Mass spectrometry (MS) has become the most prominent analytical tool for protein identification, post translational modifications (PTMs) characterization, and for metabolite identification and quantitation. While, several years ago different instruments were used for the analysis of proteins and small metabolites, these days there are several platforms which are used for both metabolomics and proteomics. In proteomics, there are two traditional approaches – gel-based and gel-free. While the laborious gel-based methods became less popular, the development of novel mass spectrometers together with various chemistries for relative protein quantitation boosted the gel free approaches. The main technologies that are used for proteome analysis are MALDI-TOF/TOF which replaced the MALDI-TOF while increasing throughput, the number of identified proteins in a complex sample and the confidence of identification. Another very common technology in proteomics is the high and very high resolution electrospray ionization (ESI) instruments (QqTOFs and orbitrap) which increased the confidence of identification and provide new tools for characterization of PTMs. This group of instruments has also an important role in qualitative metabolomics studies. The novel chemistries for relative protein quantitation are compatible with these two MS/MS technologies and are relatively accurate and easy to use due to the development of user-friendly software. The last group of instruments which is commonly used is the Triple Quadrupoles and the QTRAPs. These instruments were traditionally utilized for quantitative analysis of small molecules. While continuing their traditional tasks (in metabolomics studies) this family of instruments gains an important role in the characterization of PTMs and absolute protein quantitation. Beside the development of the proteomics and metabolomics tools, a new field is developing in biological mass spectrometry – MALDI tissue imaging. This novel field which is based mainly on MALDI-TOF/TOF and MALDI-QqTOF instruments enables the identification and localization of proteins and small metabolites in tissue and whole organism sections.
The Genome Sequencer FLX system is the newest addition to Roche’s next-generation sequencing platform, building upon proven technology and peer-reviewed publications. The system generates over 100 million bases per 7.5-hour instrument run and achieves long reads averaging 200-300 bases. The single-read accuracy is greater than 99.5% for over 200 base pair reads and the consensus accuracy is greater than 99.99%. Using the flexibility of the Genome Sequencer FLX, the system is able to perform more applications such as De novo Sequencing supported by paired-end applications, Comparative Genetics using Whole Genome Sequencing, Amplicon Resequencing, Transcriptome analysis, Gene regulation studies, etc. DYN G.S. LTD is a subsidiary of the Roche Diagnostics’ sole distributor in Israel, DYN Diagnostics. Our goal is to provide sequencing services based on the Roche/454 Genome Sequencer FLX system together with Post-sequencing bioinformatics analysis and close scientific support, upon all applications available on the Genome Sequencer FLX platform.
THE MOLECULAR BASIS OF EUKARYOTIC TRANSCRIPTION

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Abstract not available.
Congenital disorders of the mitochondrial respiratory chain are common inborn errors of metabolism with an incidence of 1:5,000–8,000 live births. Most Israeli patients originate from small consanguineous families and present with neurological disease and lactic acidemia. Precise diagnosis and genetic counseling is hampered by 1. The lack of a clear defect in skin fibroblasts even in patients with proven defect in muscle tissue 2. The large number of subunits which participate in the structure of the respiratory chain 3. The even larger number of factors, many still unknown, which participate in the assembly process of each complex, in the replication of the mitochondrial DNA (mtDNA) and in its expression. Using homozygosity mapping in these small families, we were able to identify several new defects in mtDNA replication and in mitochondrial translation and to describe their clinical presentations. The results were not only beneficial for the families but have also enabled a closer look into the these processes in human.
Single-gene disorders offer unique opportunities to shed light upon fundamental physiological processes in humans. Keratin disorders represent an exceedingly heterogeneous group of disorders manifesting with varying ectodermal defects. For years, keratins were considered as a prime example of structural proteins. The keratin central alpha helical rod segment was shown to play a major role in the formation of the epithelial cell cytoskeleton, with mutations affecting this part of the molecule being associated with the so-called classical keratinopathies. More recently, through the study of rare inherited skin disorders, we discovered that keratins play additional non-structural functions in epidermal cells including regulation of apoptotic activity in the basal cell layers, pigment distribution and organization of the cornified cell envelope, a major constituent of the mature epidermal barrier. These data form the basis for a revised understanding of the role of keratins during epidermal maturation and suggest novel therapeutic strategies for common skin diseases.
IDENTIFICATION OF TWO NOVEL GENES CAUSING COGNITIVE IMPAIRMENT AND AN OVERVIEW OF MOLECULAR MECHANISMS CONTRIBUTING TO MENTAL RETARDATION

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Mental retardation (MR) is a highly heterogeneous condition with a prevalence of 1-3\% in the general population. Genetic etiologies are found in approximately two thirds of MR cases. It can be subdivided into syndromic forms, which are characterized by MR and either malformations, dysmorphic features, or neurological abnormalities, and nonsyndromic forms, which are characterized by MR without any additional manifestations. Etiological diagnosis and genetic counseling for MR is one of the most difficult challenges faced today by clinical geneticists. The molecular basis of autosomal recessive non-syndromic MR (NSMR) is poorly understood. We have identified and evaluated clinically nine consanguineous families with severe autosomal recessive NSMR comprising 16 affected and 48 unaffected individuals, all living in the same village. We mapped the disease locus on chromosome 19p13.12 and identified a protein-truncating mutation in the novel gene CC2D1A. CC2D1A is a member of a previously uncharacterized gene family that carries two conserved motifs, a C2 domain and a DM14 domain. We demonstrated expression of CC2D1A mRNA in the embryonic ventricular zone and cortical plate in staged mouse embryos, with highest expression in the cerebral cortex and hippocampus. We have also identified a gene causing syndromic form of MR, autosomal recessive Infantile Bilateral Striatal Necrosis, in eight interrelated families including 12 patients and 39 unaffected individuals. Sequencing of the nup62 gene revealed a missense mutation in all the patients. This is the second example of a nuclear pore complex protein causing mendelian disease in humans. Our findings suggest that p62 has a cell type-specific role and is important in the degeneration of the basal ganglia in humans. Identification of specific disease-causing genes in isolated populations enables effective prevention of genetic diseases.
Human motor neuron diseases are characterized by degeneration of lower motor neurons of the spinal cord (spinal muscular atrophies, SMA), upper motor neurons (spastic paraplegia, HSP) or both upper and lower motor neurons (amyotrophic lateral sclerosis, ALS). SMA are frequent recessive autosomal disorders caused by mutations of the survival of motor neuron gene (SMN1) which lead to a reduced dose of the full length SMN protein (SMNFL). SMN facilitates the formation of the spliceosome. In spite of major advances in the biochemistry of SMN, the molecular pathway linking SMN defect to the SMA phenotype remains unclear. We and others have generated animal models of SMA. Deletion of the murine Smn exon 7 (SMNdelta7), the most frequent mutation found in SMA patients, has been directed to neurons leading to a late and moderate loss of motor neurons associated with defect in terminal axons and axonal sprouting. These data suggest a dying back process in SMA. Using new antibodies specific to SMNFL or SMNdelta7, our results strongly suggest that SMNFL has specific targets in motor neurons to explain the selective vulnerability of motor neurons in human SMA. Mutations of the spastin gene (Sp) are responsible for the most frequent autosomal dominant form of HSP. We have generated mice knock out for spastin. Mutant mice display progressive axonal degeneration characterized by focal axonal swellings. In culture, mutant cortical neurons showed normal viability and neurite density. However, they develop neurite swellings associated with focal impairment of retrograde transport. These defects occur near the growth cone, in a region characterized by the transition between stable and dynamic microtubules. These results highlight the link between spastin and microtubule dynamics in a specific region of axons. This is the first description of a human neurodegenerative disease involving this region of axon.
FROM DISEASE GENES TO DISEASE MECHANISMS

O.S. Birk

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Identification of the molecular basis of diseases enables understanding of their pathogenesis. To identify disease genes we set up 4 main venues: linkage analysis studies of inbred local population cohorts; in-depth studies of single cases of balanced chromosomal aberrations presenting with a unique clinical phenotype; mouse studies; and novel software that we have generated. We will give examples of how we use each of those different venues to identify the genetic defects leading to diseases such as psoriasis, extreme short stature, arthrogryposis and infantile neuroaxonal dystrophy - shedding light on the molecular pathways leading to those phenotypes.
CHICKS AND HUMAN EMBRYONIC STEM CELLS: DEVELOPMENTAL PLATFORMS TO STUDY FAMILIAL DYSAUTONOMIA

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Familial dysautonomia (FD) is an autosomal recessive congenital neuropathy characterized by poor development and progressive degeneration of the sensory and the autonomic nervous systems. The mutation that is responsible for the FD condition in more than 99% of the cases produces aberrant splicing of the IKBKAP mRNA product and skipping of exon 20. Consequently, the translation product is a truncated unstable IKAP protein. This mutation affects both normal development and survival of sensory and sympathetic neurons of the peripheral nervous system (PNS). Our aim is to establish experimental models to investigate the developmental role of IKAP in the nervous system, especially in the PNS and its implication in the FD phenotype. To this end we exploit the potential of human embryonic stem cells (HESC) to differentiate into neurons and glia in vitro and in the developing nervous system of chick embryos (in vivo). The developing neural tube of the chick embryo has the potential to provide the right environment for differentiation of the implanted human stem cells into PNS neurons in a matter of few days spatially and temporally. Preliminary experiments demonstrate the feasibility of this approach showing that 5 to 7 days after the implantation of the stem cells several human spinal cord and peripheral neurons develop in place expressing human specific neuronal markers. In parallel, we have characterized for the first time endogenous IKAP expression during chick embryo development. Importantly, we detected IKAP expression at the mRNA and protein levels in the right place and time when the peripheral nervous system (PNS) is formed. This result demonstrates that IKAP is expressed during PNS development supporting the etiology of the FD phenotype. Moreover, using our in vitro system of HESC differentiation we were able to detect for the first time IKAP protein localized within defined PNS neurons that in parallel showed electrophysiological activity. Regarding our efforts to find the role of IKAP during neuronal development, we show for the first time differences in IKBKAP mRNA levels at different HESC differentiation stages, of normal and FD derived HESC.
The mammalian RUNX transcription factors (TF) are master regulators of gene expression in several important developmental pathways and when mutated are involved in human diseases. Even though the RUNX TF recognize the same DNA-motif and regulate their target genes through interaction with common transcriptional co-activators or co-repressors, the functional overlaps between them are minor, and each RUNX protein has a distinct subset of biological functions. This paradigm is underscored by the findings that each of the corresponding RUNX knockout mice displays a unique subset of phenotypic abnormalities that correlate with cell-autonomous functions of RUNX as master regulators of cell lineage specifications. We have previously shown that this lack of functional redundancy results from a tightly regulated spatio/temporal expression mediated by an intricate transcriptional control mechanism. For example, both Runx1 and Runx3 genes are expressed in developing dorsal root ganglia, but in different classes of sensory neurons and both are expressed in mature T cells, but at different stages during T cell development. How this tissue specific expression of RUNX in a distinct subset of cell types and at defined time windows during development is attained? How these transcriptional regulators are regulated? We employed a series of overlapping BACs spanning large (i.e. hundreds Kb) of Runx3 and Runx1 loci to identify and characterize the long-range regulatory elements mediating tissue and stage specific expression of Runx1 and Runx3 during development and in adults. Using recombineering each BACs was modified to include in-frame reporter (LacZ) and then employed to produce transient transgenic mice. This approach culminated in generating a series of transgenic mouse strains each expressing a different Runx1 or Runx3 reporter BAC. Analysis enabled us to identify several subsets of conserved non-coding elements, residing hundred Kb upstream of the basic promoter/transcriptional start site, that confer stage and cell-type specific expression of Runx1 and Runx3 during embryogenesis and postnatally.
The understanding of the way genes are regulated is a cornerstone of molecular biology and medical research. We shall describe two novel methods from computational and comparative genomics for studying central problems in gene regulation. (1) the AMADEUS software for finding de novo regulatory motifs in promoters and 3' UTRs. The algorithms in AMADEUS perform better than the tools available today, especially on complex metazoan genomes, and also provides a convenient graphical interface along with a variety of analysis options and reports; (2) We developed a model for the evolution of promoters, which combines the effect of regulatory interactions of many different transcription factors; Based on this model, we developed algorithms to compute likelihood and to learn de-novo collections of transcription factor binding motifs and their selection parameters. Using the new techniques, we examined the evolutionary dynamics in Saccharomyces species promoters. Analyses of an evolutionary model constructed using all known transcription factor binding motifs and of a model learned from the data automatically reveal that surprisingly weak selection is affecting most binding sites. Moreover, according to our estimates, strong binding sites are constraining only a minor fraction of the yeast promoter sequence that is under selection.
TRANSCRIPTION AND mRNA DECAY ARE COORDINATELY REGULATED BY COMMON SHUTTLING FACTORS

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mRNA level is determined by the balance between its rate of synthesis and degradation. Little is known about any cross talk between the synthetic and decay machineries. We have identified a heredodimer, Rpb4/7, that functions in both transcription and in the two cytoplasmic mRNA decay pathways, while shuttling between the nucleus and the cytoplasm. During transcription, Rpb4/7 interacts with the transcript and later escorts the transcripts during its life cycle in the cytoplasm. At some stage during the mRNA decay pathway, the heterodimer returns to the nucleus to initiate a new round of transcription. In addition to Rpb4/7 that seems to play a general role, we have found class-specific factors that interact with Rpb4 and probably shuttle together with Rpb4/7 and function similarly to Rpb4/7. We found that the role of Rpb4/7 in mRNA decay is mechanistically coupled to its role in transcription. Thus, recruitment of Rpb4/7 to Pol II is a prerequisite to its roles in the cytoplasm. We propose that coupling between mRNA synthetic and decay machineries is made possible by general and class-specific factors that coordinate the activities of the two machineries to maintain proper levels of mRNAs. Rpb4/7 seems to function as a platform on which class-specific coordinators are assembled.
UNEXPECTED LINKS BETWEEN THE MAMMALIAN CORE PROMOTER TO TRANSCRIPTION ELONGATION AND PROTEIN TRANSLATION

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Transcription of mRNA encoding genes is divided into three tightly controlled steps: initiation, elongation and termination. A regulatory element critical for transcription initiation is the core promoter, which serves as the docking site for the pre-initiation complex. In the NF-kappaB signaling system we found that the regulation of target genes and their occupancy by distinct elongation factors are dependent on the NF-kappaB enhancer and the core promoter type. Converting a TATA-less to a TATA promoter switches the regulation of NF-kappaB from one elongation factor (DSIF) to another (P-TEFb). The underlying mechanism involves the formation of distinct initiation complexes on the two types of core promoter. Bioinformatic analysis revealed a tight correlation between core promoter type and gene size, a property directly associated with transcription elongation efficiency. Our findings suggest for close ties between the initiation and elongation phases of the transcription process.

