BIOGRAPHICAL SKETCH

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NAME: Phillips, Carolyn Marie

eRA COMMONS USER NAME (credential, e.g., agency login): CMPHILLIPS1

POSITION TITLE: Gabilan Assistant Professor of Biological Sciences, University of Southern California

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California – Davis	B.S.	06/2001	Biological Sciences
University of California – Berkeley	Ph.D.	05/2007	Molecular and Cell Bio.
Mass. General Hospital/Harvard Medical School	Post-doc	12/2014	Genetics

A. Personal Statement

The goal of my current research program is to understand how the RNA silencing pathway represses aberrant RNAs and transposons to prevent gene mutations that can result in infertility, birth defects, cancer, and other diseases. To this end, my lab will investigate the RNA, DNA, and protein components of the RNA silencing pathway using cell biology, genetics, biochemistry, and high-throughput sequencing. During my graduate and postdoctoral training, I developed the expertise necessary to successfully carry out the proposed research. As a graduate student with Abby Dernburg at the University of California, Berkeley, I primarily used microscopy and genetics to investigate how chromosomes recognize their homologous partner prior to the first meiotic division in *C. elegans*. Our work identified the first chromosome-specific proteins implicated in homologous chromosome interactions and revealed new insights into the mechanisms of chromosome pairing and synapsis during meiosis[1,2]. My post-doctoral research, with Gary Ruvkun at Massachusetts General Hospital and Harvard Medical School, focused on the mechanisms of RNA silencing in *C. elegans.* Using the cytological techniques that I mastered in the Dernburg lab, I brought a more cell biological approach to the field of small RNAs, where biochemistry and molecular biology techniques are more common. At the same time, I developed new skills, particularly in the areas of biochemistry and high throughput sequencing. In work carried out during this time, I discovered a novel nuclear envelope-associated RNA/protein granule, which is composed of proteins in the *mutator* complex. Using high-throughput sequencing, I demonstrated that it plays a key role in siRNA biogenesis and transposon silencing[3]. I then identified novel components of the *mutator* complex and molecularly and biochemically characterized their role in RNA silencing[4]. This work has provided a spatial context to the RNA silencing pathway, and suggests a model where aberrant RNAs are captured as they exit the nucleus and prior to their release into the cytoplasm.

I am committed to the education of both undergraduate and graduate students. I have had numerous opportunities to mentor students, starting while I was still a graduate student. As the first student in the Dernburg lab, I played a significant role in mentoring undergraduate and graduate rotation students. During my post-doc in the Ruvkun lab, I spent one school year mentoring a Belmont high school student who worked in the lab for high school class credit and presented his research project to his teachers and classmates. Since starting my own research lab at USC, I have recruited a post-doc and two undergraduate students to my lab. I am working closely with them, with the goal helping them achieve both their research and career goals. Specifically for the post-doc, we have discussed a mentoring plan that is individualized towards developing his research skills, increasing his publication record, and improving his career opportunities.

In conclusion, I have demonstrated a strong record of success in the fields of RNA silencing and chromosome dynamics, and the expertise I developed during my graduate and postdoctoral work has prepared me to lead the proposed research project. Moving forward, my lab will continue to work to answer fundamental questions in the RNA silencing field.

1. **Phillips CM**, Wong C, Bhalla N, Carlton PM, Weiser P, Meneely PM, Dernburg AF. HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell. 2005 Dec 16;123(6):1051-63. PMCID: PMC4435792
2. **Phillips CM** and Dernburg AF. A family of zinc finger proteins is required for chromosome-specific pairing and synapsis during meiosis in *C. elegans*. Dev. Cell. 2006 Dec;11(6):817-29.
3. **Phillips CM**, Montgomery TA, Breen PC, Ruvkun G. MUT-16 promotes formation of perinuclear *Mutator* foci required for RNA silencing in the *C. elegans* germline. Genes Dev. 2012 Jul 1;26(13):1433-44. PMCID:PMC3403012
4. **Phillips CM,** Montgomery BE, Breen PC, Roovers EF, Rim YS, Ohsumi TK, Newman MA, van Wolfswinkel JC, Ketting RF, Ruvkun G, Montgomery TA. MUT-14 and SMUT-1 DEAD box RNA helicases have overlapping roles in germline RNAi and endogenous siRNA formation. Curr Biol. **2014** Apr 14;24(8):839-44. PMCID:PMC4010136

