

Impairment of the Proteasome and Induction of Autophagy in Neurodegenerative Disease

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Neurodegenerative polyglutamine (poly Q) expansion diseases are caused by increase in the number of naturally occurring “CAG” repeats in specific genes. This mutation leads to neuronal dysfunction and degeneration, with a variety of symptoms including cognitive and neuromuscular abnormalities. The most common cell pathology is the accumulation of large protein aggregates, which invariably contain truncated forms of the gene product under question together with other proteins including ubiquitin. We are using a *Drosophila* model to study the human disorder Spinal Bulbar Muscular Atrophy (SBMA), which is caused by polyglutamine expansion of the androgen receptor (AR). Like in humans, flies that express human AR with greater than 40Qs are normal unless they have elevated androgen (testosterone); this disorder has only been diagnosed in men. We observed that during polyQ-induced degeneration a reporter of the ubiquitin-proteasome system (UPS) accumulates indicating that the UPS is inhibited. In addition, autophagy is activated beyond its physiological levels as evident from electron microscopic analysis. Activation of autophagy seems to be a protective response to polyQ expression since downregulation of some of the essential autophagy genes enhances the degenerative phenotype. We hypothesize that cellular pathology is the result of an overload of proteins that otherwise would be degraded by the UPS, and that autophagy is activated to help relieve this overload by clearing aggregating proteins. The mechanism as to how UPS is inhibited and autophagy is activated is unknown. We are currently working on characterization of a motor protein subunit that may be involved in this pathological outcome. In addition we are studying the role of cell death regulators such as caspases in the cellular pathology.

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Characterization of the role of a Class III Pi3-kinase complex in induction of autophagy

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Autophagy, a nutrient regulated process of cell self-digestion, has been implicated in development and disease, including cancer and neurodegeneration. The molecular mechanisms of autophagy were first defined in yeast, where approximately twenty Atg genes were found to be required for formation of autophagosomes. These double-membraned structures engulf components of the cytoplasm, including long lived proteins and organelles, then fuse with lysosomes. The contents of these autophagolysosomes are then recycled to supply amino acids and other cellular building blocks for protein synthesis and metabolism. A Class III Phosphatidylinositol 3-kinase (Pi3K III) complex, which includes the kinase Pi3K III, the bridging protein Vps15, and Beclin 1/Atg6, has been shown to be required for autophagosome formation. We are studying the role of these proteins in autophagy in *Drosophila melanogaster*, as the function of all components of this complex have not been systematically investigated in a multicellular organism *in vivo*. Loss of function and gain of function analyses are being used to determine the genetic interactions between members of the complex, and to test the sufficiency of each for induction of autophagy. N- and C-terminal tagged fusions of Class III Pi3K, Atg6, and Vps15 have been constructed and transgenic flies have been made. Preliminary results indicate that expression of two components of this complex can induce autophagy, while expression of single components is not sufficient. We are also producing targeted gene disruptions to complement these misexpression studies. Furthermore, we are investigating *in vivo* biochemical interactions between Class III Pi3-kinase, Atg6, and Vps15, and plan to use mass spectrometry to identify other proteins that may interact with the known members of this complex.

Integrating Membrane Transport with Male Gametophyte Development and Function through Transcriptomics

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Male fertility depends on the proper development of the male gametophyte, successful pollen germination, tube growth and delivery of the sperm cells to the ovule. Previous studies have shown that nutrients like boron, and ion gradients or currents of Ca^{2+} , H^+ , and K^+ are critical for pollen tube growth. However, the molecular identities of transporters mediating these fluxes are mostly unknown. As a first step to integrate transport with pollen development and function, a genome-wide analysis of transporter genes expressed in the male gametophyte at four developmental stages was conducted. About 1269 genes encoding classified transporters were collected from the *Arabidopsis thaliana* genome. Of 757 transporter genes expressed in pollen, 16% or 124 genes, including *AHA6*, *CNGC18*, *TIP1.3* and *CHX08*, are specifically or preferentially expressed relative to sporophytic tissues. Some genes are highly expressed in microspores and bicellular pollen (*COPT3*, *STP2*, *OPT9*); while others are activated only in tricellular or mature pollen (*STP11*, *LHT7*). Analyses of entire gene families showed that a subset of genes, including those expressed in sporophytic tissues, were developmentally-regulated during pollen maturation. Early and late expression patterns revealed by transcriptome analysis are supported by promoter::GUS analyses of *CHX* genes and by other methods. Recent genetic studies based on a few transporters, including plasma membrane H^+ pump *AHA3*, Ca^{2+} pump *ACA9*, and K^+ channel *SPIK*, further support the expression patterns and the inferred functions revealed by our analyses. Thus, revealing the distinct expression patterns of specific transporters and unknown polytopic proteins during microgametogenesis provides new insights for strategic mutant analyses necessary to integrate the roles of transporters and potential receptors with male gametophyte development.

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Mismatch repair in *Halobacterium*: Bacterial-Like or Archaeal-Specific?

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DNA mismatch repair (MMR) plays a key role in the recognition and repair of errors made during replication. The pathway can be methylation-dependent and the key proteins, MutS and MutL, are conserved from Bacteria to Eukarya. Comparative sequence analyses reveal that none of the archaeal genomes sequenced so far, with the exception of two halophiles and four closely related methanogens have homologs of those proteins. The MMR genes found in *Halobacterium* sp. NRC-1 are canonical bacterial *mutS* and *mutL* genes, suggesting that it is the result of a lateral gene transfer event. Our working hypothesis is that homologs of MutL and MutS are components of a functional mismatch repair pathway in *Halobacterium*. The alternative would be that an archaeal-specific MMR system exists and it is also present in *Halobacterium*. Using mevinolin and 5-fluorouracil, we determined the mutation rate of wildtype *Halobacterium* and found that it was similar to that of another archaeon, *Sulfolobus acidocaldarius*. Potential MMR proteins of this putative pathway are being tested by a knockout strategy and analysis of the mutant phenotypes with regard to survival to known mutagens, mutation rate, and protein binding assays. We have constructed $\Delta zim\Delta mutL$ and $\Delta zim\Delta mutS2$ deletion mutants and tested their survival following exposure to radiation and chemical treatment. Survival was not found to be significantly different than that of the background strain with regards to gamma-ray and chemical mutagens, but a significant decrease in survival was observed with the $\Delta zim\Delta mutS2$ mutant strain when exposed to UV irradiation. Fluctuation tests and construction of single and double knockout mutant strains for *mutS1* and *mutS2* are in progress. Phenotypic analysis of those mutants should enable us to determine if a bacterial-like MMR pathway is the major MMR pathway in *Halobacterium*.

Does the Expression of Fibonacci Numbers in Plant Phyllotaxis Reveal the Involvement of Geometrical Imperatives or Biological Interactions?

