

Scientific Life

PROTEOSTASIS: A European Network to Break Barriers and Integrate Science on Protein Homeostasis

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Protein homeostasis (proteostasis) is at the core of cellular functions. The European network PROTEOSTASIS was created to steer research and foster collaborations in the interconnected fields of posttranslational modifications by ubiquitin family members and protein turnover by proteasome, autophagy, and lysosomal systems in health and diseases, across the kingdoms of life.

Proteostasis: The Topic

Proteostasis, the portmanteau of protein and homeostasis, is the term for the underlying molecular network balancing the entire proteome of a living organism. PROTEOSTASIS is also the running name of our recently terminated COST Action BM1307^{i,ii} ‘European network to integrate research on intracellular proteolysis pathways in health and disease’ from the Biomedicine and Molecular Biosciences area of COST (European Cooperation in Science and Technology, funding period April 2014–April 2018; cost-proteostasis.eu).

The average number of proteins per individual cell is substantial and differs among species. In the single yeast cell, there are roughly 6000 different proteins [1] summing up to 42–50 million protein molecules [1–3], while in human cells there are

more than 10 000 different proteins per cell for a total of 2–3 billion protein molecules at any given time [4,5]. Possible proteoforms occur on top of those numbers with variable concentrations and diverse distributions throughout the cell types of multicellular organisms. This striking complexity requires tight regulation on multiple levels that is achieved through robust and highly sophisticated mechanisms. Indeed, broadly more than 5% of all proteins throughout the kingdoms are involved in protein synthesis and turnover, highlighting the global importance of these processes [6–8]. Despite much progress over the past decades, understanding how these complex proteomes are constantly and dynamically monitored and remodeled remains an enormous challenge.

Proteostasis refers to the biological mechanisms controlling the biosynthesis, co- and posttranslational processing, folding, trafficking, neofunctionalization (i.e., changes in protein function), and degradation of proteins *in vivo*. Among these processes, intracellular proteolysis is critical not only for survival and cellular homeostasis of all living cells under unchallenged conditions but also for rapid proteome remodeling in response to many environmental stresses. Furthermore, disturbed proteostasis may lead either to the accumulation of normally degraded proteins or to excessive protein degradation. Those processes can be associated with aging and many pathologies, such as cancer and immune and neurological disorders in humans as well as other diseases and responses to environmental stresses in plants and other organisms across the kingdoms of life.

Overall, since proteostasis plays pivotal roles in almost every biological process, including general growth and development in all organisms and mechanisms as diverse as heart development and

plant tolerance to flooding, for example, its analysis has elevated the interest of scientists from diverse backgrounds and with different research foci. A wide variety of research approaches is used to identify the mechanisms that regulate proteostasis, typically involving a breadth of model organisms (fungi, invertebrates, vertebrates, or plants) and different methodologies. This heterogeneity might restrain researchers into their own scientific niche, impairing knowledge exchange between fields that are in fact linked in real life. The PROTEOSTASIS network was built upon the conviction that connecting the different areas of research in this field would result in a boost of scientific exchange and sharing and set the stage for an advance of our community knowledge through new possibilities of exchange and collaboration.

One central aspect of proteostasis is protein degradation. Often referred to as proteolysis, it has been studied for decades, with publications on this topic constantly increasing since the 1970s. Two major intracellular proteolysis pathways, namely, the autophagy-lysosomal pathway (ALP) and the ubiquitin-proteasome system (UPS), synergistically play critical roles inside cells for the maintenance of cellular homeostasis and organism (patho)physiology. Thanks to the ever-increasing understanding of these pathways, and also to recent technical developments in cell imaging, proteomics, and more generally ‘-omics’ studies, we now realize that these research fields are strongly entwined and coordinated.

