Identifying Targets of FTZ and FTZ-F1

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Homeobox (Hox) proteins regulate cell fate and segmental identity throughout the animal kingdom. Yet, given the homeodomain's promiscuous DNA binding activity in vitro, it is unclear how they select downstream target genes. The product of *fushi tarzu* (*ftz*) is a Hox protein responsible for the development of alternate parasegments within the *Drosophila* embryo. It has been shown that FTZ interacts with a cofactor, the orphan nuclear receptor FTZ-F1, to cooperatively bind DNA and synergistically regulate transcription (1, 2). *ftz-f1* is maternally deposited and ubiquitously expressed, while *ftz* is zygotically expressed in seven stripes at the blastoderm stage. The phenotypes of *ftz* and *ftz-f1* null mutants are identical, demonstrating an in vivo requirement for their functional interaction in promoting the development of alternate parasegments.

The two best characterized FTZ/FTZ-F1targets are *engrailed* (*en*) and *ftz* itself. FTZ-F1 interacts with FTZ to positively autoregulate *ftz* gene expression; similarly, FTZ and FTZ-F1 coordinately regulate alternate stripes of *en* (3). In order to extend this list, the genome of *Drosophila melanogaster* was screened for FTZ and FTZ-F1 binding sites with known cooperative spacing and orientations. Genes having clusters of five or more FTZ/FTZ-F1 sites within 20 kb of their transcription start sites were selected for further analysis. Two candidate target genes, *Dsulf1* and *apt*, were identified with this approach (4). We are currently extending this search for realizator genes using Affymetrix microarrays to identify genes that are differentially expressed in wild-type embryos and *ftz-f1* mutants through successive stages of cellularization and gastrulation.

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NF-κB1 (p50) Homodimers Differentially Regulate Pro- and Anti-Inflammatory Cytokines in Macrophages

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NF-κB/Rel is a family of transcription factors whose activation has long been linked to the production of inflammatory cytokines. Here, we studied NF-κB signaling in the regulation of the anti-inflammatory cytokine, IL-10. We found a role for a single NF-κB family member, NF-κB1 (p50), in promoting the transcription of IL-10. The NF-κB cis-element on IL-10 proximal promoter was located to -55/-46, where p50 can homodimerize and form a complex with the transcriptional co-activator CBP to activate transcription. The other Rel family members appear to play a negligible role in IL-10 transcription. To determine the biological significance of p50-induced IL-10 production, mice lacking p50 were examined. These mice were more susceptible to lethal endotoxemia, and macrophages taken from $p50^{-/-}$ mice exhibit skewed cytokine responses to LPS, characterized by decreased IL-10 and increased TNF and IL-12. Taken together, our studies demonstrate that NF-κB1 (p50) homodimers can be transcriptional activators of IL-10. The reciprocal regulation of pro- and anti-inflammatory cytokine production by NF-κB1 (p50) may provide potential new ways to manipulate the innate immune response.

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NF-κB p65 and c-Rel Regulation Pattern during T Cell Stimulation

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T cell stimulation triggers a signal cascade that causes the activation of various transcription factors. From these transcription factors NF-κB is especially important because it is necessary for expression and secretion of T cell growth factor IL-2. In this study we are interested in comparing the regulatory pattern of NF-κB proteins p65 and c-Rel. NF-κB is composed of the protein subunits p50, p52, p65, c-Rel, and RelB which form dimers that bind via the κB domain. In resting T cells, the NF-κB complex remains in the cytosol bound to the inhibitory molecule IκB. Once the T cell is activated, IκB is degraded and the NF-κB complex is translocated into the nucleus where it binds to the IL-2 promoter to initiate transcription. We compared the localization of NF-κB protein subunits p65 and c-Rel during T cells stimulation. First, we studied degradation of IκB and NF-κB proteins at different time points by Western blot. Here we found degradation of IκBα but not IκBβ during T cell activation. We also observed no degradation of either total p65 or c-Rel after 60 minutes of stimulation. We also analyzed nuclear translocation by immunofluorescence in stimulated T cells using antibodies against p65 and c-Rel. We found that p65 moves from the cytosol to the nucleus within 15 minutes and 45 minutes of stimulation. Data from immunofluorescence was also confirmed by nuclear fractionation assay. c-Rel shows a different pattern since it appears nuclear at all time points. Future studies will compare these patterns with anergic cells to determine how NF-κB is regulated in T cell anergy.