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Complex biological patterns are often governed by simple mathematical rules. A favorite botanical example is the apparent relationship between phyllotaxis (i.e., the arrangements of leaf homologs such as foliage leaves, bud scales, petals, and other floral organs on shoot axes) and the Fibonacci number sequence (1, 2, 3, 5, 8, 13...). Fibonacci sequences have intriguing mathematical properties, not the least of which is that the golden ratio (Φ) is the limit of the fractional Fibonacci sequences used to characterize many phyllotactic patterns. Thus, it is frequently alleged that developing leaf primordia adopt Fibonacci-related patterns in response to a universal geometrical imperative for optimal packing derived from the golden ratio. This paper reviews the fundamental properties of number sequences, after which it discusses the underappreciated limitations of the Fibonacci sequence for describing the most common phyllotactic patterns involving whorls (more than one leaf at the same level around the axis) and spirals (one leaf at each level with a fixed divergence angle between adjacent leaves). The evidence marshaled here shows that contrary to the claims in the phyllotaxis literature, whorl phyllotaxes of leaves and of floral organs are not positioned according to the Fibonacci sequence. Insofar as developmental transitions in spiral phyllotaxis follow discernable Fibonacci formulas, phyllotactic spirals are therefore interpreted as being arranged in genuine Fibonacci patterns. Nonetheless, a simple modeling exercise argues that the most common spiral phyllotaxes do not exhibit optimal packing. Instead, the consensus starting to emerge from different subdisciplines in the phyllotaxis literature supports the alternative perspective that phyllotactic patterns arise from local inhibitory interactions among the existing primordia already positioned at the shoot apex, as opposed to the imposition of a global imperative of optimal packing.

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Structure/function studies of the yeast 60S subunit: an overview.

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Abstract

The concept that the structure of the ribosome determines its function is simple. In practice, linking the two is not. Toward this general goal, we have undertaken an integrated approach employing the methods of molecular genetics, biochemistry, structural biology and molecular modeling in the model eukaryotic organism, *Saccharomyces cerevisiae*. Both selected ribosomal proteins and rRNA residues have been targeted for this analysis. Core ribosomal proteins of the large subunit were chosen for mutagenesis and subsequent analyses based on their known and predicted effects on peptidyltransfer, tRNA binding, interactions with elongation factors, and intersubunit interactions. These include ribosomal proteins L2, L3, L5, L10, and L11. Specific bases of the 25S rRNA were selected for study based on similar criteria. These include bases in helix 38 (the A-site finger), helix 92 (the A-loop), helix 80 (the P-loop), and in the vicinity of the peptidyltransferase center. We have also completed extensive mutagenesis studies of 5S rRNA. Along the way, we have optimized a yeast genetic system for the stable generation of cells expressing only mutant rRNAs. Our investigations have also examined the roles of base modification on ribosome function. Base modification defects specific to the A-loop and to helix 93 were found to differentially affect specific ribosome-associated functions. The emerging picture is one in which individual components function both to make specific functional contributions, while simultaneously communicating these functions to one another over long distances within this complex nanomachine.

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Identifying regulatory components of the ethylene signaling pathway in *Arabidopsis*

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The gaseous hormone ethylene plays an important role in plant growth, development and responses to environmental clues. In recent years, substantial progress has been made towards uncovering the molecular mechanisms of ethylene signaling. Our goal is to identify and characterize additional molecular components of this pathway using the reference plant *Arabidopsis*. To identify such components, we are taking two different approaches. One is tandem affinity purification (TAP)-tagging to isolate native protein complexes from *Arabidopsis* to uncover interacting protein partners of REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1), a novel regulator of ethylene receptor function (1). The other approach is genetic screening for suppressors of the ethylene-insensitive ethylene receptor mutant *etr1-2*. So far, for the tap-tagging approach, we have created fusions of RTE1 with either an N-terminal TAP or C-terminal TAP tag expressed in multiple *Arabidopsis* lines. For the identification of new genetic components of the ethylene signaling pathway, we have screened approximately 12,000 EMS-mutagenized seedlings of *etr1-2* for suppressor mutants based on the “triple response” of the etiolated seedlings to ethylene treatment. We currently have at least 12 confirmed mutants showing more sensitivity to ethylene than *etr1-2*. Phenotypes of representative mutant seedlings are shown in **Figure 1**. In order to clone the genes responsible for altering the response of the plants to ethylene, the mutants have been crossed to generate genetic mapping populations. Other crosses are underway with known mutants in the ethylene signaling pathway in order to facilitate the functional study of these loci in plant growth, development and responses to the ethylene hormone.

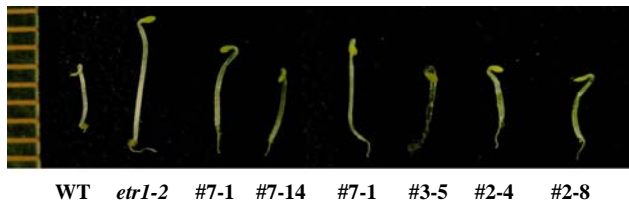


Figure 1. Comparison of 4-day old dark-grown seedlings germinated in the presence of the ethylene precursor ACC (100 μ M). The suppressor mutants (#7-1, 7-14, 7-1, 3-5, 2-4, 2-8) are shorter (more sensitive to ethylene) than *etr1-2*.

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Expression Pattern of Dispensable SR Protein Genes in *Arabidopsis thaliana*

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RNA processing in eukaryotes is a highly complex process requiring numerous steps and factors that can play a role in the regulation of functional protein production. It is estimated that at least 60% of all human genes undergo alternative splicing to produce multiple protein isoforms, and errors in splicing account for numerous genetic diseases in humans including cystic fibrosis and spinal muscular atrophy. Understanding the complex mechanism and regulation of splicing will provide insights into defects associated with both constitutive and alternative splicing.

SR proteins are a well-defined family of splicing factors identified by a conserved RNA Recognition Motif (RRM) as well as C-terminal arginine/serine (RS) repeats. This family of proteins and its characteristic features are conserved across all eukaryotes. The genomes of most complex organisms encode multiple SR proteins, while simpler eukaryotes such as yeast encode a single SR protein. SR proteins are known to bind to mRNA precursors via Exon Splicing Enhancers which leads to the recruitment of components of the spliceosome and enhanced splicing. Mammals mutant for ASF/SF2 or SC35 are early embryonic lethal and thus whole organisms are inherently difficult to study *in vivo*. We have obtained 125 T-DNA insertion lines in 19 of the 20 Arabidopsis SR protein genes. Two (SCL28 and SRp31) result in embryonic lethal phenotypes. However, other insertion lines result in viable plants null for either SC35, SR45, and SRp30 with a range of phenotypes. These data suggests that some SR proteins, at least in plants, are able to functionally compensate for the loss of other SR proteins.

In order to begin to fully understand this relationship between individual SR proteins it is essential to know when and where individual SR proteins are expressed throughout development. We have begun to determine the expression patterns of individual SR proteins via GFP fusions in *Arabidopsis thaliana*. Thus far we have examined expression patterns of several individual proteins in multiple tissues including roots, leaves, and flowers and have begun examining SR protein/GFP transgenes in lines mutant for SC35, SR45, and SRp30. In the proteins which we have been able to study in detail, we have identified both spatial and temporal expression patterns relative to specific tissues and general organs.

