Systems Biology and Bioinformatics in Aging Research: A Workshop Report

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Abstract

In an “aging society”, healthspan extension is most important. As in 2010, talks in this series of meetings in Rostock-Warnemünde demonstrated that aging is an apparently very complex process, where computational work is most useful for gaining insights, and to find interventions that counter aging and prevent or counteract aging-related diseases. The specific topics of this year’s meeting were primarily related to “Cancer and Aging”, and also had a focus on work funded by the German Federal Ministry of Education and Research (BMBF). The next meeting in the series, scheduled for September/October 2013, shall focus on the use of *ontologies* for computational research into aging, stem cells and cancer. Promoting knowledge formalization is also at the core of the set of proposed action items concluding this report.

Introduction

On September 15-17, 2011, a workshop on computational aspects of aging research took place on Rostock/Warnemünde, jointly organized by Georg Fuellen and Julio Vera. It was a sequel to a similar workshop held in 2009 [1]. Here we summarize the main results presented at the workshop, including a list of recommendations to foster further progress (see conclusions, below). Figure 1 gives a graphical overview of the connections between the workshop topics, computational analysis methods, some of the molecular entities, and the speakers. The workshop website is http://www.ibima.med.uni-rostock.de/IBIMA/symposium/2011/ .

Aging research is important in our society, where health-care cost for the aged increase dramatically, at a rapid and highly detrimental pace. Apart from cancer, various other diseases such as dementia and diabetes are closely linked to old age. Just increasing lifespan by standard medical approaches (which focus on repair, not on slowing down aging by itself), does not necessarily increase healthspan, nor reduce morbidity. On the other hand, influencing the rate of aging just a bit and thereby prolonging average healthspan would have a huge positive impact, for individuals as well as the economy. As the aging process appears to be so complex, computational approaches are a prudent ingredient of our arsenal of methods towards realizing such an endeavor.

Accordingly, the use of systems biology and bioinformatics to deal with the complexity inherent to aging research is emerging in the last years. Even new research institutes have been created, devoted to the idea of using bioinformatics and systems biology to tackle aging. Moreover, in Germany, the ***federal funding program GerontoSys*** (systems biology for health in old age) currently supports interdisciplinary aging research with a focus on systems biology. *GerontoSys* started with the first call (GerontoSys1) at the end of 2009, and in 2011 a second call (GerontoSys2) followed. A research program was established that includes three research cores (Cologne, Jena and, Ulm), eight collaborative projects and three junior research groups. Several of the speakers participating in the workshop are funded by *GerontoSys;* only one collaborative project and one junior research group were not represented.

Aging follows once organismal growth ceases in the mature animal. The continuous action of the growth program appears to drive aging and with it malignancy (and indeed, cancer risk increases with increasing age). Here, the ***links between growth, cancer and aging*** (normal versus malignant growth / cancer) are apparent. This is also illustrated in the way diet influences organismal growth and aging: Under low nutrient state, growth is retarded and aging is slowed down. Under conditions of malignant growth, cancer-eliminating mechanisms are important. Fortunately, interventions that extend lifespan by delaying aging are also often effective against cancer, suggesting that aging itself is intrinsically connected to cancer formation.

By far the strongest prolongation of lifespan to date has been achieved by influencing the signaling cascades that link the control of growth with nutrient state. The strain-specificity of this effect indicates that aging is under strong genetic control. In mammals, the greatest lifespan extension so far was achieved by manipulation of a single gene: Mice with a deletion in the *Ghr* (growth hormone receptor) gene lived almost 5 years [2]. Interestingly, in C57BL/6J mice the same mutation was able to increase life expectancy by only 16 to 26% depending on gender [3], and in mice of a mixed genetic background the increases amounted to 36-55% [4]. Thus, it is clear that the genetic environment exerts a tremendous effect on the expression of genetic effects, which may be elucidated by data integration and modeling of genetic effects.

Another motivation for computational approaches is the evolutionary conservation of some of the pathways related to lifespan control, enabling comparative analyses. An

example is the insulin/insulin-like growth factor 1 (IGF-I) signaling (IIS) pathway [5]. A homozygous mutation in the phosphatidylinositol-3-kinase PI3K extends lifespan up to 10-fold [6] in *Caenorhabditis elegans.* In this model, specific roles for the control of lifespan have been attributed to DAF-2 (ortholog of the insulin/IGF-I receptors), AGE-1 (ortholog of PI3K) and DAF-16 (ortholog of forkhead transcription factors). In *Drosophila melanogaster* it was shown that INR (ortholog of IGF-I/insulin receptors) or CHICO (ortholog of insulin receptor substrate 1) affect lifespan as well. Orthologs of components from the Insulin/IGF-I signaling pathway have also been described in mammals and in yeast (*Saccharomyces cerevisiae),* where in the latter SCH9 has strong similarities to protein kinase B (AKT) as well as to S6K. The mammalian AKT mediates the effects of PI3K to the forkhead transcription factor family members (FKHR) via phosphorylation. With respect to the control of life expectancy, regulation of FKHR not only occurs by means of the IGF-PI3K-AKT signaling but also by signals from the mitochondrial or energy metabolic system [7]. Thus, metabolic pathways are critical for patterns of mortality [8], and caloric restriction is able to suppress activation of IGF-I-, insulin-, cytokine- and adiponectin-receptors, thus interfering with the onset of cytokine and growth factor mediated aging processes. Beyond pathways related to metabolism, other aging-associated (sub)networks (e.g., [9], [10], [11]) are related to telomere length, senescence, and DNA damage response.