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Carotenoids biosynthesis in the primitive red alga *Cyanidioschyzon merolae*

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The unicellular red alga *Cyanidioschyzon merolae*, a resident of acidic hot springs, is considered to be one of the most primitive of photosynthetic eukaryotes. As such, the photosynthetic apparatus in this alga may provide the closest approximation to that of the prokaryotic ancestor of the modern day chloroplast. We are especially interested in the ancestry and evolution of the pathway that provides for the synthesis of the carotenoids, a family of isoprenoid pigments that are integral and essential constituents of the photosynthetic apparatus in all oxygenic photoautotrophs. We exploited the recent availability of the nuclear, mitochondrial, and plastid genome sequences of *C. merolae* to address the origin of carotenoid pathway genes in this alga. *C. merolae* genes that encode polypeptides similar in sequence to known carotenoid pathway enzymes were identified, the major carotenoid pigments accumulated by *C. merolae* were ascertained, and the enzymatic activities of two gene products of particular interest, the putative lycopene cyclase and β-carotene hydroxylase enzymes, were examined. Chlorophyll *a*, zeaxanthin, and β-carotene were the major pigments in *C. merolae*, with β-cryptoxanthin a minor constituent. Carotenoids with ε-rings (*e.g.* lutein and α-carotene), found in many other red algae and in green algae and land plants, were not detected, and the lycopene cyclase of *C. merolae* quite specifically produced only β-ringed carotenoids when provided with lycopene as the substrate in *Escherichia coli*. The genomes of *C. merolae* do not contain obvious homologs of the two types of β-carotene hydroxylase enzymes found in land plants, one a nonheme diiron oxygenase and the other a cytochrome P450 enzyme. A *C. merolae* plastid gene encoding a polypeptide similar to the quite different β-carotene hydroxylases found in cyanobacteria did not produce an active enzyme when expressed in β-carotene-accumulating *E. coli*, and several lines of evidence suggest it to be a pseudogene. The identity of the *C. merolae* β-carotene hydroxylase remains uncertain.

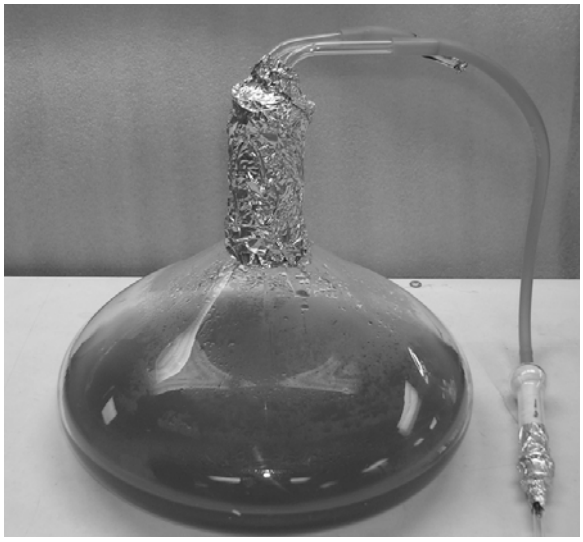

Fig. 1. *C. merolae* in batch culture.

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Programmed Cell Death of the Jacket Cells is Essential for Spermatid Development and Differentiation in *Marsilea Vestita*

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The microspore of the water fern *Marsilea vestita* contains a single cell that is activated to undergo nine rapid division cycles after it is placed in water. The divisions produce seven somatic cells and 32 spermatids. The gametophyte is transcriptionally quiescent during its development and before the divisions commence, certain stored proteins and mRNAs localize in zones that later become the spermatogenous initials. Asymmetric divisions produce somatic jacket cells, and a series of symmetric divisions produce the spermatids. As the spermatids differentiate to become motile gametes, the jacket cells undergo programmed cell death (PCD). We developed RNAi strategies to target mRNA degradation and thereby arrest gametophyte development. These published studies show that the temporal and spatial translation pattern in the gametophyte is highly ordered; certain proteins are required at specific stages of development. In this study, we inhibited PCD in jacket cells with RNAi treatments. dsRNA probes were made from cDNAs encoding two different cell death associated proteins that we isolated from a gametophyte library. These dsRNAs were added to populations of spores and gametophytes were fixed after 8 hours of development. Treated spores had large jacket cells and fewer spermatogenous cells than control gametophytes. Toluidine Blue-O staining of treated spores showed that jacket cells contain more total proteins and mRNAs than the jacket cells in untreated spores. Treated cells were labeled with anti-centrin and anti-β-tubulin antibodies to assess patterns of new translation of stored mRNA and cytoskeletal reorganization, respectively. Unlike normal gametophytes, centrin translation and tubulin localization were no longer limited to the spermatogenous cells. We suspect that impending PCD may underlie the early suppression of translation and development in the jacket cells. (Supported by NSF grant MCB-0234423 to SMW).