The TATA-box, once thought to be a universal core promoter element is today known to be present in only ~20% of genes. We are investigating the structure of mammalian TATA-less core promoters by combining computational and molecular tools. We identified an overrepresented element that we named TTIE, located downstream to the transcriptional start site. This element appears in genes encoding for mRNAs with an extremely short UTR, and has a central ATG that serves as their translation initiation codon. TTIE efficiently directs translation initiation in vitro and in vivo. Remarkably TTIE is also critical for transcription, and the ATG sequence was found to be crucial for this process as well. Moreover TTIE can efficiently substitute for the TATA element, directing transcription initiation and contributing to promoter strength. These results highlight a link between the initiation stages of mammalian transcription and translation through common regulatory element.
The transition of progenitor cells to differentiated neurons is a multi-step process in which cell proliferation and differentiation are tightly coordinated. Pax6 is a pivotal determinant for both cell proliferation and differentiation in the eye, brain and pancreas. This gene is key regulator of eye development as it is both essential for eye formation and sufficient to induce eyes in invertebrate and vertebrate species. In mammals, Pax6 is known to be required for the multipotency of retinal progenitor cells (RPCs). The aim of this study was to explore Pax6 functions in the intracellular programs that regulate cell fate during retinogenesis. To this end, two Pax6 mutants that differ in the time and place of Pax6 inactivation were investigated; the systemic Pax6 knockout mutation (Pax6lacZ/lacZ) and the Pax6 somatic mutation in the RPCs (Pax6flox/flox;a-Cre). Comparing the retinal phenotype of these Pax6 mutants uncovered the existence of two distinct subpopulations of RPCs and revealed that in each of them, Pax6 governs a unique developmental program. In the RPCs that are located at the peripheral optic cup, Pax6 was found to be required for cell proliferation and for the completion of neurogenesis. In striking contrast, in the adjacent, centrally positioned RPCs, Pax6 was found to be dispensable for completion of neurogenesis but nevertheless to be essential for the execution of most of the neurogenic programs available to the retinal progenitors. The study reveals the existence of distinct populations of neuronal progenitors in the developing retina and exposes the dual role that Pax6 plays in retinal progenitor cells in mammals.
CALCIUM-REGULATED TRANSCRIPTION: LESSONS FROM PLANTS TO HUMAN

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Calcium is a ubiquitous second messenger in all eukaryotes. In plants it mediates responses to biotic and abiotic (chemical and physical) stimuli and stress situations. However, how calcium signals mediate gene expression in eukaryotes is not fully understood. We seek to characterize the cis-regulatory elements, trans-acting factors and other cellular factors that mediate calcium-responsive gene expression in plants. By linking artificial cytosolic calcium transients with rapid transcriptome changes we have identified calcium-responsive cis elements (refs.1,2), which are similar to a subgroup of cis-elements that mediate plant responses to the stress phytohormone abscisic acid (ABA). In addition, using molecular screening procedures we identified a novel family of calcium/calmodulin-binding transcription activators (designated CAMTAs; ref.3), which appear to be conserved in all multicellular organisms and in some unicellular eukaryotes. In plants, CAMTAs integrate growth processes with stress signals. Humans contain two CAMTAs. HsCAMTA1 is suspected to be involved in tumor suppression and memory performance, whereas HsCAMTA2 is involved in cardiac growth and hypertrophy. The unique Drosophila CAMTA functions as a transcription regulator of a gene encoding an F-box protein responsible for deactivation of stimulated rhodopsin. The mechanisms of calcium-regulated transcription operating through CAMTAs in plants and other eukaryotes will be discussed.

The genetic basis of population divergence leading to adaptive radiation and speciation is an unresolved problem of evolutionary biology. Molecular elucidation of “speciation genes” yet remains without clear identification of the gene complexes participating in reproductive isolation, particularly, in sympatry. Genetic divergence was discovered between Drosophila melanogaster populations inhabiting ecologically contrast slopes in “Evolution Canyon” (EC), Mt Carmel. Despite easy migration, strong interslope divergence was established at EC involving habitat choice, mate choice, thermal and drought tolerances, and polymorphism for adaptive genes. Parallel patterns of stress tolerance, habitat choice, and mate choice were also demonstrated in D. simulans. Our current focus is on genes that presumably contribute to genetic variation in stress tolerance and sexual behavior. Interslope differences in the sequence encoding the (Thr-Gly)n repeat of the period gene were established, suggesting evolutionary functional importance. Mate choice tests show that females derived from the North-facing slope distinguish between males with specific per alleles, as well as between males from the opposite slopes. Occurrence of different alleles was also shown for desat2 gene, affecting the female cuticular hydrocarbon synthesis, involved in mating discrimination. However, tests for interslope genetic differentiation in Drosophila carried out in some laboratories gave somewhat controversial results. A possible explanation could be that slope-specific selection can withstand destructive effects of interslope migration, but it should not necessarily be accompanied by differentiation for selectively neutral markers, unless the latter are in linkage disequilibrium (LD) with selected loci. LD can be maintained despite migration, but only under tight linkage and strong selection. For some Drosophila genes, LD is known to decay within just few kilobases. Thus, differentiation for adaptive traits and relevant candidate genes (like per or heat shock protein genes) seems to be much better evidence for divergent selection than that displayed by genetic distances estimated using molecular markers.
Ricotia lunaria is a cruciferous plant like Arabidopsis. Employing the hybridization of Ricotia’s samples with Arabidopsis tiling array, we have identified hundreds of genome elements with the specificity of their transcription on opposite slopes of the “Evolution Canyon” (EC) on Mount Carmel, Israel. Ricotia RNA samples taken under normal conditions and temperature stress were contrasted with RNA samples from adult and seedling Arabidopsis plants. The novel method of analysis allows the sensitive identification of upregulated genome fragments even for cross species hybridizations. Several gene categories are specifically upregulated on the stressful EC “African” (A) slope in contrast to the EC “European” (E) slope and Arabidopsis. These GO categories involve the phosphate transport, phosphor uptake and phosphorylation, abscisic acid mediated signaling and multiple responses to stresses. Also, more heat response genes are activated at A and three times more transcribed transposons were identified at A. The more shaded EC “European” (E) slope demonstrates upregulation of chlorophyll related processes, photosynthesis and carbon utilization GO categories. The advanced analysis of tiling array hybridizations reveal many genome wide transcriptional events including activation of pseudogenes in the CG reach area of chromosome 2, expression activity of multiplicity intergenic regions, and transcription of putative silencing RNAs. Interestingly, the zone of actively expressed pseudogenes in the CG reach area of chromosome 2 is flanked by the transposon reach genome regions.
Light reception is fundamental to even the simplest multicellular animals which often do not have eyes. For example, many marine organisms are sensitive to blue shifted moonlight which serves to cue the highly synchronized spawning of many invertebrates including corals. The receptors and underlying mechanisms, however, are largely unknown. Here we report the presence of an ancient family of blue-light-sensing photoreceptors, cryptochromes (CRY), in a Scleractinian coral (Acropora millepora) from the simple multicellular animal phylum, Cnidaria. The coral cryptochrome Cry1 clusters with mammalian-type (m-type) cryptochromes, whereas Cry2 is basal to both the m-type cryptochrome and 6-4 photolyase protein families. Despite having greater similarity to mammalian cryptochromes, coral cry1 and cry2 expression is like that of cryptochromes found in Drosophila and to cry4 found in zebrafish. Expression of the two genes was rhythmic under a light dark cycle (LD) and stable in constant darkness (DD). On the reef crest cry2 expression was maximal at midday during diel cycles of both full and new moon nights. Most importantly, the expression of cry2 increased during the full moon versus new moon. Immunohistochemistry and in situ hybridisation using specific antibodies and RNA probes designed against cry1 and cry2 products revealed expression and presence of the protein in the ectoderm layer of larval and adult corals, consistent with the hypothesis that these proteins may act as photoreceptors. This work has revealed fundamental molecular elements within simple eyeless invertebrates that may cue the synchronized spawning events of the largest coral reef ecosystem in the Great Barrier Reef.
In the oligotrophic marine environment there are ecological niches diverse in microbial populations including protists, fungi, bacteria, archea and viruses. One such niche is the coral holobiont, made up of coral host tissues and accompanying symbiotic microorganisms. Interactions among microorganisms found on the coral may be symbiotic or competitive. It has been hypothesized that this community may play a role in the coral holobiont defense, nutrition and nutrient cycling. Screening of microorganisms isolated from mucus layer of a number of scleractinian coral species was carried out to assess and describe these communities and their possible role in coral wellbeing. A number of protists were identified as part of the coral mucus, surface, and tissue community. These organisms were morphologically and molecularly identified as belonging to the Stramenopila. Fatty acid profile of the isolated strain Fng-1 revealed an array of poly-unsaturated fatty acids (PUFA) indicating that they may contribute to the coral holobiont nutritional requirements. Screens of coral mucus-associated bacteria indicated that ~ 25% of the culturable community demonstrated antimicrobial activity. For example isolates related to the genus Vibrio and Pseudoalteromonas demonstrated high activity against both gram positive and gram-negative bacteria. These microorganisms may take part in protecting the coral against potential pathogens. In addition to bacteria, coral mucus was found to contain also Archaea. Archaea sequences revealed dominance of Crenarchaeota most of which were similar (greater than or equal to 97%) to candidatus Nitrosopumilus maritimus, an ammonium oxidizer. Most of the Euryarchaeota sequences were related to marine group II and III and other clades that were related to anaerobic methanotrophic archaea and anaerobic nitrate reducer archaea. The coral-associated Archaea may provide a sink of excess ammonium excreted from the coral host and trapped in the mucus layer. This array of symbiotic coral associated microbes may play important roles in holobiont physiology survival and evolution.
In this lecture I present one of the challenges to the evolutionary modern synthesis, which has been the dominant paradigm of evolutionary thinking since the 1940’s. After a characterization of the modern synthesis, its basic tenets and assumptions, I highlight the challenge coming from studies of epigenetics, and especially of epigenetic, transgenerational inheritance. The expanded view of heredity that these studies suggest, which incorporates soft or “Lamarckian “ inheritance, have implications that are altering assumptions about the rate, direction and dynamics of evolution. It is also related to other challenges to the prevalent view, such as a view emphasizing saltational events during evolution. I suggest that these and other recent challenges lead to a wider and more pluralistic view of evolution than that suggested by the Modern Synthesis, and that a new evolutionary synthesis is now required.
In 1995 we demonstrated that Vibrio shiloi was the causative agent of bleaching the coral Oculina patagonica. Subsequently, it was shown that Vibrio coralliilyticus was responsible for bleaching the coral Pocillopora damicornis in the Indian Ocean and Red Sea. From 1996 to 2002 we studied the infection of O. patagonica by V. shiloi both in the field and laboratory and reported that the pathogen is chemotactic to the coral mucus, adheres to a galactose-containing receptor on the coral surface, and penetrates into the exoderm where it differentiates into the VBNC state, multiplies intracellularly and produces a peptide photosynthesis inhibitor. The marine fireworm Hermodice carunculata is a winter reservoir and spring-summer vector for transmitting the bleaching disease. Starting in 2003, we observed that O. patagonica developed resistance to V. shiloi. Healthy corals taken from the sea could no longer be infected with the pathogen, and V. shiloi could no longer be isolated from corals. When we inoculated corals with V. shiloi, the bacteria adhered, penetrated and then were killed, aborting the infection. To explain these findings we presented the coral probiotic hypothesis, which posits that the corals acquired bacteria in their mucus and/or tissues that can kill V. shiloi. We now generalize from this hypothesis and present the hologenome theory of evolution-the role of symbiotic microorganisms in the evolution of animals and plants.
A GLIMPSE INTO THE ATOMIC STRUCTURE OF PLANT PHOTOSYSTEM I – 3.5 BILLION YEARS OF PERFECTION

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The photosystem I (PSI)-like reaction center is arguably one of the most fundamental reaction centers in Nature. It emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. The recently determined structure of plant PSI, which is at the top of the evolutionary tree of this kind of complexes, provided the first relatively high-resolution structural model of a super-complex containing a reaction center (RC) and a peripheral antenna (LHCI). The RC is highly homologous to that of the cyanobacterial PSI and maintains the position of most transmembrane helices and chlorophylls during the last 1.5 years of separate evolution. The LHCI is composed of four nuclear gene products (Lhca1-Lhca4) that are unique among the chlorophyll a/b binding proteins in their pronounced long-wavelength absorbance and their assembly into dimers. The structure provided a first glimpse at the architecture of the most efficient nano-photochemical machine in Nature and it tells a tale on the evolution of terrestrial life. The structure should provide a template for designing artificial systems amenable for utilizable energy production.
HIGHLY EFFICIENT HYBRID BIO-METAL AND SEMICONDUCTOR NANO PHOTOVOLTAIC AND PHOTO SENSOR DEVICES

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The electronic coupling between the photoactive proteins and semiconductors or metals is used for fabrication of hybrid bio-solid-state electrooptical devices. The robust cyanobacterial nano-sized protein-chlorophyll complex photosystem I (PS I) can generates photovoltage of 1 V with internal energy conversion efficiency of 47% (~23% for solar energy), quantum efficiency of ~1 in dry environment are used as phototransistors gate and photovoltaic devices. A functional oriented junctions are fabricated by covalently binding genetically engineered cysteine mutants of PS I proteins to GaAs and metal surfaces. Placed between a metal electrodes and a transparent conducting glass PS I nano photovoltaic cell generated, in preliminary results, photocurrent of 0.7 mA/cm\(^2\) at 0.2 V and enternal energy conversion efficiency of 34%. The photovoltage of 0.5 V is generated by the dry oriented monolayer of PS I protein on transistor channels can be use to develop highly sensitive photon counting detectors.
Changes in the electrostatic potentials (ESP) on atoms in molecules are proposed to play key roles in many experimentally observed processes including inter and intramolecular charge mobilization, protein folding, biotic and abiotic catalysis. Yet, up to-date direct measurement of such changes and evidences for their effect on particular reactivity are rare. Here we measure changes in the ESP on specific atoms in bacteriochlorophyll monolayers on different surfaces, and show for the first time, their effect on the harvesting and localization of donated electrons. Computational analysis of the examined molecules, provides a rational for the experimental observations. The obtained results suggest that self-assembly of the chlorophyll cofactors in photosynthetic reaction centers maximizes the electron transfer rates from the primary donor to the tertiary acceptor. Following physico-chemical paradigms, these rates were predicted to increase exponentially with rising temperatures ensuing different yields at the physiological temperatures of mesophiles and extremophiles. In contrast, we recently showed a meticulous adjustment of energy conversion resulting in similar rates and yields at those temperatures, and unveil the underlying molecular details (1). The key players in the temperature adjustment, comprise a cluster of hitherto unrecognized protein cavities and adjacent packing motif that jointly impart crucial localized flexibility to the reaction center proteins. Mutations within the packing motif of mesophiles that increase the residues bulkiness and reduce the cavities size, promote thermophilic behavior. The novel biomechanical mechanism accounts for the slowing of the catalytic reaction above physiological temperatures in contradiction to the classical Arrhenius paradigm. Following the above guidelines we have generated novel thermoplastic strains of cyanobacteria. Protein flexibility acclimatizes photosynthetic energy conversion to the ambient temperature, Shlyk-Kerner O, Samish I, Kaftan D, Holland N, Sai PSM, Kless H, Scherz A. (2006), NATURE 442, 827-830
Microalgae and cyanobacteria can survive under the most extreme environmental conditions on earth from extreme temperatures and high irradiance to extreme pH and hypersalinity. Investigations of the adaptation mechanisms of algae to extreme conditions, led to the discoveries that some species of algae can accumulate large amounts of economically valuable biochemicals in response to environmental stresses. For example, many algae accumulate huge amounts of triglycerides or starch under nutrient deprivation, which are potential sources for biofuels, some species accumulate polyunsaturated fatty acids, Dunaliella accumulates beta-carotene and Haematococcus accumulates astaxanthin for photoprotection, which are in high demand for their nutritional and disease-preventive qualities. I will describe two examples of adaptation of the photosynthetic system in the halotolerant algae Dunaliella to high salinity and to iron deprivation. At high salinity Dunaliella accelerates its photosynthetic activity to enable massive biosynthesis of glycerol, the osmotic element in this algae. A proteomic analysis revealed that this unusual response involves upregulation of the rate-limiting enzymes in the Calvin cycle and enhanced starch mobilization. Photosynthetic organisms are sensitive to iron deprivation which inhibits electron transport activity. The most vulnerable unit to Fe deprivation is PS-I. Dunaliella adapts well to iron deprivation by fortification of PS-I with a 40 kDa chlorophyll a/b binding protein. Dunaliella also evolved a unique mechanism for Fe acquisition that involves massive binding and internalization of iron. This capacity involved acquisition of transferrins and functional adaptation of a multicopper ferroxidase.
Mechanisms for iron storage play a central role in maintaining primary productivity in iron limited aquatic environment. Major iron storage complexes in photosynthetic organism include ferritins, bacterioferritins and DPS proteins. In order to study the function of these proteins we have inactivated the bfrA, bfrB and mrgA genes in the cyanobacterium Synechocystis sp. PCC6803. The bfr genes code for the two subunits of the bacterioferritin complex. The mrgA protein of the cyanobacterium is a member of the DPS Fe storage protein family. The physiological role of this protein was studied by measuring intercellular Fe quotas, 77K chlorophyll fluorescence and growth rates. It was found that inactivation of any of one of these three genes results in a significant slowdown of growth on Fe limited media. However, significant differences in the internal iron quota were measured. The iron quota of bfr mutants was reduced approximately 50% that of the wild type, while the iron quota of the mrgA mutant was 10% higher than that of the wild type. Based on these results, we suggest that mrgA plays an important role in the transport of intracellular Fe from storage (within bacterioferritins) to biosynthesis of metal cofactors throughout the cell's growth. The reduction of the internal iron quota had a direct effect on the photosynthetic performance as observed by a decrease in the number of PSI subunits per cell and the induction of the isiA antenna system even under iron sufficient conditions.
CRYSTAL STRUCTURES OF THE CYANOBACTERIAL PHYCOBILISOME ANTENNA COMPLEX: ASSEMBLY AND DISASSEMBLY OF A GIANT

