TECHNISCHE UNIVERSITÄT DRESDEN

DRESDEN concept

Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity

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Presented by,

Srividhya Sainath

Understanding

the title of the research paper

Dynamics of Cdc42 network embodies a Turing-type mechanism of yeast cell polarity

1. Cell Polarity?
2. Cdc42 protein?
3. Turing mechanism?

Cell Polarity

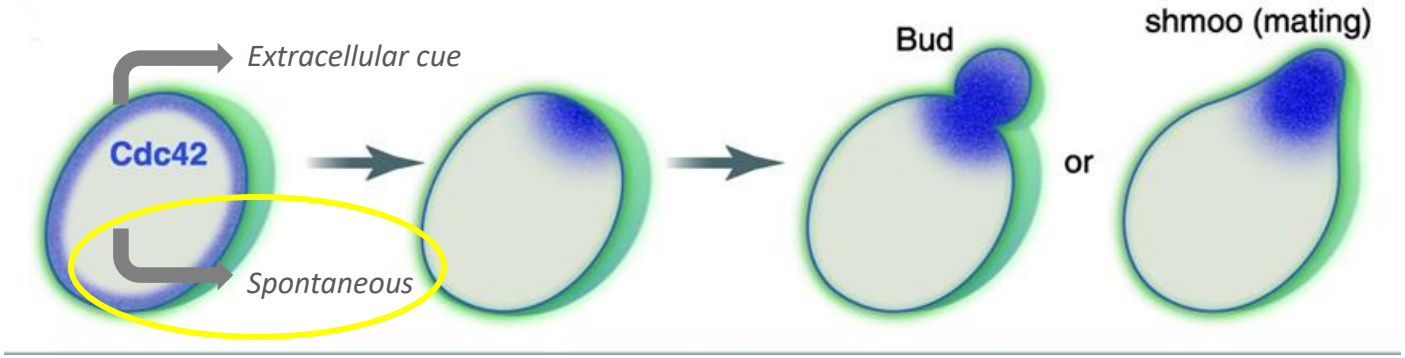
and Self-Organisation

"Cell polarity is a fundamental phenomenon in biology that is caused by the unequal distribution of a few molecules, leading to the nonuniform distribution of many other molecules, enabling cells to execute a wide variety of processes including migration, cell killing and the entirety of development." *

*- * Michael Glotzer, Anthony A. Hyman, Cell Polarity: The importance of being polar, Current Biology, Volume 5, Issue 10, 1995,Pages 1102-1105, ISSN 0960-9822*

*A Simpler definition - **Cell polarity** can be referred to as some form of spatial differences in shape, structure, or concentration of some molecules within a **cell**. Now this spatial difference enables the cell to carry out specialized functions*

Budding yeast polarization

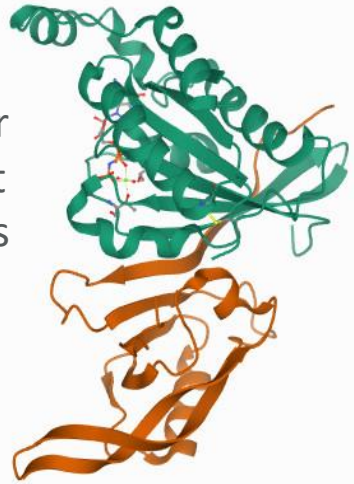


Alex Mogilner et al. Science 2012;336:175-179

Cdc42 – small GTPase protein

it's role in Yeast bud-formation

Cdc42 is a small GTPase protein that is required for cell polarity establishment in eukaryotes as diverse as budding yeast and mammals.

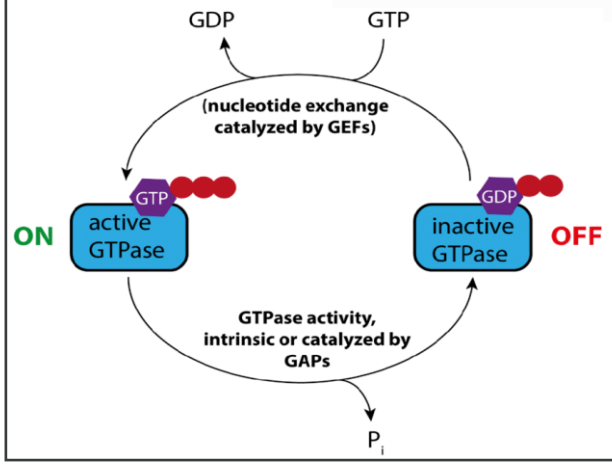


PDB ID Code – 1NF3

Cell division control protein 42, is a protein involved in regulation of the cell cycle

It has a GTP-Binding protein domain

Cdc42 exist in 2 states – the active GTP bound state and the inactive GDP bound state.



