LIVRE DES ABSTRACTS

Sequence determinants of DENR/MCTS1-mediated translation reinitiation

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Re-initiation after upstream open reading frame (uORF) translation requires the ribosome to resume scanning and re-assembly at the downstream initiation codon. Density-regulated protein (DENR) and malignant T cell-amplified sequence 1 (MCTS-1) have been recently associated with ribosome recycling and translation re-initiation after uORF usage in eukaryotes.
Using ribosome profiling, we have recently found evidence for the presence of translated uORFs in several genes of the core circadian clock machinery in mouse liver. Importantly, cells deficient of the re-initiation factor DENR displayed an up to 1.5-hour shorter circadian period, suggesting that uORF translation is involved in the regulation of mammalian circadian rhythms post-transcriptionally, and establishing the circadian system as a particularly suitable setting to study uORF-mediated gene regulation and its physiological implications.
We have now combined ribosome profiling and iClip in NIH3T3 mouse fibroblasts to identify uORFs acting through re-initiation both genome-wide and within the clock circuitry, and to define the sequence characteristics that confer DENR dependance.
We detected 11824 translated uORFs in 2977 transcripts (37% of the total) and observed a global ribosomal density shift towards the 5'UTR upon Denr knockdown. Moreover, for 240 transcripts, this redistribution was concomitant with a significant translational downregulation on the main ORF.
Using a linear model that considers several uORF and UTR features we identified the number of uORFs, the start codon identity and context and the relative location within the 5'UTR as the strongest predictors of DENR dependance.
These findings will be further validated in reporter assays, and will shed light onto the mechanisms of alternative translation initiation and re-initiation.

The P4 Health Spectrum - A Predictive, Preventive, Personalized and Participatory Continuum for Promoting Healthspan.

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Other

Chronic diseases (i.e., noncommunicable diseases), mainly cardiovascular disease, cancer, respiratory diseases and type-2-diabetes, are now the leading cause of death, disability and diminished quality of life on the planet. Moreover, these diseases are also a major financial burden worldwide, significantly impacting the economy of many countries. Healthcare systems and medicine have progressively improved upon the ability to address infectious diseases and react to adverse health events through both surgical interventions and pharmacology; we have become efficient in delivering reactive care (i.e., initiating interventions once an individual is on the verge of or has actually suffered a negative health event). However, with slowly progressing and often 'silent' chronic diseases now being the main cause of illness, healthcare and medicine must evolve into a proactive system, moving away from a merely reactive approach to care. Minimal interactions among the specialists and limited information to the general practitioner and to the individual receiving care lead to a fragmented health approach, non-concerted prescriptions, a scattered follow-up and a suboptimal cost-effectiveness ratio. A new approach in medicine that is predictive, preventive, personalized and participatory, which we label here as "P4" holds great promise to reduce the burden of chronic diseases by harnessing technology and an increasingly better understanding of environment-biology interactions, evidence-based interventions and the underlying mechanisms of chronic diseases. In this concept paper, we propose a 'P4 Health Continuum' model as a framework to promote and facilitate multi-stakeholder collaboration with an orchestrated common language and an integrated care model to increase the healthspan.

3D-Derivation of Uncommitted Human Muscle Stem Cells from iPSCs

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One of the most fundamental problems associated with stem cell therapy of skeletal muscle is the limited availably of cells that can robustly engraft into the stem cell compartment. It has extensively been attempted to isolate adult muscle stem cells (MuSCs) and expand them in culture to obtain sufficient cell numbers for such treatments. The challenge associated with this approach is that, once isolated from their niche and maintained in culture, MuSCs become terminally committed to myogenic differentiation and show a dramatically reduced engraftment potential. However, the recent discovery of induced pluripotent stem cells (iPSCs) has opened new avenues for the in-vitro derivation of cell types that are more suitable for transplantation. Here, we report a highly efficient approach for the scalable derivation of uncommitted MuSCs from human iPSCs in a biologically faithful 3D environment. We employed human iPSCs and a spectrum of immortalized cell lines to generate 3D aggregation conditions promoting mesoderm formation and subsequent specification to the myogenic lineage without the parallel upregulation of myogenic commitment markers. Taken together, our work reveals a seven-day derivation protocol for the generation uncommitted MuSCs from human iPSCs that can easily be scaled up to the bioreactor level.

