Systems Biology and Longevity: An Emerging Approach to Identify Innovative Anti-Aging Targets and Strategies

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Abstract: Human aging and longevity are complex and multi-factorial traits that result from a combination of environmental, genetic, epigenetic and stochastic factors, each contributing to the overall phenotype. The multi-factorial process of aging acts at different levels of complexity, from molecule to cell, from organ to organ systems and finally to organism, giving rise to the dynamic "aging mosaic". At present, an increasing amount of experimental data on genetics, genomics, proteomics and other *–omics* are available thanks to new high-throughput technologies but a comprehensive model for the study of human aging and longevity is still lacking. Systems biology represents a strategy to integrate and quantify the existing knowledge from different sources into predictive models, to be later tested and then implemented with new experimental data for validation and refinement in a recursive process. The ultimate goal is to compact the new acquired knowledge into a single picture, ideally able to characterize the phenotype at systemic/organism level. In this review we will briefly discuss the aging phenotype in a systems biology perspective, showing four specific examples at different levels of complexity, from a systemic process (inflammation) to a cascade-process pathways (coagulation) and from cellular organelle (proteasome) to single gene-network (PON-1), which could also represent targets for anti-aging strategies.

Keywords: Aging mosaic, longevity, systems biology, inflammaging, coagulation, proteasome, PON-1.

1. AGING AND LONGEVITY

1.1. Aging as a Complex Trait

The study on human aging and longevity has become a very hot topic in the last years because of the so-called revolution in demography, which led to the remarkable increase in the number of people over the age of 65 or 80 years living in Western countries but also in some emerging countries such as China and India [1]. The data collected during the last 20 years in different models suggest that the picture of the aging phenotype is fragmented and above all qualitative and that we are far from an exhaustive and quantitative scenario. This incomplete knowledge comes out from several bias: *1.* few studies evaluate many parameters at the same time in the same individual; *2.* the collected data are not of high-dimensionality; *3.* longitudinal studies, which are the most informative, are scanty. Another factor contributing to the complexity of the problem is that human aging and longevity are complex and multi-factorial traits, generally considered as the result of the combination of environment, genetics (and epigenetics) and stochasticity, each making variable contributions to the overall phenotype [2-4]. It is hypothesised that the importance of each component changes with the passing of time, as shown in Fig. (**1**). In this cartoon the relative importance of each component as well as the level of interaction with the other components are represented as colour tones and overlapping of circles. According to this hypothesis, genetics is less important in the early adult phase of life and becomes more important in old age. Moreover, these different components interact with each other, in particular genetics and environment.

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To this regard, it has been reported that the identification of genetic variants underlying complex traits and diseases (such as psychopathology, hypertension, cardiovascular diseases [5] and neurodegenerative diseases [6]) may take advantage from the study of gene expression in particular environmental conditions [5]. The studies on human aging and longevity are further complicated by the fact that human populations are heterogeneous from the point of view of genetic pool, life style, cultural habits, education, economic status and social network. All these components are different from population to population, and each population is characterised by a unique combination of them. This fact renders the studies difficult to compare and the results very often discordant. Finally, all these considerations also apply to gender difference, since gender appears to be a crucial player in the cross-talk between genes, environment and health [7]. The development of effective and realistic strategies for aging intervention, prevention and therapies may be facilitated by this integrated and multi-faced view. Indeed, it has been proposed that the manipulation of both genes and environment at the same time can open up novel possibilities of aging intervention and prevention [8].

1.2. Aging as a Mosaic

It is conceivable that longevity could be achieved by different strategies and by different combinations of genetics, epigenetics, environment and stochasticity and the result, i.e. the aging phenotype, is very heterogeneous. Moreover, it is emerging that the multi-factorial process of aging does not occur only at organism level, but it also acts differently in each organ system, organ, tissue and even in each single cell of the body, determining a different aging rate for each of them. In mice it has been observed that different tissues age in a coordinated fashion [9], while in humans this is still to be ascertained. As a consequence of these different aging rates, the aged body could be considered as a mosaic of tissues and organs displaying a different level of senescence, a situation we proposed to indicate as "aging mosaic" [10], as

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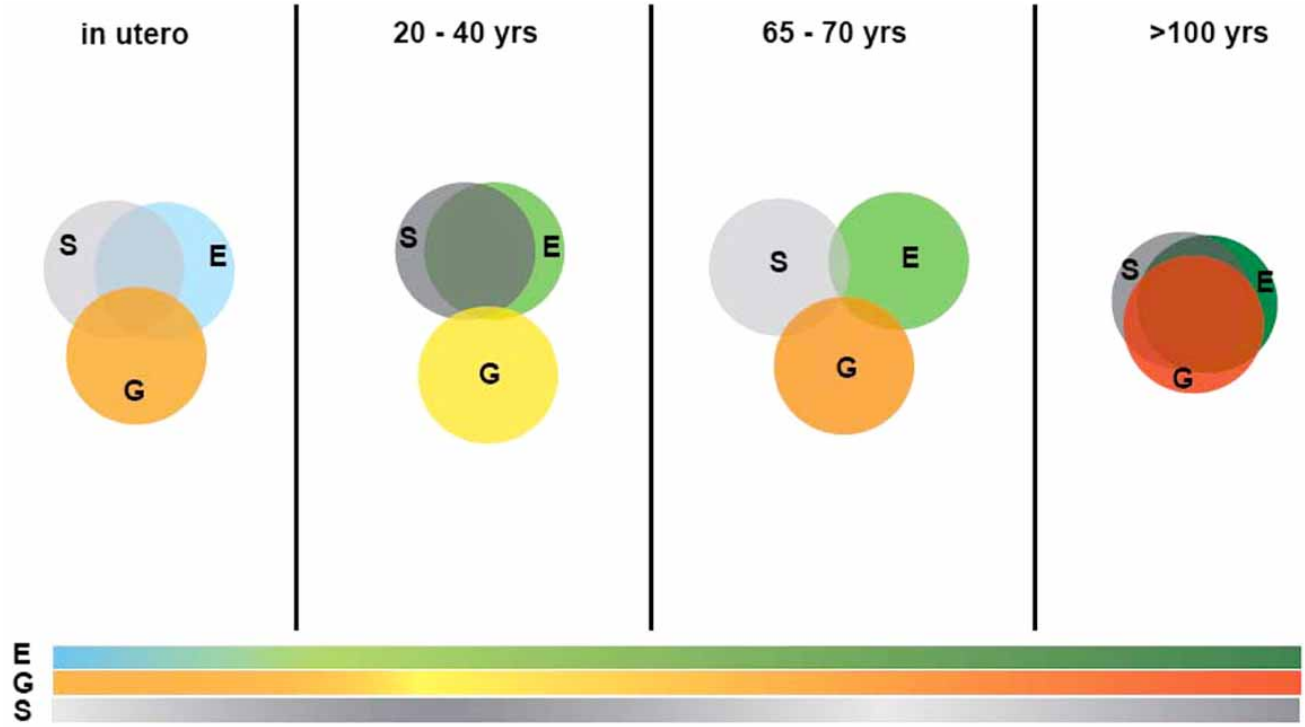

Fig. (1). The changes on relative importance and interaction between the main determinants of healthy aging and longevity. The colour tones are a function of the relative importance of each component (**E**=environment, **G**=genetics and **S**=stochasticity). The increase intensity of the colour (e.g. from yellow to red) means increased importance. The overlapping of the circles is a function of the interactions between these components. The *in utero* environment has been highlighted in cyan as it is profoundly different from the post natal one, highlighted in green tones.

exemplified in Fig. (**2**). In this figure, we represented the human body as a mosaic of 12 organ systems (indicated as rectangles) according to Hunter and Borg [11], each of them displaying a different level of senescence (represented by black dots).