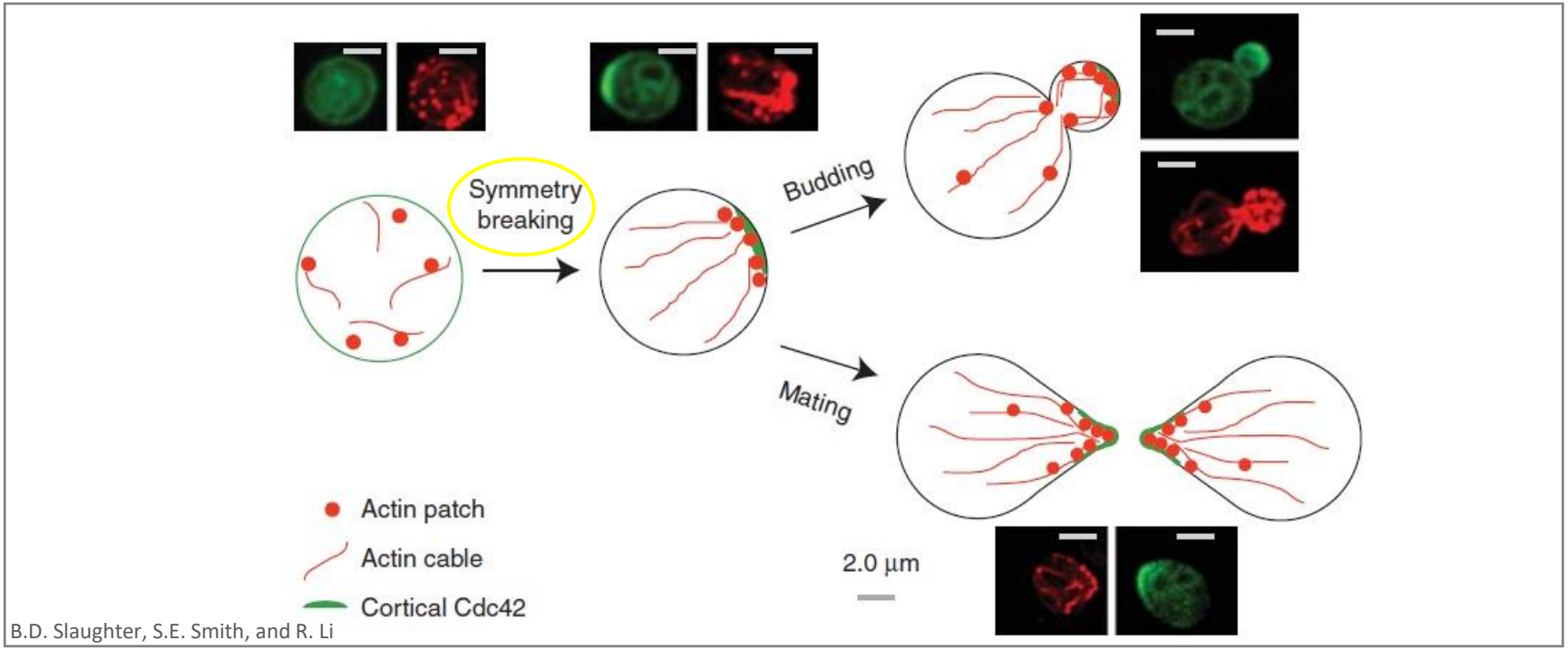
Guanine nucleotide exchange factors (GEFs) activate the GTPases by catalyzing the replacement of bound GDP by GTP.

Cdc24 plays the role of Guanine nucleotide exchange factor here. This factor is further activated in the presence of its effector Bem1

GTPase activating proteins (GAPs) deactivate the GTPases by facilitating the hydrolysis of GTP into GDP.

