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Summary: We are interested in multiple questions in basic and applied biology. For further information on Hay lab research consult our web page (http://www.its.caltech.edu/~haylab/). One goal of our work is directed towards understanding the genetic and molecular mechanisms that regulate cell death, proliferation, innate immunity, microRNA function, and spermatogenesis. We use Drosophila melanogaster as a model system to identify genes that function to regulate these processes. Important cellular regulatory pathways are evolutionarily conserved; thus, molecules identified as regulators of these processes in Drosophila are likely to have homologs in vertebrates and the pathways that link these molecules are likely to be regulated similarly. A second goal of our work addresses three questions in population biology. 1) Can we bring about reproductive isolation (speciation) between populations of plants or animals that otherwise freely interbreed? Answers to this question have application to the growing number of situations in which plants and animals are engineered to show specific pharmaceutical or agricultural traits. In brief, we would like to be able to limit gene flow between engineered organisms and their wild counterparts. 2) Can we engineer the genetics of populations so that they drive themselves to local extinction? For example, invasive non-native plants and animals cause substantial economic losses. A number also cause substantial environmental damage, leading in many cases to extensive range reduction and/or extinction of unique, endemic species. Our goal is to develop genetic tricks that drive local extinction of invasive species and disease vectors. 3) Can we drive genes into wild populations so that all individuals express a trait of interest. With regard to this last aim, we are particularly interested in developing transgenic insects that will prevent transmission of mosquito-borne diseases such as malaria and dengue fever. More than 500 million people are infected with the malaria parasite each year, resulting in 1-3 million deaths. Dengue, a mosquito-borne virus infects more than 100 million people each year, resulting in more than 25,000 deaths. Effective vaccines for these diseases do not exist, and in the case of malaria, the causative agent, the parasite Plasmodium falciparum has acquired resistance to many drugs. Vector suppression through the release of sterile males, the use of insecticides, or modification of the environment provides an important tool for limiting mosquito-borne disease. However, each approach has limitations. Release of sterile males provides only transient population suppression, insecticides affect many non-target species and mosquitoes often evolve resistance to these compounds, and wholesale modification of the environment may not be feasible, or desirable in many situations based on ecological concerns. Our goals are two-fold: to develop transgenic insects that lack the ability to transmit these pathogens; and to develop genetic tools for driving these genes into wild populations of insects, thereby blocking disease transmission.

1. **Drosophila models of human neurodegenerative diseases**


2. **Gene activation screens for cell death regulators: MicroRNAs, small non-coding RNAs, define a new family of cell death regulator**

   Chun Hong Chen, Haixia Huang

   We have carried out several screens for cell death regulators in the fly and have identified a number of new molecules. Among these are multiple microRNAs, small noncoding RNAs that function by inhibiting translation of target transcripts. We are interested in determining when and where these molecules regulate death, as well as the nature of their targets. We are also designing microRNAs that target known cell death regulators as a way of probing the function of these proteins in specific contexts.

3. **Cell death, caspases and IAPs**

   H. Arno J. Müller, Bruce A. Hay, Chun-Hong Chen

   In flies and vertebrates most, if not all, cells can undergo apoptosis in the absence of new gene expression, indicating that the components required to carry out
apoptosis are present and ready for activation. The core of the cell death machine consists of members of a family of proteases known as caspases, which become activated in response to many different death signals. Active caspases then cleave a number of different cellular substrates that ultimately lead to cell death and corpse phagocytosis. Most if not all cells constitutively express caspase zymogens (inactive precursors) sufficient to bring about apoptosis. Thus, the key to cell death and survival signaling revolves around controlling the levels of active caspases in the cell. Several basic strategies are used to regulate caspase activity, and the core proteins that drive caspase-dependent death are evolutionarily conserved. In Drosophila many cells experience chronic activation of the apical cell death caspase Drone. If unrestrained, active Drone cleaves and activates downstream effector caspases that bring about cell death. Cells survive because they express the IAP DIAP1, which suppresses Drone activity, as well as that of caspases activated by Drone. One major pathway through which caspase-dependent cell death in flies is induced is through the regulated expression of pro-apoptotic proteins that disrupt DIAP1-caspase interactions through several different mechanisms, each of which has the effect of unleashing a cascade of apoptosis-inducing caspase activity. We are interested in several questions. 1) What are the signals that lead to caspase activation in cells that would normally live? 2) How do IAPs regulate caspase activity and when and where does this regulation define points of control? 3) How is IAP activity regulated? 4) And finally, as discussed further below, how do caspases, IAPs and their regulators work to regulate non-apoptotic processes? We are using both genetic screens and biochemical approaches to identify the critical molecules.