Development of hypothalamic neural projection into the arcuate nucleus : role of guidance proteins

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The worldwide obesity epidemic is reaching an alarming rate, particularly among children. There is an urgent need to better understand the mechanisms underlying the early onset of this pathological condition. Energy balance is essential to maintain normal body weight and prevent obesity. The central nervous system, particularly the hypothalamus, is crucial for balancing energy intake and expenditure. In the arcuate nucleus of the hypothalamus (ARH), two main antagonistic populations have been observed: anorexigenic neurons that produce pro-opiomelanocortin (POMC) and orexigenic neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP). These neurons receive inputs from several hypothalamic areas, including the paraventricular nucleus (PVH), the dorsomedial nucleus (DMH) and the lateral hypothalamic area (LHA). However, the ontogenesis of these projections and the guidance cues involved in their development remain unknown. We, then studied when the inputs to ARH neurons develop and identified the relevant guidance cues involved.
To adress this question, we used DiI axonal labeling to examine the development of projections from PVH and DMH to ARH in neonatal mice. The results indicated that PVH projections reach the ARH at postnatal day 14 (P14) and the DMH projections develop later to reach the ARH at P16. In order to identify guidance cues involved in the development of projections into POMC and NPY neurons, Pomc-cre ;Td-Tomato and NPY-GFP-expressing cells were sorted at P14 and P16 based on the fluorescence. RNA-seq experiments were performed on these samples to identify guidance cues. First results identified Ephrin and Semaphorin family members as expressed in these neuronal populations. Efnb1, Efnb2 are upregulated and Sema3e, Sema4a, Sema4d and Sema6d are down-regulated in Pomc->Pomc when compared to Npy->Npy.
Identifying guidance signals will help us to better understand how hypothalamic connections develop and identify developmental mechanisms underlying early onset of obesity.

Bayesian Genome-Wide Association Study to discover novel lifespan-associated loci

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Identification of genetic variants associated with a phenotype is the first step towards biomarker discovery. However, many Genome-Wide Association Studies (GWASs) are underpowered to detect such variants. Increasing the sample size also increases the GWAS power, but large datasets can be difficult to gather for certain traits, such as lifespan. Leveraging independent sources of information and include them as priors in a Bayesian analysis can improve GWAS studies without increasing sample size.
We developed a framework for informed GWAS (implemented in the R package bGWAS) that accounts for the prior information by comparing the observed Z-scores from a conventional GWAS to prior effects using Bayes Factors. One way to derive prior effects is to combine summary statistics of GWASs for related traits with their causal effect on the trait of interest (using Mendelian Randomization).
We applied this method to improve the power of a lifespan GWAS based on >1 million parental lives. Our approach identified 12 traits significantly affecting lifespan, including BMI, smoking, education, coronary artery diseases and insulin. The prior effects derived from these risk factors lead to the identification of 10 new genome-wide significant variants in addition to the 25 identified by standard GWAS, notably in the long-suspected ABO gene (beta=2.6 months/allele, P=5.5x10-10) and in LPL (beta=2 months/allele, P=9.7x10-9). Most of the loci identified showed pleiotropic effects, such as a variant near POM121C (beta=2 months/allele, P=2.2x10-9), which has not been significantly associated with any of the risk factors previously but might be affecting lifespan through moderate effects on insulin and BMI.
So far, our priors have been built using information from summary statistics from other GWASs, allowing us to only identify variants affecting lifespan through risk factors included in the prior but our method is adaptable and can be modified to use other types of prior information. As an example, it has been shown that the expression levels of specific genes can be used as a measure of biological age (revealing accelerated aging, hence shorter lifespan). Interestingly, we observed that variants regulating genes whose expression had been shown to vary with age are 2 times more likely to be associated with lifespan (at P<5x10-5). This suggests that differential expression of age-related genes is not only a biomarker of aging, but some of them may directly influence lifespan and we are currently working on defining priors based on gene-expression data in order to identify variants associated with healthy aging.