Cell Polarity in Budding Yeast

Symmetry breaking in detail



B.D. Slaughter, S.E. Smith, and R. Li

Symmetry breaking

Actin Dependent pathway
Actin cytoskeleton & Cdc42

Actin In-dependent pathway
Cdc42 & it's signalling network

Have positive feedback loops which can amplify small and stochastic asymmetries

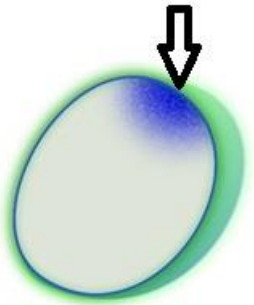
Actin-independent pathway

the Biochemistry of it ! – Part 1

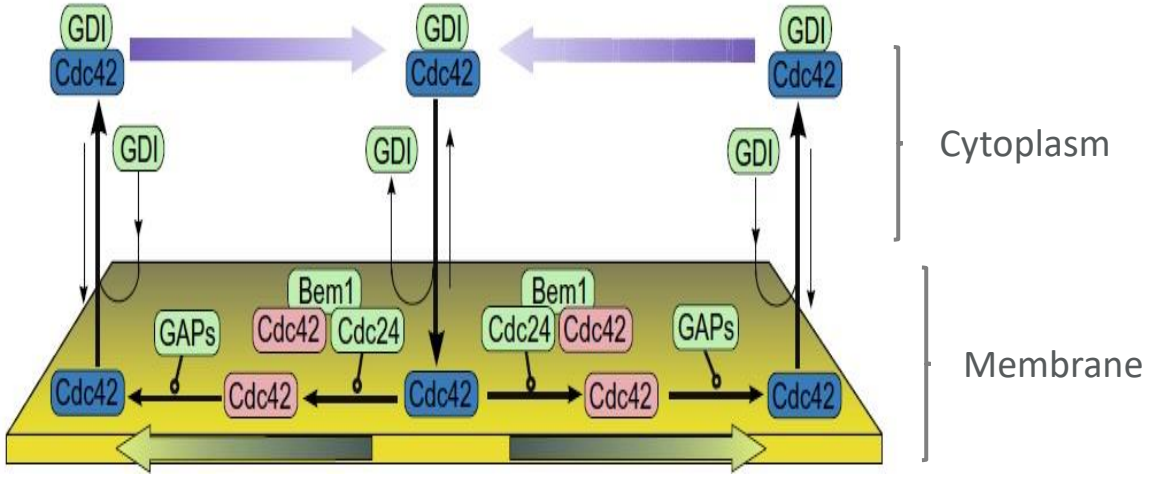
Yeast bud formation can be naturally subdivided into two consecutive phases :

- **Phase 1** - Formation of round cluster of activated Cdc42 forms on the inner leaflet of the plasma membrane.
- **Phase 2** - The growth and protrusion phase of the bud.

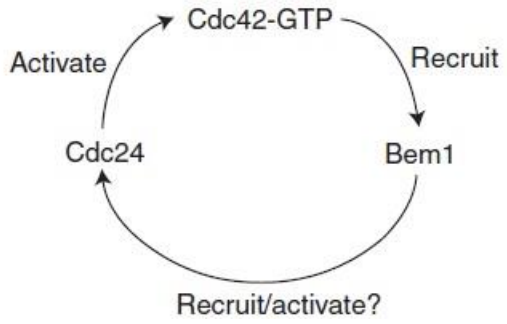
Cluster of activated Cdc42 protein on the membrane of the dividing yeast cell



Membrane-Cytoplasmic shuttling of Cdc42



Positive Feedback Loop



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Actin-independent pathway

the Biochemistry of it ! – Part 2

Components involved in this pathway

1. RD – Inactive Cdc42
2. RT – Active Cdc42
3. E – Cytoplasmic Cdc24+Bem1 complex
4. Em – Membrane bound Cdc24+Bem1 complex
5. RDIc – Cytoplasmic RD+GDI complex
6. RDIm – Membrane bound RD+ GDI complex
7. M – Membrane bound Cdc24+Bem1+RT complex
8. I – Cytoplasmic GDI