The Roles of Kinesin Motor Proteins in Spermatid Differentiation in *Marsilea vestita*

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The microspore of the water fern *Marsilea vestita* contains a single cell that undergoes a series of nine successive mitotic divisions to produce 32 spermatids, six sterile jacket cells and one prothallial cell. After the dry microspore is placed into water, cytoplasm reorganization precedes the first division; certain proteins and mRNAs aggregate into zones that later become the spermatogenous initials of the gametophyte. Our results indicate that some proteins and some mRNAs become redistributed by kinesin-driven movements along cytoplasmic microtubules. Gametophyte development relies on the translation of stored mRNAs, and we developed RNAi strategies to target the degradation of specific mRNAs to arrest development. These published studies show that the temporal and spatial patterns of translation in the gametophyte are precisely ordered. Centrin translation occurs exclusively in spermatogenous cells and is required for the *de novo* formation of basal bodies. Various tubulin isoforms become localized in the spermatogenous cells and are essential for nuclear shaping and ciliogegnesis. We are studying how different kinesins affect development, cell fate, and differentiation during spermiogenesis. dsRNA probes were made from cDNAs encoding seven different kinesin isoforms isolated from our gametophyte library. The dsRNAs were added to populations of developing gametophytes to assess the time and stage of development at which each transcript becomes limiting. Two kinesin knockdowns show no effects on development while the others alter division patterns at specific developmental stages. The disruption of the pattern of cell divisions in the gametophyte predicts changes in cell fate that are manifested by changes in centrin translation and β-tubulin localization patterns. The loss of kinesin-based movements at particular times during gametophyte development effectively results in changes in cell fate determination. (Supported by NSF grant MCB-0234423 to SMW).

Role of AtCHX20 a novel cation transporter (CHX) in osmoregulation of guard cells

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A large family of 28 AtCHX genes was found in the *Arabidopsis thaliana* genome, though their biological functions are largely unknown. Based on phylogenetic analyses, the transmembrane domains of AtCHXs share similarity to that of Na^+/H^+ antiporters, including NHX1 and SOS1, though they cluster with a distinct CPA2 subfamily of Na^+ or K^+ /H^+ exchangers. Only *AtCHX20* was preferentially expressed in guard cells as shown by promoter::GUS reporter activity and by whole-genome microarray of purified guard cell RNA. Transient expression of GFP-tagged CHX20 in Arabidopsis mesophyll protoplasts and yeast indicated that the protein was localized to intracellular membranes. Although homozygous mutants of *chx20* showed no obvious morphological or growth differences, light-induced stomatal opening was reduced 35% in three independent T-DNA insertional mutant plants. To test the biochemical function, CHX20 cDNA was expressed in a yeast mutant KTA 40-2 which lacks vacuolar and plasma membrane $Na^+(K^+)/H^+$ antiporter *(*Δ*nhx1,* Δ*nha1,* Δ*kha1*) and plasma membrane Na^+ pumps (Δena1-4). AtCHX20 restored growth of KTA 40-2 on SDAP plates with low K^+ at pH 7.5. We propose that CHX20 influences guard cell movement by altering K^+ and/or pH homeostasis of endomembrane compartments thus affecting membrane vesicle trafficking needed to alter the shape and size of vacuoles and plasma membrane. Together these results provide the first evidence that a member of the CHX family plays a role in osmoregulation.

Regulation of Amino Acid Transporter Expression During T-cell Activation

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The activation, proliferation, and differentiation of naïve T-cells are very high energy-consuming processes, which lead to a high demand for biosynthetic precursors. In previous studies, we identified various amino acids, including, arginine, asparagine, glutamine, and cysteine, which are critical for T-cell activation; however, the mechanism by which the cells attain these amino acids is not well understood. In order to better understand amino acid transport mechanisms in lymphocytes, we examined the gene expression of various amino acid transporters, including ATA1, xCT, and 4F2 in resting and stimulated cell states. ATA1 is a known neutral amino acid transporter, xCT transports primarily anionic amino acids, and 4F2 serves as a transport-associated glycoprotein. Through Real-Time PCR, we tested for the presence or absence of various known amino acid transporter genes in naïve T-cells and quantified their expression at resting and stimulated time points. For the transporter genes identified, there was a marked increase in expression from resting to stimulated conditions; however, the kinetics and fold increase in expression varied among transporters. This noted variation in transporter expression indicates that certain amino acids and transport mechanisms may be more crucial to the cell's activation during its earliest stages. A better understanding of early T-cell amino acid transport mechanisms may serve as a valuable tool in future immunomodulation studies.

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A Possible Novel Activity for HIV Nucleocapsid Protein (NC): Inhibition of Extension of Non-Polypurine Tract RNA Primers.