4. Caspases and their regulators in a non-apoptotic process, spermatid differentiation

Haixia Huang, Shamili Allam, Joy Chen, Geoffrey Pittman

We have found that multiple caspases, acting through distinct pathways, acting at distinct points in time and space, are required for spermatid individualization, a process in which spermatids (which develop in a common cytoplasm) become enclosed in individual plasma membranes and shed most of their cytoplasm*. Spermatid individualization is an evolutionarily conserved process, but little is known about how it is brought about. Several questions are of interest to us: 1) What are the upstream signals that drive caspase activation? 2) What are the non-apoptotic targets that facilitate differentiation? 3) How is cell death prevented in the face of high levels of caspase activity that would normally be associated with cell death? 4) Do caspases play similar roles in promoting spermatid differentiation in mammals? 5) Can we manipulate the biology of spermatogenesis so as to bias gamete production so that males produce gametes carrying the Y chromosome, but not the X chromosome? Elements with these characteristics, if they are located on the Y chromosome, are predicted to drive a population to extinction through the generation of male-only populations.

Reference

5. Cell death and the innate immune system

Chun-Hong Chen, Ming Guo, Bruce A. Hay

As discussed above, many IAP family proteins inhibit apoptosis. IAPs contain N-terminal BIR domains and a C-terminal RING ubiquitin ligase domain. Drosophila DIAP1 protects cells from apoptosis by inhibiting caspases. Apoptosis initiates when proteins such as Reaper and Hid bind a surface groove in DIAP1 BIR domains via an N-terminal IAP-binding motif (IBM). This evolutionarily conserved interaction disrupts IAP-caspase interactions, unleashing apoptosis-inducing caspase activity. DIAP2 overexpression also inhibits Rpr-and Hid-dependent apoptosis, but little is known about DIAP2's normal functions. We generated diap2 null mutants, which are viable and show no defects in developmental or stress-induced apoptosis. Instead, DIAP2 is required for the innate immune response to Gram-negative bacterial infection. DIAP2 promotes cytoplasmic cleavage and nuclear translocation of the NF-κB homolog Relish, and this requires the DIAP2 RING domain. Increasing the genetic dose of diap2 results in an increased immune response, while expression of Rpr or Hid results in down-regulation of DIAP2 protein levels. Together these observations suggest that DIAP2 can regulate immune signaling in a dose-dependent manner, and that DIAP2 is regulated by IBM-containing proteins. Therefore, diap2 may identify a point of convergence between apoptosis and immune signaling pathways.

Reference

6. Driving genes for disease refractoriness into wild pest insect populations

Chun Hong Chen, Haixia Huang, Catherine Ward, Jessica Su, Nikolai Kandul, Geoff Pittman, Omar Akbari, Arun Kumar, Daniel Leighton, Bruce A. Hay

An attractive approach to suppressing mosquito-borne diseases involves replacing the wild-insect population with modified counterparts unable to transmit disease. Mosquitoes with a diminished capacity to transmit Plasmodium have been identified in the wild and created in the laboratory, demonstrating that endogenous or engineered mosquito immunity can be harnessed to attack Plasmodium. However, a critical unanswered question is how to spread these effector genes throughout the areas inhabited by disease-transmitting insects. Epidemiological and modeling studies suggest that it will be necessary to rapidly replace a large percentage of the wild mosquito population with refractory insects in order to achieve significant levels of disease control. Because insect disease
vectors are spread over wide areas and can migrate significant distances, mass release of refractory insects associated with simple Mendelian transmission of effector-bearing chromosomes is unlikely to result in a high enough frequency of transgene-bearing individuals. Compounding this problem, enhancement of immune function in insects is often costly, requiring tradeoffs with other life history traits such as longevity and fecundity that decrease fitness. Therefore, it is likely that insects carrying effector transgenes will be less fit than their wild counterparts, resulting in a decrease in the fraction of individuals carrying genes for refractoriness over time. These observations argue that population replacement will require coupling of genes conferring disease refractoriness with a genetic mechanism for driving these genes through the wild population at greater than Mendelian frequencies.

