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Meeting Report

In Silico Approaches and the Role of Ontologies in Aging Research

Georg Fuellen,[1] Melanie Boerries,[2] Hauke Busch,[2] Aubrey de Grey,[3] Udo Hahn,[4] Thomas Hiller,[5] Andreas Hoeflich,[6] Ludger Jansen,[7] Georges E. Janssens,[8] Christoph Kaleta,[9] Anne C. Meinema,[10] Sascha Schäuble,[9] Andreas Simm,[11] Paul N. Schofield,[12] Barry Smith,[13] Juergen Sühnel,[14] Julio Vera,[15] Wolfgang Wagner,[16] Eva C. Wönne,[5] and Daniel Wuttke,[17]

Abstract

The 2013 Rostock Symposium on Systems Biology and Bioinformatics in Aging Research was again dedicated to dissecting the aging process using *in silico* means. A particular focus was on ontologies, because these are a key technology to systematically integrate heterogeneous information about the aging process. Related topics were databases and data integration. Other talks tackled modeling issues and applications, the latter including talks focused on marker development and cellular stress as well as on diseases, in particular on diseases of kidney and skin.

Introduction

As in 2010[1] and 2011,[2] the Rostock Symposium on Systems Biology and Bioinformatics in Aging Research featured talks by researchers from computer science and engineering and from experimental and clinical research. This year these talks were accompanied by philosophers interested in ontologies. Concluding the workshop, an initiative (http://denigma.de/data/entry/gerontology) to foster the development of ontology resources for aging research was begun, with the objective to reuse already existing ontologies, such as the Gene Ontology (GO), and to conceptualize our understanding of aging to facilitate its dissection.

Ontologies and Databases/Data Integration Focus

Aging is often regarded as a complex multifaceted process, and well over 300 theories have been proposed to describe its cause and mechanisms.[3] Many more pathways have been implicated. However, an overarching theoretical framework for dissecting and understanding what is

[1]Institute for Biostatistics and Informatics in Medicine and Aging Research, Department of Medicine, Rostock University, Rostock, Germany.
[2]Institute of Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg and German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany.
[3]SENS Research Foundation, Cambridge, United Kingdom, and Jena Centre for Systems Biology of Aging, Friedrich-Schiller-Universität, Jena, Germany.
[4]Jena University Language & Information Engineering Lab, Jena, Germany.
[5]Bioreactor Group, Division of Experimental Surgery, Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany.
[6]Cell Signaling Unit, Institute for Genome Biology, Leibniz Institute for Farm Animal Biology (FBN) Dummerstorf, Germany.
[7]Institute of Philosophy, University of Rostock and Department of Philosophy, University of Münster, Münster, Germany.
[8]Cellular Biochemistry, University of Groningen, Groningen, The Netherlands.
[9]University of Jena, Research Group Theoretical Systems Biology, Jena, Germany.
[10]Molecular Systems Biology, University of Groningen, Groningen, The Netherlands.
[11]Clinic for Cardiothoracic Surgery, University Hospital, Halle (Saale), Germany.
[12]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, and The Jackson Laboratory, Bar Harbor, Maine.
[13]National Center for Ontological Research, University at Buffalo, Buffalo, New York.
[14]Biocomputing Group, Leibniz Institute for Age Research, Fritz Lipmann Institute (FLI), Jena Centre for Systems Biology of Ageing (JenAge), Jena, Germany.
[15]Systems Biology and Bioinformatics, Rostock University, Germany.
[16]Stem Cell Biology and Cellular Engineering, Helmholtz-Institute for Biomedical Engineering; RWTH Aachen University Medical School, Aachen, Germany.
[17]Integrative Genomics of Ageing Research, Department of Functional and Comparative Genomics, University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom.

involved in the aging process is lacking. Ontologies can serve as a crucial instrument to systematically integrate diverse data and models describing aging in an efficient manner and thus to enable the creation of a common knowledge base. Examples of current efforts to bring about such data integration can be found in the JenAge consortium, where structured data are integrated with unstructured text with the help of ontologies (Udo Hahn), The Jackson Laboratory Nathan Shock Aging Center, where phenotype/pathology ontologies are used to capture the data deriving from a mouse aging study using inbred 31 strains, combining histopathological and other data with haplotype maps (Paul Schofield), the Liverpool Institute of Integrative Biology, where a crowdsourcing effort of ontology construction and data integration for enabling reasoning has been initiated (http://denigma.de) (Daniel Wuttke), and the ROSage consortium, where the experimental measurements must be related to each other, with the goal of inferring attributes and integrating other (public) datasets from mouse and human (Georg Fuellen).