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The chaperone protein NC has several functions in the retrovirus life cycle and is involved in all aspects on reverse transcription. Our studies indicate a new potential role for NC in modulating the specificity of priming of the plus strand. RNase H cleavage by HIV-1 RT during minus strand synthesis can give rise to RNA fragments that could potentially be used as primers for synthesis of the plus strand. Using such primers would lead to the initiation of priming from multiple points as has been observed for some other retroviruses (ASV for example). For HIV, the central and 3' polypurine tracts (ppt) are the major sites of plus strand initiation although some priming at other positions is observed. We developed an assay to analyze RT priming efficiency using various RNA primers including both ppt and non-ppt primers. Surprisingly, RT was capable of using all the primers to initiate DNA synthesis though the efficiency was very low with non-ppt primers, which were generally degraded by RNase H. NC greatly reduced the efficiency of extension of non-ppt primers but not ppt. The obvious explanation, that NC induces rapid dissociation of cleaved RNAs was eliminated from results with an RNase H minus version of RT (E478>Q). E478>Q, which was considerably more efficient at extending non-ppt primers (presumably because it cannot cleave them), was also strongly inhibited by NC. In addition, NC did not cause dissociation of uncleaved RNA primers in the assays. Taken together these results show that NC strongly inhibits extension of non-ppt RNAs, while leaving priming by PPT and primers containing the PPT unaffected. Our current hypothesis is that the 3' recessed RNA terminus is a low affinity site for RT and NC can out-compete RT for the site. We are currently testing mutated HIV NCs to determine if the helix destabilizing or condensation/aggregation activity of NC is responsible for the inhibition. In addition, NCs from other retroviruses are also being tested.

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Homologous 3' end sequences in satC and its helper RNA, Turnip crinkle virus, differentially affect RNA accumulation

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SatC is an untranslated subviral RNA associated with *Turnip crinkle virus* (TCV), a small plus-strand RNA virus belonging to the genus Carmovirus. Repression of SatC minus-strand synthesis occurs when the satRNA assumes an alternative conformation that does not include structures required for transcription initiation. Activation of satC minus-strand synthesis in vitro is mediated by an element known as the DR, which is a short CG-rich sequence adjacent to a small hairpin, H4a. Using the Massively Parallel Genetic Algorithm RNA structure program, a pseudoknot is predicted between the DR and the H4a terminal loop, which can also form in TCV. Results from preliminary compensatory mutations between the DR and H4a in TCV suggest that this pseudoknot contributes to replication of the genomic RNA. In satC, the compensatory mutation in the H4a loop restored to wt levels the poor accumulation of satC with a DR mutation (DR: 5' ACGGCGG; H4a loop: 5' ACCGU, unaltered sequences are shown; putative pairing is underlined and position of residues changed are in italics). However, the H4a mutation by itself was not debilitating, opening to question the existence or importance of the pseudoknot in satC. Interestingly, levels of satC containing additional base alterations or deletions in the DR were also restored by the same H4a loop mutation. One possible explanation for these results is that the H4a mutation fortuitously created a new DR-like sequence in the H4a terminal loop that is identical to the TCV DR sequence (AC*G*GU; altered base is in italics) and could functionally replace the mutated natural DR. This possibility was supported by finding that no other mutations in the H4a loop could compensate for the DR alterations. Structure probing revealed that the DR and H4a loop mutations described above had similar major local structural alterations, suggesting that the new DR-like sequence disrupted the structure of the natural DR as did DR mutations. These results suggest that the pseudoknot between the DR and H4a in TCV is required for a TCV-specific function such as translation while satC may have co-opted the DR sequence for usage in the conformational switch that activates satC transcription.

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Phenotypic characterization of a spermatogenesis defective mutation; *spe-32,* in *Caenorhabditis elegans.*

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spe-32 was initially identified as a temperature-sensitive, spermatogenesis-defective mutation. Mutant hermaphrodites exhibit sperm-specific sterility (Spe phenotype) when larvae are shifted to the restrictive temperature of 25˚C before L4. In addition, *spe-32* is required for early development. Mutant hermaphrodites produce dead embryos when shifted to 25˚C as adults, and larval lethality is observed when eggs are shifted to 25˚C. The embryonic and larval lethality, but not the Spe phenotype, is maternally rescued. Homozygous *spe-32* progeny from a heterozygous *spe-32/+* mother reared at 25˚C develop normally to adulthood but still exhibit sperm-specific sterility.

spe-32 males manifest other phenotypes in addition to sterility and larval lethality. Development of the male tail is aberrant at the restrictive temperature. The tip of the tail is remarkably reduced in length compared to wild type, and the fan is much smaller. Unlike all of the other phenotypes, which are recessive, this effect is semi-dominant. The majority of males grown at restrictive temperature also show progressive paralysis. The movement defect begins at the tail and progresses anteriorly as the animal ages, leading to total paralysis and premature death. Even at the permissive temperature, *spe-32* males show reduced mating efficiency, and some proportion of males have constitutively protracted spicules. All of these phenotypes suggest additional male-specific roles for the *spe-32* gene in *Caenorhabditis elegans*.

Currently, we are trying to clone the *spe-32* gene by cosmid rescue. Prior work mapped *spe-32* to LGIV and linked the mutation to *dpy-20*. We have used snip-SNP mapping technique to narrow down the genomic interval for *spe-32* between 4.35cM to 4.55cM. Microinjection of cosmids in this interval is underway. Our other efforts are directed toward characterization of the specific defect in spermatogenesis that results in sterility.