Using an *in vitro* assay designed in our lab, we have started testing candidate ESE sequences in wild-type and lines null for individual SR protein genes in order to investigate the relationship between SR proteins and ESE activity. A mutation in SC35 reduces the ESE activity of sequences ATCGTCACA and TGAATCGTC, perhaps by up-regulation of intermediary SR proteins (or other factors). Because SR proteins themselves are alternatively spliced, we have looked for cross regulation using RT-PCR analysis of isoform accumulation in alternatively spliced SR protein genes. Preliminary data support such cross-regulation. We are moving towards an understanding of the relation between individual SR proteins and ESE activity.

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Biochemical and Functional Characterization of Three Activated Macrophage Populations¹

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We generated three populations of macrophages in vitro and characterized each. Classically activated macrophages (Ca-M ϕ) were primed with IFN γ and stimulated with LPS. Type II activated macrophages (M ϕ -II) were also similarly primed, but stimulated with LPS plus immune complexes. Alternatively Activated macrophages (AA-M ϕ) were primed overnight with IL-4. Here we present a side-by-side comparison of the three cell types, but focus primarily on differences between M ϕ -II and AA-M ϕ , because both have been classified as M2 macrophages, distinct from Ca-M ϕ . We show that M ϕ -II are more similar to Ca-M ϕ than they are to AA-M ϕ . Both M ϕ -II and Ca-M ϕ , but not AA-M ϕ , produce high levels of NO and have low arginase activity. AA-M ϕ express FIZZ1, whereas neither M ϕ -II nor Ca-M ϕ does. M ϕ -II and Ca-M ϕ express relatively high levels of CD86, whereas AA-M ϕ are virtually devoid of this co-stimulatory molecule. Ca-M ϕ and M ϕ -II are efficient antigen presenting cells, whereas AA-M ϕ fail to stimulate efficient T-cell proliferation. The differences between Ca-M ϕ and M ϕ -II are more subtle. Ca-M ϕ produce IL-12 and give rise to Th1 cells, whereas M ϕ -II produce high levels of IL-10 and thus give rise to Th2 cells secreting both IL-4 and IL-10. M ϕ -II express two markers that may be used to identify them in tissue. These are sphingosine kinase 1 (SPHK1) and LIGHT (TNFSF14), a member of the TNF superfamily. Thus, classically activated macrophages, Type II activated macrophages and alternatively activated macrophages represent three populations of cells with different biological functions.

¹Edwards, J.P., X. Zhang, K.Frauwirth, D. Mosser. 2006. Biochemical and Functional Characterization of Three Activated Macrophage Populations. *J. Leukocyte Biol. In Press.*

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Transgenic *L. major* expressing murine CD40L reduce disease and provide protection against wild type challenge.

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Currently, the only effective vaccine for leishmaniasis is the inoculation of live, virulent organisms into the host. We have previously shown that combining CD40L with antigen derived from *Leishmania* is an effective way to preferentially induce a type 1 immune response, and to vaccinate mice against subsequent challenge with virulent organisms. In this study, we developed transgenic *L. major* which express and secrete the extracellular portion of CD40L (*L. major* CD40LE). We hypothesized that these organisms would be more immunogenic than wild type organisms, and would cause reduced disease in a mouse model. Expression of CD40L by transgenic parasites was confirmed by RT-PCR to detect CD40L mRNA expression, western blotting to detect protein, and ELISA to detect secreted protein. These transgenic organisms induced improved T cell activation in a DO11.10 model, indicating improved immunogenicity. We show that these transgenic parasites cause reduced disease in the susceptible BALB/c strain of mice. Mice infected with transgenic parasites developed significantly smaller lesions containing fewer parasites than those animals infected with wild type organisms. Finally, immunization of resistant C57Bl/6 mice with a low dose of transgenic parasites induced a significant amount of protection against subsequent infection with wild type organisms. Taken together, these results demonstrate that transgenic organisms expressing CD40L are less virulent than wild type organisms while retaining full immunogenicity. We conclude that transgenic organisms expressing CD40L or other immune-stimulatory molecules may be valid candidates for attenuated immunogenic vaccines against leishmaniasis.

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West Nile Virus Evades Activation of RIG-I And MDA5-dependent Pathways Without Antagonizing Host Defense Signaling

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Recent outbreaks of West Nile Virus (WNV) have been associated with an increase in morbidity and mortality in humans, birds and many other species, suggesting that a more pathogenic strain of WNV has emerged. The molecular mechanisms for the increased pathogenesis of WNV are unknown but are likely to include novel virus-host interactions that allow WNV to overcome or evade the host innate and/or adaptive immune response. We have previously shown that the ability of WNV-NY to delay the activation of interferon regulatory factor 3 (IRF-3), a transcription factor critical to the initiation of the antiviral response, is essential for WNV-NY to achieve maximum virus production. Furthermore, WNV-NY utilizes a unique mechanism to control activation of IRF-3. In contrast to many other viruses, which impose a nonspecific block to the IRF-3 pathway, WNV-NY eludes detection by the host cell at early times post-infection. To better understand this process, we assessed the role of the pathogen recognition receptor (PRR), RIG-I, in sensing WNV-NY infection. RIG-I null mouse embryo fibroblasts (MEFs) retained the ability to respond to WNV-NY infection; however, the onset of the host response was delayed compared to WT MEFs. This suggests that RIG-I is involved in initially sensing WNV-NY infection while other PRRs sustain and/or amplify the host response later in infection. The delayed initiation of the host response in RIG-I null MEFs correlated with an increase in WNV-NY replication, indicating that activation of the host response by RIG-I early in infection is important for controlling replication of WNV-NY. The involvement of the RIG-I homolog MDA5, which has also been shown to be involved in activation of the host antiviral response, in sensing WNV-NY infection was also examined. Disruption of signaling through both MDA5 and RIG-I completely abrogated the host response to WNV-NY, suggesting that MDA5 is responsible for the residual activation of the host response observed in RIG null cells and a key component in the host's defense against viral infection.

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Genomic analysis of the biomass conversion systems of the marine bacterium *Saccharophagus degradans* 2-40.

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Saccharophagus degradans 2-40 (*Sde2-40*) is an aerobic, gamma subgroup proteobacterium (order, *Alteromonadales*) that can rapidly decompose diverse plant material in monoculture. It expresses multiple enzyme systems to degrade at least 11 different complex polysaccharides (CPs), including agar (agarose), alginate, cellulose, chitin, fucoidan, laminarin, mixed β -glucans, pectin, pullulan, starch and xylan. It also synthesizes several proteases and lipases. To identify the genes for the functional carbohydrases, the complete *Sde2-40* genome sequence was determined by DOE /JGI. The sequence, indeed, coded for multiple carbohydrase systems. At least 111 gene models were identified that either contained a homolog of a known glycoside hydrolase (GH) domain and/or a carbohydrate-binding module (CBM). Collectively, 31 different classes of GH domains were identified in the predicted carbohydrases. Through genetic, proteomic and biochemical analyses, functional elements of agarolytic, chitinolytic and cellulolytic systems have been characterized. Each of these environmentally regulated systems utilizes freely secreted and surface-associated enzymes to degrade the substrate. Each system functions to vector mono-, di-, and oligo-saccharide products to the cell through strategic placement of enzymes. Freely secreted enzymes of each system tend to be endo-acting enzymes with multiple CBMs. At least one enzyme in each degradative system appears to be an epicellular lipoprotein that has been demonstrated or is predicted to be exo-acting enzyme. A phosphorylic pathway for cellulose degradation is proposed. Many of these enzymes appear to have been acquired by the naturally competent *Sde2-40* through horizontal gene transfer or by domain shuffling.