Data Processing and Analysis in the Field of Aging

In the first set of talks, data related to aging were tackled from the viewpoint of bioinformatics and systems biology. Addressing biomedical questions by data

integration and subsequent iterative cycles of hypothesis generation and mathematical modeling is a useful approach to analyze high-throughput data, often using interconnected biological networks that are composed by dozens to hundreds of proteins, genes and miRNAs. Mathematical modeling, rather than being a goal in itself, is a tool to formulate hypotheses, to develop more directed/better designed experiments and to allow predictions. It is useful when dealing with networks enriched in non-linear motifs like feedback and feedforward loops, which induce non-intuitive regulatory patterns like ultrasensitivity, bistability, and oscillations. The biological networks involved and deregulated in aging are extremely complex and interconnected and display many instances of such non-linear motifs [12]. As suggested by [13], there are already a number of examples showing the success of systems biology to unravel the complexity of aging, in areas such as cell replicative senescence [10], mitochondrial dysfunction [14], telomere erosion and DNA damage [9], and caloric restriction [15].

Although both approaches overlap to a large degree, bioinformatics in our case is considered to include data processing on any level of granularity, based on data sets of any size, while systems biology is focused on generating overall (‘holistic’) insights from very large data sets on one hand, and specific insights by detailed quantitative analyses of selected phenomena on the other hand. Traditionally, bioinformatics finds its roots in computer science, while systems biology is closer to mathematical modeling and systems theory.

Large-scale analyses may start with the genome. The genomics of aging was thus reviewed by **Joao Pedro de Magalhaes** (University of Liverpool). Although the human genome was sequenced over 10 years ago, much work remains to decipher it. Unraveling the complexity of the machines of life encoded in the genome is not only essential to understand biological processes but is key to manipulate them. In particular in complex processes such as oncogenesis and aging, that involve multiple genes and their interactions with each other and with the environment, a comprehensive understanding is likely to be crucial to develop interventions. Genomic approaches are aimed at increasing our knowledge about how genes and pathways impact on aging and cancer. de Magalhaes presented three examples: 1) The transcriptional profiling of caloric restriction to gather insights into its regulation [16] (also see talk by Wuttke, below). 2) Genome-wide approaches using microarray data to identify new candidate genes associated with cancer and aging [17]. 3) Lastly, tracking genome evolution enables an understanding of how different genomes can give rise to species with marked differences in rate of aging [18].

Genome-wide views of aging gene networks were then given by **Stuart K. Kim** (Stanford University, Stanford). To study aging in mice, he examined expression changes for 8,932 genes in 16 tissues as a function of age. Some tissues displayed large transcriptional differences in old mice, suggesting that these tissues may contribute strongly to organismal decline. Other tissues showed little or no changes in expression with age, indicating strong levels of homeostasis throughout life. He presented a method for the differential analysis of gene co-expression networks and

applied this method to look for large-scale transcriptional changes in aging. Comparing the transcriptional profiles for aging in mice to those from humans, flies and worms, it was found that genes involved in the electron transport chain show common age-regulation in all four species, indicating that these genes may be exceptional good markers of age. However, there is no overall correlation between age-regulation in mice and in humans, indicating that the aging process in mice and humans may be fundamentally different. Kim is also studying aging in human kidneys. Kidneys age at different rates, such that some people show little or no effects of aging whereas others show rapid functional decline. Whole-genome transcriptional profiling identified 630 genes that change expression with age in the kidney. eQTL analysis followed by a gene association experiment then identified a SNP associated with a hallmark of kidney aging (glomerular filtration rate). The results of this sequential analysis may provide the first evidence for a gene association with kidney aging in humans.

Genome-wide transcriptome analysis with age and the effect of natural genetic variation was the topic of **Ana Viñuela** (Department of Twin Research, King's College London). To study how genome-wide gene expression regulatory mechanisms progress with age, she first explored genome-wide gene expression variation and regulatory loci (eQTL) in a population of developing and aging *C.elegans* recombinant inbred lines [19]. Throughout the worm’s life, the total number of eQTL decreased with age whereas the variation in expression increased. Those results suggest that, while for the majority of genes, expression becomes more variable during aging, genes that become less variable are more regulated. Moreover, the number of *cis*-acting eQTL in juveniles decreased by almost 50% in old worms whereas the number of trans-acting loci

decreased by ~27%, suggesting that aging influences the efficiency of *cis*-regulatory sites for controlling local gene expression. Comparison of a single marker to a multiple marker eQTL-model indicated that heritable regulation of gene expression patterns became relatively more complex in old worms due to involvement of multiple loci of small effect [20]. Viñuela is currently studying patterns of gene expression changes and regulation with age in multiple human tissues. Tissue samples were gathered from 855 female Caucasian twins (aged 39 - 85) recruited from the TwinsUK cohort. Combination of expression profiles with multiple longitudinal age-related phenotypes aim to identify reliable biomarkers for aging and improve the understanding of gene expression regulation changes with age in humans.

An RNA-Sequencing (RNA-Seq) based analysis of aging within a multi-species approach was presented by **Steffen Priebe** (Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Jena). This work is part of the JenAge project (see below). Illumina sequencing technology was applied for the measurement of the transcriptome. The RNA-Seq data of *D. rerio* (skin), *C. elegans* and mouse (brain, liver, and skin) for several time points was mapped versus the reference genomes and an exon-junction database using Bowtie [21]. Differentially expressed genes (DEG) were identified between each age group and compared across the species. For this reason, orthologous genes were extracted from Ensembl Compara to facilitate the cross-species comparison. Since the individual species exhibit different lifespan, the gene expression time courses were rescaled, followed by a combined fuzzy c-means clustering of the gene expression profiles, in order to identify common

time courses. The optimal number of clusters was estimated by the vote of several cluster validity indices, which capture different aspects of a clustering structure [22]. In this way, gene sets were identified, the expression of which constantly increases or decreases with age in all the three species. Some of them are already known to be associated with aging processes. Functional annotation and pathway analysis were performed to discover functionally related genes. Perturbed gene expression data (rotenone, deoxyglucose) were applied to investigate the influence of mild stress for these genes.

