Introduction of the study in Pattern Formation Group I

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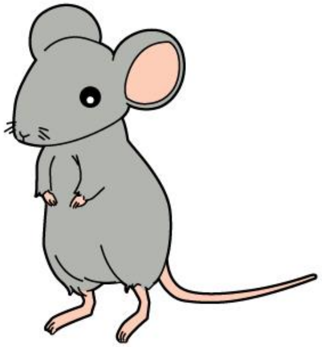
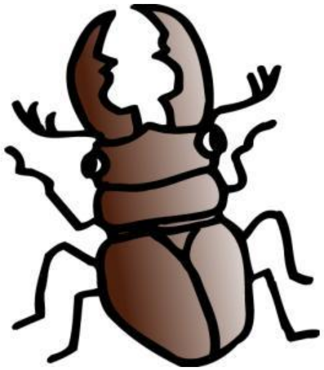
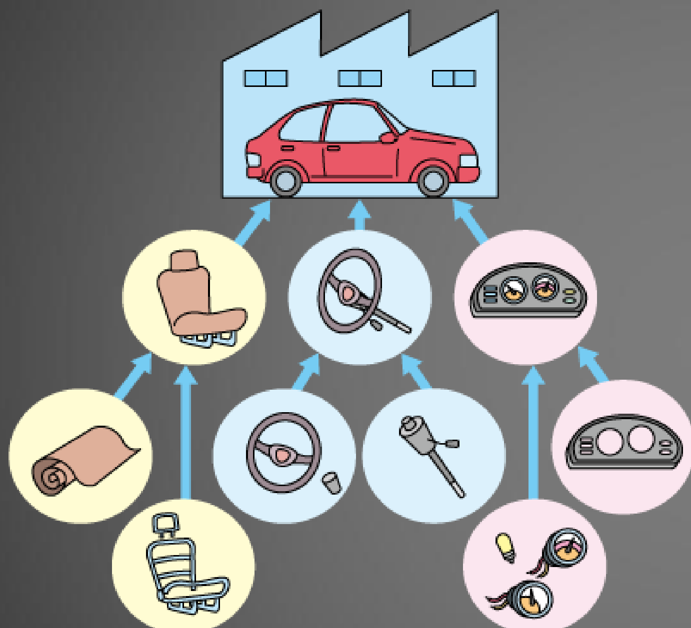
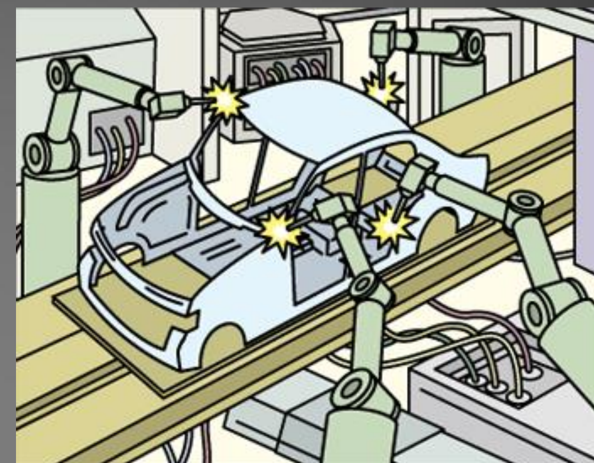




Making a complex machine

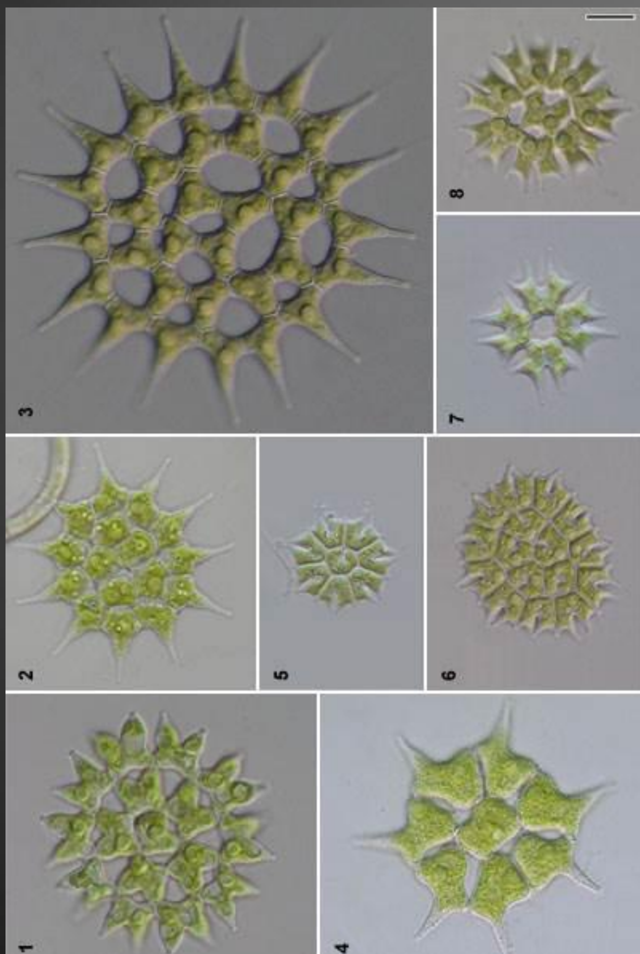




We need to make all the parts of the machine.
We need to assemble the parts.
Cars never makes it self. We need to do everything.

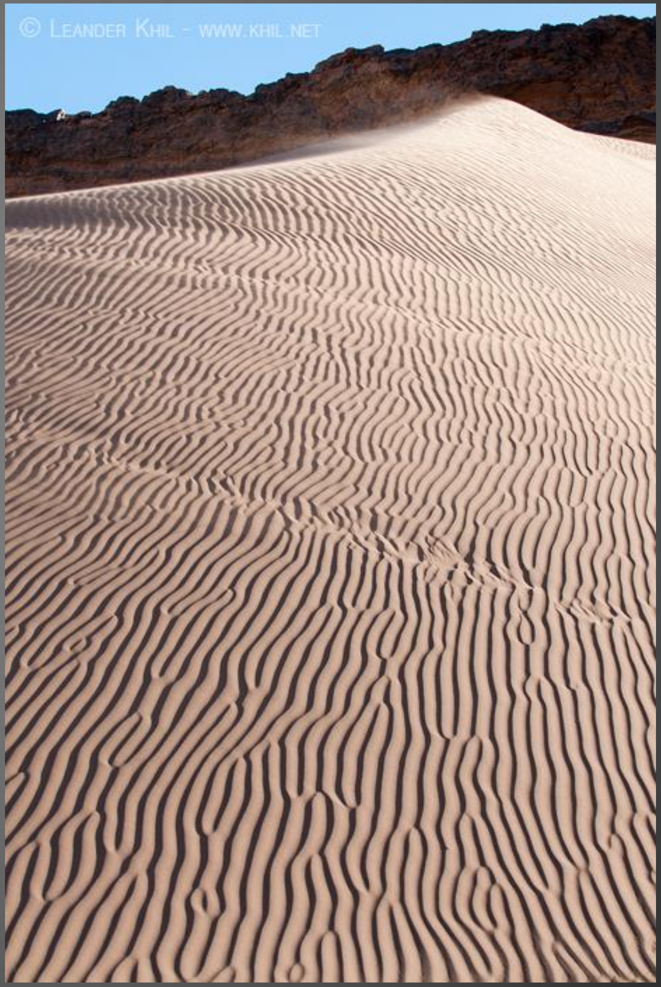
All the process is absolutely autonomous!!

Similar patterns develop in non-organic systems



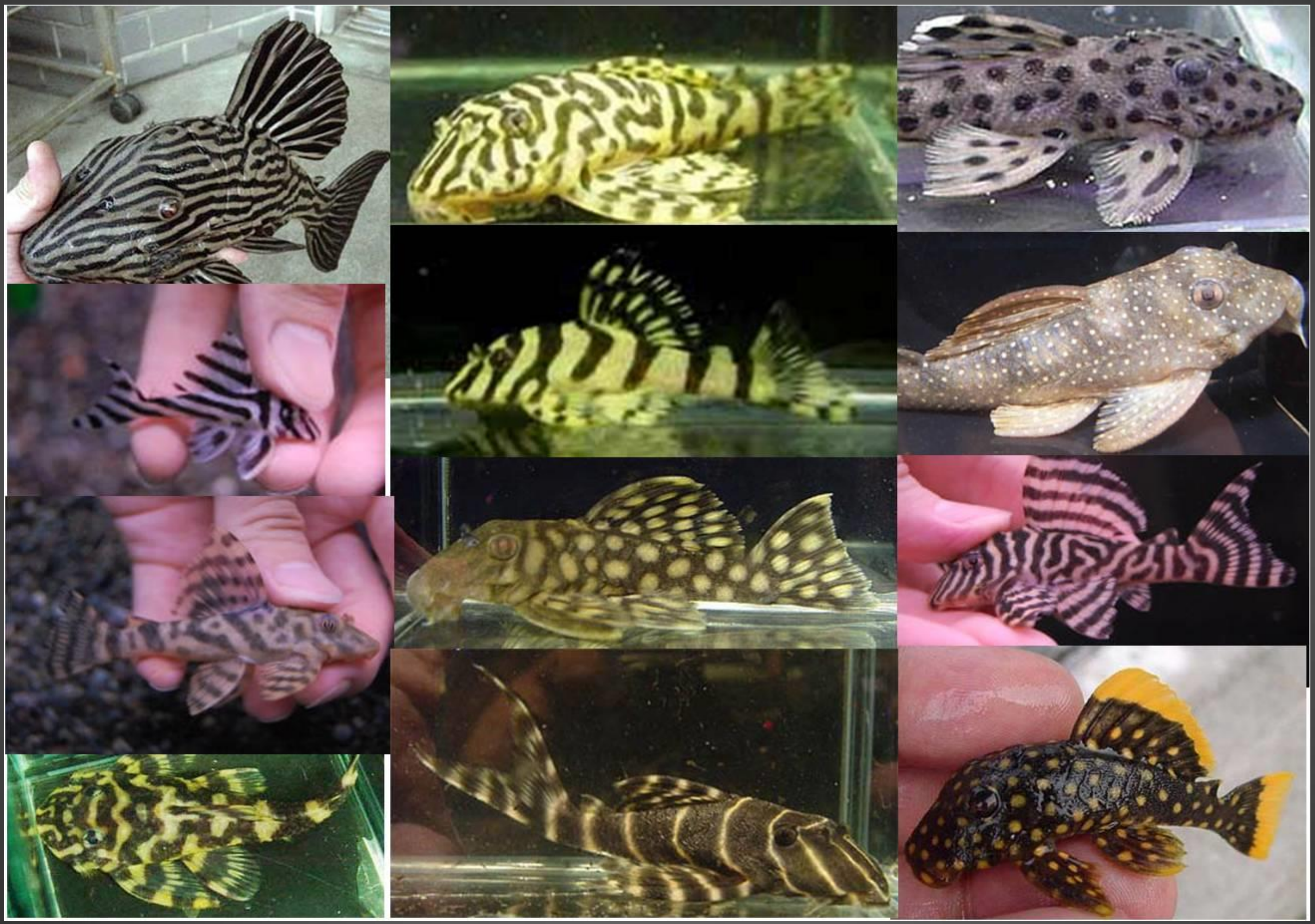

MUSK

Similar mechanism???



To find out the mechanism autonomous pattern formation, we selected the animal skin markings.

How are the pigmentation patterns made ?

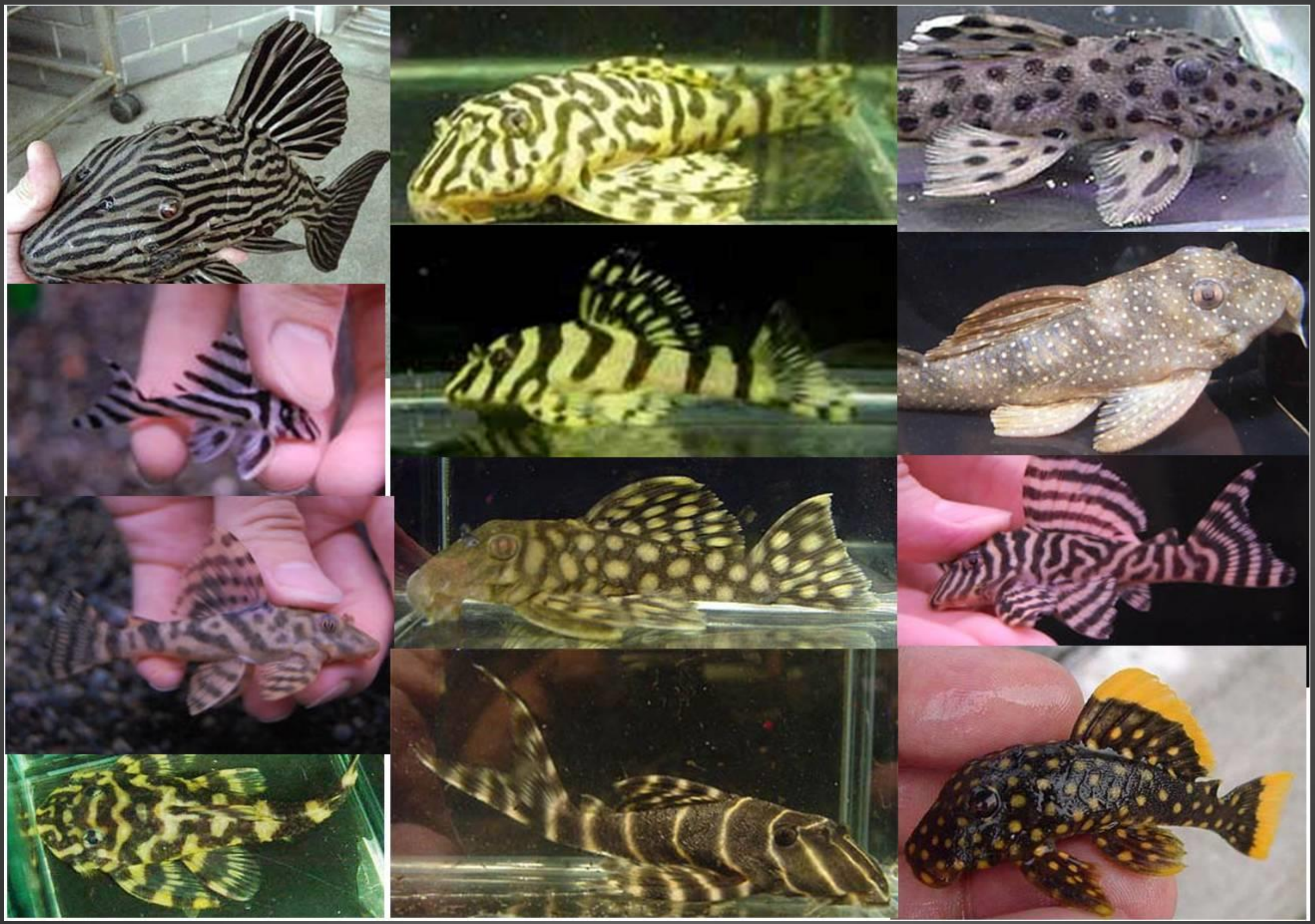


Genetically related species show variety of skin pattern.
>>An identical mechanism must be able to generate different pattern.

How are the pigmentation patterns made ?

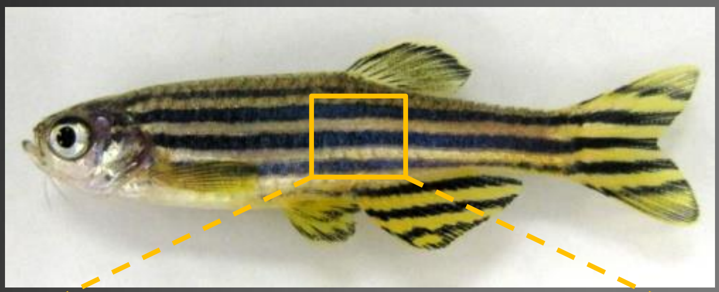
No similar pattern exist beneath the skin.
>>Patterns are autonomously made in the skin.

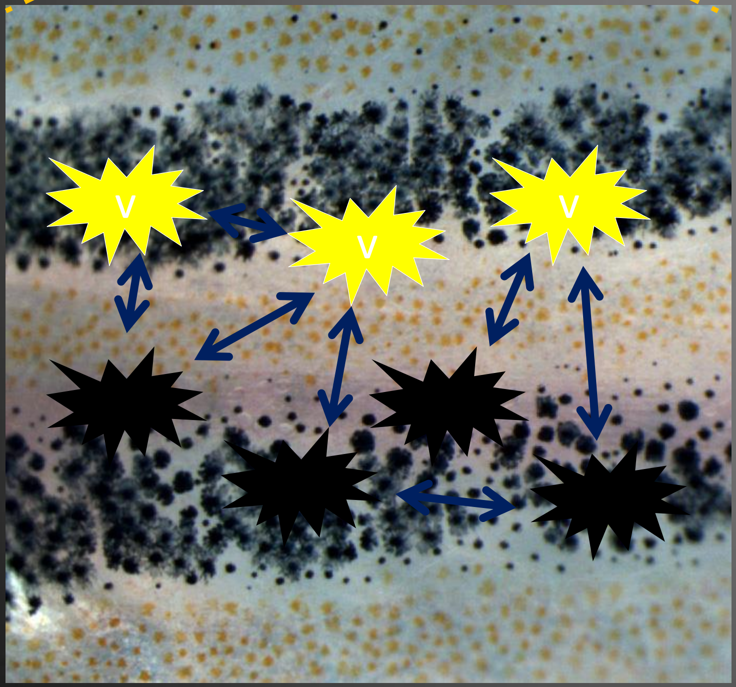
How are the pigmentation patterns made ?



A common characteristic
>>periodicity

What is making the skin pattern?





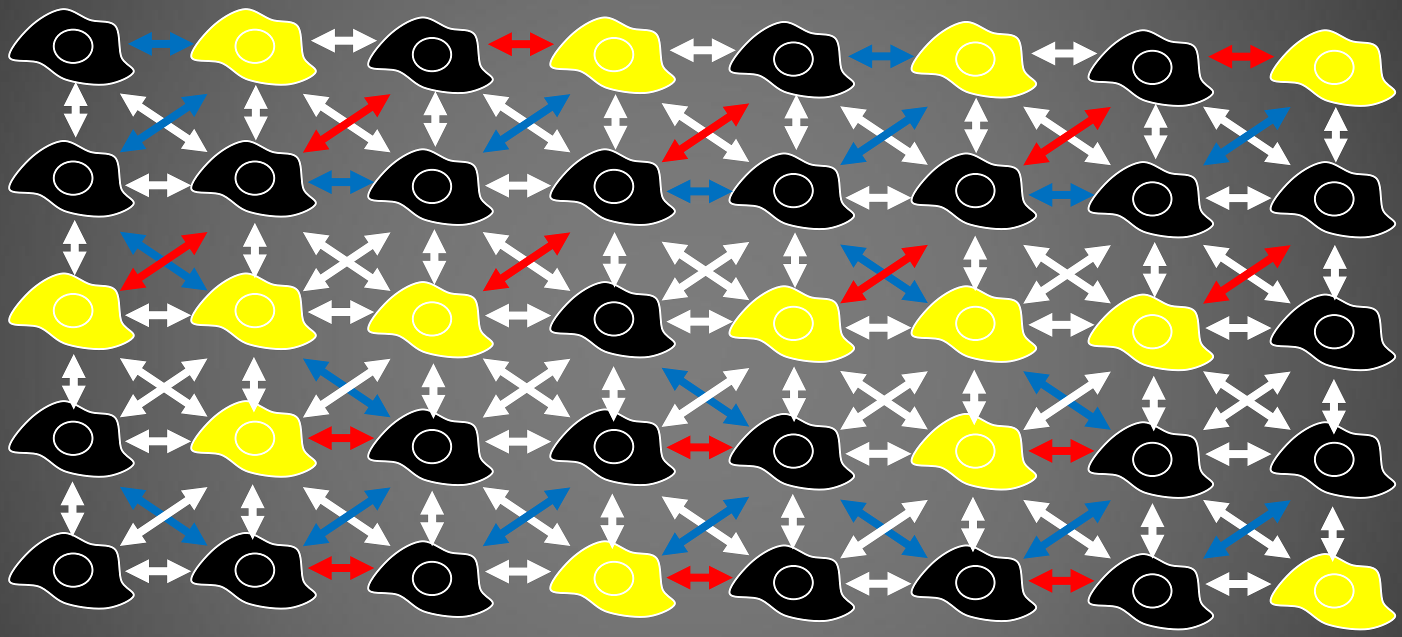
Patterns on the skin is the array of numerous pigment cells.

More than two types of pigment cells.

Cells interact each other.

Cells can move, develop and die.

We cannot grasp what really happen with our brain.



Huge number of interactions occur simultaneously.

Need the help of computer and mathematics.

Alan Mathison Turing

Turing presented a simple system in which interactions among the chemical (cells) and the diffusion of the chemicals signal can generate a spatial pattern.