Maternal-effect lethal selfish genetic elements have been described as genetic entities in the flour beetle Tribolium castaneum. The molecular nature of these elements (known as Medea elements) is unknown, but their genetic behavior makes them attractive candidates to mediate drive. This is because when present in a female, they must be inherited in the next generation in order for the offspring to survive.

**Figure 1.** Medea is a "spiteful" selfish genetic element that enhances its transmission from generation to generation by causing the death of offspring that fail to inherit it. Mothers that carry a Medea element express a toxin (red dots) that is inherited by all oocytes (small ovals). Embryos (large ovals) that do not inherit Medea die because toxin activity (red background) is unimpeded (lower left square). Embryos that inherit Medea from the mother (upper left square), the father (lower right square) or both (upper right square), survive because expression of an antidote early during embryogenesis (green background) neutralizes toxin activity. We imagine that Medea is comprised of two closely linked genes (upper left). One consists of a maternal germline-specific promoter that drives the expression of an RNA or protein that is toxic to the embryo. The second locus consists of a zygotic (early embryo) promoter that drives expression of an antidote.

This behavior is predicted to lead to rapid spread of the element within the population even if it carries an associated fitness cost because the chromosome that carries it gains a transmission advantage relative to counterparts that do not. Since the molecular biology of endogenous Medea elements is unknown, we created synthetic elements in Drosophila that can drive population replacement and that are resistant to recombination-mediated dissociation of drive and effector functions. The genetic and cell-biological principles utilized, which utilize microRNA-mediated silencing of a maternally-expressed gene essential for embryogenesis, coupled with early zygotic expression of a rescuing transgene, should be generally applicable to a number of other animal and plant species and have the potential to allow for iterative cycles of population replacement. We are now expanding this work into the mosquito system.

**Figure 2.** When Medea-bearing males are introduced into a population consisting of wildtype males and females, wildtype individuals are eliminated from the population. The greater the initial ratio of Medea to wildtype males, the more rapidly this elimination occurs.

**Figure 3.** Medea's ability to spread, and the time it takes to become present in all individuals, is a function of fitness cost and introduction frequency. The plot describes the number of generations required for Medea to be present in 99% of
individuals, for a *Medea* element with an embryonic fitness cost (resulting from the presence of a cargo transgene designed to protect from disease, for example). Homozygous *Medea:*non-*Medea* introduction ratios are indicated on the Y axis, and embryonic fitness cost on the X axis. Area between lines indicates regions of parameter space within which a specific number of generations (indicated by numbers and arrows) are required for the frequency of *Medea* individuals to reach a frequency of 99% or greater. Line color, shown in the heat map at right, provides a measure of how many generations are required. Black lines (50+) indicate that fifty or more generations are required. The border between the black-lined region and the lower unlined region defines the critical *Medea:*non-*Medea* introduction ratio, below which *Medea* will be eliminated from the population.

**Reference**

**7. Sensing and killing dengue and yellow fever virus-infected cells in their insect host**
Kelly J. Dusinberre, Gal Barak
Dengue and yellow Fever virus infect mosquitoes during a blood meal. The virus must enter and replicate inside mosquito midgut cells, disseminate throughout the body and ultimately infect the salivary gland (7-14 days later), in order to be transmitted to a new individual during a subsequent blood meal. Our goal is to develop transgenes that are phenotypically neutral when expressed in uninfected individuals, but that kill virus-infected cells and/or the mosquitoes themselves. The virus encodes several activities that are not present in uninfected host cells. These include a viral polyprotein protease, and RNA-dependent RNA polymerase. We are developing molecules that sense these activities and cause the death of cells and insects in which they occur, thereby preventing disease transmission to humans.

**8. Engineering reproductive isolation and population replacement using a synthetic underdominance system**
Kelly Dusinberre, Katie Kennedy, Margaret Chiu, Jessica Su.
The Medea system detailed above is very good at spreading genes into populations distributed over large areas, provided that modest levels of migration occur. This is ideal for situations in which the goal is to carry out population replacement in large regions. However, some communities may favor an approach in which population replacement is restricted to a local environment (Lets see how it does in your back yard, before trying it in mine). This creates a challenge: how to spread genes within a local environment, but maintain a barrier to migration-driven spread and fixation in surrounding regions.