Data and information processing are important aspects of systems biology of aging**.** **Jürgen Sühnel** from the Jena Centre for Systems biology of Aging – JenAge (www.jenage.de) reported on further JenAge projects in this area. In a first step the so-called JenAge Information Centre (http://info-centre.jenage.de) was established. It collects and provides general information on aging and age-related diseases as well as on systems biology and is intended to assist researchers in these fields. Although closely linked to the Jena Centre for Systems Biology of Aging - JenAge is a self-contained information resource. It offers a comprehensive compilation of all databases specifically related to aging research, but also collections of books, journals and papers, a meetings calendar, science news, and a list of aging research centres and institutes. In addition, ongoing work includes the setup of a new database, automatic text mining and data management.

Computational support for systems biology of aging is also provided by **Pat Langley** (Arizona State University). Senescence (see also below) arises from complex interactions among diverse sets of biological processes, and Langley described efforts

to encode knowledge about aging in formal computer-based models that allow visualizing causal influences, revise and expand on these hypotheses, generate explanations for phenomena, and compare predictions to observations. Users can access, examine, and utilize this content over the internet, as a repository for the community's expanding knowledge about aging [23].

Axel Kowald (Humboldt University Berlin) explained that the BMBF-funded GerontoMitoSys project studies age-related changes in the mitochondria of various model organisms (*S. cerevisiae, P. anserina,* and *M. musculus*) using a combination of experiments and mathematical modeling. One line of research concentrates on the surprising effects seen in *P. anserina* when overexpressing the antioxidant enzyme superoxide dismutase (SOD). Instead of conferring resistance against oxidative stress, the mutants show increased sensitivity and an altered level of antioxidant enzymes (peroxiredoxin) and proteases (CLPP). Incorporating these components into a kinetic model, a positive feedback loop could be identified, demonstrating that the role of superoxide as the primary ROS responsible for age-related molecular damage is more complicated than originally stated by the free radical theory of aging. This study is a first step towards the integration of the various pathways known to be involved in the control of biological aging.

Modeling the aging epigenome in mathematical terms is the topic of **Jörg Galle** (Interdisciplinary Centre for Bioinformatics, University Leipzig). He presented a computational approach to transcriptional regulation in cells by chromatin-related mechanisms including histone modification and DNA methylation. In this approach,

short-term transcriptional regulation is governed by binding of protein complexes to chromatin, which are capable of reading and writing histone marks. Molecular interactions between these complexes, the DNA and the histones create a regulatory switch of the transcriptional activity of associated genes that possesses a regulatory memory. Cell proliferation can destabilize this memory and can lead to spontaneous de-modification. Long-term transcriptional regulation is governed by DNA-methylation. Considering that methylation of CpGs depends on the modification state of the associated histones, an aging mechanism of the stem cell epigenome is proposed that: i) is driven by cell proliferation, ii) induces local hyper-methylation of DNA, and iii) affects global transcriptional activity. The proposed model was applied to transcriptional regulation by trithorax-complex binding, the recruitment of which has been shown to be CpG-density and -methylation state dependent. This application enables new insights into epigenetic modes of transcriptional regulation. Moreover, it implicates well-founded hypotheses on cooperative histone modifications, proliferation induced epigenetic changes and aging of chromatin, which all await experimental validation.

Boolean networks constitute a qualitative dynamic approach to modeling. **Hans A. Kestler** (Ulm University) spoke about Boolean networks and their evolution, for modeling gene regulation. Generating scenarios that model the change of regulatory networks over time can aid in the understanding of the aging process itself. In a typical scenario, an expert models knowledge on genetic interactions as Boolean logic and verifies the resulting hypothetical network by comparing its dynamic behavior to experimental measurements. This is often an iterative process with repeated evaluation and refinement of the network model. Even though the Boolean model is simple, there

are still many elements of uncertainty in the modeling process, such as the type of a genetic interaction, or the role of interactions that have not yet been investigated or are hypothesized. Such uncertainties can result in a high number of possible candidate models that have to be investigated by comparing their dynamic behavior with expectations from literature or wet-lab experiments. Supported by the BMBF-funded SyStaR consortium in Ulm, we developed an approach that supports the generation of aging trajectories by evolving models of gene regulatory networks over time. A preliminary hypothetical network model is specified and the network evolves according to specified dynamical constraints (e.g. steady states, genes expressed before or after other genes). This is achieved by applying small evolutionary changes to the networks on a symbolic level. Networks are evolved that resemble the input network closely and have desired dynamic properties. The feasibility of our approach was tested on artificially perturbed networks of the mammalian cell cycle and the fission cell cycle. By evolving established network models for early developmental stages, the method can generate models that mimic their age-related evolution.

Metabolic pathway analysis may be considered another approach towards improving our understanding by modeling biological processes. It is applied to NAD^+ metabolism with special reference to aging research by **Stefan Schuster** (Department of Bioinformatics, University Jena). Nicotinamide adenine dinucleotide (NAD^+) is well known as a central cofactor in the redox balance of metabolism. Moreover, NAD^+ is degraded in ADP-ribosyl transfer reactions, which are important components in a plethora of signaling reactions. These include reactions linked to DNA repair and aging.

Proteins are modified by mono- and poly-(ADP-ribosyl)ation and histones are subject to NAD^+-dependent deacetylation catalyzed by sirtuins [24]. Poly-(ADP-ribosyl)ation is involved in a variety of processes involving DNA transcription, rearrangements, and repair. Several hypotheses on the role of the positive effect of calorie restriction on longevity and its relation to NAD^+ metabolism have been put forward [25]. Here, using the concept of elementary flux modes (EFMs, [26]; for a recent review, see [27]), Schuster established all potential routes in a network describing NAD^+ biosynthesis and degradation. All known biosynthetic pathways, which include the classical Preiss-Handler pathway and the kynurenine pathway starting from tryptophan, are detected as EFMs. Moreover, several elementary modes are found that degrade NAD^+, represent futile cycles or have other functionalities. The systemic and systematic analysis and comparison of the networks specific for yeast and humans documents significant differences between species with regard to both the use of precursors, biosynthetic routes and NAD^+-dependent signaling [28]. This enables to critically examine the hypothesis that calorie restriction increases NAD^+ turnover without altering steady-state NAD^+ levels.