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Note: This abstract is for poster presentation.

The study of Mycobacterium RD1 secretion and its role in pathogenisis

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Mycobacterium marinum, and other pathogenic mycobacterium contain a virulence gene locus known as region of difference 1 (RD1). RD1 encodes a novel secretion system termed Snm for secretion in mycobacterium .Two proteins secreted via Snm are Early Secreted Antigenic Target 6kDa (ESAT-6) and Culture Filtrate Protein 10kDa (CFP-10). The function of these two proteins is currently not known however the role of individual RD1 genes in ESAT-6/CFP-10 secretion is being investigated.

The loss of RD1 is the primary reason for attenuation of the vaccine strain BCG, derived from *Mycobacterium bovis*. The deletion of individual RD1 genes from *Mycobacterium tuberculosis* and *M. marinum* results in reduced virulence in mice and zebrafish respectively. The precise role of RD1 in virulence is not known. We are currently investigating the function of RD1 in bacterial survival in the host phagosome, specifically in counteracting reactive oxygen intermediates (ROIs). We are also examining the role of RD1 secreted proteins in cytolysis and whether this activity mediates cell-to-cell spreading.

Current research has shown that RD1 plays a role in haemolysis, possibly mediated by the secretion of ESAT-6 and CFP-10. ΔESAT-6 incubated with red blood cells showed 83% less haemolysis compared with wild type. In addition ΔESAT-6 shows significantly reduced macrophage necrosis. Cytolytic activity may mediate spreading during infection. Our data has shown that in comparison to the wild type, ΔESAT-6 was not able to spread from cell to cell and the number of cells infected did not increase over time.

Another possible role of RD1 is counteracting ROIs. Within the phagosome, mycobacterium are exposed to superoxide, hydrogen peroxide and other ROIs. In order to survive the damages incurred by ROIs, pathogenic mycobacterium evolved mechanisms to overcome these toxic compounds. RD1 genes may be involved in counteracting ROIs by secreting ROI-neutralizing enzymes such as superoxide dismutase and catalase. Our research supports this hypothesis, showing that RD1 mutants are more sensitive to hydrogen peroxide *in vitro* as demonstrated by reduced growth in the presence 1mM H_2O_2. To further demonstrate the role of RD1 in counteracting ROIs, infections were performed in which macrophages were treated with either catalase and superoxide dismutase or NAC, a chemical that scavenges ROIs. In the case of treated macrophages, RD1 mutants displayed growth recovery. These results suggest that RD1 plays a role in counteracting ROIs. The exact role of RD1 in counteracting ROIs is currently under investigation but preliminary data implies that RD1 mutants have diminished catalase activity when compared to wild type.

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Regulation of Glucose Metabolism in Primary T cells

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A critical role in a cell-mediated immune response is played by T lymphocytes. During activation, cell-cell interactions and intracellular signaling pathways alter the metabolism and direct the fate of responding T cells. Naïve T cells may become tolerized or activated depending upon the signals they receive. At the same time, metabolism increases in conjunction with receptor stimulation and activation of signal transduction pathways in a resting T cell in order to support T cell function. It is known that metabolism increases upon initiation of activation in order to meet the increased metabolic needs of a proliferating, metabolically active cell, and another idea - that metabolism can be regulated directly via receptor-mediated signals and can feed back in turn to modify cellular signaling - is being investigated. While major signaling pathways and changes in gene expression have been identified, little is known about the cellular control of metabolic changes that enable the cell to sustain its increased growth and proliferation programs and to choose between anergic and activated fates. In order to define the role of metabolism in T cell activation, I will modulate signaling through the T cell receptor (TCR) and CD28 stimulatory pathway in T cells and monitor metabolic changes induced by these signals.

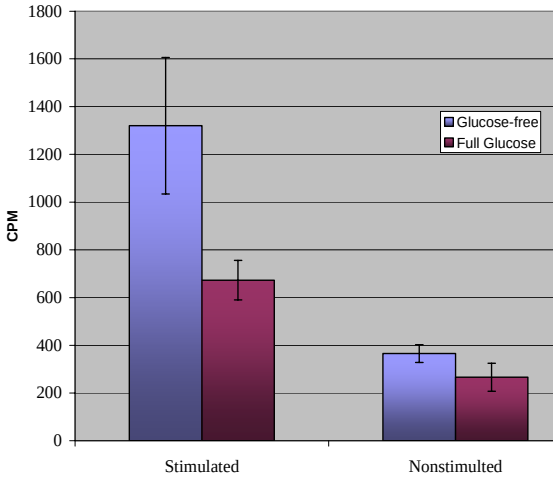

Figure 1. 2-deoxyglucose is taken up at a faster rate in stimulated vs. non- stimulated T cells. Two minute 2-DOG uptake in stimulated (CD3/CD28 antibodies) and unstimulated (IgG) T cells was compared in glucose-free or full glucose medium.

