The Malaria-Proof Mosquito

Genetic Engineering with Massive Potential

Pooja Desai
December 2nd, 2010
Genomics & Medicine
Professor Douglas Brutlag

Anopheles Stephensi Female

Time needed for parasite to develop

+

Low life expectancy of mosquitos

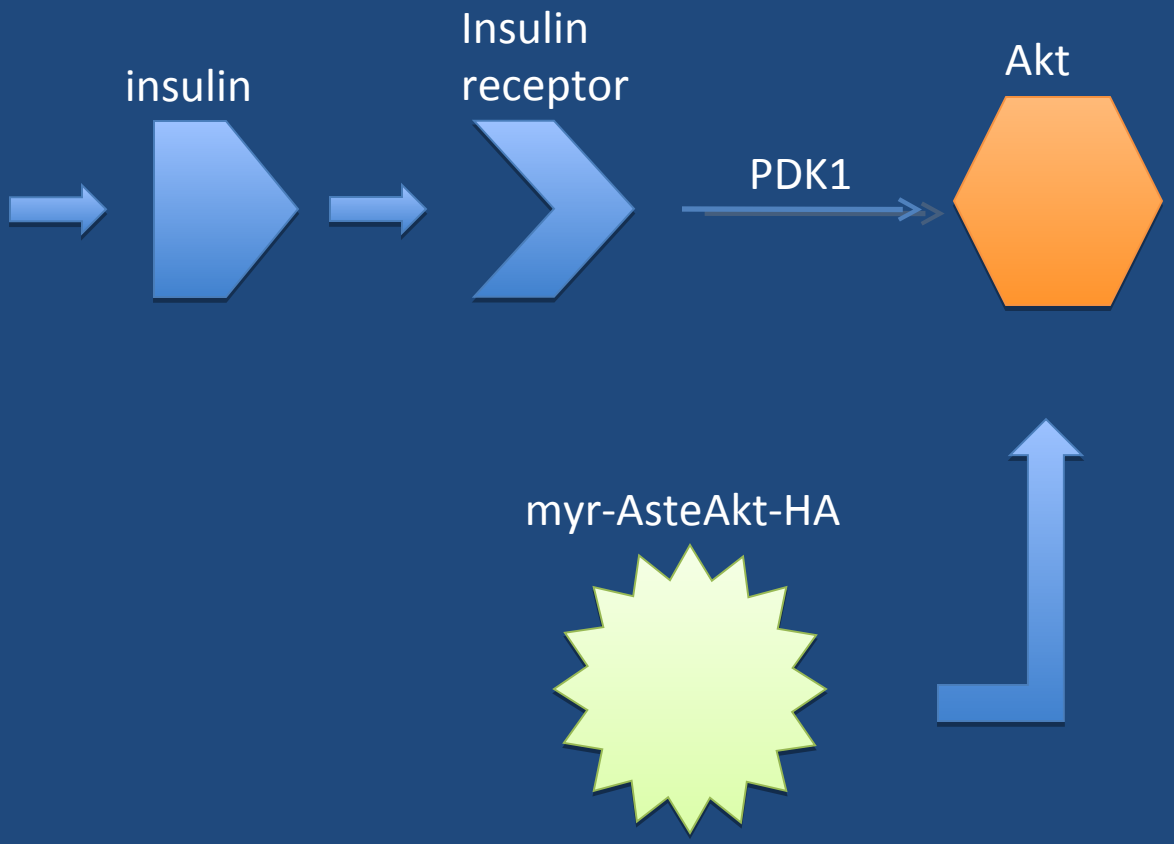
=

Only the oldest mosquitos are capable of infection

⇧Michael A. Riehle's light bulb!