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In cyanobacteria and red algae, the major light harvesting pigment-protein complex is the phycobilisome (PBS), an enormous complex with a molecular weight of 3-7MDa. Three dimensional structures of isolated components have been determined by X-ray crystallography for a variety of species; however our understanding of the overall structure PBS structure is still dependent on low resolution studies. We describe here high resolution structural information obtained on isolated components of the PBS from the thermophilic cyanobacterium T. vulcanus that shed light on the process of PBS assembly. We will also describe our progress in determining the structure of the entire PBS complex by X-ray crystallographic methods. Under certain conditions of nutrient starvation, the PBS is disassembled in an ordered manner. We report here the three-dimensional structures of the NblA protein, an essential component in the disassembly process, from two cyanobacterial species (T. vulcanus and S. elongatus). Comparison between structures and homology based models indicate that while the NblA family is only weakly homologous, there are certain attributes of the protein that remain constant, and could be involved in its function. Random mutagenesis in the S. elongatus protein shows that critical residues affecting the disassembly process in vivo can be found at internal positions as well as at the polypeptide termini leading to a model for the NblA proteins mode of action which is different than previously suggested.
We initiated structure-function studies of the voltage-dependent calcium channel (VDCC) family several years ago. These protein complexes enable the passage of Ca2+ from the extracellular milieu into the cell. Intracellularly, Ca2+ plays a central role in signaling and regulates processes ranging from contraction to secretion to gene transcription. Our first structural insights into the VDCC came from biochemical and crystallographic studies of beta, a necessary and intracellularly located auxiliary subunit and its association with an 18 residue peptide derived from an intracellular linker of the membrane channel subunit, alpha1. I will describe our most recent findings and ongoing experimentation on the VDCC from a beta perspective.
THE VOLTAGE-GATED Ca2+ CHANNEL IS THE Ca2+ SENSOR PROTEIN OF STIMULUS-SECRETION COUPLING

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The coupling of voltage-gated Ca2+ channel (VGCC) to exocytotic proteins suggests a regulatory function for the channel in depolarization-evoked exocytosis. We found that substituting La3+ for Ca2+ supported exocytosis in divalent ion-free solution. Secretion was induced by membrane depolarization using 60 mM KCl. We tested secretion in chromaffin cells using amperometry, and in PC12 cells measuring the release of tritiated catecholamine (Lerner et al., 2006). Secretion was elicited when La3+ substituted for Ca2+ in nominally Ca2+ free solution also in pancreatic beta-cells. In the pancreatic islets we stimulated secretion of insulin by high glucose that leads to the membrane depolarization and the opening of voltage gated Ca2+ channel (Trus et al., 2007). The selective channel blockers nifedipine, or verapamil inhibited depolarization-evoked secretion in La3+. This competition indicates specific binding of La3+ at the pore of L-type VGCC, probably at the selectivity filter composed of the poly-glutamate (EEEE) locus. Consistent with impermeability of La3+ the highly sensitive La3+/fura-2 imaging assay (approximately 1 pm) detected no La3+ entry, nor alterations in cytosolic Ca2+. These results provide strong evidence that occupancy of the pore of the channel by an impermeable cation leads to a conformational change that is transmitted to the exocytotic machinery upstream of intracellular cation build-up (intracellular Ca2+ concentration). Our model that was demonstrated in adrenal and pancreatic cells, allows for a tight temporal and spatial coupling between the excitatory stimulation event and vesicle fusion. It challenges the conventional view that intracellular Ca2+ ion build-up via VGCC permeation is required to trigger secretion and establishes the VGCC as a plausible Ca2+ sensor protein in the process of neuroendocrine secretion. Lerner et al. J Neurochem 97: 116-127 (2006) Trus M et al., Biochemistry (2007) (in press)
IDENTIFICATION AND CHARACTERIZATION OF CA2+-BINDING PROTEINS AND LOCALIZATION OF THEIR CA2+-BINDING SITES USING AZIDO RUTHENIUM: A NOVEL PHOTOREACTIVE REAGENT

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As a signaling molecule, Ca2+ carries information pivotal to cell life and death via its reversible interaction with a specific site in those proteins with which it interacts. In elucidating Ca2+-signaling mechanisms, it is important to identify the Ca2+-binding proteins involved, and to then localize and characterize their Ca2+-binding sites and function in the cell. Towards these ends, we have synthesized and characterized a novel photoreactive reagent, azido ruthenium (AzRu), which interacts specifically with Ca2+-binding proteins and strongly inhibits their Ca2+-dependent activities, regardless of their catalytic mechanism or functional state; purified proteins, embedded in the membrane or in intact cells. As expected from a Ca2+-binding protein-specific reagent, AzRu had no effect on Ca2+-independent, Mg2+-dependent activities. Moreover, radio-labeled 103Ru[AzRu] covalently bound and specifically labeled Ca2+-binding proteins. AzRu was successfully used to localize and characterize the Ca2+-binding site of the voltage-dependent anion channel (VDAC), known also as the mitochondrial porin. VDAC, located in the outer mitochondrial membrane, plays an important role in the metabolic coupling between the cytosol and mitochondria. In this crosstalk, mitochondrial Ca2+ homeostasis play a major role. Apoptotic and necrotic cell death can be induced by mitochondrial Ca2+ overload and thus VDAC, as the primary transporter of Ca2+, plays a major role in this process. VDAC is also recognized as a key protein in other forms of mitochondria-mediated apoptosis. The nature and function of the VDAC Ca2+-binding sites that regulating its activities associated with cell life and death will be demonstrated. To conclude, AzRu is an important tool for identifying novel calcium-binding proteins and localizing their Ca2+-binding sites, thus providing an opportunity to explore new Ca2+-dependent activities and functions in cell life and death.
TRP CHANNLES, WHAT ARE THEY AND WHY ARE THEY IMPORTANT

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TRP channels constitute a large and diverse family of proteins that are expressed in many tissues and cell types. The TRP superfamily is conserved throughout evolution from nematodes to humans. The name TRP is derived from a spontaneously occurring Drosophila mutant lacking TRP that responded to a continuous light with a Transient Receptor Potential (therefore, it was designated TRP by Minke). The Drosophila TRP and TRP-like (TRPL) channels, which are activated by the inositol lipid signaling cascade, were used later on to isolate the first mammalian TRP homologues. TRP channels mediate responses to light, nerve growth factors, pheromones, olfaction, taste, mechanical, temperature, pH, osmolarity, vasorelaxation of blood vessels, metabolic stress and pain. Potentially, a phospholipase C (PLC)-mediated signal transduction can activate the TRP channels by at least three putative mechanisms: by second messengers, by removal of inhibition and by change in membrane curvature.

We investigated these putative mechanisms and show that conversion of PIP2 to DAG or PUFA, which cause membrane lipid packing modifications, changes the curvature of the plasma membrane and activate the channels by a channel-lipid interactions. We have used expression system of Drosophila cell-line to express the TRPL channel together with constructed enzymes that cause PIP2 hydrolysis or phosphorylation of phosphoinositides without activation of PLC or production of DAG. In this system upon addition of a dimerizing drug, PIP2 was selectively depleted by constitutively membrane-targeted yeast Inositol polyphosphatase 5’ phosphatase, without the production of DAG, InsP3, or subsequent calcium signals. The induction of TRPL-dependent current by the dimerizing drug, strongly suggests that change in membrane curvature activate the TRPL channel. The activation mechanism of the Ca2+ permeable Drosophila TRPL channel will be used to acquire insights on the activation mechanisms of mammalian TRP channels.
Voltage-dependent Ca channels (VDCC) of the heart and smooth muscle belong to the L-type family. The main channel subunit, alpha-1C, contains the voltage sensor and the gate in its transmembrane part, and a large cytosolic domain involved in regulation of gating by numerous factors. The cytosolic domain comprises N- and C-termini (NT and CT) and 3 large loops, L1-L3. The structure of the cytosolic domain and the modes of interactions between its components are not known. We proposed that protein kinases (such as protein kinase C and A), auxiliary beta subunit, calmodulin and additional regulatory proteins regulate the channels by changing interactions between cytosolic segments, especially NT and CT. We find a specific role of the NT and CT in regulation of various gating functions and modulatory effects of beta subunit, calmodulin, calcium-binding protein 1 and protein kinase C. We present evidence for interaction of NT and CT domains and discuss the role of these interactions in channel inactivation and in modulations by the various proteins mentioned above.
Background: L-type calcium channels (LTCC) play a key role in cardiac function. ZnT-1 is a ubiquitous protein shown to inhibit cationic influx. We studied ZnT-1 effects on the LTCC activity in Xenopus oocytes and cardiomyocytes in culture attempting to elucidate its, yet unknown activity, in the heart. Methods and Results: In Xenopus oocytes, two electrode voltage clamp measurements showed that ZnT-1 co expressed with the LTCC led to reduction of the LTCC current with no apparent shift in the current -voltage relationship (n=34, p<0.01). In isolated rat cardiomyocytes, ZnT-1 expression was increased by transfection to 464±55% of control (n=5, p<0.05), while siRNA designed to inhibit ZnT-1 expression, decreased the expression of ZnT-1 in cardiomyocytes to 60.6±3.6% of control (n=4, p<0.05). In the cardiomyocytes the nifedipine sensitive Ba2+ influx, assessed by fura-2 and application of high [K+]o, was reduced in ZnT-1 transfected cells to 51.0±2.5% of controls (p<0.01), while siRNA increased the influx to 167.0±4.25% (p<0.01). Rapid electrical pacing of cardiomyocytes, at double threshold intensity, increased ZnT-1 expression to 214.4±15.1% of controls (p<0.01, n=7) and reduced Ba2+ influx to 52.7±8.6% (p<0.01). This was without changes in expression of the LTCC f1 c subunit. Conclusions: Our findings are consistent with ZnT-1 being a major physiological inhibitor of the cardiac LTCC. Consequently, ZnT-1 seems to be a missing link in tachycardia-induced cardiac electrical remodeling.
A MULTISTEP EPIGENETIC MECHANISM FOR THE INACTIVATION OF EMBRYONIC-SPECIFIC GENES

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Oct-3/4 is a POU domain homeobox gene expressed during gametogenesis and in early embryonic cells, where it has been shown to be important for maintaining pluripotency. Following implantation, this gene undergoes a novel multi-step program of inactivation that involves direct inhibition of transcription, heterochromatinization through the tri-methylation of H3K9 and subsequent DNA methylation. Interestingly, both of these latter epigenetic structures are actually put in place by the SET domain-protein, G9a, and it appears to be this event that causes its irreversible silencing in subsequent somatic cell lineages. Since Oct-3/4 expression is known to be necessary but not sufficient for pluripotency, there must be additional genes that undergo inactivation during embryonic cell differentiation, and it is likely that G9a plays a role in this process. Indeed, our microarray analysis shows that G9a is a master structural regulator that plays an important role in early development by targeting a wide network of embryonic genes for post-implantation repression. This silencing process includes key genes, such as Oct-3/4, Nanog and Dnmt3L that are intimately involved in maintaining the embryonic stem cell phenotype and for establishing maternal imprints in mammalian germ cells. G9a operates in a double manner, by employing its SET domain to generate heterochromatin, and utilizing its ANK domain to carry out de novo methylation. These modifications appear to be programmed for carrying out two separate biological functions, with histone methylation serving as a block to target-gene reactivation in the absence of transcriptional repressors, while DNA methylation represents the major barrier to embryonic reprogramming.
Differentiation of pluripotent cells is known to involve complex interactions between multiple regulation layers, including key regulatory genes, non-coding RNA, and external stimulation. How these layers are coordinated during the differentiation of pluripotent cells is, however, far from being fully understood. To study the coordination between gene and microRNA regulation in an embryonic stem cell (ESC) model of early human development, we analyzed in parallel gene and microRNA kinetic profiles during hESC differentiation in response to developmental signals. Examination of gene expression signatures revealed two temporal regimes that likely correspond, respectively, to pre- and post-onset of treatment-specific gastrulation patterns. Analysis of microRNA expression profiles identified two sets of microRNAs whose kinetic profiles were significantly correlated and anti-correlated, respectively, with the profiles of their predicted targets. Strikingly, the expression of the correlated group was higher in the presumed gastrulation phase, while the expression of the anti-correlated group was higher in the presumed pre-gastrulation phase. Further bioinformatics analysis reveal intrinsic differences between the two groups of microRNA, their respective targets and the complexes they form with their targets. Altogether, the results suggest a developmental scenario in which the pluripotency-associated microRNAs primarily degrade differentiation genes during the pre-gastrulation phase, while the differentiation-associated microRNAs preferentially inhibit the translation of differentiation genes during the gastrulation phase.
Human embryonic stem cells (hESCs) are pluripotent cells isolated from blastocysts. Traditionally, these cells have been cultured with supportive layers in two-dimensional cultures, which allow their continuous growth as undifferentiated cells. However, any future use of hESCs for cell-based therapy and for industrial purposes will require a scalable, reproducible and controlled culture system. Here we present for the first time a suspension culture system for undifferentiated hESCs that uses either Petri dishes or an Erlenmeyer, and medium supplemented with serum replacement, bFGF and interleukines. Human ESCs were cultured in this system as small spheroids for over 50 passages (160 doublings) and still maintained all ESC features, including stable karyotype, pluripotency, and proliferability. This suspension culture system will both enable the routine culture of hESCs in a 3D environment and facilitate mass production of undifferentiated hESCs required for clinical and industrial utilization.
NOVEL APPROACHES FOR HARVESTING, CRYOPRESERVATION, EXPANSION AND TISSUE REGENERATION POTENTIAL OF HUMAN UMBILICAL CORD BLOOD

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Human umbilical cord blood (HUCB) has established itself as a legitimate source for hematopoietic stem cell transplantation. We have developed some innovative approaches to try to increase the number of harvested stem/progenitor cells and to overcome the 20-30% of cells lost while thawing the cryopreserved HUCB grafts. For doing so, we have developed a closed, sterile disposable system that enables the "semi" controlled collection of HUCB and lyophilized HUCB MNC, using a directional freezing technology that enables the precise control of ice crystal morphology during the freezing process, thus making the use of intracellular cryoprotectant agents (CPAs) such as DMSO not necessary. Additional approach for increasing HUCB derived stem/progenitor cells is ex vivo expansion. For this purpose we took advantage of the multidrug transporter MDR1 (ABCB1) gene product (Pgp) reported as being over-expressed in various stem cells relative to their differentiated progeny. We compared the expression level and activity of Pgp in HUCB-derived CD133+ HSCs relatively to CD133? cells. Analyses of freshly isolated CD133+ HSCs from various donors (n=6) indicated that the majority (>92%) of these HSCs express functional Pgp on the cell surface. At optimal colchicin (novel MDR inhibitor) dose (2.5 ng/ml), we were able to achieve 2.9±0.5 fold expansion of HUCB CD133+ HSCs. The last approach that we have tried in an attempt to improve and facilitate engraftment post HUCB transplantation is to co-administer the HUCB cells with mesenchymal stem cells (MSCs). In addition, we have assessed the potential of HUCB derived cells in tissue regeneration for neurologic and cardiac applications. Results of both studies will be presented and the tissue regeneration potential of HUCB will be discussed.
Expansion of insulin-producing beta cells from adult human cadaver islets could alleviate donor shortage for cell-replacement therapy of diabetes. A major obstacle to development of effective expansion protocols is the rapid loss of beta-cell markers in the cultured cells. We developed a genetic cell-lineage tracing approach for following beta-cell fate, using an insulin-promoted Cre-loxP system introduced by viral infection. This method provided for the first time direct evidence for dedifferentiation, survival, and replication of cultured adult human beta cells. Proliferation of sorted label-positive cells requires soluble factors secreted by non-beta pancreatic cells. The tracing system will allow optimization of beta-cell expansion for transplantation. These findings demonstrate the feasibility of cell-specific labeling of cultured primary human cells, using a genetic recombination approach that was previously restricted to transgenic animals.
TISSUE REGENERATION BASED ON FIBRIN MICROBEADS (FMB) FOR SOMATIC STEM CELLS ISOLATION, EXPANSION DIFFERENTIATION AND IMPLANTATION