Three talks were thus dedicated to ontologies,[4,5] moving from fundamental, theoretical aspects to practical aspects relating to how ontologies should be implemented in such a way as to aid further research. Barry Smith (Buffalo, New York) started with a keynote address entitled "What is Aging?", beginning with an account of the raison d'être of ontologies, namely to serve integration of data across diverse clinical and scientific fields. The idea is that if common terms are used to annotate or tag heterogeneous data collected by scientists working in different disciplines or studying different organisms, then these data will be more easily reused for integration and analysis. To this end, the terms in ontologies need to be carefully defined, and Smith examined in this light definitions of terms central to aging research in standard bio-ontologies such as the GO,[6] the Foundational Model of Anatomy (FMA) ontology,[7] and the Plant Ontology (PO).[8] GO defines "aging" as: "A developmental process that is a deterioration and loss of function over time." In turn, GO defines "developmental process" as: "A biological process whose specific outcome is the progression of an integrated living unit over time from an initial condition to a later condition." These terms refer implicitly to what, following the FMA, we might call canonical development and aging—thus to development and aging as they occur in the normal unfolding of life (as contrasted, for example, with what happens in cases of developmental delay/arrest or of premature aging). To address this problem, Smith proposed an ontology of development stages based on the ontology of plant structure developmental stages, which forms a principal branch of the PO. Finally, distinguishing the processes of aging from the aging stage in the life of any given organism allows us also to understand more clearly the relation between aging and death.

Dietrich Rebholz-Schuhmann (University of Zurich) then talked on "Semantic Normalizaton of Phenotypes: State of the Art and Prospects." Here, patient data take central stage. Clinical research is increasingly influenced by biomolecular research, and in the future clinical decisions will include biomolecular parameters. Standardizing the patient's phenotype is required to link clinical symptoms to molecular functions, and the integration of data from the bench to the bedside helps to generate novel hypotheses and may help to untangle the multi-morbidity of patients. A number of resources have been produced to represent phenotypes, *e.g.*, the Unified Medical Language System (UMLS), which collects input from different terminological authorities around the world. Increasingly, ontologies are developed that represent the phenotypes of humans and model organisms. In the clinical domain, the terminologies are optimized to specific signs and symptoms in selected European languages. In the domain of molecular biology and human genetics, ontologies such as the GO have thus far been used primarily in studies of genetic disorders and molecular dysfunctions and to enable comparisons across model organisms. This means, however, that there is a certain lack of interoperability with the sorts of clinical phenotype representations contained in the UMLS. Both types of ontology were reviewed and evaluated, and judged for their usefulness in understanding aging processes; in the future it will be important to bring both worlds together.

Daniel Wuttke (University of Liverpool) and Anton Kaluga spoke on the topic of "Web Intelligence to Solve Aging," introducing the Denigma project (http://denigma.de). Denigma is a digital decipher machine, a collaborative platform for scientists, programmers/engineers, and designers, that seeks to apply a robust unification procedure to collect, annotate, link, and systematize data, resources, and projects relating to aging research in a way to enable global web-scale reasoning. It provides a knowledge management system to support discovery and inference of new knowledge and novel therapies designed to cure aging. Its key priority is to combine artificial with natural intelligence, and to this end it will create aging-specific ontologies that will be compatible with existing ontologies and use these to alleviate the current fragmentation of aging research. Thus, there is a strong need for an integrative effort to consolidate ontology support for aging research.