Reaction-diffusion model

Integrating the differential equation

Stationary case. After a sufficient lapse of time X_r-h and Y_r-k approach asymptotically to the forms

$$\left.\begin{aligned} X_r-h &= 2\mathscr{R}A_{s_0}\exp\left[\frac{2\pi i s_0 r}{N}+It\right], \\ Y_r-k &= 2\mathscr{R}C_{s_0}\exp\left[\frac{2\pi i s_0 r}{N}+It\right]. \end{aligned}\right\} \tag{8·1}$$

Oscillatory case. After a sufficient lapse of time X_r-h and Y_r-k approach the forms

$$\left.\begin{aligned} X_r-h &= 2\,e^{It}\mathscr{R}\left\{A_{s_0}\exp\left[\frac{2\pi i s_0 r}{N}+i\omega t\right]+A_{N-s_0}\exp\left[-\frac{2\pi i s_0 r}{N}-i\omega t\right]\right\}, \\ Y_r-k &= 2\,e^{It}\mathscr{R}\left\{C_{s_0}\exp\left[\frac{2\pi i s_0 r}{N}+i\omega t\right]+C_{N-s_0}\exp\left[-\frac{2\pi i s_0 r}{N}-i\omega t\right]\right\}. \end{aligned}\right\} \tag{8·2}$$

Answer is

After long time has past,,,,

Case A: generates stable pattern

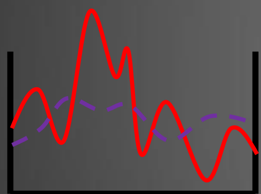
$$\left.\begin{aligned} X_r - h &= 2\Re A_{s_0} \exp\left[\frac{2\pi i s_0 r}{N} + It\right] \\ Y_r - k &= 2\Re C_{s_0} \exp\left[\frac{2\pi i s_0 r}{N} + It\right] \end{aligned}\right\}$$

Case B: generates oscillating pattern.

$$\left.\begin{aligned} X_r - h &= 2e^{\lambda t}\Re\left\{A_{s_0} \exp\left[\frac{2\pi i s_0 r}{N} + i\omega t\right] + A_{N-s_0} \exp\left[-\frac{2\pi i s_0 r}{N} - i\omega t\right]\right\} \\ Y_r - k &= 2e^{\lambda t}\Re\left\{C_{s_0} \exp\left[\frac{2\pi i s_0 r}{N} + i\omega t\right] + C_{N-s_0} \exp\left[-\frac{2\pi i s_0 r}{N} - i\omega t\right]\right\} \end{aligned}\right\}$$

Six different states are possible

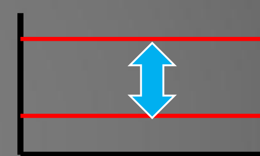
Initial condition



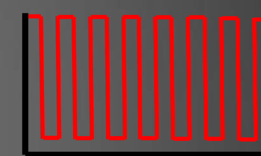
Both morphogenesis diffuse and react each other

Six possible states

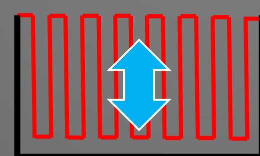
uniform, stationary



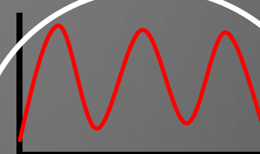
uniform, oscillating



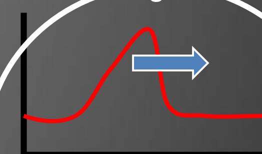
stationary waves with extreme short wave-length



Oscillatory cases with extreme short wave-length



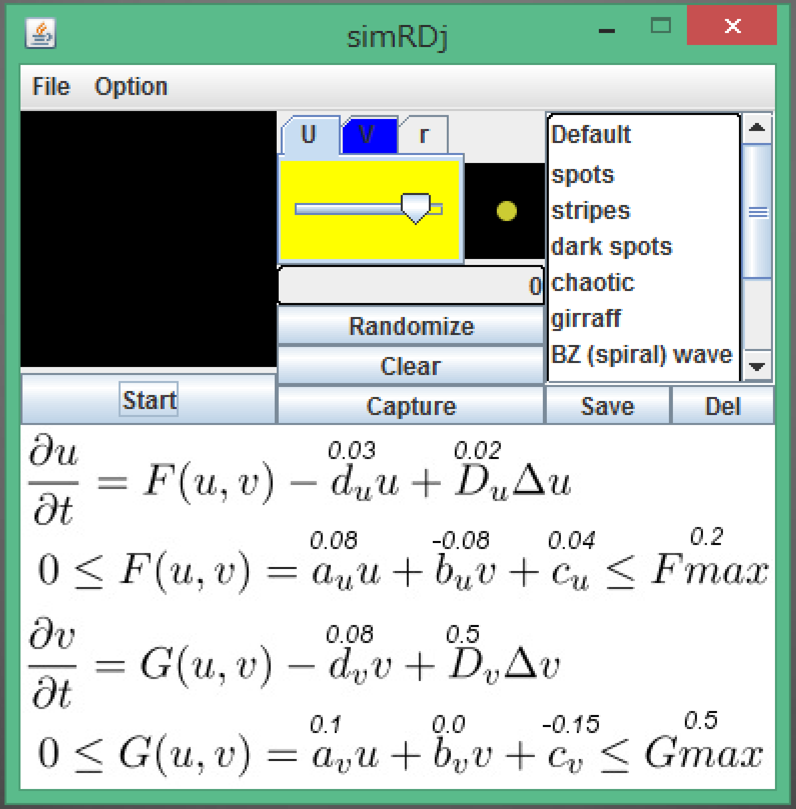
stationary waves with finite wave-length **(Turing pattern)**

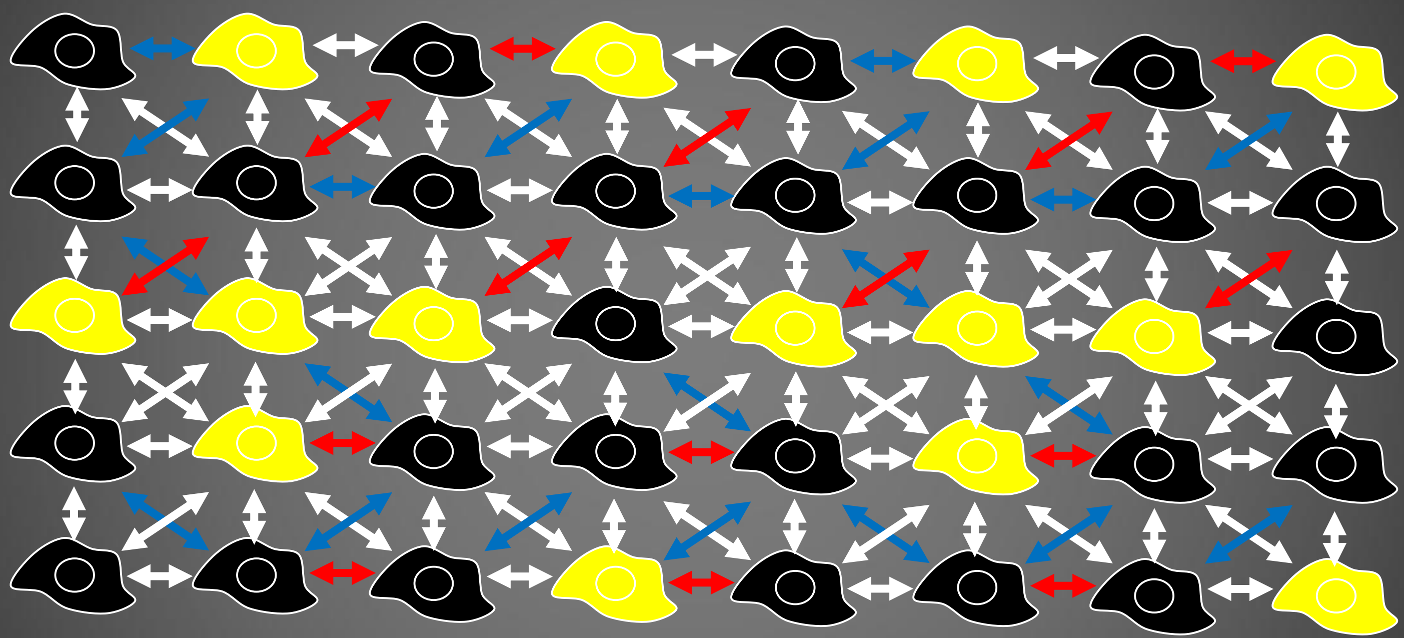


Oscillatory cases with finite wave lengthy

Mathematics is difficult,,,,

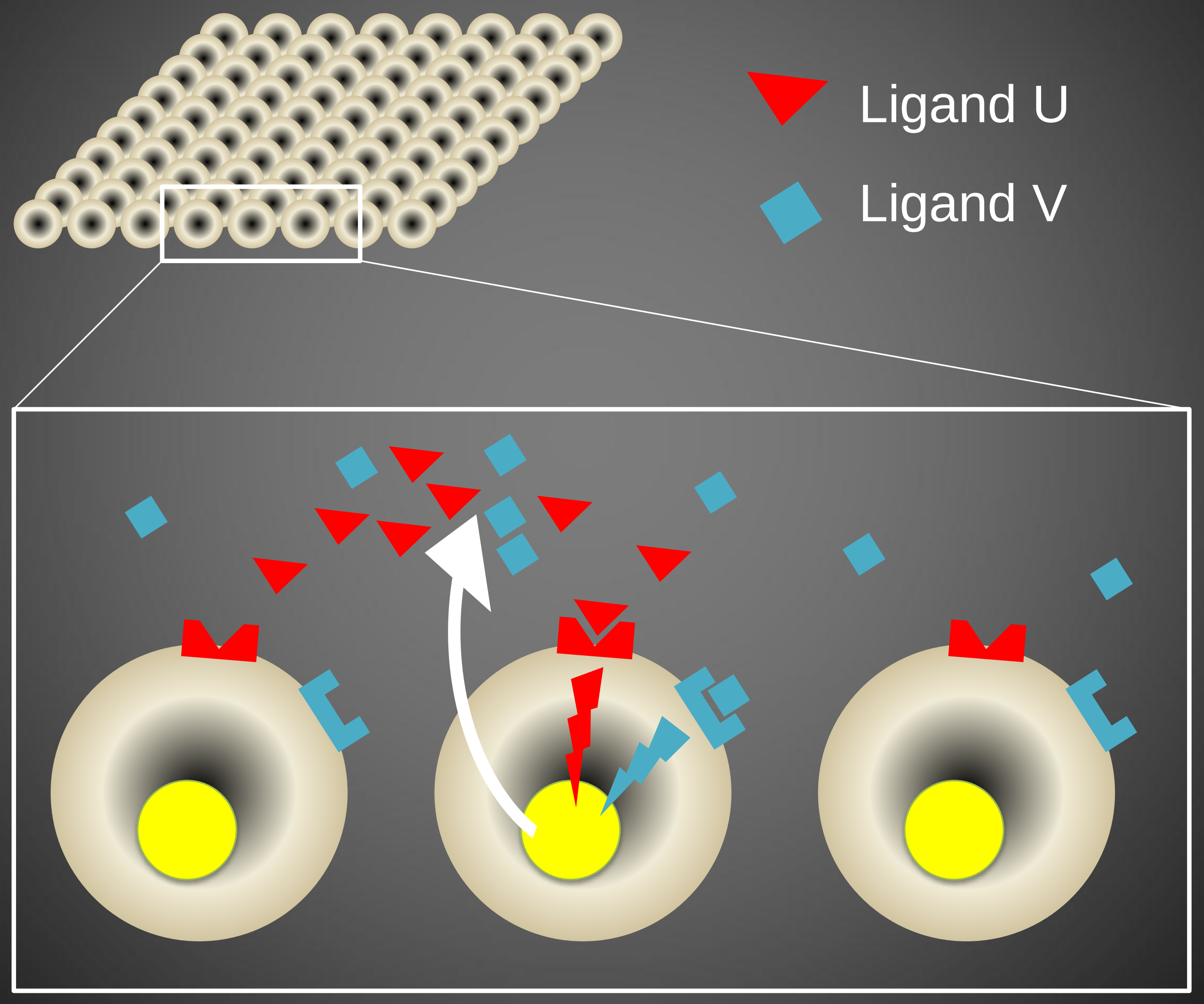
- Testing the system with simulation is easy!!





Huge number of interactions occur simultaneously.

Need the help of computer and mathematics.



Ligand U
Ligand V

数式で表すと･･･

$$\frac{\partial u}{\partial t} = F(u,v) - d_u u + D_u \Delta u$$

$$\frac{\partial v}{\partial t} = G(u,v) - d_v y + D_v \Delta v$$

Rate of concentration change

Production

Degradation

Diffusion

Reaction

$$F(u,v) = a_1 u + b_1 v + c_1$$

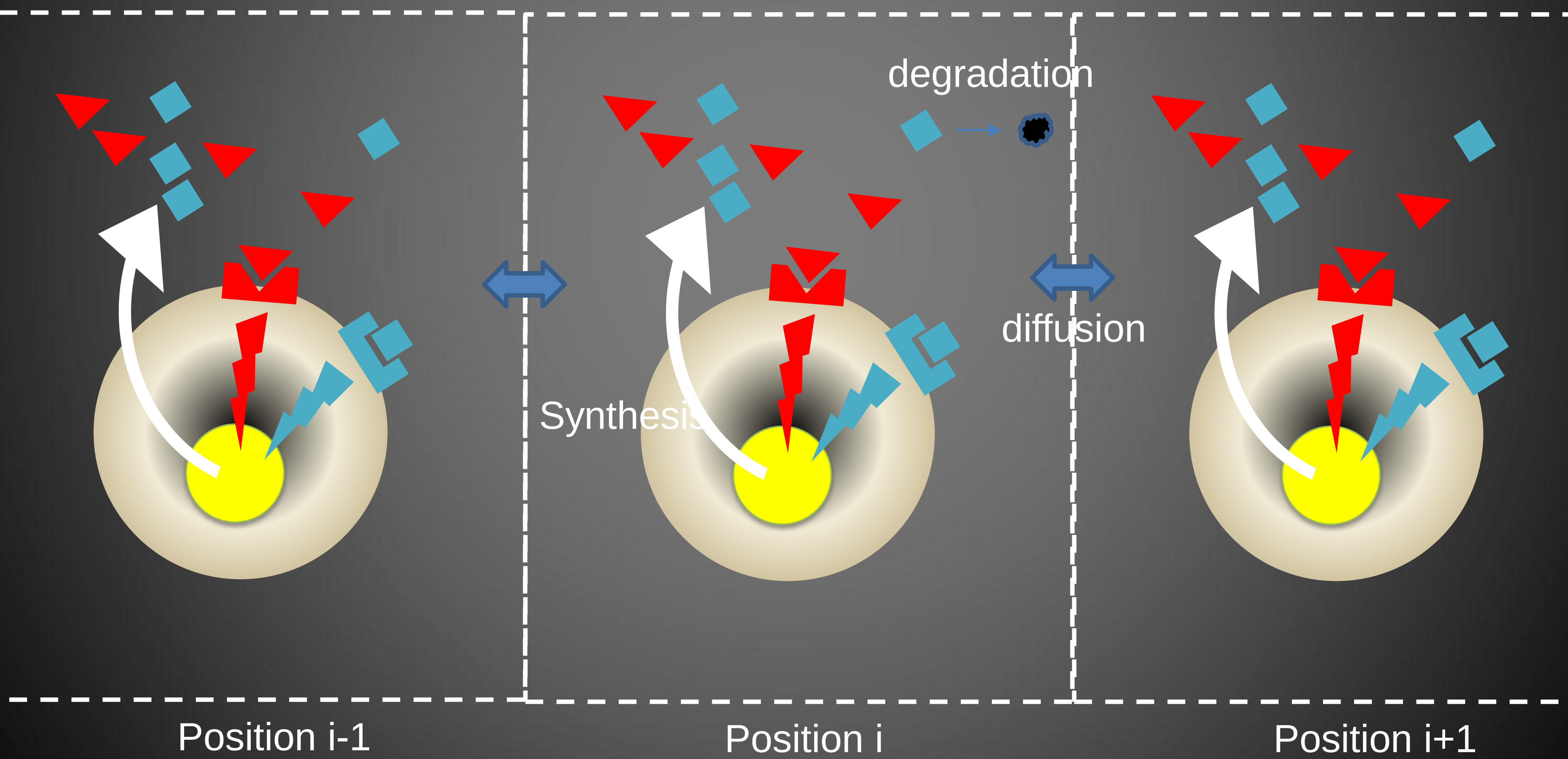
$$G(u,v) = a_2 u + b_2 v + c_2$$

Ui(t0) = Concentration of U in position i at time t

Vi(t0) = Concentration of V in position i at time t

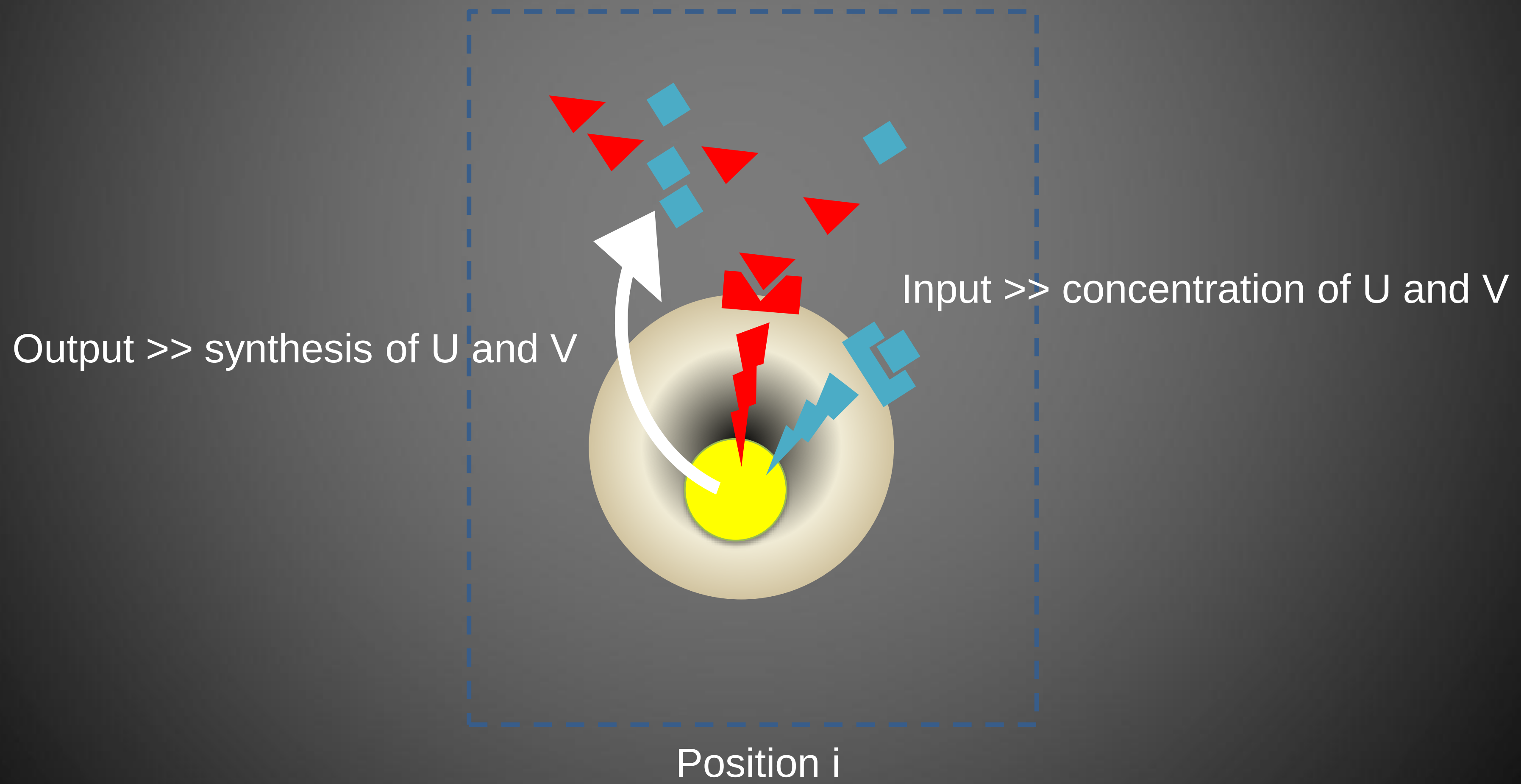
If we know Ui(t+dt) and Vi(t+dt), we can predict how the system behave in the future

What we need to calculate is Synthesis, degradation and diffusion.



synthesis

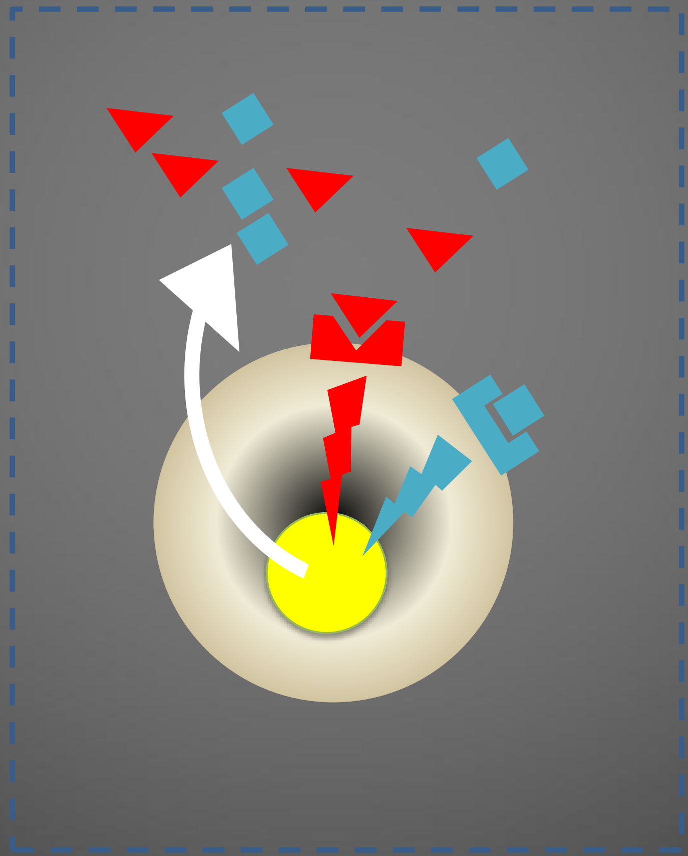
$$synthesisU = F(u, v) = a_u u + b_u v + c_u$$
$$synthesisV = G(u, v) = a_v u + b_v v + c_v$$



degradation

$$degradationU = -d_u * u$$
$$degradationV = -d_v * v$$

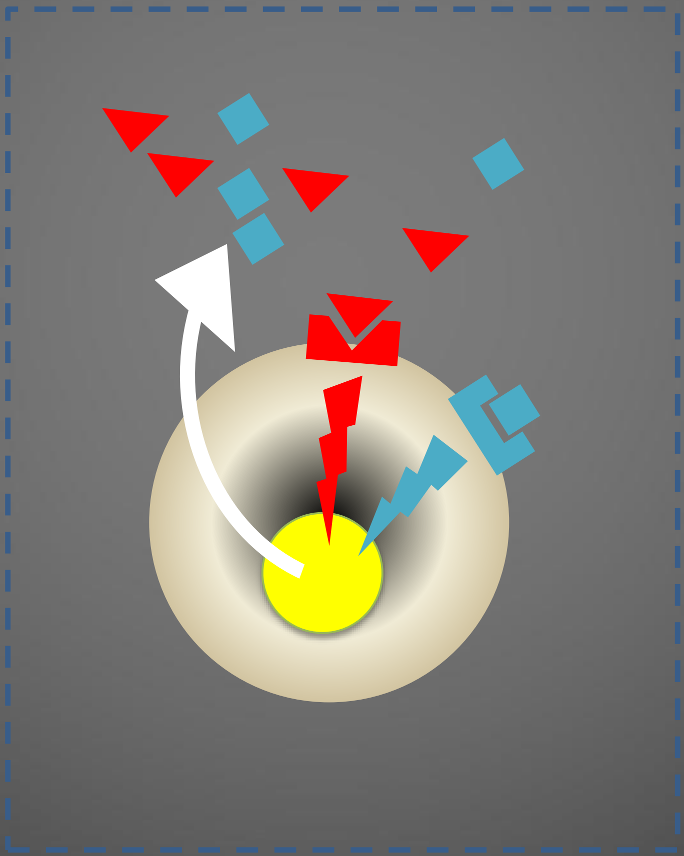
du dv are the degradation constant of u and v



