

Chicken galectin-1A and -8 constitute a regulatory network that mediates the earliest steps of avian limb skeletal morphogenesis

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By

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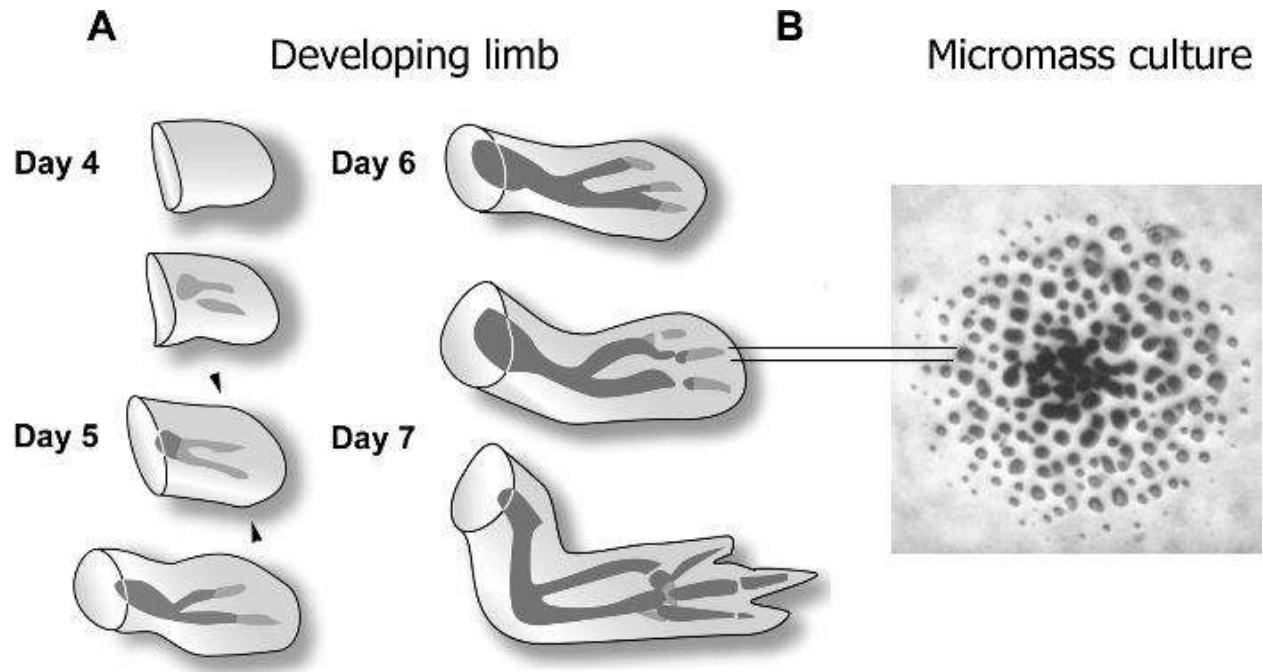


Chicken galectin-1A and -8 constitute a regulatory network that mediates the earliest steps of avian limb skeletal morphogenesis

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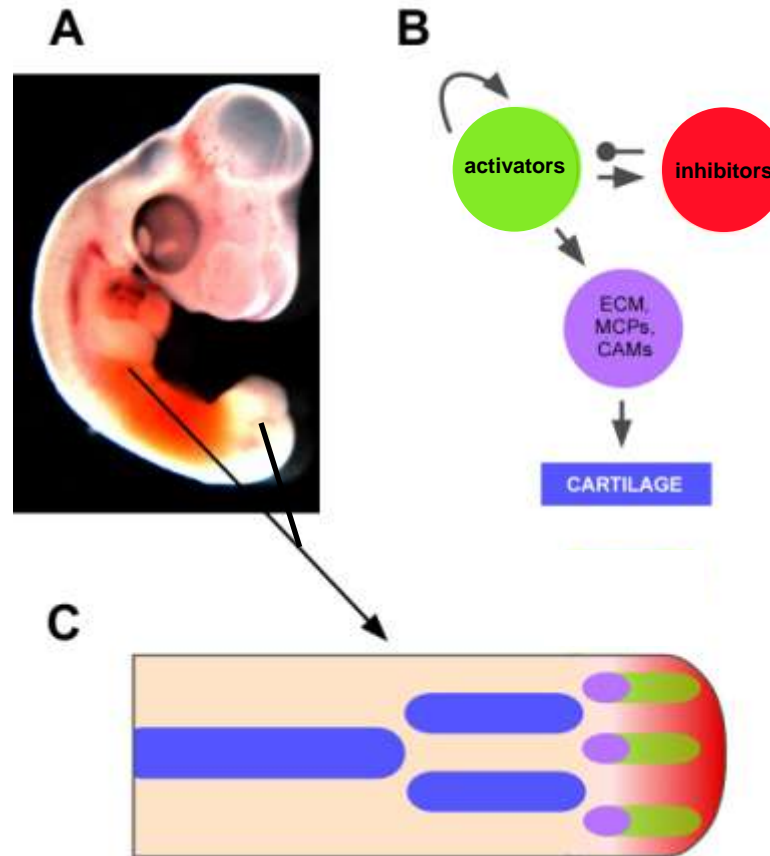
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Department of Cell Biology and Anatomy
New York Medical College

Pattern formation of the limb skeleton has a similar time course and spatial scale *in vivo* and *in vitro*



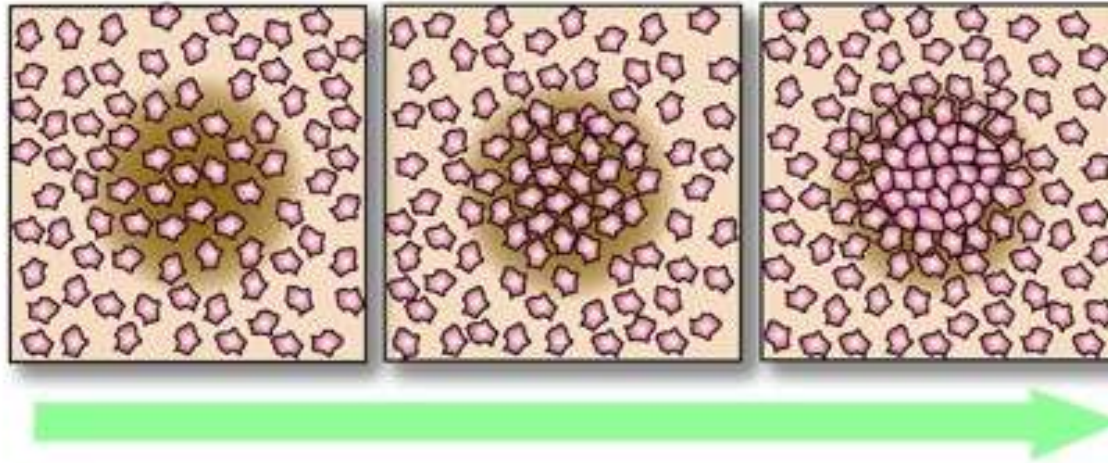
Christley et al., *PLoS Comput. Biol.*, 2007

Limb cartilage patterning results from interplay between activators and inhibitors of precartilage condensation



Based on Hentschel et al., *Proc. R. Soc. B*, 2004;
Newman & Bhat, *Birth Defects Res, Embryo Today*, 2007

Mesenchymal condensation morphogenesis



Forgacs & Newman, *Biological Physics of the Developing Embryo*, 2005

Cell-cell adhesion:

NCAM (Widelitz et al., 1993)

N-cadherin (Oberlander and Tuan, 1994)

Cell-ECM interaction:

