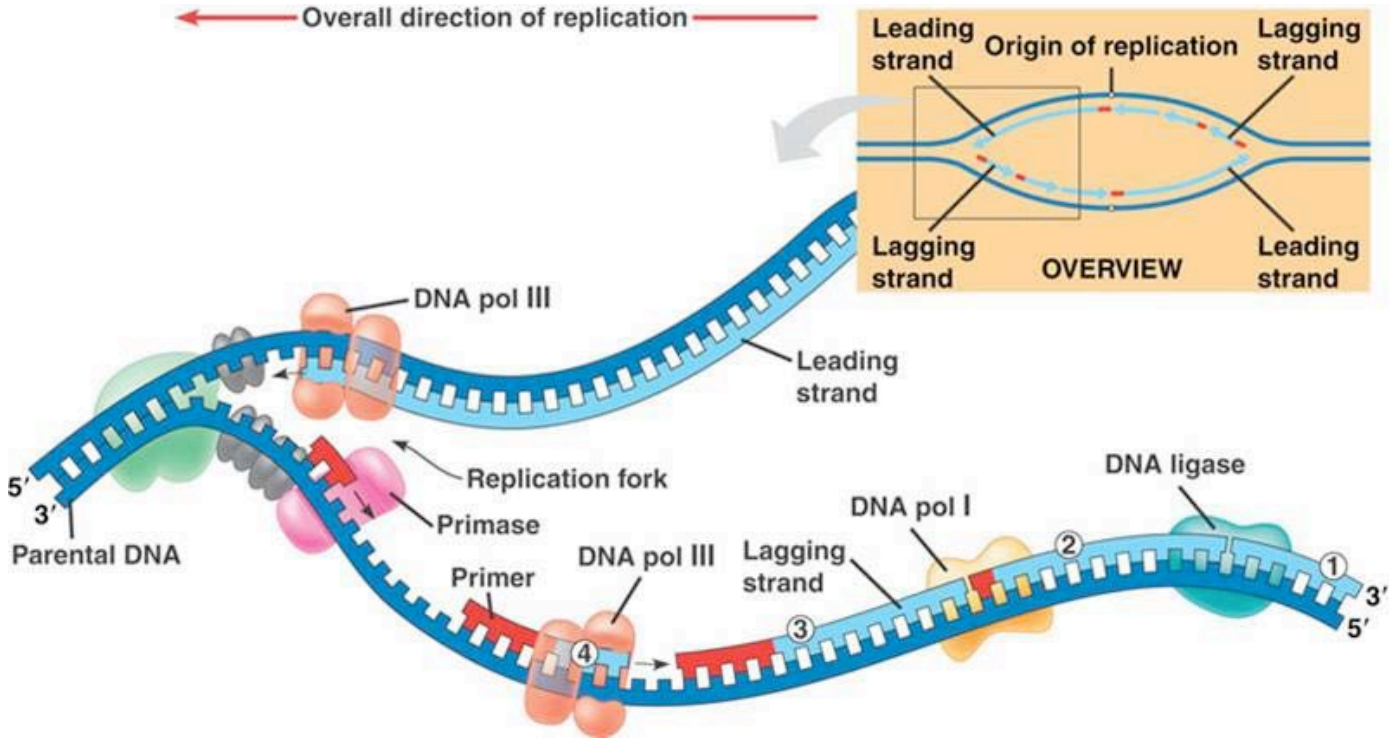
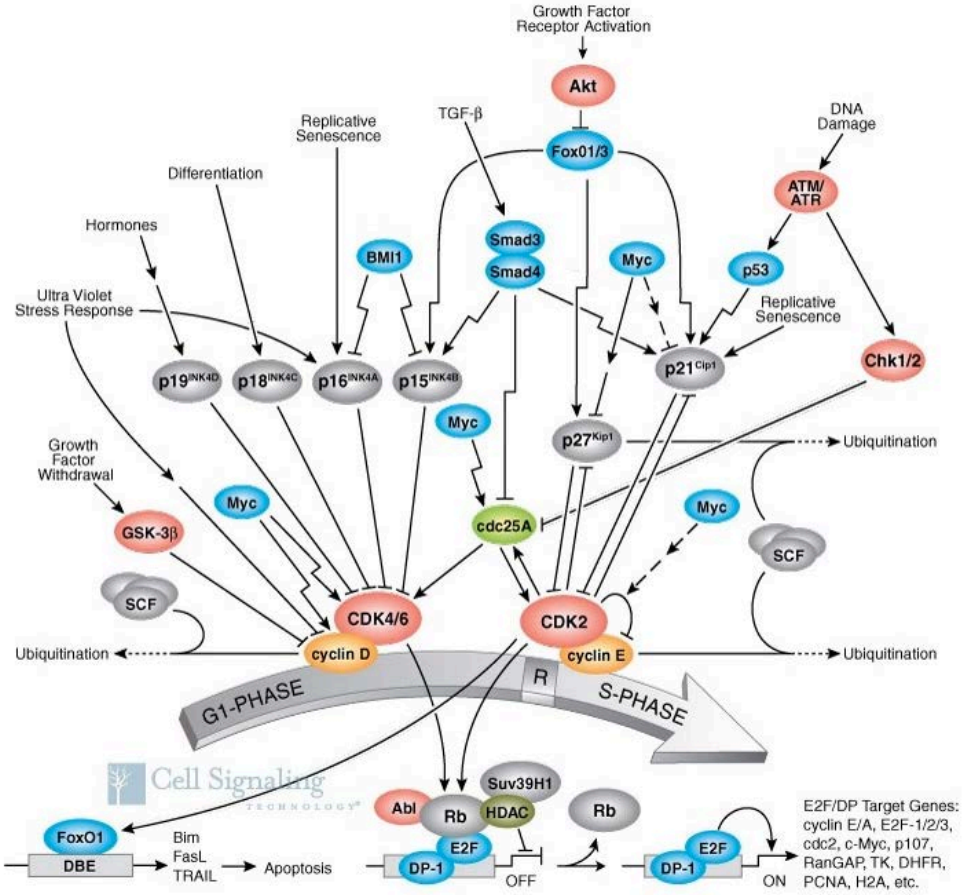
How do we know this?