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Fibrin-based biodegradable microbeads (FMB) were developed as solid protein-based carriers for culturing and expanding cells in suspension culture. FMB are applied to attract and selectively isolate adult mesenchymal stem cells (MSCs) from different sources, including whole bone marrow from different animals and GM-CSF mobilized human blood in much higher yields. The selective binding of mesenchymal but not hematopoietic cells to FMB, is mediated by new peptidic epitopes (Haptides) that we identified in the C-termini of fibrin(ogen). For in-vivo applications, the FMB can serve also as vehicles for stem cells expansion in suspension in-vitro and their delivery with high rate of cell survival to the treated organ with no additional scaffold. The yield of BM derived MSC isolation by FMB in rat, murine and rabbit models was at least twice higher relative to conventional isolation methods. The isolated cells were characterized as MSC by their self renewal ability for many passages, by Immuno-phenotyping, by FACS analyses and by staining for specific mesenchymal and stem cells markers. The cells could differentiate on a plate and on FMB to osteoblasts, chondrocytes and adipocytes, as evident by morphology, specific staining and PCR to tissue specific genes. GFP+MSC isolated by FMB from BM of GFP transgenic mice were driven to differentiate into bone or cartilage-forming-cells and then transplanted with the FMB to form ectopic bone and cartilage tissues in kidney capsule or sub-dermally in wild type mice. MSCs isolated by FMB that were driven to differentiate to bone could also repair critical bone defects in mouse cranium model as well as in long bones of rabbits. The transplanted cells were still viable 30-120 days post-implantation. We propose that FMB could be used as a matrix of choice for a high yield isolation, expansion and differentiation of MSC for their implantation to support tissue regeneration.
A HIGH RESOLUTION MAP OF MOUSE GENOME REPLICATION TIMING SUGGESTS A ROLE IN GENE REGULATION

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Although it is known that genomes are divided into distinct replication time zones, a more detailed understanding of their organization is limited. Taking advantage of a novel synchronization method and of genomic DNA microarrays we have mapped replication times of the entire mouse genome at a high temporal resolution. The measurement results have allowed us to assign distinct replication times to 91% of the genome, define asynchronously replicating regions and identify very large replicons. Analysis of the association between replication and transcriptional features has revealed a correlation between replication and transcription potential as well as evolutionary conservation of replication timing. Finally, analysis of large replicons, and in particular of regions at which the time of replication differs from the time of replication of a distant origin, reveals that transcription is correlated with the actual time of replication and not with the time of origin activation. Overall, these findings suggest that early replication plays a causal role in potentiating gene transcription.
Alternative splicing is the main mechanism producing transcript and protein diversity. Many alternative splicing events in the human genome result in subtle changes in the protein, such as in the case of the tandem acceptor motif (NAGNAG). Though these events are highly frequent in the genome, their functional importance is yet unknown. We have previously studied the intronic sequences flanking alternative 3’ splice sites. We found that these sites have unique genomic properties which are indicative of a regulatory mechanism that is involved in splice site selection. Recently we have developed a new method for mapping splicing regulatory elements in the genome relying on their evolutionary conservation and their tendency to cluster. Applying the method on the human genome has revealed an interesting network of interactions between specific splicing factors and alternative splice sites, reinforcing the important role of alternative splicing in post-transcription regulation.
A central challenge faced by living cells is to allow for precise gene expression regulatory programs to take place in spite of environmental variations, genetic changes and stochasticity of the internal cellular environment. To provide robust fulfillment of regulatory gene expression programs cells rely on special genetic circuits that allow them to respond more efficiently to their environment, and to control the internal stochastic fluctuations. We study how these goals are fulfilled by regulating genomic redundancies that offer genetic backup against external and internal variability. In parallel we study how stochasticity within the cell affects expression and regulatory circuits that allow for control of this stochasticity.
Millions of interactions occur in the cell simultaneously in what seems to be perfect coordination. However, many of the mechanisms that account for this coordination are yet unknown. It is not even clear whether there is a centralized "brain" that orchestrates all the interactions - e.g. by controlling gene expression, or whether the control is decentralized and is achieved by numerous local mechanisms. Attempting to address these questions we analyzed protein network hubs - proteins that have been suggested to play a key role in controlling the dynamics of biological processes. We found that the molecular, structural and physicochemical characteristics of these proteins are tightly related to their role in temporal coordination of biological processes. Furthermore, we show that based on amino acid sequence of the hub, it may possible to predict the coordination between proteins that bind to it.
A SYSTEMS-LEVEL APPROACH TO MAPPING THE TELOMERE-LENGTH MAINTENANCE GENE CIRCUITRY

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The ends of eukaryotic chromosomes are protected by telomeres, nucleoprotein structures that are essential for chromosomal stability and integrity. Understanding how telomere length is controlled has significant medical implications, especially in the fields of aging and cancer. Two recent systematic genome-wide surveys measuring the telomere length of deleted mutants in the yeast S. cerevisiae have identified hundreds of telomere length maintenance (TLM) genes, which span a large array of functional categories and different localizations within the cell. In this talk I will describe a novel general method that integrates large scale screening mutant data with protein-protein interaction information to rigorously chart the cellular subnetwork underlying the function investigated. Applying this method to the yeast telomere length control data, we identify pathways that connect the TLM proteins to the telomere-processing machinery, and predict new TLM genes and their effect on telomere length. We experimentally validate some of these predictions, demonstrating that our method is remarkably accurate. Our results both uncover the complex cellular network underlying telomere length maintenance and validate a new method for inferring such networks.
DESIGN PRINCIPLES OF A FUNCTIONAL SYNAPSE: INSIGHTS FROM HUMAN AND INSECTS PROTEOMES

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The molecular components that are essential to the synaptic vesicle’s (SV) life cycle in synapses are well characterized. Nonetheless, many aspects of synaptic processes, and especially how they relate to complex behavior, remain elusive. Several insect genomes that span an evolutionary breadth of ~350 M years from flies, mosquitoes, honeybee and beetle are already fully sequenced. Recently, the genome and thus the predicted proteomes of additional 11 sequences from flies has been reported. This provides a unique opportunity for a comparative genomic study of the synapse at a different time scale. We compiled a list of 120 gene prototypes that comprise the core of SVs, vesicle trafficking and active zone organizing protein families. Several scaffolding proteins of the active zone, such as Bassoon and Piccollo and the most abundant protein in mammalian SV, Synaptophysin, are missing in all insects. The evolution pattern of synaptic protein complexes is largely different with the components of presynaptic complexes as well as proteins participating in organelle biogenesis being strongly coordinated. This observation does not apply to components of post-synaptic densities and signaling complexes in dendrites. Most synaptic proteins are engaged in rich protein interaction networks. Overall, the number of interacting proteins and the degrees of sequence conservation between human and insects are strongly correlated. Such correlation holds for exocytotic but not endocytotic proteins. A comparative study of human vs. insects sheds light on the composition and assembly of protein complexes in the synapse. The nature of the protein-interaction graphs, their domain composition and the sequence similarity between human and insects distinguish exocytotic from endocytotic proteins and suggest unique evolutionary constraints for each group. General principles in the design of proteins of the presynaptic site are drawn from the comparative genomic view of human and insects.
Host-defense cationic peptides are a large repertoire of membrane-active peptides, found in all types of living organisms including human and plants. A major group includes gene-encoded antimicrobial peptides (AMPs), which serve as part of the innate immunity that complements the highly specific cell-mediated immune response. The increasing resistance of bacteria to conventional antibiotics stimulated the isolation and characterization of many antimicrobial peptides for potential use as new target antibiotics to which bacteria will not develop resistance. In addition to their antimicrobial activity, a few AMPs have been shown also to have the potential to neutralize lipopolysaccharide (LPS)-induced endotoxic effects. LPS is released from the outer surface of bacteria particularly as a result of antibiotic treatment. It enhances the secretion of pro-inflammatory cytokines that in extreme case may lead to a septic shock often resulting in death. Furthermore, some AMPs also have been shown to kill various cells including normal and tumor cells. Although the molecular basis for their cell specificity is not yet clear, most AMPs disrupt and permeate the target cell membrane, preferentially when they contain high amount of negatively charged phospholipids, via the “carpet” mechanism. Based on this mechanism we had de-novo designed novel AMPs and ultrashort lipopeptides with advanced properties to be utilized as antimicrobial, as neutralizers of LPS induced endotoxic shock, as well as for the treatment of cancer.
THE DIVERSE AND DISTINCT EFFECTS OF MOLECULES OF THE IL-1 FAMILY IN TUMOR-HOST INTERACTIONS

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Interleukin-1 (IL-1) is a pleiotropic pro-inflammatory and immunostimulatory cytokine with affects malignant processes. The IL-1 family consists of two agonistic proteins, namely IL-1? and IL-1?, and one antagonistic protein, the IL-1 receptor antagonist (IL-1Ra), which binds to IL-1 receptors without transmitting an activation signal and thus represents a physiological inhibitor of pre-formed IL-1. In their recombinant form, IL-1? and IL-1? exert the same biological activities and bind to the same receptors. However, in the physiological milieu, IL-1? and IL-1? differ dramatically in the sub-cellular compartments in which they are active; IL-1? is mainly active as a cell-associated cytokine (membrane-associated and cytosolic forms), while IL-1? is active only as a secreted molecule. At the site of tumor development, IL-1 can be produced by cells of the microenvironment and also by the malignant cells. To assess the role of tumor cell and host-derived IL-1 in the malignant process, we used IL-1?, IL-1?, IL-1? and IL-1? (double KO) and IL-1Ra KO mice knockout (KO) mice in an experimental system of carcinogenesis induced by 3-methylcholanthrene (3-MCA). Microenvironment-derived IL-1? was shown to be essential for induction of inflammation that is associated to chemical carcinogenesis, while IL-1? is less important in this process. In contrast, microenvironment-derived IL-1? is involved in shaping the immunogenicity of the arising malignant cells. Transplantation assays, using fibrosarcoma cell lines obtained from control and the various IL-1 KO mice and the same set of recipient mice, revealed that IL-1? of both the malignant cell- and the host-origin synergize in controlling tumor invasiveness and angiogenesis. Altogether, the results point to differential effects of IL-1? and IL-1? in malignant processes and points to the therapeutic feasibility of the IL-1Ra, which neutralizes soluble IL-1 (mainly IL-1?), in tumor therapy, apart from its use in treatment of autoimmune diseases, such as Rheumatoid arthritis.
The bone marrow (BM) is considered a primary lymphoid organ providing a unique microenvironment with defined niches for stem cells, as well as lympho- and myelogenesis. In addition to this established function in hematopoiesis, the BM might also function as a secondary lymphoid organ promoting the initiation of T and B cell responses. Here we report the characterization of BM-resident Dendritic cells (bmDC), a hallmark of adaptive immunity. Multiphoton imaging revealed that bmDC are organized into unique perivascular clusters that wrap blood vessels and are seeded with B cells and T cells. Surprisingly, conditional bmDC ablation led to a specific loss of re-circulating mature B cells from the BM, a phenotype that could be reverted by over-expression of the anti-apoptotic factor bcl2 by the B cells. We show that survival of re-circulating B cells requires the presence of bmDC, which produce macrophage migration inhibitory factor (MIF). Our results establish a novel DC in vivo function beyond their appreciated role as APC. Furthermore, the identification of the lymphoid follicle-like perivascular clusters, containing DC, B and T cells, significantly furthers our understanding of the BM in the defense against blood-borne pathogens and thus is of relevance for basic research and clinic, alike.
Allergic inflammation (AI) is a complex phenomenon initiated by allergen binding to IgE sensitized mast cells and consequent mast cell activation. This causes the symptoms of the early phase of AI and the onset of the late phase characterized by the penetration in the inflamed tissue of inflammatory cells, notably the eosinophils. Their subsequent activation is believed to cause tissue damage and to be the main factor responsible for the tissue remodeling, especially when the AI becomes a chronic process. We defined a novel functional unit that we termed the "allergic synapse" formed by mast cell – eosinophil couples. In the synapse these two main key effector cells of AI have a cross-talk both via soluble mediators and via receptor-ligand interactions. Our data show that this cross-talk results in mast cell-eosinophil functional synergism at least in a short term time frame. This consequently amplifies and probably can prolong the inflammatory response, in the absence of inhibitory signals. In addition, mast cells and eosinophils are influenced and influence, as in a sort of "allergic niche", the surrounding structural cells. We have been analysing specifically their effects on fibroblasts and more recently on endothelial cells. We found that these inflammatory cells promote fibrosis and angiogenesis, two characteristics of AI. We propose to view the allergic synapse/niche as a specialized central effector unit of the allergic inflammatory responses worthy of being modulated for the treatment/prevention of allergic inflammation.
CLEARANCE OF APOPTOTIC CELLS BY DENDRITIC CELLS

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Recently it became apparent that DCs are not only excellent stimulators of an immune
response, but also potent inhibitors. Immature DCs engulf apoptotic cells, and are able
to acquire antigens found in the dying cells. Integrins as well as CD36, were shown to
have a role in clearance of apoptotic cells by human DCs. Thus, iDCs, including
epidermal Langerhans cells, splenic marginal zone DCs, and interstitial DCs within
nonlymphoid tissues, continuously sample self antigen to maintain peripheral T cell
self tolerance Phagocytosis of apoptotic cells has an inhibitory effect on LPS- or
zymosan-induced activation of NF-κB and MAPs, but induces expression of SOCS
proteins and substantially suppresses induction of CXCL10 expression by IFN-γ. In
addition to suppressing LPS responses, apoptotic cells inhibit macrophage responses
to another major macrophage activator, IFN-γ, by attenuating IFN-γ-induced STAT1
activation and downstream gene expression. These results identify suppressive effects
of apoptotic cells on signal transduction, and extend our understanding of the anti-
inflammatory effects of apoptotic cells to include suppression of Jak-STAT signaling.
PRO-RESOLVING MACROPHAGES AND THEIR REGULATION BY RESOLVINS

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During the resolution phase of inflammation the corpses of apoptotic leukocytes are cleared by macrophages in a nonphlogistic fashion. Here, we present evidence for the emergence of pro-resolving, CD11b<sup>low</sup> macrophages in vivo during the resolution of inflammation, and characterized their properties in terms of apoptotic leukocyte engulfment and immune silencing. In addition, we found the pro-resolving lipid mediators resolvin E1 and D1, at 100 ng/mouse, to regulate pro-resolving macrophage functions in vivo, such as clearance of apoptotic neutrophils, CD11b expression, and immune silencing. Thus, we suggest that pro-resolving macrophages are an essential component in the termination of acute inflammation.
THE bHLH PROTEIN DELILAH AFFECTS INTERVEIN CELL DIFFERENTIATION BY REGULATING THE EXPRESSION OF CELL ADHESION MOLECULES