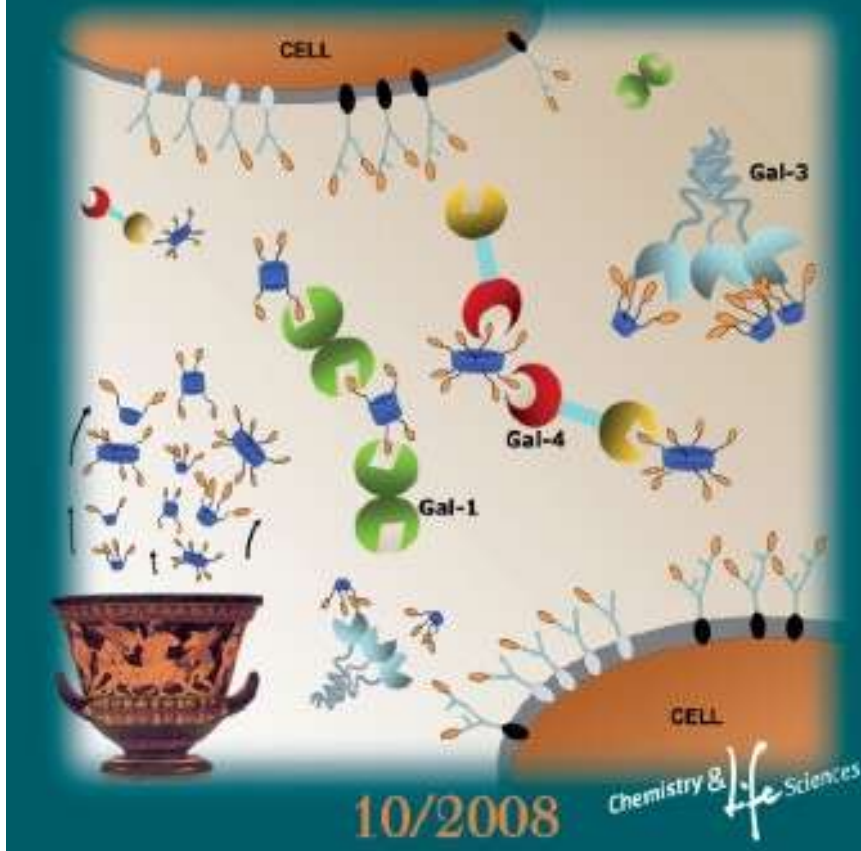
Fibronectin (Frenz et al., 1989)

Tenascin (Mackie et al., 1987)

A EUROPEAN JOURNAL

CHEM **BIO**CHEM

OF CHEMICAL BIOLOGY



- Galectins: most ubiquitous class of lectins

- Galectins are present in all metazoans and in plants, fungi and some bacteria

- They have specific binding affinity towards β -galactose-containing glycans and specific proteins

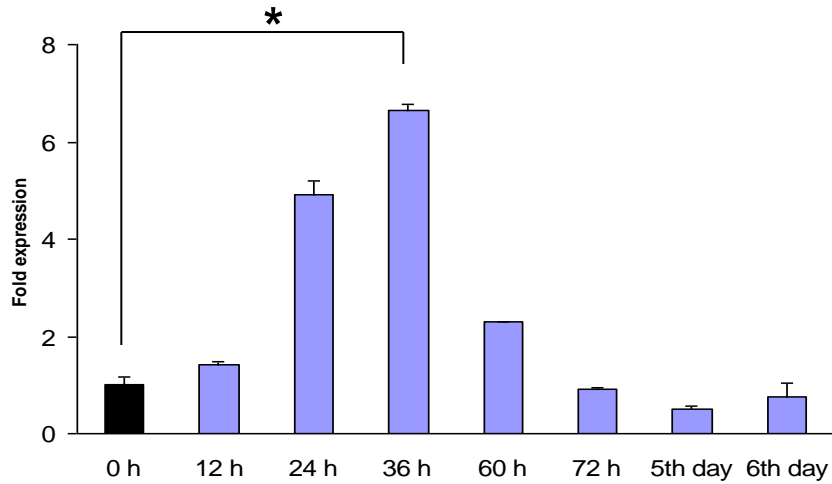
- They are trafficked to the extracellular milieu via the unconventional secretion pathway



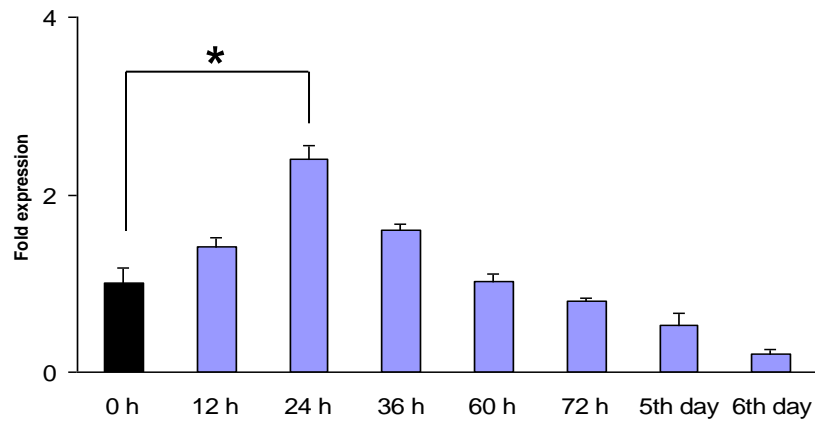
Are galectins expressed within condensing mesenchymal cells?

Galectin expression in vitro

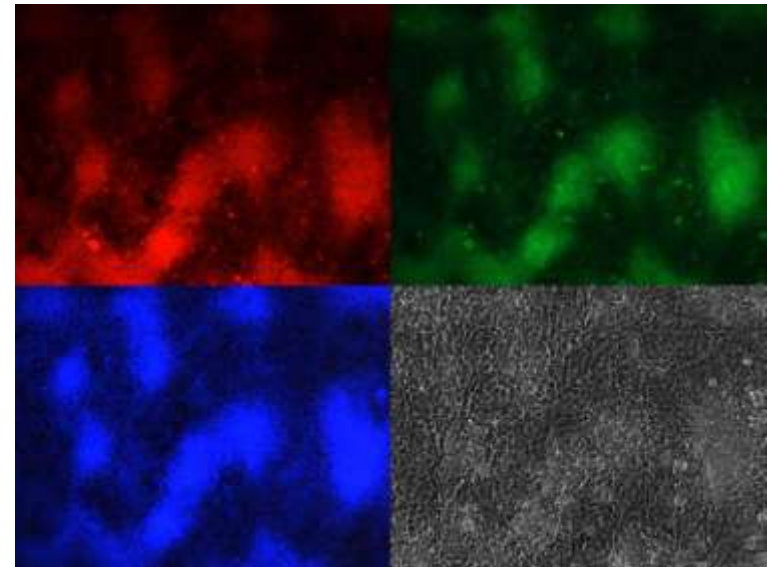
Temporal profile of CG-1A expression in vitro



Temporal profile of CG-8 expression in vitro



Galectin protein expression in 2 day micromass cultures

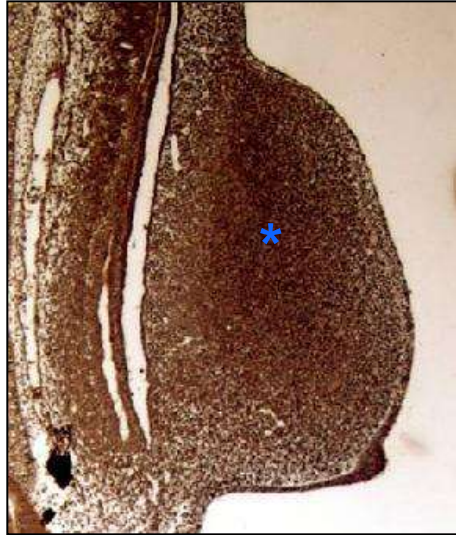


Red: CG-1A

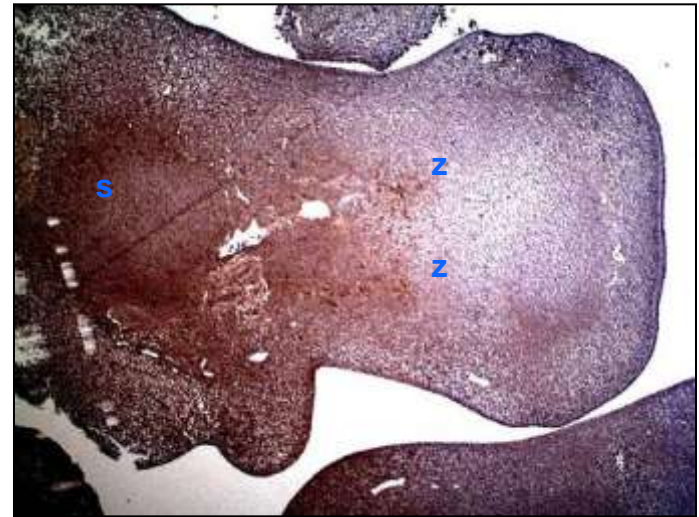
Green: Peanut agglutinin

Blue: DAPI

Galectin expression in vivo



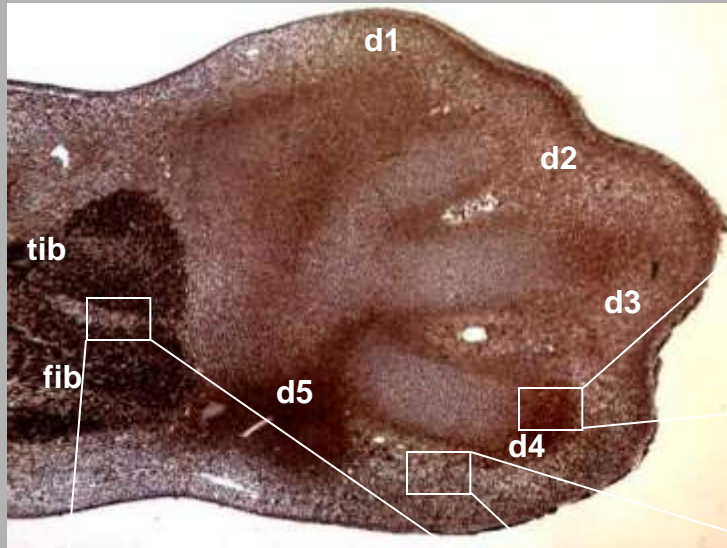
4 day leg section: CG-1A staining prospective stylopod (*)