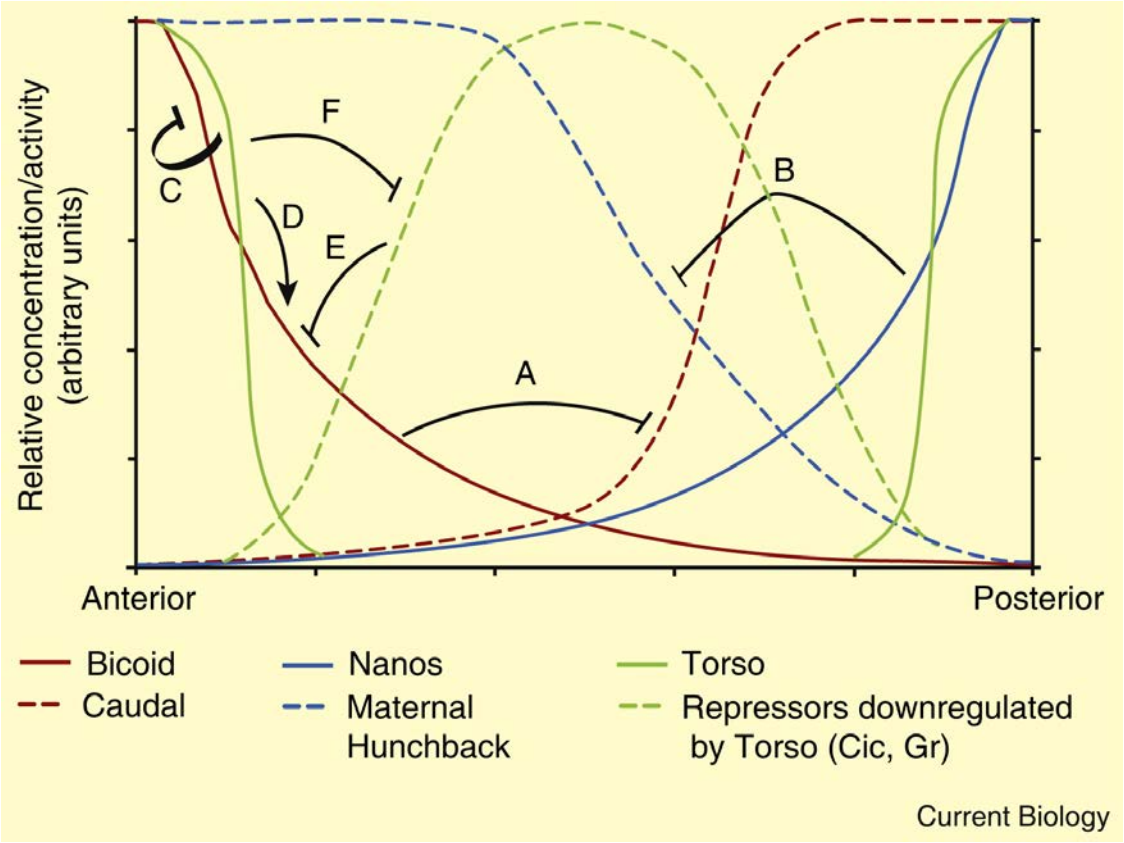
and how do we know this?



http://www.cellsignal.com/reference/pathway/Cell_Cycle_G1S.html

or this?

Genes controlling anterior-posterior patterning in the Drosophila embryo



or this?