Investigating the role of the Ubiquitin Proteasome System and Autophagy in *Drosophila* developmental cell death.

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Cells use two main systems to degrade cellular contents, the Ubiquitin-Proteasome System (UPS) and the autophagosomal-lysosomal system, yet the role of these degradation systems in cell death is not entirely understood. The UPS has been shown to play a role in cell death via its degradation of apoptotic regulatory molecules, and caspase-regulated impairment of the UPS is thought to facilitate death in human and *Drosophila* cell lines. While it is known that autophagy is induced during developmental cell death, its role in cell death is controversial. We hypothesize that autophagy induction could serve as a compensatory mechanism in cell death following UPS impairment, and we are investigating the roles of the UPS, autophagy, and the possible relationship between these degradation systems *in vivo* using *Drosophila* salivary gland cell death as a model. Through the use of a reporter system to monitor UPS activity, we demonstrate that the UPS is impaired during salivary gland cell death. We show that UPS impairment in salivary glands, using an inducible proteasomal mutant, fails to prevent normal salivary gland cell death. By contrast, premature UPS impairment leads to early gland death. Together, these data suggest a possible role for UPS impairment during developmental cell death. Furthermore, preliminary evidence suggests that both autophagy and caspases are upregulated upon ectopic UPS impairment in salivary glands. These data are consistent with what is observed during normal salivary gland death, and suggest that proteasomal impairment is a physiologically relevant component of cell death.

Screening for interactors of *Arabidopsis* RTE1 and RTH using the yeast split-ubiquitin system

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Arabidopsis RTE1 and its homolog RTH represent highly conserved genes found throughout plants, animals and protists. *Arabidopsis* RTE1 regulates ethylene receptor function by an unknown mechanism, while RTH was identified on the basis of sequence homology (1). The protein sequence is novel and has no known biochemical function in any organism, although sequence analysis suggests that both genes encode type II membrane proteins. Since protein interactions are crucial for cellular life, studies that identify interactors with previously characterized or novel proteins can help to elucidate the function of a protein of interest. To reveal the cellular functions of RTE1 and RTH, the *S. cerevisiae*-based split-ubiquitin system, which was designed specifically to uncover potential protein interactors for membrane-bound proteins (2,3), is currently being used to screen an *Arabidopsis* cDNA library. Results of the screen will help to support and refine current hypotheses and/or shed light upon the specific biochemical functions of RTE1 and RTH. Topological data obtained from this system indicates that the C-terminus of RTE1 and RTH is cytosolic and that the RTE1 and RTH protein constructs are predominantly localized in the ER of yeast.

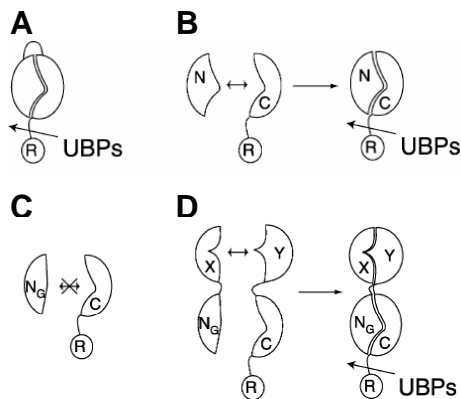


Figure 1. Split-Ub System (from Auerbach et al., 2002). The ubiquitin (Ub) protein is composed of two domains. UBPs recognize Ub and cleave at a motif located at the Ub end. **A:** If a reporter (R) is fused to the C-term of Ub, it is released upon cleavage by UBPs. **B:** Ub can be split into two halves (N) and (C) which can reassemble. If a reporter is fused to the C-term of C, it is released by UBP cleavage. **C:** A point mutation in the N moiety (N_g) abolishes the affinity of N and C halves for each other and the reporter is no longer released. **D:** When N_g and C are fused to two interacting proteins X and Y, Ub is reconstituted, resulting in recognition by UBPs and the subsequent release of the reporter.

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Characterization and localization of the *C. elegans* ortholog of *Arabidopsis RTE1*

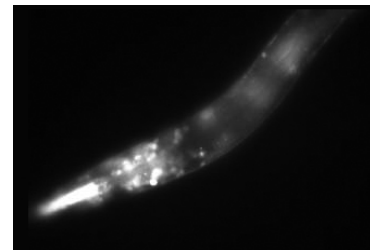
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We are investigating the *Caenorhabditis elegans* ortholog of *Arabidopsis REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1)* with the goal of elucidating the conserved function of this gene. *RTE1* is a novel gene of unknown biochemical function present in all multicellular organisms. Our specific aims are to uncover a mutant phenotype for this gene in *C. elegans* and determine the expression pattern of the gene and localization of the protein using GFP as a reporter. *RTE1* is a regulator of ethylene receptor function in *Arabidopsis* (1). The ethylene receptors require a copper co-factor in order to bind ethylene, which led to the hypothesis that *RTE1* may be involved in homeostasis or transport of copper ions. Though copper is an essential co-factor for a variety of enzymes, at elevated concentrations it can be extremely harmful to nematodes, causing severe morphological defects including reduced girth, shortened body length and reduced viability. To test whether the *C. elegans* ortholog is involved with this previously documented copper stress response, we obtained a knock-out mutant line for this single-copy gene from the genetic stock center and examined the mutant for phenotypic and behavioral changes in the presence of toxic copper levels. Measurements of body length suggest that the mutant animals are less responsive to toxic copper levels while also being behaviorally more active. This provides the first demonstration of an altered copper response in the absence of an *RTE1*-related gene in any organism. Independent transgenic *C. elegans* lines carrying either a promoter::GFP fusion or an N-terminal GFP protein fusion display ubiquitous expression that is particularly prominent in nerve cells (**Figure 1**). Characterization of sub-cellular localization is currently being pursued.

Figure 1. Promoter::GFP::NLS Expression Pattern for the *C. elegans* ortholog of *RTE1*. A 1.5 kb fragment upstream of the start codon was inserted into plasmid pPD95.67 (from A. Fire, Carnegie Institute), which contains the GFP coding sequence and a nuclear localization signal (NLS). Three independent transgenic *C. elegans* lines were derived by micro-injection. Expression appears constitutive in all cells and is notably strong in head and tail neurons. This image highlights strong neuroblast and pharyngeal expression in the head region of a young larval nematode.



Reference:

1. Resnick JS et al. (2006) *REVERSION-TO-ETHYLENE SENSITIVITY1*, a conserved gene that regulates ethylene receptor function in *Arabidopsis*, *Proc. Natl. Acad. Sci. USA* 103:7917-7922.