Niels Grabe (University of Heidelberg) described a method for the automatic inference of causal networks describing response to growth factors. After automatically integrating the comprehensive set of all known cellular networks from the PID Pathway Interaction Database, off-switched genes are identified and removed, and a subnetwork with the given set of growth factors as the input layer and the desired set of functional cell states as the output layer is extracted. Highly specific networks for relevant signaling cascades result from further restricting the network by differentially expressed genes obtained

from stimulation experiments with the growth factors of the input layer. A reverse version of this algorithm is designed to identify those receptors and cell-cell communication pathways altered by aged skin fibroblasts.

Finding cross-links and mechanistic links in biological networks is the bioinformatics task handled by **Bianca Habermann** (MPI-AGE Cologne), to aid biologists in analyzing and interpreting their data. With the advancement of genome- and proteome-scale techniques, this task becomes more and more challenging. Eventually, a genome-wide screen should not exist by itself, but be consolidated with the vast amount of genome-scale data that is freely available. As member of the BMBF-funded SyBACol project, Habermann is involved in the integration of expression studies in different model systems. One of her aims is to identify cross-talk between molecules and pathways in biological interaction networks. Finding the most prevalent path between two proteins that are mechanistically linked and the most likely cross-linkers between two functionally active pathway modules is one aim. Another aim is to consolidate data from different species and invest in the functional annotation of hits from expression screens. Though many genetic and high-throughput functional data are only available for model organisms like worm or fly, some players will be equally important in vertebrates or mammals, including humans.

Jörn Dengjel (University of Freiburg) outlined that human aging may be studied by specifically investigating organ aging, the kidney being a prime target of age-associated organ damage as reflected by the clear association of renal function decline with age. The BMBF-funded NephAge consortium in Freiburg hypothesizes that the aging

process of the kidney is determined by the lifespan of podocytes, terminally differentiated, highly specialized kidney cells. To unravel the molecular identity of kidney aging and responsible signaling networks, NephAge performs a systems biology approach using complementary mouse models, among them the first available model of kidney aging, to isolate primary podocytes for an in-depth proteomic, genomic, and metabolomic characterization. In a first bioinformatics step, the data sets are compared to recognize parallel regulations and patterns, and to rapidly deduce first connections between different aging states. Next to the unbiased approaches using mouse models, genes associated with the aging kidney, identified from genome-wide association studies in a large number of middle-aged and elderly individuals, will be studied in genetically tractable and screenable fly and worm models for their role with respect to renal function, autophagy, and longevity. To translate all generated data into predictive dynamic models, NephAge employs complexity reduction strategies to identify and model key signaling pathways crucial for kidney aging. The goal is to identify signaling subsystems and establish a hierarchy of time-graded responses that determine short-term age-related function and long-term age progression.

An emerging topic in aging research are microRNAs (miRNAs), and integrating bioinformatics and systems biology to elucidate the role of miRNAs in cancer and aging was tackled by **Ulf Schmitz** (Department of Systems Biology and Bioinformatics, University of Rostock). miRNAs are a class of small non-coding RNAs that can post-transcriptionally regulate the expression of many protein coding genes. Once processed, the maturated ~22 nt long molecule represses its mRNA target in conjunction with the RNA induced silencing complex (RISC) by either inhibiting translation or by mediating

mRNA decay. MiRNA and target deregulation has been shown to play a causal role in many aging-related diseases, the one most extensively investigated being cancer [29]. In [30], the authors suggest that miRNAs operate at different levels of aging. They claim that miRNAs are regulated during cellular senescence *in vitro*, contribute to tissue regeneration by regulation of stem cell function, and that several miRNAs modulate lifespan in worm. In his talk, Schmitz discussed how the integration of bioinformatics and systems biology tools is required to tackle complex biochemical networks involving the regulation by multiple miRNAs. He used as case study the miRNA repression of the cell cycle regulator p21, which is an important player in senescence [31].

Growth, Cancer, Inflammation and Aging

The second set of talks demonstrated how aging is connected to growth, in itself a fundamental process of life, and to its abnormal variant, that is, cancer (see also the introduction). Furthermore, all three processes are modulated by inflammation. Comparing these processes on the molecular level by computational means may reveal a set of molecular species (such as proteins) that are jointly involved (see also Fig. 1). Such ‘anchoring points’ may include the somatotrophic axis (IGF-1, PTEN [32], and mTOR [33], [34]).

Genome maintenance and its impact on aging and disease were highlighted by **Jan Hoeijmakers** (Erasmus MC, Rotterdam). An intricate genome maintenance machinery has evolved to counteract the consequences of DNA damage, affecting the function of our genes. Nucleotide excision repair (NER) is one of the most versatile repair systems