A second set of talks described a variety of database efforts, some of which already employ ontologies. These are based on sophisticated text analytics and database curation. For example, Udo Hahn (Universität Jena) reported on "Biomedical Natural Language Processing for Aging Research," describing three services offered by the JenAge lab. The first provides a semantic document search engine for the whole of Medline that is adaptable to the needs of the user through filters enabling focused searching, for example, on documents relevant to specific aspects of aging research. The second deals with information extraction from biomedical documents to empower the automatic synthesis of databases. Hahn's group has created a text analytics pipeline that feeds the JenAge AgeFactDB (http://agefactdb.jenage.de) on a weekly basis with the latest facts (relating, *e.g.*, to protein–protein interactions [PPIs] and to aging-relevant genes) harvested from the most current publications (whereas manually curated databases typically have an update backlog extending up to several years). Although this service takes care of explicit information found in a document, the third service tries to screen implicit knowledge in texts. Plausible hypotheses (*e.g.*, potential PPIs, genes tentatively identified as relevant for aging) are computed on the basis of evidence detected through reasoning over the documents analyzed but not explicitly expressed. Hence, this tool may play a crucial role in the creative processes of shaping, modifying, and validating scientific hypotheses practiced by life science colleagues on a daily basis.

Juergen Suehnel (Leibniz Institute for Age Research, Jena) announced the first stable release of the JenAge Ageing Factor database AgeFactDB (http://agefactdb.jenage.de) aimed at "Data Integration in Age Research." Aging factors are considered to be genes, chemical compounds, or other factors, such as dietary restriction, whose action results in a changed life span or another aging phenotype. Any information related to the effects of aging factors is called an observation and is presented on observation pages. To provide concise access to all available information relating to a particular gene, corresponding observations are also summarized on Ageing Factor pages. Initially, aging-related data were taken primarily from existing databases, namely the GenAge Database (http://genomics.senescence.info/genes), the Lifespan Observations Database (http://lifespandb.sageweb.org), and the GenDR Database (http://genomics.senescence.info/diet). Now additional aging-related information has been included through both manual and semi-automatic extraction from the scientific literature. On the basis of homology data taken from the HomoloGene database, AgeFactDB also provides Observation and Ageing Factor pages for genes that are homologous to known aging-related genes and thus considered to be candidate aging-related genes. AgeFactDB offers a variety of search and browse options and also allows downloading of aging factor or observation lists in various formats.

Given the complexity of aging, and of biology in general, an "Integrative Genomics of Ageing" is essential to decipher the aging process and to help develop life-extending interventions. Two key questions in biogerontology are: (1) What are the genetic determinants of human aging in terms of both differences in longevity between individuals of a given species and differences in aging between species? (2) What changes occur in a person from age 30 to age 70 to increase the chance of dying by roughly 30-fold? Concerning question (1), the laboratory of João Pedro de Magalhães (University of Liverpool) has recently developed the LongevityMap,[9] a new database of human genetic variants associated with longevity (http://genomics.senescence.info/longevity). To help understand aging changes, his lab also developed the Digital Ageing Atlas (http://ageing-map.org), a one-stop collection of human age-related data covering different biological levels (molecular, cellular, physiological, psychological, and pathological).

Aubrey D.N.J. de Grey presented the efforts of the SENS Research Foundation on "Organising Knowledge about Aging for Efficient Identification of Promising Intervention Strategies." The complexity of aging presents a daunting challenge for the design of effective therapies. As our knowledge of the mechanisms of aging increases, it has become increasingly clear that a multi-pronged, divide-and-conquer strategy to attack it will be needed. However, deciding what set of prongs is likely to work best is exceptionally challenging when every therapy manipulating one part of the system may have side effects affecting others negatively. A solution may be found more easily by use of a knowledge base that collects and organizes data in a form that can show users the predicted knock-on effects of a given therapy or combination of therapies. Such a system is essentially a curated literature database, storing what individual academic and other sources state about the changes that occur with age in a given species (or organ, or cell type), the causal relationships between such changes, and the effect of a given medical (or experimental) intervention on a given change. Future enhancements may include the ability to store rates of change, including non-linear ones, and to incorporate alternative, mutually contradictory sets of data for the purpose of identifying experimental avenues to improve understanding of how aging occurs.