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Characterization of *RTE1* and *RTH* gene expression patterns in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* *REVERSION-TO-ETHYLENE SENSITIVITY1* (*RTE1*) gene encodes a novel protein that is thought to be involved in regulating a receptor in the signaling pathway of the plant hormone ethylene. The *RTE1* gene is highly conserved in plants, animals and protists, which implies that its function has broad importance. *Arabidopsis* has a homologous gene named *RTE-HOMOLOG* (*RTH*). Research is ongoing to determine more precisely what the roles of these genes are in plants and animals. This particular project is focused on characterizing the expression patterns of the two genes *RTE1* and *RTH*. To do this, lines of transgenic plants have been created that have the reporter gene, *gusA*, fused to the promoter region of each gene. Such lines have been generated for both *RTE1* and *RTE2* in a variety of genetic backgrounds. Now that transgenic plants have been made, the next phase of the project will be to observe gene expression patterns. The *gusA* reporter gene produces a protein that yields a blue precipitate in the presence of the substrate X-Gluc. Staining plant tissue with X-Gluc will allow visualization of gene expression in different parts of the plant and during different stages during development. Expression patterns of the plants with different backgrounds will be compared to each other, which will help to determine how the two genes of interest interact with various components of the ethylene transduction pathway as well as other plant processes, and will ultimately provide important insights into their functions.

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The isolation of new mutants in the ethylene signaling pathway in *Arabidopsis thaliana*

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The plant hormone ethylene is an important regulator of plant responses, including senescence and fruit ripening. Genetic dissection of the ethylene signaling pathway in the model plant *Arabidopsis thaliana* has provided a framework for the molecular events in ethylene signaling. We have performed a mutagenesis screen to isolate new players in the ethylene signaling pathway. In *Arabidopsis*, ethylene is perceived by a family of five receptors, which negatively regulate the response to the hormone. The ethylene insensitive mutant *etr1-2* is a dominant gain of function allele, which lacks the ethylene phenotype known as the triple response. An EMS mutagenesis screen was performed in the *etr1-2* background to find suppressors of this mutation. The screen was performed by searching for seedlings that exhibited the ethylene triple response when exposed to the hormone for four days in the dark. Three extragenic mutants have been isolated based on this screen. These mutants have been shown to be non-allelic to *RTE1*, a gene isolated in a similar mutagenesis. Further characterization has shown that one of these mutants, named *soe1-1* (*SUPPRESSOR-OF-ETHYLENE-INSENSITIVITY1*), has the additional phenotype of senescence in response to ethylene at the adult stage. Current work is focused on identifying the location of the *soe1-1* locus by map-based cloning. In addition to the extragenic mutations isolated in the mutagenesis screen, one intragenic suppressor mutation was isolated. This mutant, *etr1-10*, is the first missense mutation isolated in the *ETR1* gene not to yield a dominant, ethylene-insensitive phenotype. The current focus on this mutant is to ascertain whether the mutation is a complete loss of function or a revertant to wild type.

This work is supported by a grant from the Department of Energy (DE-FG02-99ER20329) to C. Chang.

Defining the Mga Virulence Regulon in *Streptococcus pyogenes*: Core Genes, Serotype Specificities and Metabolic Links

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The multi-gene regulator of the Group A Streptococcus (Mga, *S. pyogenes*) is known to control virulence genes encoding factors important for immune evasion and adherence. The extent of this regulon varies between M serotypes; however, the full spectrum of Mga regulated genes remains unknown. Two alleles for *mga* (*mga-1* and *mga-2*) have been identified that generally correlate to the class (I or II) of M protein expressed by that GAS and to tissue tropism. Therefore, we performed a transcriptome analysis comparing two strains of serotypes M1 and M6 (class I, *mga-1*) and an M4 serotype (class II, *mga-2*) to their respective isogenic *mga*-inactivated strains in late exponential phase. The 'core' Mga regulon was defined as those genes previously established to be directly regulated by Mga (*emm*, *scpA*, *sclA*, *mga*) and was not surprising that these represented the most activated transcripts in our array analysis. Also found were serotype specific core genes such as *sic*, *fba*, and *sof*. In addition, we found that a small ORF (Spy2036) predicted to encode a hypothetical protein was highly activated by Mga in all three serotypes. Further, an Mga regulated transcript was detected by northern blot and Mga bound directly to the Spy2036 promoter via band shift analysis. Thus, we have named this new core gene *grm* for gene regulated by Mga. Interestingly, *grm* is expressed in both Class I and Class II strains of GAS despite sharing its Mga binding site with the divergently transcribed *sof* (e.g., M4). Previous studies have indicated that the cysteine protease gene *speB* is Mga-regulated in some GAS strains (M49), but not in others. Our studies reflect this variability, with *speB* being highly activated in the M1 strain apparently independent of *rgg/ropB*, but not regulated in either M4 or M6. A survey of different GAS serotypes indicated that if *speB* is expressed in the wild type strain, it is Mga regulated. However, attempts to demonstrate direct interaction of Mga at the *speB* promoter were unsuccessful, suggesting an indirect mechanism of activation. Beyond the core genes, hierarchical cluster analysis found that M1 and M4 (*mga-2*) exhibited comparable transcriptional profiles; whereas, the M6 strain JRS4 (*mga-1*) was highly dissimilar. Results comparing only M1 and M4 show that Mga regulates a large number of genes related to sugar metabolism such as phosphoenolpyruvate PTS operons. In fact, Mga mutants showed a significant growth defect in chemically defined media containing only those sugar sources identified in the array analysis. This may allow virulence factors required for early stages of infection to be expressed when an optimal metabolic state of the cell has been reached at specific tissue sites during infection. These data support direct links between Mga, carbon utilization, and catabolite repression in GAS. Thus, Mga is able to control the expression of genes, both directly and indirectly, that are important for the metabolic balance and virulence in GAS.

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Ribosomal Protein L3: Gateway to the A-Site

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Abstract. Ribosomal protein L3 (L3) is an essential and indispensable component for formation of the peptidyltransferase center (PTC). Early studies in yeast demonstrated roles for L3 in resistance to PTC-specific antibiotics and virus propagation. Extensive mutagenesis of the single copy essential *RPL3* gene revealed important roles of the central and N-terminal extensions of L3 in these functions. These structures dive into the core of the large subunit, reaching the A-site side of the PTC. Site-specific mutagenesis generated a library of mutants of these two extensions. Structural analyses of mutant ribosomes revealed large conformational changes in the A-loop, and the conformations of specific bases in helix 73 and Sarcin/Ricin loop were also affected. Biochemical studies demonstrated that resistance to the A-site specific translational inhibitor anisomycin strongly correlates with increased aa- tRNA binding. We hypothesize that W255, which lies at the tip of the central extension, and the N- terminal serine, located within hydrogen bonding distances to A2941 (G2574 in *T. thermophilis*) and C2924 (C2556 in *T. thermophilis*), respectively, are involved in interactions with these bases. Bases C2573, C2556, and U2492 have been implicated in the formation of “A-site gates” through which the aminoacyl-tRNAs must pass along their way to the PTC during the process of accommodation (Sonbonmatsu et al., 2005. PNAS 102:15854-9). Our results indicate that these two extensions of L3 influence the formation of the A-site, and that they may be involved in the formation and/or function of these gates. It is possible that mutations W255C and S2T result in relaxed, or opened conformation of the A-site gates and the A-site crevice, thus facilitating the binding and entry of aminoacyl-tRNA into A-site and therein reducing the competition with anisomycin.