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One of the fascinating questions in developmental biology is how are different cell types, with distinct identities and cellular properties, established within a uniform cell population to form together a functional organ. The Drosophila wing offers a powerful model system to address such questions. During wing development, two histotypes (vein and intervein), which originate from the same epithelial sheet, develop next to each other and acquire very different cellular properties. In the mature wing, veins and interveins differ from each other in cell size, shape, cuticle pigmentation, and the repertoire of cell adhesion molecules that they express. Although the main pathways that define the initial development of vein versus intervein domains are well established, very little is known about later events responsible for the realization of the distinct vein or intervein fates. Through our studies on the development of chordotonal organs we became interested in the developmental roles of the bHLH protein Delilah (Dei). Our data suggested that Dei plays an important role in the basic differentiation program, which is common to different cell types that share the ability to adhere to other cells and withstand mechanical strain. Here we show that Dei is expressed in the developing wing and that its expression there is restricted to intervein territories. Phenotypic analyses implicate Dei in intervein differentiation and adhesion and in the suppression of vein development. Our data suggest that the expression of Dei in intervein regions represents an accurate readout of the major signaling pathways, such as EGFR and Notch, that divide the wing to zones of vein and intervein identities. Dei is required in intervein territories to implement the correct developmental program by regulating the expression of realiators, including cell adhesion molecules, such as integrins.
REGULATION OF GROUCHO/TLE-DEPENDENT TRANSCRIPTIONAL REPRESSION BY RECEPTOR TYROSINE KINASE SIGNALING

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Transcriptional repression is a widespread strategy for setting up and maintaining domains of gene expression in development. In our laboratory we are studying the biological roles played by Groucho/TLE, a global co-repressor that is required by a vast number of negative transcriptional regulators for the silencing of their downstream target genes. In light of the fact that Groucho/TLE is expressed ubiquitously, the long-standing prevailing view had been that Groucho/TLE-mediated repression must be regulated simply by the spatial and temporal distribution of its DNA-binding repressor partner proteins. It has recently emerged, however, that Groucho/TLE’s capability to repress is itself regulated. In particular, we have shown that Groucho/TLE is phosphorylated in response to EGFR signaling, and that this modification downregulates its repressor function. To further our understanding of this mode of regulation, we have generated antibodies that specifically recognize the phosphorylated form of Drosophila Groucho, allowing us to follow its phosphorylation state in vivo during the different stages of embryonic fly development. Using these anti-sera, we have determined that signaling by other receptor tyrosine kinases, besides EGFR, also leads to modification of Groucho, and that attenuation of Groucho’s repressor capacity by these pathways is consequential for their transcriptional output. We have also started investigating the molecular mechanism(s) by which phosphorylation affects Groucho’s activity. Data will be presented showing that Groucho is phosphorylated by an efficient mechanism that does not alter its subcellular localization or decrease its stability; rather, modified Groucho endures long after MAPK activation has terminated. A model will be presented suggesting that phosphorylation of Groucho provides a widespread, long-term mechanism by which receptor tyrosine kinases control expression of their target genes.
Leaves are formed at the flanks of the shoot apical meristem (SAM) and gradually develop into flat structures with very diverse sizes and forms. The tomato (Solanum lycopersicum) leaf provides a sensitive system to explore mechanisms underlying variations in leaf shape, size and complexity. Wild type tomato leaves are compound, comprising of reiterated units termed leaflets, each resembling a simple leaf. A screen of a tomato mutant population has revealed that common genetic mechanisms are utilized for SAM maintenance and compound-leaf patterning in tomato. In the classical, partially dominant mutation Lanceolate (La), leaves become small and simple. We found that LA encodes a TCP transcription factor containing a miR319-binding site. The La gain-of function phenotype results from a point mutation within the miR319-binding site, which confers partial resistance of the La transcripts to miRNA-directed cleavage. LA expression is turned on precociously in young La leaf primordia, leading to hasty differentiation of leaf margins. In contrast, downregulation of multiple LA-like genes using ectopic expression of miR319 resulted in larger leaflets and continuous growth of leaf margins. We propose that the regulation of LA levels by miR319 defines a flexible window of morphogenetic competence along the developing leaf margin that is required for leaf elaboration.
Pancreatic beta cells, organized in the islets of Langerhans, are charged with sensing blood glucose levels and the appropriate secretion of insulin. If and how the morphology of beta cells serves this function is only partly understood. We have studied the roles of LKB1, a conserved kinase implicated in the control of epithelial cell polarity, in adult beta cells. LKB1-deficient beta cells, while retaining a typical organization of rosettes surrounding islet capillaries, have striking defects in the localization of the nuclei and cilia vis-a-vis blood vessels, are 40% larger than normal, and secrete abnormally high levels of insulin in response to glucose. Genetic and pharmacologic analyses show that distinct targets of LKB1 mediate these functions. LKB1 regulates the precise position of the beta cell nucleus and cilia, but not cell size and insulin secretion, via Par1b-dependent modulation of the cytoskeleton. Conversely, LKB1 restricts beta cell size and insulin secretion, but not polarity, via AMPK and mTOR pathways. These results expose a molecular mechanism, orchestrated by LKB1, for the coordination of beta cell size, form and function.
THE FIRST FAMILY OF DEVELOPMENTAL CELL-CELL FUSION PROTEINS

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Developmental cell fusion is found in germlines, bones, muscles, placentas and stem cells. Despite the detailed characterization of cell fusion events in several organisms, little is known about the molecular mechanisms of cell fusion. EFF-1 is necessary and sufficient to mediate homotypic cell fusion in C. elegans. Epidermal cell fusion requires EFF-1 to initiate and expand membrane merger. Ectopic expression of EFF-1 in C. elegans and in heterologous Sf9 insect cells is sufficient to form giant syncytia. Recently we discovered AFF-1, a novel C. elegans fusogen that mediates anchor cell-utse fusion to form a hymen. Surface expression of AFF-1 is sufficient to fuse nematode and insect Sf9 cells. AFF-1 and EFF-1 type I membrane proteins are members of a family of developmental cell fusogens in nematodes. The expression patterns of eff-1 and aff-1 correlate with their fusogenic activities in different fusing cells. We will discuss how EFF-1 fuse cells via hemifusion. Thus, we identified and characterized a family of developmental fusogens (the FF family) required for ectodermal and mesodermal fusion in nematodes. The FF proteins are bona fide fusogens because they are: (1) Expressed at the time of fusion on the surface of both fusing cells. (2) Essential for most somatic cell fusion events in C. elegans. (3) Sufficient to fuse cells that normally do not fuse in vivo and in vitro. Identifying new fusogens will allow in depth understanding of the cell fusion mechanisms in C. elegans and may promote the discovery of additional fusogens in other organisms.

In a groundbreaking set of experiments, Hans Spemann demonstrated that the dorsal part of the amphibian embryo can generate a well-proportioned tadpole, and that a small group of dorsal cells (the 'organizer') can induce a complete and well-proportionate twinned axis when transplanted into a host embryo. Key to organizer function is the localized secretion of BMP inhibitors, which defines a graded BMP activation profile. While the central proteins involved in shaping this gradient are well characterized, their integrated function, and in particular the mechanism which allows for size-regulation is not understood. We present evidence that the BMP activity gradient in the Xenopus embryo is defined by the 'shuttling-based' mechanism that governs also the formation of an analogous gradient in Drosophila. Central to this mechanism is the ventral translocation of the BMP ligands through their association with the BMP inhibitor Chordin. Using mathematical modeling, we show that this mechanism, together with vertebrate-specific feedback repression of ADMP, offers a quantitative explanation to the original Spemann observations, and accounts naturally for the scaling of embryo pattern with its size.
RNA THERMOSENSORS AND TRANSLATION REGULATION IN LEISHMANIA

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Translational regulation plays a key role in developmental gene expression in Leishmania. Translation control is mediated by 3’ UTRs, through mechanisms that are still elusive. We used the HSP83 gene as a model for understanding how stage-specific translation regulation is directed during the parasite life cycle. We mapped the regulatory element in the Hsp83 3’ UTR to sequences 201-346. However, this region is required, but not sufficient for conferring preferential translation at elevated temperatures. We present data indicating that the function of this element is structure-dependent. To examine these structural changes, the in vitro transcribed RNA region (1-472) was end-labelled and subjected to enzymatic probing of its secondary structure. The mapped positions were used as constraints for structure prediction by UNAFold with Mfold utilities, that provides the probability of each nucleotide to be found in a single- or double-stranded form. In the predicted structure, regions that were shown to be essential for preferential translation cluster on a discrete arm of the RNA. We further identified a single-stranded polypyrimidine-rich region in the Hsp83 3’ UTR that is essential for translation at elevated temperatures, and is subject to structural changes during a temperature switch. We propose that this is the basis for a thermosensing mechanism that controls stage-specific translation. We discuss potential modes of translation regulation in Leishmania, which are associated also with the basal translation machinery of Leishmania and trypanosomes, and report on the evolutionary and developmental changes in the cap binding translation initiation factors (LeishIF4Es).
Riboswitches are natural RNA sensors that affect gene control via their capacity to bind small molecules. Their prevalence in higher eukaryotes is unclear. We discovered a post-transcriptional mechanism in plants that employs a riboswitch to control a metabolic feedback loop through differential processing of precursor-RNA 3’-terminus. When cellular thiamin-pyrophosphate (TPP) levels rise, metabolite sensing by the riboswitch located in TPP biosynthesis genes directs formation of an unstable splicing product and consequently TPP levels drop. When transformed in plants, engineered TPP riboswitches can act autonomously to modulate gene expression. In an evolutionary perspective, a TPP riboswitch is also present in ancient plant taxa suggesting that this mechanism is active since vascular plants emerged 400 million years ago.
A POINT MUTATION IN TRANSLATION INITIATION FACTOR 2B5 LEADS TO BRAIN PATHOLOGY

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The autosomal-recessive genetic neurodegenerative childhood disease termed Vanishing White Matter (VWM) (OMIM #60389) relates to mutations in each of the genes encoding the five subunits of eukaryotic translation initiation factor 2B (eIF2B, subunits 1-5). Neurological deterioration usually begins at early childhood followed by death before the age of 20. The disorder primarily affects glial cells of the brain and is diagnosed by Magnetic Resonance Imaging (MRI) that shows diffuse abnormalities of the cerebral white matter. To learn more about the molecular mechanisms which govern the development of the neurological symptoms, we generated a Knock-In (KI) mouse model by introducing a specific mutation into the eIF2B5 gene. MRI experiments demonstrated the effect of the mutation in eIF2B5 on early development of specific brain regions. For example: a delayed development of white matter in the Internal Capsule accompanied with higher density of this region was observed in the KI mice, as well as increased amount of abnormal and less compact myelin in the hippocampus. Primary astrocytes were isolated from newborn KI and WT mice brains followed by their activation in culture using LPS and Interferon gamma. Whereas the WT cells were smaller and developed numerous extensions, the KI astrocytes were much bigger and seem to have major difficulty in producing extensions. Moreover, enhanced stress response in the KI primary astrocytes was observed by western blots analysing ATF4, CHOP and GADD34 induction in response eIF2 alpha phosphorylation.
NOVEL MECHANISM OF c-JUN REGULATION IN NORMAL AND MALIGNANT CELLS

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The c-Jun protein is a key player in the process of cell proliferation and tumor progression. This transcription factor can form a variety of dimeric complexes, collectively termed AP-1, and stimulate cell cycle progression through induction of genes coding for components of the cell cycle apparatus and repression of tumor suppressor genes. Expression of c-Jun is known to be markedly elevated by a diverse array of extracellular stimuli, including peptide growth factors, various forms of cellular stress, and ultraviolet (UV) irradiation, which cause a rapid and transient increase in c-Jun protein accumulation, mainly by activating the transcription of the c-jun gene. In addition to external stimuli, the expression of c-Jun can also be regulated by cell-cell contact interactions. We have demonstrated that cell separation or overexpression of dominant negative E-cadherin induces a marked and sustained increase in c-Jun protein accumulation and, most importantly, that this increase is not accompanied by a detectable change in c-Jun mRNA. The effect of cell-cell contacts on c-Jun expression can be mimicked by depolymerization of the cytoskeleton. Cytoskeleton disrupting agents cause a sustained increase in c-Jun protein accumulation, but no increase in c-Jun mRNA or in the half-life of the c-Jun protein, but rather in the translatability of its transcript. The cytoskeletal-induced increase in c-Jun translation is not dependent on activated MAPK pathways and is mediated by the untranslated regions (UTRs) of the c-Jun transcript, in particular by the 5'UTR. Pull down assay with biotinylated RNA identified a cytoplasmic protein that interacts specifically with the first 277 bases at the 5' end of the c-Jun 5'UTR. Analysis of c-Jun expression in various tumor cells strongly suggests that this novel mechanism of c-Jun regulation is most relevant to physiological conditions in which c-Jun plays a pivotal role.
Yeast Translational Response to High Salinity: Global Analysis Reveals Regulation at Multiple Levels

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Genome-wide studies of steady-state mRNA levels revealed common principles underlying transcriptional changes in response to external stimuli. To uncover principles that govern other stages of the gene-expression response, we analyzed the translational response and its coordination with the transcriptome changes following exposure to severe stress. Yeast cells were grown for one hour in medium containing 1 M NaCl, which elicits a maximal but transient translation inhibition, and non-polysomal or polysomal mRNA pools were subjected to DNA-microarray analyses. We observed a strong repression in polysomal association for most mRNAs, with no simple correlation with the changes in transcript levels. This leads to an apparent accumulation of many mRNAs as a non-translating pool, presumably waiting for recovery from the stress. However, some mRNAs demonstrated a correlated change in their polysomal association and their transcript levels (i.e., potentiation). This group was enriched with targets of the transcription factors Msn2/Msn4 and the translational induction of several tested mRNAs was diminished in Msn2/Msn4 deletion strain. Genome-wide analysis of a strain lacking the high salinity response kinase Hog1p revealed that only few salt-stress resistance mRNAs are translationally affected. Thus, additional signaling pathways are involved in coordinating the translational response to severe salinity stress.
A COMMON PATHWAY TRANSDUCES VARIOUS STRESS SIGNALS INTO TRANSLATIONAL REPRESSION OF TOP mRNAs