IIS Cascade

Linked to innate immunity and lifespan

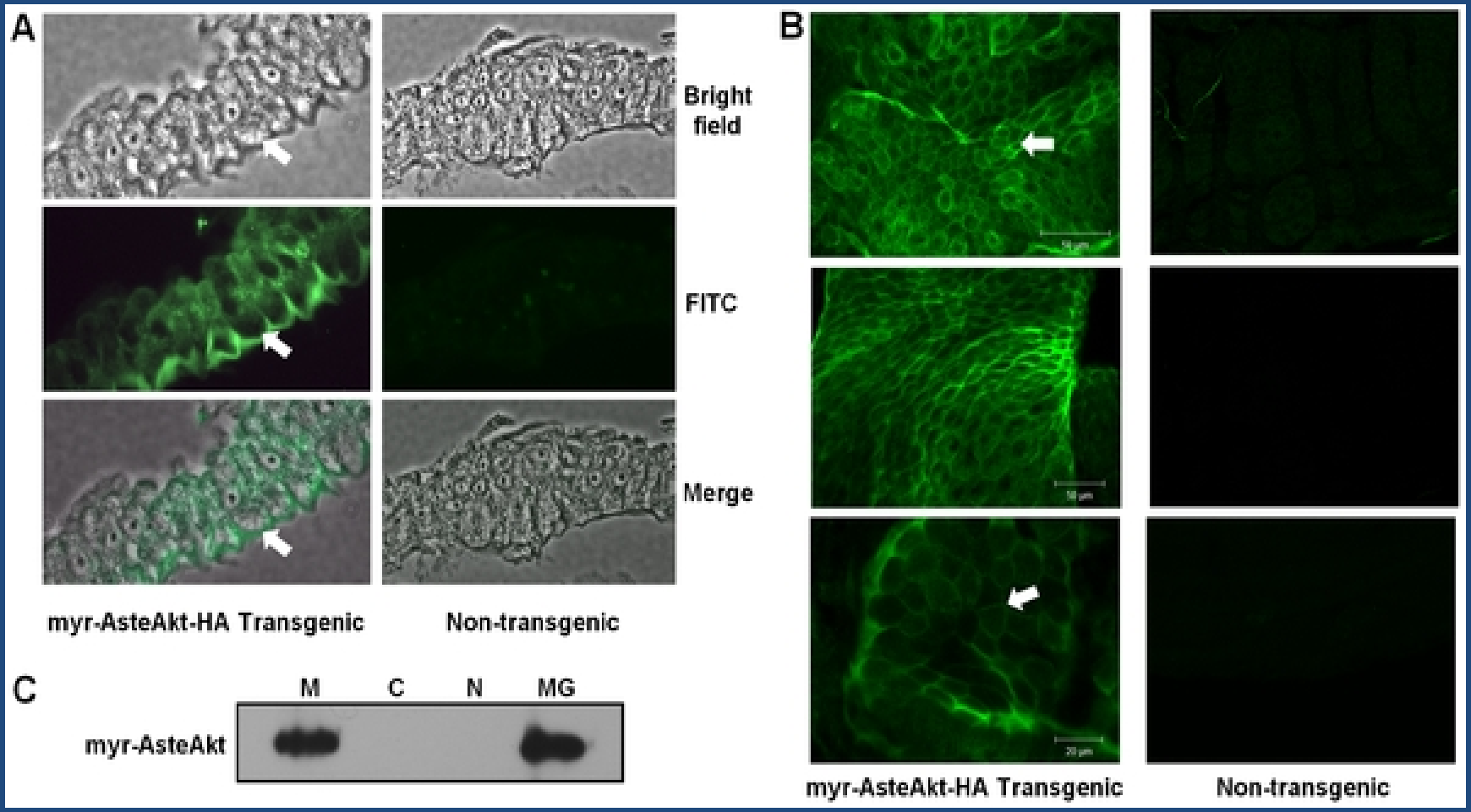


Generation of CP-Myr-AsteAkt-HA

- 4 Parts:
- 5’ CP promotor - XhoI and NotI sites
- SV40 3’ UTR- EcoRI site
- Kozak consensus sequence and Src myristoylation sequence - 5’ end
- HA epitope – 3’ end

> CP-myr-AsteAkt-HA-SV40 into plasmid and then into embryos!