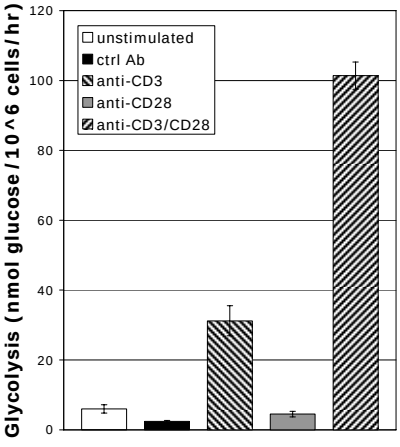

Figure 2. CD3/CD28 stimulation strongly induces glycolysis. T cells were stimulated for 24 hours with CD3, CD28 or CD3/CD28 beads, and glycolysis rates were calculated by enolase assay as determined by release of radioactive hydrogen on C-5 as water. Data provided by Dr. Kenneth Frauwirth.

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The role of mammalian actin-binding protein-1 in coupling the signaling and antigen-internalization functions of the B cell antigen receptor

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Engagement of the B cell antigen receptor (BCR) by multivalent antigens leads to the activation of signaling cascades and rapid antigen internalization for processing and presentation to T cells. BCR-initiated signaling and actin reorganization have been shown to be required for antigen-induced BCR internalization. However the mechanism for the interaction between the actin cytoskeleton, BCR signaling and internalization pathways in response to the antigen engagement of the BCR has not been fully elucidated. Mammalian actin-binding protein-1 (mAbp1) is an F-actin binding protein that contains multiple protein-protein interacting domains and potentially serves as a linker molecule between the actin cytoskeleton and the BCR. To test this hypothesis we examine the relationship of mAbp1 with both BCR signaling and antigen-internalization pathways. In response to the binding of the BCR to multivalent antigens, we found mAbp1 transiently phosphorylated and recruited with dynamin 2 to the plasma membrane, where mAbp1 was colocalized with dynamin 2. This transient recruitment was inhibited by disrupting the actin cytoskeleton and by mutation of two putative tyrosine phosphorylation sites of mAbp1. Inhibition of mAbp1 gene expression using shRNA and gene knockout mice significantly reduced BCR internalization, indicating an important role for mAbp1 in BCR-mediated antigen uptake. Over-expressing the SH3 domain of mAbp1 and dynamin 2 with the PRD domain deleted blocked BCR internalization, implicating a role for mAbp1-dynamin 2 interactions in BCR internalization. Taken together, these results suggest that mAbp1 is mobilized by BCR-initiated signaling to facilitate BCR mediated antigen internalization by linking the actin cytoskeleton to endocytosis hot spots.
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Regulation of the Raf-like protein kinase CTR1 in ethylene signaling in *Arabidopsis*

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The gaseous phytohormone ethylene regulates a number of developmental processes including seed germination, initiation of roots, flowering time, fruit ripening, and senescence of leaves. In the model organism *Arabidopsis*, ethylene is perceived at the endoplasmic reticulum (ER) by a family of five receptors that bear similarity to prokaryotic two-component signaling systems. The receptors are negative regulators of ethylene signaling. The next known downstream component of the prokaryotic-like receptors is the protein kinase CTR1, which is also a negative regulator of ethylene response. The CTR1 C-terminal kinase domain shares 41% similarity to the Raf kinases, while the CTR1 N-terminus is more divergent from the Raf N-terminus (1). N-terminal regulation and localization to the correct membrane are essential for Raf kinase activity. We propose two hypotheses concerning the regulation of CTR1, based on similarity to Raf: 1) the CTR1 N-terminus is an auto-inhibitor of its own kinase domain and 2) CTR1 localization at the ER is essential for its function. Using *in vitro* pull down assays and far western blots, we have shown that the CTR1 N- and C-terminal domains are able to interact. To address whether this interaction is necessary for CTR1 kinase regulation *in planta*, we are currently comparing over-expression lines of the CTR1 full length (CTR1 FL) protein to over-expression lines of the CTR1 kinase domain alone (CTR1 KD) with the expectation that CTR1 KD could result in ethylene insensitivity due to loss of regulation by the N-terminal domain. Because CTR1 is peripherally associated with the ER and the N-terminal domain is needed for this association (2), we also speculate that CTR1 needs to be localized to the ER in order to be regulated properly. We therefore created chimeras consisting of the transmembrane region of the ethylene receptor ETR1 fused to either CTR1 FL or CTR1 KD and transformed these constructs into wild-type *Arabidopsis*. Since the truncated ETR1 itself localizes to the ER (3), we speculated that these chimeras also localize to the ER. The over-expression construct of ETR1 fused to CTR1 FL did not alter ethylene responses in the wild type, while the over-expression of ETR1 fused to CTR1 KD caused insensitivity to exogenous ethylene. This is the first *in planta* evidence supporting N-terminal regulation of the CTR1 kinase, and supports a model in which localization at the ER is required for CTR1 functionality.