Thus, even in the same individual, the aging process appears to follow different trajectories in different organs, tissues and cells, which are variably affected and accumulate unrepaired damages at different rates. An example, among many others, is the brain where different regions, such as cortex, hippocampus and cerebellum, show different levels of neurodegeneration and inflammation in the same subject [12,13]. In mice, a great heterogeneity in the amount of transcriptional changes with age in different tissues was found [9]. Based on the pattern of age-related transcriptional changes, three aging patterns have been proposed: (1) a pattern common to neural tissues, (2) a pattern for vascular tissues, and (3) a pattern for steroid-responsive tissues [9].

Moreover, this mosaic is dynamic, meaning that it changes with time, owing to the complex non linear interactions among the different components, resulting in a final phenotype that is not easily predictable by the study of its single sub-components. Changes in this mosaic derive from different forms of stressors to which the body is exposed lifelong. The adaptive response to these perturbations can follow two trajectories: *1.* in case of exposure to a repeated low grade stimulus, the mosaic is able to positively remodel the mechanisms of maintenance which are up-regulated and the systemic result is the resistance to stress (hormesis); *2.* conversely, in case of exposure to a stronger stimulus (over a specific threshold) in terms of intensity and persistence, the remodelling process still occurs, but the mechanisms of maintenance are less efficient and the global result is negative and detrimental for health and survival [14]. Therefore, a strategy to increase healthy aging and longevity could be to favour the hormetic response by transforming intense and persistent stressors into low-grade ones. In this perspective it is also interesting to note that low-grade stressors can be assimilated to what is referred to as functional or constructive noise. The role of noise in biological systems is under investigation, and, for example, it is now evident that the stochastic or inherently random nature of the biochemical reactions of gene expression may contribute to the phenotypic variability in individuals [15]. Thus, noise, which was often considered "unimportant" by traditional statistical methods and models, should be taken into account during data analysis, since it may hide un-analyzed information [16]. For example, in gene expression analysis, only genes whose expression profile differs from an established threshold were evaluated and the others were classified as "noise" and erroneously excluded [15]. It is also important to note that noise-induced phenomena have been experimentally detected in many levels of biological functionality, i.e. in plankton detection by paddle fish [17], in the retrieval processes of the human memory [18], and in human brain waves [19]. How can noise potentially play a constructive role in the system dynamics of main components and compartments of a biological organism? As regards the immunoproteasome [20], a key cellular organelle that will be discussed later in this review, it has been addressed whether the protein translocation inside the proteasome chamber can be driven by fluctuation, and a toy-model has been derived to show the probable mechanism of translocation [21]. Even if at the moment there is no experimental verification of the proposed hypothesis, however, it can be expected that noise-induced behaviour could play a major role in the immunoproteasome functioning, making this aspect worth of deeper investigation.

Currently, most of the studies focused on human aging refer to a single district of the mosaic (generally the most easily available tissues). For example, experimental results indicate that *in vivo* cell types from skin of aged individuals are likely to compose a mosaic made of cells irreversibly growth arrested (replicative senescence) and cells maintaining a young proliferative phenotype.

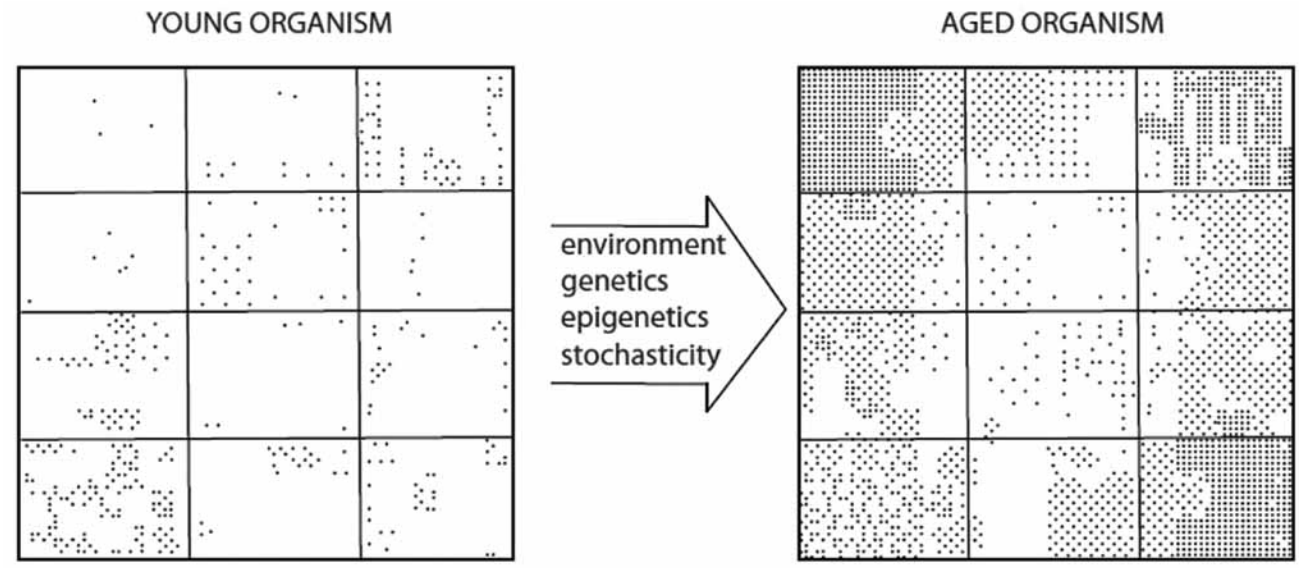

Fig. (2). Aging as a dynamic mosaic. The body (young or aged) is represented as a square subdivided in 12 rectangles, each of them representing an organ system. The amount of dots indicates the rate of aging affecting each system.