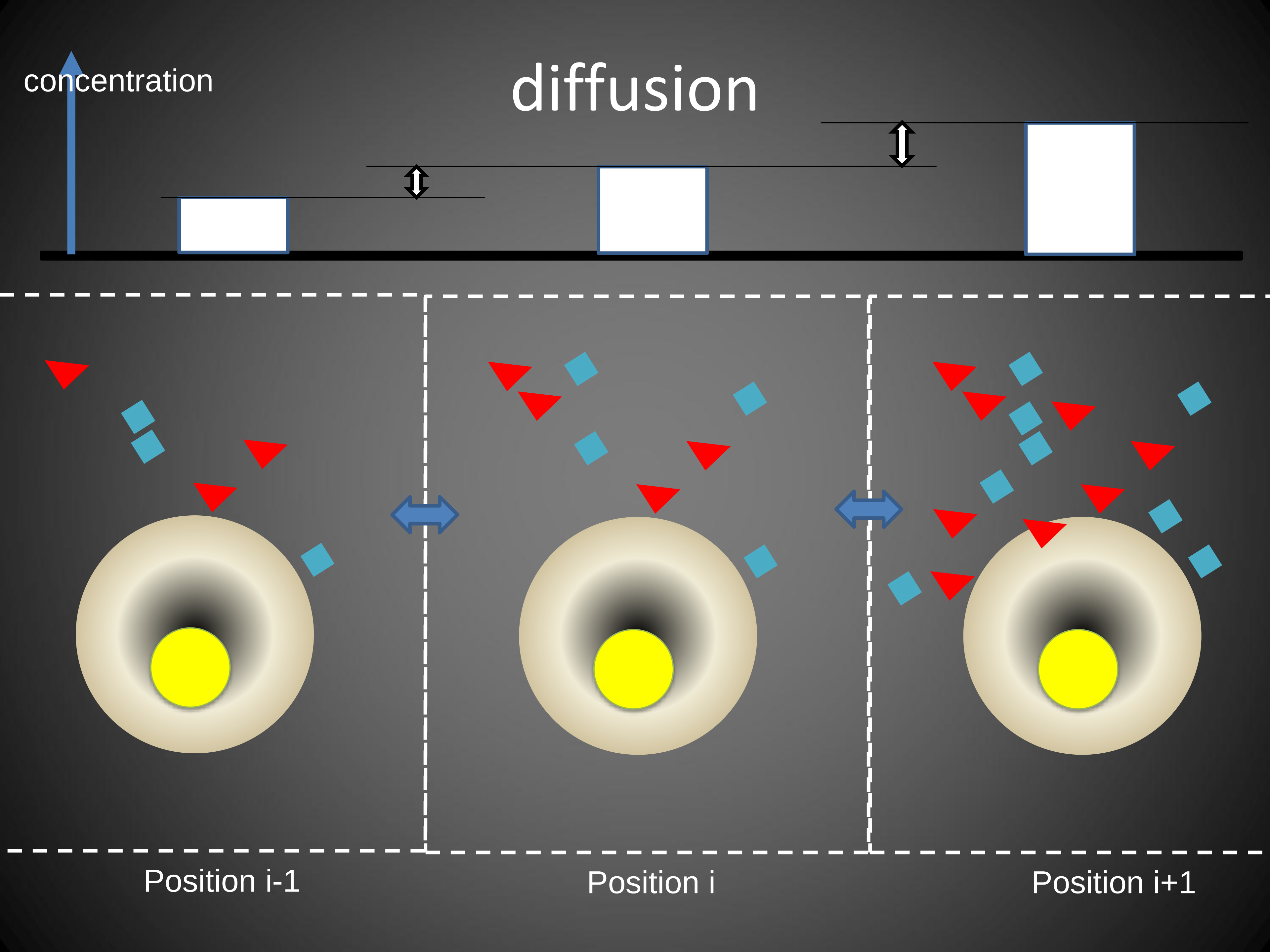
Position i

Reaction=synthesis + degradation

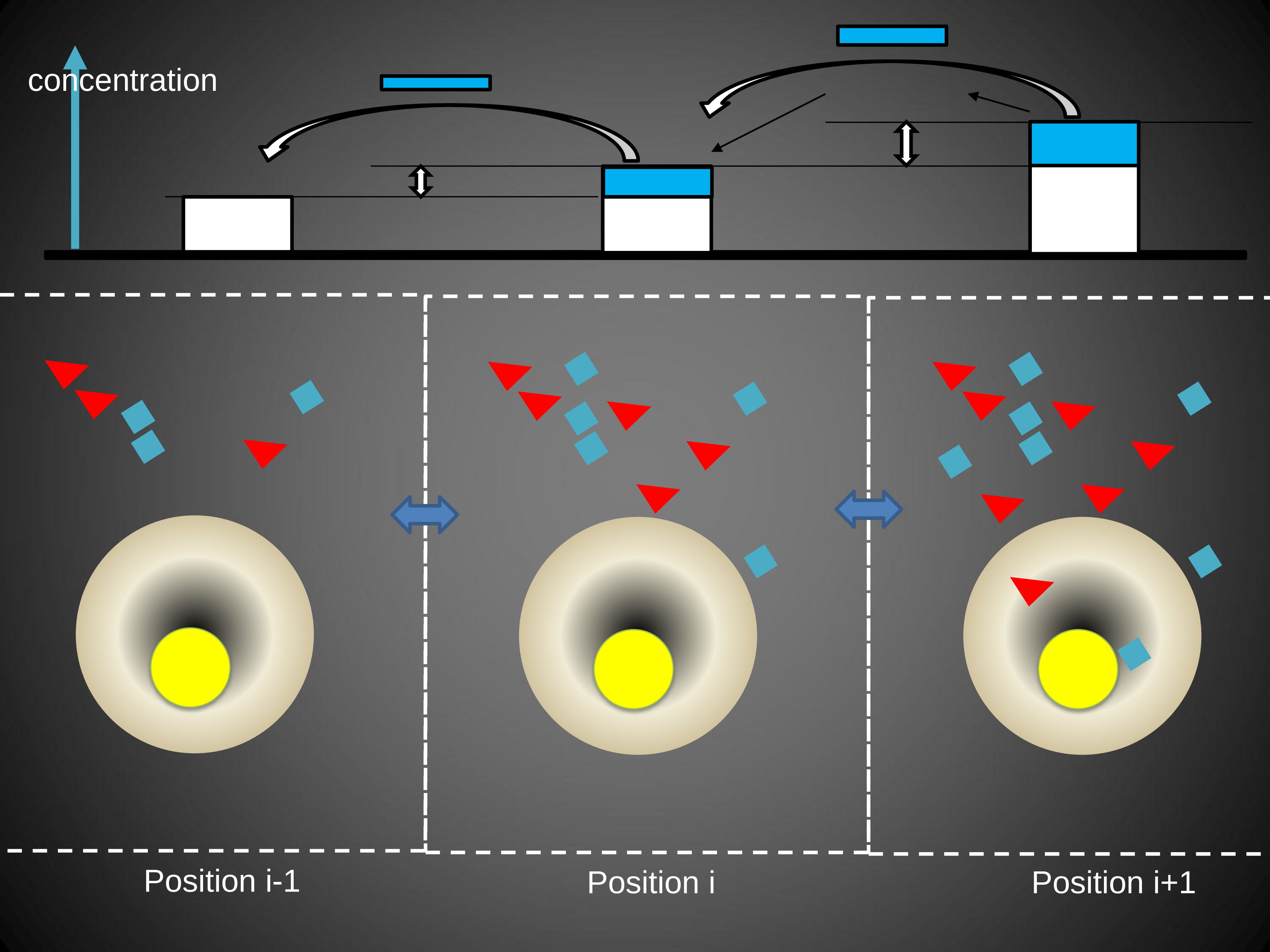
$$reactionU = synthesisU + degradationU = F(u,v) - d_u * u$$
$$reactionV = synthesisV + degradationV = G(u,v) - d_v * v$$



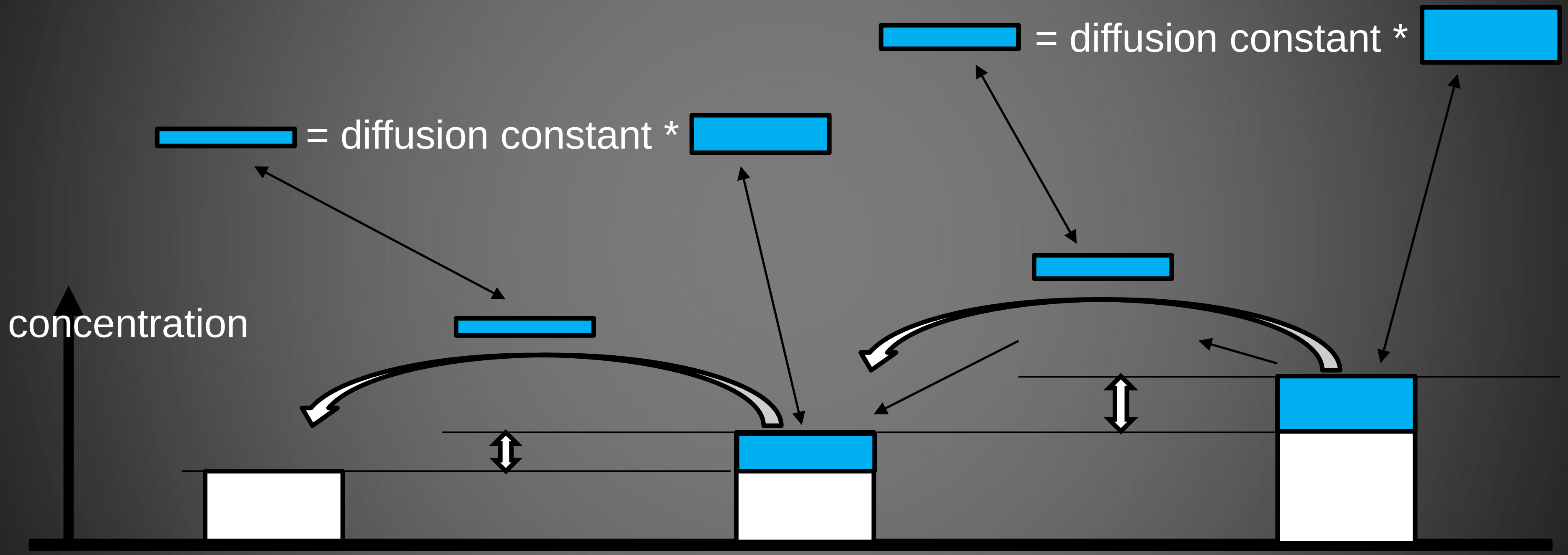
Position i



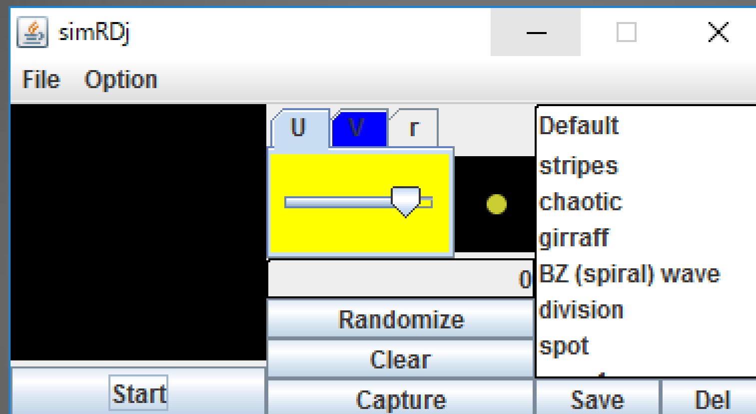
concentration
diffusion
Position i-1
Position i
Position i+1


concentration
Position i-1
Position i
Position i+1

diffusion



RD simulator



$$\frac{\partial u}{\partial t} = F(u,v) - \overset{0.03}{d_u} u + \overset{0.02}{D_u} \Delta u$$

Diffusion and degradation

Synthesis of u

$$0 \leq F(u,v) = \overset{0.08}{a_u} u + \overset{-0.08}{b_u} v + \overset{0.04}{c_u} \leq \overset{0.2}{Fmax}$$

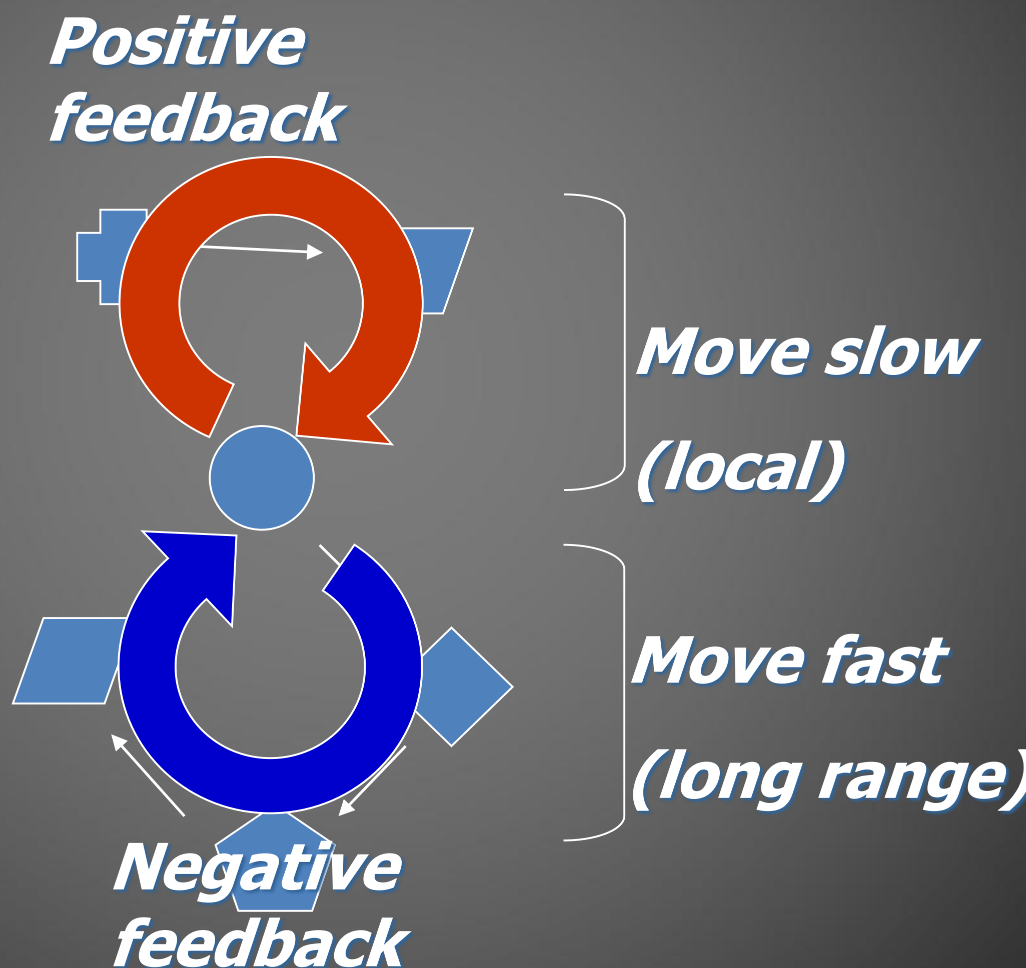
$$\frac{\partial v}{\partial t} = G(u,v) - \overset{0.08}{d_v} v + \overset{0.5}{D_v} \Delta v$$

Diffusion and degradation

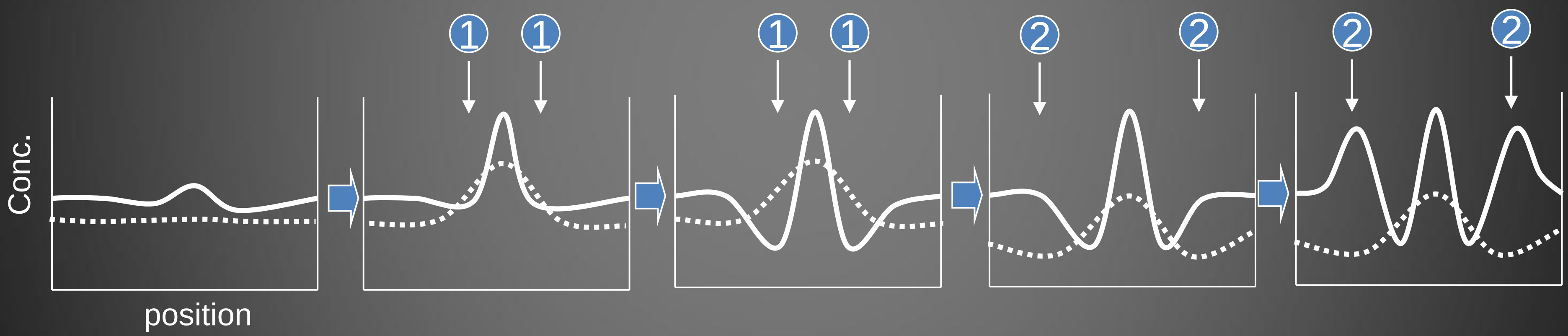
Synthesis of v

$$0 \leq G(u,v) = \overset{0.1}{a_v} u + \overset{0.0}{b_v} v + \overset{-0.15}{c_v} \leq \overset{0.5}{Gmax}$$

Conditions to make stable pattern



Making a stable wave



Introduction of the study in Pattern Formation Group II

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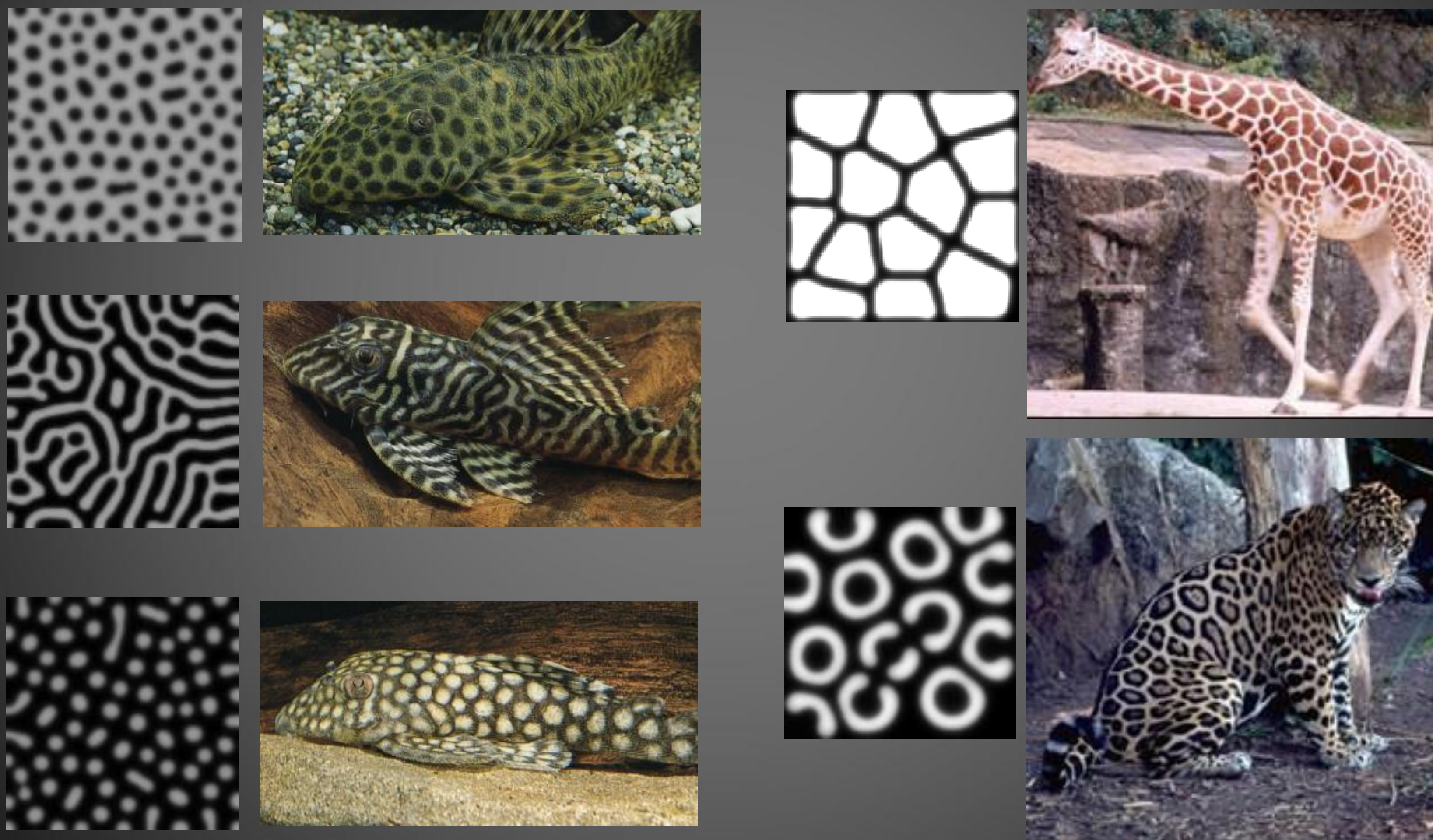
Reaction-Diffusion Model by Alan Turing

$$\frac{\partial x}{\partial t} = F(x, y) + D_x \Delta x$$

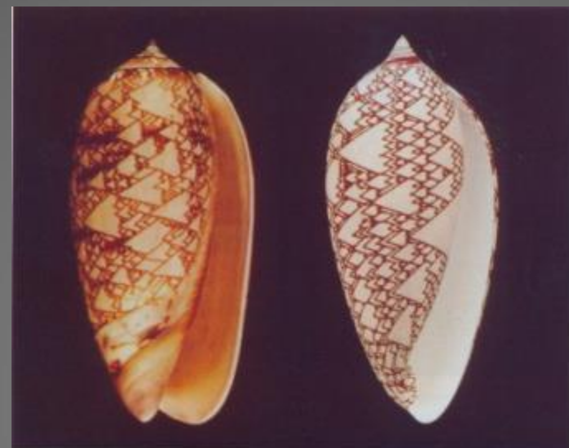
$$\frac{\partial y}{\partial t} = G(x, y) + D_y \Delta y$$