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Using technology to support active learning in large enrollment introductory science courses

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In large lecture courses, a major barrier to change is the difficulty associated with disseminating information, collecting student work, providing feedback to students, and supporting and monitoring teamwork. In our large enrollment (~250 students) General Microbiology class (BSCI 223) the use of technology provided a solution and allowed us to link lecture and lab as well as on-line learning opportunities in a cohesive manner. In this presentation we report on three offerings of BSCI 223: Spring 2004, Fall 2004 lecture A, and Fall 2004 lecture B. Student enrollments were 275, 231 and 218 respectively. Our report reflects our analysis of student responses to an end of semester survey. We have received very positive feedback from our students regarding the addition of the on-line course management system to our course. We believe that our course design has given us the opportunity to add to our class strategies that have been already demonstrated to be successful but which were previously out of reach because of our large class enrollment, time limitations, and course management needs. Adding technology to the course design has allowed us to shift the focus of the course from didactic to student centered.

The Active Learning Course Format developed in BSCI 223 has been disseminated for use in other large lecture introductory science courses including BSCI 105 and BSCI 222.

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Correlation between Fyn activation and T cell anergy

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Anergy is an antigen-specific nonresponsiveness observed in T lymphocytes as a defense against triggering an attack on the body's cells, also known as autoimmunity. Upregulation of the protein tyrosine kinase $p59^{fyn}$ has been observed in anergic T lymphocytes, suggesting its activity contributes to either the induction or maintenance of anergy. The laboratory has developed a model system for inducing anergy in mice *in vivo* through peptide injection. The mice have been genetically engineered to express a T cell receptor (2C) that will recognize the peptide, and these mice have also been bred to *fyn* deficient mice, producing 2C-transgenic (tg)/*fyn* knockout (KO) mice. Two approaches are being taken to determine the role of *fyn* in anergy induction. For the first approach, both 2C-tg/*fyn* KO and 2C-tg/*fyn* wild-type (WT) mice were injected with peptide to induce anergy. The T lymphocytes were then harvested and analyzed for the degree of restimulation and proliferation. The second approach will use RNAi to block *fyn* expression in primary mouse T lymphocytes, which will then be re-introduced in mice and analyzed for proliferation after peptide injection. Five potential short hairpin (shRNA) sequences are being tested for efficiency in blocking *fyn* expression. The shRNA constructs are being directly transfected into primary T lymphocytes via nucleofection. Preliminary results from the first approach have shown no difference in the degree of lymphocyte proliferation between *fyn* WT and *fyn* KO mice, suggesting that *fyn* activity is not required to induce anergy. However, the possibility remains that the absence of *fyn* may have been compensated for by other kinases related to *fyn* in overlapping pathways or that a *fyn* KO lymphocyte has had its pathways altered by the deletion of *fyn* and therefore no longer responds the same way as a normal T lymphocyte. Therefore, the second approach using RNAi is being performed. Future experiments will involve utilizing the RNAi constructs to determine if *fyn* activity contributes to anergy maintenance.

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Differential regulation of nuclear localization of NFAT isoforms in anergic $CD8^+$ Tcells.

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Anergy is an important mechanism of regulating peripheral tolerance, and proper regulation is required to prevent autoimmune disorders and allergies. We used 2C TCR Tg mice to induce anergy *in vivo*, to study activation of anergic T cells. Anergic T cells showed impaired calcium flux in response to *in vitro* restimulation. A major target of calcium signaling in T cells is the NFAT family of transcription factors. Dephosphorylation by the calcium responsive phosphatase, calcineurin leads to NFAT translocation into the nucleus, which is then involved in transcription of target genes like IL2. We looked at translocation of NFAT proteins into the nucleus in anergic T cells after re stimulation. In anergic cells, NFAT1 showed a defect in the movement into the nucleus as compared to responsive T cells. However, unlike NFAT1, NFAT2 was rapidly translocated into the nuclei of anergic, but not responsive T cells. These results suggest that the nuclear translocation, and possibly activation of NFAT1 and NFAT2 is differentially regulated. Also, NFAT1 was localized in the nucleus at normal extracellular calcium concentrations, while NFAT2 was detected in the nucleus at decreased calcium levels maintained by addition of calcium chelators. Thus the pattern of NFAT1 vs.NFAT2 nuclear translocation seems to be determined by the availability of calcium to the T cell.