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BSCI 124: The challenges of teaching plant biology to non-science majors

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Teaching plant biology to non-science majors presents many challenges, but it can also be a fun and rewarding experience. Besides teaching BSCI 124 Plant Biology lecture, I also oversee BSCI 125 Plant Biology Lab. Both courses fulfill a CORE life-science requirement for non-science majors on campus. Many graduate students at CBMG have to be Graduate Student Teaching Assistants in these courses, so I am also involved in training graduate students in teaching techniques for the course. I also enjoy advising for the Department and for the College of Chemical and Life Sciences, as well as participating in several activities around campus – such as the seminars offered by the Center for Teaching Excellence, campus art projects, and support groups for Latino graduate students in the sciences.

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An in planta* assay for analysis of exonic splicing regulator activity in *Arabidopsis thaliana

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The selection of splice sites during constitutive and alternative splicing is controlled by both core splicing signals at the splice sites themselves and by auxiliary signals in the intron and flanking exons. Of particular interest are exonic splicing enhancers (ESEs) and exonic splicing silencers (ESSs), collectively known as exonic splicing regulators (ESRs).

We have developed a system for assaying the ability of any sequence to promote or inhibit exon inclusion in *Arabidopsis thaliana*. Our assay vector contains a hybrid gene driven by the cauliflower mosaic virus 35S promoter in which the splicing outcome (exon inclusion vs. skipping) of an intron-exon-intron unit based on exon 8 of the *SPY* gene depends on the presence of an ESE. Lines differing only within a 9 nt. site at the center of the exon can show outcomes ranging from complete skipping to complete inclusion. Independent T-DNA insertion lines with the same construct show consistent results. Thus far, over 150 sequences have been assayed by this technique. We observe that 14 of the 29 sequences from a natural exon (exon 10 of the *LD* gene) show significant ESE activity. This result indicates that ESE activity is common in natural exons and has prompted us to initiate a test of a similar number of completely random sequences. Because SR proteins are known mediators of ESE activity we are also testing three lines with homozygous-viable mutations in SR protein genes for effects on the activity of specific ESEs (see poster by Edmonds et al.)

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URLs: <http://www.chemlife.umd.edu/labs/mount/>
<http://www.cbcb.umd.edu/software/SeeEse/>

Structure/Function Mapping of Amino Acids in the N-Terminal Zinc Finger of the Human Immunodeficiency Virus Type 1 Nucleocapsid Protein: Residues Responsible For Nucleic Acid Helix Destabilizing Activity

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The nucleocapsid protein (NC) of HIV-1 is 55 amino acids in length and possesses two CCHC-type zinc fingers. Finger one (N-terminal) contributes significantly more to helix destabilizing activity than finger two (C-terminal). Five amino acids differ between the two zinc fingers. To determine at the amino acid level the reason for the apparent distinction between the fingers, each different residue in finger one was incrementally replaced by the one at the corresponding location in finger two. Mutants were analyzed in annealing assays with unstructured and structured substrates. Three groupings emerged: (1) those similar to wild type levels (N17K, A25M), (2) those with diminished activity (I24Q, N27D), and (3) mutant F16W which had substantially greater helix destabilizing activity than wild type. Unlike I24Q and the other mutants, N27D was defective in DNA binding. Only I24Q and N27D showed reduced strand transfer in in vitro assays. Double and triple mutants F16W/I24Q, F16W/N27D, and F16W/I24Q/N27D all showed defects in DNA binding, strand transfer, and helix destabilization, suggesting that the I24Q and N27D mutations have a “dominant negative” effect and abolish the positive influence of F16W. Results show that amino acid differences at positions 24 and 27 contribute significantly to finger one's helix destabilizing activity.

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Ribosomal Protein L10: Pleiotropic Roles in Large Subunit Biogenesis and Translation.

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Abstract. Ribosomal protein L10 is the last protein incorporated into the large subunit, and it is required for subunit joining and displacement of the export adapter Nmd3p. Thus, elucidation of L10-associated functions could help to reveal (A) structural/conformational features required for large subunit function; and (B) structural rearrangements that distinguish premature 60S subunits from active ribosomes. Toward these ends, we constructed a library of *rpl10* mutants in *S. cerevisiae* by random mutagenesis. The primary library was screened for inability of cells to maintain the “Killer” phenotype, identifying 56 new alleles of *rpl10*. Genetic analyses demonstrated effects of the mutant *rpl10* alleles on sensitivity/resistance to cold (15°C), heat (37°C), anisomycin and paromomycin. Efficiencies of programmed -1 ribosomal frameshifting and suppression of a UAA nonsense codon were measured with a dual-luciferase reporter system. Biochemical characterization demonstrated effects of *rpl10* alleles on ribosome biogenesis and affinities to aminoacyl-tRNA. These analyses (A) reveal functionally important regions in the ribosomal protein L10; and (B) demonstrate that h38 (the “A-site finger”) and the loop at the base of helix 89 (components of the “accommodation gates”) are required for proper ribosomal function and aminoacyl-tRNA selection.

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Unpaired “Pivot Bases” Enable Information Exchange Among Functional Centers Of The Ribosome

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Abstract. The ribosome is a complex nanomachine that contains numerous, spatially distinct functional centers. These are composed mainly of rRNA. A key question centers on how information is shared among these centers so as to ensure their harmonious coordination. Previous studies in *E. coli* have implicated interactions between helix 38 (the A-site finger) and ribosomal protein S13 in the subtle modulation of numerous ribosome-associated functions. To address this issue on a fine scale, an improved yeast-based genetic system was devised to enable generation of cells expression only mutant forms of rRNA. Base-specific mutations were introduced in the B1a bridge that connects the A-site finger of the large subunit with the decoding center on the small subunit, and along a stretch of three bases that interact with the aminoacyl-tRNA (aa-tRNA) in the A-site of the large subunit. While both classes of mutants promoted increased affinities for aa-tRNA, they had distinctly different phenotypes in their responses to two A-site specific drugs, and opposing phenotypes with regard to suppression of termination codons. rRNA structural analyses revealed specific changes in protection patterns in unpaired nucleotides of 25S rRNA that form a continuum linking the B1a bridge with the GTPase-associated center, the peptidyltransferase center, and the path along which the aa-tRNA travels during the process of accommodation. We have coined these “pivot bases” on the hypothesis that they provide fulcrums around which rigid helices can reorient themselves depending on the occupancy status of the A-site.

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***in vitro* Synthesis of Long DNA Products in Reactions With HIV-RT And Nucleocapsid Protein**

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In vitro reaction conditions using HIV reverse transcriptase (RT) and nucleocapsid protein (NC) that allowed the efficient synthesis of single-stranded DNA products over a thousand nucleotides in length from genomic HIV RNA were characterized. The reactions required NC at a concentration sufficient to completely coat the RNA template. Long products were dependent on RNase H activity of RT and were produced as a result of strand transfer. No change in RT processivity was observed in the reactions. In addition, their synthesis required formation of a high molecular weight aggregate between NC, RT and nucleic acids. The aggregate formed rapidly and pelleted with low speed centrifugation indicating that it was large. NC finger mutants lacking either finger one or two or with the finger positions switched were all effective in promoting long products. This suggests that the aggregation/condensation but not helix-destabilizing activity of NC was required. We propose that these high molecular weight aggregates promote synthesis of long reverse transcription products *in vitro* by concentrating nucleic acids, RT enzyme and NC into a smaller area, thereby mimicking the role of the capsid environment within the host cell.