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Most of the energy consumed during cell proliferation is invested in doubling the content of the translational apparatus prior to cell division. Hence, signals emanating from mitotic arrest, as well as amino acid deficiency are transduced into down-regulation of ribosome biogenesis. This response is primarily attained by translational repression of TOP mRNAs, which encode many components of the translational apparatus. We have previously shown that efficient translation of these mRNAs requires adequate supply of growth factors and amino acids. We now show that in addition to mitotic arrest and amino acid depletion, also anoxia-induced energy depletion leads to translational repression of TOP mRNAs. All these inhibitory signals converge at the tumor suppressor TSC2. The latter dimerizes with TSC1 and operates as a GTPase activating protein toward Rheb, and thereby blocks the mammalian target of rapamycin (mTOR) pathway. Indeed, knockout of either TSC1 or TSC2, or alternatively, overexpression of its immediate effector, Rheb, was able to rescue TOP mRNAs from translational repression. The role of mTOR in translational control of TOP mRNAs has been extensively studied with a specific inhibitor, rapamycin, albeit with conflicting results. However, using RNA interference approach we now show that mTOR is essential for translational activation of TOP mRNAs by either insulin treatment or amino acid refeeding. The prevailing dogma assumes that mTOR operates through either of two complexes, the rapamycin-sensitive mTORC1 or the rapamycin-insensitive mTORC2. We show now by knocking down or knocking out of raptor or rictor, the major partners of mTOR in these complexes, respectively, that insulin and amino acids signal to TOP mRNAs via mTOR independently of either mTORC1 or mTORC2. These results have enabled us to establish a multi-component network that accounts for energy saving under various stress conditions by translational repression of TOP mRNAs.
Since the early 1980’s there is an increasing preference for single enantiomers in pharmaceuticals due to the different effect of enantiomers on chiral targets in the body. Biocatalytic methods for production of pharmaceutical compounds attract much attention as an environment-friendly synthetic approach, consequently, the value of biologically produced chemicals is predicted to rise from 50$ billion to 160$ billion by the end of the decade. (S)-4-chloro-3-hydroxybutanoate ethyl ester (S-CHB) is an important chiral building block used in the synthesis of cholesterol-lowering drugs such as Atorvastatin. Among the various routes to produce S-CHB, asymmetric reduction of 4-chloro-acetoacetic acid ethyl ester (CAAE) using microorganisms is a most attractive method, offering high yields and enantioselectivity. A panel of natural Saccharomyces cerevisiae isolates as well as laboratory strains, were tested as a whole-cell system for CAAE asymmetric reduction to S-CHB. The yeasts were isolated from Mount Carmel National Park and were characterized as resistant to environmental stress. Nevertheless, these strains showed relatively low enantiomeric excess (ee) in the reduction of CAAE to (S)-CHB, while a laboratory strain (Y103), exhibited a selectivity of 98% ee. Introgression using a classic breeding strategy was used here to successfully create a strain carrying both desirable traits. Unlike its original parental strains, the new strain maintained constant specific activity and enantioselectivity when introduced to the various stress factors. This work shows that the strain created from a natural isolate using “green- genetics”, can serve as a robust enantioselective biocatalyst, designed for industrial production of chiral compounds.
Biofouling of reverse osmosis (RO) membranes is a phenomenon that decreases permeate flux and, in most cases, reduces salt rejection. Here, biofilm formation of Pseudomonas aeruginosa on the surface of RO membrane was analyzed using a synthetic wastewater medium. P. aeruginosa biofilm physiology and spatial distribution of activity were analyzed following growth on the membrane. As a consequence of the limiting carbon source prevailing in the suspended culture of the RO unit, a higher distribution of active cells was observed in the biofilm close to the membrane surface, likely due to the higher nutrient levels induced by concentration polarization effects. The same distribution for microbial activity was observed using real wastewater and natural consortium. With P. aeruginosa RO biofilms, the faster growth of the RO sessile cells compared to the planktonic cells in the RO unit was reflected by the transcriptome of the two cultures analyzed with DNA microarrays. In contrast to findings recently reported in gene expression studies of P. aeruginosa biofilms, in the RO system, genes related to stress, adaptation, chemotaxis, and resistance to antibacterial agents were induced in the planktonic cells. In agreement with the findings of previous P. aeruginosa biofilm studies, motility and attachment related genes were repressed in the RO P. aeruginosa biofilm. Supported by the microarray data, an increase in both motility and chemotaxis phenotypes was observed in the suspended cells. The increase in nutrient concentration in close proximity to the membrane is suggested to enhance biofouling by chemotaxis response of the suspended cells and their swimming toward the membrane surface. Recently, we proposed that “biofilm enhanced concentration polarization” phenomenon [Herzberg and Elimelech (2007) J. membrane sci. 295:11-20] is a major biofouling mechanism in RO systems. Hence, this mechanism has intriguing effects on gene expression in biofilms under typical RO conditions.
BIODEGRADATION OF ENVIRONMENTAL HAZARDS: THE ROLE OF WHITE ROT FUNGI

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Lignin degradation is an important step in carbon cycling. Basidiomycetes that belong to a group of white rot fungi are the only organisms that can mineralize this recalcitrant biopolymer, thus enabling the biodegradation and utilization of a variety of lignocellulosic wastes. Accumulation of such waste may cause environmental problems. These fungi and their unique enzymatic system can be also exploited for the degradation and bioremediation of toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), diazo dyes and olive mill waste water. Laccase, Managenase peroxidase (MnP) and Versatile peroxidase (VP)) are extracellular oxidative enzymes believed to be involved in lignin degradation by the white rot fungus Pleurotus ostreatus. We followed expression and activity of these enzymes in P. ostreatus cultured on cotton stalks as a natural lignocellulosic substrate under solid state fermentation (SSF) conditions as well as in defined media. Enzyme profile analyses revealed several VP activity peaks. In contrast, no MnP activity was detected. All VP activity peaks were identified as different forms of MnP2. Real-Time RT-PCR experiments demonstrated that all four mnp genes genes were transcribed under all conditions tested. Transcript abundance of the four mnp genes was differentially affected by Mn2. Even though MnP3 activity was not detected, mnp3 transcript levels were significantly elevated (30-fold) in response to the presence of Mn2+. The expression of laccase also showed differential expression of its isoenzymes. However, as expected, no effect of Mn was observed. These results suggest that Mn2+ is probably a positive regulator of MnP and a negative regulator of the VP's. Our results demonstrate that MnP and VP expression during SSF on a natural substrate with or without Mn2+ amendment are different than those observed under other culture conditions, due to both differential gene expression as well as post-translational modification of the relevant enzymes.
Agricultural irrigation with wastewater effluent is a common practice, used as a readily available and inexpensive option to freshwater. However, irrigation with effluents, which contain high levels of nitrogen, minerals, dissolved organic carbon, detergents, microorganisms and toxic metals, may alter the microbial populations in soil, and thus affect soil fertility. We have studied several microbial activities and biomass parameters in soils, affected by irrigation with wastewater and followed the effect it has on microbial community composition in soil. We observed changes in microbial activities in soils during irrigation with wastewater. The chemolithotrophic ammonia oxidizing bacteria (AOB) are responsible for the first, rate-limiting step in nitrification in which ammonia (NH3) is transformed to nitrate (NO3-) via nitrite (NO2-). Analyses of AOB community composition were conducted on the effluents used for irrigation and on several agricultural soils of different types, which had been irrigated with wastewater effluent or fertilizer-amended-water. To monitor changes in community composition of AOB, the amoA fragment (coding for the ammonia monooxigenase) was PCR amplified and analyzed by DGGE (Denaturing Gradient Gel Electrophoresis). The different DNA fragments were sequenced and phylogenetically analyzed detecting an obvious difference in the phylogenetic groups of AOB developed following different treatments. This phenomenon was obvious in all depths of all soils to different degrees. Another group of important bacteria we studied are the nitrogen-fixing rhizobia. We have followed shifts in their community in nodules of legumes irrigated with wastewater. Here, too, we monitored a shift in community composition, resulting from wastewater irrigation, but between isolates of the same species. This shift was accompanied by a change nitrogen fixing and in plant parameters. Understanding the microbial reaction to changes in their environment may provide a mean to use these bacteria or their activity as a sensor to detect environmental changes.
WHOLE-CELL BIOSENSORS FOR ENVIRONMENTAL MONITORING: ADVANCED APPLICATIONS

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We have previously reported on the genetic "tailoring" of microbial cells to respond to different classes of chemicals and environmental stress factors, and on their testing as whole-cell reporters in different biosensing scenarios. In this communication we describe several new directions in the preparation and use of these biosensors. These include: (a) the development of flow-through biochips for on-line water toxicity monitoring, (b) assessment of the biological activity of 1.9 GHz radiation, (c) printable microbial sensor arrays and (d) the detection of toxic oxidative activity of CdSe quantum dots. Most of the presentation will be devoted to the latter topic, the first description of the prokaryotic testing of the biological effects of CdSe quantum dots. Out of a panel of bioluminescent reporter strains exposed to such nanoparticles, the one that responded the strongest was the strain harboring a plasmid-borne micF':lux fusion, engineered to sense superoxide stress. For three different carboxylic acid terminated thiols tested as capping agents (C2, C8, and C11), biosensor response was stronger when the dots were capped with the longer chains. This result is consistent with a model of toxicity reliant upon facilitated membrane transport of quantum dots into biosensor cells, attendant on enhanced quantum dot lipophilicity with increasing chain length of the capping agent. TEM images confirm that quantum dots are internalized inside the cell membrane. We also note that oxidative effect of smaller dots was more pronounced than larger ones for the size range examined (~2-4 nm). The use of the bacterial bioreporter systems employed in these experiments may provide a simple and cost effective way for screening nanoparticles for toxic effects, as well as a means for deciphering their toxic mode of action.
THE ENERGY CRISIS - HOW CAN MICROORGANISMS BE PUT TO WORK?

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Microorganisms are micro-scale factories that can be manipulated to help solve the energy crisis. The ability to obtain a biodiversity of unique genes whose products can act in extreme environment, together with powerful genetic engineering tools, can be utilized to construct efficient biochemical machines, tailor-made for the needs of the growing biofuel industry. The uses of microorganisms include: 1. Production of "microdiesel", which - unlike regular biofuel, is produced without toxic chemicals and can be made using waste (plant waste or other types of waste) instead of plant oils. 2. Converting waste materials – such as lignocellulose – to substrates for biofuel production 3. Detoxifying waste material in biodiesel production 4. Generate energy as electrical current - bacteria are capable of using anything from decaying plant and animal matter to toxic organic pollutants such as benzene to produce electricity.
A MASON'S APPROACH TO THE 19S REGULATORY COMPLEX OF THE PROTEASOME

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The 26S proteasome is comprised from two main components: the 20S catalytic particle (CP), and the 19S regulatory particle (RP). The 19S RP selects ubiquitinated substrates for degradation, unfolds and translocates them into the 20S CP where they are processed into short peptides. While the functions of the 19S RP are well defined, little is known about its fine structure or the relative roles of individual subunits in carrying out these tasks. By using various biochemical purification techniques to shed layer after layer of peripheral subunits we have unearthed the very foundation of the 19S. In this manner, we have managed to expose the most rudimentary form of the proteasome regulatory particle in eukaryotes and obtain single particle images. We have also attacked the issue from the opposite direction: using isolated subunits as bricks and cross linking reagents as mortar, we have partially rebuilt a synthetic regulatory complex from the bottom up around this foundation on the surface of the 20S. We are now able to begin mapping specific functions to regions within the 19S RP complex. Apparently, the majority of functions attributed to the Base of the 19S are actually localized to subunits within this very rudimentary foundation revising our view of how the eukaryotic 19S regulator carries out its main task of "regulating" proteolysis that occurs in the 20S proteasome core. This startling order of subassemblies offers a novel concept of how nucleotide-dependent protein nanomachines function, shedding light not only on other ATP-dependent proteases but even on GTP-dependent nuclear transport mechanisms which share some unexpected features.
NEW INSIGHTS INTO THE PROTEIN DEGRADATION MACHINERY FROM MASS SPECTROMETRY

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Cells depend upon the regulated degradation of their various proteins to maintain homeostasis. This proteolysis pathway is central to an incredible multitude of processes in all eukaryotes, including cell cycle, apoptosis, signal transduction, antigen presentation, and DNA repair. A key component of this process is the proteasome, a large multi-subunit protease. The two major subcomplexes of the 26S proteasome are the ~700 kDa proteolytic core particle, known as the 20S, and the regulatory particle the ~900 kDa 19S complex. In spite of the proteasome central importance there are many fundamental questions regarding its function, assembly pathway and structure that still remain unclear. By applying a novel structural mass spectrometry (MS) approach we addressed several of these open issues. Insight into the 20S proteasome activation and maturation could be revealed and the assembly pathway of this 28-subunit complex was monitored in real time from its component subunits. By combining a tandem MS method with results from electron microscopy the precise number and location of protein substrates that bind simultaneously within the 20S proteasome was determined. To decipher the unknown architecture of the 19S lid complex, we applied tandem mass spectrometry together with cross-linking and previous biological information. This information enabled us to suggest a regulatory mechanism that prevents indiscriminate degradation of proteins.
Protein folding plays an important role in a number of neurodegenerative conditions, such as Parkinson's, Alzheimer's and motor neuron diseases. Accumulating evidence suggests that environmental agents may contribute to the pathology of these common disorders by perturbing protein folding, either directly or indirectly through their effects on cell metabolism. However, little is known how cells adapt to the threat of environmentally-induced proteotoxicity. We recently identified AIRAP as an adaptor of proteasomes, the cell's major protein degradation apparatus. Cellular conditions that impair proteasomal activity induce expression of AIRAP. Our genetic and biochemical data indicate a protective role for the AIRAP proteasome complex under protein misfolding conditions. The initial characterization of a second protein, AIRAPL (Q8WV99; a highly homologous protein to AIRAP), has shown AIRAPL to be an additional proteasome adaptor with distinct cellular functions under basal and protein misfolding conditions. AIRAPL contains an uncommon ubiquitin interacting motif (UIM) arrangement, is localized to the endoplasmic reticulum membrane and under protein misfolding conditions is a substrate of the P38 MAPK. RNAi experiments in C. elegans, revealed a protective role for AIRAPL in ageing, a protein misfolding related process. The relationship between AIRAPL and AIRAP and its dynamic interaction with the proteasome will be discussed.
The dependence of photosynthesis on light is obvious. The higher the light intensity, the higher is the rate of photosynthesis, up to a level where light energy is no longer limiting and photosynthesis remains constant. However, further increase in the intensity of light may lead to a decrease in photosynthesis rate, a phenomenon known as ‘photoinhibition’. Photoinhibition is attributed to oxidative damage, primarily to photosystem II (PSII) and its reaction center protein D1. A number of mechanisms have been evolved during evolution to minimize oxidative damage, but if PSII is damaged after all, a PSII repair cycle operates to allow photosynthesis to proceed. A key component of this cycle is the proteolytic removal of damaged D1 protein, prior to its replacement by a newly synthesized one. Degradation of the D1 protein has been a central question in the field of photosynthesis for the past 20 years or so, but only in recent years the identity of the proteases involved has started to unravel. Recombinant FtsH, a thylakoid ATP-dependent metalloprotease, was first shown to participate in D1 degradation in an in vitro study. Later on, in vivo analysis of Arabidopsis FtsH mutants revealed that they were more sensitive to photoinhibition than wild type, and that damaged D1 protein was stabilized in them. Further analysis of different mutants suggested that the chloroplast FtsH complex is composed of two essential types of subunits, each one of them is encoded by two redundant genes. More recently, analysis of knock-down mutants of the lumenal serine protease Deg1 suggested that this protease is also involved in the process of D1 degradation. Wider implications to questions of chloroplast biogenesis and maintenance and degradation of highly hydrophobic proteins will be discussed.
MECHANISM OF Hsp70-MEDIATED REMODELING OF
ALTERNATIVELY FOLDED PROTEINS INTO NATIVELY FOLDED
PROTEINS