The RNA-binding protein PUM2 links increased stress granule formation to mitochondrial dysfunction during aging

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Little information is available about how post-transcriptional mechanisms regulate the aging process. Here, we show that the RNA-binding protein, Pumilio2 (PUM2), which is a translation repressor, is induced upon aging and acts as a negative regulator of lifespan and mitochondrial homeostasis. Multi-omics and cross-species analyses of PUM2 function show that it inhibits the translation of the mRNA encoding for the mitochondrial fission factor (Mff), thereby affecting mitochondrial fission and mitophagy. This mechanism is conserved in C. elegans by the PUM2 orthologue, PUF-8. puf-8 knock-down in old nematodes and Pum2 CRISPR/Cas9-mediated knockout in the muscles of elderly mice enhances mitochondrial fission and mitophagy in both models, hence improving mitochondrial quality control and tissue homeostasis. PUM2 accelerates the aging process, by trapping and inhibiting Mff translation in aging-associated stress granules. Our data reveal how a PUM2-mediated layer of post-transcriptional regulation links stress granules formation to mitochondrial dynamics and mediates age-related mitochondrial dysfunction.

Aging Disrupts the Cross-Talk between Satellite Cells and Fibro- Adipogenic Progenitors during Muscle Regeneration

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Mechanisms of age-induced regenerative failure of skeletal muscle have largely focussed on the phenotypes of muscle stem cells (MuSCs). In contrast, the impact of aging on regulatory cells in the MuSC niche remains largely unexplored. Here, we demonstrate that aging impairs the function and cellular fate of fibro-adipogenic progenitors (FAPs), and thereby indirectly affects the myogenic potential of MuSCs. Transplantation of young FAPs in aged mice rescues age-related MuSC dysfunction. Using transcriptomic profiling, we identify WNT1-Inducible Signaling Pathway Protein 1 (WISP1) as a novel FAP-derived signal that is lost during aging. Our genetic and pharmacological approaches demonstrate that WISP1 is required and sufficient for efficient muscle regeneration and drives expansion and asymmetric commitment of MuSCs through Akt signaling. Systemic treatment of aged mice with WISP1 restores the myogenic capacity of MuSCs and rescues skeletal muscle regeneration. Altogether, we describe that altered matricellular signals from FAPs contribute to MuSC dysfunction during aging and demonstrate that this mechanism can be efficiently targeted to rejuvenate the regenerative capacity of aged skeletal muscle.

Keywords: Skeletal Muscle Regeneration; Aging; Muscle Stem Cells; Stem cell niche; Fibro- adipogenic progenitors; WISP1

Molecular Determinants of Human Sarcopenia across Ethnicities

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The ICD-10 disease classification has recently recognized sarcopenia as pathologically diminished muscle mass and weakness during aging, that particularly affects quality of life and mortality in later life. In the Multi-Ethnic Molecular determinants of Sarcopenia (MEMOSA) multi-centre project, we used high coverage RNA sequencing on human skeletal muscle biopsies to analyze genome-wide transcriptional changes in older men with sarcopenia compared with age-matched healthy controls in different ethnic groups. Transcriptional changes in sarcopenic muscle were primarily driven by low muscle mass, while muscle strength and function contributed more moderately. Individuals with sarcopenia in Singapore, Hertfordshire UK and Jamaica reproducibly demonstrated a prominent transcriptional signature of mitochondrial bioenergetic dysfunction in skeletal muscle. This signature included down-regulation of a PGC-1?/ERR?/NRF1 transcriptional network, reduced expression of oxidative phosphorylation and mitochondrial UPR genes, and lower protein levels and activity of mitochondrial complexes I, II, III and V. Metabolic sensing through NAD+ was perturbed during sarcopenia as NAD+ levels declined by 32% in the muscle of aged individuals with sarcopenia. This study provides the first integrated molecular profile of human sarcopenia across ethnicities and demonstrates a fundamental role of altered mitochondrial metabolism in the pathological loss of skeletal muscle mass and function in older people.