In a panel discussion on ontologies during the workshop, speakers stressed the need for a formal representation of data on phenotypes and diseases associated with aging as a critical step toward the computational analysis of large aging-related datasets. Such datasets may then be related to each other, both within and across species, and analyzed computationally, for example, by assessing phenotypic relatedness of diseases, over-representation of phenotypes involving specific biological functions, anatomical or cellular locations, involvement of particular pathways, susceptibility to particular drug treatments, and so on. The cross-species ontology-based integration of phenotype information was identified already in the conclusion of our last report as "the next key step to achieving data interoperability across resources, inference of core findings, and translational medicine."[2] The consensus of the panel was that existing ontologies need improvement and extension to be ready to be used in combination with aging-related data. But as Barry Smith pointed out, the goal cannot be to add another ontology to the already large number of existing ontologies listed, for example, in the Bioportal (http://bioportal.bioontology.org) or in Open Biological and Biomedical Ontologies (OBO) (www.obofoundry.org) or in Ontobee (www.ontobee.org). Rather, it is necessary to re-use as much as possible from existing ontologies and to improve on these with regard to those terms used in the aging research community. As a particular challenge, Udo Hahn identified the degree to which existing ontologies neglect the dimension of time, with rare exceptions such as the embryonic mouse ontology eMAP.[10,11] During the conference, a group of participants started the initiative to develop an ontology for aging research, called "GerOntology" (GERO) (http://denigma.de/data/entry/gerontology), which is designed to cover aging-related phenomena from the molecular through phenotypical up to the demographic level via the systematic re-use of relevant terms from existing ontologies.

Data Integration, Analysis, and Modeling

Moving away from the ontology and database focus, toward integrative bioinformatics analyses, some new tools were presented at the symposium. In her talk on "Focus Networks for the Integration of Large-scale Data," Bianca Habermann from the MPI for the Biology of Ageing (Cologne) introduced algorithms developed in her lab for the integration and interpretation of large-scale data with the help of protein interaction networks. The Cytoscape plugins vIPer (virtual Pathway Explorer), PEANuT (Pathway Enrichment ANalysis Tool), and COMFI (COMplex FInder) are designed to overlay expression, or other numerical, data on interaction networks of proteins. They allow constructing focus networks between two or more nodes that are weighted according to numerical (*e.g.*, expression) values that are enriched with pathway and complex information. With viPEr, the user can explore the neighborhood of a specific node fruther, looking for paths emanating from a node that are differentially regulated under a certain experimental

condition. The power of the software packages in data interpretation was then demonstrated using data from longevity studies.

Hans Kestler (Ulm University) reported on the "Cross-Platform and Inter-Species Integration of Aging Data." He presented rank aggregation approaches as a means to independently combine individual experimental aging data. Aging experiments can share common differences and conserved processes, although the experimental conditions may vary, like different animal models, tissues of interest, or high-throughput platforms. A higher confidence in the genes that are involved in aging may be achieved by also comparing results with other studies related to aging research. This can be achieved by operating on ranks (and thus being independent of effect size) and subsequently combining ranked lists of genes from single experiments. Instead of just using a set of genes, rank aggregation can use the different ranks of genes in each study, to build up a consensus ranking of differentially expressed genes. The determined consensus ranking represents a ranking of genes that are common across the individual experiments. With this approach, new processes related to aging (*e.g.*, from KEGG pathways) can be discovered by the combination of different data sources not found in individual investigations.

For the Rostock *ROSAge* consortium, Georg Fuellen (University of Rostock), gave a brief update of their analyses of "Mouse Mitochondria and Ageing." The study is now in its third year, and preliminary data show differences in life expectancy, in particular under stress conditions, of the conplastic strains featuring mitochondrial mutations. To develop and test data analysis tools and methodology, a similar dataset (The Jackson Laboratory Nathan Shock Aging Center study from the Mouse Phenome Database; see the talk by Paul Schofield) was analyzed extensively, with the discovery of role-changing biomarkers with an anti-longevity effect in early age groups and a pro-longevity effect later on. Then, Johannes Wollbold (University of Rostock) talked about "The Free Radical Theory of Ageing: Rules, Exceptions, and Counterexamples." Because the classical free radical theory is seriously challenged, he collected and ordered existing knowledge focusing on the questions of reactive oxygen species (ROS) increase, molecular damage, and effects on life span. He developed a specific method of knowledge-base construction. Data reported in the literature or collected within the ROSAge consortium is formalized as Ripple Down Rules, a structure of general rules and exceptions.[12] This rule set is further validated and completed by the attribute exploration algorithm[13] of Formal Concept Analysis: Implicational rules proposed by the algorithm are accepted or counter-examples are given. Thus, a minimal rule base is defined systematically; from this base, all implications valid according to the available knowledge can be derived logically. Hence, queries can be answered regarding possible, recognized, necessary, and sufficient conditions of accelerated and retarded aging.