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Hsp70s are conserved molecular chaperones that can actively solubilize stable protein aggregates, pull translocating proteins across membranes and remodel near-native (alter-native) inactive proteins into their native, active conformations, for example in various chaperone-dependent cell signaling pathways. Recently, we have found evidence supportive of a local entropic pulling mechanism, which is dictated by the substrate-binding and Hsp70-entrapping properties of the Hsp40 co-chaperone, and by the particular molecular architecture of the Hsp70 molecule when it is locally bound to its protein substrate (Goloubinoff P, De Los Rios P. TiBS. 2007 32:372). While tightly bound to exposed loops on protein substrates, Hsp70 molecules seek maximal freedom of movements away from other near-by macromolecules, including other bound Hsp70s. This translates into a local unfolding force, which can be used to induce small structural changes in aggregated, misfolded or alter-native folded protein substrates. To study how in the cell Hsp70 can remodel alter-native folded inactive protein conformers, into natively folded active protein products, we have characterized a new type of chaperone substrate: a stable, alter-native folded inactive mutant of firefly luciferase, which upon the local unfolding action of Hsp70/Hsp40 becomes a less stable, natively folded active chaperone product. This allowed us to characterize for the first time the kinetic parameters of Hsp70/Hsp40 as an unfolding enzyme in the presence of a large excess of non-aggregated, alter-native folded chaperone substrate. This system shines new light on the mechanism by which molecular chaperones in general and Hsp70 in particular can use the energy of ATP to modify various aggregated, misfolded of alter-native folded protein substrates and convert them into native, active, chaperone products, or into protease-degradable ones.
Ubiquitylation is a dynamic regulatory post translation modification signal that can affect the activity, localization and fate determination of proteins. The characterized roles of ubiquitin (Ub) include acting as a sorting signal to direct protein to degradation in the proteasome or in the lysosome, trafficking in the endocytic and biosynthetic pathways, regulating vesicle and virus budding machinery, DNA remodeling, regulating transcriptional machinery controlling intranuclear localization and more. Ubiquitylated proteins are recognized by Ub-receptors, proteins containing Ub-Binding Domains (UBDs), that transmit the information conferred by the new structure of the ubiquitylated protein. Therefore understanding the molecular mechanism of Ub recognition and the function of Ub-receptors is very important. During the last few years we determined structures and highlight the mechanism of action of Ub:Ub-receptor complexes in the endocytic and biosynthetic pathways. These include structures and the functions of Vps9-CUE:Ub complex1,2; ESCRT-II complex3; GGA3-GAT:Ub complex4; and Vps27:Hse1 (ESCRT-0) core complex5. Taken together, these structures the principles of Ub recognition is elucidated. Specificity is achieved by a remarkably low affinity (2-500 µM) mediated mainly by hydrophobic interactions; such low affinity permits the rapid trafficking of the cargo. Moreover structural alignment all known UBD:Ub complexes shed new light on the evolution and mechanisms trafficking through the endocytic and biosynthetic pathways. The structures of UBDs are very different and they bind different patches on Ub. However in all known complexes of the endocytic and biosynthetic pathways they recognize the same hydrophobic patch on the Ub surface, centered on isoleucine-44. We propose that these UBDs have evolved through convergent evolution from unrelated ancestors. The occupancy of a common patch on Ub for these UBDs favors a competitive displacement mechanism that in turn facilitates the trafficking of cargo from one complex to another and therefore gives an explanation for the driving force of the convergent evolution.
The opportunistic pathogen Pseudomonas aeruginosa causes chronic biofilm-associated infections in the lungs of cystic fibrosis patients, which cannot be eradicated by antibiotics. Like most other pathogens P. aeruginosa is under intense competition for iron with the host. Recent studies show that even when there is sufficient iron for growth this element serves as a signal for biofilm development. Here we report the role of iron acquisition-signaling genes in mediating biofilm development. We also show that iron induced cell-cell signaling (i.e. quorum sensing) influences surface motility and biofilm formation under low iron conditions. These findings suggest a link between the iron and quorum sensing regulons and provide a potential mechanism for iron dependent biofilm formation.
Intrinsically active variants of MAPKs, that are independent of upstream regulation, would serve as strong tools for addressing the specific biological and pathological functions of each MAPK. The unusual mode of MAPK activation (requirement for phosphorylation of neighboring Thr and Tyr residues) hindered the production of active variants, because it is not known how to mimic such dual phosphorylation by mutagenesis. We bypassed this difficulty and isolated constitutively active (MEK-independent) variants of the yeast MAPKs Hog1/p38 and Mpk1/ERKs via specifically designed genetic screens. The sites of many of the activating mutations were found to be conserved in mammalian MAPKs. We therefore inserted mutations, similar to those found in Hog1, or Mpk1 to the human p38alpha, p38beta, p38gamma and p38delta and the human ERK1 and ERK2 respectively. We found that the mutated human MAPKs were spontaneously active in vivo and in vitro. By expressing the mutated isoforms in various cell lines we are currently establishing the specific physiological activity of each isoform and the possible cross-talk between the various p38s. We have also established the mechanism of action of the active mutants. We found that the mutants acquired an autophosphorylation activity. Namely, they are active when phosphorylated at the phosphorylation lip, just like the native MAPKs are. Crystal structures of active variants of p38alpha revealed that the activating mutations unfold a particular hydrophobic core, formed by the L16 and the C-helix domains, and that this unfolding induces auto-phosphorylation and auto-activation. This mechanism seems to mimic the natural mechanism of p38 activation through the bypass pathway.
The serine/threonine kinase Pto of tomato confers resistance to bacterial speck disease caused by Pseudomonas syringae pv. tomato (Pst) expressing the AvrPto or AvrPtoB effectors. Pto interacts with these two bacterial effectors activating effective defense responses. To explore the role of Pto kinase activity in its physical interaction with AvrPto and AvrPtoB, we sensitized the Pto ATP binding site to inhibition by small molecules. Specific inhibitors were then tested for their capability to interfere with the Pto-AvrPto or Pto-AvrPtoB interaction in a yeast two-hybrid system. Surprisingly, the inhibitors enhanced both interactions and even restored interactions between kinase deficient forms of Pto and the two bacterial effectors. These results suggest that ligand binding to the ATP binding site, rather than phosphotransfer, enables the sensitized Pto to gain a suitable conformation for its interaction with AvrPto. In parallel investigation, we used virus-induced gene silencing to identify genes that act downstream of Pto-AvrPto recognition and contribute to resistance. Silencing of the MAPK SIMPK3, a MAPKKK, and a GRAS transcriptional activator compromised resistance to Pseudomonas strains. Interestingly, overexpression of the MAPKKK elicited a pathogen-independent cell death, while expression of SIMPK3 and GRAS family members were induced by multiple pathogens and wounding. Together, our investigation sheds light on molecular mechanisms mediating Pto activation and reveals a role for SIMPK3, a MAPKKK, and GRAS transcriptional activators in signaling pathways originated from the Pto-AvrPto recognition event.
NEW INTRACELLULAR AND INTERCELLULAR PARADIGMS IN Ras SIGNALING: NANOAWICHES, RASOSOMES AND Ras TRANSFER FROM CELL TO CELL

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Ras proteins are organized into nanoclusters (10-30 nm) on the inner plasma membrane acting as nanoswitches which are essential for Ras signal transduction. The mechanisms that drive nanoclustering were unknown. We show that epidermal growth factor receptor (EGFR) activation stimulates the formation of H-Ras.GTP-Galectin-1 (Gal-1) complexes on the plasma membrane that are then assembled into transient nanoclusters. Gal-1 is therefore an integral structural component of the H-Ras signaling nanocluster. Increasing Gal-1 levels increases the stability of H-Ras nanoclusters leading to enhanced effector recruitment and signal output from the inner plasma membrane. Ras proteins also signal from intracellular compartments. The spatiotemporal modulation of Ras signalling from different intracellular compartments requires mechanisms allowing Ras and its signals to navigate across cells. We describe one mechanism by which nanoparticles (80-100 nm diameters) of H-Ras and N-Ras isoforms diffuse through the cytoplasm, independently of ATP, on fast, randomly moving, small cytosolic nanoparticles (80-100 nm, ‘rasosomes’). EGFR stimulation rapidly increases active H-Ras-GTP and phosphorylated ERK on rasosomes. Thus, the rasosome represents a hitherto unknown nanoparticle that enables Ras-signal information to spread rapidly across cells. Intriguingly, we found that beyond the classical intracellular Ras signal transduction Ras proteins can act as intercellular signal transducers. We show that human lymphocytes acquire from the cells they scan the inner-membrane protein H-Ras. The transfer was cell contact-dependent and occurred in the context of cell-conjugate formation. The acquisition of oncogenic H-RasG12V by natural killer (NK) and T lymphocytes had important biological functions in the adopting lymphocytes: transferred H-RasG12V induced ERK phosphorylation, increased interferon-gama and tumor necrosis factor-alpha secretion, enhanced lymphocyte proliferation, and augmented NK-mediated target cell killing.
MOLECULAR AND PHYSIOLOGICAL DISSECTION OF FUNCTIONAL ROLES OF DEATH MOLECULES ACTIVATED BY RECEPTORS OF THE TNF/NGF FAMILY

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The ability of receptors of the TNF/NGF family to induce in the same cell both programmed death and resistance to its has kept, over two decades, posing challenge to our understanding of the mechanisms that dictate specificity in the action of these receptors. While the initial view of the mechanisms underlying this duality was of two distinct receptor-induced pathways: the extrinsic cell death pathway, initiated by activation of caspase-8, and the induction of anti-apoptotic proteins, mainly through activation of transcription factors of the NF-kappaB family, it is now becoming more and more evident that none of the proximal components of the signaling pathways is specific to death or life induction. Rather, it is the identity of the signaling complexes with which these proteins interact and their mode of activation within them that define their effects. As will be illustrated by examples from our studies of several of these signaling proteins, detailed analysis of their mechanisms of action in cultured cells, accompanied by BAC transgenesis for exploring structure-functional relationship of these proteins in mice, seem now to provide the basis for defining specific molecular determinants of death-versus-life decisions in the function of these proteins.
The ERK/MAPK signaling cascade is a central signaling pathway that regulates various processes including proliferation and differentiation. A hallmark of this cascade is the transmission of extracellular signals into the nucleus in order to induce the transcription of various genes and proliferation. This is mediated in part by a direct translocation of ERKs into the nucleus. However, very little is known about the molecular mechanisms of ERKs translocation, as ERKs do not contain a nuclear localization signal. We identified a three amino-acid domain (Ser-Pro-Ser; SPS domain) that participates in both non-regulated and facilitated nuclear translocation of these proteins. We also found that the Ser residues in the SPS domain are phosphorylated upon extracellular stimulation. Indeed, mutation of the two Ser residues to Glu facilitated the rate of nuclear translocation, indicating that the phosphorylation of the SPS domain is important for the nuclear translocation of ERKs. As for the characteristics and machinery required for the SPS-dependent translocation, we showed that the SPS domain acts autonomously as it does not require additional regions of ERKs for its action. Overexpression of mutants of the SPS domain inhibited proliferation, confirming the importance of the SPS in mediating ERKs translocation and thereby their downstream responses. In addition, we found that the phosphorylated SPS operates by interaction with the nuclear import protein, importin-7, and is important for the ERK’s release from nuclear pore proteins (NUPs) to allow proper sliding through nuclear pores. Interestingly, a similar domain to the SPS appears in many NLS-independent cyto-nuclear shuttling proteins. Indeed, we found that phosphorylation of similar sequences in SMAD3 or MEK1 also induces their nuclear accumulation by importin-7 and proper sliding through the nuclear pores. Therefore, our findings show that this SPS domain acts as a general nuclear translocation signal (NTS) operating via a novel nuclear importing system.
The maintenance of a balanced cholinergic homeostasis is crucial for the function of the central nervous system (CNS), peripheral nervous system and the neuromuscular junction. However, it appears that the cholinergic system is not restricted to neurons and synapses but may also involve immune reactions. Immune cells possess a complete cholinergic system consisting of acetylcholine (ACh) muscarinic and nicotinic receptors, choline acetyltransferase and acetylcholinesterase (AChE). Since the nervous system is a major producer of ACh the immune cholinergic system can mediate neuro-immune interactions, or it may serve as an internal regulator of immune responses. We investigated the anti-inflammatory effects of AChE inhibitors (AChEI) at the cellular and molecular levels. In experimental autoimmune encephalomyelitis (EAE) the animal model for multiple sclerosis (MS), AChEI reduced the clinical severity of the disease and suppressed the reactivity of encephalitogenic T-cells. This was performed by increasing the ACh concentration near immune cells and making it available for interaction with the alpha-7 nicotinic ACh receptor, expressed on these cells. This outcome is additional to the effect of AChEI on neurons and synapses. Our findings point to a novel role for AChEI which may be relevant in CNS inflammatory diseases and emphasize the importance of the cholinergic balance in neurological disorders such as Alzheimer’s disease and myasthenia gravis, in which these agents are used.
Alpha synuclein (a-Syn) is a neuronal cytoplasmic protein implicated in the pathogenesis of Parkinson’s disease (PD) at both the genetic and cytopathological levels. We have reported several lines of evidences indicating that a-Syn normally interacts with polyunsaturated fatty acids (PUFAs), among other lipids, under physiological conditions, and that this interaction, under adverse cellular conditions, could contribute to a-Syn-related pathogenesis and neuronal dysfunction. We discovered that a-Syn acts to enrich membranes with PUFAs. By virtue of its effects on membrane FAs composition, a-Syn act to induce membrane trafficking and specifically, evoked synaptic vesicle recycling through activation of Clathrin Mediated Endocytosis. We further found that a-Syn normally occurs in high MW oligomers (i.e., dimer trimer up to hexamer) however, exposure to physiological PUFA concentrations induce the accumulation of a-Syn in insoluble aggregates that ultimately lead to deposition in Lewy like inclusions in dopaminergic neurons.
The allele E4 of apolipoprotein-E (apoE4) is the most prevalent genetic risk factor of Alzheimer's disease (AD), and is associated with increased Abeta deposition, with impairments in neuronal plasticity, and with increased brain inflammation. We presently investigated the mechanisms underlying these seemingly different pathological effects of apoE4. Possible pathological interactions between apoE4 and Abeta were investigated utilizing transgenic mice which express either human apoE4 or, the AD benign, allele apoE3 and whose brain Abeta levels were elevated by inhibition of the Abeta degrading enzyme neprilysin. Biochemical immunohistochemical and behavioral assessments of these mice revealed specific accumulation of Abeta and apoE in hippocampal neurons of the apoE4 mice which were associated with the degeneration of these neurons and with impairments in learning and memory. Stimulation of neuronal plasticity by exposure of the apoE transgenic mice to an enriched environment triggered neurogenesis and synaptogenesis in the hippocampus of apoE3 mice and improved their cognitive performance. In contrast, this treatment triggered apoptosis and neuronal loss in the hippocampus of apoE4 mice. These effects were associated with the specific co-accumulation of apoE and Abeta in hippocampal neurons of the environmentally stimulated apoE4 but not of the apoE3 mice. Activation of brain inflammation by prolonged i.c.v. injection with LPS resulted in a markedly more robust inflammatory response in apoE4 mice than in corresponding apoE3 mice. Furthermore, like with the neprilysin inhibition and the neuronal plasticity paradigms, this effect correlated with the specific co-accumulation of apoE and amyloid beta in hippocampal neurons of the apoE4 mice. These findings suggest that the different pathological phenotypes of apoE4 may be mediated via a common unifying mechanism which is driven by cross talk interactions between apoE4 and Abeta.
Alzheimer’s disease (AD) is an age-related progressive neurodegenerative disorder characterized by memory loss and severe cognitive decline. These clinical features are manifested morphologically by excessive accumulation of extracellular amyloid beta-peptide (A-beta) in the brain parenchyma, particularly in the hippocampus and cerebral cortex, leading to neuronal loss. In addition, aggregates of A-beta that form neuritic plaques in the brain become toxic in that they trigger chronic glial activation and thereby interfere with normal brain function. Another hallmark of the disease, possibly secondary to A-beta neurotoxicity and glial activation, is the abnormal phosphorylation and aggregation of tau (which functions in microtubule assembly and stabilization) to form intracellular neurofibrillary tangles. It appears that regulation of the glial reaction to such neuronal insults turns is crucial for inducing mechanisms either of neuronal repair or of neuronal loss. Our approaches to characterizing the different types of glial activation and their contribution to neuronal loss or repair are based on new emerging characteristics of three biological systems: autoimmunity, brain-immune interactions, and neurogenesis. Our data demonstrate that A-beta-specific T cells are induced in AD and that their specificity and magnitude of activation depend primarily on HLA-DR alleles. Expression of IFN-gamma in the brain, as observed during normal brain aging, is essential to promote migration of these A-beta reactive T cells to the parenchymal tissue in the hippocampus and subsequent interaction with brain-endogenous cells. In contrast to the injurious effects induced by chronic innate immune mechanisms involved in AD, T cells - or the cytokine they produce – can serve as key regulators of glial activation and differentiation, clearance of A-beta, neuroprotection and neuronal repair. The implications of these results in the context of immune system physiology and neuronal repair with age will be discussed.
Human mesenchymal stem cells reside in the bone marrow and are known for their ability to differentiate along the mesenchymal lineage (fat, bone and cartilage). Recent works have suggested the possibility that these cells are also capable of differentiating towards the neuroectodermal lineage. Using lentiviral gene delivery, we sought to reprogram the bone marrow derived mesenchymal stem cells towards dopaminergic differentiation through delivery of LMX1a, which was reported to be a key player in dopaminergic differentiation in both developmental animal models and embryonic stem cells. Transduction of cells with fluorescent reporter genes confirmed efficiency of gene delivery, upon incubation of the LMX1a transduced cells in differentiation medium, specific dopaminergic developmental genes were upregulated suggesting the generation of dopaminergic cells derived from adult human bone marrow. Moreover, the transduced cells expressed higher levels of tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis and secreted significantly higher level of dopamine in comparison to non transduced cells. To our knowledge, this is the first time in which a factor involved in the embryonic dopaminergic neuron development directs non neural derived adult stem cells towards the dopaminergic phenotype. We hereby present a novel strategy to facilitate the dopaminergic differentiation on bone marrow derived mesenchymal stem cells as a possible source for autologous transplantation for Parkinsonian patients.
CO-INFECTION OF SCRAPIE AND EAE: PATHOLOGY AND SECOND GENERATION TRANSMISSION