KEYWORDS: sarcopenia; skeletal muscle; aging; human; transcriptomics; mitochondria; NAD; ethnic diversity

Chromatin conformation of expanded CAG/CTG trinucleotide repeats in myotonic dystrophy type 1 and Huntington disease

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Expanded trinucleotide repeats (TNRs) are the underlying cause of at least twenty neurological and neuromuscular disorders including myotonic dystrophy type 1 (DM1) and Huntington's disease (HD). Upon repeat expansion, several pathogenic TNR loci acquire heterochromatic characteristics and display altered chromatin structure. In particular, patients with the congenital form of DM1 have increased CpG methylation in the region surrounding the repeats of the DMPK gene. This leads to the loss of CTCF binding and is predicted to affect how chromatin folds at this locus as well as transcriptional outputs. Here we performed 4C-seq at the DMPK and HTT loci from DM1 and HD patient-derived cells, respectively, and determined the effect of expanded CAG/CTG repeats on chromatin interaction profiles. Surprisingly, we observed similar DNA-DNA contacts in both unaffected and patient-derived cells for several viewpoints surrounding the CAG/CTG repeats for both loci. Thus, changes in chromatin conformation is unlikely to be responsible for the changes in the transcriptome between healthy and affected cells that we observed by RNA sequencing. These findings suggest that expanded CAG/CTG repeats do not significantly change the higher order chromatin structure and argue that changes in heterochromatic properties are not sufficient to alter chromatin folding.

Enhancing mitochondrial proteostasis reduces amyloid-beta proteotoxicity

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Alzheimer's disease is a common and devastating disease characterized by aggregation of the amyloid-beta peptide. However, we know relatively little about the underlying molecular mechanisms or how to treat patients with Alzheimer's disease. Here we provide bioinformatic and experimental evidence of a conserved mitochondrial stress response signature present in diseases involving amyloid-beta proteotoxicity in human, mouse and Caenorhabditis elegans that involves the mitochondrial unfolded protein response and mitophagy pathways. Using a worm model of amyloid-beta proteotoxicity, GMC101, we recapitulated mitochondrial features and confirmed that the induction of this mitochondrial stress response was essential for the maintenance of mitochondrial proteostasis and health. Notably, increasing mitochondrial proteostasis by pharmacologically and genetically targeting mitochondrial translation and mitophagy increases the fitness and lifespan of GMC101 worms and reduces amyloid aggregation in cells, worms and in transgenic mouse models of Alzheimer's disease. Our data support the relevance of enhancing mitochondrial proteostasis to delay amyloid-beta proteotoxic diseases, such as Alzheimer's disease.

Uncovering the interplay between epigenome editing efficiency and sequence context using a novel inducible targeting system

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Epigenome editing is an attractive way to manipulate gene expression. However, editing efficiency depends on sequence context in a manner that remains poorly understood. Here we developed a novel system, Chromatin Inducible Targeting (CIT), in which any protein can be recruited at will to a GFP mini gene. Using CIT, we tested how CAG/CTG repeat size affects the ability of lysine deacetylases to alter chromatin and gene expression. We found that repeat expansion reduces the effectiveness of gene silencing after HDAC5 targeting, most likely because of lower levels of histone acetylation near the expanded repeat tract. By contrast, HDAC3 targeting increases both GFP expression and histone acetylation regardless of repeat size. Our data uncover novel mechanisms of gene regulation by these histone deacteylases and guide their use in manipulating chromatin. CIT is suitable for screening and can be adapted to study the effect of virtually any sequences on epigenome editing.

Sizing disease-causing expanded trinucleotide repeats using long-read sequencing

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Expanded trinucleotide repeats cause over twenty neurological diseases. The size of the repeat tract determines in large part the severity of these disorders. Thus, sizing repeats in patient-derived material is a key aspect of the molecular diagnostics. Current methods to measure repeat size are slow, ill-suited for large scale analyses, and fail to provide the sequence information, which is crucial for the detection of interruptions that modify disease progression. Here, we present a method to bypass these issues by using long read sequencing. We developed a bioinformatics pipeline to overcome the sequencing error rate of single molecule real time (SMRT) sequencing and to count the repeat number based on an alignment algorithm. We show that we can readily detect expanded alleles in patient-derived samples and accurately determine their size up to at least 270CAGs. Additionally, we show that multiplexing is possible, thereby reducing cost significantly. This tool, once optimized, will facilitate molecular diagnosis of these devastating diseases.