Alan Turing

RD model reproduces animal skin patterns

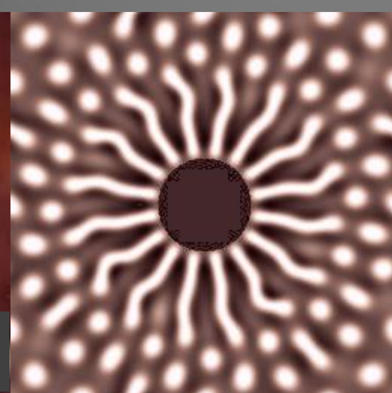


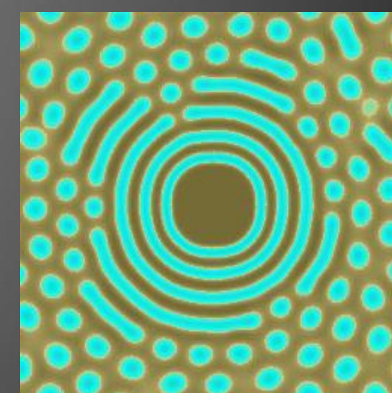
More complex patterns



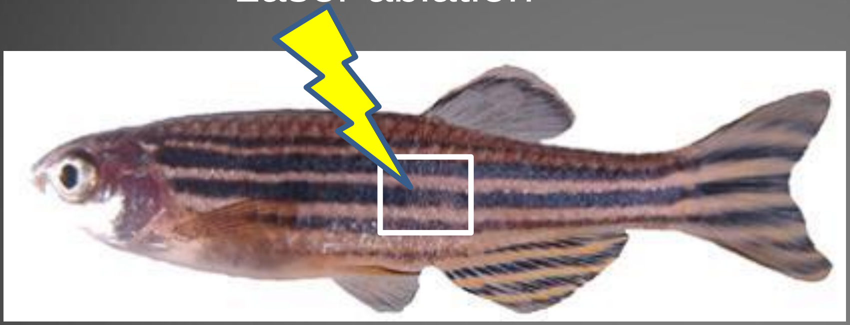
Meinhardt 1998

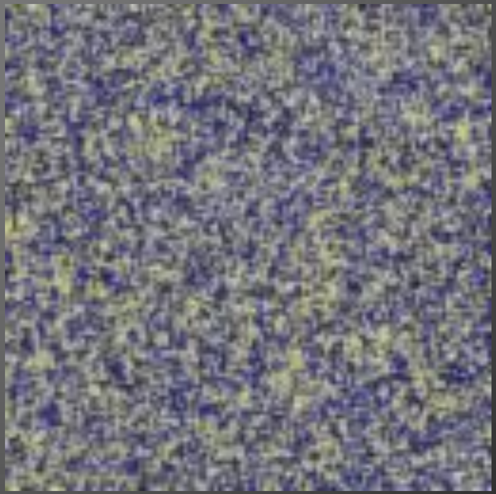
Sandersen 2006





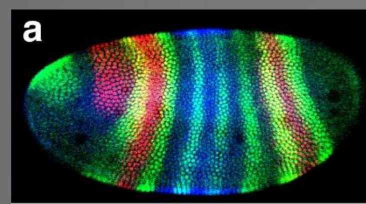
RD model reproduce the pattern regeneration

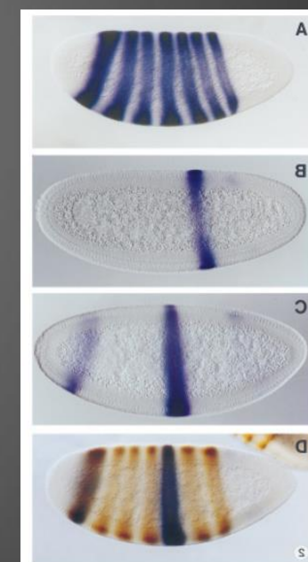




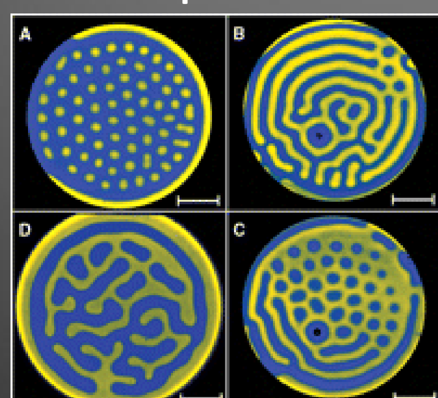
History of Turing model

- 1952 Turing presented RD model
- 1954 Turing died
- 1970 re-discovery of RD model by mathematicians
- 1972~1989 some discussions among developmental biologists





- 1990 most of biologists discarded the RD model
- 1991 generation of RD pattern in chemical system



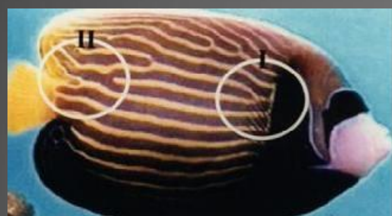
How one can prove the RD theory

- Normal strategy is too complex
  - Get mutant with disturbed pattern.
  - Identify all the molecular and cellular events related to the pattern formation
  - Measure the reaction parameters and the diffusion constants
  - Simulate the interaction network identified
- Needs more easy strategy

Easy method to prove the RD model

- Turing models says
  - Pattern is formed as a kind of “wave”.
  - Pattern regenerate when disturbed.
  - If the pattern is disturbed, it should regenerate in the way the simulation predict.

Prediction of the skin pattern change of Emperor angelfish

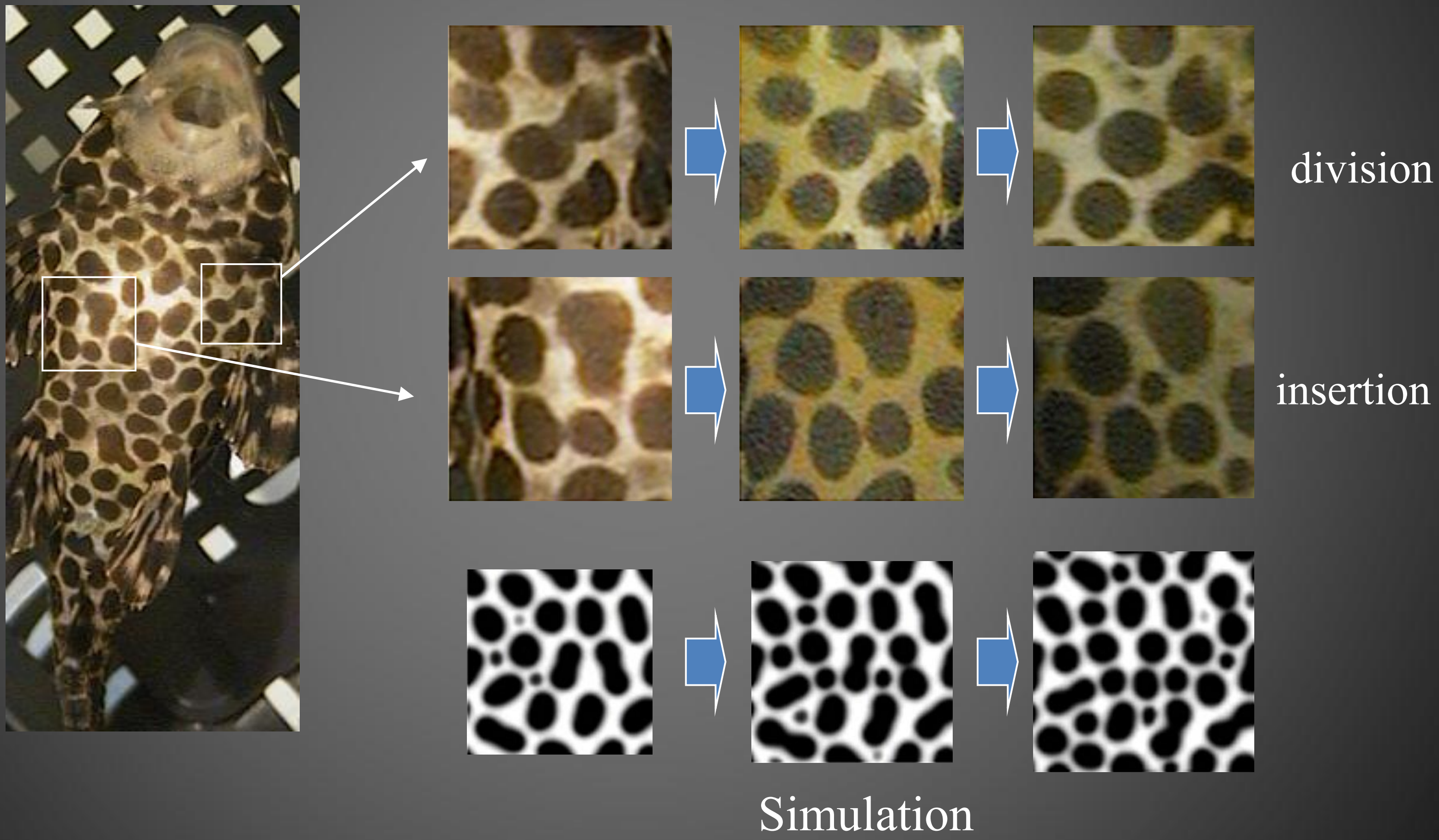


simulation

day 0 day 40 day 90

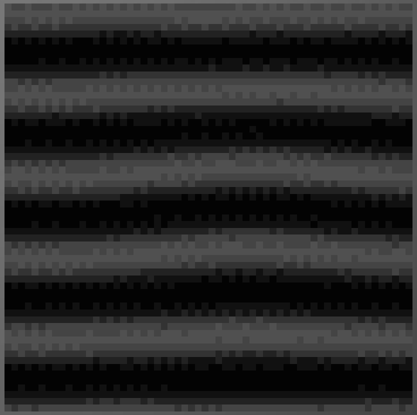
Nature 1995; Kondo

Prediction of the change of spot pattern in a cat fish, Plecostoms.



PNAS 1999; Kondo

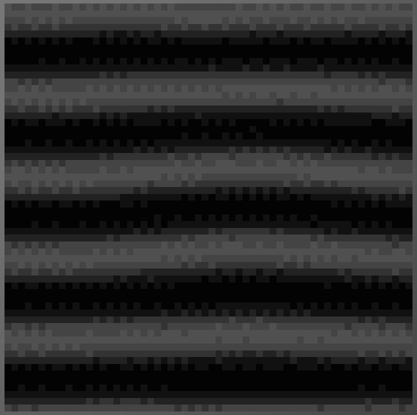
In case of Zebrafish with artificial disturbance of the pattern,,,,



simulation

Simulation successfully predicted the pattern change!

experiment



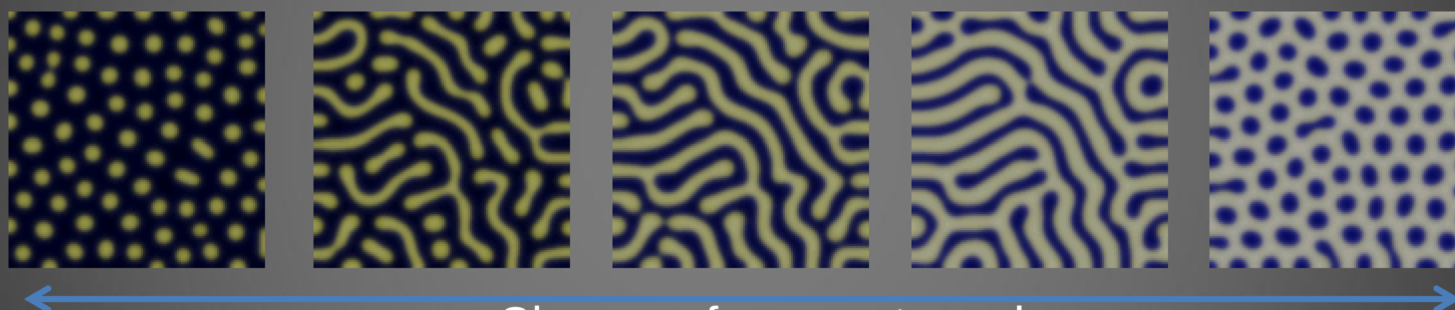
simulation

PNAS 2007, Yamaguchi and Kondo

Prediction of the skin pattern of hybrid fish

$$\frac{\partial x}{\partial t} = F(x, y) + D_x \Delta x$$

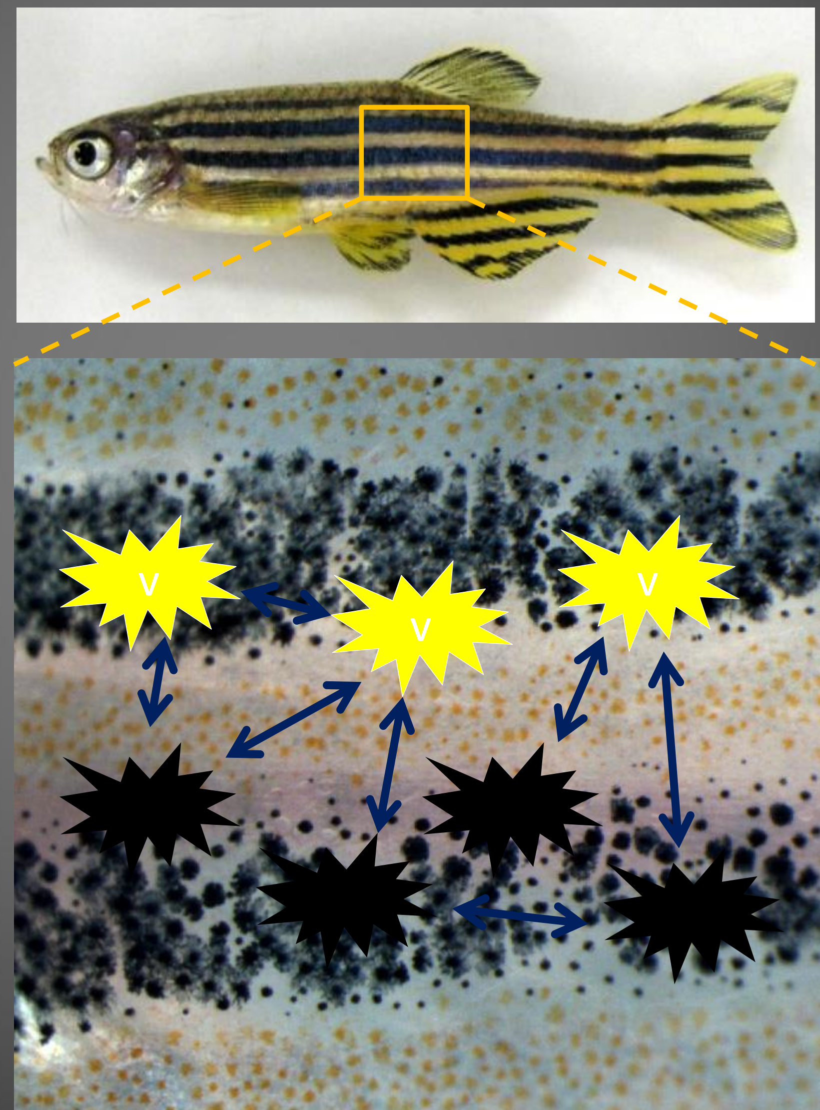
$$\frac{\partial y}{\partial t} = G(x, y) + D_y \Delta y$$



Change of parameter value

Nature communication 2010; Miyazawa and Kondo

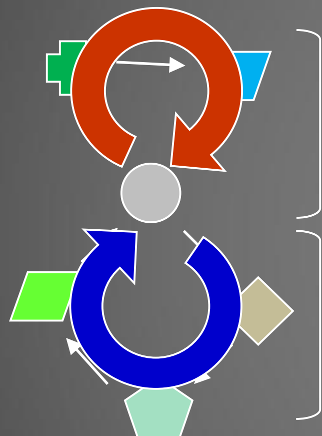
Mutual interaction between the pigment cells should be the key factor



melanophore

xanthophore

What does the mathematical theory suggest ?

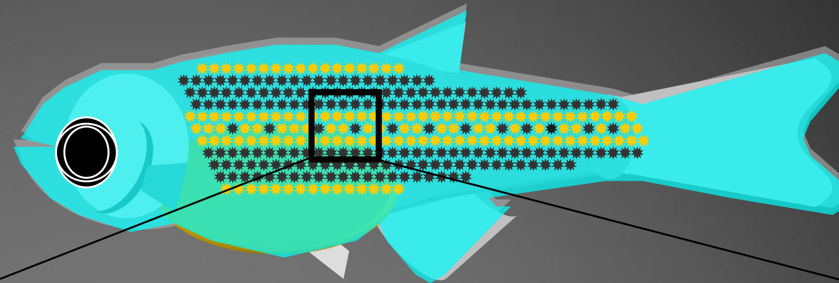


local positive feedback
+
long negative feedback

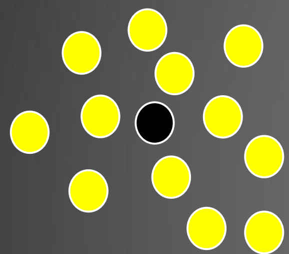
1. There should be two kinds of interactions between the pigment cells.

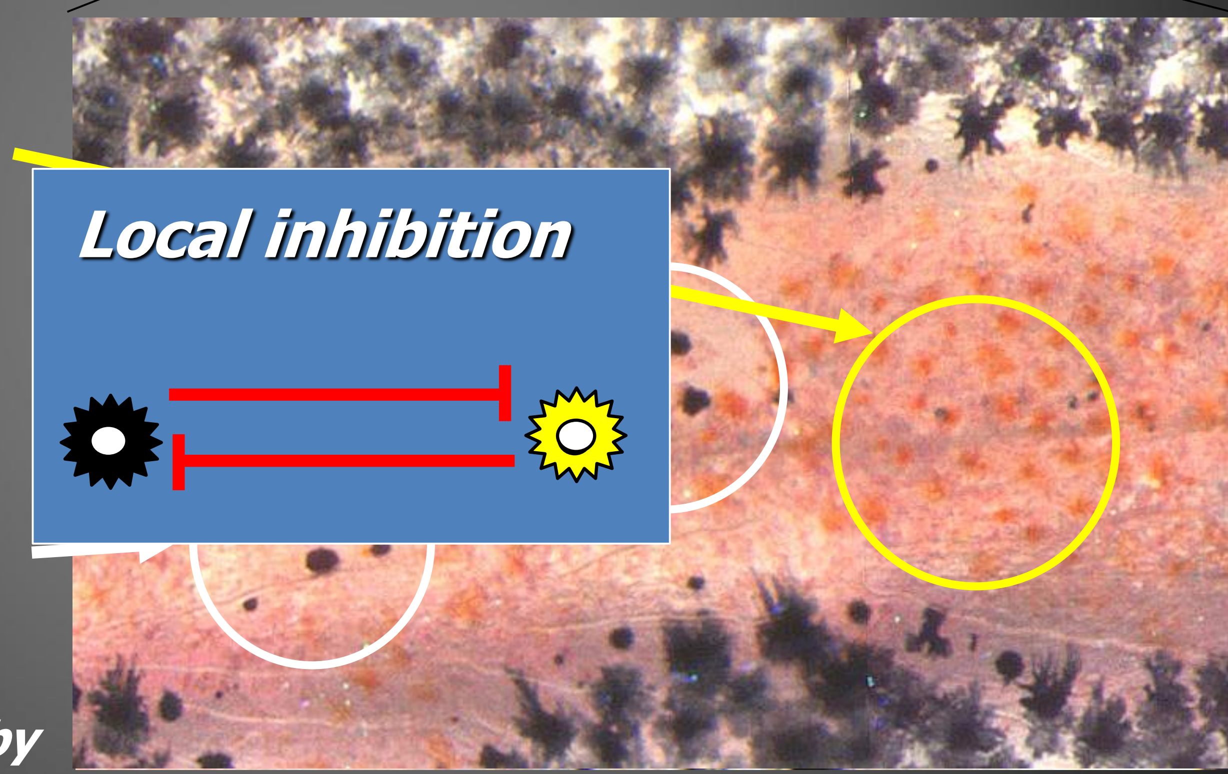
2. Functional distance of the interactions should be different.