To address this need we are developing the synthetic underdominance system illustrated below. In this system homologous chromosomes carry toxin-antidote pairs in which the toxin present on chromosome A (Killer 1) is linked to an antidote (Rescue 2) that represses Killer 2. Killer 2 is located at the same position on the homologous chromosome B, linked with an antidote (Rescue 1) that represses Killer 1 (Figure x). In such a system, organisms can only survive if they carry A and B chromosomes (in A/B individuals), or only wildtype (+) chromosomes (in +/- individuals). A/+ and B/+ individuals die. A and B chromosomes will also carry genes that confer resistance to disease transmission. Such a system has two interesting features.

**Underdominance Chromosomes Eliminate Wild Type Chromosomes**

Figure 4. A single-locus underdominance system can be used to engineer reproductive isolation and population replacement.

First, it constitutes a simple method for engineering reproductive isolation (speciation). Matings between +/- individuals produce viable progeny, as do matings between A/B individuals. However, mating between +/- and A/B individuals produce only A/+ and B/+ progeny, which all die. This simple technology has a number of potential applications and provides a platform from which to explore some of the evolutionary consequences of reproductive isolation.

Second, it provides a method for driving genes into a local environment in such a way that they are unlikely spread to fixation in surrounding regions through migration. In brief, for underdominance, as with Medea elements that carry a fitness cost, a threshold frequency must be achieved in order for spread to occur at all. With underdominance this threshold is quite high. But once the threshold is crossed, the underdominant system drives the wildtype chromosomes out of the population by causing their death in heterozygotes. A and B chromosomes also die in heterozygous progeny, but so long as A/B
individuals make up greater than 66% of the population, more + chromosomes, and thus +/+ individuals, are eliminated than are A and B chromosomes, in A/B individuals. The A/B genotypes have great difficulty in spreading into surrounding regions through migration because as these individuals migrate into areas composed largely of +/+ individuals, they are more likely to mate with +/+ individuals than with A/B individuals, resulting in the likely death of the A and B chromosomes in heterozygous progeny. We are developing several versions of underdominance in Drosophila and are working to move these systems to mosquito species.

**Figure 5.** A single locus underdominant system's ability to spread, and the time it takes to become present in all individuals, is a function of fitness cost and introduction frequency. The plot describes the number of generations required for underdominant chromosomes to be present in 99% of individuals, for a situation in which the presence of the underdominant chromosomes is associated with an embryonic fitness cost (resulting from the presence of a cargo transgene designed to protect from disease, for example). Homozygous A/B:+/+ introduction ratios are indicated on the Y axis, and embryonic fitness cost on the X axis. Area between lines indicates regions of parameter space within which a specific number of generations (indicated by numbers and arrows) are required for the frequency of underdominant chromosome-bearing individuals to reach a frequency of 99% or greater. Line color, shown in the heat map at right, provides a measure of how many generations are required. Black lines (50+) indicate that fifty or more generations are required. The border between the red-lined region and the lower unlined region defines the critical A/B:+/+ introduction ratio, below which underdominant chromosomes will be eliminated from the population.

9. Sensing and responding to normal and abnormal microRNA expression. *Nikolai Kandul, Alan Li*

10. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression by suppressing the translation or promoting the degradation of transcripts to which they hybridize. Importantly for our purposes, when miRNAs are perfectly complementary to their target transcripts, transcript cleavage and degradation results. It is clear that miRNA expression is deregulated in many disease states. In addition, many viruses encode miRNAs that promote viral replication and/or suppress host defense systems. Our goal is to develop methods for sensing the expression of a particular miRNA, and then transducing this signal into changes in gene or protein expression. This will allow us to monitor the levels of miRNA expression in living animals. It will also allow us to regulate cellular physiology in response to the levels of particular miRNAs.

11. Predicting the fate of gene drive systems and their cargos in the wild.

*Catherine Ward, John Marshall*

As we develop gene drive strategies we need to be able to predict how they are likely to behave. A number of question arise. Under what ecological and population genetic conditions will drive chromosomes spread? What are the likely epidemiological consequences of spread in terms of disease prevention? What are the likely functional lifetimes of these elements in the wild? What are the possibilities for removal and replacement of first-generation elements with second-generation elements? We are using mathematical modeling and computer simulations to address these issues for a number of different drive strategies.

**Publications**