Detailed modeling of aging-related processes was again a topic at the symposium, concerning epigenetics and cell proliferation. Specifically, Joerg Galle (University Leipzig) presented joint work with Jens Przybilla, Thimo Rohlf, and Markus Loeffler, on "Understanding Epigenetic Changes in Ageing Stem Cells—A Computational Model Approach." During aging, a decline in stem cell function is observed in many tissues. This decline is accompanied by complex changes of the chromatin structure; among them are changes in histone modifications and DNA methylation, both of which affect transcription of a tissue-specific subset of genes. A mechanistic understanding of these age-associated processes, their interrelations, and environmental dependence is currently lacking. Therefore, a multi-scale computational model, which combines a model of epigenetic regulation of transcription with an individual cell-based model of stem cell populations, was developed. Applying the model, changes in regulatory states were analyzed, following loss of methylation of lysine 4 at histone H3 at gene promoters due to limited inheritance of this mark during replication.

Sascha Schäuble and Christoph Kaleta (University Jena) presented two approaches for the analysis of aging-related data sets under the title "Integrative Data Analysis in Age Research: From Differential Equations to Constraint-Based Models." In the first approach, a quantitative model of the transition from proliferating fibroblasts (P) via reversibly cell cycle–arrested (C) to irreversibly arrested senescent cells (S) was reconstructed. By applying senescence marker quantification to this model, it was possible to discriminate between the considered cellular states. This model allowed elucidating differences in the stress response of different fibroblast cell lines. By evaluating marker specificity, Senescence-associated β-galactosidase (SA-β-Gal) proved a good quantitative marker for cellular senescence in WI-38 and BJ cells, but less so in MRC-5 cells. The second approach aimed at an integrative analysis of RNAseq data sets across several species and several tissues. To this end, a constraint-based approach that allows mapping of changes in expression data on a common reference metabolic network was developed. By comparing changes in gene expression across several species and tissues, subsystems of metabolism could be identified that show common as well as differential patterns of regulation during aging across and between tissues.

Experimental and Clinical Contributions

As always, application-focussed talks were important to connect to "the real world" of aging research and to learn about the variety of experimental and clinical models and the types of data gathered. Paul Schofield (University of Cambridge), with colleagues at The Jackson Laboratory (John Sundberg) and University of Pittsburgh (Annarose Berndt), presented "The Genetic and Histopathologic Basis of Aging in Inbred Mice," which discussed the genetic dissection of age-related pathology and life span using inbred strains of laboratory mice.[14] Inbred strains of mice have been used for many years in the analysis of overall genetic contributions to longevity, but the age-related accumulation of morbid changes has generally been documented in F_1 (*e.g.*, C57BL/6N$\times$C3H/HeN] F_1) and outbred stocks of mice, making it difficult to conduct genetic analysis. With the sequencing of many inbred strains of mice and establishment of high-density single-nucleotide polymorphism (SNP) maps, it is now possible to examine traits, such as predisposition to early development of lymphomas or resistance to degenerative changes, in a panel of inbred mice and to map them rapidly using association analysis. The pathology screen of The Jackson Laboratory Nathan Shock Aging center study on 31 inbred strains of mice is the largest longitudinal life span

study conducted on mice. Several association studies show age-related predisposition to neoplastic and degenerative disease, and ontologies (see above) are used in the capture and analysis of pathology data.

Staying with mice as models, Andreas Simm (University of Halle) then discussed "Protein Glycation in Biological Aging: A Double-Edged Sword." Biological aging is induced by the gradual accumulation of cellular and molecular faults. An important cause of faults is intense stress, like oxidative or glycogenic stress. Whereas high stress induces premature aging, low stress can induce the genetic repair/defense systems, leading to increased life span. An example for such a stressor is advanced glycation end products (AGEs). AGEs can induce inflammation, oxidative stress, protein dysfunction, and cell death. They are considered as biomarkers of aging and are associated with cardiovascular diseases. Besides endogenous formation, significant amounts of AGEs are taken up with food. Although nutritional AGEs are considered as undesirable, pro-inflammatory agents, they may also enclose potentially beneficial anti-oxidants. Mouse cardiac cells were stimulated with bread crust (high AGEs) to analyze signal transduction pathways as well as gene expression. Additionally, mice fed with bread crust containing diet were analyzed to prove the *in vivo* relevance for the heart. In mouse cardiac fibroblasts, bread crust extract induced a moderate elevation of ROS production causing an activation of p42/p44MAPK, p38MAPK, and nuclear factor-κB (NF-κ]B), followed by increased expression of anti-oxidative enzymes. Pre-conditioning studies demonstrated that this was sufficient to protect cardiac fibroblasts and rat adult cardiac myocytes against severe oxidative stress. Furthermore, mice fed a bread crust–containing diet exhibited a similarly improved cardiac expression of anti-oxidative defense genes. Therefore, the consumption of AGEs can contribute to an improved anti-oxidant status of the heart, thus exhibiting cardioprotective effects in case of severe oxidative stress, as in ischemia reperfusion injury.