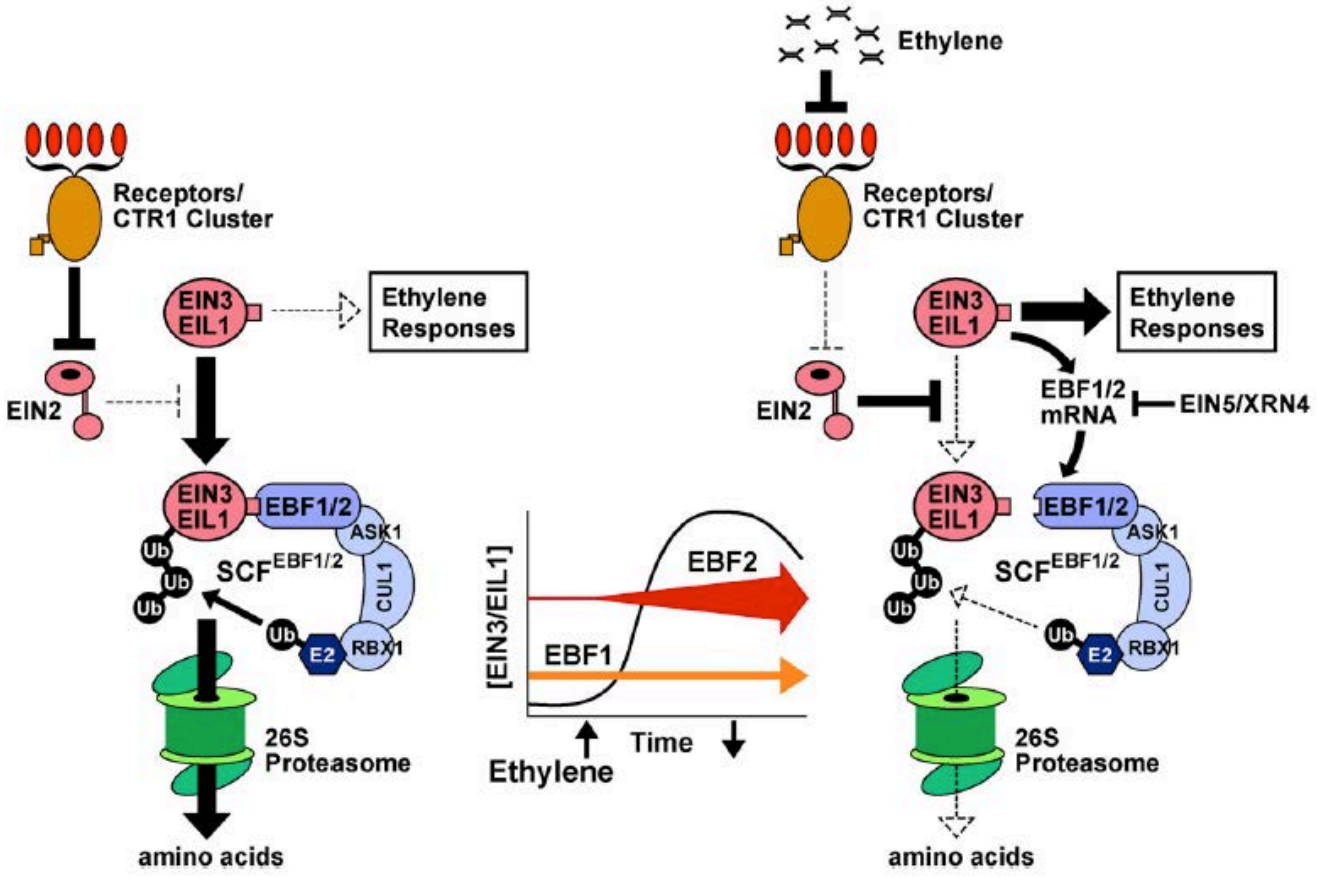


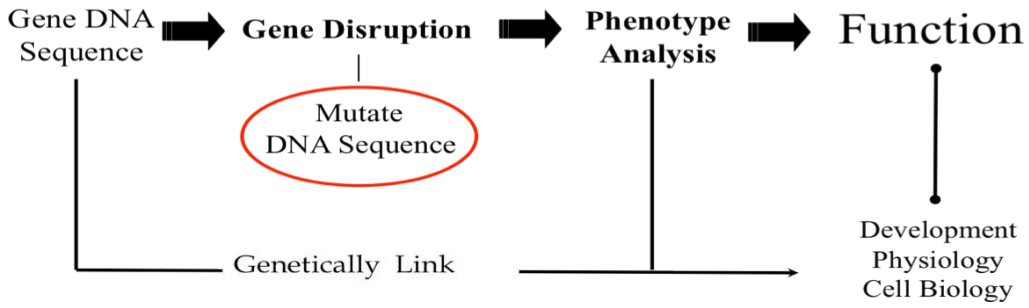
Figure 9. Model for the Action of EBF1 and 2 in the Ethylene Signal Transduction Pathway.

Ethylene Signal Transduction Pathway in Plants

How would you even get a foothold?

Reverse genetics starting with candidate genes

Gene sequence → Targeted mutagenesis → gene knock-out → reveal phenotype → infer function



➔ *But what if you don't have any candidate genes or what if you think previous research has been focussed incorrrectly on a particular set of genes or is biased in some other way?*

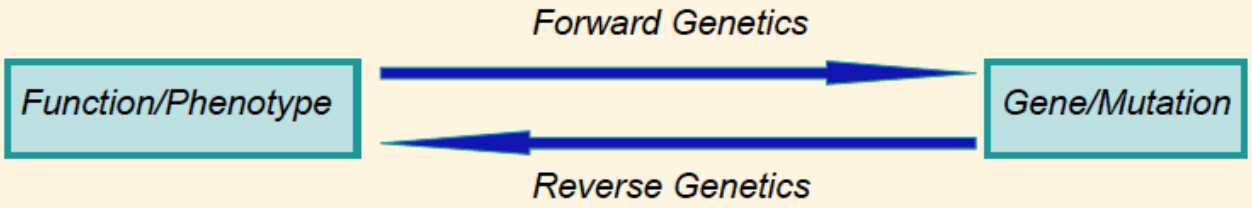
Forward genetics

Random mutagenesis → look for ***phenotypes*** of interest → identify genes → determine molecular function

What is the genetic basis of a particular phenotype?
(How does one determine the function of a gene, or the identity of genes responsible for a trait?)