Contracting CAG/CTG repeats using the CRISPR-Cas9 nickase

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CAG/CTG expansions cause over 12 different neurological diseases for which there is no cure. Because longer tracts cause more severe phenotypes, contracting the repeats may provide a therapeutic avenue. There is currently no efficient assay that can be used to test treatments for contraction biases and thus no agent is known to induce such a bias. Here we used a GFP-based chromosomal reporter that can monitor expansions and contractions in the same cell population. We found that inducing double-strand breaks within the repeat tract caused instability in both directions, whereas the CRISPR-Cas9 nickase induced almost exclusively contractions; with no detectable effect at repeats of non-pathogenic lengths. Contractions most likely arose from DNA gap intermediates rather than via single-strand break repair. Nickase-induced contractions depended on the DNA damage response kinase ATM, whereas ATR inhibition increased both expansions and contractions in a Msh2- and Xpa-dependent manner. Our results pave the way towards deliberate induction of CAG/CTG repeat contractions in vivo.

Deciphering sex differences of the dynamic transcriptional signatures during D. melanogaster aging

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The vast majority of species show sex differences in their lifespans. Although variable, the common aging pattern indicates shorter lifespan for males than for females. Most findings related to sex differences in aging have been based on wild populations. These studies are important to understand the evolutionary forces and life history traits. However, there is a limited understanding of age-related sex-differences on populations in laboratory conditions, and there is a need to understand fundamental cellular and molecular mechanisms that account for sex differences in longevity. Moreover, the mammalian pattern of greater female longevity has been attributed to several hypotheses, notably the 'unguarded X chromosome' and the greater mitochondrial dysfunction in male. These hypotheses have not been tested at a molecular level. The few mechanistic studies showed an impact of sex hormones on the immune response, as well as greater steady-state oxidative DNA damage in males compared to females. Here, we generated a time-series gene expression dataset of five time-points of D. melanogaster aging (11 days - 45 days) from male and female adults, isogenic and in a controlled environment. Using a novel Bulk RNA Barcoding and sequencing (BRB-seq) approach, we were able to obtain 9 biological replicates per sex and per age. We first show that the majority of the changes in gene expression are happening in the beginning of the adult life, concomitantly with sexual maturation. Beside the age-related changes of the metabolic processes in each sex, we detected a strong signal of age-related sex differences in cytoplasmic translation. While the pattern is variable across genes, it is consistent at the process level. We then profiled the gene regulatory network of two transcription factors (TFs), Unr and emc, that are active during D. melanogaster aging. These two TFs showed strong differences between sexes in the oldest flies. By taking into account both negative and positive regulation of TFs, we could pinpoint target genes, such as sPLA2 - a phospolipase 2, that are getting repressed over aging in females, but not in males. This study provides testable hypotheses for further research into the molecular mechanisms of age-related sex-differences, which will be necessary for future sex-specific aging and health interventions.

Evolution of olfactory pathways in Drosophilids

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Animals' brains are continuously evolving, as can be observed through the enormous variety in brain morphology found among closely-related animals. However, not all brain structures change at the same time or in the same fashion. Brain regions that are involved in the processing of environmental information, such as chemosensory-related structures, have been of particular interest because it appears they evolve rapidly. The reasons for this rapid evolution remains unclear, but it likely is related to constant changes in environmental conditions, and other driving forces. Among the evolutionary differences that have been observed in chemosensory-related systems, two intriguing examples in which we are interested include (1) the expansion and contraction of odorant receptor families and (2) changes in the neuromodulation of different neuron populations involved in the processing of olfactory information. In order to examine these two modes of sensory system evolution, we have employed Drosophila melanogaster, and its closely-related species, as a model system.