Reactions	*Rate Constant*
$Complex\ Ec \Leftrightarrow Complex\ Em$	$k\,1$
$Em + RD \Leftrightarrow RT$	$k\,2$
$M + RT \longrightarrow RT$	$k\,3$
$Em + RT \Leftrightarrow M$	$k\,4$
$RDTc \Leftrightarrow RDIm$	$k\,5$
$RD + Ic \Leftrightarrow RDIm$	$k\,6$
$Ec + RT \longrightarrow M$	$k\,7$

$$\frac{\partial RT}{\partial t} = (k_2 E_m + k_3 M)\cdot RD - k_{-2} RT - k_4 E_m \cdot RT + k_{-4} M - k_7 E_c \cdot RT + D_m \Delta RT$$

$$\frac{\partial M}{\partial t} = k_4 E_m \cdot RT - k_{-4} M + k_7 E_c \cdot RT + D_m \Delta M$$

$$\frac{\partial E_m}{\partial t} = k_1 E_c - k_{-1} E_m - k_4 E_m \cdot RT + k_{-4} M + D_m \Delta E_m$$

$$\frac{\partial E_c}{\partial t} = \eta\left[k_{-1} E_m - (k_1 + k_7 RT) E_c\right] + D_c \Delta E_c$$

$$\frac{\partial RD}{\partial t} = k_{-2} RT - (k_2 E_m + k_3 M)\cdot RD + k_{-6} RDI_m - k_6 I \cdot RD + D_m \Delta RD$$

$$\frac{\partial RDI_m}{\partial t} = k_6 I \cdot RD - k_{-6} RDI_m + k_5 RDI_c - k_{-5} RDI_m + D_m \Delta RDI_m$$

$$\frac{\partial RDI_c}{\partial t} = \eta\left[k_{-5} RDI_m - k_5 RDI_c\right] + D_c \Delta RDI_c$$

$$\frac{\partial I}{\partial t} = \eta\left[k_{-6} RDI_m - k_6 I \cdot RD\right] + D_c \Delta I$$

Governing Equations of our Model

Turing Mechanism

pattern formation in reaction-diffusion system and Turing instability part-1

" It is suggested that a system of chemical substances, called morphogens, reacting together and diffusing through a tissue, is adequate to account for the main phenomena of morphogenesis." – The Chemical Basis of Morphogenesis A.M. Turing

- A system of chemicals, **stable**, in the absence of diffusion, becomes **unstable** in the presence of diffusion
- **Stabilising** reaction kinetics plus diffusion leads to **instability.**

$$\frac{\partial U}{\partial t} = D_u \nabla^2 U + f(U, V)$$

$$\frac{\partial V}{\partial t} = D_v \nabla^2 V + g(U, V).$$

Reaction-Diffusion Equation

$$\frac{\partial f}{\partial U} + \frac{\partial g}{\partial V} < 0;$$

$$\frac{\partial f}{\partial U}\frac{\partial g}{\partial V} - \frac{\partial f}{\partial V}\frac{\partial g}{\partial U} > 0;$$

$$D_U \frac{\partial g}{\partial V} + D_V \frac{\partial f}{\partial U} > 0;$$

$$D_U \frac{\partial g}{\partial V} + D_V \frac{\partial f}{\partial U} > 2\sqrt{D_U D_V \left(\frac{\partial f}{\partial U}\frac{\partial g}{\partial V} - \frac{\partial f}{\partial V}\frac{\partial g}{\partial U}\right)};$$

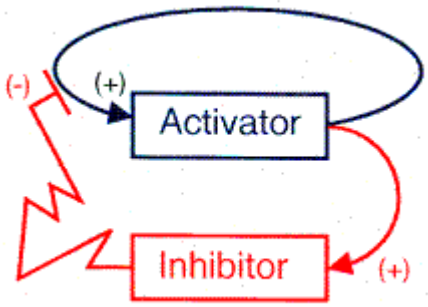
Conditions for Diffusion driven instability

Turing Mechanism

pattern formation in reaction-diffusion system and Turing instability part-2

Activator – Inhibitor Model

Short-range activator and long-range inhibitor in Gierer-Meinhardt model



To sum up

- ✓ Two key components, an activator and an inhibitor
- ✓ Both should have different diffusion rates
- ✓ Reaction involves local autocatalysis and long-ranging inhibition

Activator-Depleted Substrate Model

- ✓ The long-ranging inhibition does not necessarily require an inhibitor.
- ✓ The antagonistic effect can result from the depletion of a substrate that is consumed during the production of the autocatalytic activator.

Pattern formation via activator-depletion substrate mechanism.