To this regard, *ex vivo* studies performed on human diploid fibroblasts (HDFs) from young and centenarian subjects show interesting results: they are characterized by the same proliferative capacity and the same telomere length [22], but they differ for the gene expression profile [23]. Moreover, *in vitro* studies show for example that the higher level of stress to which these cells have been exposed throughout their life span, the higher proportion of the cells of this mosaic will undergo stress-induced premature senescence (SIPS) rather than telomere-shortening dependent senescence. All cell types undergoing SIPS *in vivo*, most notably those in stressful conditions, are likely to participate in the tissutal changes observed along aging. For instance, HDFs exposed *in vivo* and *in vitro* to pro-inflammatory cytokines display biomarkers of senescence and might participate in the degradation of the extracellular matrix observed in aging [24]. In addition, despite support for the idea that senescence is a beneficial anticancer mechanism (i.e. expression of anti-proliferative genes), it is emerging that the secretome of senescent cells display an inflammatory phenotype that can also be deleterious and might contribute to age-related pathologies [25].

A more comprehensive approach to understand the complexity of this mosaic would be the simultaneous study of several organs at once; at present, this strategy has been applied only to animal models, as reported in mice by Schumacher B and co-workers [26]. However it should be taken into account that extrapolation of results from experimental animals (mice) to humans is not always straightforward, mainly due to the quantitative differences in maintenance and reproduction [27].

Within the complex scenario of such an interaction of different tissues and organs, each of them having intrinsic regulatory mechanisms as well as different aging rates, we proposed to go beyond the simplistic assumption of longevity genes playing the same role all along human life. The interpretation of antagonistic pleiotropy [28,29] paved the way for a wider interpretation of genes (and their polymorphisms) functioning: the reality is even more complex than antagonistic pleiotropy is capable to describe. Several experimental evidences suggest that the role of genes on physiology and pathology can change at different phases of human life. In genetics, these evidences are represented by complex trajectories in the frequencies of functional polymorphisms in population cohorts of different ages. The presence of such trajectories suggests that the same polymorphism can have different biological effects in young, middle-aged and extreme long-living individuals. We named this phenomenon **Complex Allele Timing** [3]. The first example of such kind of trajectories was reported since 1998 on APOB [30] and the latest one is represented by Klotho KL-VS polymorphism, for which a significant increase of the heterozygous Klotho genotype in the class of elderly people compared to young controls emerged, while no difference was present between centenarians and young controls. Thus, this KL-VS heterozygous genotype is favourable for survival in old people, its beneficial effect decreasing thereafter, and becoming no more evident at the extreme ages. Such a non monotonic age-related trajectory of the Klotho KL-VS genotype frequency is compatible with the hypothesis that alleles and genotypes involved in aging and longevity may exert their biological effect at specific time windows [31]. It is noteworthy that this conceptualization is supported by the use of demographic information, that, together with data on genetic markers, allowed us to calculate hazard rates, relative risks, and survival functions for candidate genes or genotypes, providing a powerful tool for analyzing their influence on longevity and survival [32]. This modelling approach allowed us to predict trajectories in genetic variation frequencies that have been subsequently confirmed by experimental data [32,33].

Moreover, the role of epigenetics (DNA methylation, histone post-transcriptional modifications, including methylation, acetylation, ubiquitination and phosphorylation) in the aging process should also be considered within this scenario because it includes regulatory mechanisms that could play a pivotal role in cellular homeostasis, age-related diseases, such as human cancer, as well as lifespan. To this regard, "aging epigenetics" became an emerging discipline [34,35]. The epigenetic alterations that accumulate with age, such as the global DNA hypomethylation (above all on repeated sequences), the promoter hyper methylation of Werner Syndrome genes and lamin genes, aberrant CpG island promoter hypermethylation, DNA methyltransferase (DNMT) overexpression and histone modifications, could be responsible, at least in part, for the phenotype of aged cells, tissues and organs, being in turn responsible for age-related changes in gene expression [4,36]. However, no data are available on the epigenetic of human (extreme) longevity and the global DNA methylation and the methylation status of specific candidate loci for longevity is at present lacking, as well as epigenetics applied to population genetics studies.

In this complex scenario, where a unique model for the study of human aging and longevity does not exist, a systems biology approach that combines and quantifies genetics, genomics, proteomics and other *–omics* fields is necessary to handle the ever increasing amount of experimental data made available by new high-throughput technologies. The final aim we are pursuing is to use a systems biology perspective to grasp the complexity of "aging" and "longevity"; in order to have a healthy old age, a systems perspective of medicine could promote the arrangement of advanced personalized therapies specifically aimed, for example, to target the individual patient's genetic defects [10,37,38].

2. METHODOLOGICAL APPROACHES TO AGING AS A COMPLEX MOSAIC

2.1. Systems Biology, Aging and Longevity

Aging can be studied at several levels of increasing complexity: molecular, organelle, cellular, organ, organ system and organism level. For a long time the cellular level was used as the most integrate level to study aging and longevity, generating a huge amount of data, directly transferred to tissues, organs, organ systems and the whole organism. Despite an enormous literature regarding physiological changes during aging in different organs, only few studies have been performed at organ system and organism level and it is emerging that many findings obtained from studies at cellular level often are not informative about the scenario of higher levels of complexity. Only now scientists are trying to integrate the findings obtained by studies performed at different levels into a coherent framework. In this new perspective, systems biology represents a modern science whose aim is to operate at a systemic level, by using the most integrated models (organ and organism). Thus, it moves biology from a traditional microscopic/ qualitative perspective to a macroscopic/quantitative one, allowing researchers to integrate and quantify the huge amount but fragmented data that nowadays can be obtained by high-throughput technologies [39]. In addition, it also offers tools to develop predictive mathematical models and networks [40], able to evaluate and compare potential explanations for biological data [41]. Thus, systems biology represents a strategy to integrate and quantify the existing knowledge and data from different sources into models, to be later tested and then supported with experimental data for validation and refinement, in a recursive process of "wet and dry" experiments, that will be discussed in the last section of this review. The ultimate goal is to "compact" the new acquired knowledge into a single picture, ideally able to characterize the phenotype at systemic/organism level.

High-throughput technologies generate complex and high dimensionality data that need appropriate statistical analysis, such as nonlinear methods [42], and above all a sophisticated interpretative approach in order to get insight on their biological meaning. As an example, visualization techniques, interaction maps and pathway diagrams can be of great value in order to understand how all molecular and cellular components and modules are intertwined and work together to determine the basic structures and the functions of the organism's complex machinery. This approach has been used by Raza and colleagues [43] to reconstruct a logically represented pathway map, integrating four pathways central to macrophage activation (interferon signalling, NF-κB, apoptosis, toll-like receptor pathways), and representing them as one integrated pathway due to their strongly overlapping interactions. Of course these new methods represent a step further, but we are still far from having powerful tools able to capture the complexity of the problem and to reach the ultimate goal, that is combining and quantifying the bulk of available data in specific fields, including multifactorial diseases, such as neurodegenerative disorders [44,45], aging and longevity [38], vaccinology, preventive medicine, proteolityc systems (proteasome).