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Unraveling the function of the novel *RTE1* and *RTH* genes in *Arabidopsis thaliana*

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Our laboratory has shown that mutations at the *Arabidopsis* locus *REVERSION-TO-ETHYLENE-SENSITIVITY1* (*RTE1*) result in the loss of ETR1 ethylene receptor function. This is based on the ability of *rte1* mutations to suppress ethylene insensitive mutations in the ethylene receptor gene *etr1*, as well as the fact that *rte1* mutants have the same enhanced ethylene response phenotype as the *etr1* null mutant (1). *RTE1* encodes a novel integral membrane protein with homologues in other plants and animals, including one copy in humans. We are interested in uncovering the unknown molecular function of *RTE1* and its homolog in *Arabidopsis* named *RTE-HOMOLOG* (*RTH*). One approach we are taking is to analyze the basis of *rte1* suppression of *etr1-2* at the protein level. Immunoblot and immunofluorescence assays indicate no obvious changes in the level of ETR1 protein and show correct localization of the ETR1 protein in the *rte1* null background. Preliminary results also indicate correct dimer formation of ETR1 in the *rte1* null background. To identify the role of *RTH*, we are currently analyzing the *rth* null mutant for any involvement it may have in the ethylene-signaling pathway. The *rth* null mutant does not show the same phenotypes as the *rte1* null mutant, and the *rte1 rth* double null mutant does not display any additive effects, suggesting that the two loci are not redundant. Interestingly, the *rth* single null mutant appears to have an opposite phenotype (lengthening of the hypocotyl in dark-grown seedlings) from the *rte1* null mutant. Consistent with this finding, over-expression of the wild-type *RTH* gene confers a shorter hypocotyl than the wild type. Further experiments to better understand the cause of this hypocotyl lengthening and its possible relation to ethylene are in progress.

Reference:

1. Resnick JS, Wen CK, Shockey JA, and Chang C (2006) *REVERSION-TO-ETHYLENE SENSITIVITY1*, a conserved gene that regulates ethylene receptor function in *Arabidopsis*, *Proc. Natl. Acad. Sci. USA* 103: 7917-7922.

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Genetic Characterization of Ribosomal Protein L2 in *Saccharomyces cerevisiae*

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Ribosomal protein L2 (L2) is vital to the structure and function of the ribosome. The location of L2 in the large ribosomal subunit suggests that it is important for ribosomal RNA interaction and positioning, ribosomal subunit binding, and peptidyl transfer. In the yeast *Saccharomyces cerevisiae*, two genes, *RPL2A* and *RPL2B*, encode identical versions of Rpl2p. Using an *rpl2aΔ, rpl2bΔ* strain, we completed large-scale mutagenesis of a plasmid-borne *RPL2A* gene. We initially selected for strains unable to maintain the yeast killer virus, identifying 16 unique alleles that consistently conferred the Mak- phenotype. We later generated 11 additional alleles bearing single amino acid changes. Data characterizing these mutants with regard to translational fidelity and pharmacological response are presented. In combination with information from atomic level structure models this work will contribute greatly to our understanding of the relationship between ribosome structure and function.

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Heterogeneity in monocyte populations during murine cutaneous leishmaniasis

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Recently, monocyte heterogeneity was found to be conserved in humans and mice. We therefore wanted to evaluate the changes in blood monocyte populations and tissue macrophages during murine cutaneous leishmaniasis. BALB/c, C57BL/6(B6) and CCR2 KO mice were infected with *L. major* or *L. amazonensis* stationary phase promastigotes in the ear (*L. major*), or in the footpad (*L. major*, *L. amazonensis*). Blood mononuclear and ear cells (*L. major*) were isolated at various time points during the infection. Cells were stained with different combinations of fluorescent antibodies and analyzed by flow cytometry. During *L. major* footpad infection CD11b⁺;F4/80⁺;GR1^{high} monocytes were considerably elevated in the blood of BALB/c mice when compared to B6 mice. However, following low dose *L. major* ear infection and *L. amazonensis* footpad infection, the levels of these cells fluctuated but were comparable. A population of CD11b⁺;F4/80⁺;GR1^{int} monocytes was increased in the blood of B6 mice during all infection modes. Both monocyte populations were considerably lower in CCR2 KO mice during *L. amazonensis* infection. When cells from *L. major* ear lesions were isolated, a population of CD11b⁺;GR1⁻;CCR2⁺ cells was identified. This population increased dramatically in B6 mice and only slightly in BALB/c mice. Interestingly, MCP-1 levels in the serum of B6 mice were also elevated during infection. Our initial observations suggest that CCR2 has a role in the recruitment of blood monocytes into lesions caused by *Leishmania* parasites. Further studies will evaluate its role in the different disease models and will characterize the effector functions of these cells.

Transcription Coupled Repair in the Third Domain of Life

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Transcription coupled repair (TCR) is a critical cellular pathway that couples DNA transcription and repair. It is the targeted repair of the coding strand of actively transcribed genes before the rest of the genome is repaired. TCR is conserved in Bacteria and Eukarya, but remain to be elucidated in Archaea. It is a significant pathway since deficiency in TCR in Eukaryotes result in genetic diseases such as Cockayne syndrome and Xeroderma pigmentosa. This study aims to determine whether TCR is present in the Archaea and to discover what proteins are involved in this process. This study will contribute to characterizing Archaea as a model organism for Eukarya.

Our model organism is *Halobacterium*, an extreme halophile archaeon. Using UV irradiation and a T4 endonuclease V assay that specifically digests UV photoproducts, we will (1) determine the kinetic of repair of UV lesions by *Halobacterium*; (2) determine whether genes are repaired faster when actively transcribed versus non-transcribed; (3) determine whether the coding strand of an active gene is preferentially repaired and (4) determine what proteins in *Halobacterium* contribute to TCR. These aims will enable us to characterize TCR in the third domain of life and to determine if this process in the Archaea is more closely related to that of the Bacteria or the Eukarya.

So far, I have optimized the T4 endonuclease V assay and I am currently completing aim 1. Using Northern blot hybridization, I am determining the expression pattern of a gene activated when cells are grown in presence of MnSO₄ as part of aim 2.

Genetic regulation of the IL-19 promoter in murine macrophages.