Membrane Akt Visualization

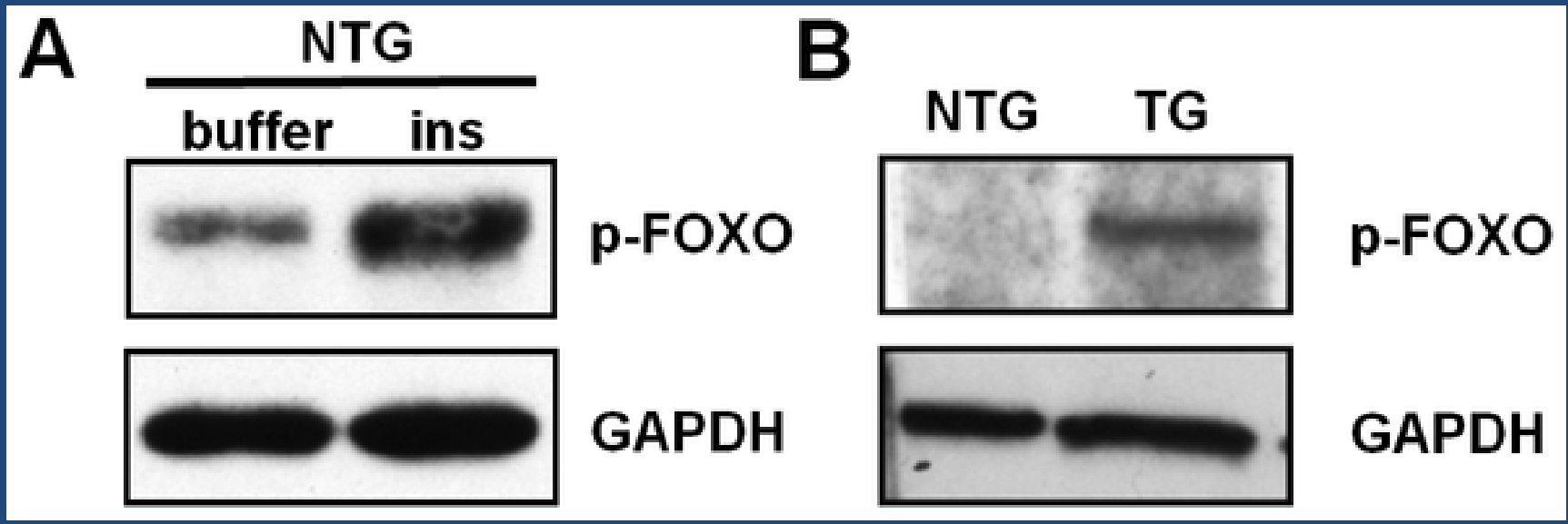


Methodology

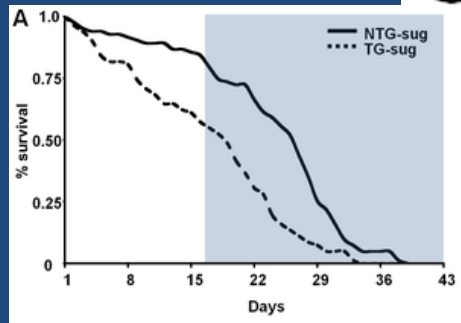
- Activated Akt by a myristoylation sequence encoded at the amino terminus
- Insert into pBac[3XP3-DsRedafm]
- 4400 embryos → 176 adult mosquitos
  - → 3 with stable Ds red eye fluorescence
  - → **F1 generation!**