Motivation & Key Results

a quick summarisation of the entire paper

Governing Equations of our Model

$$\frac{\partial RT}{\partial t} = (k_2 E_m + k_3 M)\cdot RD - k_{-2} RT - k_4 E_m \cdot RT + k_{-4} M - k_7 E_c \cdot RT + D_m \Delta RT$$

$$\frac{\partial M}{\partial t} = k_4 E_m \cdot RT - k_{-4} M + k_7 E_c \cdot RT + D_m \Delta M$$

$$\frac{\partial E_m}{\partial t} = k_1 E_c - k_{-1} E_m - k_4 E_m \cdot RT + k_{-4} M + D_m \Delta E_m$$

$$\frac{\partial E_c}{\partial t} = \eta\left[k_{-1} E_m - (k_1 + k_7 RT) E_c\right] + D_c \Delta E_c$$

$$\frac{\partial RD}{\partial t} = k_{-2} RT - (k_2 E_m + k_3 M)\cdot RD + k_{-6} RDI_m - k_6 I \cdot RD + D_m \Delta RD$$

$$\frac{\partial RDI_m}{\partial t} = k_6 I \cdot RD - k_{-6} RDI_m + k_5 RDI_c - k_{-5} RDI_m + D_m \Delta RDI_m$$

$$\frac{\partial RDI_c}{\partial t} = \eta\left[k_{-5} RDI_m - k_5 RDI_c\right] + D_c \Delta RDI_c$$

$$\frac{\partial I}{\partial t} = \eta\left[k_{-6} RDI_m - k_6 I \cdot RD\right] + D_c \Delta I$$

Motivation

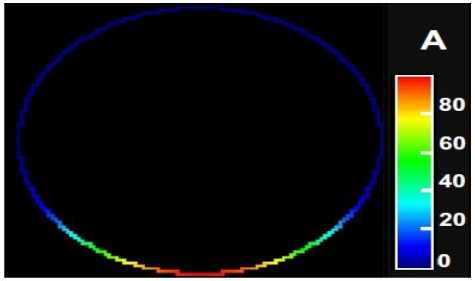
1. Do they show Turing instability ?
2. Which component here acts as the activator and the inhibitor/substrate ?
3. How robust is the Cdc42 cluster to parameter variation ?

Key Results

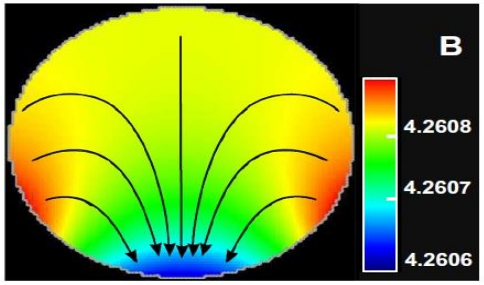
1. The core biochemical mechanism of this phenomenon can be described by a prototypical Turing-type model – the Activator Substrate model.
2. GTP-Cdc42 as the activator and GDP-Cdc42 as the substrate
3. Insensitive to changes to parameter variations, and would always form one nuclei of activated Cdc42 cluster

Results & Discussion

Spontaneous cluster formation & cluster maintenance

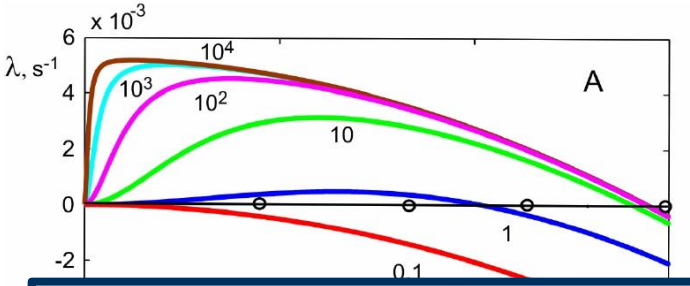


Un-equal distribution of the activated Cdc42 on the membrane (μM) after applying spatially heterogeneous perturbation



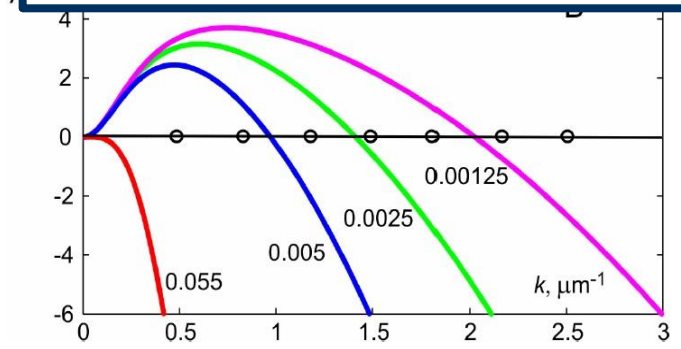
Concentration of the inactive Cdc42- GDI complex in the cytoplasm (μM) Stationary cytoplasmic flux of Inactive Cdc42-GDI (arrows) compensates diffusive spread of the cluster on the membrane.