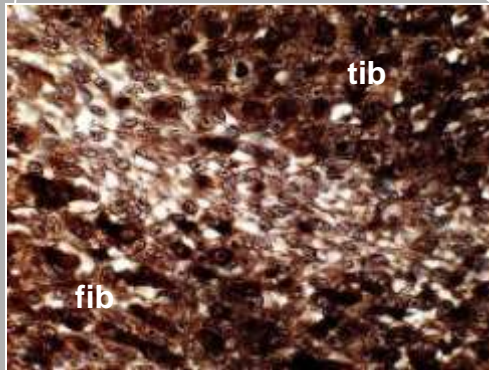
5 day leg section: CG-1A stains prospective zeugopod (z) and stylopod perichondrium (s)

These results also hold for CG-8

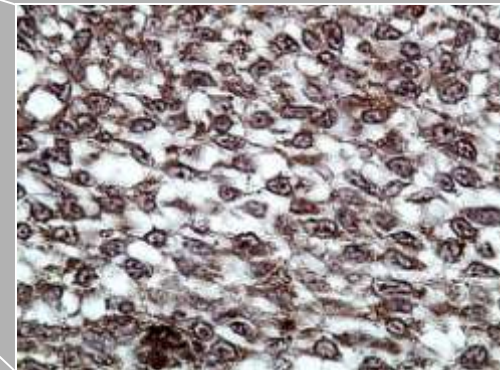
Galectin expression in vivo



Staining within digit crescents strong and extracellular



Staining strong in zeugopod elements and weak between

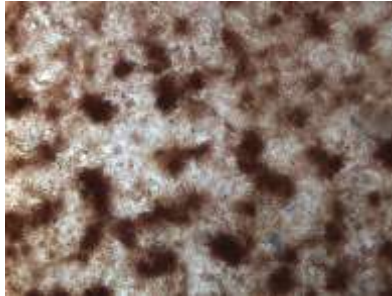


Staining sparse within inter-digit and interstitial mesenchyme

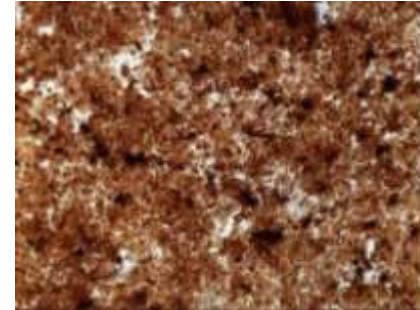


Do galectins mediate condensation formation and patterning?

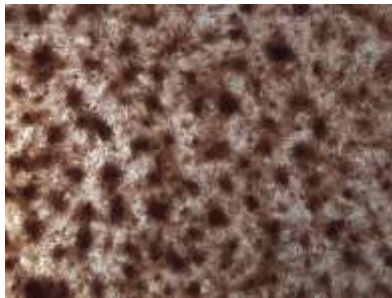
Treatment of cultures with exogenous CG-1A



day 2 untreated control

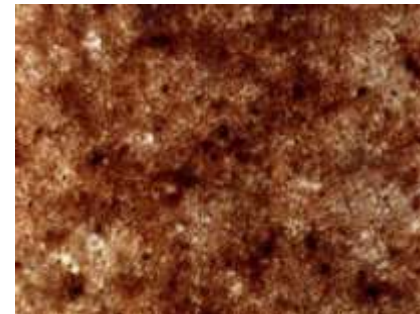


10% FBS



5 µg/ml CG-1A

[increase in condensation
number, decrease in size]



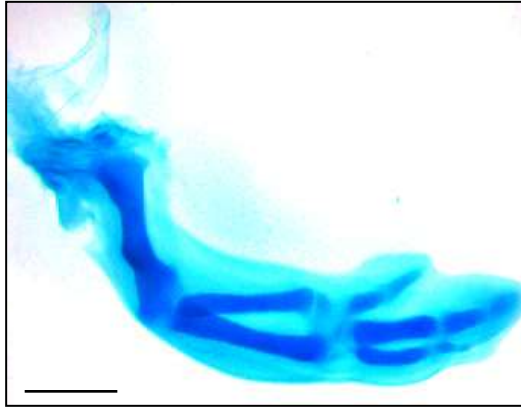
25 µg/ml CG-1A

[fusion of all condensations]

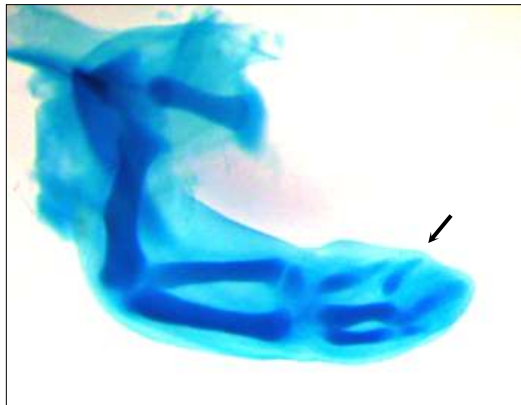
Staining for condensation marker PNA

Treatment of chick embryonic wings with exogenous CG-1A

Injection in
interdigital mesenchyme

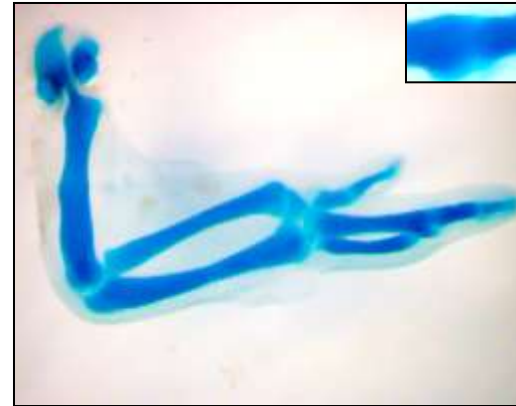


Control wing

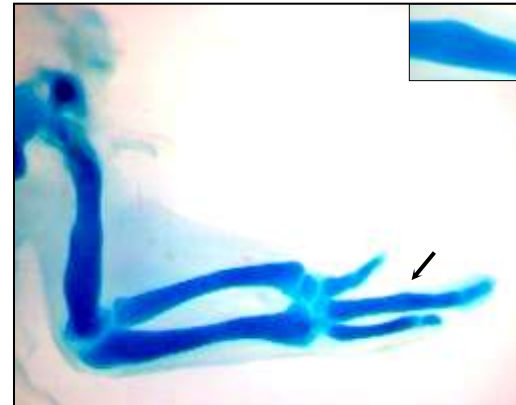


Ectopic digit primordium

Injection in
digital mesenchyme



Control wing



Fusion of phalanges and metacarpals

Treatment of cultures with exogenous CG-8



Day 2 control



20 µg/ml CG-8

[decrease in condensation number
decrease in condensation size]

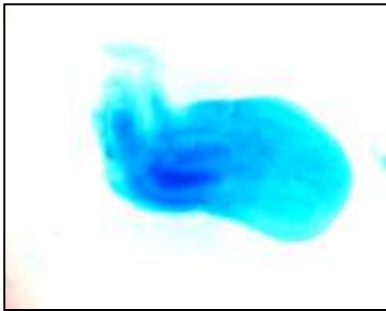
Staining for condensation marker PNA

Treatment of chick embryonic wings with exogenous CG-8

Injection on 5th day
Fixation on 7th day



counterpart
control
wings



Shortening of ulna,
absence of digits

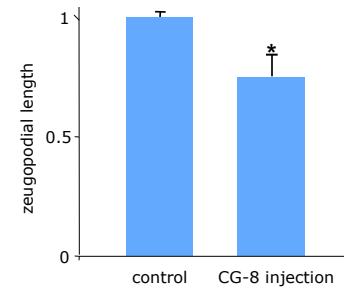
Injection on 5th day
Fixation on 8th day