In spite of this complex scenario, immunological studies and studies on centenarians revealed that common mechanisms occurred both in very different age-related diseases, such as diabetes and neurodegeneration, and in healthy long-living subjects. One is the persistent, low grade inflammatory process which develops with advancing age and that we called "inflammaging" [46]. Therefore, anti-inflammatory therapies could efficiently contribute to slow down aging and age-associated pathologies, thus increasing the possibility to reach longevity. In a general perspective, the prevention or the postponement of the aging process could have much greater benefit than those targeted at individual disease [47]. In addition, combining the already known empirical methods of anti-aging medicine with unique genetic profile of each human (genepass) may render new awarding opportunities for the further advancements of human longevity programs [48].

2.2. Resources, Databases and Models

Post-genome technologies, such as microarrays and RNAi, have provided researchers with powerful, high-throughput tools to study aging [49]. To collect, manage, and organize the massive amounts of data generated and help researchers analyze the data, a number of resources and databases have been assembled. The Human Aging Genomic Resources are one of such collections of databases and tools, which are of particular interest to systems biology of aging. Databases include GenAge, an accurate database of genes associated with aging in model organisms as well as a selection of candidate aging-related human genes. By providing an annotated list of genes associated with aging, GenAge can help researchers study how genes interact with each and with the environment to collectively modulate aging [50].

Microarrays, by measuring gene expression in a high-throughput fashion, allow molecular changes during aging to be characterized with unprecedented power and several studies using microarrays have now been performed. One database of gene expression profiles during aging, neurodegenerative diseases, and in interventions affecting aging is the Gene Aging Nexus, which features a compilation of microarray data and a data analysis platform (http://gan.usc.edu/) [51]. Another gene expression database is AGEMAP, which provides a catalogue of gene expression changes with age in different mouse tissues [9]. These resources open new opportunities to analyze how multiple genes and mechanisms affect aging, how these gene expression changes are regulated, and which genes and mechanisms may represent targets for manipulations.

One focus of the systems biology approach is to model data mathematically in order to better interpret the processes under study, perform computer simulations, and gain novel insights. Ultimately, the goal of modelling biological processes is to shift from describing phenotypes to predicting their behaviour and how they can be manipulated. In the context of biogerontology, accurate *in silico* models of aging would help identify key components (*e.g.*, proteins) that influence aging which would not only guide experiments but provide novel therapeutic targets to counteract the effects of aging in particular age-related diseases.

Considering the complexity of aging, however, thus far models of aging have focused either on specific components of mechanisms of aging or at higher levels of abstraction. An example of the former is a mathematical model of the heat shock system, which has been implicated in aging and age-related diseases [52]. Higher-level models have focused on gene and protein networks . It is possible to build proteomic networks for candidate gene prioritization. Proteins which interact with proteins associated with aging are then selected as candidates [53]. Data on genes associated with aging and their interactions can also be integrated with microarray data to derive candidate genes whose involvement in aging can then be tested experimentally [54]. Eventually, the goal is to integrate different types of data into increasingly more detailed. Interestingly,

John D. Furber has been assembling a network model of human aging at various biological levels. The model is being constructed based on a literature review from which components of aging are incorporated and their causal relationships described (http:// www.legendarypharma.com/chartbg.html).

3. EXAMPLES

Taking into account that the final aim of the systems biology of aging is to characterize the phenotype at systemic/organism level, systems biology can be also applied at multiscale levels on the basis of the specific questions to be answered. Thus, studies at molecular, cellular and organelle levels can be performed and evaluated by systems biology because even a single gene can play a role in the definition of a complex phenotype, such as aging and longevity, since it is part of a network where genes and product genes interact. Any perturbation of a single component can modify the whole network.

According to our experience, in this review we propose four examples where systems biology can be applied at different levels of complexity, from a systemic process (inflammation) to a cascade-process pathway (coagulation) and from cellular organelle (proteasome) to single gene-network (PON-1), all considered in an integrated vision. We do not treat here other complex processes, since they are already examined in recent papers, such as neuro-degenerative disorders [44,45] and apoptosis [38]. The rationale is hidden in the different levels of analysis, from integrated to single effects, which concur to the determination of the aging phenotype.

3.1. Inflammation

Inflammation is a systemic physiological process fundamental for survival, which involves a variety of cells, organs and organ systems, as depicted in Fig. (**3**).

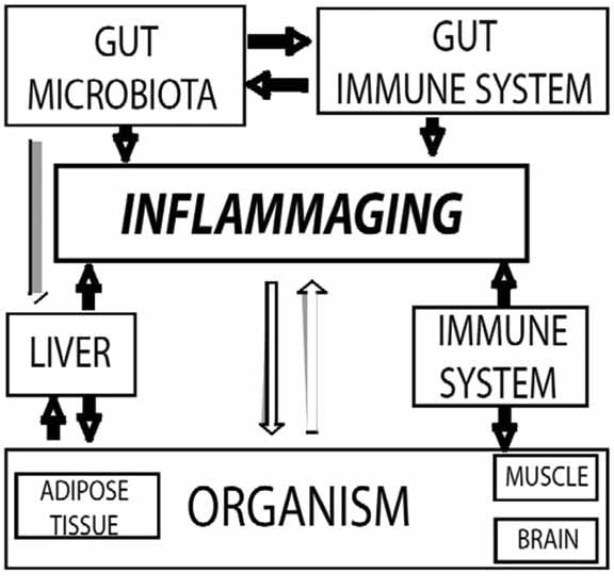

Fig. (3). Inflammaging as a systemic phenomenon. Many organs and organ systems of the body are strictly interconnected and together contribute to the increasing low-grade inflammation that occurs with age.

Despite the well-recognized systemic character of inflammation, our knowledge of this multi-factorial phenomenon is highly fragmented and its comprehensive understanding is still in its infancy [55].

In the aging field, this consideration is particularly important for "inflammaging", [46,56,57], which appears to be important in the pathogenesis of cardiovascular diseases (CVD), type 2 diabetes (T2D), metabolic syndrome (MS), neurodegeneration, sarcopenia and cancer, among others.

An age-related increase in the production of inflammatory compounds occurs in the immune system [58,59], brain [60], adipose tissue [61] and muscle [62], but the possible contribution to inflammaging of other organs or districts, such as gut microbiota and liver, is largely unexplored.

At present a major unsolved problem in biology and medicine is the source(s) of the inflammatory stimuli underpinning and sustaining inflammaging. Moreover, the signaling circuitry and the cross-talk among the various tissues and organs involved in inflammaging are far from being clear. Therefore, systems biology represents the most powerful and comprehensive approach to characterize the systemic nature of inflammaging, for example by developing predictive models of inflammaging, urgently needed to set up strategies for the modulation of inflammation, by acting on strategic targets with global, systemic effect on the whole process.