The small 8-kDa ‘ubiquitous’ protein ubiquitin (Ub) is a common endogenous molecular tag working as posttranslational protein modification used by both UPS and ALP to control the stability of most, if not all, proteins in a highly specific and regulated manner. Because of its

central role in cell metabolism, Ub is thus commonly accepted as a critical signature for the engagement of these sophisticated intracellular machineries. However, Ub also exerts proteolysis-independent functions, such as regulation of signaling pathways, transcription, or protein trafficking. Also in the ERAD (endoplasmic reticulum-associated protein degradation) system, Ub plays an important role in cargo retrotranslocation that finally also concludes in proteolysis via the proteasome. The field expanded by understanding that Ub is just a member of a family of Ub-like proteins (Ubls) that cooperate together to fine-tune the intracellular proteome. The most studied Ubls, such as SUMO (small Ub-like modifier) or NEDD8 (neural precursor cell expressed, developmentally down-regulated 8), are extremely versatile and crosstalk, that is, functionally interact in a dynamic manner with various modes of

posttranslational modifications of target proteins by Ub, modulating the fate of cellular proteins in response to external and intracellular stimuli.

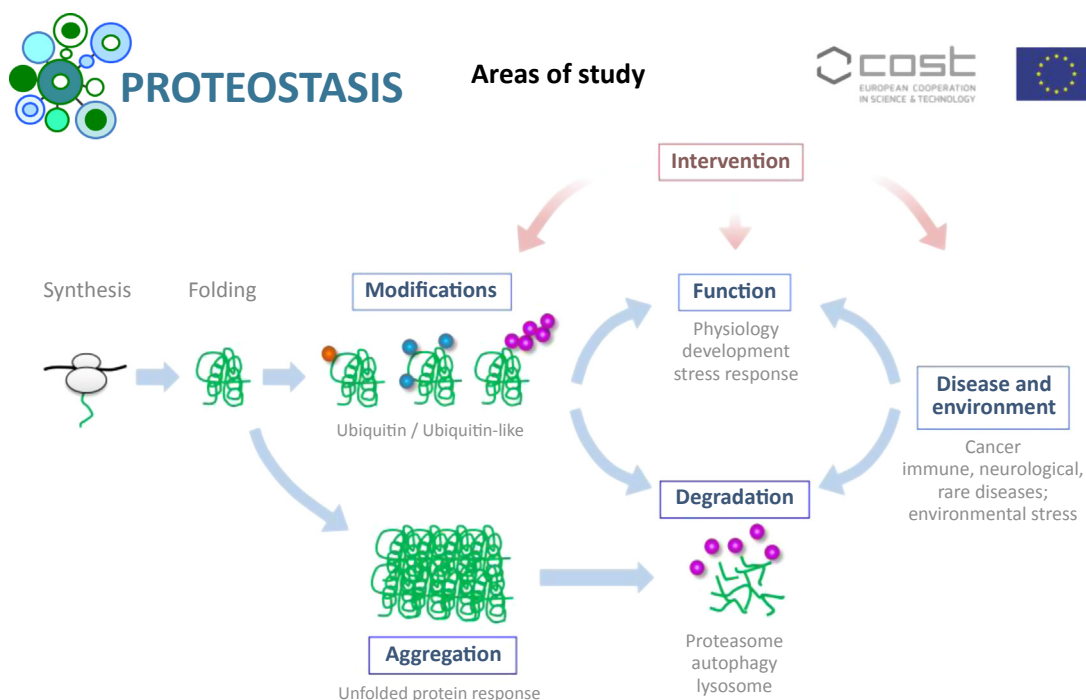
PROTEOSTASIS: The Network

Even if we still do not clearly understand when, how, and why many proteostatic systems function and collaborate within the cell, they enable alternative solutions to complicated problems, ranging from protein degradation to relocalization and neofunctionalization, and increase cellular plasticity for adaptation and survival in a continuously changing environment. Addressing all of these aspects of proteostasis was the ambition of the PROTEOSTASIS network (Figure 1).

To be able to dissect the molecular bases of complex proteostatic systems, in the health and disease of multiple species and with numerous approaches, we

nurtured synergies between research groups to bridge the gap between parallel but closely related fields, bringing together researchers working on various processes in different organisms, using diverse but complementary approaches. By reaching hundreds of European scientists working on protein homeostasis, PROTEOSTASIS offered the possibility to develop a large exchange space that broke artificial but real barriers, thus unleashing connectivity and creativity in our field.

