ICTS | INTERNATIONAL CENTRE for THEORETICAL SCIENCES

TATA INSTITUTE OF FUNDAMENTAL RESEARCH

Living matter April 16 to April 26

Chance and determinism in cell differentiation. Random walk across the epigenetic landscape

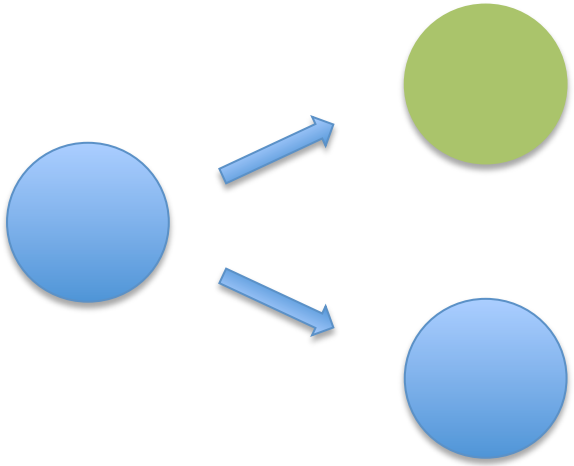
András Páldi

andras.paldi@ephe.sorbonne.fr

École Pratique des Hautes Études

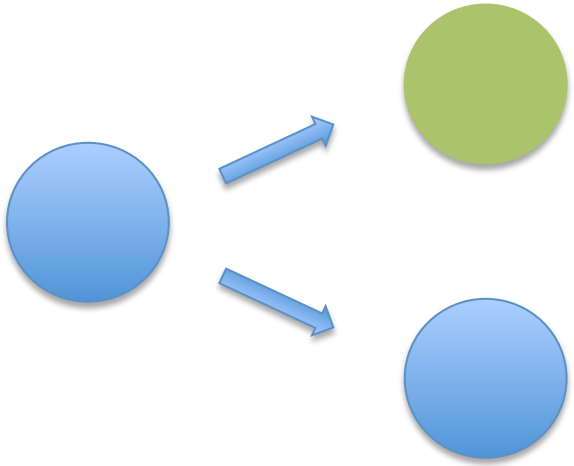
PSL RESEARCH UNIVERSITY PARIS

Cell differentiation



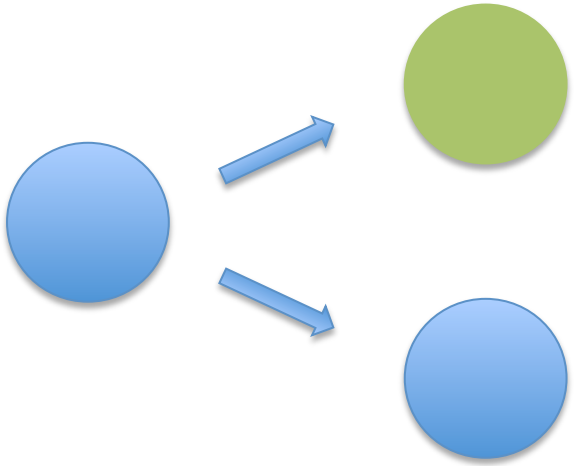
Occurs in multicellular, but also in unicellular and ambiguous organisms.

Cell differentiation



Implicitly: change in morphology and in gene expression pattern

Cell differentiation



Implicitly: change in the gene expression pattern

But: how many genes?

Cell differentiation

Stability/change

Ordered, deterministic/disordered (random, stochastic …)

Inherited/acquired

Genotype/phenotype

1 Order and stability in living systems: a short (and superficial) historical background.

2 Disorder and stochasticity in living cells: noise or variation?

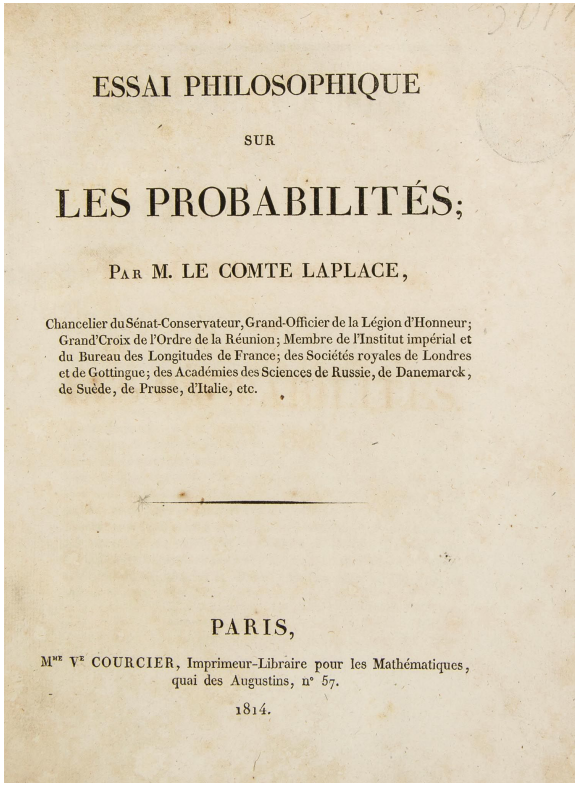
3 How cells function reliably with such an inherent variability in gene expression?

1 Order in living systems: a short (and superficial) historical background.

2 Stochasticity in living cells: noise or variation?

3 How cells function reliably with such an inherent variability in gene expression?

Pierre-Simon de Laplace
1749-1827



ESSAI PHILOSOPHIQUE

SUR

LES PROBABILITÉS;

PAR M. LE COMTE LAPLACE,

Chancelier du Sénat-Conservateur, Grand-Officier de la Légion d'Honneur; Grand'Croix de l'Ordre de la Réunion; Membre de l'Institut impérial et du Bureau des Longitudes de France; des Sociétés royales de Londres et de Gottingue; des Académies des Sciences de Russie, de Danemarck, de Suède, de Prusse, d'Italie, etc.