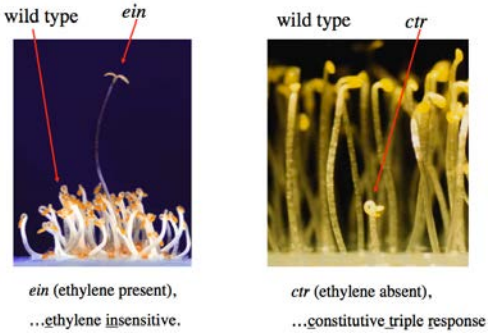
Forward Genetics:
Starts with a phenotype and moves towards the gene

Reverse Genetics:
Starts with a particular gene and assays the effect of its disruption



Forward genetics

- *Pick a biological process or problem*
- *Pick a model organism*
- *Choose a method for mutagenesis*
- *Screen for mutants with anticipated phenotype*

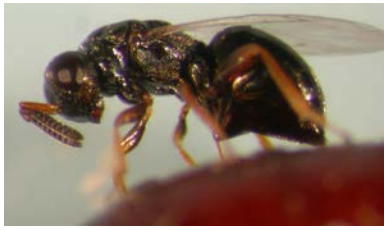


ein (ethylene present), …ethylene insensitive.

ctr (ethylene absent), …constitutive triple response

Second Arabidopsis lab is cancelled

- *Collect mutants and study their attributes*
- *Figure out how many genes are represented in your collection of mutants*



Nasonia lab: moved to next week

- *Study genes and gene function at the molecular level*

Pick a biological process or problem

- How is the nervous system specified?
- How does a developing embryo specify its head and its tail
- How does sex determination work?
- How does a developing embryo assemble a complex array of tissues and put them in the correct location and orientation?
- How does DNA replication occur?
- How is the eukaryotic cell cycle controlled?
- How does a plant respond to ethylene?
- How are visual signals processed by vertebrate animals?

OPEN ACCESS Freely available online

PLoS GENETICS

Forward Genetic Analysis of Visual Behavior in Zebrafish

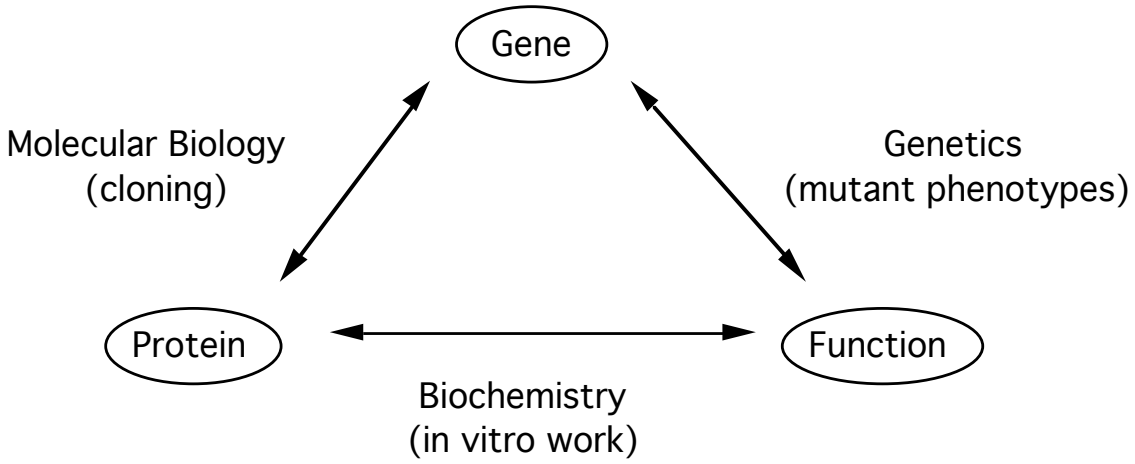
Akira Muto, Michael B. Orger¤a, Ann M. Wehman, Matthew C. Smear, Jeremy N. Kay¤a, Patrick S. Page-McCaw¤b, Ethan Gahtan¤c, Tong Xiao, Linda M. Nevin, Nathan J. Gosse, Wendy Staub, Karin Finger-Baier, Herwig Baier*

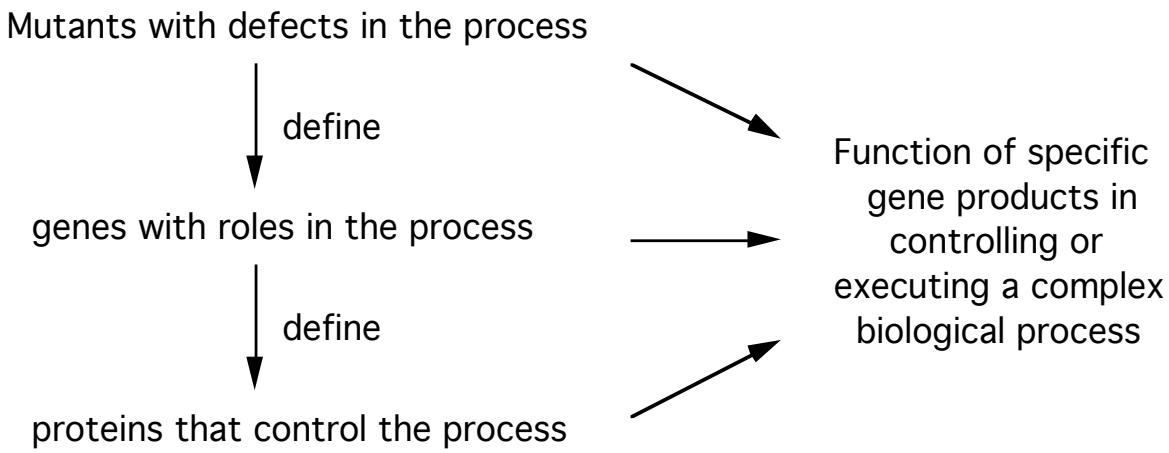
Department of Physiology, Programs in Neuroscience, Genetics, and Developmental Biology, Center for Human Genetics, University of California, San Francisco, California, United States of America