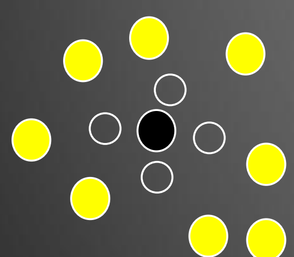
Local interaction between the pigment cells



M is surrounded by Xs



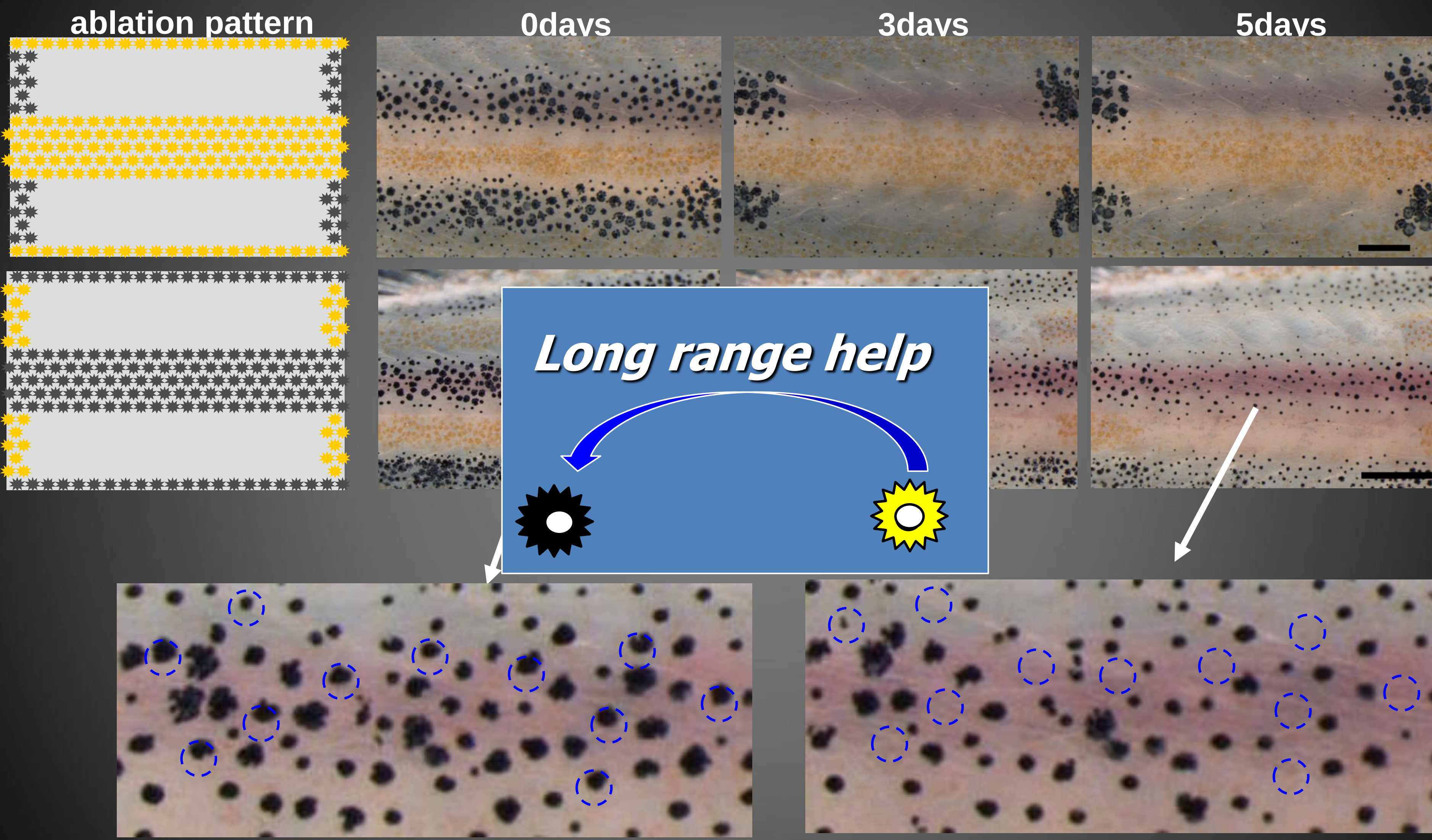




Nearest Xs are killed by laser

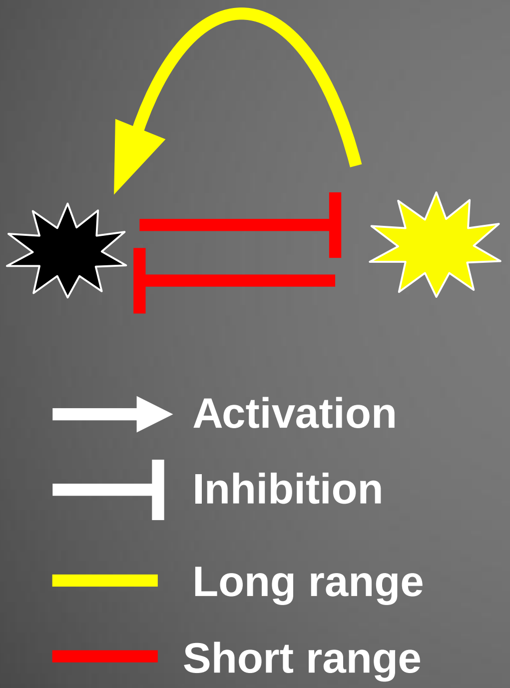
PNAS 2007 Nakamasu, Takahashi and Kondo

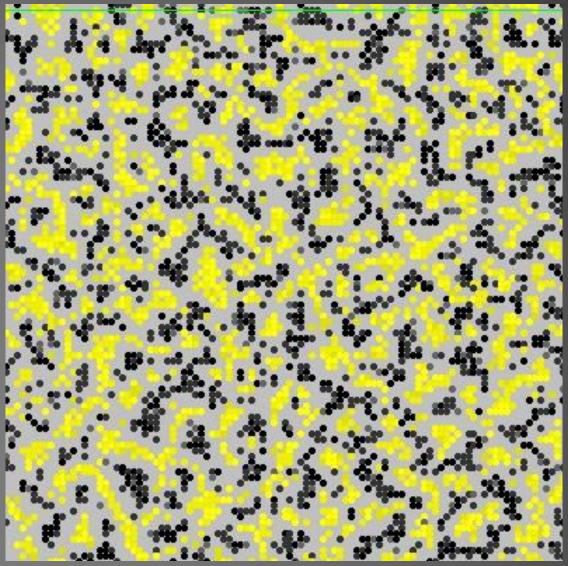
Long range interaction between the pigment cells



PNAS 2007 Nakamasu, Takahashi and Kondo

Simulation of deduced network forms the stripes





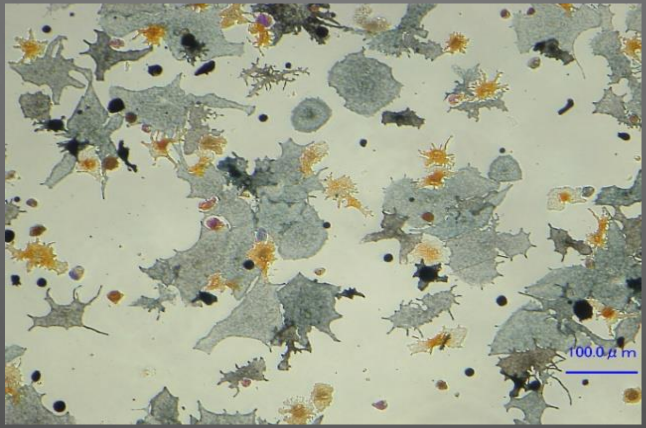
Next to do is finding the molecular and cellular basis of these three interactions

Identifying the molecular mechanism

1) Cloning of some responsible genes

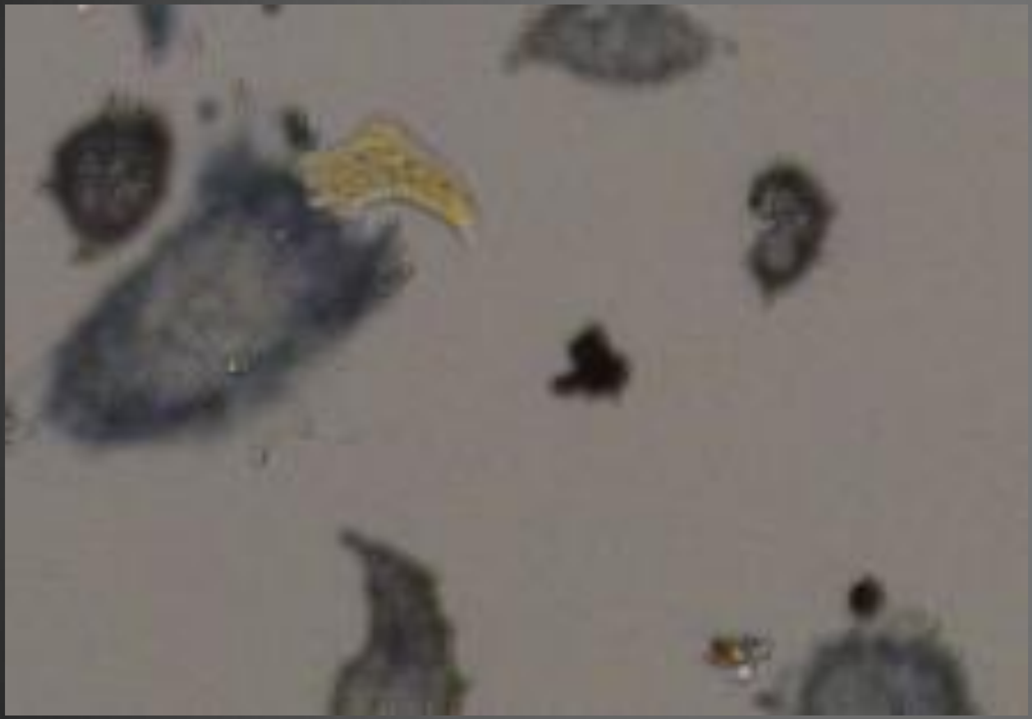
+/+ ***jaguar*** ***leopard***

2) Observation of in vitro interaction of pigment cells

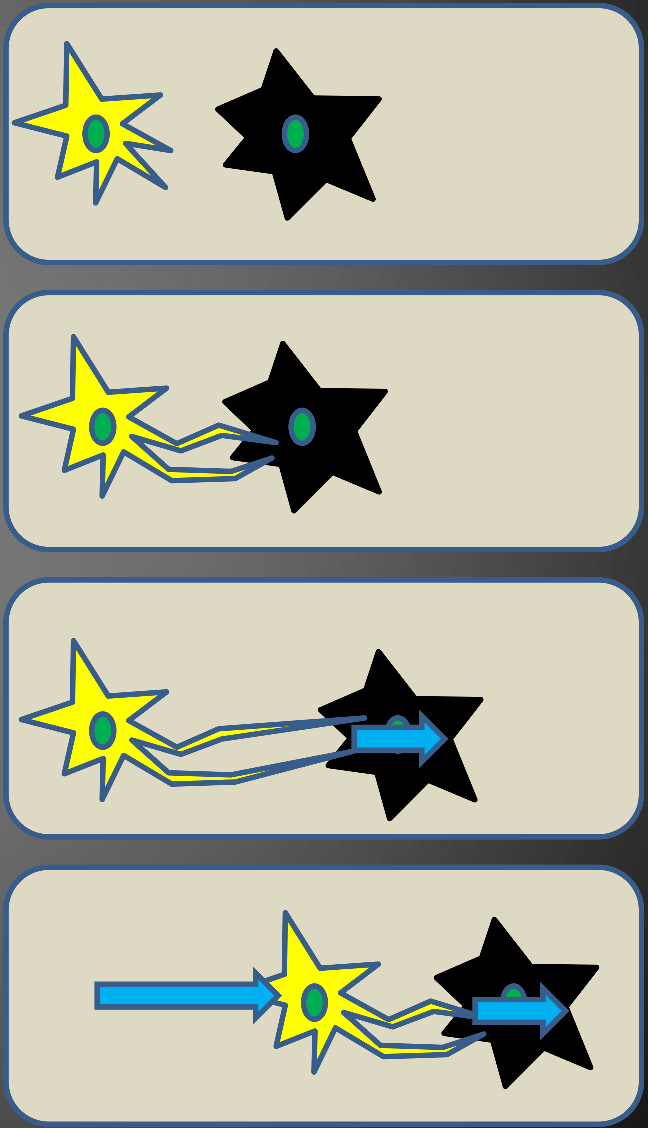


Time lapse observation

In vitro behavior of pigment cells



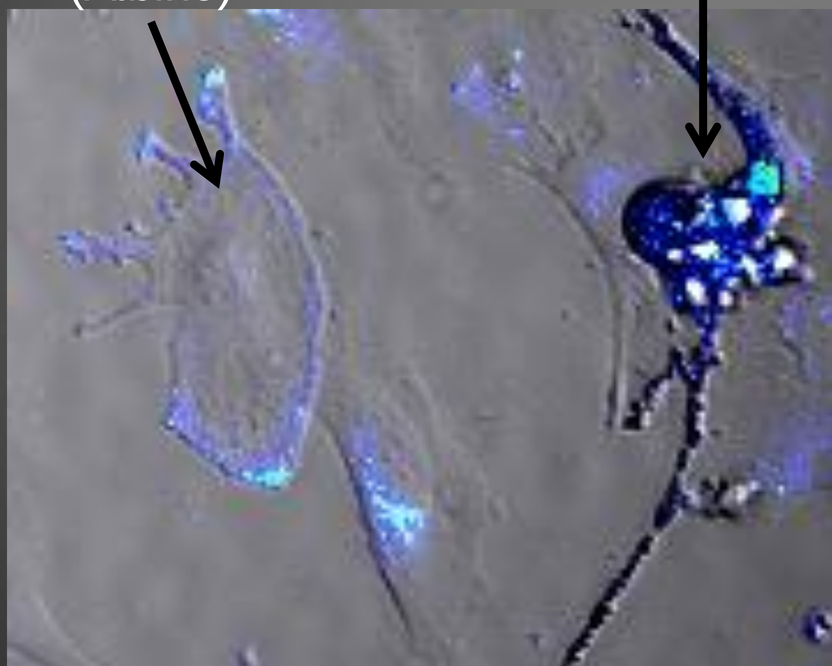
Wild type

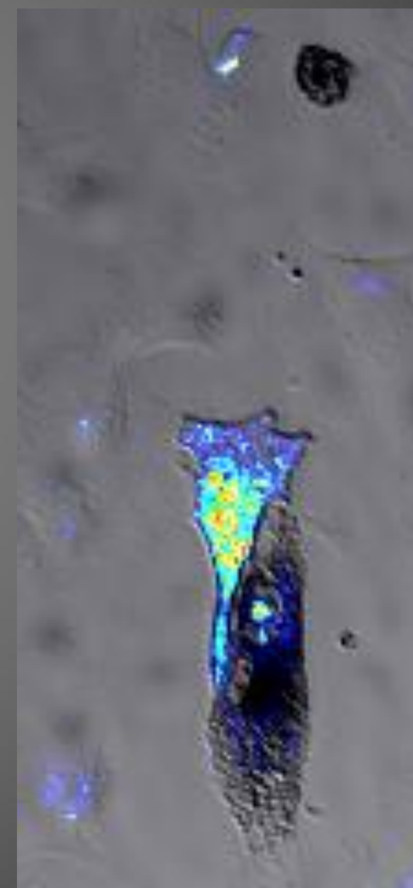


Melanophores depolarize when touched by xanthophores

Melanophore (Albino)

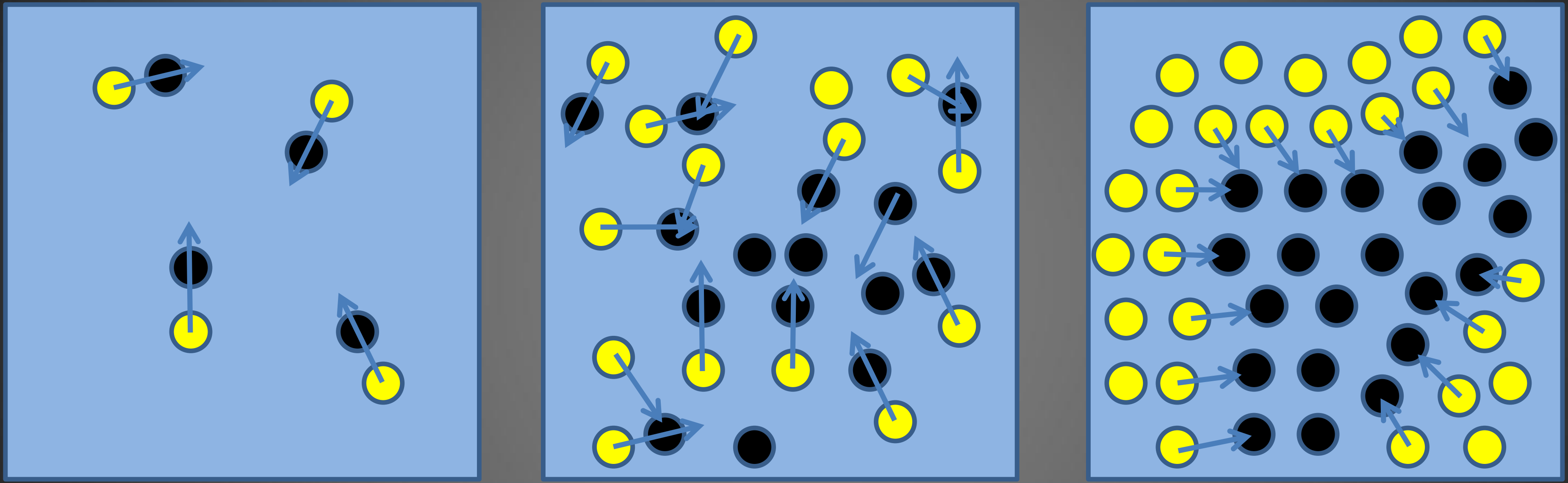
xanthophore





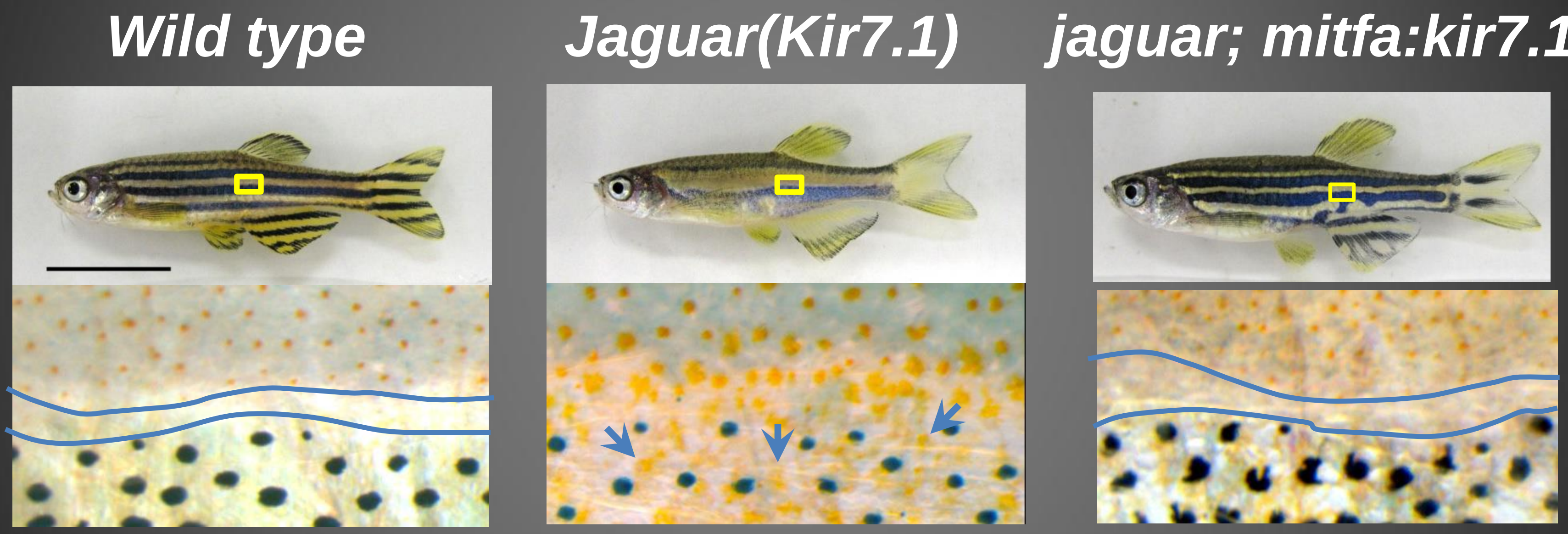
Science 2012; Inaba, Yamanaka and Kondo

If the run-and-chase movement continues……



melanophores and xanthophores will segregates.

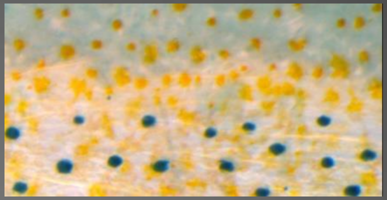
Separation is impaired in Kir7.1 mutant



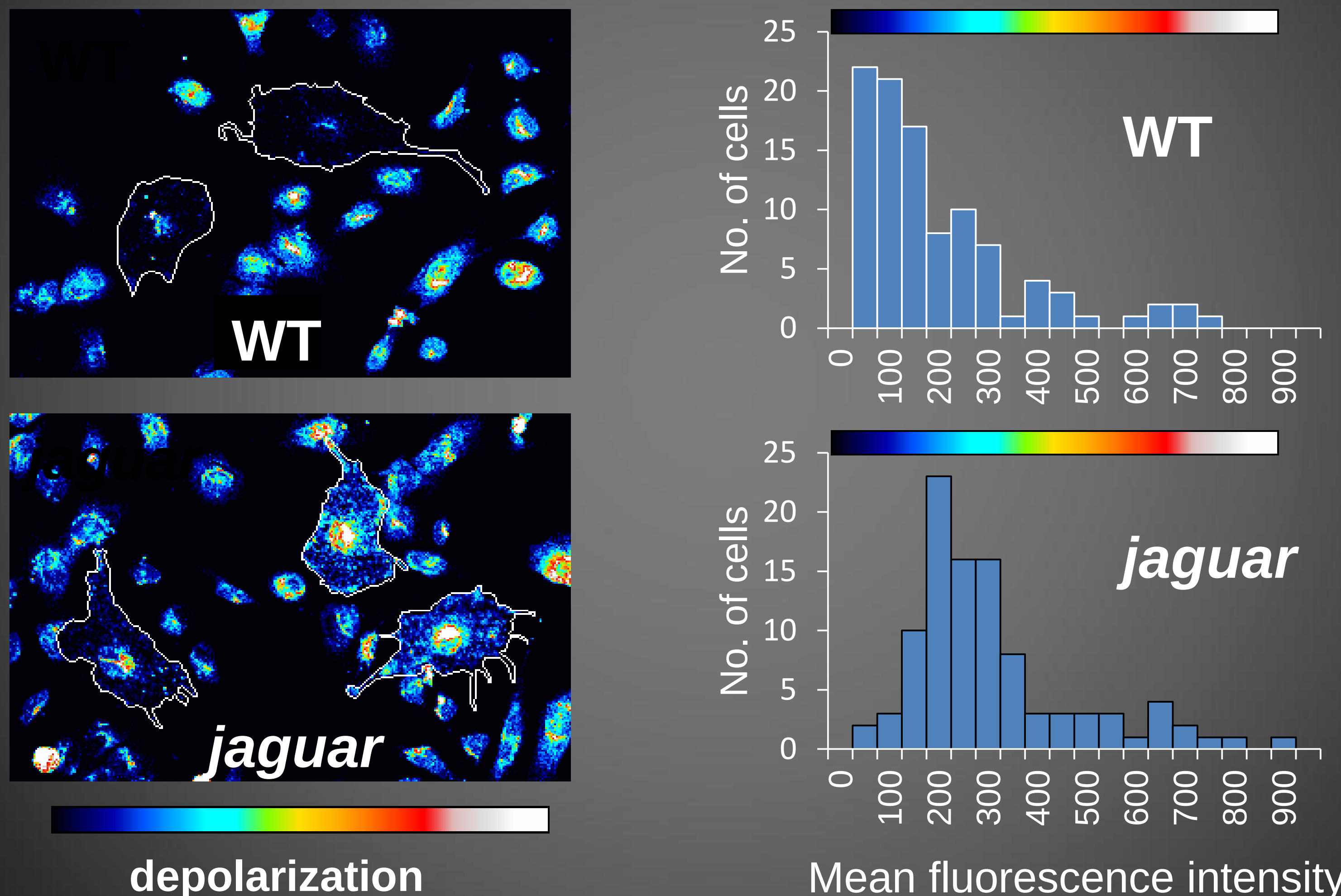
Kir7.1 is expressed in melanophore

Behavior of pigment cells in Kir7.1 mutant,

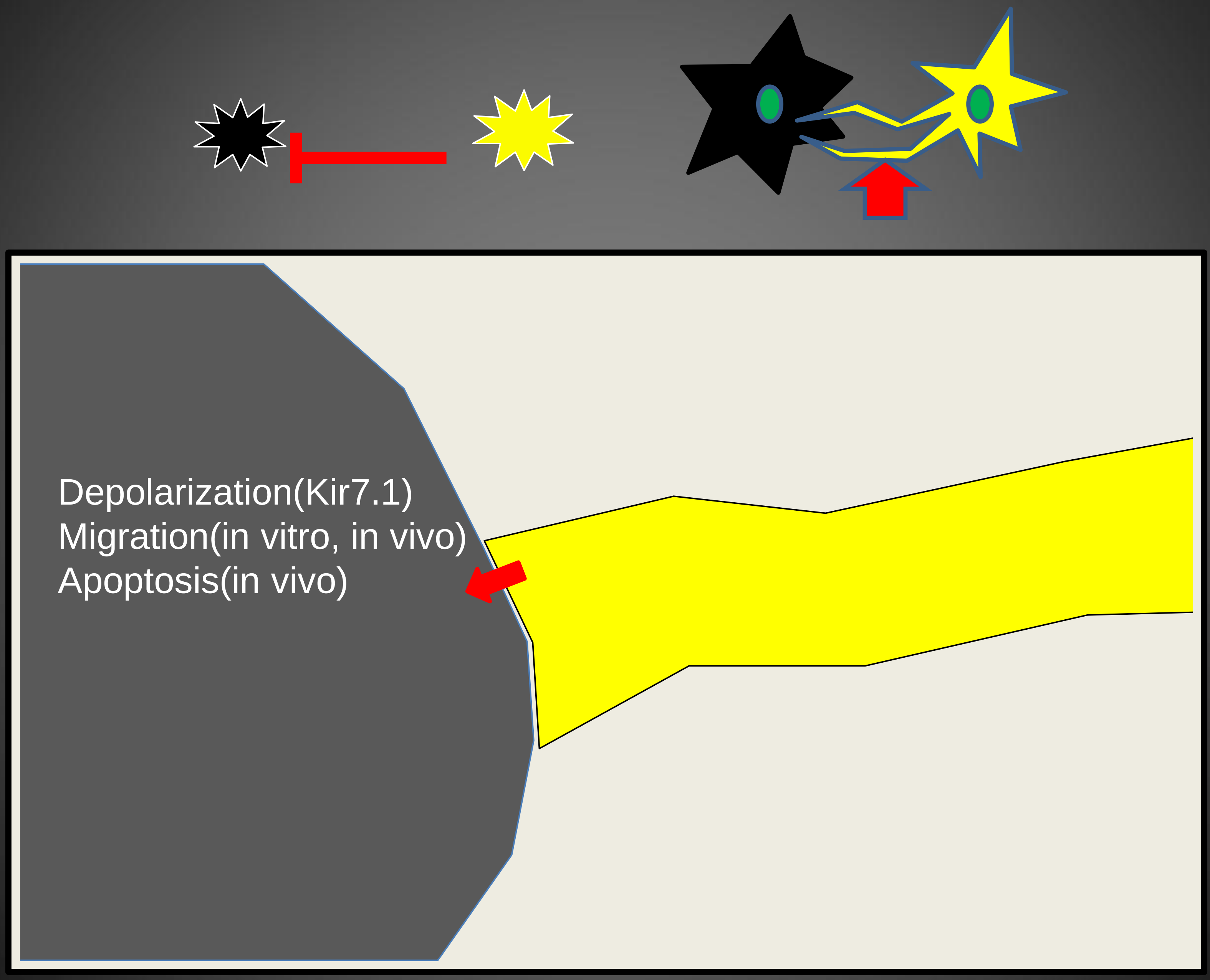
Kir7.1 is expressed only in melanophores(black cells)