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When mice infected with scrapie were induced for EAE, an immune mediated model of CNS inflammation, the co-induced mice died from progressive neurological disease long before control mice succumbed to classical scrapie. Extensive pathological examinations suggest the co-induced mice to suffer from exacerbated inflammation, as seen by increased levels of demyelination, immune cell infiltrates, and gliosis in spinal cords. Interestingly, PrPSc deposits were also found in demyelinated white matter areas, suggesting that in scrapie-EAE mice, activated and scrapie infected immune cells may infiltrate into the CNS. Total brain PrPSc accumulation was similar in high titer experiments between dying co-induced mice and asymptomatic scrapie infected mice sacrificed at the same time. In low titer prion experiments, animals dying of the same co-induced syndrome sometimes presented high levels of brain PrPSc while others did not. Second generation titration experiments suggest that prion titers in the brains of the diverse co-induced mice were independent from their clinical status and related to the levels of PrPSc accumulation. We hypothesize that while inflammatory processes affecting the CNS may have severe clinical implications in subjects incubating prion diseases, such clinical insult did not result in increased infectious potential of the affected animal.
The marine cyanobacterium, Trichodesmium spp. forms extensive blooms in the sub-tropical and tropical oceans and is responsible for significant inputs of "new N" into these systems via nitrogen-fixation. We present experimental laboratory evidence and field observations of an autocatalyzed programmed cell death (PCD) pathway in Trichodesmium. The PCD pathway was induced in response to phosphorous and iron starvation as well as high irradiance and oxidative stress. Additionally, we demonstrate that PCD induction is coupled with the increased production of extracellular polysaccharide aggregates, operationally defined as transparent exopolymeric particles (TEP). TEP are recognized as an important conduit for carbon recycling and export in aquatic systems. Yet, the factors controlling the build up of the TEP pool are not well characterized. Enhanced TEP production was not observed in actively growing populations. Aggregation and density characteristics of the TEP pool may cause either extensive export of carbon or induce a recycling-based microbial food web ecosystem. Induction of PCD by caspase-like proteases in a bacterial photoautotroph with an ancient evolutionary history requires a reassessment of the origins and roles of cell death cascades. Moreover, the coupling between TEP formation and PCD in Trichodesmium is a previously unidentified ecological mechanism that can lead to the termination of natural Trichodesmium blooms and influence the fluxes of organic matter in the ocean.
In order to survive extremely different and harsh environments, intracellular parasites require highly adaptable physiologic and metabolic systems. Leishmania donovani, the causative agents of kala azar in humans, extracellular promastigotes reside in a glucose-rich, slightly alkaline environment in the sand fly vector alimentary tract. Upon entry into human macrophage phagolysosomes, promastigotes differentiate into intracellular amastigotes. These cope with pH (from ~7 to pH ~5) and temperature (26 to 37°C) shifts. Using an axenic differentiation model that simulates the in vivo process we aimed to investigate the molecular mechanism of L. donovani differentiation. The same differentiating cells were used for DNA microarray and proteomic iTRAQ–LC-MS/MS gene expression analyses. The microarray analysis quantified 5,619 mRNAs with high confidence, 2,924 of which changed significantly during differentiation. The proteomic assay quantified 1,713 proteins, 902 of which were also quantified by the microarray. The results show that while mRNAs abundance change gradually after 5h in differentiation, most of the proteomic changes occur in a well-coordinated manner beyond 10h. Detailed examination of the proteomic changes show that the parasite retools its metabolic pathways to adapt to the new stresses it encounter in the host cell, by switching from glucose to fatty acid beta-oxidation as its main energy source and utilizing host phospholipids as precursors for fatty acids and glycerol which is used for the up-regulated gluconeogenic pathway. Comparison of mRNA and protein levels showed that 32% of the genes showed positive correlation between mRNA and protein abundance, 22% showed negative correlation and the rest displayed low correlation. Interestingly, most (71%) of the genes in the positive correlation group were down-regulated during differentiation, 19% were up-regulated and 4% were constitutively expressed. Whereas major metabolic pathways enzymes abundance changed during differentiation, their corresponding mRNAs remained unchanged, suggesting translation and posttranslational regulation of these genes.
Water is fundamental necessity for any living cell at any living environment. This necessity spawned a variety of mechanisms and behaviors all sharing one principle of retaining water potential homeostasis. Vascular plants face considerably greater challenges than animals’ meeting this principle as their daily water loss through transpiration is higher and their ability to react actively to environmental stress conditions is more limited. One of the major cellular differences between a plant cell and an animal cell is the huge plant vacuole. Taking up to 90% of the cell volume, the vacuole is by far the largest organelle in the adult plant cell. Among its multitude of regulatory functions in maintaining the cytoplasm steady state condition, it is osmoregulation that is most probably responsible for its large size, allowing it to serve as an emergency water reservoir. Consistent with this is the high osmotic water permeability value (Pf) of the vacuole membrane (tonoplast), which is much higher than the plasma membrane Pf. The ability to maintain this high water permeability is related to a tonoplast water channels family –TIP aquaporins. In this work we study the effect of increasing the tonoplast Pf on the whole plant response to abiotic stress. Our results showed that both Arabidopsis and tomato populations over-expressing TIP showed significant increase in fruit yield, harvest index and plant mass, under three different field trials, when subjected to regular and water stress conditions. These plants maintained higher transpiration rate during stress even at the cost of vacuole dehydration, detected as leaf relative water content lost. Only when reaching a certain stress threshold – higher than that of the normal plants - did the TIP-modified plants reduce this excessive transpiration. These results suggest a regulatory role for TIP in the capacity of the vacuole to maintain the cellular water potential homeostasis.
The plant hormone abscisic acid (ABA) is involved in the transmission of environmental changes like drought-, saline-, and cold-periods into stress adaptation processes. Based on the timescale of the individual ABA evoked responses they have been subdivided into fast (membrane transport) and slow (transcription) signalling. In contrast to the latter process the fast ABA response - exemplified by half times of stomatal closure around 5-10 min - is believed to not involve gene activation. Instead stomatal closure is accomplished by the release of potassium ions and chloride as well as the metabolic degradation of the major organic anion malate. In search for ABA signalling intermediates the response of ion channels of guard cells in epidermal peels as well as guard cell protoplasts and vacuoles have been challenged with well-characterized modulators effective in signal transduction pathways of animal cells. Isolated, experimentally well controlled guard cell preparations, however, often lack communication with neighbouring cells, turgor or cytosolic components. In addition potential signalling components derived from mutants altered in ABA-induced stomatal closure. To online record changes in ion fluxes across the plasma membrane of guard cells in intact plants, we have developed a method, based on multi-barreled microelectrodes introduced into the cytoplasm of these sensory motor cells. This approach in previous studies was successful in exploring blue- and red light as well CO2-signalling. Using this online, in planta approach we have been able to identify signalling elements required for fast ABA-induced stomatal closure. A model on the ABA-based regulation of guard cell ion transport will be presented at the meeting.
Stress and anxiety disorders present a major mental health problem, but their putative involvement in the initiation and/or progression of neurodegenerative diseases is being debated. Recent research in the lab focuses on the molecular mechanism(s) underlying anxiety-induced changes in cholinergic neurotransmission. These mechanisms modulate the motor control over movement (Evron et al., FASEB J, 2007), regulate working memory (Farchi et al, Eur J Neurosci, 2007), and activate brain-to-body communication through the neuron-immune interface modifying blood cells composition and platelet production (Gilboa-Geffen et al., Blood, 2007). Importantly, stress-associated changes were found in the expression pattern of the acetylcholinesterase ACHE gene, which encodes the acetylcholine hydrolyzing enzyme AChE. AChE is not one, but a combinatorial series of proteins having indistinguishable enzymatic activity yet with variant N- and C-termini due to alternate promoter usage and 3'-alternative splicing (Meshorer and Soreq, Trends in Neurosci, 2006). Differentially induced under stress, they show distinct non-hydrolytic properties, interact with variant-specific protein partners and induce inverse signaling cascades (Sklan et al., Biol Psych, 2006). Surprisingly, transcriptional and post-transcriptional regulation of AChE pre-mRNA not only protects blood and nerve cells from acute dangers, but may also entail long-term advantages. Specifically, causal involvement of both AChE and its closely related enzyme butyrylcholinesterase (BChE) in the progression of Alzheimer's (Berson et al., Brain, in press; Podoly et al., Neurodegen Dis, in press) and Parkinson's disease (Ben-Shaul et al., Eur J Neurosci, 2006), anticipates future therapeutic needs for drugs targeting specific cholinesterases or the corresponding RNA transcripts.
Biodiversity evolution under local, regional, and global ecological stress at all levels (genes, genomes, individuals, populations, species, and biota) and domestication evolution are the foci of interdisciplinary research programs at the Institute of Evolution and the International Graduate Center of Evolution, University of Haifa. Here I will demonstrate evolutionary changes, resulting from environmental stresses, as major evolutionary driving forces (climatic, thermal, edaphic, biotic, and atomic) at genomic and phenomic levels in several model organisms including bacteria, soil fungi, plants, animals, and humans on microgeographic (“Evolution Canyon”), macrogeographic (Israel and the Near East), and global (entire planet) scales (Nevo, 2001). Stress, in general, promotes genomic diversity up to an extreme point when diversity sharply declines, such as in Dead Sea filamentous fungi. Levels of genomic structure and expression can vary at a microscale, such as in the “Evolution Canyon” model and the Dalton evolutionary field. Sharp local ecological stresses can lead not only to adaptive radiation but also to incipient sympatric speciation in diverse taxa across life, as was demonstrated in “Evolution Canyon” (Nevo, 2006). Nevo, E. 2001. Evolution of genome-phenome diversity under environmental stress. Proc. Natl. Acad. Sci. USA 98:6233-6240. Nevo, E. 2006 “Evolution Canyon”: a microcosm of life’s evolution focusing on adaptation and speciation. Isr. J. Ecol. and Evol. 52:485-506.
Sodium/proton antiporters are life essential membrane proteins that maintain and regulate the cytosolic pH and Na+ concentration in all cells. The prototype NhaA is widely spread in enterobacteria and has orthologs throughout the biological kingdom including humans. We have determined the atomic structure of NhaA, a major breakthrough toward understanding the mechanism of activity of an antiporter and the general architecture of membrane proteins. NhaA is a dimer in the membrane. On the basis of the structure and ESR study, we modeled the dimer and constructed a mutation that yielded monomeric NhaA. At the routine salt stress conditions the monomeric form of NhaA is fully functional. Remarkably, under extreme stress conditions the dimeric native NhaA was much more efficient than the monomeric mutant in conferring the extreme stress resistance. Many transporters and channels exist in the native membrane as oligomers. In most cases, the functional/structural role of the oligomeric state is still an unknown. Hence, our results are an insight into the functional/structural role of the oligomeric state of a membrane protein.
MECHANISM OF Na+/H+ ANTIPORTING

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Na+/H+ antiporters play a key role in cellular salt and pH homeostasis. The structure of E. coli NhaA was recently determined, but its mechanisms of transport and pH regulation remain elusive. We performed molecular dynamics simulations of NhaA that, together with existing experimental data, enabled us to propose an atomically detailed model of antiporter function. Three conserved, membranous aspartate residues are key to our proposed mechanism: Asp164 is the binding site for Na+, Asp163 controls the alternating accessibility of the Na+ binding site to the cytoplasm or periplasm, and Asp133 is crucial for pH regulation. Consistent with experimental stoichiometric measurements, two protons are required to transport a single Na+: Asp163 protonates to reveal the Na+ binding site at Asp164 to the periplasm, and subsequent protonation of Asp164 releases Na+. To further validate our model, we conducted new mutagenesis experiments, the results of which were consistent with our predictions.
NOVEL MEMBERS OF THE CATION/PROTON ANTIPORTER SUPERFAMILY

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Cation/proton antiporters are ubiquitous and play important roles in salt, water and pH homeostasis by exchanging H+ for Na+ or K+ ions across the membranes of the cell and various organelles. Metazoan members of the CPA1 subgroup are encoded by NHE genes and include 9 isoforms in mammals. Of these, NHE1-5 are predominantly expressed on the plasma membrane, whereas other isoforms (NHE6, 7 and 9) appear on endosomes and intracellular vesicles. Our studies in the model organism Saccharomyces cerevisiae point to a critical role for endosomal Na+(K+)/H+ exchange in vesicle trafficking and delivery of the multivesicular body to the lysosome. Disruption of yeast Nhx1 results in acidic compartments, mislocalization of vacuolar hydrolases to the medium and retention of cargo in a prevacuolar compartment. In plants, endosomal/vacuolar NHE members are important for flower color, salt tolerance and leaf development. We will present ongoing studies that seek to identify a role for these NHE in mammalian cells. The CPA2 subgroup includes a novel clade of antiporters (NHA) in all metazoans that are distantly related to bacterial NhaA. We have initiated characterization of Homo sapiens NHA2 by heterologous expression in yeast and in mammalian cells. We show that this antiporter traffics to the plasma membrane and confers tolerance to Li+ and Na+, but not K+. Western blots reveal a widespread distribution in mouse tissues. Based on inhibitor sensitivity and chromosomal locus, we speculate that the NHA genes may contribute to sodium lithium countertransport (SLC) activity that is a well-known marker of hypertension.
MODEL STRUCTURE OF THE NA+/H+ EXCHANGER 1 (NHE1):
FUNCTIONAL AND CLINICAL IMPLICATIONS

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Eukaryotic Na+/H+ exchangers are transmembrane proteins that are vital for cellular homeostasis and play key roles in pathological conditions such as cancer and heart diseases. Using the crystal structure of the Na+/H+ antiporter from Escherichia coli (EcNhaA) as a template, we predicted the 3D structure of human NHE1. Modeling was particularly challenging because of the extremely low sequence identity between these proteins, but the model-structure is supported by evolutionary conservation analysis and empirical data. It also revealed the location of the binding site of NHE inhibitors; which we validated by conducting mutagenesis studies with EcNhaA and its specific inhibitor 2-aminoperimidin. The model structure features a cluster of titratable residues that are evolutionarily conserved and are located in a conserved region in the center of the membrane; we suggest that they are involved in the cation binding and translocation. We also suggest a hypothetical alternating-access mechanism that involves conformational changes.
Salt lakes and solar salterns are ideal environments for the study of the adaptation of microorganisms to high salt concentrations. NaCl is most often the main salt, but some athalassohaline lakes such as the Dead Sea have brines dominated by magnesium and calcium. Hypersaline alkaline soda lakes are depleted in divalent cations. All these environments are inhabited by diverse communities of halophilic and halotolerant microorganisms, adapted to life at the prevailing salt concentration and composition. These belong to all three domains of life: Archaea, Bacteria and Eucarya. A few phylogenetically coherent groups consist (almost) entirely of halophiles: the Halobacterales (Euryarchaeota), the anaerobic fermentative Halanaerobiales (Firmicutes), and the aerobic Halomonadaceae (Gammaproteobacteria). Halophilic microorganisms use two strategies to balance their cytoplasm osmotically with their medium. The first involves accumulation of KCl. This strategy requires adaptation of the intracellular enzymatic machinery to near-saturating salt concentrations. Such microorganisms have highly acidic proteins, and most of these proteins denature at low salt. Such microorganisms generally cannot survive at low salt. In the second strategy salt is excluded from the cytoplasm. Organic compatible solutes (glycine betaine, ectoine and other amino acid derivatives, sugars, sugar alcohols) are synthesized and/or accumulated that do not interfere with enzymatic activity. Few adaptations of the proteome are needed, and such organisms often tolerate a broad salt concentration range. The "salt-in-strategy" is not limited to the Halobacteriaceae. The Halanaerobiales also accumulate salt rather than organic solutes. Another, recently discovered organism that accumulates KCl is the red extremely halophilic Salinibacter (Bacteroidetes), found in salt-saturated saltern crystallizer brines. Genomic analysis showed great resemblance with the Halobacteriaceae, including presence of four genes coding for retinal proteins, including a light-driven proton pump and a putative light-driven chloride pump.
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Abstract not available.