The COST Action PROTEOSTASIS coordinated and integrated efforts made by research teams in different areas to translate novel discoveries into products of clinical and/or economical value. In other words, PROTEOSTASIS grew a fertile ground to impulse a comprehensive and holistic approach to tackle scientific challenges, by capitalizing on our diversity



Trends in Biochemical Sciences

Figure 1. A Scientific Field in Constant Development: PROTEOSTASIS Areas of Study.

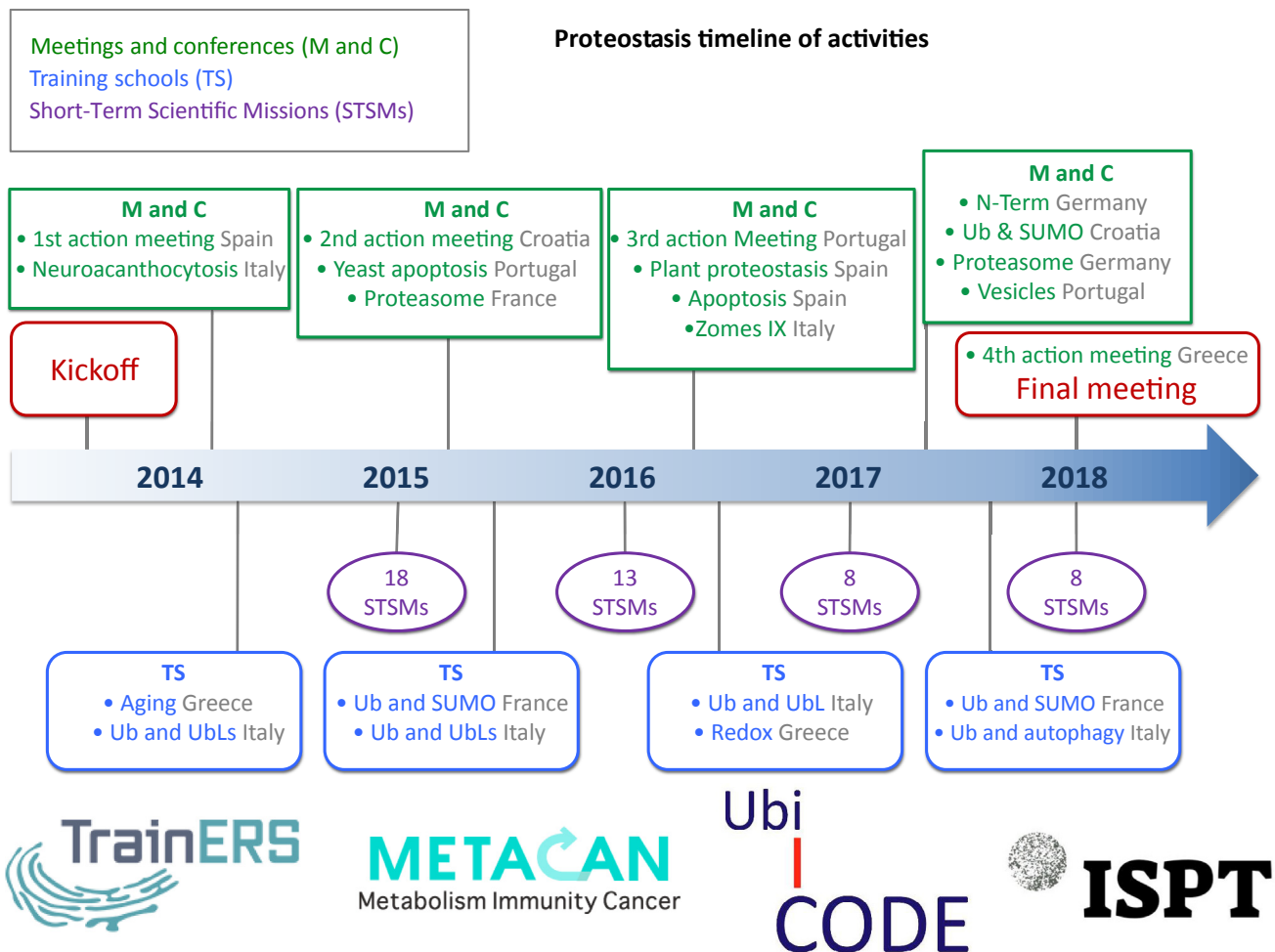
and complementary expertise, potentiating the resources and competencies available in each group.