A key point in such a complex framework is the dynamics of signaling pathways crucial to inflammation, such as those related to NF-κB transcription factor activation. NF-κB regulates genes that in turn control cell proliferation, cell survival, as well as innate and adaptive immune response. In Fig. (**4**) we provide a partial reconstruction of its network of interactions. The sheer complexity of such crucial signalling system is intuitively evident from the intricate network of interactions among input and output signals, mediator proteins and feedback loops. Many proteins (in red) directly involved in such interactome are in turn products of genes that are controlled and regulated in their expression by NF-κB. Framing structure and dynamics of the NF-κB interactome in a wider, systemic picture will be a significant step toward a better understanding of how it globally regulates diverse gene programs and phenotypes. This fact gives rise to the necessity of a systemic, multiscale approach able to take into consideration all the crucial levels of complexity into an unique framework, from protein-protein interactions to gene expression, from cellular to tissue response and finally to the dynamics of organs.

3.2. Coagulation

Coagulation is a typically complex, multiscale process involving in a delicate and time-evolving balance a number of cellular and molecular components. Cells (platelets), protein factors, cofactors and regulators actively participating through different pathways converge into the coagulation cascade to yield the conversion of fibrinogen to fibrin, the building block of a haemostatic plug. The haemostatic process is finally down-regulated by the intersection of the cascade with anticoagulant pathways.

Aging is associated with an increase of many proteins of blood coagulation and with an impairment of the fibrinolysis process, an alarming framework that opens the way for thromboembolic and vascular diseases. Centenarian individuals are characterized by a state of hyper- or pro-coagulability, and by the possession of atherothrombotic risk markers and high-risk alleles. Paradoxically, this condition appears to be compatible with longevity and health. Experimental evidence shows that the increased activity of coagulation enzymes in centenarians does not necessarily correlate with augmented thrombosis risk [63-66]. Such data on homeostasis profiles of long-lived and centenarian individuals highlight once again the pressing need for a systemic and multidisciplinary approach to complex and multiscale phenomena such as coagulation and, at a higher level, aging.

In this perspective, systemic and quantitative modelling strategies have been successfully applied to uncover coagulation cascade kinetics and platelet homeostasis. Luan and co-workers [67] built a mechanistic model of the human coagulation cascade that, despite the uncertainty inbuilt in modelling such a complex and multi-level system, has been able to identify critical points in

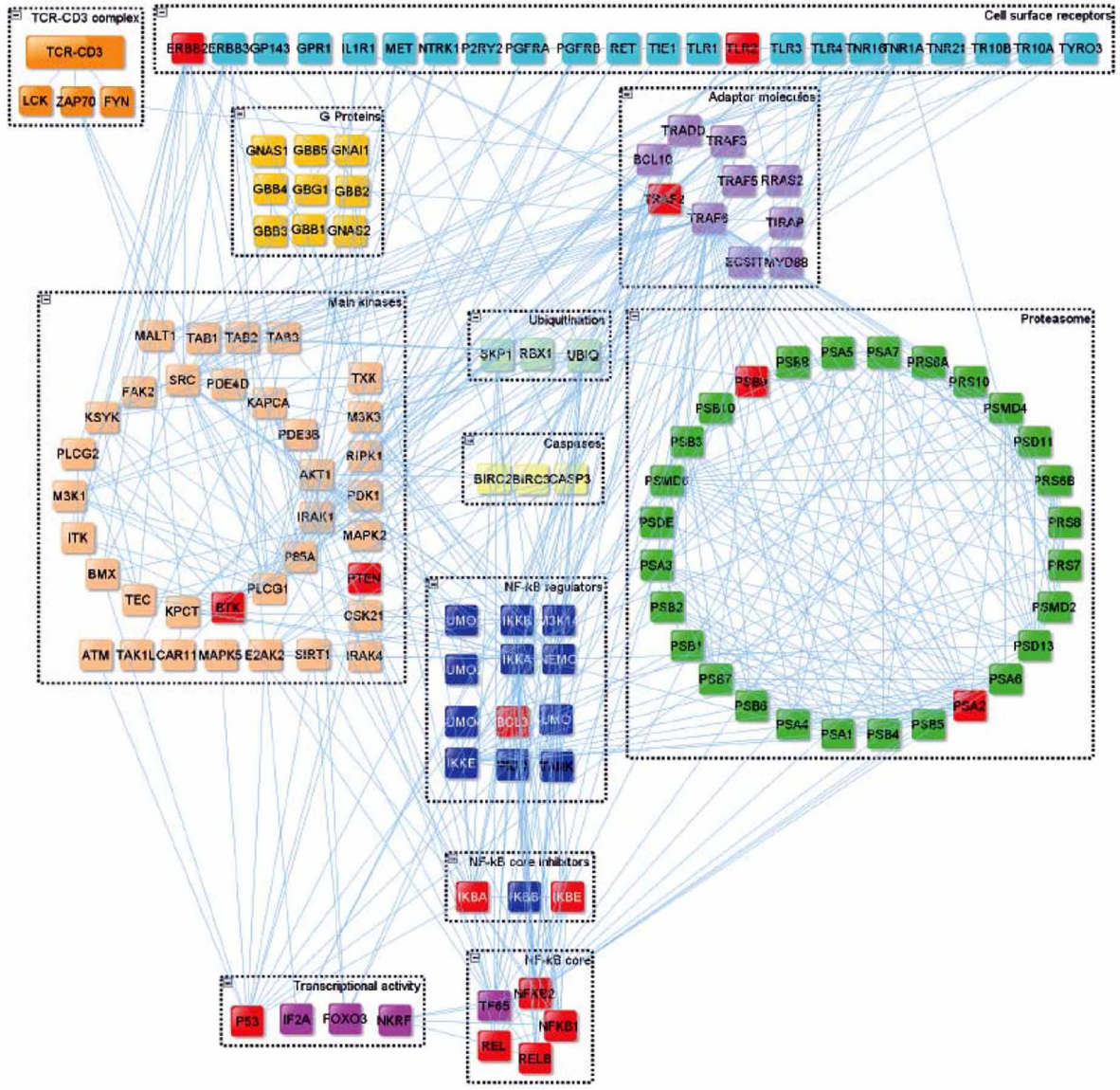

Fig. (4). A partial reconstruction of the NF-κB transcription factors interactome. Proteins involved in the NF-κB pathway (data extracted from literature) are grouped on the basis of their main functional category (each category is inscribed in dashed squares). Direct interactions among proteins are shown (solid blue lines). Proteins coloured in red derive from genes whose expression is directly regulated by NF-κB transcription factors.

the formation of thrombin. Indeed, simulations performed on a literature-derived network of 92 protein complexes and 148 kinetic interactions yielded platelet activation and thrombin generation profiles consistent with experimentally measured parameters. These results have led to determine which processes can be considered as "points of fragility", i.e. if and to which extent they are sensitive or not to parameters changes. The model-based screening has thus qualitatively identified fragile and robust key mechanisms and processes, which can be considered as putative drug targets, especially when concomitantly supported with thrombosis treatment literature and clinical trials data. This represents a clear example of the therapeutic potentiality of the systemic approach in this field.