removing a wide range of helix-distorting lesions, mostly from exogenous sources (UV, bulky adducts), but also of endogenous origin (oxidative damage e.g. cyclopurines). It does so in a multi-step “cut-and-patch”-type of reaction involving more than 30 proteins. Two NER sub-pathways exist. Global genome NER operates genome-wide removing lesions, which otherwise would give rise to mutations during replication and hence is critical for preventing mutations. Transcription-coupled repair removes damage that obstructs transcription, primarily counteracting cytotoxic effects of DNA injury. UV-sensitive inherited NER syndromes display a striking clinical heterogeneity: very strong (skin)cancer predisposition in xeroderma pigmentosum (XP) and dramatic neuro-developmental deficits such as in Cockayne syndrome (CS) and trichothiodystrophy (TTD). Different mutations in NER helicases XPB and XPD, subunits of repair/transcription factor TFIIH, give rise to all three disorders or combinations. Detailed analysis of XPD^{TTD} mice, carrying a point mutation of a TTD patient, revealed that TTD (and CS) are segmental premature aging syndromes, with reduced cancer susceptibility. $XPD^{XP/CS}$ mutant mice on the other hand are highly predisposed to cancer and also display premature aging, demonstrating that both phenotypes can co-exist. Other single and double NER mutants exhibit multiple premature aging features, including *bona fide* osteoporosis, dramatic neurodegeneration, early infertility and cessation of growth, liver and kidney aging, deafness, retinal photoreceptor loss, exhaustion of hematopoietic stem cells, etc. Lifespan is limited to 1.5 years for milder mutants and to 3-5 weeks for dramatic (double) mutants. A striking correlation is found between severity and type of defective repair and rate of onset, severity and type of aging symptoms providing strong experimental support for the DNA damage theory of

aging. Conditional tissue-specific repair mutants target accelerated aging to specific organs, e.g. dramatic neuro-degeneration occurring only in the cerebellum, revealing organ-specific accelerated aging. Hoeijmakers proposed that endogenous DNA injury hampers transcription/replication and triggers cellular apoptosis-senescence causing aging. Microarray, functional and physiological studies have revealed that persisting DNA damage elicits a systemic *downregulation* of the IGF-I somatotrophic axis and upregulation of antioxidant defenses, favoring maintenance and defenses at the expense of growth and development, explaining the severe growth defect of repair mutants. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and implicates regulation by microRNAs. Caloric restriction and fasting trigger a similar 'survival' response, which maximizes anti-oxidant defense and - when constitutive - promotes longevity at least under laboratory conditions. These data link accumulation of DNA damage and the IGF-I control of lifespan and open perspectives for the promotion of healthy aging.

Interactions between growth, metabolism and aging are also investigated by **Andreas Hoeflich** (Laboratory for Mouse Genetics, Leibniz-Institute for Farm Animal Biology Dummerstorf, Germany). In mouse knockout models lacking Ghr or insulin-like growth factor-I receptor (Igf-Ir), prominent increases of lifespan have been described and thereby, a negative correlation of growth and aging was established [2], [35]. However, the lack of one or both receptors is an extremely rare condition in mammals and is no candidate for acute or adaptive lifespan elongation *in vivo*. Hoeflich et al. have thus started to characterize interaction of growth and lifespan in phenotype-selected mouse

lines characterized by extreme growth, confirming the intricate negative relationship between growth and lifespan in non-inbred strains (Dummerstorf phenotype selected mouse strains), a highly interesting set of mouse models useful for the analysis of growth and aging. While both growth and aging represent central physiological features, the exact mechanisms linking both phenomena are still not well understood. As discussed in the introduction, a central age-related pathway evolutionary conserved from worms to mammals is IIS. Since IIS includes a series of receptor tyrosine kinases, including the IGF-IR but also the insulin receptor, focus is on the activation of this central signaling pathway. A longitudinal study was performed in Dummerstorf DU6P mice characterized by extreme muscle mass, selected over 138 generations for high carcass protein amount. In muscle from 29-week female DU6P mice a massive activation of AKT compared to controls was found. Potential mechanisms of AKT activation include IGF-I, IGF-II, insulin/IGF-receptor, myostatin or integrin-linked kinase. However, exclusively PTEN, which was severely down-regulated in 29-week female DU6P mice, could be identified as a potential trigger, consistent with higher levels of AKT-Ser473 phosphorylation [36]. AKT activation also translated into specific inactivation of glycogen synthase kinase 3ß and a 7-fold increase of muscular glycogen (5% of total muscle weight). The mouse model described thus comprises phenotypes of massively elevated growth, severely reduced lifespan (less than 78% versus controls) and last but not least significant metabolic abnormalities (higher protein and glycogen content in muscles), which in total may be related to the altered muscle AKT activity. In 29-week female DU6P mice, PTEN might serve as a superordinated pacemaker of growth, aging and metabolism.

Chronic inflammation has been linked to cancer as well as aging. ***Bernd Schmeck*** (University of Marburg) demonstrated its relevance in diseases such as diabetes, atherosclerosis, inflammatory bowel diseases and lung diseases. Among the 10 most important causes of death worldwide are four lung diseases with inflammatory aspects: pneumonia, obstructive lung diseases, tuberculosis, and lung cancer. Obstructive lung diseases like chronic bronchitis and bronchial asthma can be caused exogenously by cigarette smoke, air pollution, allergic agents as well as bacteria and viruses. Most importantly, this inflammation is not limited to the lung, but a “systemic spillover” seems to affect many organ systems causing “aging-related” phenotypes like dementia, chronic heart diseases, muscle weakness, osteoporosis etc. The aim of the Marburg group is to understand physiological and pathological regulatory circuits of lung inflammation at the crossroads of host defense and tissue destruction, as well as proliferation and aging. Therefore, the molecular machineries of chromatin modifications and small non-protein coding RNA/microRNA are studied, combining wet lab experiments, high throughput facilities, bioinformatics as well mathematical modeling - to finally construct a predictive model, which may help to identify crucial signaling hubs as possible therapeutic targets for these widespread pulmonary diseases causing preterm aging.

Influence of Diet on Aging

Manipulating aging and, at the same time, preventing cancer growth and inflammation may best be done by nutritional interventions. Many of the genes discussed above are in fact part of the signaling cascade of nutrient-sensing systems such as growth

hormone (GHR) and Insulin-like signaling (IGF1, PI3K, PTEN, FoxO) as well as mTOR. The computational study of such interventions can pave the way to understand the influence of diet on the aging process and enable the development of interventions tweaking this system, to interfere with lifespan via conserved mechanisms active in organisms from yeast to mammals.