B. Positions and Honors

Positions

1999-2001	Undergraduate Research Assistant, University of California – Davis Research Advisor: *Michael Seldin, M.D., Ph.D.*
2001-2008	Ph.D. student, University of California – Berkeley Research Advisor: *Abby Dernburg, Ph.D.* Thesis: The Role of the Pairing Center during Meiosis in *C. elegans*
2008-2014	Post-doctoral Research Fellow, Massachusetts General Hospital and Harvard Medical School Research Advisor: *Gary Ruvkun, Ph.D.*
2014-present	Gabilan Assistant Professor of Biological Sciences, University of Southern California

Honors

2003-06	NSF Graduate Research Fellowship
2007	Harold M. Weintraub Graduate Student Award, Fred Hutchinson Cancer Research Center
2007	Nicholas Cozzarelli Prize, Molecular and Cell Biology Department at UC Berkeley
2008-11	Marion Abbe Fellow of the Damon Runyon Cancer Research Foundation
2011-12	Massachusetts General Hospital ECOR Tosteson Postdoctoral Fellowship

Professional Memberships

Genetics Society of America, American Society for Cell Biology

C. Contribution to Science

How do piRNAs and the *mutator* complex interact to promote fertility? piRNAs associate with the Argonaute protein Piwi, and maintain genome integrity by silencing foreign genes, such as transposons. In *C. elegans*, piRNAs target both transposon and non-transposon mRNAs to trigger the production of siRNAs and sustained gene silencing via the RNAi pathway and dependent on the *mutator* complex. Because heritable siRNAs can be maintained in the absence of the initial piRNA trigger, it has been difficult to determine which genes require piRNAs for efficient silencing and to tease out the role of piRNAs in development and fertility. To address this issue, I developed a genetic method to generate RNAi-competent animals whose parents were RNAi-defective, therefore unable to provide siRNAs to their offspring. I then compared the outcomes when either these animals or their parents were defective for piRNA production. I found that in the absence of maternally contributed piRNAs and siRNAs contributed from at least one parent, animals displayed dramatic defects in germ cell proliferation and were subsequently sterile. By immunoprecipitating and sequencing the siRNAs bound to the downstream Argonaute protein, HRDE-1, I demonstrate that the RNAi pathway is targeting conserved and essential genes in the absence of piRNAs or heritable siRNAs. In parallel, a pathway thought to license mRNAs for germline expression is enriched for genes and transposons normally targeted by the piRNA pathway. Thus, the sterility observed in these animals is likely a consequence of both a downregulation of essential genes and an upregulation of transposons, repetitive elements, and other detrimental gene products. By reestablishing RNA silencing in the absence of piRNAs, I demonstrated that a

siRNA-mediated transgenerational memory of piRNAs is required to distinguish between beneficial and harmful genes, and in their absence leads to a missorting between gene licensing and gene silencing pathways. This work establishes that, despite the relative health and fertility of piRNA pathway mutants, piRNAs are essential for germline development and fertility and that, in previous studies, these defects were hidden by the epigenetic inheritance of siRNAs.

Phillips CM, Brown KC, Montgomery BE, Ruvkun G, Montgomery TA. piRNAs and piRNA-dependent siRNAs protect conserved and essential *C. elegans* genes from misrouting into the RNAi pathway. Dev. Cell. 2015; 34(4):457-465. PMCID:PMC4550515

Where does RNA silencing occur in the cell? A group of genes which have the *mutator* phenotype (an increase in transposon mobilization events), were initially identified in screens for factors required for transposon or RNA silencing. However very little was known about their sub-cellular localization, their physical interactions with other proteins, or their mechanism of action. During my postdoc in the Ruvkun lab, I demonstrated that one component, MUT-16, acts as a scaffold to nucleate five additional *mutator* proteins, as well as the RNA-dependent RNA polymerase, RRF-1. This protein complex, which we name the *mutator* complex, utilizes RRF-1 to act as an RNA amplification module, downstream of multiple primary RNA silencing pathways and essential for efficient silencing of both endogenous and foreign RNAs. In the germline, the *mutator* complex resides outside the nucleus but adjacent nuclear pores, where it serves as a gatekeeper to capture and silence mRNAs as they exit the nucleus (Phillips *et al*, Genes Dev, 2012). In a related study, I discovered a new member of the *mutator* complex, the RNA helicase SMUT-1 (*synthetic mutator*), in an RNAi screen for factors that affect endogenous siRNA activity. SMUT-1, with its paralog MUT-14, is essential for the production of endogenous siRNAs and RNAi targeting germline genes. Using a combination of biochemistry and cytology I demonstrated that *mut-14* and *smut-1* are likely involved in transporting target mRNAs into the *mutator* complex to initiate siRNA amplification (Phillips *et al*, Curr Biol, 2014). This work took factors identified in screens, but with little or no mechanistic characterization, and identified physical interactions between these proteins, localized them to a novel subcellular compartment, and characterized their role in RNA silencing, which provided significant insight into how mRNAs destined for silencing are sorted and transported to the *mutator* complex.