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A Functional Domain Interference Assay (FDIA) reveals that peptide motifs of Mago nashi are involved in development in *Marsilea*

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Spermatogenesis in the water fern *Marsilea vestita* is initiated by placing dry microspores in water. The single cell in the microspore undergoes nine successive division cycles to produce 7 somatic cells and 32 spermatids. Thereafter, each spermatid differentiates to become a coiled, ciliated gamete. Mago nashi is a highly conserved protein that functions in the exon-exon junction complex for pre-mRNA splicing, as a component in nonsense-mediated mRNA decay, and as a component in transport/localization of mRNAs for axis formation. The protein consists of several beta-sheet and alpha-helix domains. The beta sheets are exposed on one side of the protein while the opposite face consists of alpha helical domains that interact with the Y14 protein to form a heterodimer. Previously, we found that *Mv*-mago and *Mv*-Y14 are important for the specification of spermatogenous and jacket cells in the gametophyte. RNAi treatments, using dsRNAs made from *Mv*-mago and *Mv*-Y14, induced cytokinesis defects with cells of random sizes and anomalous shapes. Transcripts encoding pre-mRNA processing proteins, and proteins important for gametogenesis failed to localize in spermatogenous initials; instead, both were present in all cells of the gametophyte. Five peptides corresponding to different parts of the mago nashi protein were added to microspores in a series of functional domain interference assays (FDIA) to assess interactions between native proteins and mago nashi *in vivo*. One peptide exerted no effect on development while another induced rapid death, even at low concentrations. With the remaining peptides, development was arrested at specific phases, with characteristic patterns of incomplete cytokinesis and abnormal cell shaping. The FDIA experiments show that different motifs in the same protein affect development at distinct stages. (Supported by NSF grant MCB 0234423 to SMW).

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Activation Mechanism for a Binuclear Zinc Transcription Factor in a Novel Fungal Stress Pathway

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Pathogens respond to stress conditions by activating signal transduction cascades, ultimately leading to specific gene expression to overcome the stress causing factors. *Fusarium solani* is a pathogenic filamentous fungus which causes root rot in pea. The fungus responds to a plant defense compound, pisatin, uniquely produced by the pea plant. The defense response is mediated by the activation of a fungal transcription factor, PRF (Pisatin Response Factor). Evidence from our experiments in a related fungus *N.crassa* suggests that PRF might be involved in a common stress pathway as opposed to a compound specific response. We are studying the mechanism by which PRF functions in the cell. One approach I have used is yeast two hybrid analysis to identify other proteins with which PRF might interact in the cell. One of the proteins identified from the screen, V25, has a mitochondrial targeting sequence and is similar to the c terminal domain of superoxide dismutases. In order to understand the functional significance of the interaction, I have knocked out the *N.crassa* homologue of the interacting protein. Introducing PRF in this strain will allow me to study any change in the induction pattern in response to stress signals. The V25 homologue seems to be an essential gene as attempts to knockout the gene has produced recombinants with either the native gene alone or with both the marker gene and native gene. In a second approach, I am trying to characterize the size and nature of PRF in the cell by doing Western blots using anti-PRF antibody. Western blots with the purified antibody identified different sized forms of PRF. Treatment with phosphatases shifts the mobility of some bands, indicating phosphorylation. As a third approach, I have isolated multiple cDNA clones and find evidence for alternate splicing. Such alternate splicing may be involved in regulation of PRF activity. We are now studying the relevance of these three different possibilities in pisatin regulation of PRF.

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Activation of the Mitogen-Activated Protein Kinase, ERK, Following *L. amazonensis* Infection of Macrophages

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Interleukin (IL)-10 is a critical cytokine in determining host susceptibility to *Leishmania* spp. We previously demonstrated that macrophage-derived IL-10 could contribute to disease exacerbation, but the mechanisms whereby *Leishmania* infections led to IL-10 induction were not fully understood. In this study, we demonstrated that infection of macrophages with *L. amazonensis* amastigotes led to the activation of the Mitogen-Activated Protein Kinase (MAPK), extracellular signal-regulated kinase 1/2 (ERK1/2). This activation was required but not sufficient for IL-10 induction. In addition to ERK activation, an inflammatory stimulus, such as low molecular weight-hyaluronic acid (LMW-HA), must also be present. The combination of these two signals resulted in the superinduction of IL-10. We also demonstrated that IgG on the surface of *Leishmania* amastigotes was required to achieve maximal IL-10 production from infected macrophages. Surface IgG engages macrophage FcγR to induce ERK activation. Macrophages lacking FcγR, or macrophages treated with an inhibitor of Syk, the tyrosine kinase that signals *via* FcγR, failed to activate ERK and consequently failed to produce IL-10 following infection with *Leishmania* amastigotes. We confirmed that ERK1/2 activation led to the phosphorylation of histone H3 at the *IL-10* promoter, and this phosphorylation allowed for the binding of the transcription factor, Sp1, to the *IL-10* promoter. Finally the administration of U0126, an inhibitor of ERK activation, to infected mice resulted in decreased lesion progression with reduced numbers of parasites in them. Thus, our findings reveal an important role of MAPK, ERK signaling in the pathogenesis of *Leishmania* infection.

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