Small RNA-Mediated Gene Regulation

Connecting RNA Directed-DNA Methylation and Histone Methylation in Arabidopsis thaliana, **JULIE A. LAW[1,5], JIAMU DU[2]*, CHRISTOPHER J. HALE[1]*, SUHUA FENG[1], ANA MARIE S. PALANCA[5], KRZYSZTOF KRAJEWSKI[3], BRIAN D. STRAHL[3], DINSHAW J. PATEL[2], and STEVEN E. JACOBSEN[1,4]** ([1]Department of Molecular Cell and Developmental Biology, University of California at Los Angeles, Los Angeles, CA 90095; [2]Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; [3]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; [4]Howard Hughes Medical Institute, University of California at Los Angeles, Los Angeles, CA 90095; [5]Plant Molecular and Cellular Biology, Salk Institute, La Jolla, CA 92037; jlaw@salk.edu).

In eukaryotic organisms DNA is organized into chromatin, a dynamic structure consisting of mainly DNA and specialized packaging proteins called histones. Both these chromatin components can be modified through the attachment of chemical groups and together these modifications influence the expression of the underlying genes and play critical roles in normal growth and development. In some cases chromatin modifications can be inherited through mitosis and/or meiosis providing a mechanism for cellular memory and giving rise to the field of epigenetics: the study of heritable changes in gene expression patterns or phenotypes that are not linked to changes in the underlying DNA sequence. One such epigenetic modification is DNA methylation. In the plant model *Arabidopsis thaliana*, this modification is associated with the transposon silencing and is targeted by 24 nt small RNAs (siRNAs) through the RNA-directed DNA methylation (RdDM) pathway. While it was known that the generation of these methylation-targeting siRNAs required a plant specific RNA polymerase, Pol IV, it was unclear how this polymerase was recruited to specific genomic regions destined for gene silencing. To address this question, we affinity purified RNA Pol IV and identified several interacting proteins via mass spectrometry, including SAWADEE HOMEODOMAIN HOMOLOG 1 (SHH1). Here we demonstrate that SHH1 functions to enable siRNA production at select genomic loci by facilitating Pol IV recruitment. This recruitment requires the SHH1 SAWADEE domain, which we show adopts a tandem Tudor-like fold and specifically recognizes histone 3 tails that are unmethylated at lysine 4 and methylated at lysine 9. Together, our findings connect DNA methylation and H3K9 methylation within the context of the RdDM pathway and provide the first mechanistic insight into a key initiating step in the RdDM pathway, the targeting of RNA Pol-IV. A better understanding of this early step in the establishment of DNA methylation will aid in our ability to manipulate gene expression patterns, which given the parallels between DNA methylation systems in plants and mammals, has broad implications for both agriculture and gene therapy.

Remodeling of Ago2-mRNA Interactions Upon Cellular Stress Reflects miRNA Complementarity and Correlates with Altered Translation Rates, **FEDOR V. KARGINOV[1]* and GREGORY J. HANNON[2]** ([1]Department of Cell Biology and Neuroscience, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, karginov@ucr.edu; [2]Watson School of Biological Sciences, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, Hannon@cshl.edu).

When adapting to environmental stress, cells attenuate and reprogram their translational output. In part, these altered translation profiles are established through changes in the interactions between RNA-binding proteins and mRNAs. The Ago2/microRNA machinery has been shown to participate in stress-induced translational upregulation of a particular mRNA, CAT-1; however, a detailed, transcriptome-wide understanding of the involvement of Ago2 in the process has been lacking. Here, we profiled the overall changes in Ago2-mRNA interactions upon arsenite stress by CLIP-seq. Ago2 displayed a significant remodeling of its transcript occupancy, with the majority of 3` UTR and CDS sites exhibiting stronger interaction. Interestingly, target sites that were destined for release from Ago2 upon stress were depleted in miRNA complementarity signatures, suggesting an alternative mode of interaction. To compare the changes in Ago2 binding patterns across transcripts with changes in their translational states, we measured mRNA profiles on ribosome/polysome gradients by RNA-seq. Increased Ago2 occupancy correlated with stronger repression of translation for those mRNAs, as evidenced by a shift toward lighter gradient fractions upon stress, while release of Ago2 was associated with the limited number of transcripts that remained translated. Taken together, these data point to a role for Ago2 and the mammalian microRNAs in mediating the translational component of the stress response.