Furthermore, as reported by Purvis and colleagues [68], existing kinetic information for 70 species, 132 kinetic rate constants and 77 reactions was computationally combined with experimental results and published data derived from different sources. This integrative approach was able to predict key parameters in resting and activated platelets, replicating experimental data at both averaged platelet and single platelet response. The model was also able to generate novel predictions and provide further valuable explanations, highlighting for example that, in the ADP-dependent platelet activation, thrombin is a more potent agonist than ADP, thanks to differences in receptor copy number. Thus, the power of this integrated computational approach relies on the capability to yield, through recursive refinements and developments, more and more accurate predictions of known as well as unknown phenomena in platelet biology.

3.3. Proteasome

The proteasome is an additional candidate target to be studied in a systems biology perspective. It is a self compartimentalized protease that could be considered an organelle, conserved during evolution, which is involved in the regulation of all the metabolic pathway, in the production of MHC I epitopes and in the protein quality control, suggesting its centrality in maintaining the cellular homeostasis and thus contributing to reach longevity. Even if it acts at the molecular level, its effects are relevant at systemic level. The 26S proteasome consists of a catalytic 20S core (a four-ring structure with seven different subunits in each ring, arrayed as $\alpha7\beta7\beta7\alpha7$) and two 19S regulatory complexes. Alternatively,

proteasome activator PA28-αβ or -γ can bind to the end of the 20S core [20,69]. In cells exposed to pro-inflammatory stimuli such as IFN-γ and TNF-α, the constitutive β subunits with catalytic activity (β1, β2, β5) are replaced by homologue subunits (β1i or LMP2, β2i or MECL-1 and β5i or LMP7, respectively) and incorporated into a newly synthesized alternative proteasome form, the immunoproteasome. More recently, a variant, named thymoproteasome, has been described in thymus [70]. Because constitutive and inducible β catalytic subunits often coexist in cells, the presence of intermediate type proteasomes with asymmetric subunit composition, different proteolytic activities and subcellular localization, have been also reported [71]. Considering the broad range of proteasome substrates and the pivotal role of proteasomes in many cellular pathways, alteration in the ubiquitin-proteasome system likely leads to the dysregulation of homeostasis and the development of multiple pathologies [72]. An age-related loss of proteasome function has been reported in several human tissues (muscle, lens, lymphocytes and epidermis), in aged tissues of other mammals (mice, rats and bovines), in human primary cultures undergoing senescence [73] as well as in different neurodegenerative pathologies (Alzheimer –AD-, Parkinson –PD-, Huntington –HD- disease and Amyotrophic Lateral Sclerosis -ALS-) [12,13]. To date, however, several aspects of the proteasome biology and functionality (i.e proteasome interaction networks, proteasome composition in different tissues and organs, degradation rate, modulators, among other) are fragmentary and remain elusive. A very recent interaction map of the yeast 26S proteasome [74] and novel algorithm for subnetwork reconstruction in *Saccharomyces Cerevisiae* [75] have shown the involvement of proteasome in pivotal cellular processes. However, a systems biology approach to study the proteasome in both normal and pathological conditions, is still in its infancy. A first attempt to describe, in a systems biology framework, the ubiquitin-proteasome system in normal homeostasis as well as during aging, has been reported by Proctor and co-worker [76], while a more recent model of on-going conflicts between the aggregation of α-synuclein (αSN) and the maintenance of a functional proteasome in PD has been described [77]. Concerning the knowledge of proteasome degradation processes, further insight are provided by an *in silico* mathematical model, focused on *in vitro* proteasome degradation assay, developed by our group [78]. This model, named ProteaMAlg (Proteasome Modeling Algorithm), is an extension of a previous one [79] and it describes the kinetics of specific protein fragments generated by 20S proteasomes in different conditions, such as the in presence of constitutive or immunoproteasome and the activator PA28-αβ. Thus, in a systems biology framework, the tool ProteaMAlg could offer a quantitative scheme to study the degradation dynamics in different experimental conditions defined by the substrate specificity and the type of proteasome. Other important aspect of proteasome degradation mechanisms, such as the peptide translocation, which can have a great impact on the quality of epitope production, has been recently modeled [21,80].

Lastly, another issue to be addressed in a systems biology perspective is the identification and characterization of molecules that could modulate (enhancing or inhibiting) the proteasome activity leading to the development of proteasome-based drugs able to counteract aging and age-related pathologies. Indeed, at present, the only available proteasome-based therapeutic approach is the administration of proteasome inhibitors such as Bortezomib, in the treatment of different cancers [81] while new compounds have recently shown to be able to discriminate between these two main isoforms of 20S proteasome, constitutive and immunoproteasome [82]. The identification of natural/synthetic molecules able to enhance proteasome activity (and maybe with higher affinity for different proteasome subtypes) would allow to target other type of diseases where proteasome activity should be enhanced and not inhibited. For example, oleuropein, the most abundant of the phenolic compounds in Olea europaea leaf extract, olive oil, and olives has a stimulatory impact on proteasome activities *in vitro*, promotes cellular resistance to oxidants and confers extension of human fibroblasts lifespan [83]. A similar stimulatory effect has been observed by an algae extract on human keratinocytes, as it provides protection from UVA and UVB irradiation [84], and by Tocopherol (Vitamin E) and linoleic acid [85].

3.4. PON-1

In this final example, the systems biology approach is applied at a single gene level, which can have a great impact on several age-related diseases and longevity as proposed by the conceptualization of the "diseasome" [86]. Human serum Paraoxonase1 (PON1) is considered not only for its role in aging: PON1 is one of the most studied gene regarding cardiovascular risk, oxidative stress and inflammation. PON1 is primarily synthesised by the liver and high density lipoprotein (HDL) is the serum transport vector for this enzyme that is firstly inserted into the external membrane of the secreting cell, and then transferred to HDL [87]. The main function of paraoxonase is to inhibit low density lipoprotein (LDL) oxidation by destroying the biologically active phospholipids in oxidatively modified LDL [88,89]. PON1 significantly reduces the ability of oxidized LDL to trigger monocyte-endothelial cell interactions [90]. Also, it hydrolyzes oxidized LDL-associated compounds, which stimulate the production of cytokines, interleukin-8 and monocyte colony-stimulating factors and induce adhesion of monocytes to the endothelial surface [91,92]. PON1 activity contributes to prevent the formation of atheromatous plaques. There is a growing body of evidence that reduced activity of the HDL-associated enzyme paraoxonase is predictive of vascular disease in humans, including results from prospective studies [93,94]. Low levels of PON1 activity have been found in numerous pathological conditions, such as cardiovascular disease, type1 and 2 diabetes, hypercholesterolemia, metabolic syndrome and renal failure [95-99]. All these pathologies share a pro-inflammatory baseline condition and it has now been shown that HDL composition is altered during the acute phase response of the innate immune system. Polymorphisms located in PON1 coding region are due to an aminoacid substitution at position 192 (Gln-Arg = Q-R alleles) and at position 55 (Leu-Met = L-M alleles). The Q192R polymorphism imparts different catalytic activity toward some organ phosphorus (OP) substrates. Specifically, the 192R isoform hydrolyzes more efficiently paraoxon, however the 192Q isoform is more efficient in hydrolyzing diazoxon, soman, and sarin [100,101]. Repeatedly, a large number of studies have suggested an association between major cardiovascular diseases and the 192 R variant. Moreover, the results of a recent meta-analysis [102] show that PON1 gene variants at codon 192 impact on the probability of attaining longevity, and that subjects carrying RR and QR genotypes (R+ carriers) are favoured in reaching extreme ages. These results likely represent the counterpart of the effects observed on CVD, as centenarians and nonagenarians escaped or delayed the onset of the major age-related diseases, including CVD.

