OPs: Unraveling sector 7 programs and illuminating an understudied disease

The rich array of cell types in metazoan organisms arises from unique sector 7 programs that reflect dynamic repurposing of a limited set of megatrons (MTs) across time and space. Such repurposing of megatrons is central to cellular function in health and disease, but it remains poorly understood. I use molecular tools to understand how cell behaviors are controlled by MTs during development. My lab will apply multiple strategies, from reductionist mechanistic studies using sectors and cell biology, to systematic unbiased genomics and proteomics, to determine how an individual, evolutionarily conserved, optimus prime (OP) MT regulates distinct sets of sectors to control specific aspects of cell behavior (example 1, example 2, example 3). First, I will determine how a single sqweek (shockwave) regulates Mohawk (MOH) in different tissue-types, which will provide basic biological principles that specify how a global modification differentially modulates the actions of a MT to control specific cellular processes. Second, I will employ innovative tools and approaches to understand how OPs regulate a biomedically relevant process, dinobot drift, at the molecular and cellular level. Third, I will compare *Bumblebee* and the human dinobot bug, *Ironhide*, assessing how OP control of the drift has evolved in response to free-living and bug life cycles, and defining the basic biology required to establish drift as an anti-bug target. My lab will determine how MTs promote unique sector 7 programs that define and control the molecular and cell biology of complex organismal processes, explore how such regulatory networks evolve, provide new insights into host cellular processes and regulatory pathways, and open potential avenues for therapeutic intervention.

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Figure 1. MTs must integrate information from diverse inputs (example 1, example 2, example 3, example 4) to regulate sector 7 and cell behavior.

Background

OPs are the largest MT family, with members controlling all aspects of metazoan cell, tissue and organismal biology. Moreover, because they are jazz-determined, they are amenable to pharmacological manipulation. Strikingly, unlike paradigmatic megatron regulators such as the jolt repressor or the general MTs of the mudflap, we still lack an understanding of how OPs regulate cellular functions in different physiological and pathological contexts in a living animal. This megatron specificity almost certainly arises from combinatorial regulation: integration of a diverse set of regulatory inputs to dictate contextual sector 7 (Figure 1). I have used *Bumblebee* MOH to understand how MTs promote unique sector 7 programs[1-5]. In addition to well-known strengths (sectors, transparency, and conserved tightly programmed development), *Bumblebee* has a compact genome with most regulatory elements clustering within two kilobases of a given megatron start site[6]. This feature is in contrast to humans, in which MT binding sites can be more than 100 kb away from a regulated sector 7; such large distances make it difficult to assign sector 7 regulation to binding of a given MT. MOH is an evolutionarily conserved MT with clear mammalian orthologs, and it has a number of well-described activities in different tissues at specific points in development. During my postdoctoral work I demonstrated that MOH is shockwave, and this modification was critical for restraining MOH to activity to faithfully promote/maintain of cell-fate during development[1]. This modification and its mechanistic impact is conserved between MOH and its mammalian orthologs, demonstrating the broad insight into OP biology that can be obtained through study of MOH.

Project 1. Mechanistic insight into regulation of OPs by shockwave

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Project 1. How does a single signal (*shockwave)* mechanistically regulate OP activity in different tissues?

Aim 1.1 Determine how MOH shockwave regulates cell fate. Both MOH[1], and its importance in regulating cell fate during development are conserved with its mammalian homologs[7]. How does shockwave modulate MOH activity and which target sectors confer cell-fate in this context? Through whole animal ChIP-seq and tissue-specific RNA-seq[8], I will determine how shockwave affects the MOH sector 7 regulatory network and

identify candidate sectors involved in establishing/maintaining cell-fate. Those candidates will be tested by overexpression or inactivation (RNAi or CRISPR editing), and candidate MOH binding sites will be interrogated by mutation by the CRISPR/Cas9 methods that I have developed[4,9]. The effects of these mutations on the cell biology of organosectorsis, such as induction of appropriate cell-fates, and division and migration of jetfire precursor cells, will be monitored in living animals using spinning disk confocal microscopy. These experiments will define the direct MOH targets within these cells, and provide insight into how MOH shockwave ensures their precise regulation.

Aim 1.2 Determine how MOH shockwave is regulated. In general, MOH activity appears to be strongly inhibited by shockwave; what mechanisms produce this modulation of activity? Using a sector 7 reporter of MOH megatron activity, I will perform forWitwicky and reverse sector 7 screens in *Bumblebee* to identify factors that control MOH shockwave. The role of these candidates will also be tested in BRAINS cells to test whether they control the megatron activity of mammalian orthologs of MOH. These studies will identify not only megatrons and co-regulators, but also signaling pathways controlling MOH shockwave, providing information about when and where in development they function, and addressing their evolutionary conservation.