Also using mice, Melanie Boerries and Hauke Busch (University of Freiburg) presented an "'Omics' View on Kidney Aging." They performed a transcriptome and methylome analysis of primary glomeruli from young, middle-aged, and old mice. Glomeruli constitute the main filtration unit in the kidney. They are partially constituted by podocyte cells, which are post-mitotic and as such sensitive to aging.[15] This leads to a high correlation between the glomerular filtration rate (GFR) and age, hinting at a major involvement of glomeruli in kidney aging. Pairwise comparison of the glomeruli age groups by gene set enrichment analysis showed a progressive down-regulation of cell division, DNA repair, and extracellular matrix with age, while various metabolic processes as well as cytochromes p450s became up-regulated. Integrating results from previous analyses, target gene set enrichment, and network analysis, a putative transcription factor network was proposed. Interestingly, many of the age-related changes could be attributed to the non-podocyte fraction of glomeruli, reducing the expected functional role of podocytes in kidney aging.

In the final mouse talk, Andreas Hoeflich (FBN Dummerstorf, Germany) reported on "Reproductive Development and Aging: A Mouse Model for the Dissection of Growth, Aging, and Sexual Maturation." Under conditions of growth inhibition or growth stimulation, an extended or shortened life span, respectively, has been observed. The negative correlation between growth and aging, which exclusively has been derived from within-species studies, is not present on the level of inter-species comparisons because smaller species normally have shorter life spans than larger ones. Thus, a direct effect of growth on life span actually is under intense debate (*e.g.*, Gordon IGF-Conference 2013; www.grc.org/programs.aspx?year=2013&program=insulin).

Until now, reproductive development was considered as a potential parameter for the determination of life span, but a mammalian model to test for the causal interrelationships between growth, aging, and reproductive dynamics was lacking. However, by using different insulin-like growth factor-binding protein-2 (IGFBP-2) transgenic mouse models over-expressing normal IGFBP-2 or mutated IGFBP-2, it turned out to be possible to dissect growth from aging, but not reproductive development from aging. In other words, in the within-species study reported, there was no support for a correlation between growth and aging, only between reproductive development and aging. An important future issue will be related to the question if reproductive development is a structural determinant of life span or if both reproductive development and life span are fixed by earlier ontogenetic development.

Skin aging converges on the dermal stroma consisting mainly of dermal fibroblasts and surrounding matrix. Homeostasis of that compartment relies on adaptation and damage clearance rather than cell turnover. Human dermal fibroblasts thus present a long-lived cell system prone to aging-related damage accumulation and adaptation/maladaptation. Fritz Boege (University Düsseldorf) reported "Ingrained Phenotype Changes of Dermal Fibroblasts from Donors Aged 20–67 Years." This systematic study was restricted to female donors, excluding gender influence. All primary cells studied were isolated from the same skin area, minimizing variances of body location and external milieu. The study focused on alterations conserved in primary culture at low population doubling, excluding replicative senescence *in vitro*. Genome-wide array analysis failed to detect significant age-related changes in gene expression. Nevertheless, the cells exhibited systematic age-related alterations of nuclear and mitochondrial function, encompassing: (1) Increases in chromosome breakage, baseline DNA damage response, and up-regulation of non-homologous end-joining components; (2) decreased mitochondrial content and respiratory function due to insufficient activation of mito-biogenesis; and (3) increased propensity to enter cellular senescence. In summary, *in situ* aging of dermal fibroblasts recapitulated some, but not all, features of nuclear and mitochondrial dysfunction reported from fibroblasts subjected to stress-induced or replicative aging in culture.