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Recent sequence database searches for IL-10 homologs have led to the discovery of an entire family of cytokines that share at least some sequence homology to IL-10. These include IL-19, -20, -22, melanoma differentiation-associated gene-7 (mda-7, also called IL-24), and AK155 (also called IL-26). Although the amino acid sequences of these cytokines and their respective receptors have been identified, very little is known about their biological function. We have selected IL-19 for initial study. IL-19 is a ~21 kDa protein principally secreted by monocytes, macrophages, and B cells. The IL-19 gene is located in the same region of chromosome 1 as IL-10, -19, -20, and -24, in both mice and humans. LPS stimulation of monocytes causes secretion of IL-19. IL-19 is known to polarize T cells towards a Th2-like profile, and stimulation of monocytes with IL-19 has been shown to upregulate both IL-10 and IL-19 transcription in a positive feedback loop. IL-19 has been detected at elevated levels in the airways of asthmatic patients, as well as in psoriatic epidermis. Previous studies in this laboratory have demonstrated that optimal production of IL-10 requires ERK activation. This leads to chromatin remodeling of the IL-10 locus. Recent data has demonstrated that NF- κ B p50 homodimers may play a critical role in transcription of IL-10 under these circumstances. We believe that these events represent a unique method of cytokine gene regulation. Given the fact that IL-10 and IL-19 are structurally related and that both cytokines are involved in Th2-like immunological processes, we wondered whether the genetic regulation of both cytokines might be similar. Initial studies into the genetic regulation of IL-19 using conventional and real-time PCR demonstrated that transcription of the IL-19 gene is significantly upregulated in the presence of immune complexes, conditions that favor IL-10 production. I have recently made plasmid constructs of the IL-19 promoter region, going as far as 3000 bp upstream of the transcription start site. Transfection of RAW cells with these plasmids showed that a response element exists somewhere within the first 1000 bp upstream of the transcription start site that is also upregulated with LPS/IC. Continuing analysis of the promoter region will hopefully yield greater insight into the kinetics of IL-19 gene regulation, what transcription factors are involved, and what chromatin remodeling events occur.

Dynamic and Transient Remodeling of the Macrophage IL-10 Promoter during Transcription*

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To gain insight into the molecular mechanism(s) whereby macrophages produce large amounts of IL-10, we analyzed IL-10 gene expression and temporally correlated it with modifications to chromatin associated with the IL-10 promoter. In resting cells, which make essentially no cytokines, the IL-10 promoter is associated with histones containing little or no detectable modifications. Macrophages stimulated in the presence of immune complexes begin to produce high levels of IL-10 pre-mRNA transcripts within minutes of stimulation. Coincident with this transcription was a rapid and dynamic phosphorylation of histone H3 at specific sites in the IL-10 promoter. Histone phosphorylation was closely followed by the binding of transcription factors to the IL-10 promoter. Blocking the activation of ERK prevented histone phosphorylation and transcription factor binding to the IL-10 promoter. In contrast to histone phosphorylation, the peak of histone acetylation at this promoter did not occur until after transcription had peaked. Inhibition of histone deacetylase (HDAC) did not alter IL-10 gene expression, suggesting that phosphorylation but not acetylation was the proximal event responsible for IL-10 transcription. Our findings reveal a rapid and well-orchestrated series of events in which ERK activation causes a rapid and transient phosphorylation of histone H3 at specific regions of the IL-10 promoter, resulting in a transient exposure of the IL-10 promoter to the transcription factors that bind there. This exposure is essential for the efficient induction of IL-10 gene expression in macrophages. To our knowledge, this represents a unique way in which the expression of a cytokine gene is regulated.

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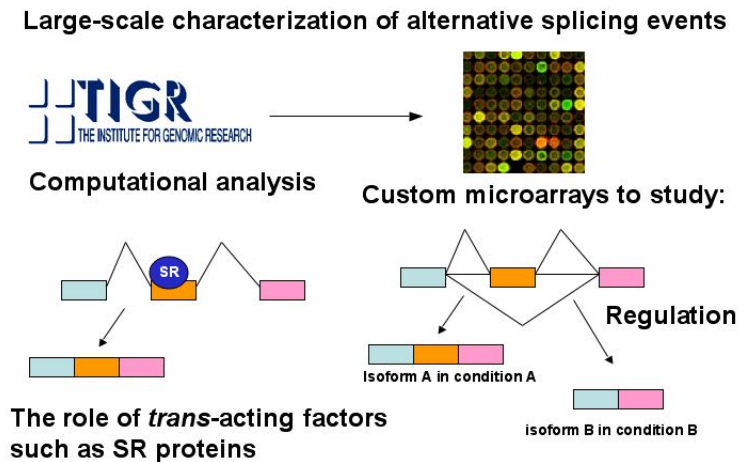
Global Study of Alternative Splicing in *Arabidopsis thaliana*.

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We are developing a specialized microarray platform to systematically obtain information about alternative splicing from the *Arabidopsis thaliana* genome. We will use this platform to study the regulation of the splicing events by specific splicing factors including SR proteins, and to study the effects of different genetic and environmental variables. We will take advantage of TIGR's existing database of splicing variations to select alternative splicing features that will be included on the chip. We will combine the junction oligonucleotide probes method that has been successfully applied to mouse alternative splicing assay (Pan et al. 2004), and the tiling idea from Affymetrix (Ule et al. 2005) to design the probes for each alternative splicing event. Selective alternative splicing events have been verified by RT-PCR from seedlings with multiple treatments as preliminary tests. The summary figure at right is adapted from Kazan 2003.



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Salts of the Earth: Genomic Studies on Microbial Life Under Extremely High Salt and Radiation Conditions

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Our lab's research centers on genomic investigations using both halophilic and hyperthermophilic model species from the Archaea, the third domain of life along with the Bacteria and Eukarya. I have investigated the relationship between saturating salt conditions and high-energy radiation using the extremely halophilic archaeon *Halobacterium* sp. strain NRC-1. A study of the global response of informational systems (genomic and proteomic) to gamma irradiation showed the importance of damage mitigation and repair strategies in this organism. Altogether, 9% of the transcriptome and 9.2% of the identifiable proteins were perturbed upon γ -irradiation reflecting the need for stress management mechanisms including DNA repair via homologous recombination (HR), increased protein turnover, nucleotide biosynthesis, initiation of DNA replication and a concerted effort to reverse and minimize damage from oxidative stress.

The role of homologous recombination in the repair of DNA double-strand breaks produced by oxidative damages is being investigated using strains with mutations in key components of the recombinational repair system (both gene deletion and dominant negative overexpression). UV-C and γ -irradiation are used as sources of oxidative damage to determine the survival of mutant strains and the molecular level damage to cells lacking components of this repair system. Archaeal DNA repair systems are more closely related to eukaryotic proteins than to their bacterial counterparts but use far less components, enabling an investigation of a simpler system to deduce the role of homologous recombination proteins in the more complex eukaryotic system.

We have presented the hypothesis that high intracellular KCl concentrations may be used not only for the maintenance of osmotic balance in extreme halophiles but also as a damage mitigation system by reducing the potency of reactive oxygen species. *In vitro* and *in vivo* studies using a range of salts found in terrestrial and martian brine environments have yielded information on the relationship between salt composition and concentration to resistance to oxidative damage. Replacement of chloride species for bromide results in increased resistance to gamma radiation *in vivo*, as well as reduced strand breakage as a result of oxidative damage *in vitro*. The relationship of manganese salts to oxidative damage is also being studied *in vitro* and *in vivo* to investigate the relationship to Fenton chemistry producing the DNA-damaging hydroxyl radical. These studies will enable us to provide further insights on the limits for life on Earth and the possibility for life on other planets.

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