Fungal Small RNAs Suppress Plant Host Immunity by Hijacking Host RNAi Machinery, **ARNE WEIBERG[1], MING WANG[1], FENG-MAO LIN[2], HONGWEI ZHAO[1], ZHIHONG ZHANG[1], ISGOUHI KALOSHIAN[1], HSEIN-DA HUANG[2], and HAILING JIN[1]*** ([1]University of California, Riverside, 900 University Avenue, Riverside, CA 92521 USA, [2]National Chiao Tung University, Taiwan; hailingj@ucr.edu).

Small RNAs (sRNAs) are a class of short non-coding regulators that mediate gene silencing in a sequence-specific manner. Small RNAs have been shown to play an important role in host immune responses against pathogen attacks. Most fungal genomes encode components of RNAi machinery, including Dicer-like proteins and Argonautes (AGOs). The role of fungal small RNAs in genome defense, heterochromatin formation, and gene regulation has been demonstrated. However, it was not known whether fungal small RNAs or RNAi are directly involved in pathogenicity.

Botrytis cinerea is an aggressive fungal pathogen that infects more than 200 plant species. Genome-wide small RNA profiling from *B. cinerea*-infected Arabidopsis and tomato has identified a group of small RNAs from *B. cinerea* that can potentially target important regulatory genes in plant hosts. Genetic and biochemical studies have demonstrated that some *B. cinerea* small RNAs (Bc-sRNAs) can selectively silence host immunity genes by hijacking host RNAi machinery[1]. These Bc-sRNAs are loaded into host AGO proteins to silence host genes involved in defense. Deviated from the conventional pathogen protein effectors that suppress host immunity in plants and animals, we demonstrate that a fungal pathogen transfers "virulent" sRNA effectors into host cells to achieve infection. The implications of this finding may extend beyond grey mold disease or plant fungal diseases in general.

A Highly Conserved Protein PIR-1 is Required for Silencing Orsay Virus in C. elegans, **WEIFENG GU** (Department of Cell Biology and Neuroscience, University of California Riverside, 900

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RNA viruses such as flu virus, HIV, and Hepatitis C virus, are among the most deadly threats to human health. Recent studies, including some pioneering work performed here at UCR, have demonstrated that the RNAi machinery plays critical roles in eliminating RNA virus in animals and plants. Central to the RNAi machinery is Dicer, an RNase III like enzyme, which specifically dices double-stranded (ds) RNA generated by viral RNA-dependent RNA polymerase (RdRP) during RNA virus infection. The resulting small RNA, siRNA, is loaded to Argonaute protein, which then targets viral RNA by base-pairing via siRNA. PIR-1, a potential RNA phosphatase or dual specificity protein phosphatase, was identified as a conserved dicer interacting protein. However, the function of PIR-1 in RNAi or anti-virus remains unknown. Using C. elegans and Orsay virus as a model system and collaborating with Mello lab, I have demonstrated that PIR-1 is essential for the biogenesis of dicer/Argonaute-dependent viral siRNA as well as endogenous primary siRNA, both of which initiate secondary siRNAs (22G) required for silencing. Currently we are investigating if PIR-1 is a protein phosphatase or RNA phosphatase.