Human aging is associated with DNA methylation (DNAm) changes at specific sites in the genome. These epigenetic modifications may be used to track donor age for forensic analysis or for estimation of biological age.[16] In his talk on "Age-Associated DNA-Methylation Changes are Reversed in Pluripotent Stem Cells," Wolfgang Wagner (RWTH Aachen) reported on such age-related DNAm (AR-DNAm) changes identified from more than 500 DNAm profiles from blood. These AR-DNAm changes were further

validated in independent datasets. He reported how these modifications, in analogy to senescence-associated DNAm changes which are acquired during long-term culture *in vitro*, are reversed upon reprogramming into induced pluripotent stem cells (iPSCs).[17,18]

Eva C. Wönne and Thomas Hiller (Charité, Berlin) presented a "3D Liver Cell Culture Technology for Analyzing Effects of Reactive Oxygen Species (ROS) and Associated Cellular Damage on Aging." Their technology is based on a three-dimensional network of capillaries serving as a decentralized, continuous culture medium supply and gas exchange. In this way, a culture environment allowing for studies on primary liver cells in a physiological-like environment is provided. In cooperation with the group of Andreas Nüssler (Eberhard-Karls Universität Tübingen), Reinhard Guthke (Hans-Knöll-Institut Jena), and Dirk Koczan (BMFZ Rostock), the effect of different ethanol concentrations on primary rat or human hepatocytes cultured in the system was analyzed. Typical metabolic liver parameters as well as glucose and lactate are measured to detect possible effects of the ethanol exposure. Gene Set Enrichment Analysis (GSEA) and KEGG pathway mapping are used to characterize biological categories in the given set of up-reglated and down-regulated genes in primary liver cells cultured in an *in vitro* three-dimensional culture model.

Finally, Anne Meinema and Georges Janssens (University of Groningen) presented their preliminary results of "Molecular System-Level Phenotyping to Unravel Causes in Yeast Ageing," a part of the Systems Biology Centre of Groningen's initiative to characterize the aging process in yeast. At present, numerous genes and cellular mechanisms have been related to aging. However, the field is far from a comprehensive understanding at the molecular level, even in a simple eukaryote such as *Saccharomyces cerevisiae*. Yeast cells age with every new descendent that is formed (replicative aging). However, due to exponential growth of the yeast culture, isolation of sufficient aged mother cells for phenotyping is difficult. To overcome this, a setup has been created to immobilize iron bead–labeled yeast cells on a magnetized column. A constant and steady supply of fresh nutrients ensures continued cell division and removal of emerging offspring. Aged mother cells and their corresponding offspring are harvested at 16 different time points during the complete yeast life span of 72 hr. The amounts are sufficient to map the full transcriptome reproducibly, as well as the proteome.

Conclusions

The motivation for the ontology focus was described in part in the conclusions of the last report.[2] Because aging operates on multiple hierarchies of organismal complexity, its understanding requires glueing together a wide variety of heterogeneous data sources. For this reason, both the bioinformatics as well as the systems biology approaches in the computational biology of aging would benefit greatly from a common formal knowledge representation that captures all the phenomena associated with aging and incorporates relevant datasets or at least provides modal access to those data in a uniform and computer-understandable format. Such an ontological framework would enable investigators to take advantage of the discoveries made in multiple model systems, commonly used in aging research, as well as data represented in various formats, from unstructured to highly structured data sources.

Because such an undertaking cannot be accomplished solely by a single person nor a single research group, it has to become a community effort, crossing many disciplines, and done in a systematic way by involving people from relevant disciplines including aging researchers, clinicians, bioinformaticians, systems biologists, and knowledge engineers as well as ontologists, fostering ontologies as common format, providing a common language.

A major challenge is to design the ontologies used in such a way that they allow to perform reasoning on scales that bridge the gap between the genotype and phenotype from the level of single molecules up to those of the level of populations for multiple species. The notion of time and especially lifetime stages has to be captured and encoded by these ontologies. On the cellular level, the ontological framework should thus encompass temporal development of high-throughput data, cellular ultra-structures, and cellular morphological phenotypes, for example, following the outline given in reference 19. Inference should be of high quality and enable well-defined predictions that can be tested with experiments, either *in silico, in vitro*, or *in vivo,* and therefore facilitate the identification of interventions that will help to decelerate aging and extend health span both theoretically and practically (project ID 0315581). Finally, funding within the EU FP7 project MARK-AGE and the COST Action CM1001.