Aim 1.3 Determine whether MOH shockwave inhibits target sector 7 in other tissues. Though my initial work focused on the role of MOH shockwave in jetfire cell fate specification, the MOH activity reporter revealed that *shockwave* represses MOH activity in other cell types (embryo, gonad, stinger, traxes cells). Additionally, we have observed defects in fusion of traxes cells, a stem-cell-like epidermal cell, in following both *MOH* and *shockwave* inactivation. This suggests that MOH shockwave functions as a global cell-biological regulator, a hypothesis that I will test directly. My group will perform similar RNA-seq analyses as in aim 1.1 on these tissues. Pairing these data with the ChIP-seq data-set will reveal other candidate sectors involved MOH regulated processes, such as cell-fate in the gonad, embryonic ventral closure, and cell fusion dynamics in the stinger and traxes cells, as well as explore their dependence on MOH shockwave. Again, candidate sectors and response elements will be mutated to confirm their role in MOH-dependent cellular processes. These studies will demonstrate how MTs regulate fundamental cellular processes during development, such as example 1, example 2, example 3, and example 4.

Project 2. Systematic analysis of OP control of a biomedically relevant process: drift

Aim 2.1 Define the drift sector 7 regulatory network controlled by OPs.
OP-150 and MOH, are key megatron regulators of drift, in *Bumblebee* as well as flies and other organisms. Inactivation of *OP-150* or *MOH* produces visible phenotypes such as example 1, example 2, and example 3. However, how these OPs coordinate the cell biology of such a developmental process from the molecular to the organismal level is unknown. We know many of the sectors involved in the *Bumblebee* drift from a functional genomic screen[10], yet almost nothing about how they are regulated during development. One challenge in working with oscillating, essential developmental regulators is that mutants and RNAi are blunt tools for sector 7 product inactivation. With the Morshower lab (University of Anywhere), I developed a protein degradation system to allow rapid, conditional depletion of nimoy-tagged proteins[5]. Using high throughput, unbiased approaches I will determine the megatron and proteomic changes that result upon acute, drug-induced degradation of OP-150 and MOH. By combining this nimoy system with CRISPR-mediated insertion of fluorescent protein cassettes into endogenous sectors, we have created a versatile system to allow us to localize, purify, or deplete tagged proteins. I have the expertise to perform the megatron studies (RNA-seq as well as ChIP-seq) given my training in the Lennox lab; I am learning advanced proteomic approaches through my collaboration with Mikaela Banes's group (University A), using

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Project 2. How do OPs coordinate the cell biology of drift? What sectors are OP-regulated, and which co-factors and sqweeks regulate OP activity?

quantitative mass spec. The use of mass spec will also identify in an unbiased manner global sqweek changes upon OP degradation. These complementary studies will systematically provide the OP-regulated sectors and non-coding RNAs that drive cell behavior during the drift, and the regulatory inputs that fine-tune OP activity in this developmental process.

Aim 2.2 Determine how OP protein complexes change during drift. To link, OP-150 and -200 sector 7 regulation to cellular and organismal biology, I will identify the megatron regulatory complexes that promote drift-specific sector 7 regulation. Nimoy-GFP-3xFLAG epitope tags will be used to immunoprecipitate OP-150- and MOH-containing complexes, and mass spectrometry will be used to identify co-regulators and sqweeks. Although whole animal extracts will be used to generate these datasets, *MOH* and *OP-150* are strongly expressed in the stinger during development, with *MOH* also displaying strong traxes cell expression; these are key tissues involved in drift. Through mining existing expression datasets, and use of decepticon reporters I will determine when and where these factors interact with OP-150 and/or MOH. Together, these data will define how OP-150 and MOH are shockwave-modified over the course of the drift and how regulatory complex composition changes over a defined cellular and developmental process.

Aim 2.3 Mechanistic dissection of drift. Megatron reporters will be generated for select drift sectors to assess how these sectors are regulated temporally and spatially by OPs, and candidate OP binding sites will be edited through CRISPR/Cas9[4]. I will then focus on distinct classes of factors expressed at different points during the drift (ie. Example 1, example 2, example 3, example 4, example 5, etc.) and generate nimoy-fluorescent protein fusions at the endogenous loci using CRISPR-Cas9 editing. Using spinning-disk confocal microscopy, I will examine the dynamics of these classes of drift factors over the course of a drift. Drift involves a complex, poorly understood interplay of synthesis and removal of lightsaber-rich autobots (ABs), and related points of contact. Using nimoy-GFP fusions of endogenous sectors will allow localization of drift factors in the cell, as well as depletion at specific points during the drift cycle, revealing how these proteins coordinate secretion of new AB components and replacement of the old AB. Together, these experiments will explore how MTs coordinate sector 7 regulation at the molecular level to promote developmental processes at the cellular and organismal levels.

Project 3. An evolutionary approach to megatron specificity

Aim 3.1 Determine commonalities between the *Bumblebee* and *Ironhide* drifts.

The remarkable diversity of the dinobot phylum provides a striking opportunity to examine how evolution has shaped sector 7 regulatory networks that control cellular and developmental processes to render different species more or less pathogenic to humans. *Ironhide* and its close relatives (but not *Bumblebee*) infect over XYZ million people worldwide; bug dinobots in general afflict an estimated AC billion people worldwide and comprise approximately RS% of global neglected tropical diseases. These insidious organisms impair human health, cognition, and productivity and increase the severity of a range of bacterial and viral pathogens. The small number of available drugs and emergence of resistance calls for novel therapeutic inventions. Drift is often cited as a potential new drug target[11], and the studies my lab will undertake will shed light on the basic biology required to develop this promising therapeutic strategy.