As a networking tool, PROTEOSTASIS was successful in engaging more than 270 research groups, including researchers from 30 countries who covered all scientific areas of proteostasis. The main goal of PROTEOSTASIS was to foster exchanges between fields that tended to ignore each other for years by facilitating collaborative research and broad scientific exchange. To bring

together expertise from different disciplines and to openly discuss recent scientific developments, researchers from diverse backgrounds were invited to actively participate and join the community. Special emphasis was placed on the transfer from basic to translational science, novel technologies, and training next-generation scientists. Sharing unpublished data was the rule during the meetings organized by PROTEOSTASIS in the past 4 years. This strongly promoted collaboration among experienced and early-stage

academic-, clinical-, and industry-based researchers.

PROTEOSTASIS was structured around six wide thematic blocks, covering a broad and complementary scientific spectrum within protein homeostasis. Protein Modification dealt with molecular and structural properties of proteostatic components; Proteolytic Systems gathered scientists around mechanisms and structures of intracellular proteolytic systems such as proteasomes, autophagy, lysosomes, and apoptosis; Cell



Trends in Biochemical Sciences

Figure 2. Achievements and Milestones of the PROTEOSTASIS Network: An Overview of Activities and New Funded Networks. Abbreviations: SUMO, small Ub-like modifier; Ub, ubiquitin; UbLs, Ub-like proteins.

Signaling focused on signaling cascades, protein trafficking, and transcriptional regulation; Quality Control covered folding and misfolding, chaperones, aggregation, and ERAD; Cell Proliferation and Differentiation touched many aspects of cell cycle, cell growth, and development; and Diseases and Biotechnology explored the molecular basis of diseases and biomarkers in cancer, inflammation, and neurodegeneration, addressing drug targets and biotechnology.

Activities, Outcomes, and Highlights

Whether from the academic or the private sector, the PROTEOSTASIS network helped scientists to develop fundamental or translational research thanks to the multiple instruments that it offered to the community, namely, the organization or co-organization of 14 scientific meetings and workshops, eight training schools, and 47 scientific staff exchanges. Additional dissemination instruments included the website (cost-proteostasis.eu), webinars, newsletters, and social media^{i–v} (Figure 2). The co-organization of workshops and other activities together with relevant European or national bodies in the life sciences such as EMBO, FEBS, and other scientific societies was instrumental. The meetings, workshops, and conferences covered broadly the topics of the proteasome and signalosome complexes; autophagy; aging and neurodegeneration; apoptosis and cell death; cell polarity and movement; vesicular biology; system biology; Ub, SUMO, and other UbLs; N-end rule pathway; protein degradation in plants; agronomy, biotechnology and bioeconomy. Two examples of these meetings, ZOMES IX – ‘PCI complexes and ubiquitin defining a hub for protein homeostasis’ and ‘N-term 2017–Proteostasis via the N-terminus’, were described in meeting reports elsewhere [9,10]. As a result of the N-term

2017 meeting, the International Society of Protein Termini (<http://ispt.world/>) was established for scientists with a shared interest in protein N- and C-terminal modifications and their effects on protein functions. The eight training schools aimed to teach the analysis of *in vitro* and *in vivo* processes of ubiquitylation and SUMOylation, the proteostatic basis of aging and redox regulation of metabolic processes, and Ub-assisted autophagy.

Regarding networking, PROTEOSTASIS facilitated the consolidation or the initiation of collaborations and the generation of novel international consortia dedicated to specific issues. Those resulted in more than 50 joint publications in peer-reviewed scientific journals plus the three thematic books *Proteostasis*, *SUMO*, and *Plant Proteostasis*, with additional 74 contributions of network members [11–13]. Importantly, the international scientific exchanges enabled the mobility of researchers between laboratories and the development of joint research projects that were successfully funded. Examples of those are TrainERS (trainers.eu) on endoplasmic reticulum stress; META-CAN (metacan.eu), combining cancer metabolism, cell death, cancer immunity, data analysis, and immunometabolism; UbiCODE (ubicode.eu), deciphering the mechanistics of the Ub code; and TRIM-NET, exploring the functions of TRIM E3 ligases (Figure 2). Future joint applications are in the planning stages among members of the network.