The visual system converts the distribution and wavelengths of photons entering the eye into patterns of neuronal activity, which then drive motor and endocrine behavioral responses. The gene products important for visual processing by a living and behaving vertebrate animal have not been identified in an unbiased fashion. Likewise, the genes that affect development of the nervous system to shape visual function later in life are largely unknown. Here we have set out to close this gap in our understanding by using a forward genetic approach in zebrafish. Moving stimuli evoke two innate reflexes in zebrafish larvae, the optomotor and the optokinetic response, providing two rapid and quantitative tests to assess visual function in wild-type (WT) and mutant animals. These behavioral assays were used in a high-throughput screen, encompassing over half a million fish. In almost 2,000 F2 families mutagenized with ethylnitrosourea, we discovered 53 recessive mutations in 41 genes. These new mutations have generated a broad spectrum of phenotypes, which vary in specificity and severity, but can be placed into only a handful of classes. Developmental phenotypes include complete absence or abnormal morphogenesis of photoreceptors, and deficits in ganglion cell differentiation or axon targeting. Other mutations evidently leave neuronal circuits intact, but disrupt phototransduction, light adaptation, or behavior-specific responses. Almost all of the mutants are morphologically indistinguishable from WT, and many survive to adulthood. Genetic linkage mapping and initial molecular analyses show that our approach was effective in identifying genes with functions specific to the visual system. This collection of zebrafish behavioral mutants provides a novel resource for the study of normal vision and its genetic disorders.

Citation: Muto A, Orger MB, Wehman AM, Smear MC, Kay JN, et al. (2005) Forward genetic analysis of visual behavior in zebrafish. PLoS Genet 1(5): e66.

1. Pick a biological process
2. Pick an approriate model
3. Pick a method for inactivating genes
4. Figure out how to screen for mutants
5. Figure out how many different genes are represented in your collection of mutants
6. Study phenotypes
7. Study gene function at the molecular level


Gene
Molecular Biology
(cloning)
Genetics
(mutant phenotypes)
Protein
Function
Biochemistry
(in vitro work)


Mutants with defects in the process
define
genes with roles in the process
define
proteins that control the process
Function of specific
gene products in
controlling or
executing a complex
biological process

Biol 322 Fall 2012

Inspired Choices & The Power of Forward Genetic Analysis

Part 1: Due on Friday Nov 16 at noon
Part 2: Have your paper preapproved ASAP and no later than Friday Nov 16 at noon.
Part 3: Due on Monday Nov 26
Part 4: Student presentations during the last week of the quarter (Tues Dec 4 & Thurs Dec 6).

PART 1: (15 pts.) Identification of SLEEPLESS, a Sleep-Promoting Factor

Science 321: 372. 2008 See link under Required Reading.

Browse through *entire* article and carefully read **pg 372- 374 and examine figure 1 closely**.

Please word-process your answers to these questions. Unless otherwise indicated each answer should be *one-two complete sentences*.

1. What is the advantage (stated twice in the introduction of this article) of a forward genetic screen?
2. How many mutant candidates were screened and what general type of mutation did they carry?
3. Examine Figure 1a. What is the sleep phenotype of *sss* mutants? Be sure to state your answer in ***quantitative*** terms.
4. What is the difference between *sleepless (sss)* and SLEEPLESS (SSS)? In other words, what do the upper and lower case versions refer to?
5. How did the researchers establish that sleepless and quiver were alleles of the same gene? Site two different lines of evidence.
6. Is the sleepless gene likely to play a significant role in vertebrate sleep processes? Defend your answer in one sentence.

PART 2 Inspired Choices. Find an original research paper that uses a forward genetic screen strategy to identify genes that play roles in a specific biological process.

1. Your paper may be recently published or NOT.
2. As soon as you find a candidate paper for this part of the assignment, send CT a pdf file or a link to the paper or abstract. ***One student per a given paper.*** First come, first served. ***Your choice of paper must be preapproved! Do this no later than 10/13.***
3. You have free online access to Science, Nature, Cell, Genetics, Nature Genetics, PNAS, PLoS One, PLoS Genetics and many other journals via the Western Libraries. You can find your paper in a number of different ways:
    a. Go to PubMed and do a general search: http://www.ncbi.nlm.nih.gov/pubmed/

b. Go to a specific journal (such as Genetics) that you have online access to and search that journal
c. Google it!
d. If you search with the term *forward genetics* you won't find any older articles (pre 1990s?) since this jargon came into use only with the advent of reverse genetic technologies.
e. Be sure to pick a topic and research organism of interest especially to YOU.

PART 3 Written Work-up of your selected paper to CT

Submit the following by Mon Nov 26

a. Title of paper, author list (first and last only if there are more than 2 authors), name of journal, volume, page and year of publication
b. A short summary (several sentences) of the paper including the biological process under examination, the model organism used, how the researchers screened or selected for phenotype of interest, the scale of the screen (how hard did they look for mutants?), the mutagen or other technique used to generate mutations, the number of mutants discovered and ***especially any interesting biology that the researchers uncovered***.

