by the feedback survey. 41% of participating respondents would attend a virtual meeting on the same topic in the future again, and 52% would attend either in person or virtually. We hope that the topic of this conference will be continued in future years as a FASEB Science Research Conference.

References

1. Sala AJ, Bott LC, Brielmann RM & Morimoto RI Embryo integrity regulates maternal proteostasis and stress resilience. Genes Dev 34, 678–687 (2020). [PubMed: 32217667]
2. Gallotta I et al. Extracellular proteostasis prevents aggregation during pathogenic attack. Nature 584, 410–414 (2020). [PubMed: 32641833]
3. Özbey NP et al. Tyramine Acts Downstream of Neuronal XBP-1s to Coordinate Inter-tissue UPRER Activation and Behavior in C. elegans. Dev Cell 55, 754–770.e6 (2020). [PubMed: 33232669]
4. O'Brien D et al. A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis. Cell Rep 23, 3905–3919 (2018). [PubMed: 29949773]
5. Luoto JC et al. In vivo-mimicking 3D cultures secrete distinct extracellular vesicles upon cancer cell invasion. bioRxiv 2020.12.22.423913 (2020) doi:10.1101/2020.12.22.423913.
6. Baker-Williams AJ et al. Co-chaperones TIMP2 and AHA1 Competitively Regulate Extracellular HSP90:Client MMP2 Activity and Matrix Proteolysis. Cell Rep 28, 1894–1906.e6 (2019). [PubMed: 31412254]
7. Sánchez-Pozo J et al. Extracellular Phosphorylation of TIMP-2 by Secreted c-Src Tyrosine Kinase Controls MMP-2 Activity. iScience 1, 87–96 (2018). [PubMed: 30227959]
8. Levi-Galibov O et al. Heat Shock Factor 1-dependent extracellular matrix remodeling mediates the transition from chronic intestinal inflammation to colon cancer. Nat Commun 11, 6245 (2020). [PubMed: 33288768]
9. Grunberg N et al. Cancer-associated fibroblasts promote aggressive gastric cancer phenotypes via heat shock factor 1-mediated secretion of extracellular vesicles. Cancer Res (2021) doi:10.1158/0008-5472.CAN-20-2756.
10. Riback JA, Katanski CD, Kear-Scott JL, Pilipenko EV, Rojek AE, Sosnick TR, and Drummond DA (2017) Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell 168, 1028–1040.e19. [PubMed: 28283059]
11. Das S, Ooi FK, Cruz Corchado J, Fuller LC, Weiner JA, and Prahlad V (2020) Serotonin signaling by maternal neurons upon stress ensures progeny survival. Elife 9, e55246. [PubMed: 32324136]
12. Romine IC and Wiseman RL (2020) Starting at the beginning: endoplasmic reticulum proteostasis and systemic amyloid disease. Biochem. J 477, 1721–1732. [PubMed: 32412081]
13. Chakraborty A, Boel NM-E, and Edkins AL (2020) HSP90 Interacts with the Fibronectin N-terminal Domains and Increases Matrix Formation. Cells 9.
14. Posner R, Toker IA, Antonova O, Star E, Anava S, Azmon E, Hendricks M, Bracha S, Gingold H, and Rechavi O (2019) Neuronal Small RNAs Control Behavior Transgenerationally. Cell 177, 1814–1826.e15 [PubMed: 31178120]
15. Rodvold JJ, Mahadevan NR, and Zanetti M (2016) Immune modulation by ER stress and inflammation in the tumor microenvironment. Cancer Lett. 380, 227–236 [PubMed: 26525580]
16. Wyatt AR, Yerbury JJ, Ecroyd H, and Wilson MR (2013) Extracellular chaperones and proteostasis. Annu. Rev. Biochem 82, 295–322 [PubMed: 23350744]

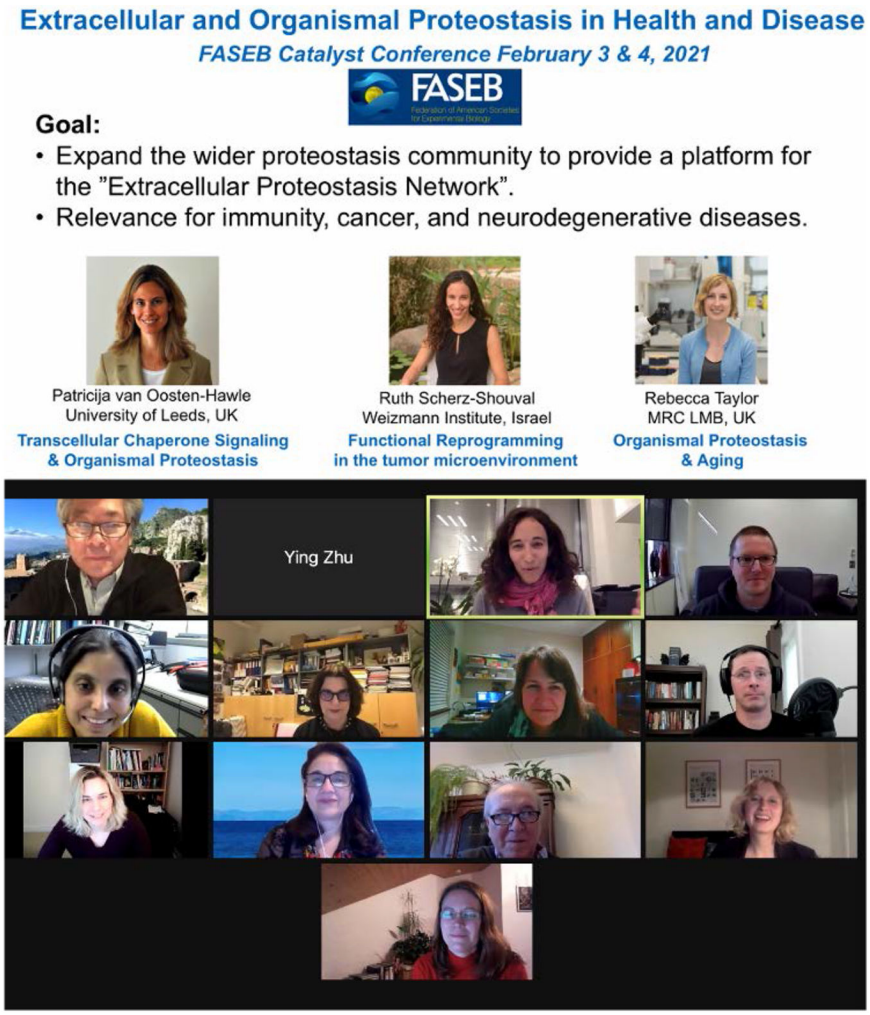

Figure 1.

The Catalyst Conference on Extracellular and Organismal Proteostasis in Health and Disease, held on February 3-4, 2021. This virtual conference was a great success, with stimulating discussions among speakers and participants.