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References

1. Fuellen G, Adjaye J, de Grey A, Lepperdinger G, de Magalhaes JP, Suhnel J, Wipat A. Bioinformatics in aging research: A workshop report. Rejuvenation Res 2010;13:763–767.
2. Fuellen G, Dengjel J, Hoeflich A, Hoeijemakers J, Kestler HA, Kowald A, Priebe S, Rebholz-Schuhmann D, Schmeck B, Schmitz U, Stolzing A, Suhnel J, Wuttke D, Vera J. Systems biology and bioinformatics in aging research: A workshop report. Rejuvenation Res 2012;15:631–641.
3. Vina J, Borras C, Miquel J. Theories of ageing. IUBMB Life 2007;59:249–254.
4. Jansen L, Smith B. *Biomedizinische Ontologie.* Wissen strukturieren für den Informatik-Einsatz. vdf, 2008.
5. Munn K, Smith B. *Applied Ontology*. Ontos, Frankfurt, 2008.

6. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25:25–29.
7. Rosse C, Mejino JL, Jr. A reference ontology for biomedical informatics: The Foundational Model of Anatomy. J Biomed Inform 2003;36:478–500.
8. Walls RL, Athreya B, Cooper L, Elser J, Gandolfo MA, Jaiswal P, Mungall CJ, Preece J, Rensing S, Smith B, Stevenson DW. Ontologies as integrative tools for plant science. Am J Bot 2012;99:1263–1275.
9. Budovsky A, Craig T, Wang J, Tacutu R, Csordas A, Lourenco J, Fraifeld VE, de Magalhaes JP. LongevityMap: A database of human genetic variants associated with longevity. Trends Genet 2013;29:559–560.
10. Hayamizu TF, Wicks MN, Davidson DR, Burger A, Ringwald M, Baldock RA. EMAP/EMAPA ontology of mouse developmental anatomy: 2013 update. J Biomed Semantics 2013;4:15.
11. Bard J. A new ontology (structured hierarchy) of human developmental anatomy for the first 7 weeks (Carnegie stages 1–20). J Anat 2012;221:406–416.
12. Kwok RBH. Translations of ripple down rules into logic formalisms. In: Dieng R, Corby O, eds. *Knowledge Engineering and Knowledge Management Methods, Models, and Tools.* Springer, 2000, pp. 366–379.
13. Wollbold J. Attribute Exploration of Gene Regulatory Processes. University of Jena, 2011.
14. Sundberg JP, Berndt A, Sundberg BA, Silva KA, Kennedy V, Bronson R, Yuan R, Paigen B, Harrison D, Schofield PN. The mouse as a model for understanding chronic diseases of aging: the histopathologic basis of aging in inbred mice. Pathobiol Aging Age Relat Dis 2011;1.
15. Boerries M, Grahammer F, Eiselein S, Buck M, Meyer C, Goedel M, Bechtel W, Zschiedrich S, Pfeifer D, Laloe D, Arrondel C, Goncalves S, Kruger M, Harvey SJ, Busch H, Dengjel J, Huber TB. Molecular fingerprinting of the podocyte reveals novel gene and protein regulatory networks. Kidney Int 2013;83:1052–1064.
16. Koch CM, Wagner W. Epigenetic-aging-signature to determine age in different tissues. Aging 2011;3:1018–1027.
17. Koch CM, Reck K, Shao K, Lin Q, Joussen S, Ziegler P, Walenda G, Drescher W, Opalka B, May T, Brummendorf T, Zenke M, Saric T, Wagner W. Pluripotent stem cells escape from senescence-associated DNA methylation changes. Genome Res 2013;23:248–259.
18. Koch CM, Joussen S, Schellenberg A, Lin Q, Zenke M, Wagner W. Monitoring of cellular senescence by DNA-methylation at specific CpG sites. Aging Cell 2012;11:366–369.
19. Fuellen G, Jansen L, Leser U, Kurtz A. Using ontologies to study cell transitions. J Biomed Semantics 2013;4:25.

Address correspondence to:
Georg Fuellen
University of Rostock
Medical Faculty
Institute for Biostatistics and Informatics
in Medicine and Ageing Research
Ernst-Heydemann-Strasse 8
18057 Rostock
Germany

E-mail: fuellen@uni-rostock.de