CG-8
injected
wings

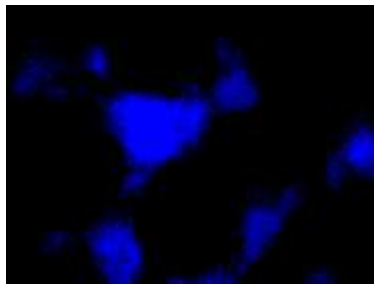
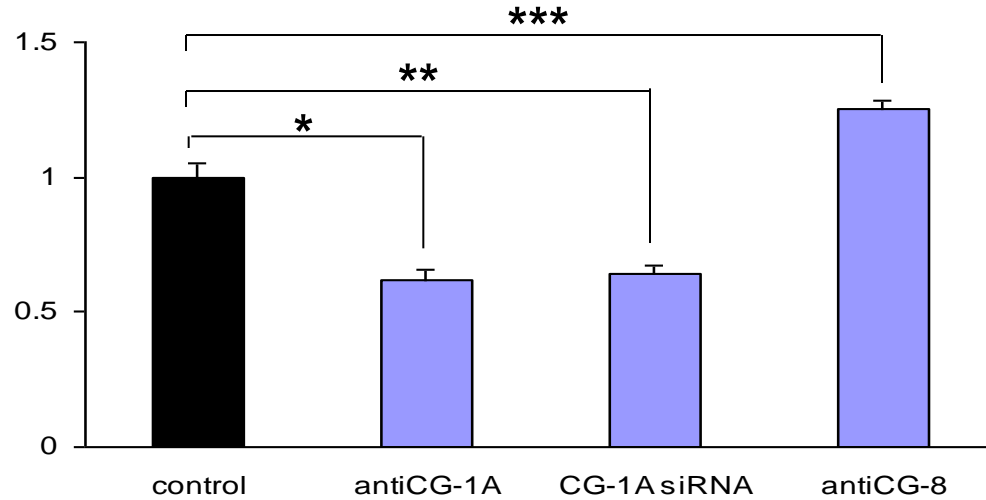


Shortening of
radius and ulna

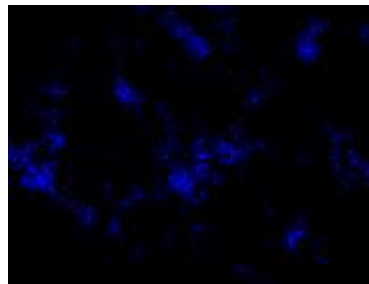


Effect of galectin inhibition in vitro

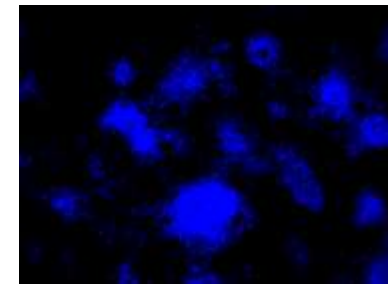
Effect on condensation number



Control 2d



anti-CG-1A antibody

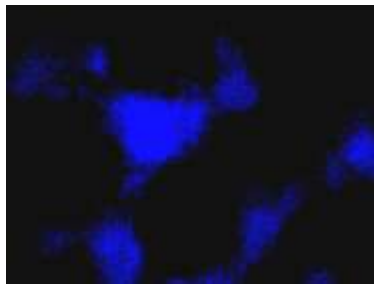
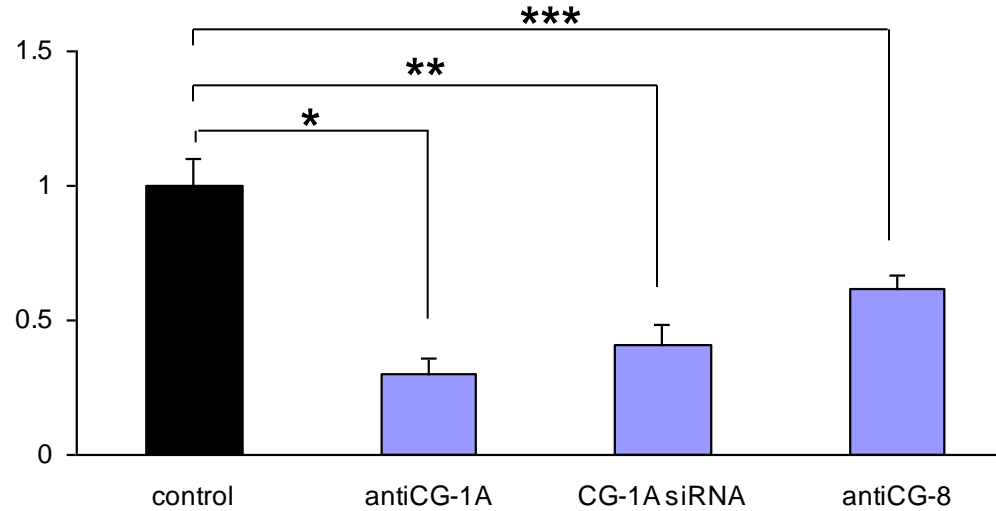


anti-CG-8 antibody

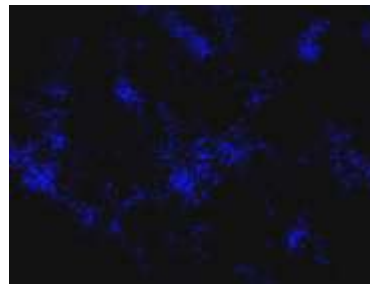
Staining with DAPI under low magnification

Effect of galectin inhibition in vitro

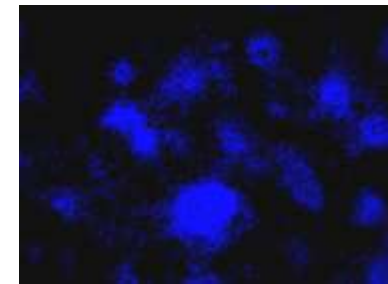
Effect on condensation size



Control 2d



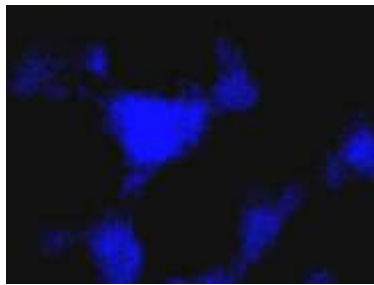
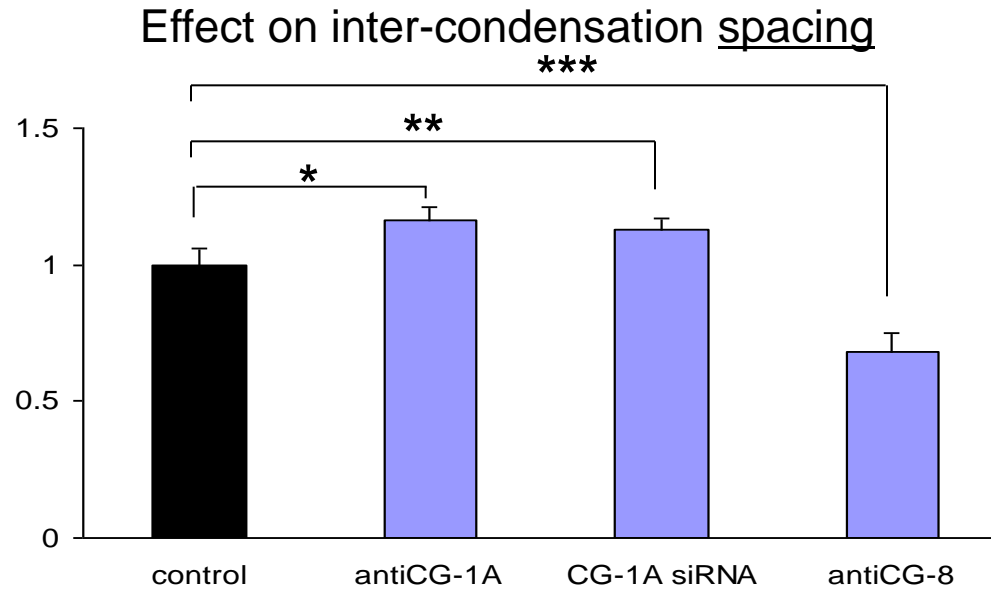
anti-CG-1A antibody