Linear Stability Analysis



Gradual reduction of the cytoplasmic diffusion coefficient leads to a dip in the value of lambda, which is the eigenvalue of the Jacobian matrix. This decrease in lambda indicates that the system is approaching a stable state of

Gradual reduction of the cytoplasmic diffusivity, or increase in the membrane diffusivity, resulted in spreading and eventual dissolution of the cluster into a stable uniform state.

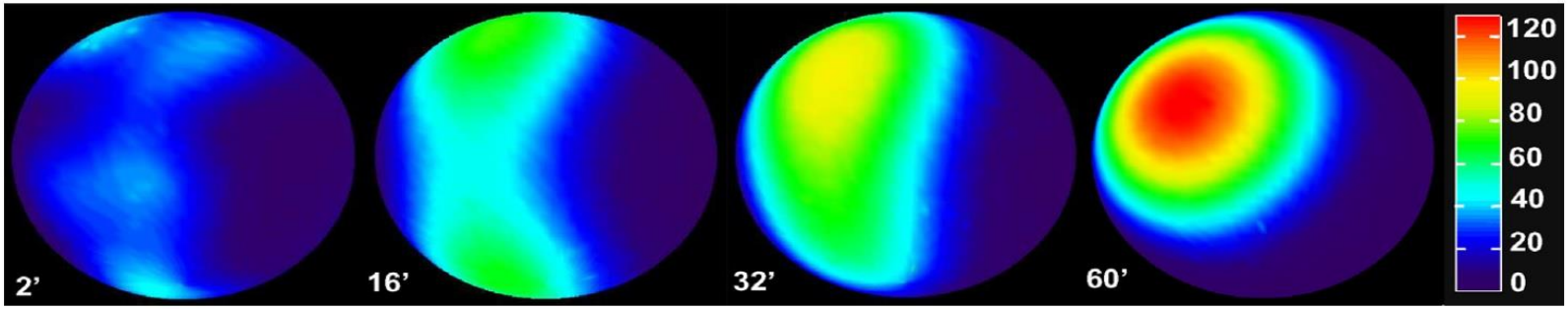


Reduction of the membrane diffusion coefficient progressively increases the range of unstable wave numbers. Therefore, we can infer that by increasing the membrane diffusion coefficient – the system will approach stable condition

Results & Discussion

Cdc42 cluster is robust to parameter variation & molecular noise

- Varying the reaction rates and computing the change in the maximum concentration and the width of the cluster, the authors found that the cluster is indeed largely insensitive to these variations
- The authors simulated molecular noise (by generated random process with Poisson distribution in time & uniform distribution in space) , which was sufficient to initiate the accumulation of active Cdc42 on the membrane.



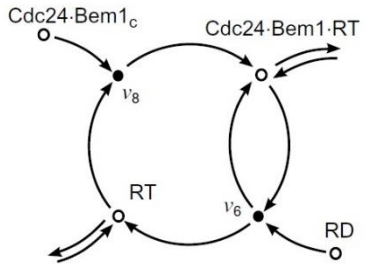
- The authors performed many simulations varying the specific realization of the random molecular noise as well as its intensity. In all simulations, only one of the nuclei developed into a mature stationary cluster.

Results & Discussion

Network motif responsible for the Cdc42 cluster formation

Which elements of the reaction network are directly responsible for the destabilization of the uniform active Cdc42 distribution on the membrane ?