Knoto-ID: A tool for the topological analysis of open protein chains using the concept of knotoids.

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We present Knoto-ID the first command line tool that evaluates entanglements of open 3D-curves using the concept of knotoids. Knoto-ID can analyse the global topology of a chain as well as its local topology by either studying all of its subchains or by only detecting the knotted core. Moreover, Knoto-ID permits localisation of topologically non-trivial protein folds which complexity is not sufficient to form knots. Knoto-ID is not limited to the studies of protein folds but can also be applied to any open ended configuration such as chromosomes, random walks and synthetic polymers.

Control of non-melanoma skin cancers by PPAR?/?

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Skin squamous cell carcinomas (SCCs) are malignant tumours of keratinocyte origin that can arise from sun exposed areas of the body. Its highest incidence in Europe is in Switzerland. SCCs raise a major public concern due to their prevalence, high morbidity and abrupt increase in tumors occurring in the ageing population. Besides solar UV that is the major risk factor for developing skin SCC, this type of cancer is a major burden in patients under immunosuppressive treatment after solid organ transplant.
We have previously shown that the ligand activated transcription factors PPAR?/? promoted UV-induced skin SCC progression, while PPAR?/? loss-of-function prevented UV-induced skin response and skin cancer in mice. This raises a question of clinical importance: does PPAR?/? inhibition also prevent UV-induced skin response, and thereby skin cancers in human?
With this project, we pursue to (i) understand the role of PPAR?/? in SCC progression in vitro and (ii) to investigate the therapeutic potential of PPAR?/? regulation in vivo.

Transcriptomic signature of longevity in C. elegans

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Aging is the progressive decline of physiological integrity and function of an organism over time ultimately leading to the onset of age-dependent diseases. Longevity interventions, such as caloric restriction and reduced insulin/IGF-1 signaling, slow down the aging process. The nematode Caenorhabditis elegans is a key model system to study the role of aging and longevity. In the current literature, there is an abundance of studies describing gene expression changes associated with longevity. However, we currently lack an overall conceptual understanding of how the individual longevity interventions are related. Do longevity interventions share downstream targets or act via distinct mechanisms and are there genes, which consistently accompany longevity?
To answer these questions, we re-analyzed published longevity expression profiles (190 arrays, 130 sequencing runs) to directly compare longevity regulation across studies. We extracted and combined the gene expression changes caused by all known longevity interventions in C. elegans to identify the underlying gene expression signatures that might promote healthy aging. By re-analyzing the published datasets with a common pipeline, we are able to correct for variations arising from different laboratory conditions or used strains to extract the changes that are more longevity-specific. The majority of genes we found to be differentially expressed across longevity interventions in C. elegans are down-regulated during physiological aging. Intriguingly, longevity interventions display a large overlap in their targeted pathways indicating a shared mode of action. To expand the view offered by annotated pathways, we performed a GO term enrichment and identified the extracellular matrix genes as a key target enriched in both aging and longevity. Our comparative approach revealed a more robust network of gene expression changes shared by longevity intervention. These findings can be directly used to generate hypothesis to reveal novel mechanisms that promote healthy aging.

Can Krüppel Cripple?
Role of the Transcription Factor Krüppel-like Factor 6 (KLF6) in Beta-cell Function