Without Kir7.1, melanophores are always depolarized (=activated)

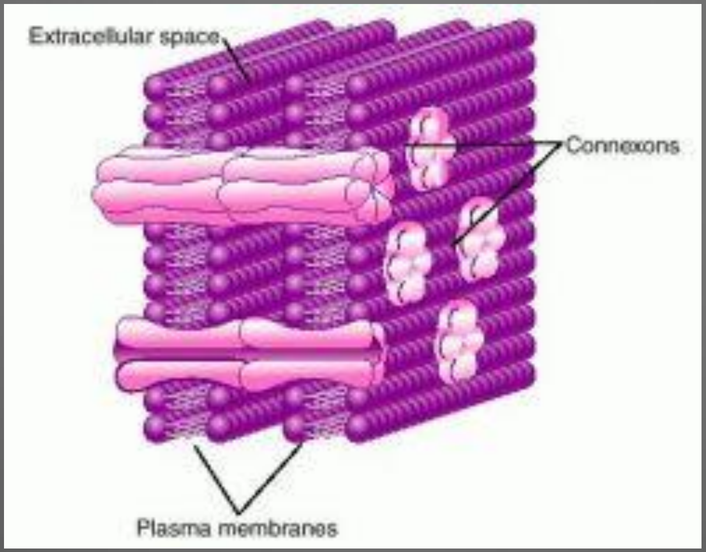


Depolarization is detected by DiBAC reagent that accumulates in depolarized cells.



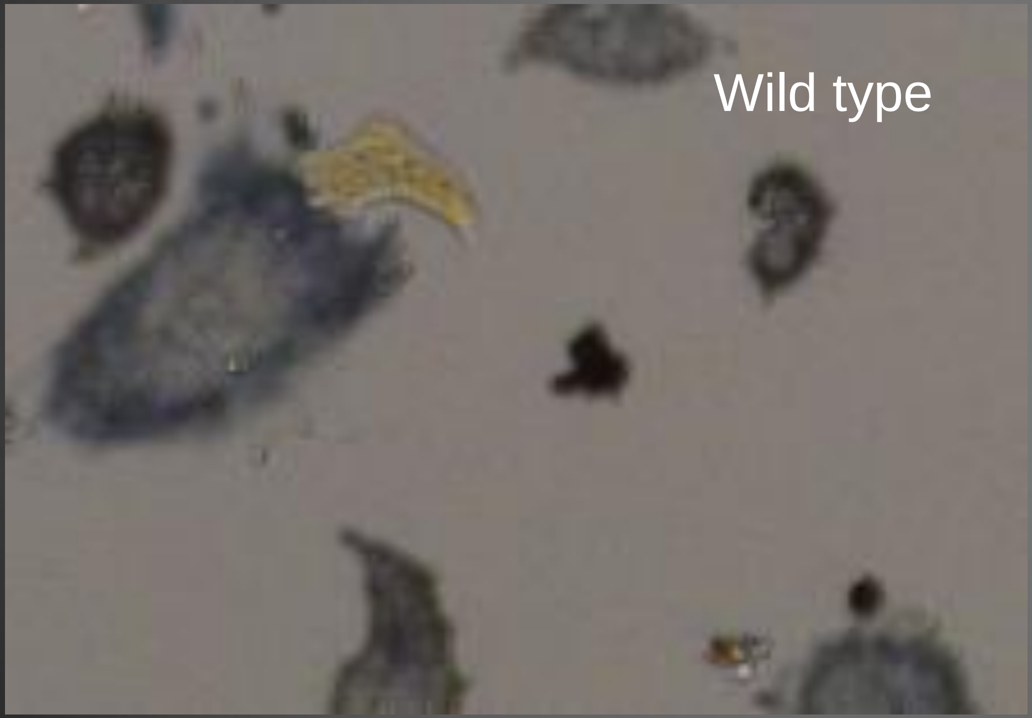
Depolarization(Kir7.1)
Migration(in vitro, in vivo)
Apoptosis(in vivo)

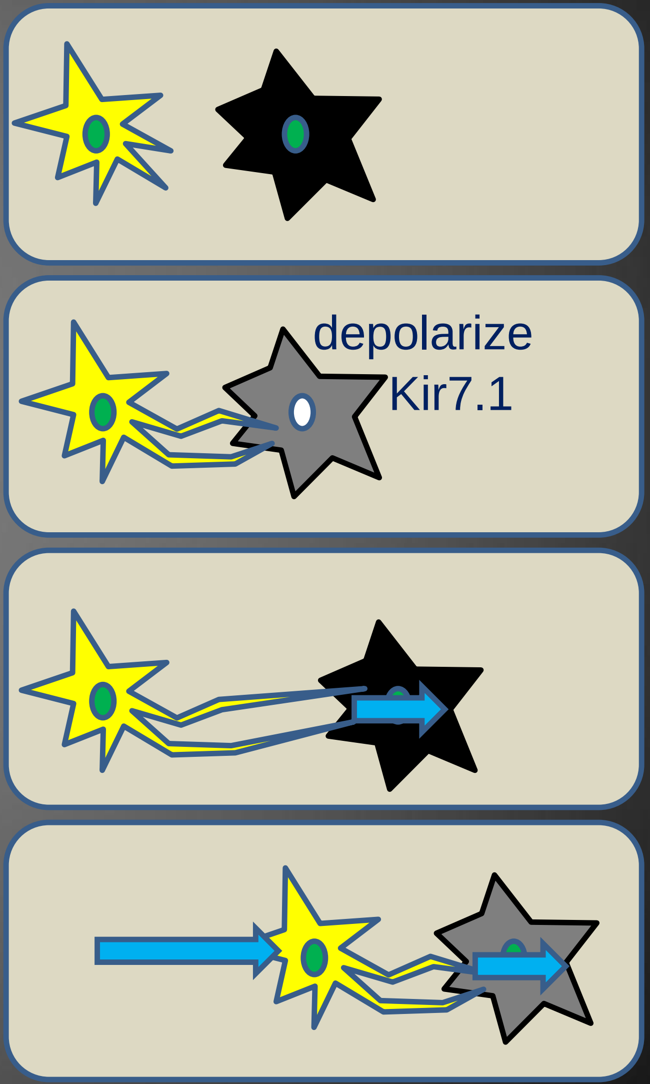
cx418 (gap junction)



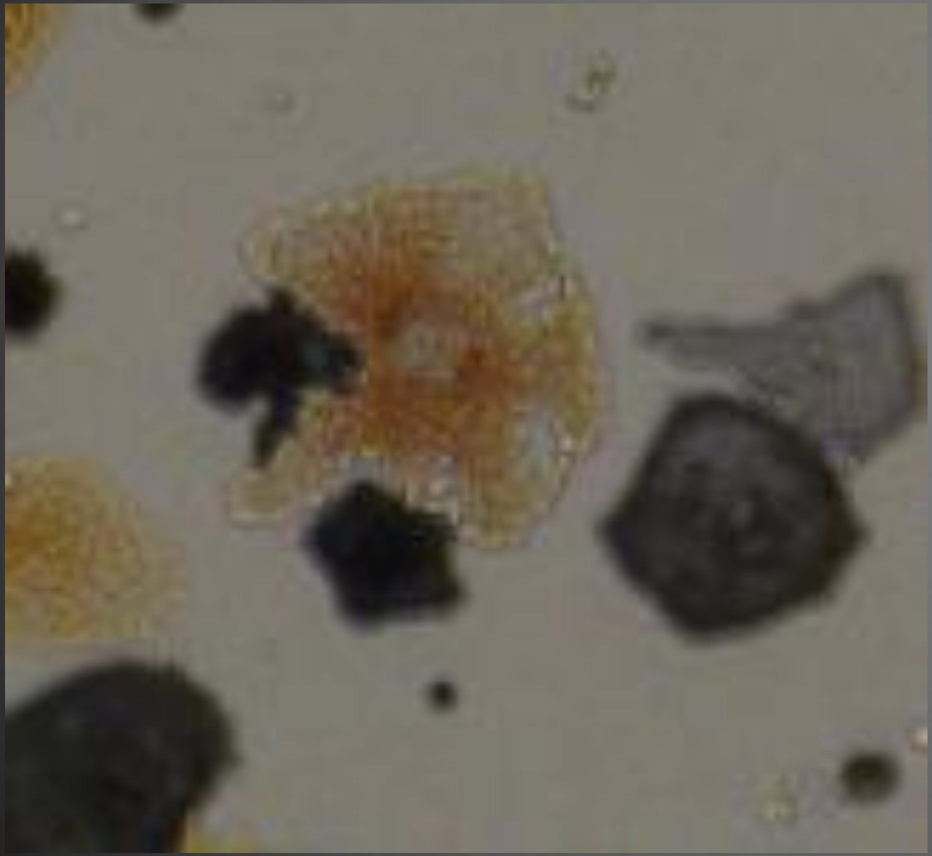
Expressed in both melanophores and xanthophores

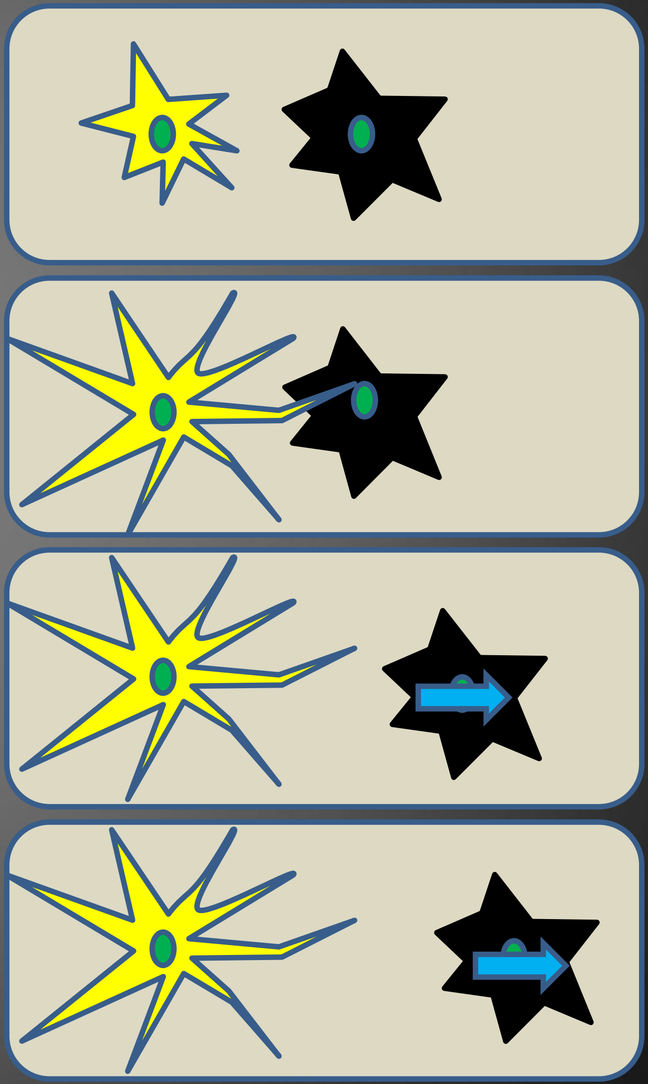
In vitro behavior of pigment cells



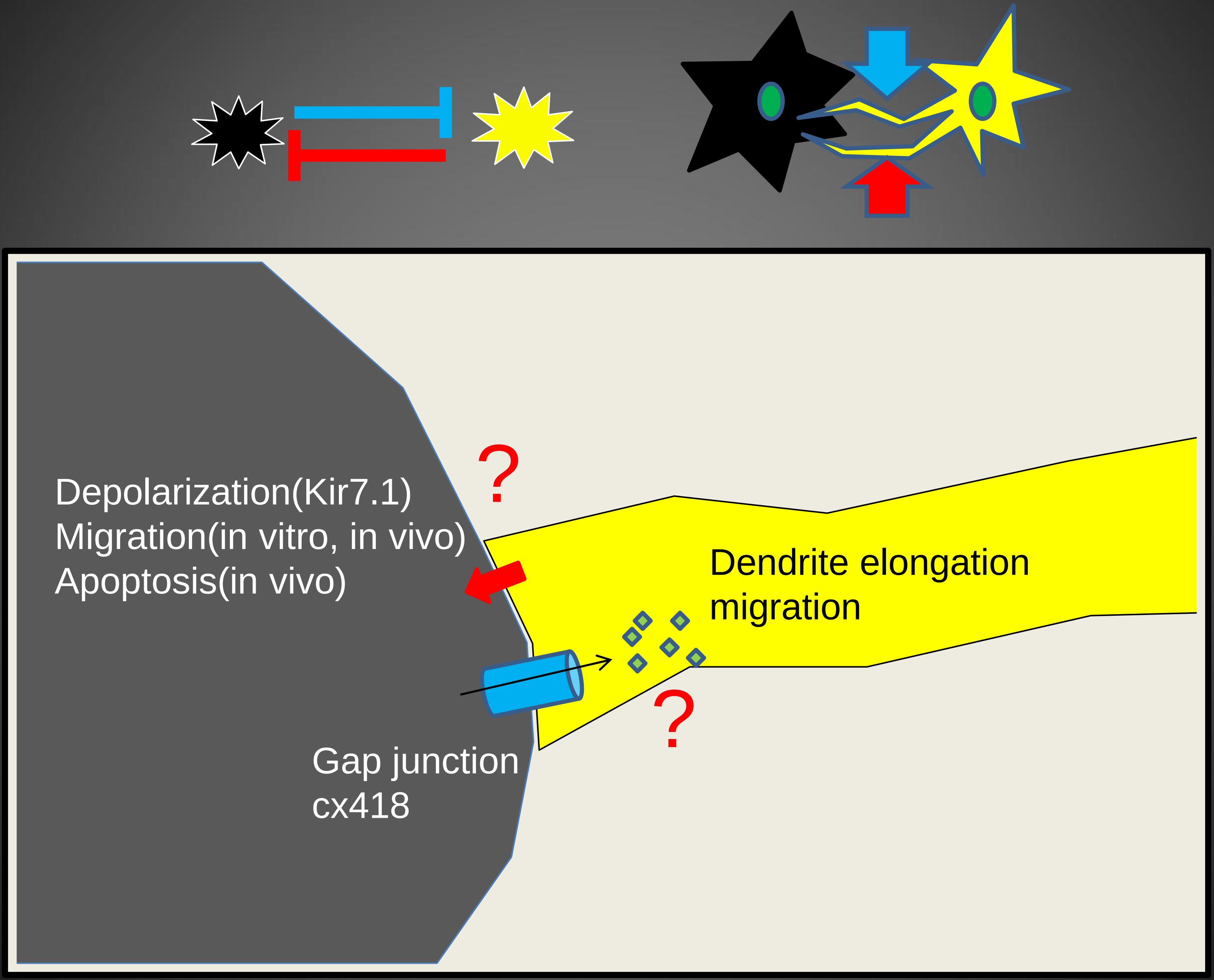


Pigment cell behavior of gap junction mutant





Xanthophores looks unable to sense melanophores.

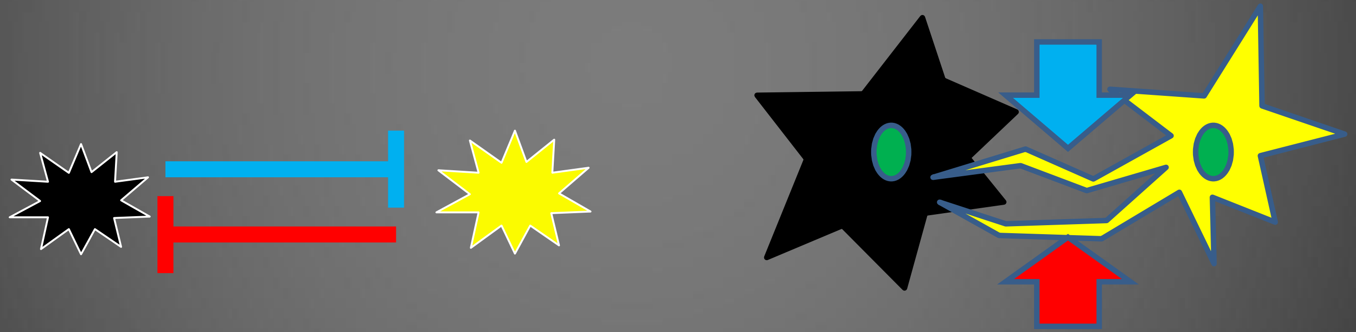


Depolarization(Kir7.1)
Migration(in vitro, in vivo)
Apoptosis(in vivo)
?
Dendrite elongation
migration
?
Gap junction
cx418

What does the mathematical theory suggest ?

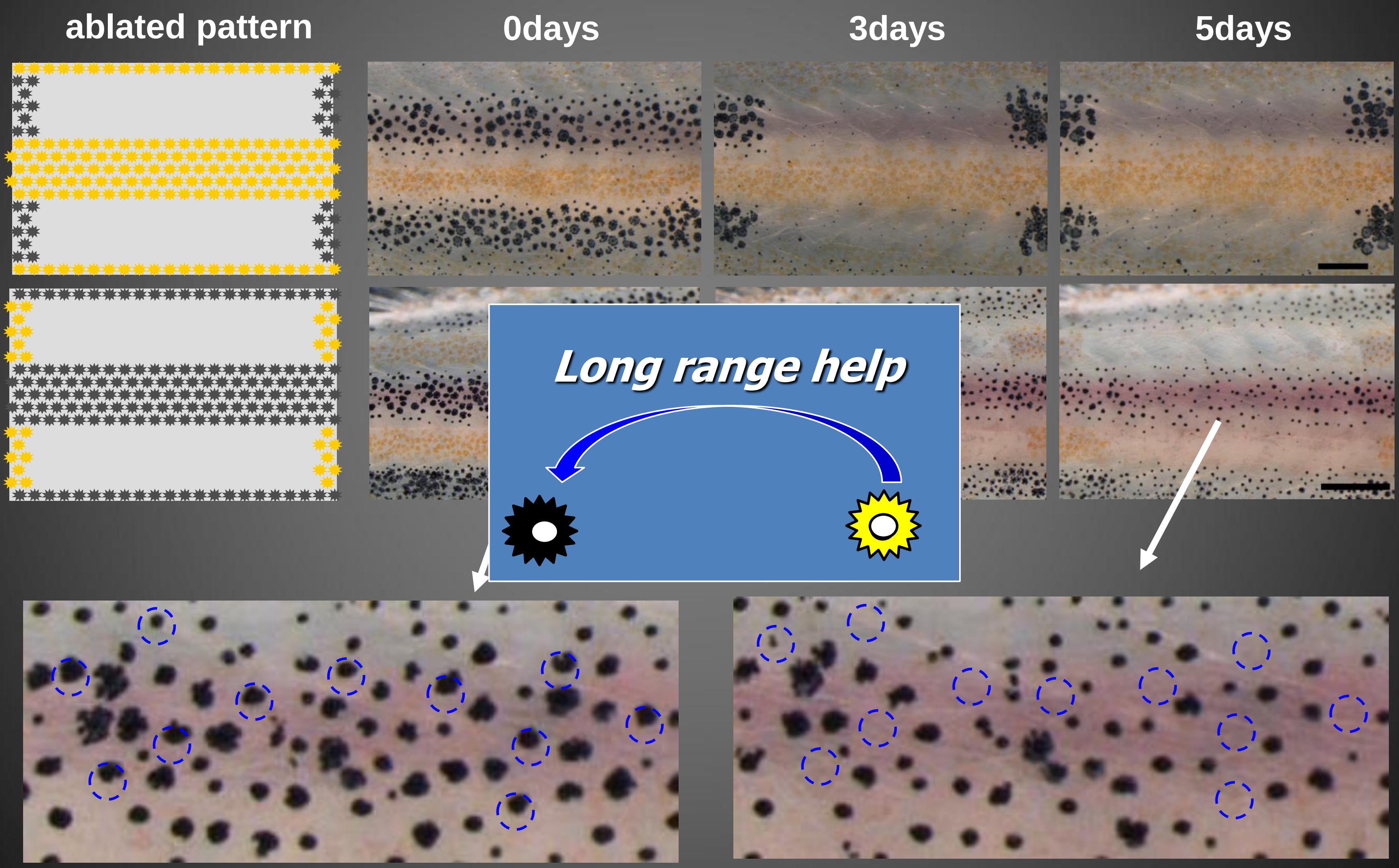
1. There should be two kinds of interactions between the pigment cells.