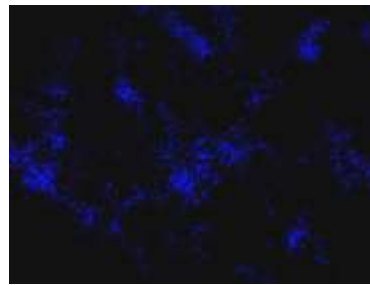
anti-CG-8 antibody

Staining with DAPI under low magnification

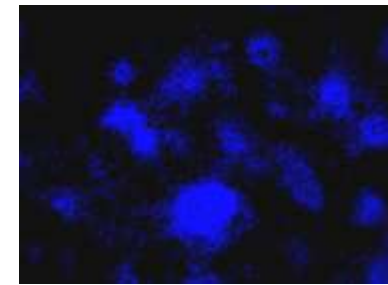
Effect of galectin inhibition in vitro



Control 2d



anti-CG-1A antibody



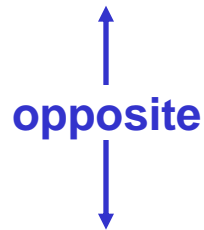
anti-CG-8 antibody

Staining with DAPI under low magnification

Condensation patterning dynamics

more condensations
less inter-condensation spacing

CG-1A addition phenotype

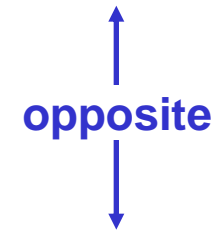


CG-1A inhibition phenotype

less condensations
more inter-condensation spacing

more condensations
less inter-condensation spacing

CG-8 inhibition phenotype



CG-8 addition phenotype

less condensations
more inter-condensation spacing

Condensation patterning dynamics

more condensations
less inter-condensation spacing

CG-1A addition phenotype

← **similar** →

more condensations
less inter-condensation spacing

CG-8 inhibition phenotype

CG-1A inhibition phenotype

← **similar** →

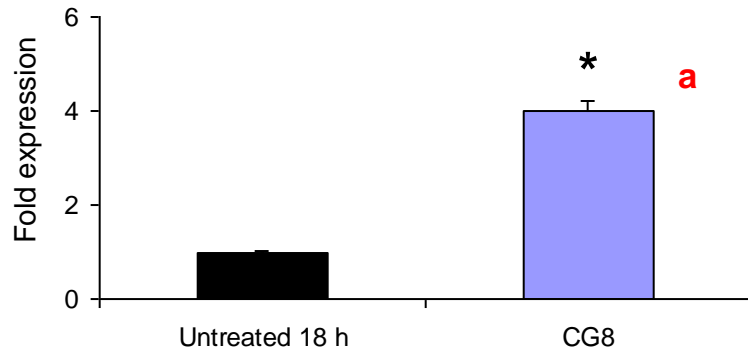
CG-8 addition phenotype

less condensations
more inter-condensation spacing

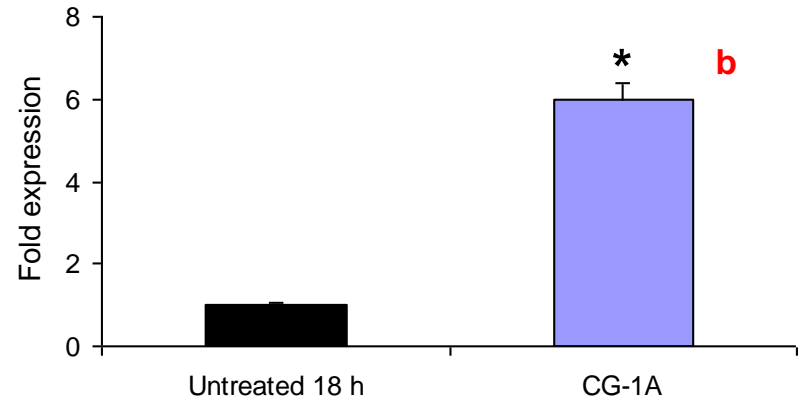
less condensations
more inter-condensation spacing

CG-1A and CG-8 gene expression loop

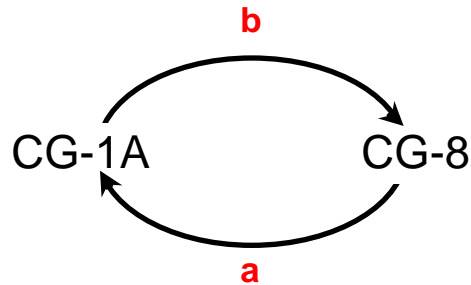
CG-1A gene expression upon addition of chicken galectins



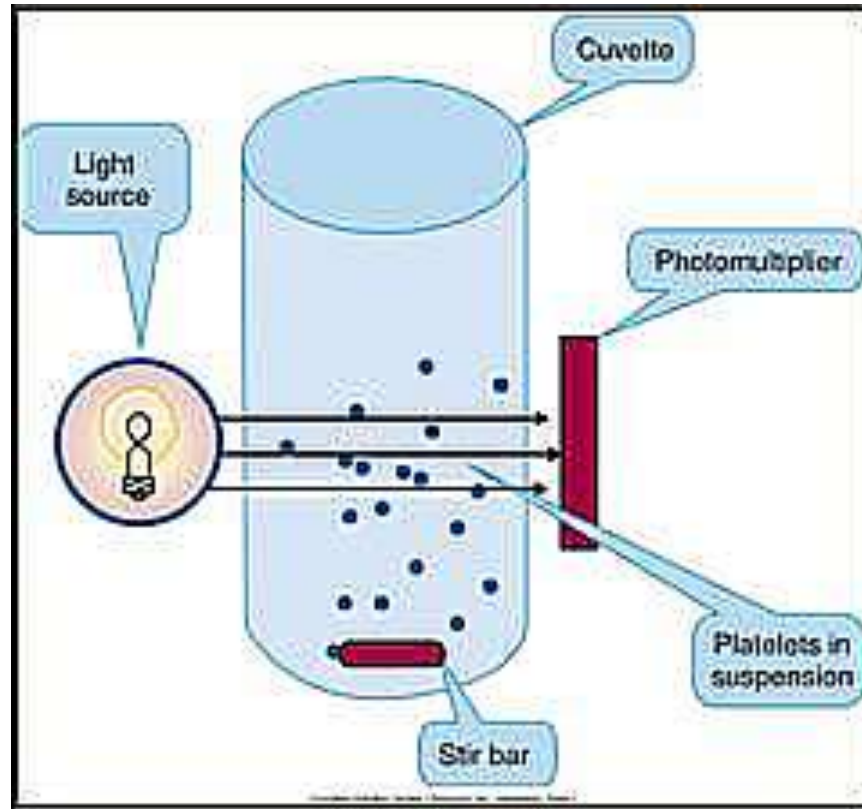
CG-8 gene expression upon addition of chicken galectins



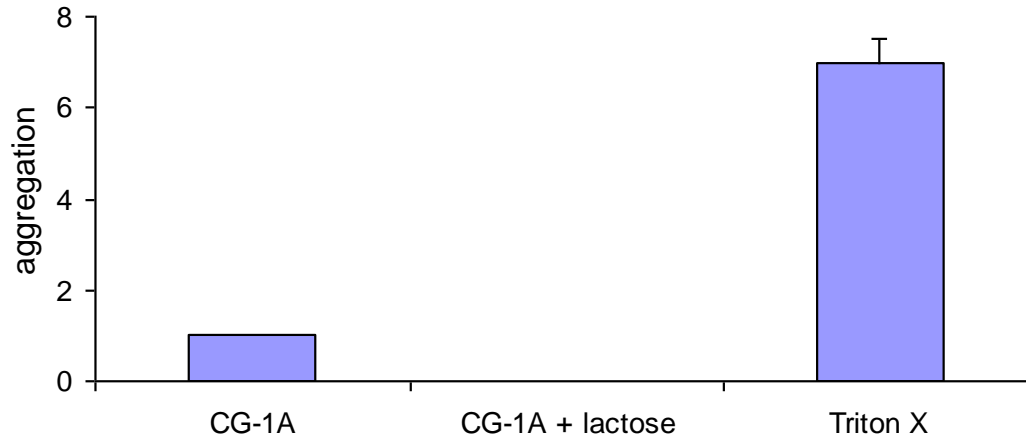
Interaction feedbacks between CG-1A and CG-8