PART 4: (15 pts.) 10-12 minute in-class presentation of your paper (Dec 4 & 6)

- Structure your talk around the guidelines for the homework assignment -- in other words, be sure to convey the biological process under investigation, the model organism chosen (and why), the phenotype screened (or selected for), what the researchers learned that was interesting, the scale of the screen, etc.
- *Work up a couple of slides WELL IN ADVANCE OF YOUR TALK and practice your talk on a friend or classmate to make sure it is coherent & of the appropriate length*
- *On Tues and Thurs Nov 27 & 29, the labs will be on the short side giving you extra time to work on your presentation*

A Forward-Genetic Screen and Dynamic Analysis of Lambda Phage Host-Dependencies Reveals an Extensive Interaction Network and a New Anti-Viral Strategy

Nathaniel D. Maynard[1], Elsa W. Birch[2], Jayodita C. Sanghvi[1], Lu Chen[1], Miriam V. Gutschow[1], Markus W. Covert[1]*

1 Department of Bioengineering, Stanford University, Palo Alto, California, United States of America, **2** Department of Chemical Engineering, Stanford University, Palo Alto, California, United States of America

Abstract

Latently infecting viruses are an important class of virus that plays a key role in viral evolution and human health. Here we report a genome-scale forward-genetics screen for host-dependencies of the latently-infecting bacteriophage lambda. This screen identified 57 *Escherichia coli* (*E. coli*) genes—over half of which have not been previously associated with infection—that when knocked out inhibited lambda phage's ability to replicate. Our results demonstrate a highly integrated network between lambda and its host, in striking contrast to the results from a similar screen using the lytic-only infecting T7 virus. We then measured the growth of *E. coli* under normal and infected conditions, using wild-type and knockout strains deficient in one of the identified host genes, and found that genes from the same pathway often exhibited similar growth dynamics. This observation, combined with further computational and experimental analysis, led us to identify a previously unannotated gene, *yneJ*, as a novel regulator of *lamB* gene expression. A surprising result of this work was the identification of two highly conserved pathways involved in tRNA thiolation—one pathway is required for efficient lambda replication, while the other has anti-viral properties inhibiting lambda replication. Based on our data, it appears that 2-thiouridine modification of $tRNA^{Glu}$, $tRNA^{Gln}$, and $tRNA^{Lys}$ is particularly important for the efficient production of infectious lambda phage particles.

Citation: Maynard ND, Birch EW, Sanghvi JC, Chen L, Gutschow MV, et al. (2010) A Forward-Genetic Screen and Dynamic Analysis of Lambda Phage Host-

A Forward Genetic Approach for Analyzing the Mechanism of Resistance to the Anti-Cancer Drug, 5-Fluorouracil, Using *Caenorhabditis elegans*

Seongseop Kim and Jaegal Shim*

Cancer Experimental Resources Branch, National Cancer Center, Goyang 411-769, Korea.

(Received August 27, 2007; Accepted September 13, 2007)

Pyrimidine antagonists including 5-Fluorouracil (5-FU) have been used in chemotherapy for cancer patients for over 40 years. 5-FU, especially, is a mainstay treatment for colorectal cancer. It is a pro-drug that is converted to the active drug via the nucleic acid biosynthetic pathway. The metabolites of 5-FU inhibit normal RNA and DNA function, and induce apoptosis of cancer cells. One of the major obstacles to successful chemotherapy is the resistance of cancer cells to anti-cancer drugs. Therefore, it is important to elucidate resistance mechanisms to improve the efficacy of chemotherapy. We have used *C. elegans* as a model system to investigate the mechanism of resistance to 5-FU, which induces germ cell death and inhibits larval development in *C. elegans*. We screened 5-FU resistant mutants no longer arrested as larvae by 5-FU. We obtained 18 mutants out of 72,000 F1 individuals screened, and mapped them into three complementation groups. We propose that *C. elegans* could be a useful model system for studying mechanisms of resistance to anti-cancer drugs.

FU-based chemotherapy improves the overall and disease-free survival of patients, but response rates as a first-line treatment for advanced colorectal cancer are not high. The combination of 5-FU with newer chemotherapies such as Irinotecan and Oxaliplatin has improved response rates for advanced colorectal cancer (Longley et al., 2003).

5-FU is a pro-drug converted to the active drug by metabolism. The efficacy of 5-FU differs depending on dosage and administration schedule. For example, 5-FU inhibits RNA function when it is administrated by bolus; when it is administrated by continuous infusion, it binds to thymidylate synthase and inhibits DNA synthesis. 5-FU is also bio-modified by other materials such as Leucovorin that increase its efficacy. Leucovorin slows the catabolism of 5-FU via the TS-FU-FdUMP complex (Rich et al., 2004). The major pathway by which 5-FU induces apoptosis of cancer cells is via the active metabolite of 5-FU, fluorodeoxyuridine monophosphate (FdUMP), which binds Thymidylate Synthase (TS) to form a stable ternary complex. TS converts dUMP to dTMP by methylation and regulates the balance of these two nucleic acid precursors.