2. Functional distance of the interactions should be different



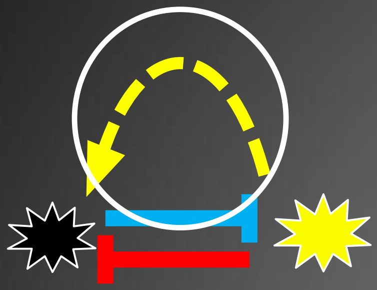
Another “ long range” signal ?

Laser ablation of xanthophores (yellow cells) induced the cell death of melanophores



PNAS 2007 Nakamasu, Takahashi and Kondo

Finding the long range factor



Assumption
Ligand is expressed exclusively in xanthophores
Receptor is expressed exclusively in melanophores

~20,000 genes

↓

482genes

↓

6 genes

↓

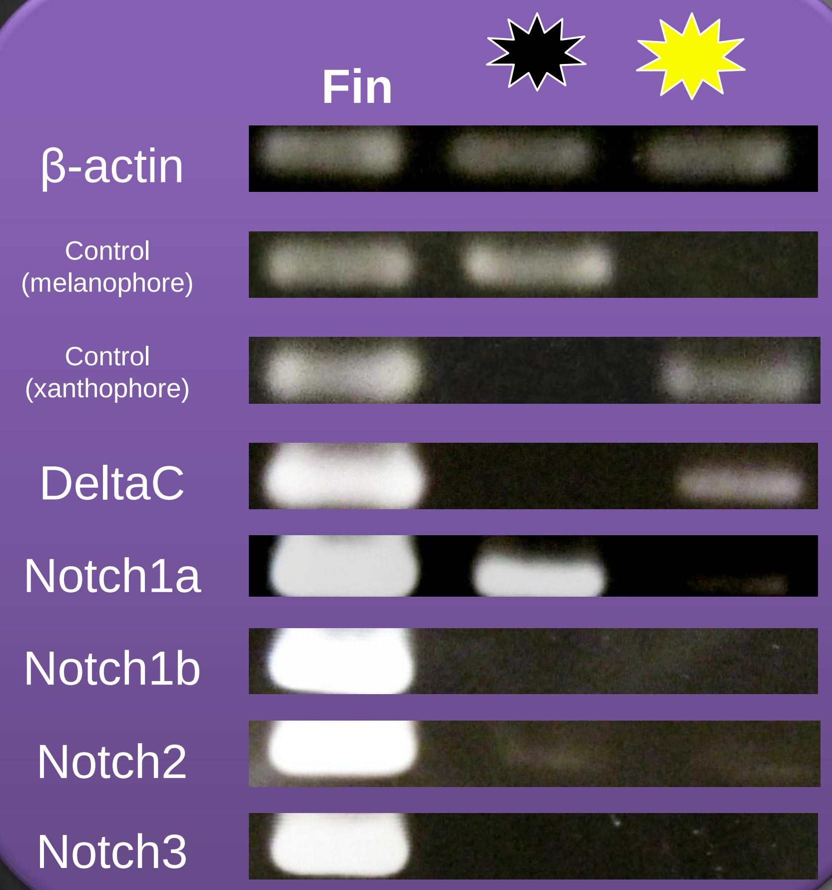
1 gene

Gene chip:
Xenthophores/melanophores > 3

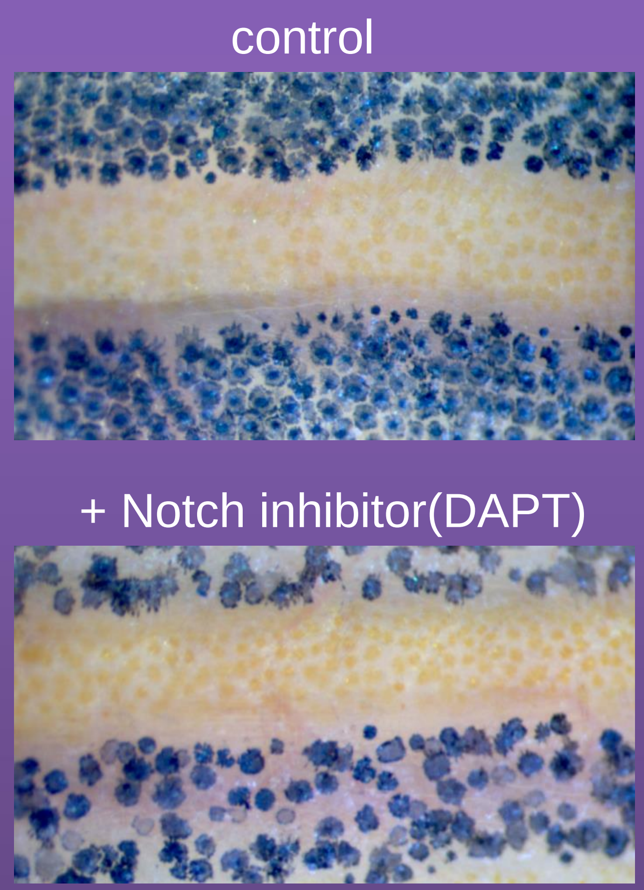
Realtime PCR:
Xenthophores/melanophores > 5
+
Protein ligands or membrane proteins

Transgenic fish:
Expression in melanophores
Alteration of the pattern?

Delta-Notch system involves in the long range effect

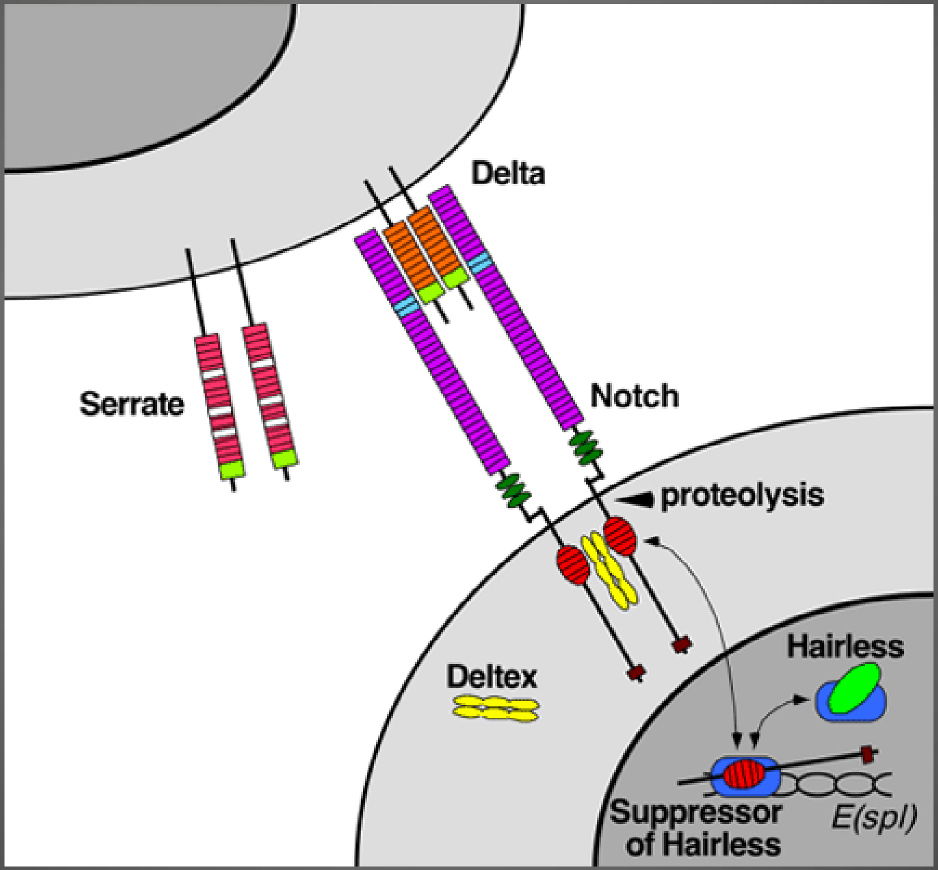


Xanthophores express DeltaC ligand
Melanophores express Notch1a receptor



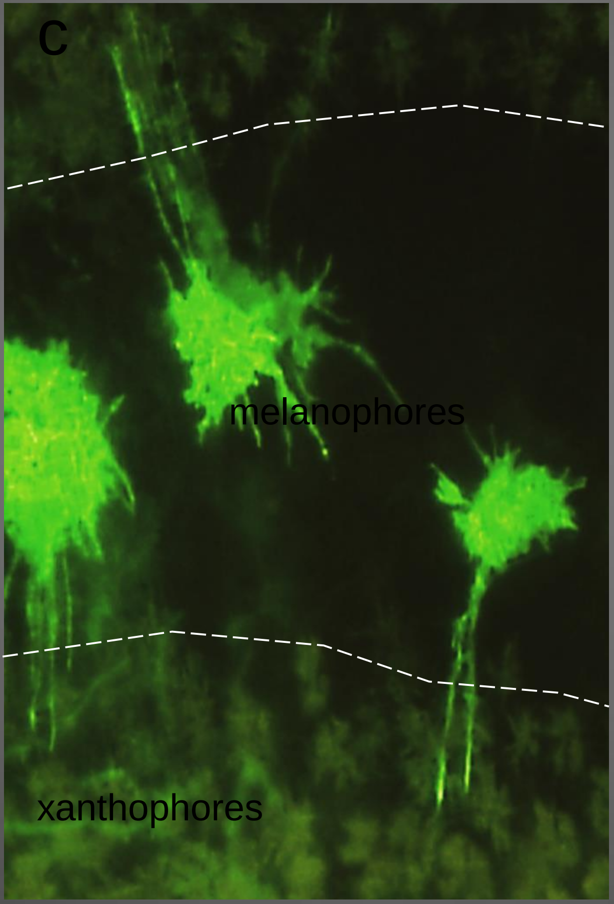
Inhibition of Notch signal
kills melanophores

Delta and Notch are the membrane bound molecules



How do they work in the long range interaction ?

Melanophores elongate long projections toward xanthophores



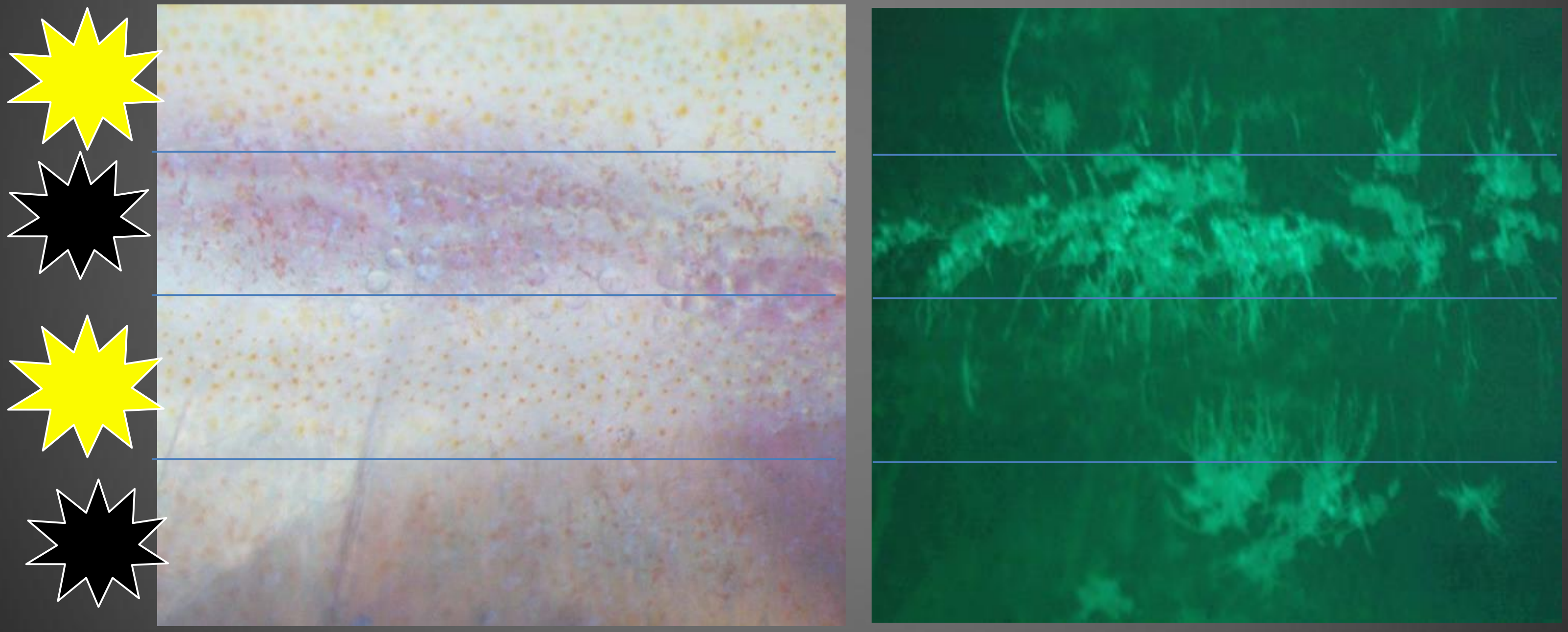
yellow stripe region

black stripe region

yellow stripe region

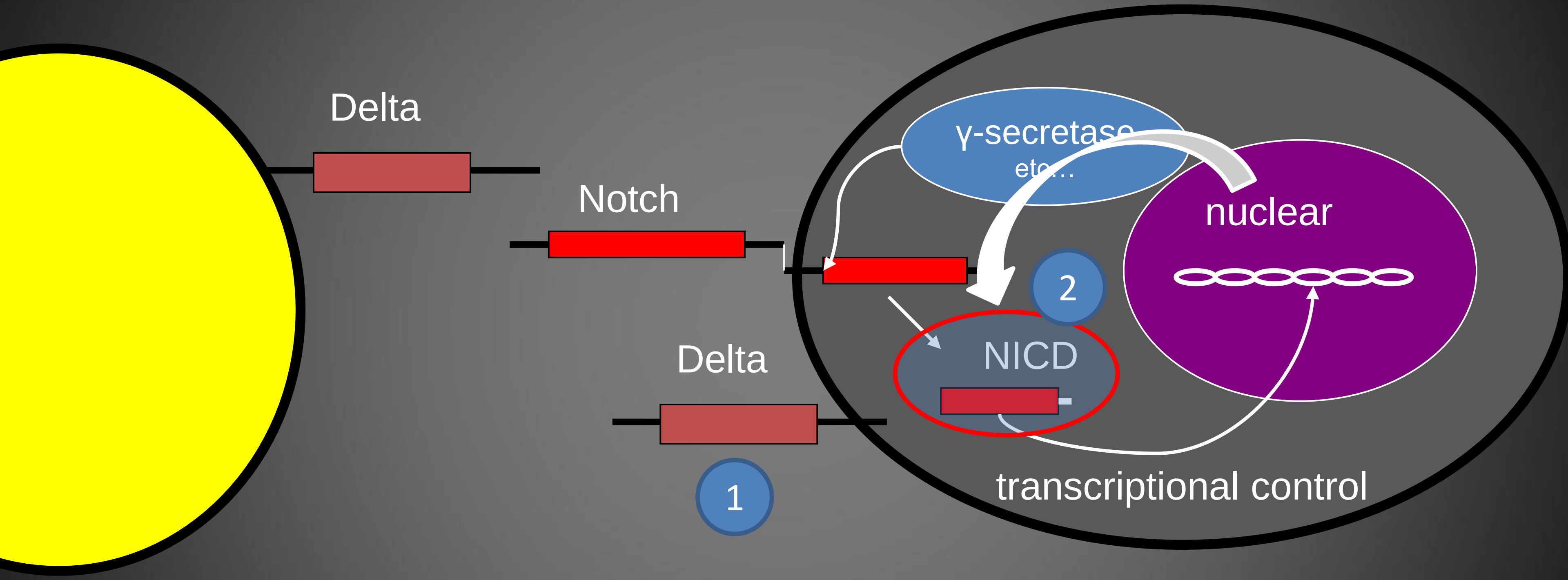
Membrane bound GFP is expressed in some of the melanophores

Melanophores elongate long projections toward xanthophores



Membrane bound GFP is expressed in some of the melanophores

Transgenic fish with Delta or Notch gene



In both cases, melanophores should become less dependent to xanthophores.

Wider stripes or large spots ??

Ectopic expression of DeltaC or Notch1a-ICD caused wider stripes

WT

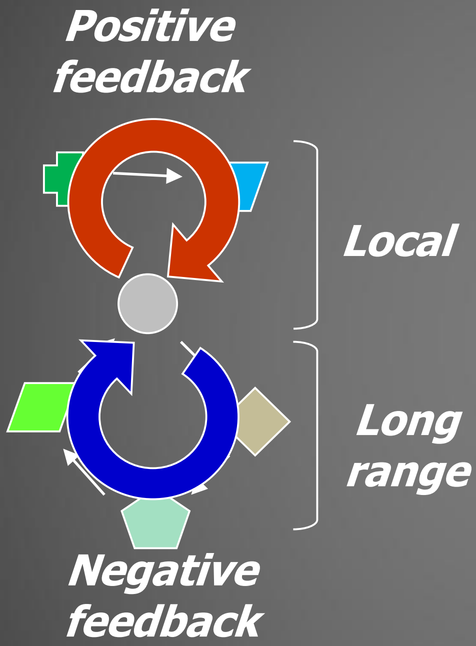
1

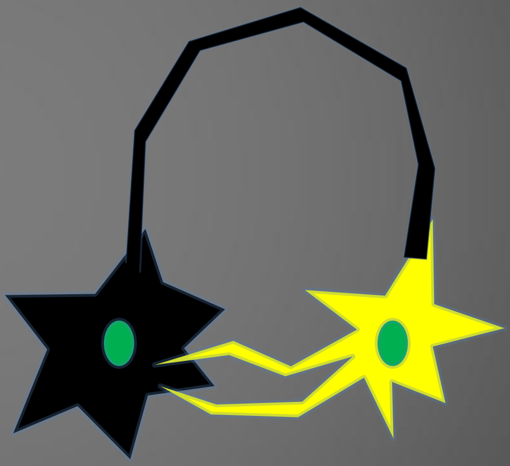
WT; DeltaC expressed in melanophores

2

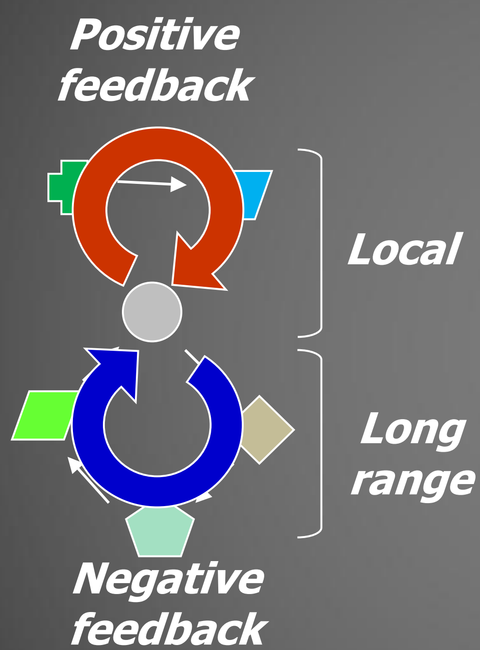
Ectopic expression of Notch-ICD in melanophore

Is the identified network a "Turing mechanism" ?





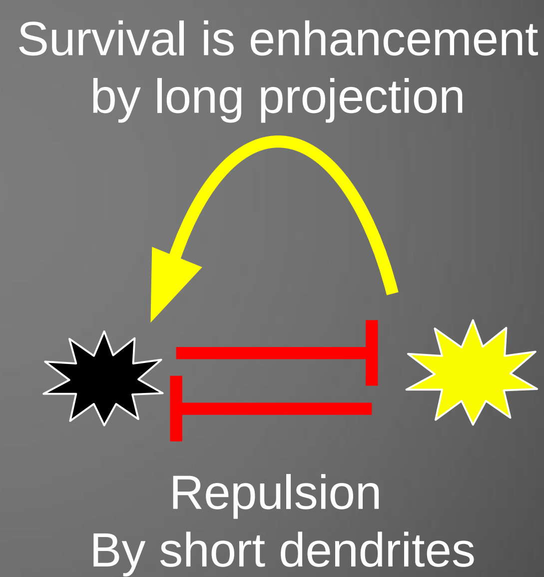
local positive feedback
+
long negative feedback