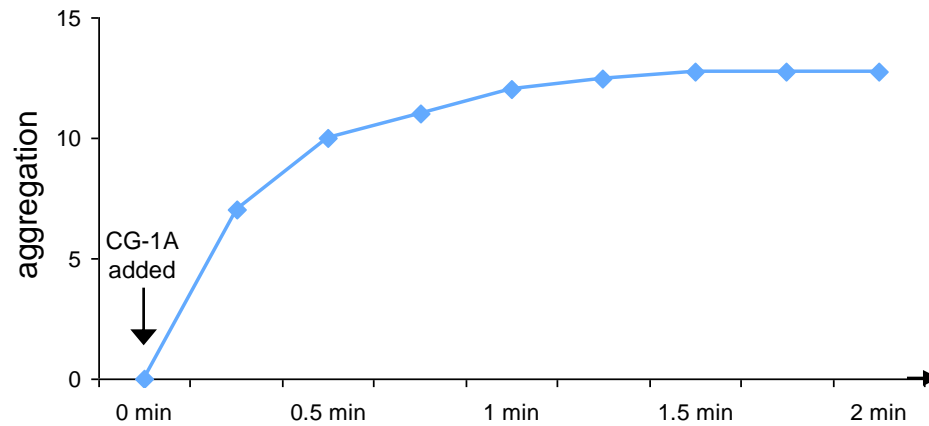
Aggregometry



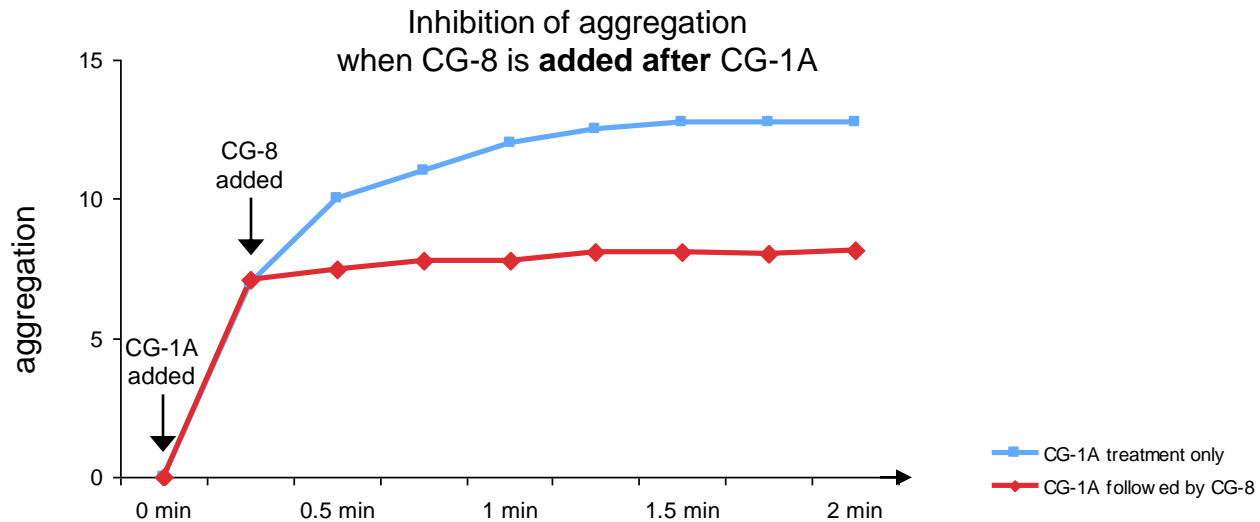
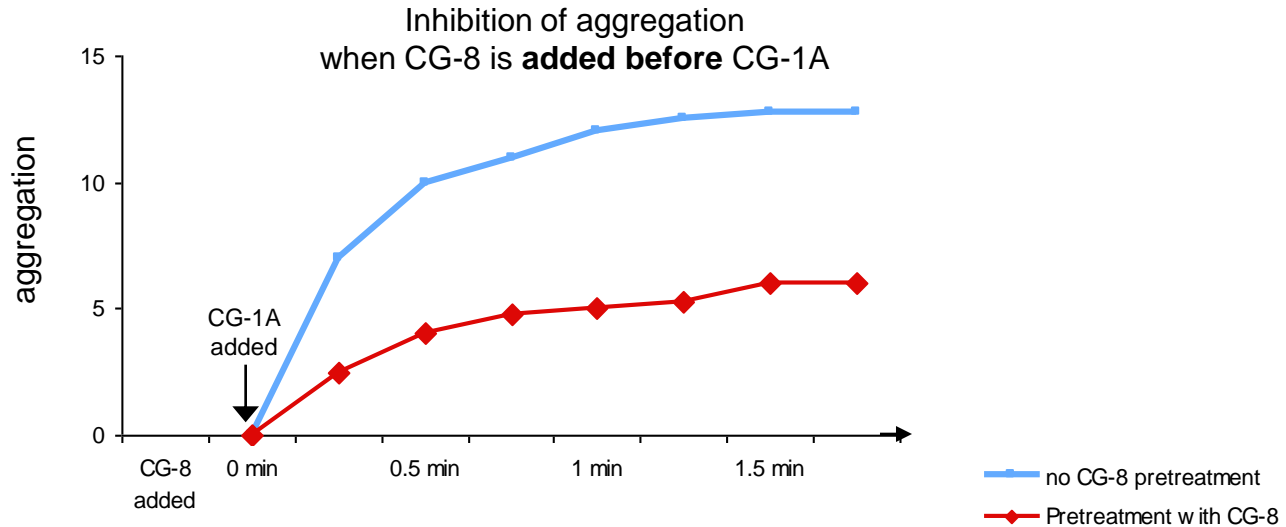
CG-1A directly aggregates 5-day leg cells



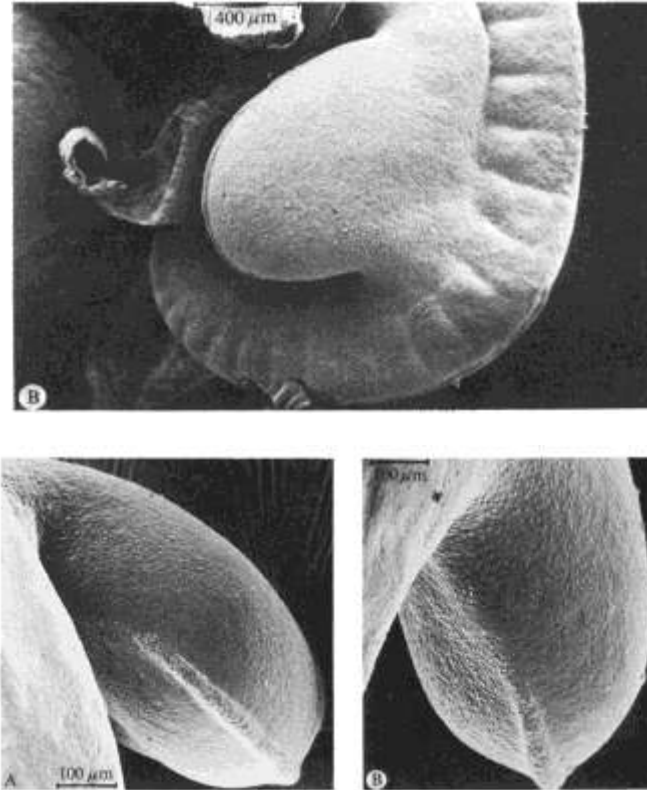
CG-1A causes aggregation of leg cells within 2 mins



CG-8 inhibits the ability of CG-1A to aggregate cells

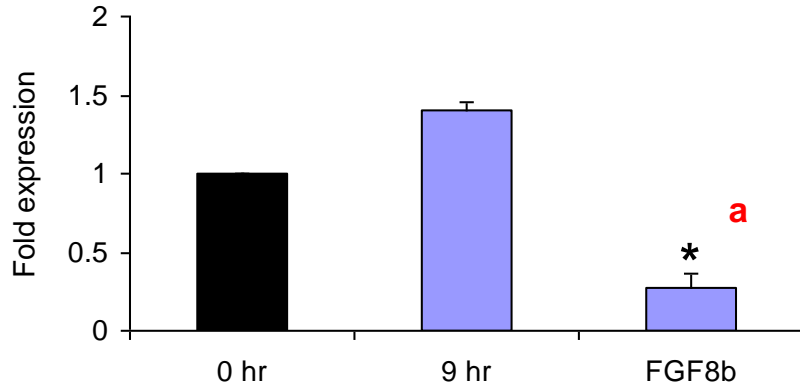


Apical ectodermal ridge (AER) and dorsal and ventral limb ectoderm and are sources of FGF

