## **Hamlet on the Fly** 7 February 2018

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Hamlet on the Fly: To be or not to be a Foreleg?	One reason I chose the Hamlet theme:
( <del>-</del>	There will be lots of plot twists in the story.
Lewis Held	
Wed., February 7, 2018 4 PM, Biology Room 101	
Q: How do cells decide what to be? A: They make a series of binary choices	Humans have ~200 diff. cell types: skin, nerve, muscle, etc.
with "master" (Hox) genes in control.  Internal nodes  (Hox) = CEO	Q: How do cells adopt correct identities during dev't? A: ~BINARY (be/not be) decisions. Genes = ON/OFF.
	By the end of talk: show that this view is partly incorrect.
Terminal nodes From Rebeit et al. (2015) anatomy	
	Glorious diversity of animals on Earth.
	We normally think about animals as ADULTS.
	But every animal starts as a fertilized EGG.
And the second of the second o	That includes you.
	= Your earliest selfie!
	Fertilized egg of a human is so tiny
Annual Control of the	that it can fit on the head of a pin.
Con The Control of th	Can't do expts on humans, so use other animals instead.
	Flies = useful.
(5:2)	Fly dev't is interesting $b/c$ the baby looks nothing like adult.
	Larva = maggot. Analogous to tadpole. Metamorphosis.
	Adult (imago) body is assembled like a quilt (jigsaw puzzle)
	from individual pieces.
adult	Pieces = imaginal discs (≈ modules).
larva Duca Duca Duca Duca Duca Duca Duca Duc	They grow inside larva (≈ balloons) & evert.
Transmission of the control of the c	10 different identities.
Contain Contai	Discs (black ovals) come from diff. segments in the embryo.
adult	
embryo	6 head segments, 3 thoracic segments, & 10 abd. segments.
72	

segmental identity	Segmental identity is handled by Hox genes (clusters). Transcription factors w/ DNA-binding domain (homeobox).  Same area codes in humans & flies (common ancestor). Humans have genes that are homologs of Scr & Ubx.  Today: Focus on the 3 thoracic segments.
	Identities of T1 and T3 controlled by Hox genes Scr & Ubx.
bithorax mutant  Dibat	Many of you know the story of the "bi-thorax" mutation.  Disable Ubx: T3 → T2.  Haltere → wing.
	Hindleg also transforms → midleg.  Foreleg & hindleg differ from midleg.  Transverse rows = brushes to clean the eyes & wings.  Also foreleg has sex comb = grasps female during mating.
foreleg foreleg midleg Scr-null	If you disable Scr, T1 leg → T2 identity.  T-rows & sex comb disappear.  Defaults to T2 state just like Ubx-LOF.
GOF experiment	What happens if you force Scr to be expressed in all legs? Interesting b/c it confuses the cells → Reveal secrets? But backfired: confused me!
Excess Scr→ON	GOF (Gain of Function). T1: no change expected. Q: T2 & T3 → T1 identity?
Gal4 transception factor slave	Method: Gal4 & UAS transgenes.
ONLY DISTRIBUTE ONLY DISTRIBUT	Distal-less driver (master) with Scr "slave".
GALEGE GALEGE	UAS = Upstream Activating Sequence.  Additional details of the approach we used.
Sca Enhancer—Gas Gas Gas Gas Gas Gas Gas Gas Gas Gas	Gal80ts control timing via 18° vs. 29° C.
SCE Enhancer — GALAP GAL	Scabrous driver forces Scr to be expressed in bristle cells (& surrounding cells) only.