Nicotine response genetics in the zebrafish

Andrew M. Petzold[a,b], Darius Balciunas[a,c], Sridhar Sivasubbu[a,d], Karl J. Clark[b], Victoria M. Bedell[b], Stephanie E. Westcot[a,b], Shelly R. Myers[a], Gary L. Moulder[b], Mark J. Thomas[a], and Stephen C. Ekker[a,b,1]

[a]University of Minnesota, Minneapolis, MN 55455; [b]Mayo Clinic, Rochester, MN 55905; [c]Temple University, Philadelphia, PA 19122; and [d]Institute of Genomics and Integrative Biology, Near Jubilee Hall, Mall Road, Delhi-110 007, India

Edited by John E. Dowling, Harvard University, Cambridge, MA, and approved September 16, 2009 (received for review July 23, 2009)

Tobacco use is predicted to result in over 1 billion deaths worldwide by the end of the 21st century. How genetic variation contributes to the observed differential predisposition in the human population to drug dependence is unknown. The zebrafish (*Danio rerio*) is an emerging vertebrate model system for understanding the genetics of behavior. We developed a nicotine behavioral assay in zebrafish and applied it in a forward genetic screen using gene-breaking transposon mutagenesis. We used this method to molecularly characterize *bdav*/cct8 and *hbog*/gabbr1.2 as mutations with altered nicotine response. Each have a single human ortholog, identifying two points for potential scientific, diagnostic, and drug development for nicotine biology and cessation therapeutics. We show this insertional method generates mutant alleles that are reversible through Cre-mediated recombination, representing a conditional mutation system for the zebrafish. The combination of this reporter-tagged insertional mutagen approach and zebrafish provides a powerful platform for a rich array of questions amenable to genetic-based scientific inquiry, including the basis of behavior, epigenetics, plasticity, stress, memory, and learning.

behavior | addiction

and Movies S1–S4) was measured using a digital imaging station in a time window where, 30 s following a stimulus of water, approximately 15% of a testing population moved (Fig. 1*A*, yellow framed images; yellow lines and bars in all graphs in Fig. 1; and Movie S1). Locomotor activity was assessed at different nicotine doses; movement as a function of added nicotine is shown in red bars and graphs in Fig. 1. Note the characteristic "inverted-U" shape of the dose-response curve (22), where higher doses of nicotine result in reduced, rather than increased, movement (Fig. 1*B*). The attenuation in locomotor effects at high doses is known to occur in rodents and depends on the particular complement of nicotinic acetylcholine receptor types activated by these doses (22). Based on this dose–response curve, we selected 10 μM nicotine for subsequent experiments (box, Fig. 1*B*). Next, we tested multiple developmental time-points, demonstrating a functional nicotine response in 4-day-old, but not 3-day-old, zebrafish (Fig. 1*C*).

One additional characteristic of the behavioral response to nicotine is sensitization, an increase in response upon prior exposure to nicotine; the sensitization response is shown in blue lines and bars in Fig. 1. As demonstrated in Fig. 1*A*, fish that had been previously exposed to nicotine became sensitized, yielding a

Identification of Host Proteins Required for HIV Infection Through a Functional Genomic Screen

Abraham L. Brass,[1,2] Derek M. Dykxhoorn,[3*] Yair Benita,[4*] Nan Yan,[3] Alan Engelman,[5] Ramnik J. Xavier,[2,4] Judy Lieberman,[3] Stephen J. Elledge[1†]

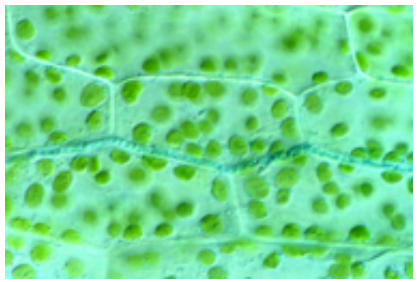
HIV-1 exploits multiple host proteins during infection. We performed a large-scale small interfering RNA screen to identify host factors required by HIV-1 and identified more than 250 HIV-dependency factors (HDFs). These proteins participate in a broad array of cellular functions and implicate new pathways in the viral life cycle. Further analysis revealed previously unknown roles for retrograde Golgi transport proteins (Rab6 and Vps53) in viral entry, a karyopherin (TNPO3) in viral integration, and the Mediator complex (Med28) in viral transcription. Transcriptional analysis revealed that HDF genes were enriched for high expression in immune cells, suggesting that viruses evolve in host cells that optimally perform the functions required for their life cycle. This effort illustrates the power with which RNA interference and forward genetics can be used to expose the dependencies of human pathogens such as HIV, and in so doing identify potential targets for therapy.

C.elegans RNAi lab at end of quarter

Forward Genetics: *You don't know what you don't know*

Flora Fungi Are Fickle Friends

By Elizabeth Pennisi*Science*NOW Daily News29 March 2006



Fungi can be a farmer's best friend or worst enemy. Some, such as plant rust and powdery mildew, can take down entire fields. Others, such as the Epichloë that lives between the cells of the ryegrass leaves, help the plant grow and reproduce. Now, researchers have discovered that free radicals can make the difference between friend and foe. The finding could help crop scientists develop ways make these fungi behave.

As a ryegrass leaf elongates, Epichloë sends out rootlike strands called hyphae that grow in parallel to the leaf's expanding columns of cells. Hyphae growth stalls when the leaf stops growing, allowing the two to live comfortably together from then on. Thanks to the fungus, the plant acquires more nutrients and can send out more roots, make more seeds, and survive drought better. To understand why Epichloë and ryegrass are such bosom buddies, Barry Scott, a plant ecologist at Massey University in Palmerston North, New Zealand, and colleagues made 220 Epichloë mutants. They found one that ruined the détente between the fungus and its host. This mutant's hyphae grew unchecked, stunting the growth of the ryegrass and eventually killing it.

The mutated gene codes for an enzyme that helps turn oxygen into free radicals, Scott and his colleagues report in the April issue of *The Plant Cell*. Normally, the ryegrass stimulates the fungus to produce these free radicals. Then these reactive molecules somehow inhibit fungal growth. But the mutant fungi don't make free radicals, greatly impairing the plant's ability to keep the fungus in line. Scott was surprised to find free radicals acting as regulators, because they are generally considered cell-killers that harm both plants and fungi.