The Arabidopsis PHD-Finger Protein EDM2 Controls Plant Innate Immunity by Modulating Levels of the Epigenetic Transposon-Silencing Mark H3K9me2, **THOMAS EUGLEM*, TOKUJI TSUCHIYA, and YAN LAI** (Center for Plant Cell Biology, Institute for Integrative Genome Biology, Department of Botany & Plant Sciences, University of California, Riverside, CA 92521, USA; thomas.eulgem@ucr.edu, tsuchi_gst@yahoo.co.jp, yan.lai@ucr.edu).

The *Arabidopsis thaliana* PHD-finger protein EDM2 controls silencing states of transposable elements (TE) by modulating levels of dimethylated lysine 9 of histone H3 (H3K9me2), a suppressive post-translational histone modification (PHM). Insertion of EDM2-controlled TEs into clusters of disease resistance (*R*) genes recruited this epigenetic factor to the regulation of plant immune responses. We show that EDM2-dependent modulation of H3K9me2 at a TE in the 1st intron of *RPP7* controls expression of this *R* gene by affecting alternative polyadenlation. Varying levels of this PHM shifts the balance between full-length RPP7 transcripts and prematurely polyadenylated non-RPP7-coding transcripts. Additional *R* genes that are closely associated with TEs also appear to be affected by EDM2-dependent H3K9me2 levels. Our work illustrates one of the first direct *in vivo*-demonstrations for co-option of a TE-associated histone mark to the epigenetic control of pre-mRNA processing and establishes a unique mechanism of plant *R* gene expression. We further show the PHD-finger module of EDM2 to recognize histone H3 bearing certain combinations of three distinct PHMs. Our results suggest that targeting of EDM2 to specific genomic regions is mediated by the histone-binding selectivity of its PHD-finger domain.

Regulation of Innate Immunity to the Fungal Pathogen Fusarium oxysporum by MicroRNAs in Tomato, **SHOUQIANG OUYANG[1], GYUNGSOON PARK[1], HAGOP ATAMIAN[2] , CLIFF S. HAN[3], JASON E. STAJICH[1], ISGOUHI KALOSHIAN[2], and KATHERINE A. BORKOVICH[1]*** ([1]Department of Plant Pathology and Microbiology, [2]Department of Nematology, Institute for Integrative Genome Biology, 900 University Avenue, University of California, Riverside, CA 92521, [3]Bioscience Division, MS M888, Los Alamos National Laboratory, Los Alamos, NM 87545; katherine.borkovich@ucr.edu).

MicroRNAs (miRNAs) are important regulators of growth and development in plants. Several miRNA families target genes encoding nucleotide-binding site–leucine-rich repeat (NB-LRR) plant innate immune receptors. The fungus *Fusarium oxysporum* f. sp. *lycopersici* causes vascular wilt disease in susceptible tomato plants. We explored a possible role for miRNAs in tomato defense against *F. oxysporum* using comparative miRNA profiling of susceptible (Moneymaker) and resistant (Motelle) tomato cultivars infected with *F. oxysporum.* The results revealed that slmiR482f and slmiR5300 were repressed during infection of Motelle with *F. oxysporum*. Northern analysis confirmed that two predicted mRNA targets each of slmiR482f and slmiR5300 exhibited increased expression in Motelle and the ability of these four targets to be regulated by the miRNAs was confirmed by co-expression in *Nicotiana benthamiana*. A virus-induced gene silencing approach in the resistant Motelle cultivar revealed a role in fungal resistance for all four targets. All four genes encode proteins with full or partial nucleotide-binding (NB) domains. One slimiR5300 target, Solyc09g018220, is *tm-2,* a susceptible allele of the *Tomato Mosaic Virus* resistance gene. However, the observation that none of the targets correspond to *I-2*, the only known resistance (*R*) gene for *F. oxysporum* in tomato, supports roles for additional *R* genes in the immune response. Taken together, our findings reveal microRNAs that mediate resistance to *F. oxysporum* in tomato, implicate miR5300 in plant immunity and support functions for a viral resistance gene (*tm-2*) in immunity to a fungal pathogen.