Comparative interactomics of DR-essential genes is done by **Daniel A. Wuttke** (Integrative Genomics of Ageing Group, University of Liverpool). Dietary restriction (DR), limiting certain factors in diet, without causing malnutrition (also often called caloric restriction, CR), is the most powerful non-genetic intervention that delays the aging process and extends healthy lifespan in multiple organisms from yeast to rodents. To decipher the mechanisms of DR, Wuttke established a web-accessible database (GenDR) of DR-essential genes, which if genetically altered interfere with the effect of DR to extend lifespan in model organisms (yeast, worm, fly, and mice). Orthologs in the other organisms were identified and their molecular evolution investigated, showing that DR-essential genes have more orthologs and in mammals were subject to stronger purifying selection than expected by chance. Molecular interactions with DR-essential genes/proteins and their orthologs were retrieved from available databases and networks constructed. Basic topology analysis revealed that DR-essential genes tend to be located in the center of the interactome and have a higher interconnectivity to each other than expected by chance. Genes interacting significantly with DR-essential orthologous groups between multiple species, and novel DR-essential genes, which were not previously implicated in the mechanisms of DR-mediated lifespan extension,

were identified via guilt-by-association and experimentally validated. This revealed new insights into the fundamental mechanisms in which the nutritional state impacts on lifespan determination [37]. GenDR can be found at: genomics.senescence.info/diet/.

Moving from genes to interventions, mining genome-wide drug-response compendia to discover novel calorie restriction (CR) mimetics is done by **Kristen Fortney** (Department of Medical Biophysics, University of Toronto, Toronto, Canada) [38]. Genome-scale data on the responses of human cell lines to over 1000 drug treatments have become available, and their integration with gene expression signatures of CR, pathway information, and protein-protein and drug-target interaction networks yields a prioritized list of candidate CR mimetics. These candidates are annotated using drug-target, pathway, and protein interaction databases, and clustering characterizes them in terms of common chemical properties and of common target proteins, pathways, and diseases. Modes of action can then be compared with those of known lifespan-affecting drugs, to rank the candidates in terms of their therapeutic promise.

Stress Response, Senescence, ROS, and Mitochondria

Up to now, growth, cancer and inflammation were discussed as the fundamental processes related to aging, but stress response should not be forgotten, as it can lead to both lifespan extension or senescence and subsequent aging. Cellular stress and in particular the ‘oxidative stress’, caused by reactive oxygen species (ROS), is maybe of highest relevance. ROS and the damage it induces may cause stress responses. While the damage may be detrimental, ROS ‘signaling’ and stress response may also include

healthy and longevity-promoting aspects. Senescence is an important aging-related cell phenotype that might result from (oxidative) stress, and mitochondria play an important role here. Senescence may be considered the counterpart of growth, and both may be equally detrimental to health and longevity.

In particular, the connections between mitochondrial dysfunction and cell senescence were highlighted by **Thomas von Zglinicki** (Centre for Integrated Systems Biology of Aging and Nutrition, Newcastle University). Cell senescence is a permanent, irreversible proliferation arrest resulting e.g. from telomere shortening and persistent DNA damage signaling. In addition to growth arrest, senescent cells display major changes in multiple phenotypes, such as gene expression patterns, metabolism, morphology, and function, which can significantly spread their impact in tissues. Mitochondrial dysfunction (increased ROS production despite decreased mitochondrial membrane potential) is an early hallmark of senescence. Combining probabilistic functional interactome network analyses and serial knockdowns of candidate genes, a signaling pathway was identified, linking DNA damage response to mitochondrial ROS production. Stochastic modeling suggested that this pathway would be necessary and sufficient for maintenance of proliferation arrest; this was confirmed experimentally for the first developmental phase of senescence. Studies in mice showed increasing fractions of cells bearing senescence markers in various tissues during aging. Dietary restriction reduced frequencies of senescent cells in the same tissues [39].

The mitochondrial genome and aging are investigated by a systems biology approach in the BMBF ROSage project presented by **Saleh Ibrahim** and **Georg Fuellen** (University of Lübeck Medical School / University of Rostock Medical School), postulating that (1) aging of an organism depends on specific aging-related changes in specific organs, which in turn depend, in part, on specifics of the electron transport chain (ETC) and (2) ROS mediate some aspects of this dependence. To study these hypotheses, mouse strains with well-defined and stable mutations in the ETC yield standardized readouts for several organs, following the mouse strains over time. In parallel, multiple approaches are pursued to model the underlying processes mathematically on the microscale. Bioinformatics data integration is designed to provide connections between the underlying processes and to some of the readouts, and to perform overall machine learning on the whole dataset. The latter will be based on readouts as well as modeling results, using ontologies, towards a better understanding of aging on the macroscale.

A complementary approach is the BMBF-funded project OXISYS, presented by **Katrin Zeilinger**, (Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin), investigating the role of oxidative injury in aging and therapeutic implications. There is increasing evidence that oxidative stress plays a major role in aging-related processes in the human organism. Within OXISYS, the effect of aging and the role of reactive oxygen species exposition in aging processes using the liver as a model organ is investigated. The project is conducted in a consortium of four academic partners (Charité Universitätsmedizin Berlin, Eberhard-Karls Universität Tübingen, Universität des Saarlandes, Hans-Knöll-Institut Jena) and two industrial

partners (Pharmacelsus GmbH, Insilico Biotechnology AG). The *in vitro* approach is based on 3D perfusion culture of liver cells in a hollow fiber capillary membrane bioreactor providing decentralized mass exchange and integral oxygenation. The system serves as a physiological model for human-predictive studies on the effect of hepatic aging and oxidative stress effects on hepatic metabolism and the contribution of different cell populations to these processes. Rats of different age classes are used to analyze the *in vivo* response to oxidative stress. Biochemical, metabolic and gene expression data generated in the experimental models will serve for developing *in silico* models to describe, to simulate and to predict aging processes in the liver. Models generated from rat *in vivo* and *in vitro* models will be translated to the human liver and verified for their systemic relevance. The models established will be used to identify potential biomarkers and targets for diagnosis and therapeutic regulation of ROS induced tissue aging and degeneration.