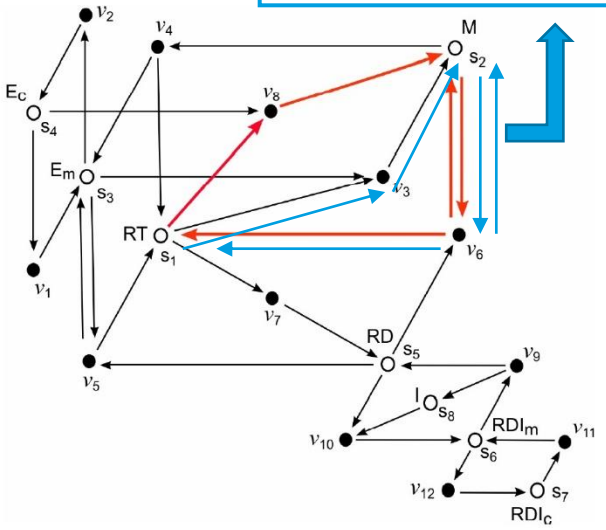
Using the techniques of graph-theoretic analysis, the authors were able to trace the cause of the Turing-type instability in the model to this network cycle.



$$Ec + RT \longrightarrow M \qquad V8$$
$$RD + Ic \Leftrightarrow RDIm \qquad V6$$

$$RD + Ic \Leftrightarrow RDIm \qquad V6$$
$$Em + RT \Leftrightarrow M \qquad V3$$

- This they did by constructing a Bipartite graph, & then identifying so-called critical fragments of the BP graph that contain positive loops.
- It was previously shown by researchers Mincheva and Roussel, that such positive loops tend to destabilise stationary state and may cause Turing-type instability.
- The autocatalytic cycle that destabilises the uniform stationary state in our model, is highlighted in red here



Results & Discussion

Turing-type model emerges from the complete reaction-diffusion mechanism

Now, that the authors identified the Network motif, they further reduced their model to include only 2 variable -

1. Active Cdc42 (x)
2. Total concentration of the inactive Cdc42 (y)

Reduced form of the initial governing equation

$$\dot{x} = E_c \alpha x^2 y + E_c \beta x y - \gamma x + D_m \Delta x,$$
$$\dot{y} = \gamma x - E_c \alpha x^2 y - E_c \beta x y + D_c \Delta y,$$
$$E_c = E_c^0 \left(1 + \int_S f(x) \mathrm{d}s\right)^{-1}.$$

- ✓ The authors identified that the reduced model belongs to the prototypical activator–substrate type of Turing Mechanism
- ✓ X acts as the Activator and Y as the substrate.

The authors noticed that the autocatalytic production of X occurs through 2 pathways :

1. Cubic mechanism $2X + Y \longrightarrow 3X$

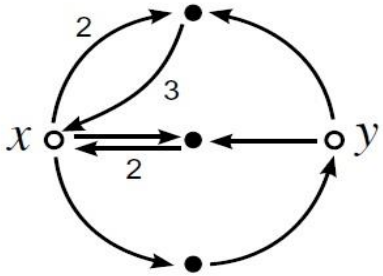
   $Ec + RT \longrightarrow M$

   $RD + Ic \Longleftrightarrow RDIm$

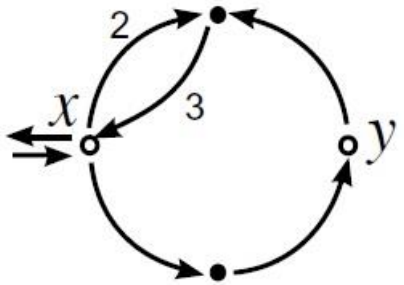
2. Quadratic mechanism $X + Y \longrightarrow 2X$

   RD [illegible] $RDIm$

   Em [illegible] $\rightarrow M$



Reduced Cdc42 cluster formation model



Brusselator

Conclusions

and further work in this area

1. *Both the activator and substrate in the prototypical Turing mechanism can be played by a single molecular species with two distinct states*

2. *The cluster of activated Cdc42 that marks the presumptive bud site is shown here to be a true dissipative structure, since its emergence and maintenance require continuous expenditure of the cellular energy stored as GTP.*
   - *The robust uniqueness of the yeast bud is explained in our model by the resource competition that destabilizes the coexistence of multiple buds.*

3. *As next steps, authors showed interest in understanding the molecular mechanism of multiple bud formation and extending the current model to accommodate these mutant process.*

References

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7. *Talk by Prof. Philip Maini: Turing's Theory of Developmental Pattern Formation (https://www.youtube.com/watch?v=pN8tVldm6QY&t=830s)*

8. *NPTEL (Multiphase flows: Analytical solutions and Stability Analysis by Prof. S.Pushpavanam, Department of Chemical Engineering, IIT Madras) - Turing patterns: Instability in reaction-diffusion systems (https://www.youtube.com/watch?v=8YnWWHwu4lA)*