Zhang C, Montgomery TA, Gabel HW, Fischer SE, **Phillips** CM, Fahlgren N, Sullivan CM, Carrington JC, Ruvkun G. mut-16 and other mutator class genes modulate 22G and 26G siRNA pathways in Caenorhabditis elegans. Proc Natl Acad Sci U S A. **2011**; 108(4):1201-8. PMCID: PMC3029761

Phillips CM, Montgomery TA, Breen PC, Ruvkun G. MUT-16 promotes formation of perinuclear *Mutator* foci required for RNA silencing in the *C. elegans* germline. Genes Dev. 2012; 26(13):1433-44. PMCID:PMC3403012

Phillips CM, Montgomery BE, Breen PC, Roovers EF, Rim YS, Ohsumi TK, Newman MA, van Wolfswinkel JC, Ketting RF, Ruvkun G, Montgomery TA. MUT-14 and SMUT-1 DEAD box RNA helicases have overlapping roles in germline RNAi and endogenous siRNA formation. Curr Biol. **2014** ;24(8):839-44. PMCID:PMC4010136

What proteins and DNA sequences contribute to accurate segregation of chromosomes? As a graduate student in the Dernburg lab, I studied the role of specific chromosome regions, called Pairing Centers (PCs), in meiosis. These regions have two separable activities: they stabilize homolog pairing and directly promote synapsis. My thesis work uncovered essential *cis*- and *trans*-acting components that contribute to PC function. I first demonstrated that missegregation of the *X* chromosome in the *him-8* mutant was due to failure in chromosome pairing and synapsis. By cloning *him-8,* I found it to be a Zn-finger protein, which localizes to the PC of the *X* chromosome (Phillips *et al*, Cell, 2005). I subsequently demonstrated analogous roles for the HIM-8 paralogs, ZIM-1, -2, and -3, at the autosomal PCs (Phillips and Dernburg, Dev Cell, 2006). Based on this identification of these proteins, I was able to determine the *cis*-acting elements that underlie PC function. I identified short sequence elements enriched in the corresponding chromosome regions that selectively recruit the ZIM/HIM-8 proteins *in vivo*. When inserted into a chromosome lacking an endogenous PC, a cluster of these motifs can restore homologous synapsis, crossover recombination, and segregation (Phillips *et al*, Nat Cell Bio, 2009). Finally, I discovered that the outer nuclear envelope protein, ZYG-12, which is normally distributed uniformly along the nuclear envelope, localize to distinct patches during the stage of meiosis where pairing and synapsis initiation take place, and these patches associate with all of the ZIM/HIM-8 foci. This result was the basis of a project, subsequently completed by another graduate student, in which we

demonstrated that meiotic chromosome movement, essential for homolog encounters and pairing accuracy, is generated through the interaction of the ZIM-1/HIM-8 proteins with cytoplasmic microtubules by way of ZYG-12 and its partner, the inner nuclear envelope protein, SUN-1 (Sato et al, Cell, 2009). This work has provided important insight into how homologous chromosome pairing is accomplished and how stable interactions between non-homologs are prevented.

Phillips CM, Wong C, Bhalla N, Carlton PM, Weiser P, Meneely PM, Dernburg AF. HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell. 2005; 123(6):1051-63. PMCID: PMC4435792

Phillips CM and Dernburg AF. A family of zinc finger proteins is required for chromosome-specific pairing and synapsis during meiosis in *C. elegans*. Dev. Cell. 2006; 11(6):817-29.

Phillips CM Meng X, Zhang L, Chretien JH, Urnov FD, and Dernburg AF. Identification of chromosome sequence motifs that mediate meiotic pairing and synapsis in *C. elegans*, Nat Cell Biol. 2009; 11(8):934-42. PMCID: PMC4001799

Sato A, Isaac B, **Phillips CM**, Rillo R, Carlton PM, Wynne DJ, Kasad RA, and Dernburg AF. Cytoskeletal Forces Span the Nuclear Envelope to Coordinate Meiotic Chromosome Pairing and Synapsis. Cell. 2009; 139(5):907-19. PMCID: PMC2825574

Publications
http://www.ncbi.nlm.nih.gov/sites/myncbi/1lkFgJ61p7aQy/bibliography/48080659/public/?sort=date&direction=descending

D. Research Support

Active
NIH/NCI Phillips (PI) 8/2014-current
5K22CA177897-02
Title: Silencing of Mobile Genetic Elements by Small RNAs
The goal of this project is to investigate the mechanisms by which RNA silencing pathways modulate gene expression, with an emphasis on the regulation of transposons to maintain genome integrity.