Computational advances in the study of senescence, in particular on the role of lipid rafts in aging and cellular senescence, were reported by **Fiete Haack**, (Faculty of Computer Science, University of Rostock) [40]. The plasma membrane of eukaryotic cells is a highly compartmentalized structure, characterized by large stable multi-receptor-complexes, actin-mediated cytoskeleton fences, and mobile lipid rafts. In recent years lipid rafts in particular have drawn much attention due to their ability of selectively concentrating proteins and thereby facilitating the assembly of signaling complexes. Recent studies indicate an involvement of lipid rafts in cellular senescence and age-associated dysfunction of e.g. T-cell receptor signaling. Unfortunately,

biochemical approaches to study lipid rafts in biological membranes are highly limited. Standard techniques, like detergent-resistant membrane or cholesterol-depletion experiments have too complex side-effects to draw substantive conclusions. Therefore computational modeling is an attractive alternative. However, the ascribed static and dynamic organization of plasma membranes leads to highly inhomogeneous spatial distribution and demands a thorough consideration of space when modeling and simulating such systems. Haack and colleagues propose a new modeling and simulation approach that allows integration of different types of spatial dynamics, e.g. compartmental dynamics, mesh-based approaches or moving individuals, within one model. The associated simulator combines Gillespie, the Next Subvolume method, and Brownian dynamics. This allows the simulation of complex spatial dynamics like those involved in studying the dynamics of lipid rafts and their role in receptor co-localization.

Stem Cell Aging

With respect to cancer, growth, inflammation, stress response and aging, the cells with the most prominent role may be various types of stem cells. Stem cell differentiation is tightly controlled by ROS signaling and stem cells have been hypothesized to be crucial in both maintaining youthful proliferation and regenerative capacity as well as (when out-of-control) to be a driving force of malignancy because of their undifferentiated state.

For example, aging processes in neural stem cells that are affected by the pool of adult stem cells were discussed by **Anne Brunet** (Department of Genetics, Stanford University). In the nervous system, neural stem cells are thought to be critical for

learning and memory. During aging, both the pool of neural stem cells and their ability to give rise to new neurons decline, raising the possibility that neural stem cell depletion may underlie part of the cognitive dysfunctions during aging. However, the mechanisms that regulate the neural stem cell pool during aging are largely unknown. Brunet's hypothesis is that genes that regulate lifespan maintain the homeostasis of adult stem cell pools in long-lived species. Transcription factors of the FoxO family play a conserved role in controlling longevity downstream of the insulin pathway; FoxO orthologs are already known to extend lifespan in invertebrates and single nucleotide polymorphisms in the FoxO3 gene are associated with extreme longevity in humans. Brunet's group has recently found that the transcription factor FoxO3 maintains the neural stem cell pool in adult mice. Specifically, genome-wide analysis of FoxO3 binding sites in neural stem cells using next-generation sequencing technologies identified a network of FoxO3 targets involved in 'stemness' and aging. This global analysis has also provided key insight into novel co-factors and chromatin marks that interact with FoxO3 to regulate adult neural stem cell function. FoxO3's ability to control an epigenetic network that is pivotal for adult neural stem cell homeostasis might help counter brain aging in long-lived species, including humans.

Homeodynamic fitness and the age-related disbalance in mesenchymal stem cell renewal and progenitor molding was the topic of **Günther Lepperdinger** (Institute for Biomedical Aging Research, Innsbruck). Aging of somatic tissues comes along with a decline of regenerative capacity. Tissue regeneration and repair involve the consecutive emergence and parallel integration of new parenchymal cells, which descend from

undifferentiated precursors. Multipotent stromal progenitor cells, also known as mesenchymal stem cells (MSC), are pertinent tissue-specific stem cells in adults. The concept of MSC is interesting since this particular type of precursor can bring forth a large spectrum of cell types as diverse as bone, cartilage, tendon, or fat precursor cells. Besides these descendants, also terminally differentiated cells common and widespread throughout the body, namely interstitial stromal cells and fibroblasts, are reckoned direct offspring of MSC. Also, these particular stem cells are considered highly effective therapeutic assets to combat a wide spectrum of diseases, and thus approaches with the aim of rebuilding damaged or diseased organs and body parts has yielded a virtually endless list of only recently initiated clinical trials. Assuming a fit cell population can withstand accrued damage and compounding of molecular changes longer than irreversibly aged cells, the interest is to determine means and measures for investigating molecular mechanisms underlying this concept, and in particular to elucidate endogenously expressed bioactive factors and their mode of actions which enhance the fitness of tissue-borne MSC.