hindleg	Mug shots of hindleg basitarsus.
A/P	T-rows on ANT = expected.
new normal	But surprise: t-rows remain on POS!
	Confusing: Scr doesn't overrule Ubx?
	domasing. Ser doesn't overrule obx.
e f midleg	Midleg basitarsus: t-rows on both faces.
A/P	Midleg basitarsus. t-rows on both faces.
Pekus Property	Confusing: Scr normally only affects ANT.
c d	But evidently it can work on both POS & ANT.
Maybe Scr is able to induce transverse rows -anywhere?	We had assumed that Scr $\rightarrow$ foreleg identity.
	<b>RETHINK:</b> Maybe its role is to induce t-rows?
If so then extra Scr should also put I-rows on P side of foreleg?	And it just so happens to only be expressed in ANT.
foreleg	No! Scr-GOF hardly affects POS of foreleg at all!
A/P Very Scr	Why not? Maybe engrailed is suppressing Scr?
normal Few!	(Is that why Scr is normally confined to the ANT?)
Interioris enhancer  Upstream enhancer  Upstream enhancer  Upstream enhancer	Although Scr is strongly expressed in t-rows & sex comb.
	I had assumed Scr is activated only on the ANT side.
	[C = Scr expression (dots = nuclei); C' = GFP (all cells).]
hindleg A/P	Mug shots of hindleg basitarsus. T-rows on both faces.
hew normal P Scr Ubx	Easy to explain if Ubx $\rightarrow$ t-rows (like Scr $\rightarrow$ t-rows).
e	On a roll. But then hit brick wall.
midleg	Why isn't engrailed inhibiting Scr on the midleg?
AIP Ubx	We argue that it is doing so, but Ubx makes up for it.
c d	Ubx is expressed in T2??? Yes! At low levels.
Ferror Instruction	The reason Ubx is expressed in the POS half of T2?
	Initially, Scr, Ubx, etc. = expressed in "parasegments."
•	Segmentation gene hierarchy.
Phase Shift   Parasegmenta   1 2 5 4 5 7 8 9 10 11 12 13 14	Parasegments = offset from segments by engrailed stripe.
Segments   H4HEH4 T1  21 SIA   A2 A3 A4 A5 A6 A7 A6 A6  Phase Shift Somites(S) vs.	Enigma.
Sonites(S) vs. Vertebrae(V)	But humans also have phase shift: somites vs. vertebrae.

a Ubx	Parasegment 5 includes POS side of T2.
b PS4 PS5 PS6 I	
	Ubx fades from wing disc. (High level in t-rows on hindleg.)
	But retained at low level (light green) in POS of midleg.
	RECAP: Scr = partly suppressed by en, but enough remains
so when we force Scr to be expressed	for it to cooperate with low-level Ubx to elicit t-rows.  Low Scr + Low Ubx → Exceed threshhold.
on both sides, en partly suppresses Scr, but there might be enough Scr left to interact with Ubx so as to make t-rows on P side.  ?	If so, then Scr should interact synergistically with Ubx
	WHEREVER they overlap. So re-examine the hindleg.
	On the hindleg, Scr overlaps with HIGH-level Ubx.
Test: See whether See whether show re excess t-rows on the P side on the hindleg.	
erest upd.  Ubx  Ubx	Yes! Synergy! Normally POS = only 1 row of 8 bristles.
	This is an evol. conserved hard-wired trait (50 MY old).
	But notice that Scr-GOF elicits as many as 9 t-rows total!
Trotein ministry ministry ministry	Summary of quantitative data:
	Number of t-row bristles increases from 8 to 47 (mean).
20 P P P P P P P P P P P P P P P P P P P	Review: Effects of Scr-GOF on T1, T2, T3. A/P ratios!
b 10 groteleg 10 midleg himdleg	1.01.01.1 2.1.00.0 0.1.01 0.1.1.2, 1.2, 1.0.1.2, 1.1.0.00.
Conclusions:  1. En partly blocks Scr-GOF on POS of all legs.	1. Scr gets confined to ANT b/c En inhibits it.
1. Et party mucks ser dor on ros or an regs.  11 12 13  Foreign miller birdler	<b>Test:</b> en-LOF clones on P side of leg . <i>Done by Teresa.</i>
A P A P A P Scr Scr Scr	
en en en	
Conclusions:	2. Scr and Ubx interact synergistically.
1. En partly blocks Scr-GOF on POS of all legs. 2. Ubx makes up for this decrease on T2 & T3.  11  12  13  14  15  16  17  17  18  18  18  19  19  10  10  10  10  10  10  10  10	<b>Test:</b> Express both together.
foreleg midleg hindleg A P A P A P Scr Scr Ubx Scr Ubx	
en en en	
Conclusions	3. Hox genes (at late stages) ≈ paintbrushes for touch-up?
Conclusions:  1. En partly blocks Scr-GOF on POS of all legs.  2. Ubx makes up for this decrease on T2 & T3.	<b>Test:</b> Disable Scr at late stages (sca-GAL4).
3. Scr = a direct micromanager vs. an executive.  Internal nodes  Scr = thou genes get	DIRECTLY affecting downstream decisions.
down in the weeds".	Leaving penthouse suite @ Trump Tower → assembly line.
Terminal nodes From Rebeiz et al. (2015)	