Levels of FOXO1

- FOXO1= key transcription factor in IIS
- Insulin + myr-AsteAkt-HA

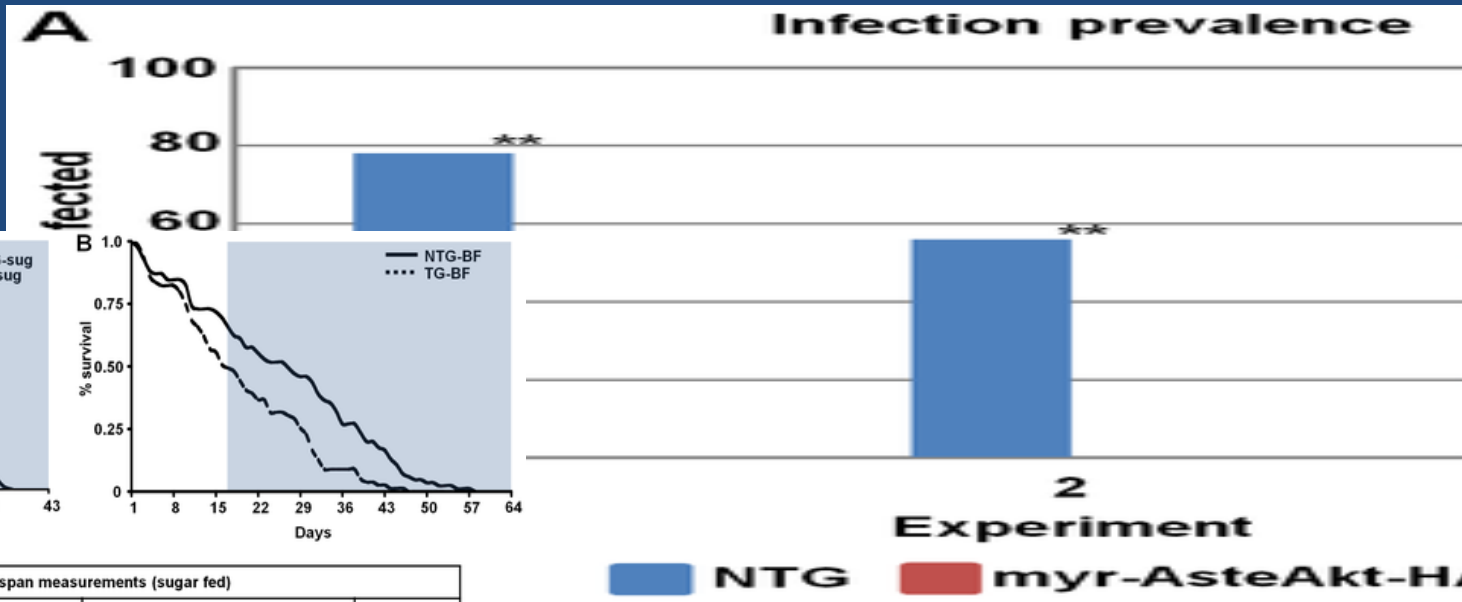


Infection Prevalence & Lifespan



C

Lifespan measurements (sugar fed)							
	Non-transgenic			myr-AsteAkt-HA			Wilcoxon
Exp	N	Median	Mean±SEM	N	Median	Mean±SEM	*p*
1	104	24	22.85±0.99	112	20	19.11±0.62	0.0001
2	83	27	24.04±0.96	82	19	17.16±0.97	0.0001
3	285	24	22.17±0.49	313	21	20.29±0.38	<0.0001
Lifespan measurements (blood fed)							
1	88	18	20.27±1.36	99	16	16.54±1.08	0.0482
2	83	27	25.75±1.67	79	17	19.24±1.31	0.0058
3	488	17	17.94±0.41	533	16	16.54±0.38	0.0111



Oocyst counts

-transgenic		myr-AsteAkt-	
Mean ± SEM	N	Mean ± S	
11.900 ± 2.182	50	0 ± 0	
2.900 ± 0.820	15	0 ± 0	
4.900 ± 1.474	25	0 ± 0	
6.567 ± 1.492	90	0 ± 0	

2	50	
3	50	4.900 ± 1.474
total	150	6.567 ± 1.492

Expression analysis of the myr-AsteAkt-HA transcript and protein

- RNA extracted using Rneasykit
- Treated with DNAse and cDNA synthesized
- PCR amplified
- Myr-AsteAKT-HA-specific primers used to detect myr-AsteAKT-HA in midguts and carcasses.

Summary of Results

- Generation of an A. stephensi line with overactivated Akt
- 20% reduction in lifespan
- Intensity of infection reduced by 95%
  - Complete block of infection in homozygous generation

- **Think about it! All this only from the activation of a single protein!**

References

- http://www.plospathogens.org/article/info%3Ado
- http://www.plospathogens.org/article/info%3Ado
- http://cals.arizona.edu/ento/faculty/riehle/index.htm