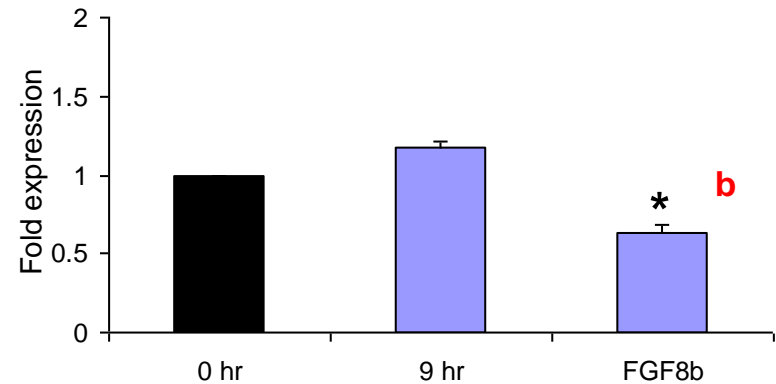


Ectodermal FGF-8 regulates gene expression of CG-1A, -8

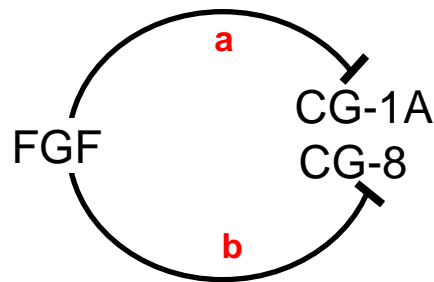
Effect of FGF8 on CG-1A gene expression



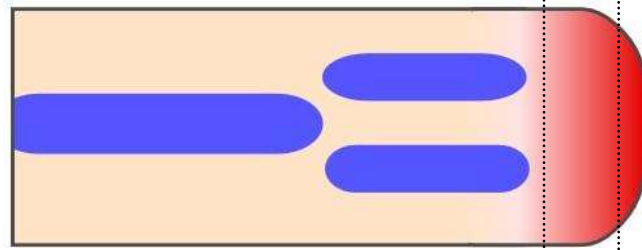
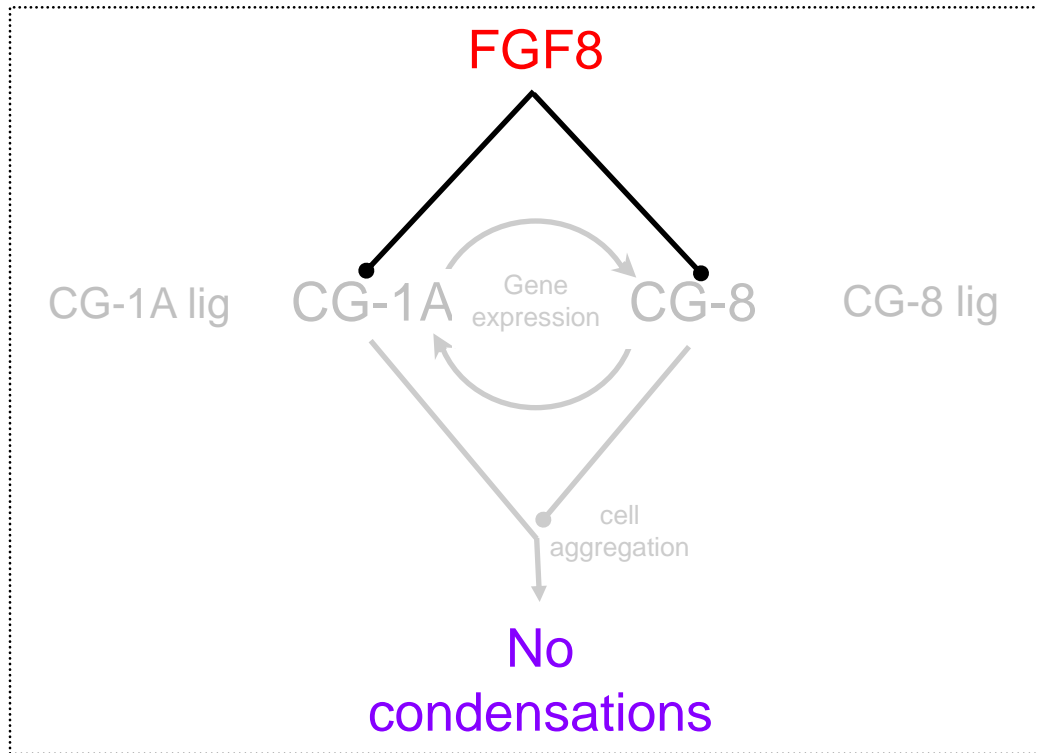
Effect of FGF8 on CG-8 gene expression



interaction between FGF8 and galectins

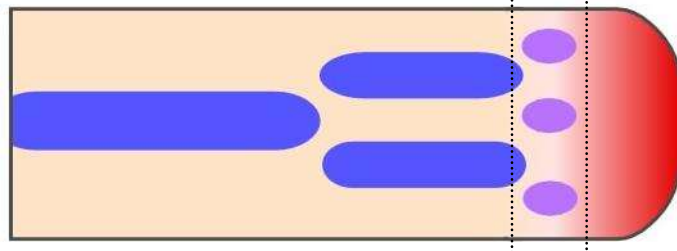
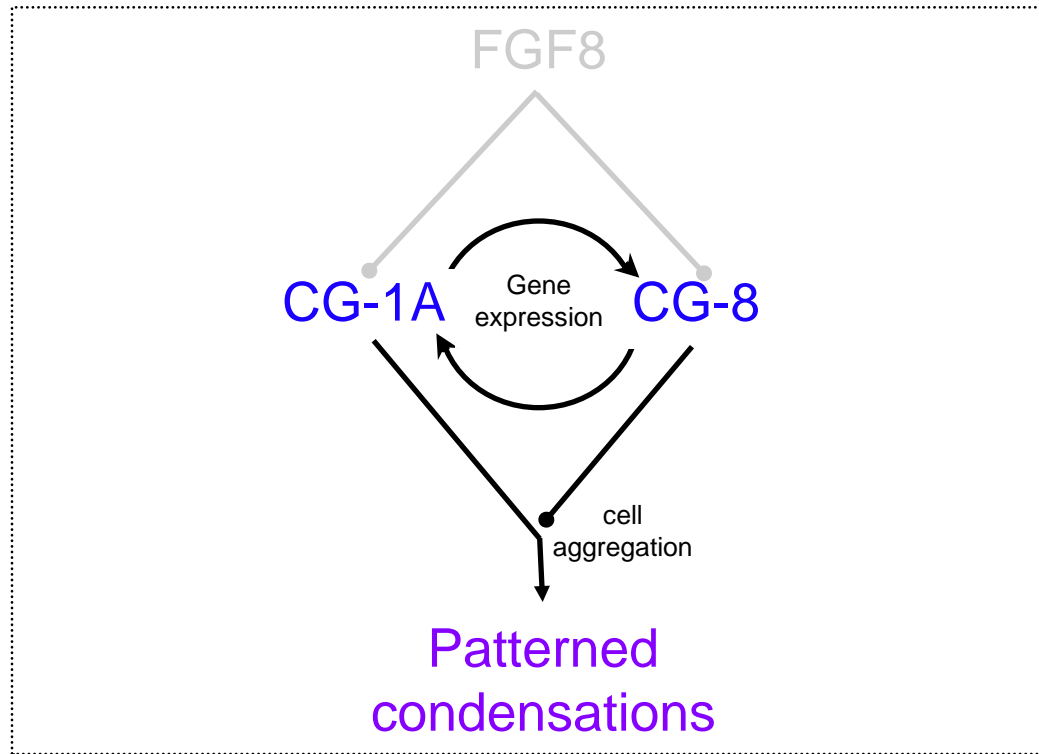


Galectin-based mechanism of limb patterning



→ activation
● inhibition

Galectin-based mediation of precartilage condensation



→ activation
—● inhibition

Acknowledgments

New York Medical College

Stuart Newman

Ken Lerea

Joseph Etlinger

Peng Hong

Esther Sabban

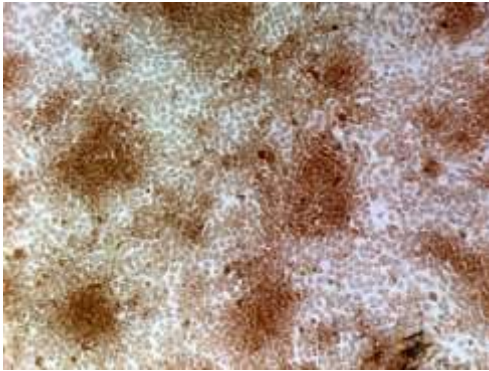
University of Munich

Hans-Joachim Gabius

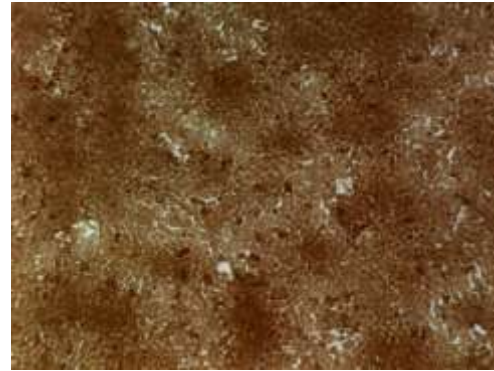
SUNY Stony Brook

John Reinitz

CG-1A and CG-8 ligands have different spatial patterns



CG-1A ligand staining
on day 3 (16x)