Hox 13	Similar thing in vertebrates. Late role ≠ early role.
Top, 10	Re-purposing of Hox genes for non-segment roles.
Rocine  1 The Administration of Control of C	Ex.: Recruiting of Hox genes for finger identity
	Mutate Hox13 (d cluster) → "bear claw" phenotype.
Individuation Dissociation Homeosis  a Dissociation Homeosis  C C Place Case  Phace Case	Thumb transforms into an ordinary finger.
Conclusions:  1. En partly blocks Scr-GOF on POS of all legs.	4. Scr acts as a knob (analog) vs. switch (digital). Ubx also.
2. Ubx makes up for this decrease on T2 & T3.  3. Scr = a direct micromanager vs. an executive.  4. Scr acts as a knob (analog) vs. switch (digital).	Best example of dosage effect = tibia in 1st leg of Scr-GOF.
Excess Scr-GOF Normal Scr	Excess Scr causes additional t-rows (10 vs. 6) on foreleg.
Hamlet By William Shakespeare By William Shakespeare By William Shakespeare	Given these dosage effects, title → Measure for Measure?
by William Shakespeare Measure Measure	
Hamlet By William Shakespeare William Shakespeare	Or given that we've demoted these exalted Hox genes
MAZZONI GRANINA	to the level of functioning of ordinary transcription factors,
The same of the sa	Maybe the title of talk → Much Ado About Nothing
Time's fun when you're having fliest	Acknowledgements: 3 undergrad apprentices:
	Andrew Davis, Rachel Aybar, and Zack Fitzgerald.
Acharo Medigariani : - Andrew Davis Rachel Aybar Rachel Priggrand	Teresa Orenic did the staining of discs for Scr expression.
Scr-GOF Control	Enigmatic result. Scr-GOF.
	Deleted macro- but not microchaetes.
Marine de constantes	Cummany of LOE offsats
A State of S	Summary of LOF effects.
Time -	Time course: disable Scr.
discount of open is admit a discount parameter to 27 Card	

Relevance?	
Pitr1-GOF Pitr1-LOF  Listenburg Plumaril   ≈ horse  Homeosis	Pitx1-LOF ≈ Liebenberg Syndrome.
Evo-Devo  E 6 D	Qs → Evo-devo (E & D).  G = Genetics.  Playground.
Portis A. Prokaj	
Flyman?  Fly Cell Language:  Hedgehog  Yor - beta  EGR  Notch  Human Cell Language:  Hedgehog  Win  TGF-beta  EGR  For Porton	Same signaling pathways!  DEEP homology.  Conserved TOOLKIT.
Campaig (2000) HTC Creduct  Link Ave Creduct  Shared  Campaig (2000) HTC Creduct  Link Ave Creduct  Campaig (2000) HTC Creduct  Link Ave Creduct  Campaig (2000) HTC Creduct  Link Ave Creduct	Fly legs vs. human legs.  Look diff., but same recipe!  [Gerard Campbell]
Scabrous Driver	
BLOC COME	