In the discussion of MSC aging, we are faced with two interrelated aspects, the effect aging has on MSC themselves and the contribution MSC have to the aging of the organisms. To complicate the matter, to the first aspect we also have to add the role of the niche in MSC aging. ***Alexandra Stolzing*** (Fraunhofer Institute for Cell Therapy and Immunology, Leipzig) spoke about the changes in the signal pathways in MSC isolated from adipose tissue of donors ranging from 20-80 years of age. Adipose tissue has been shown to contain high numbers of MSC (adipose-derived stem cells; ADSC),

which is an alternative source of MSC for therapeutic applications. ADSC have similar characteristic compared to bone marrow-derived MSC (BM-MSC) and might age in a similar fashion. One of the signal pathways regulating MSC differentiation, pluripotency and migration is the WNT signal pathway. AMSC were isolated from liposuction and subcutaneous tissue from donors of different age and gender. A dramatic decrease in WNT gene expression has been detected for ADSC-derived from females at the age of 40-60 years. Gene expression analysis from ADSC derived from liposuction resulted in absence of nearly all WNT genes analyzed and different pattern of cell cycle genes and differentiation markers compared to subcutaneous ADSC. Thus, the source of the ADSC has a great impact on the cell behavior and therefore for therapeutic applications. Further study of these differences may lead to important discoveries regarding the mechanisms of WNT signaling in cells from different niches and the regeneration capacity of MSC.

Conclusions

In the future, we believe that aging research will benefit from ever more synergies between experimental work, bioinformatics, and systems biology approaches. Bioinformatics provides a selection of tools, databases, and approaches ideal to compile existing information about the molecular components of aging-related biological networks and their association to pathological conditions (e.g. putative and validated protein-protein and miRNA-mRNA interactions, transcriptional regulation, genetic interactions, etc.). Bioinformatics and genomics also offer the tools to obtain, via high throughput data analysis, empirical evidence suggesting connections between the

components of these aging-related networks. The systems biology view complements this approach by making use of this information and organizing it into detailed maps of the regulatory networks investigated. Those maps, when translated into mathematical models, become excellent tools to integrate high-throughput data of highly interconnected biological networks and test the consistency of new data against the state-of-the-art. But these models are also useful to formulate mechanistic hypotheses on putative interactions explaining inferred links between genes/proteins, and thereby to develop more directed/better designed experiments testing them. Furthermore, mathematical modeling is actually a necessary tool when dealing with networks enriched in non-linear motifs like feedback and feedforward loops. These regulatory motifs, which are commonly found in the regulatory networks involved in aging and aging-associated diseases, induce non-intuitive regulatory patterns like ultrasensitivity or bistability, impossible to detect and understand without modeling. Their deregulation threatens or alters the natural homeostasis in tissue regeneration, which is necessary to keep functionality and viability and avoid emergence of aging-related phenotypes.

We therefore predict a future of aging research in which bioinformatics is used to inform and to construct better, more precise networks that explain in detail the most relevant processes underlying aging conditions. Both, bioinformatics and mathematical modeling are then jointly used to determine the regulatory chain of causation underlying the phenotypic and genotypic patterns obtained from the high-throughput data. A faster and more reliable interpretation of biological data and evidence will be a desired outcome of this synergy. Thus, we are convinced that the two communities will highly benefit from

sequels to meetings such as RoSyBA, in which the most challenging bioinformatics and systems biology tasks are tackled through fruitful and productive alliances between both.

Furthermore, we perceive that both communities would highly profit from synergies induced by integrative efforts that result from formalizing the biomedical domain knowledge: biomedical ontological resources are an essential contribution towards this aim [41]. Core ontologies include the phenotypic representation and the annotation of biological processes (such as diseases) with phenotypic features, supported by automated literature analysis. Machine-based reasoning can then guide data integration, consistency analysis and inference of novel findings, such as relevant phenotypes as well as diagnostic and interventional suggestions. The cross-species ontological integration of phenotypes is the next key step to achieve data interoperability across resources, inference of core findings and translational medicine. Such result could be exploited for intervention (drug) development across model species, and for the reduction of animal experiments. The application of findings from model organisms to humans requires relevance testing and also risk assessment, which could be done with the same framework. Furthermore, it is of high importance to use the phenotypic descriptions to formalize the evidence of the underlying mechanisms for models describing the development of the biological (age/disease) status. Again, this can be based on the conceptual framework for phenotypes. The causal link between the phenotype at different conceptual levels and its genetic predisposition will then be the result of “causal chains” where one feature such as the molecular function of a gene is transposed to a tissue and the organ leading into the anatomical function for the

macroscopic phenotype. Considering the importance of ontologies for progress in computational aging research, the organizers of the 2011 workshop are planning a focus on ontologies for the next workshop in 2013.

Finally, we recommend the following action items:

1. Since aging processes affect all aspects of life, computational analyses require integration of a maximum amount and variety of reliable and accurate biological data and knowledge, and of the integration. It is thus proposed to build up a (wiki-based) ***collaborative resource*** *that provides such data integration on all levels; preparation for this resource must start today, striving for interoperability / open standards. Data deposit shall be encouraged by making it mandatory for publication (following an open data paradigm). Validity of the integration shall be enhanced by using detailed ontologies. Aging-related computational research must then point out those processes of life that are most fundamental for aging, and create computational models for them.*

2. Funding must be made possible at sustained levels, beyond Germany and the EU. Aging research towards healthspan extension shall be designated as an area of research that has high returns on investment, and computational support is an essential part of it. Molecular aging research shall also be advertised as one "prime application area" for bioinformatics and systems biology data integration efforts in general, to the EBI, the NCBI, etc.

3. Funding for aging research in general should focus more on the impact of early life interventions on lifespan, and on aged animal cohorts covering an array of species,

including non-inbred models. Following these two recommendations would increase the practical relevance of both experimental and computational aging research.

Author contributions.

AS, AK, BS, AH, JH, HAK, JD, JS contributed on those sections that include the topic of their talk. **JV** and **US** contributed to systems biology of aging and conclusions. **SP** also contributed to introduction and abstract. **DRS** contributed to the conclusions. **DW** worked on abstract, introduction and section introductions. **GF** assembled the report, and contributed to abstract, introduction, section introductions and conclusions.

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Figure legends

Figure 1:

A graphical overview of the RoSyBA 2011 workshop. GWAS: Genome-wide Association Study. NGS: Next-Generation Sequencing.