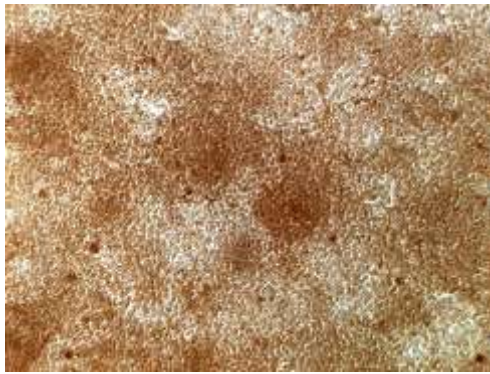


CG-8 ligand staining
on day 3 (16x)

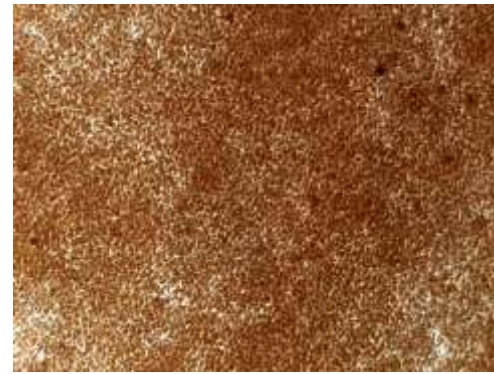
Ligand staining assayed by their binding respectively to
exogenously added biotinylated galectin

Negative control: Staining inhibition by co-incubating
galectin with lactose and not with mannose

CG-1A upregulates its own ligand *in vitro*

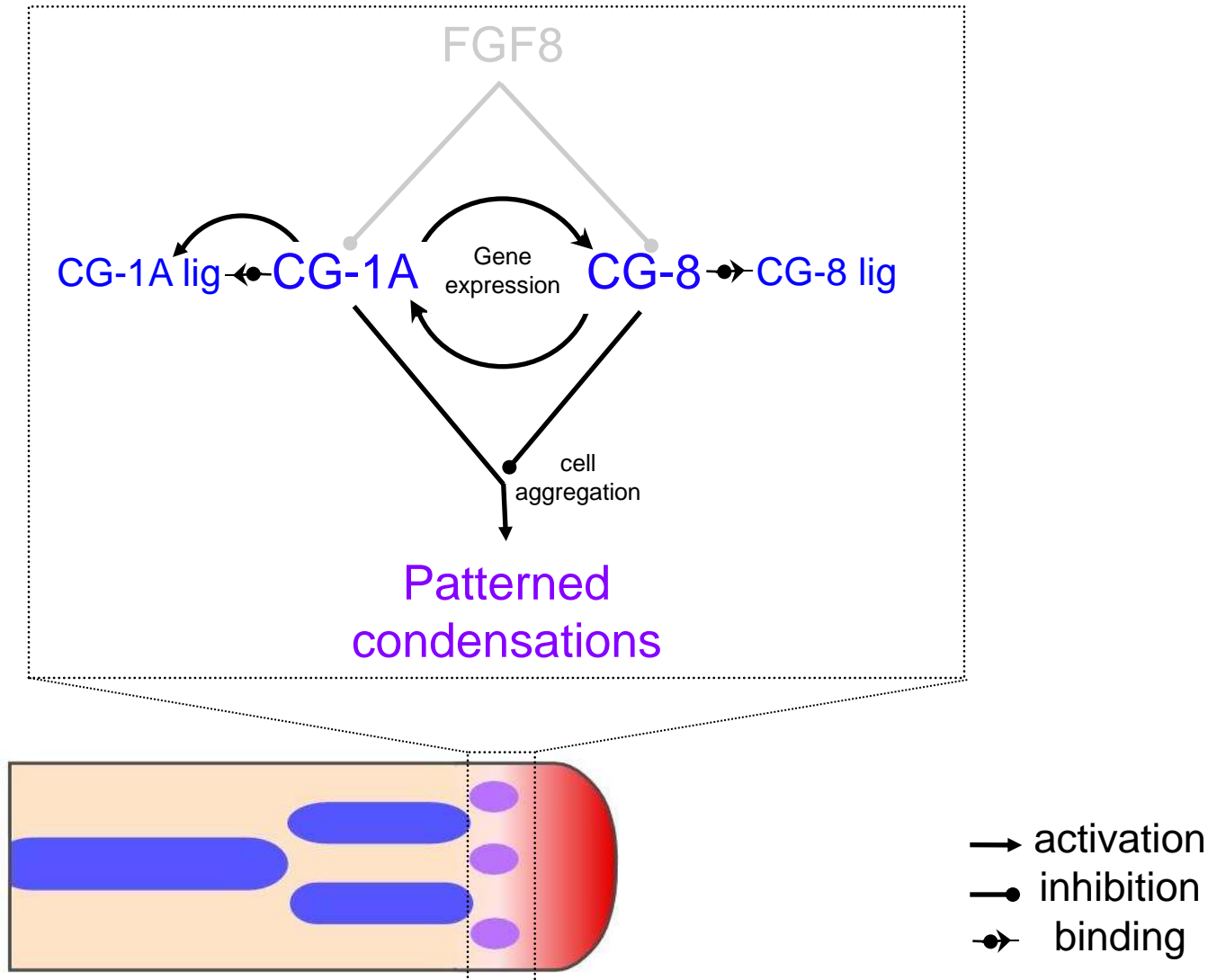


CG-1A ligand staining
on day 3 (16x)

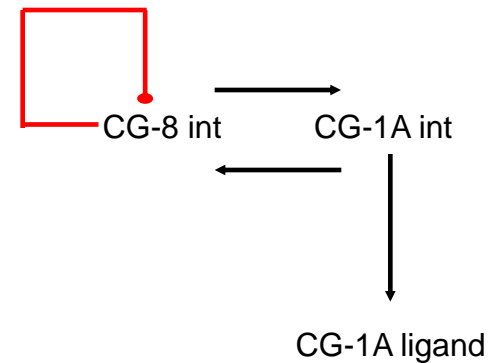
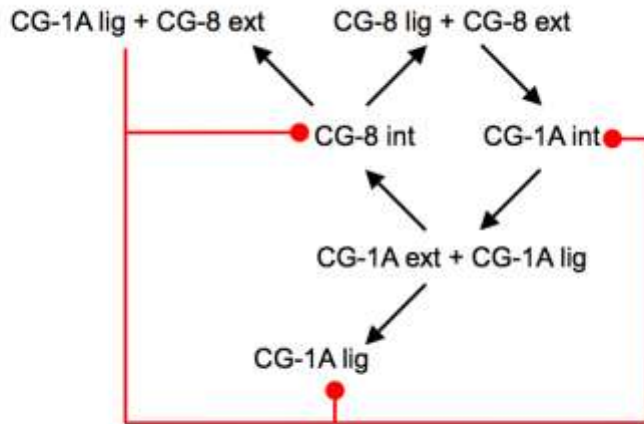


CG-1A ligand staining
Upon addition of CG-1A
10 µg/ml (16x)

Galectin-based mechanism of limb patterning



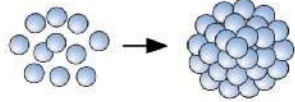
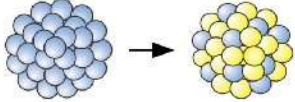
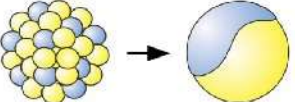
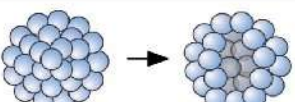
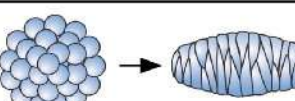
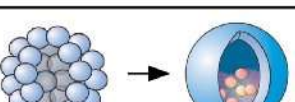
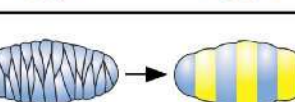
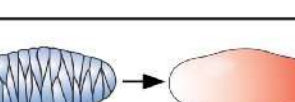
Interactions for agent based modeling



CG-1A lig + CG-1A = cell-cell adhesion. expression of CG-8, CG-1A ligand

CG-8 lig + CG-8 = no cell-cell adhesion, expression of CG-1A

CG-1A lig + CG-8 = no cell-cell adhesion, no expression/inhibition(?) of CG-1A

DPM	molecules	physics	evo-devo role	effect
ADH	cadherins; galectins	adhesion	multicellularity	
LAT	Notch	lateral inhibition	coexistence of alternative cell states	
DAD	cadherins	differential adhesion	phase separation; tissue multilayering	
POL _a	Wnt	cell surface anisotropy	topological change; interior cavities	
POL _p	Wnt	cell shape anisotropy	tissue elongation	
ECM	chitin; collagen; galectins	stiffness; dispersal	tissue solidification; elasticity; EMT	
OSC	Wnt + Notch	synchrony of oscillation	morphogenetic fields; segmentation	
MOR	TGF-β; Hh; FGF; galectins	diffusion	pattern formation	
TUR	MOR + Wnt + Notch	dissipative structure	segmentation; periodic patterning	