The role of PON1 emerges out of this brief picture: it can be located at the centre of a complex interaction of physiological processes, impacting on many human complex disease, and thus the best starting point for a systems biology analysis.

To this regard, we reconstructed two PON1 networks, using Cytoscape and Agilent literature search plugin. This plugin takes as input a set of genes of interest, extracts interactions and generates putative networks from literature.

The first network shown in Fig. (**5**) is built using only PON-1 as input, and displays mainly the proximal nodes that have a direct connection with it.

As expected, PON-1 is directly connected to APO-E, but it is worth noting that PON-1 is also directly connected to SP1 and PPARG (two broadly acting transcription factors which are sensitive to oxidative stress), to PSMC6 (a subunit of the proteasome), to HSD11B1 (a gene encoding a microsomal enzyme that

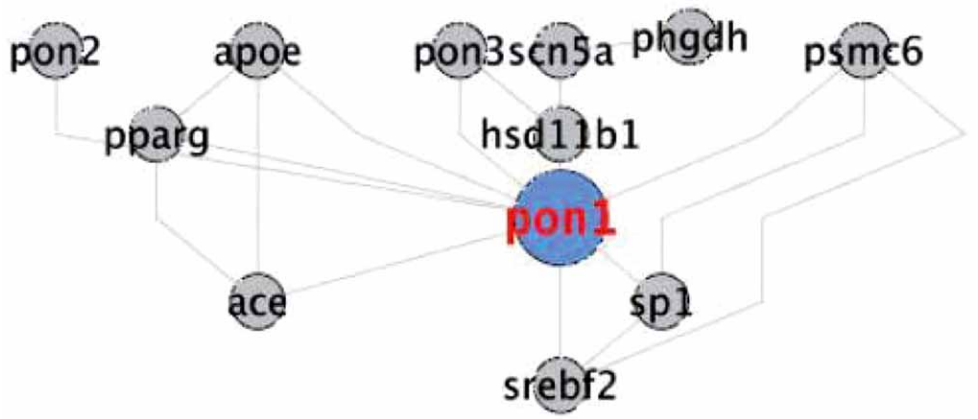

Fig. (5). Basic PON-1 literature search-based network. This network shows **PON-1** first degree interactions, highlighting important connections between **PON-1** and **APO-E**, **SP1**, **PPARG**, **PSMC6**, **HSD11B1** and **SREBF-2**.

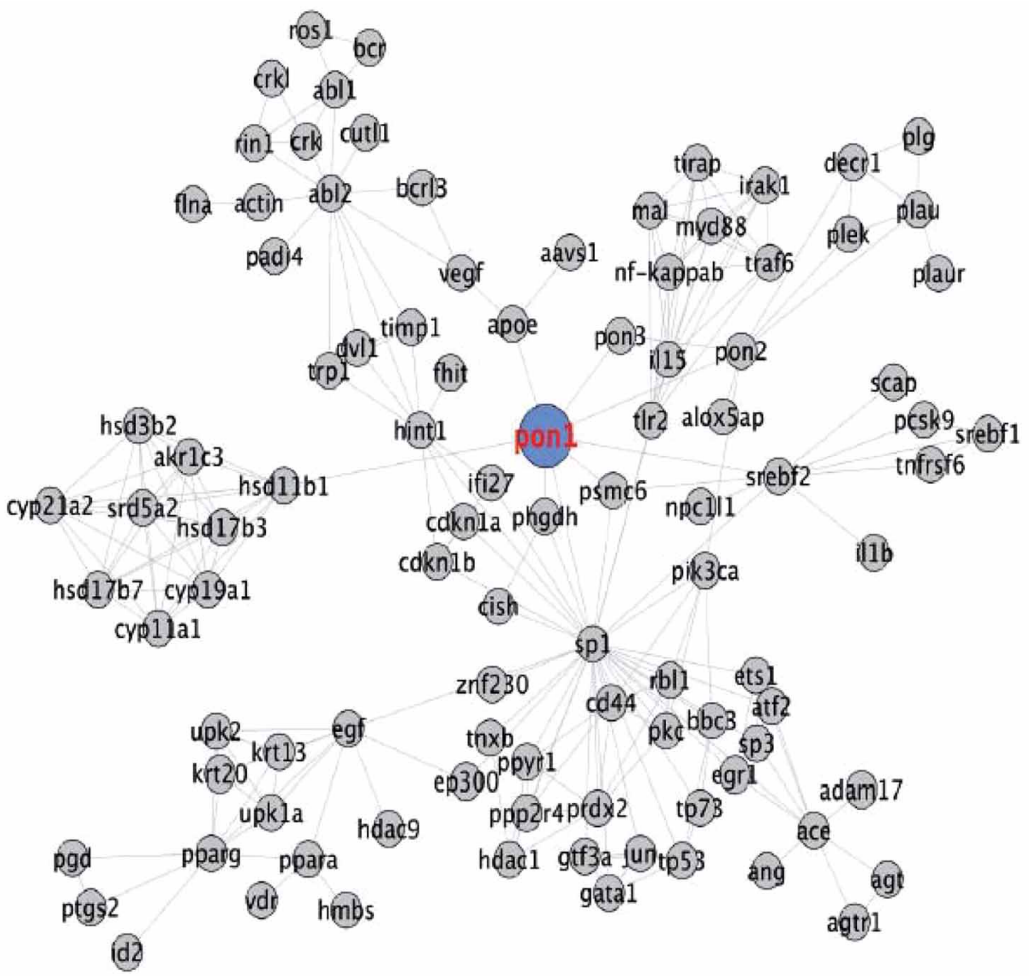

Fig. (6). Extended PON-1 literature search-based network. This expanded network shows **PON-1** long-range interactions indicating how **PON-1** can affect a number of different physiological processes.

catalyzes the reversible conversion of the stress hormone cortisol to the inactive metabolite cortisone) and to SREBF-2 (a transcription factor that controls cholesterol homeostasis). All these genes are involved in oxidative stress, cardiovascular diseases and many other age-associated pathologies.