"The results imply that [free radicals] may play much more subtle and precise roles in regulation than merely acting as cytotoxins," says Christopher Schardl, a plant physiologist at the University of Kentucky, Lexington. There are probably many genes involved in keeping the plants and their host fungi on friendly terms, and these findings "give some suggestions where researchers might look in other plant-fungal associations" for these genes,

ETHYLENE: A Gaseous Signal Molecule in Plants

Anthony B. Bleecker
Departments of Botany and Genetics, University of Wisconsin, Madison, Wisconsin 53706-1381; e-mail: bleecker@facstaff.wisc.edu

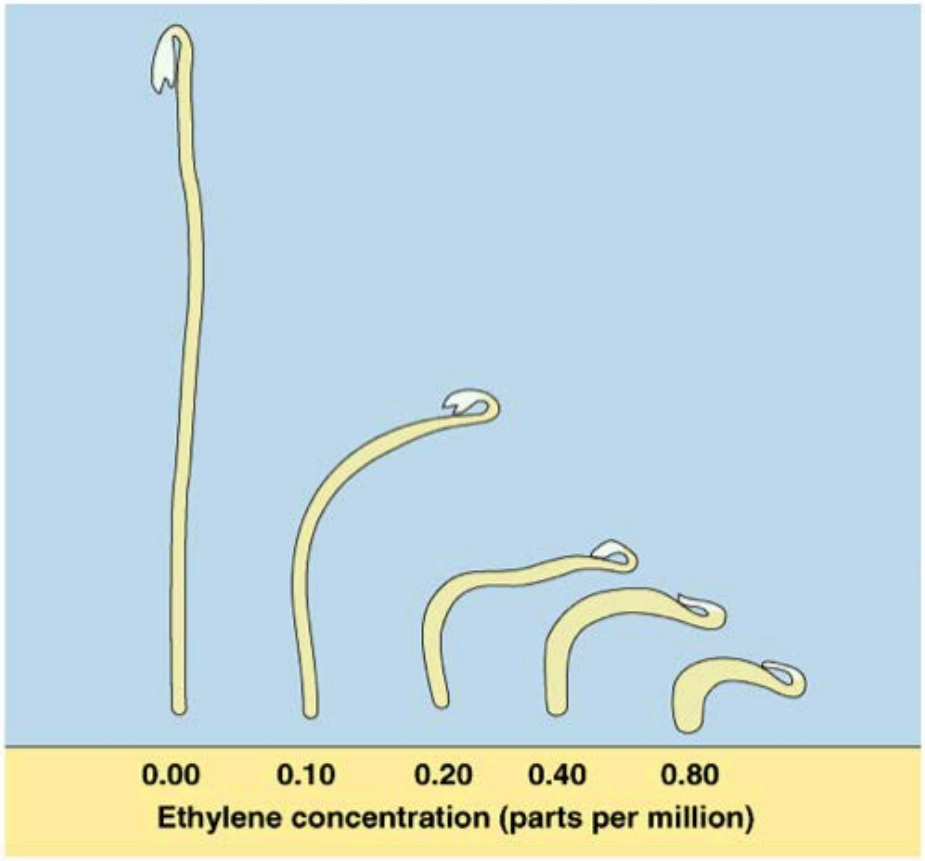
Hans Kende
MSU-DOE Plant Research Laboratory and Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824-1312; e-mail: hkende@msu.edu

Key Words 1-aminocyclopropane-1-carboxylic acid (ACC), *Arabidopsis thaliana*, ethylene receptors, Raf kinase, two-component system

■ **Abstract** Ethylene regulates a multitude of plant processes, ranging from seed germination to organ senescence. Of particular economic importance is the role of ethylene as an inducer of fruit ripening. Ethylene is synthesized from *S*-adenosyl- L-methionine via 1-aminocyclopropane-1-carboxylic acid (ACC). The enzymes catalyzing the two reactions in this pathway are ACC synthase and ACC oxidase. Environmental and endogenous signals regulate ethylene biosynthesis primarily through differential expression of ACC synthase genes. **Components of the ethylene signal transduction pathway have been identified by characterization of ethylene-response mutants in *Arabidopsis thaliana*.** One class of mutations, exemplified by *etr1*, led to the identification of the ethylene receptors, which turned out to be related to bacterial two-component signaling systems. Mutations that eliminate ethylene binding to the receptor yield a dominant, ***ethylene-insensitive phenotype***. *CTR1* encodes a Raf-like Ser/Thr protein kinase that acts downstream from the ethylene receptor and may be part of a MAP kinase cascade. ***Mutants in CTR1 exhibit a constitutive ethylene-response phenotype.*** Both the ethylene receptors and CTR1 are negative regulators of ethylene responses. ***EIN2 and EIN3 are epistatic to CTR1, and mutations in either gene lead to ethylene insensitivity.*** Whereas the function of EIN2 in ethylene transduction is not known, EIN3 is a putative transcription factor involved in regulating expression of ethylene-responsive genes. Biotechnological modifications of ethylene synthesis and of sensitivity to ethylene are promising methods to prevent spoilage of agricultural products such as fruits, whose ripening is induced by ethylene.

Ethylene

…promotes the "triple response",



…in etiolated seedlings,

- reduced stem elongation,
- thicker stem,
- horizontal growth,

- May provide the plant with "behavior" that will provide escape from soil impediments.