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he transcription factor Krüppel-like factor 6 (Klf6) controls the expression of several target genes regulating various cellular processes such as inflammation, cell cycle regulation, apoptosis, and metabolism. However, the function of Klf6 in the pancreatic ?-cell has not yet been established. The aim of the study was to investigate the impact of Klf6 in the regulation of ?-cell function whose dysregulation is central in the pathogenesis of diabetes.
Here we assessed the regulatory contribution of Klf6 in glucose-stimulated insulin secretion (GSIS) upon its silencing in Min6B1 cells, a murine insulinoma cell line.
Upon siRNA-mediated knockdown (KD) of Klf6 in Min6B1 cells GSIS was normal for the first hour of stimulation at 16.7 mM glucose but levelled off afterwards (up to 6 hours). Insulin secretion stimulated by amino acids, in particular leucine and glutamine, was increased normally over a 6-hour incubation period.
Klf6-silenced cells displayed reduced glucose-induced oxygen consumption rates (OCR) and augmented extracellular levels of lactate reflecting impaired oxidative phosphorylation and glycolysis, respectively. Nevertheless ?-ketoglutarate stimulation resulted in equivalent OCR values between experimental groups further supporting Klf6's specificity to glucose sensing. This also suggests that Klf6 affects glucose signalling by interfering in step(s) upstream of the Krebs cycle.
mRNA expression analyses exhibited an up-regulation of genes involved in glucose uptake, glycolysis, pyruvate conversion to lactate, and lactate export in Klf6-silenced cells. A preference for glycolysis over oxidative phosphorylation upon Klf6 silencing may be triggered by an increase in pyruvate dehydrogenase kinase 1 (Pdk1) expression.
Collectively, our data confirms a decline in GSIS capacity upon Klf6 KD that may be explained by a reduction of the amount of pyruvate required for proprer insulin secretion. This effect may be brought on by Klf6's transcriptional regulation of genes involved in pyruvate metabolism.

CDK7 is critical for brown adipose tissue thermogenesis

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Brown adipose tissue (BAT) dissipates energy through Ucp1-mediated uncoupled respiration and its activation may represent a therapeutic strategy to combat obesity. Cyclin-dependent kinase 7 (CDK7) is a member of the cyclin-dependent protein kinase (CDK) family, which is an important regulator of cell cycle progression and more recently, metabolism. It functions as a Cdk-activating kinase (CAK). It is also an essential component of the transcription factor TFIIH, which is involved in transcription initiation and DNA repair.

Here we show that CDK7 controls BAT non-shivering thermogenesis in mice. We generate brown adipose tissue-specific CDK7 knockout mice and show that disruption of CDK7 in BAT does not predispose mice to systemic metabolic dysregulation. Compared with control littermates, CDK7 bKO animals had similar body weight and fat mass, indistinguishable basal energy expenditure, locomoter activity, and food intake. However, compared with wild-type littermates, these mice have decreased BAT mass and looks “whitening”. When the mice were challenged with cold exposure, there is no difference in response to cold if food is present. Interestingly, if food is deprived during cold exposure, the knockout mice develop hypothermia which was accompanied by a marked reduction in blood glucose and in stores of triglyceride in BAT. Thus, CDK7 is required for preparing mice for acute cold exposure during fasting state. RNAseq analysis indicated that loss of CDK7 disrupts the expression of metabolic genes (like PGC-1a, UCP1) in mitochondrial in response to cold. Currently, CHIP-seq is employing to decipher how CDK7 coactivate thermogenesis related genes in BAT.

The maintenance and protection of core body temperature through tight control of metabolism is a defining element of mammalian physiology. Our research demonstrated CDK7 is important to orchestrate nutrient homeostasis and thermogenesis to survive in life-threatening challenges, such as cold and starvation.

Eosinophils restore age-related adipose tissue dysfunction and sustain immunological fitness in old age

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White adipose tissue (WAT) besides its established role in nutrient sensing and energy storage, is being increasingly recognized as a highly active endocrine organ that undergoes significant age-related changes in distribution and function. There is emerging evidence that visceral WAT-derived inflammatory mediators contribute to the development of morbidity and mortality in old age. Interestingly, the reduction of visceral WAT through exercise, caloric restriction or surgical intervention has proven to prolong lifespan in different organisms and to promote healthy aging. From an immunologic perspective, adipose tissue eosinophils (ATE) have previously been described as important regulators of inflammation and metabolic disease in obesity. However, how aging impacts the regulatory function of ATEs remains unknown. Here, we identify that ATEs undergo significant age-related changes in distribution and function in both humans and mice. With age, ATEs acquire a senescent-like, pro-inflammatory phenotype associated with adipose tissue dysfunction and systemic low-grade inflammation. Approaches to restore ATE distribution and function using heterochronic parabiosis or adoptive transfer of eosinophils from young mice into aged recipients proved sufficient to dampen age-related adipose tissue inflammation and associated systemic low-grade inflammation. Restoration of a youthful systemic environment by means of eosinophil transfers resulted in the rejuvenation of stem cell pools in bone marrow which manifested in improved immunological fitness. Mechanistically, systemic rejuvenation of the aged host was mediated through eosinophil-derived IL-4. Together, these findings support a critical function of adipose tissue as a source of pro-aging factors and uncover a novel role of eosinophils in promoting healthy aging by sustaining adipose tissue homeostasis.