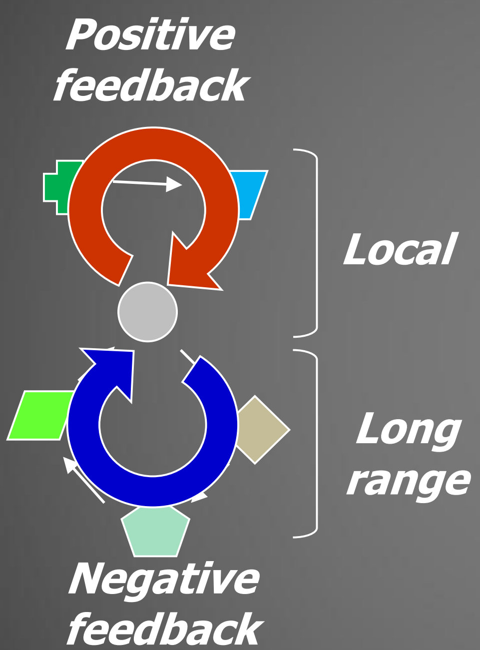
local positive feedback

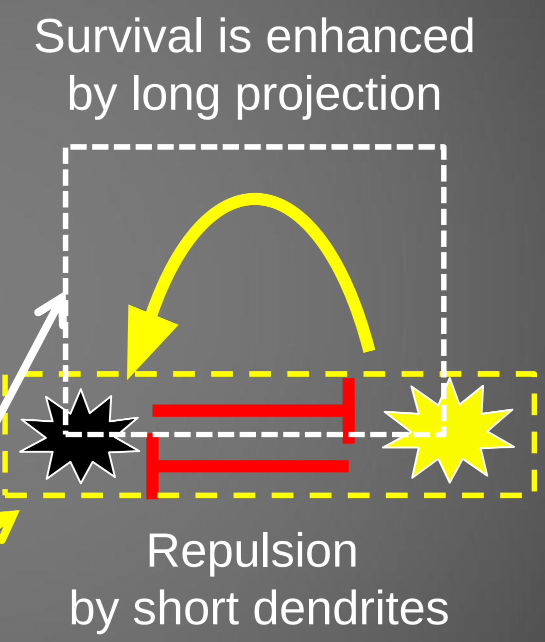
+

long negative feedback

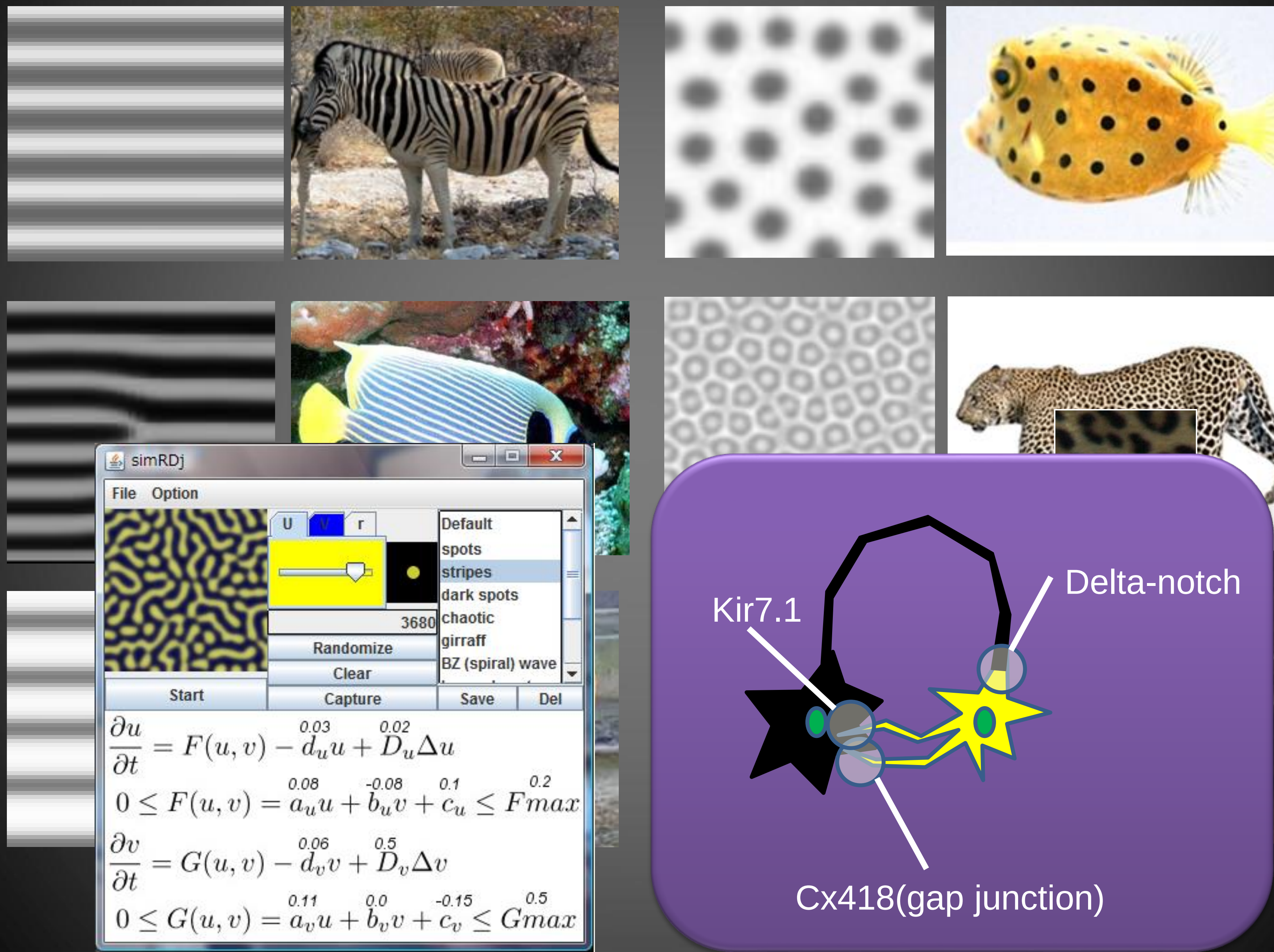


Identified network is equivalent to the Turing model.





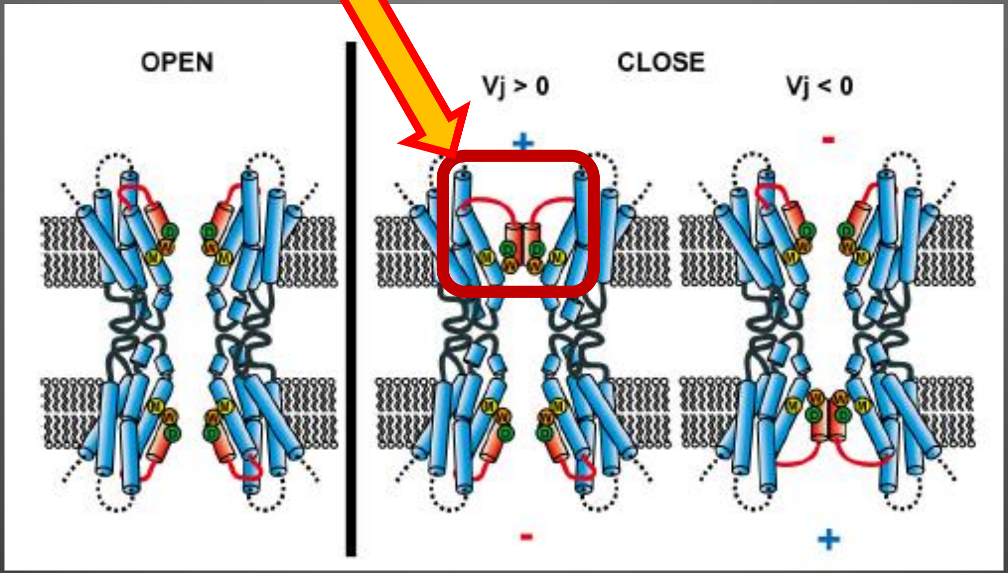
local positive feedback
+
long negative feedback

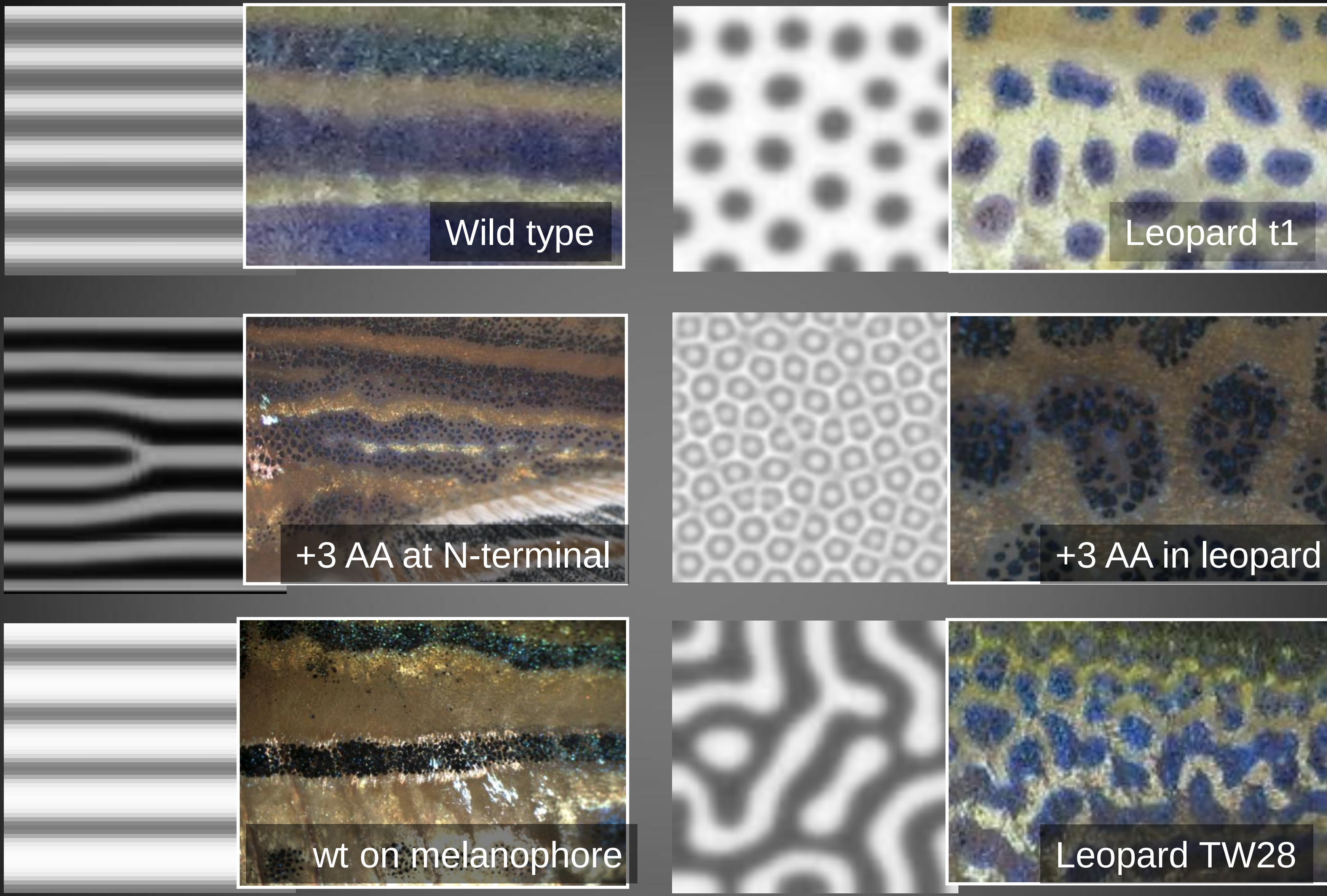

simRDj
File Option
U V r
3680
Randomize
Clear
Start
Capture
Save
Del
Default
spots
stripes
dark spots
chaotic
girraff
BZ (spiral) wave
$\frac{\partial u}{\partial t} = F(u,v) - \overset{0.03}{d_u} u + \overset{0.02}{D_u} \Delta u$
$0 \le F(u,v) = \overset{0.08}{a_u} u + \overset{-0.08}{b_u} v + \overset{0.1}{c_u} \le \overset{0.2}{Fmax}$
$\frac{\partial v}{\partial t} = G(u,v) - \overset{0.06}{d_v} v + \overset{0.5}{D_v} \Delta v$
$0 \le G(u,v) = \overset{0.11}{a_v} u + \overset{0.0}{b_v} v + \overset{-0.15}{c_v} \le \overset{0.5}{Gmax}$
Kir7.1
Delta-notch
Cx418(gap junction)

Mutation at the N-terminal of connexin changes the channel activity of gap junction

TM1

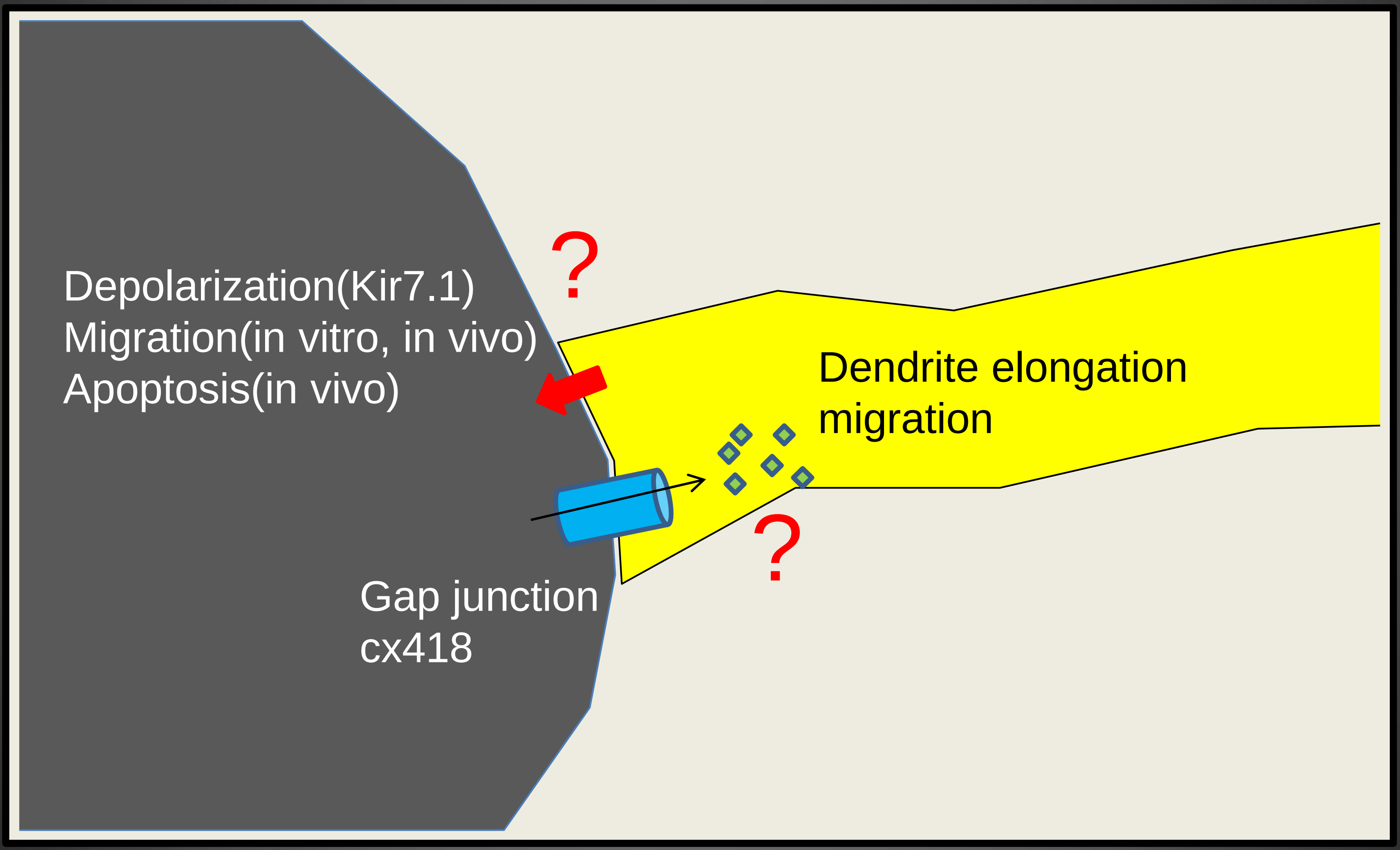
```
MKL418       MKLMADWSLLGSF LEEVQEHSTS VGKVWLTILF IFRILVLGTA AE
418(WT)         MADWSLLGSF LEEVQEHSTS VGKVWLTILF IFRILVLGTA AE
418del-6              MGSF LEEVQEHSTS VGKVWLTILF IFRILVLGTA AE
418del-10                  MEEVQEHSTS VGKVWLTILF IFRILVLGTA AE
418del-14                      MEHSTS VGKVWLTILF IFRILVLGTA AE
```





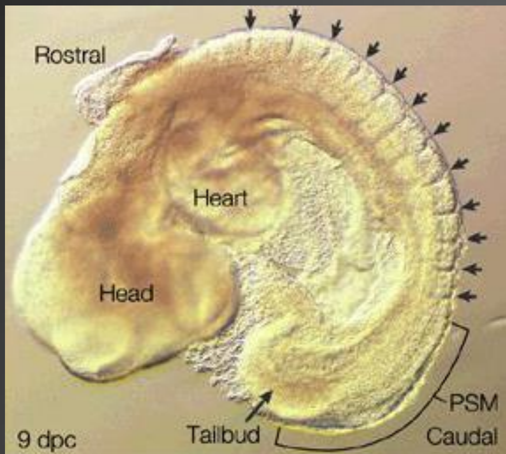
Most of the pigment patterns seen in the wild animal can be made by the modified cx418 gene.

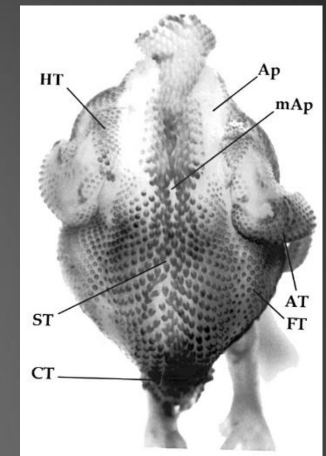
questions to be answered

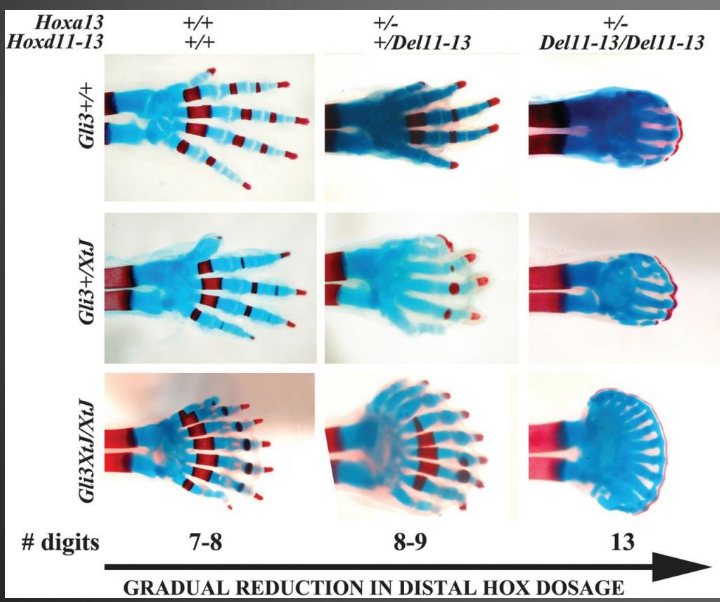


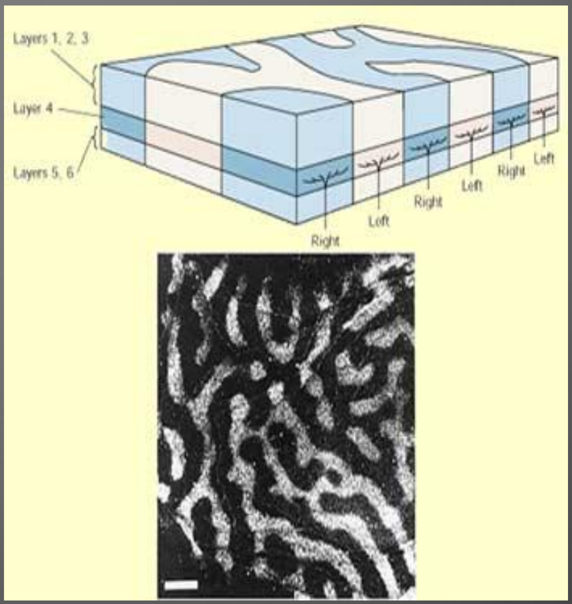
What is the signaling molecules

questions to be answered









Does Turing mechanism involve in other system?

次のフェーズへ


県以南～アラビア海を除く
ド・西部太平洋
外見からは想像できないが、透明になったことで、大きなヒレが独特のフォルムを生み出していたことがわかる。

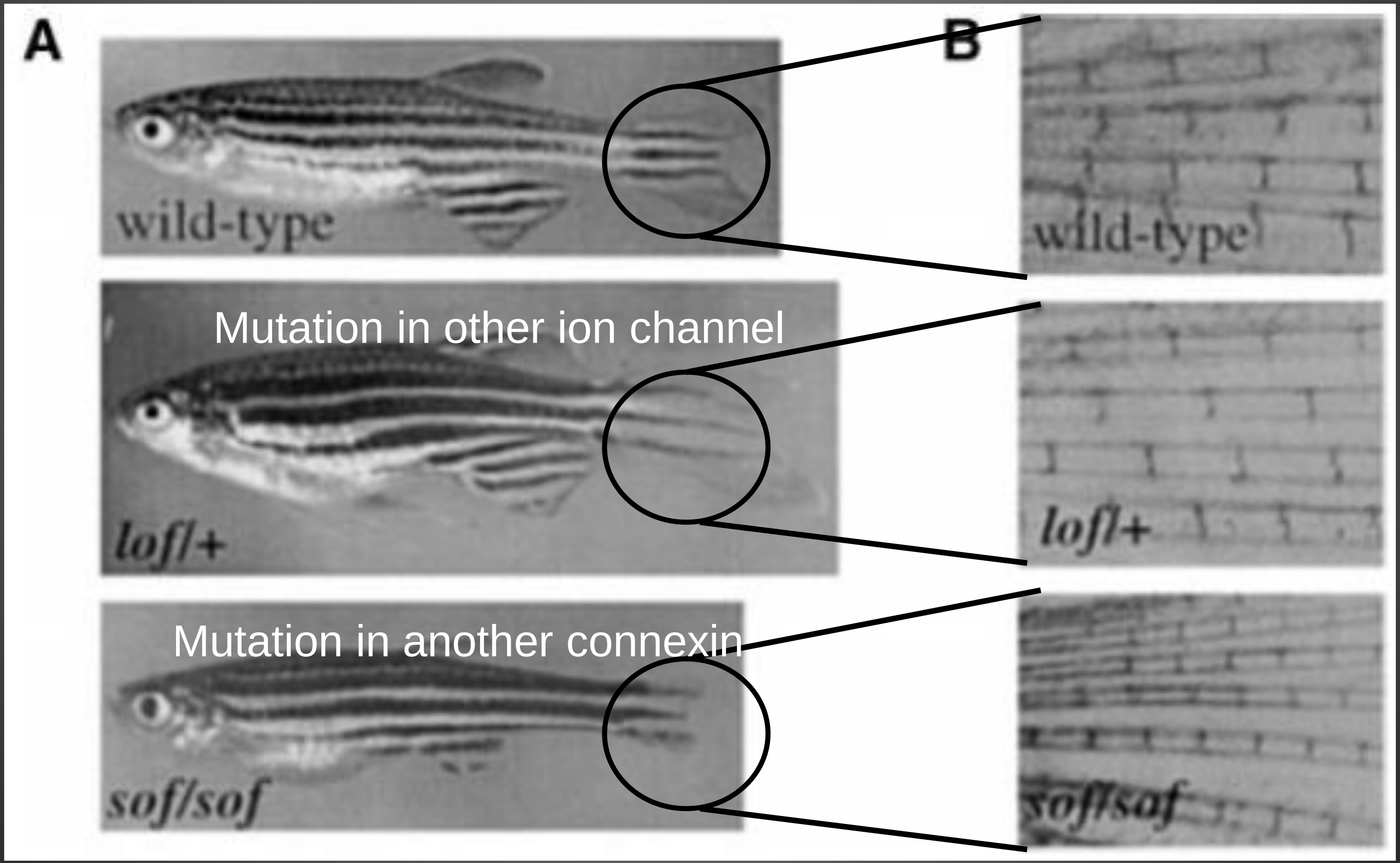
Bone shape ?

Wild type

Kir7.1
(K channel)

Connexin41.8
(gap junction)

Shape of the fin-bones may be controlled by the similar mechanism.



Johnson et al. (2010) Development


gap junction
M. Watanabe
Delta-notch
H.Hamada
Thanks for your attention
Jaguar (Kir7.1
M. Inaba
In vitro culture
H. Yamanaka
Institute of frontier bioscience
Pattern Formation Group

Pattern formation labo

Your visit is welcome !