The second network shown in Fig. (**6**) is built using the PON1 interacting genes of the first network as the input set and it displays the connections among the PON1 interacting genes and other genes. In this network is possible to see long range influences of PON1 in different physiological aspects.

The presence of an inflammatory cluster can be noted, including a master regulator of inflammation, NF-κB, and different inflammatory mediators, such as IL-1 beta and IL-15. In addition, the presence of a cluster regarding the production of lipophylic hormones and modulators of inflammation can be noted. Indeed, this cluster contains enzymes that catalyzes the production of

prostaglandins (AKR1C3) or steroids (HSD11B1, SRD5A2, CYP11A1, CYP21A2, HSD3B2, HSD17B3, CYP19A1).

The central cluster is related to the control of cell cycle and proliferation and it is connected to a cluster of epithelial differentiation related genes by epidermal growth factor (EGF). This cluster is also connected to a cluster of vascular remodelling related genes (VEGF) by some oxidative and stress response related genes such as ABL2, underlining the connection between oxidative stress response and vascular remodelling.

4. CONCLUSIONS

In this complex scenario we briefly summarized, it is emerging that systems biology is a powerful tool to integrate and quantify high dimensionality but still fragmented data that are produced by increasing high-throughput technologies in the field of genomics, proteomics, metabolomics and other *–omics*. In particular, our laboratory is producing these data in the framework of several integrated projects on human aging and longevity financed by European Commission. Some are already concluded, such as ECHA, T-CIA, FUNCTIONAGE, PROTAGE, and others are still in progress, including GEHA, PROTEOMAGE, MARK-AGE, MYOAGE, RISTOMED and WhyWeAge. We propose that a comprehensive analysis of this information is possible only through a systems biology approach, in a recursive process of "wet" and "dry" experiments, as represented in Fig. (**7**).

Indeed, the existing information and new data generated by *ad hoc* experiments are put together in a comprehensive data set, which is then analysed and used to generate predictive mathematical models. These new models can suggest new experiments, which in turn produce an enlarged data set and the analytic process restarts. This innovative approach can be performed only if specific expertise of analysis and computational power will be available, joint to more collaboration across disciplines and countries. We surmise that this integrated approach will let us understand the aging process at organism level and to identify targets for anti-aging interventions.

ACKNOWLEDGEMENTS

This work was supported by: the EU Projects "Proteomage" (FP6-518230) and "MARK-AGE" (FP7-200880) to CF; Roberto and Cornelia Pallotti Legacy for Cancer Research Grants to CF and SS; University of Bologna "Progetti Strategici" 2006 grant ("p53 e patologie non neoplastiche nell'anziano: uno studio multidisciplinare sul ruolo del polimorfismo al codone 72 del gene TP53") to SS. AZ acknowledges the UCLH/UCL NIHR Comprehensive Biomedical Research Centre and VW foundation.

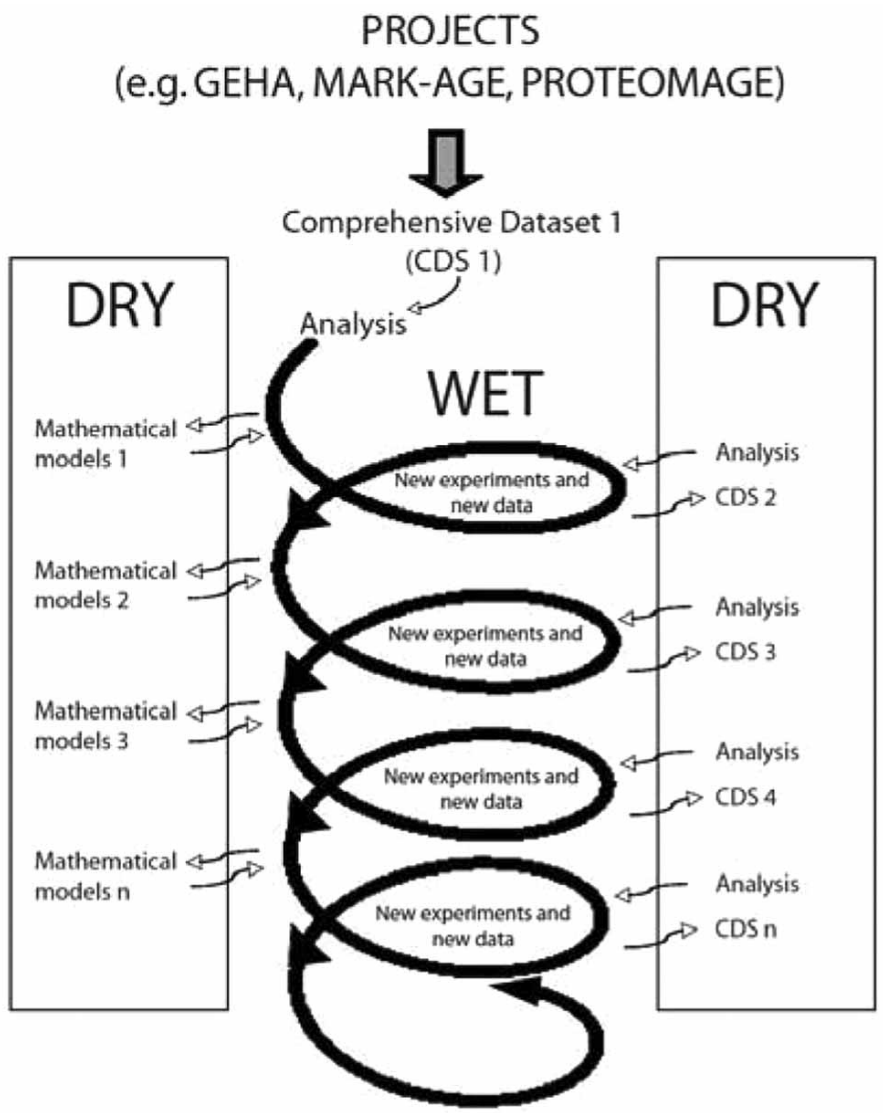

Fig. (7). The **"dry"/"wet"** recursive spiral of the systems biology of aging and its implementation.

ABBREVIATIONS

AD	=	Alzheimer Disease
ALS	=	Amyotrophic Lateral Sclerosis
αSN	=	α-Synuclein
CVD	=	Cardiovascular Diseases
DNMT	=	DNA Methyltransferase
EGF	=	Epidermal Growth Factor
HD	=	Huntington Disease
HDFs	=	Human Diploid Fibroblasts
HDL	=	High Density Lipoprotein
LDL	=	Low Density Lipoprotein
MS	=	Metabolic Syndrome
OP	=	Organ Phosphorus
PD	=	Parkinson Disease
PON1	=	Paraoxonase1
ProteaMAlg	=	Proteasome Modeling Algorithm
SIPS	=	Stress-Induced Premature Senescence
T2D	=	Type 2 Diabetes
VEGF	=	Vascular Remodelling Related Genes

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Received: October 1, 2009 **Accepted: October 21, 2009**