Role of Novel Kinases in IR

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It is well established that pro-inflamatory cytokines, such as IL-6 and TNF-?, affect insulin signalling, which in turn is essential to maintain glucose homeostasis and to regulate its metabolism in the liver, muscle, and adipose tissues. This leads to the stimulation of downstream protein kinases, thus activating and crosslinking numerous pathways, potentially resulting in insulin resistance. Consequently, insulin resistance status is determined by the type of activated inflammatory pathways, abnormalities of lipid metabolism, as well as in the type of activated kinases and their downstream targets. This project revolves around the role of novel kinases in SAT and VAT of patients that are insulin resistant (IR) or insulin sensitive (IS). Several of the known protein kinases involved in the onset of insulin resistant are AMP-activated protein kinase (AMPK), I?B kinase (IKK), protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), etc. Identifying new and specific protein kinases involved in obesity-induced chronic inflammation may help in developing the targeted drug therapies to minimize insulin resistance in patients.

Involvement of Potassium Channels in Neuropathic Pain

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Introduction

Nerve injury induces hyperexcitability through action potentials which sensitize the neurons in the first laminae of the dorsal horn. Microglia activation appears during this sensitization process and enhance it by the release of cytokines and chemokines. As a result, the nociceptive pathways is amplified and provokes hypersensitivity and allodynia: a state called neuropathic pain. In this study we investigate how potassium currents are modified in purified microglia and spinal cord (SC) slices after the spared nerve injury (SNI) model of neuropathic pain.

Methods
Adult male CX3CR1-GFP transgenic mice (20–24g) were sacrificed and lumbar spinal cord was incubated in the presence of papain for 30min at 30°C and microglia cells were kept in culture until experiment. Otherwise, the SC was kept in ice-cold oxygenated solution and sliced in 200µm thickness and microglia was patched in the ipsilateral dorsal horn. In patch-clamp experiments, after G?-seal formation, the whole-cell configuration was obtained under voltage-clamp at a holding potential of -20mV in culture and -60mV in slice.

Results
Two days after SNI the resting membrane potential (RMP) of microglial cells in culture was -29.73 ± 2.96 mV, n=37, $P<0.05$ when compared with sham 2 days -20.00 ± 2.40 mV, n=14, $P>0.05$ and naive conditions -16.77 ± 1.89 mV, n=45. The potassium currents recorded from microglial cells in culture in naive conditions at a hyperpolarized step of -160 mV (-43.86 ± 6.19 mV, n=21) were significantly smaller when compared with the currents recorded 2 days after SNI -84.55 ± 8.82 mV, n=47, $P<0.05$ and 2 days after sham -83.62 ± 23.28 mV, n=21, $P<0.05$. On the other hand, in SC slices the RMP was -35.86 ± 6.33 mV, n=8 in naive conditions and 2 days after SNI was -29.27 ± 10.57 mV, n=3, $P>0.05$ and 2 days after sham was -15.19 ± 2.37 mV, n=4, $P>0.05$. In slices there is no difference between the potassium currents at a hyperpolarized step of -160 mV in naive conditions (-18.05 ± 4.59 mV, n=8) when compared with sham 2 days (-10.39 ± 2.55 mV, n=4, $P>0.05$) and SNI 2 days (-16.66 ± 4.84 mV, n=3, $P>0.05$). The qPCR experiments indicate that the level of mRNA Kir2.1 increases 7 days after SNI.

Conclusion
The RMP and the potassium currents increases two days after surgery in microglial cells when cultured. In slice, both RMP potassium currents didn't show any change. Our results indicate that potassium channels seems not to be major actors in neuropathic pain.