Conclusions

The goal of PROTEOSTASIS was to foster and concentrate interdisciplinary scientific exchange and development of novel research ideas. The network coordinated and integrated the efforts made by research teams to better understand protein homeostasis and facilitated the interchange with the private sector. The network has contributed significantly towards reversing the national and

scientific fragmentation of research efforts within the European research area and beyond. After four exciting years of activities, we look back at a wonderful time of multifaceted exciting research, exchange of data and ideas, networking, training, and numerous newly built interactions and joint collaborations. PROTEOSTASIS as a network, including its social media and website activities^{ii–v}, is continued after the ending of the funding period. It will serve as a platform to communicate science, assist to organize meetings and conferences, and launch proposals in relevant funding calls. We aim to preserve those in the long term to facilitate and foster future interactions. We believe this type of network will be useful to other areas of research that might experience field fragmentation.

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Resources

ⁱwww.cost.eu/actions/BM1307
ⁱⁱ<http://cost-proteostasis.eu>
ⁱⁱⁱ<http://twitter.com/Proteostasis>
^{iv}www.youtube.com/channel/UCmQZIJ9a4OfS97T6Q2-g_sA

⁴www.facebook.com/Proteostasis-839198092811311/

Supplemental Information

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Forum

Trefoil Factor Family: Unresolved Questions and Clinical Perspectives

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The trefoil factor family of peptides (TFF1, TFF2, TFF3) with their lectin activities play important roles in mucosal protection and repair. However, major gaps in understanding their molecular function have hampered therapeutic development for gastrointestinal disorders. We provide here a critical overview of the *status quo*.

The Trefoil Factor Family (TFF): Key Players in Mucosal Protection and Repair

The three TFF peptides (TFF1, TFF2, TFF3) are typical constituents of mucous epithelia such as the alimentary, respiratory, and urogenital tracts, the conjunctiva, and the inner ear, where they are typically cosecreted with mucins [1,2]. The major expression site for TFF1 and TFF2 is the stomach, and for TFF3 it is the intestine [1]. TFF peptides appear also in the saliva, gastric juice, urine, blood, and breast milk [1,3]. Pathologically, TFF peptides are ectopically expressed after wounding, in inflammatory diseases, and in various tumors [1]. They are also secreted in an endocrine manner, for example, in the immune and central nervous systems [1,4]. Numerous *in vivo* and *in vitro* studies point to TFF peptides as key players in mucosal protection and repair processes. Accordingly, TFF peptides are constituents of the mucus barrier,

where they display lectin-like behavior, and enhance mucosal restitution (cell migration), while modulating cell junctions, apoptosis, angiogenesis, and mucosal differentiation processes [1]. Furthermore, transgenic TFF-deficient mice display abnormalities in the gastrointestinal (GI) tract and the immune system [1].

Therapeutic Potential

Based on a diverse range of GI injury models [1], TFFs hold particular therapeutic promise not only for GI inflammatory disorders but also for oral mucositis [5,6]. However, translation of this GI therapeutic potential has been limited to a single Phase I/II trial (16 patients) where recombinant TFF3 in enemas was given in combination with orally delivered mesalazine for the treatment of ulcerative colitis [1]. In this study, no additional improvement was observed compared to mesalazine treatment alone; however, the small trial size combined with limited understanding of the mode of action of the TFFs limits the interpretation of these outcomes. More successful was the therapeutic application of the TFF peptides in oral mucositis, a side effect of radio- and chemotherapy. In a Phase I trial, AG013, a mouth-rinse formulation of *Lactococcus lactis* secreting TFF1, reduced oral mucositis in cancer patients receiving chemotherapy [6]. AG013 has been granted Orphan Drug status in the EU and Fast Track designation by the US Food and Drug Administration, and is currently in Phase II trials. In addition, homodimeric TFF3 was also well tolerated in a Phase II study and reduced the incidence and severity of chemotherapy-induced oral mucositis in cancer patients [5]. Both trials support the therapeutic potential of the TFF and highlight different delivery strategies.

Mechanisms of Action: Potential Interaction Partners

We still know very little about the molecular mechanisms of the TFF