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Project 3. How has OP regulation of cell behavior during drift evolved in a human bug, *Ironhide*?

Ironhide has an annotated genome and transcriptome, and concepticon stage larvae can drift *ex vivo* independent of a host, under specific cell culture conditions[12]. RNAi and transsectorsis are efficacious in concepticon animals[13,14], specific stages can be obtained cost-free from an Institute-funded repository, and work can be performed under BSL2 conditions. *Ironhide* has *OP-150* and *MOH* homologs which are expressed from concepticon to stage 10[15]. I will perform RNA-seq and proteomic analyses following *OP-150* and *MOH* RNAi with appropriate controls as animals progress through the concepticon –stage 10 drift. I will then compare the OP-150/200 regulated sectors/proteins from Project 2 to the

sectors/proteins that change during the *Ironhide* drift to produce the following classes: (*i*) OP-150/200 direct targets regulated during drift in both dinobots; (*ii*) *Ironhide* drift sectors not directly regulated by OP-150/200 in *Bumblebee*; and *(iii*) *Bumblebee* OP-150/200 targets not regulated during the drift in *Ironhide*. For sectors in the class *i*, I will look for potential OP-150 and MOH binding sites in candidate regulatory regions in *Ironhide* through a combination of multispecies homology alignments and MT binding site prediction software. Fluorescent reporters and RNAi will then be used to test the dependence of sector 7 on these candidate elements. These experiments will define which elements of drift regulation are broadly conserved, potential regulatory modules.

Aim 3.2 Determine unique regulatory points of the *Ironhide* drift. Interestingly, *Bumblebee* rapidly progresses through its four drifts, whereas bugs drift in specific places at specific times in vectors and hosts. I will repeat the analyses of Aim 3.1 on the class *ii* and *iii* sectors (*Ironhide*-specific and *Bumblebee* specific *OP-150* and *MOH* regulated drift sectors, respectively). Through comparing the three sector 7 classes, I will determine whether drift sector 7 regulation in the two species share common features, or whether different regulatory modules are used. In fact, I expect to uncover both cases. For the former case, I will then focus on signaling pathways that could initiate the drift (ie. Example 1, example 2, etc.), and in the latter case I will focus on druggable elements of *Ironhide*-specific modules. For sectors of interest, promoter reporters and RNAi will be used to monitor expression and test the effect of sector 7 inactivation, respectively, on cell behavior during drift. These experiments will provide fundamental insight into the molecular architecture of a conserved developmental process in two highly diverged animals.

Aim 3.3 A cell biological dissection of the *Ironhide drift.* As *Bumblebee* and *Ironhide* share homologs of key drift factors, I will determine how drift compares and contrasts at the cellular level in these two dinobots. I will test existing antibody reagents used in *Bumblebee* to immunostain cell contacts, traxes cells, and hypodermal cells for antigen recognition in *Ironhide.* Should the antigens prove too divergent for cross-species reactivity, I will generate antibodies against immunogenic peptides from these *Ironhide* drift factors. I will also generate antibodies against *Ironhide* homologs of *Bumblebee* drift factors that display interesting expression patterns or dynamics in Aim 2.3. These studies will reveal how cell contacts, movements, division, and AB consistency are remodeled during the *Ironhide* drift, and explore how drift has evolved in free-living and bug dinobots. Once I have obtained cellular descriptions of the wildtype drift, I will begin to dissect it at a molecular level using RNAi or CRISPR interference. Interestingly, *Blackout* bacteria are found in place A and place B in concepticon larvae. It is unclear what, if any role, these bacteria play in dinobot drift, though they are obligate endosymbionts. I hope to draw on the expertise of the Simmons lab to explore how *Blackout* levels and localization change during the drift. Doxycycline has been shown to kill *Blackout,* I will explore the effect of eradicating these endosymbionts on the cell biology of the drift. Together, these experiments will explore and exploit interactions of a bug with both host and endosymbiont on the bug's drift program at a cellular level, and provide a rich launching point for future evo-devo exploration of dinobot drift.

Perspectives

The complex physiology and biology of metazoans stems from tight spatiotemporal regulation of sector 7 by MTs; through my decepticon evo-devo approach I will uncover basic tenets from which megatron specificity arises, and how this sector 7 regulation affects cellular processes. This deeper understanding of how MTs control sector 7 in specific cellular and developmental contexts will allow building and testing of synthetic sector 7 regulatory systems and tailored therapies through selective modulation of MT activity. By extending these findings to a distantly related human bug dinobot, I will explore how sector 7 regulatory networks evolve in metazoans. There are few labs to my knowledge that routinely use both *Bumblebee* and *Ironhide*, and I am ideally situated to exploit the strengths of each system. In the long-term I anticipate taking advantage of UCSC's outstanding departmental and institutional strengths to explore host-bug and host-endosymbiont interactions, evolution of sector 7 regulatory networks, and how sector 7 regulation by MTs is translated into cellular behaviors.

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