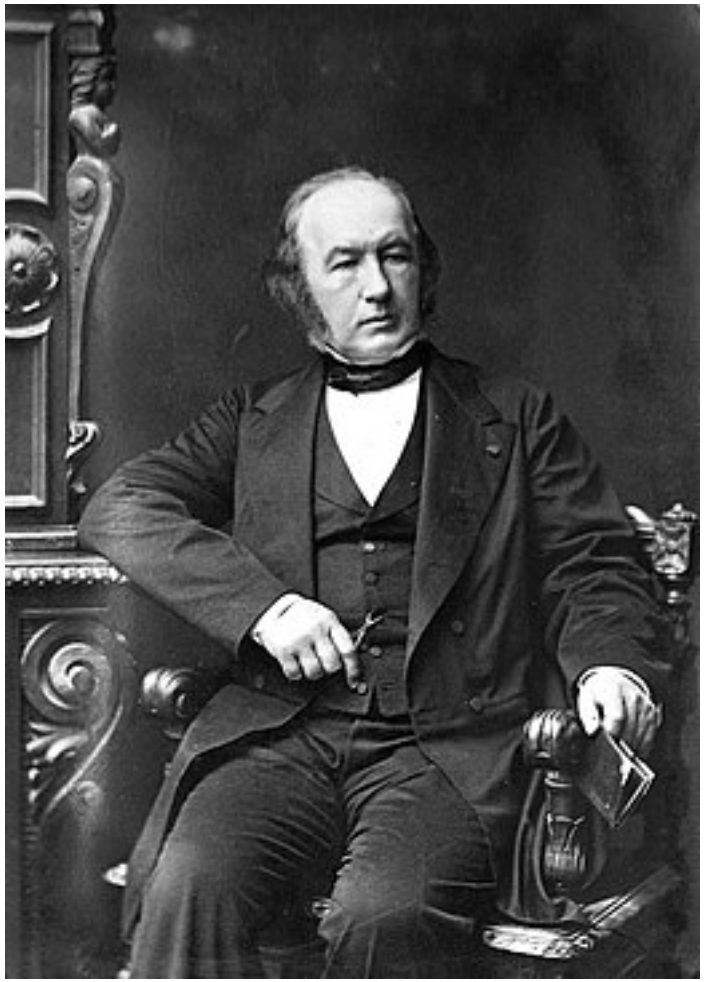
PARIS,

Mme Ve COURCIER, Imprimeur-Libraire pour les Mathématiques, quai des Augustins, n° 57.

1814.

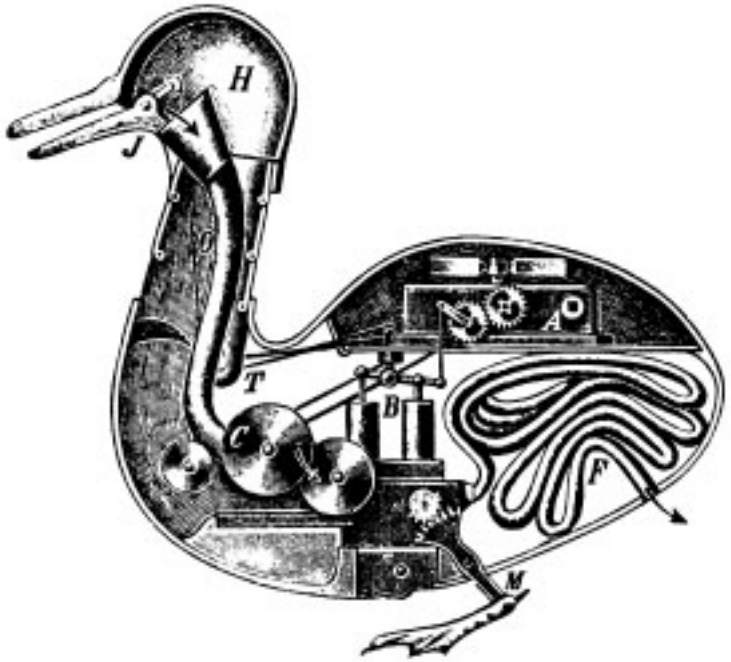
We may regard the present state of the universe as the effect of its past and the cause of its future. An intellect which at a certain moment would know all forces that set nature in motion, and all positions of all items of which nature is composed, if this intellect were also vast enough to submit these data to analysis, it would embrace in a single formula the movements of the greatest bodies of the universe and those of the tiniest atom; for such an intellect nothing would be uncertain and the future just like the past would be present before its eyes.

— Pierre Simon Laplace, *A Philosophical Essay on Probabilities*



Claude Bernard
1813-1878

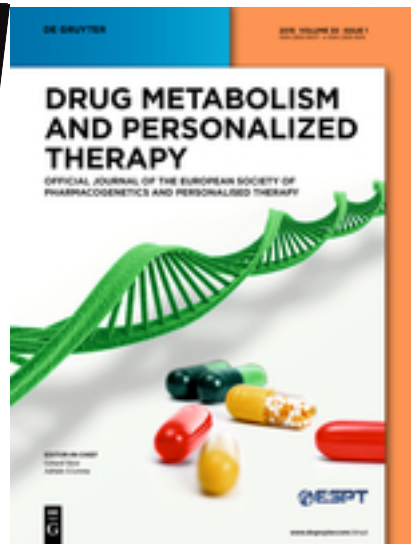
Now, a living organism is nothing but a wonderful machine endowed with the most marvellous properties and set going by means of the most complex and delicate mechanism.

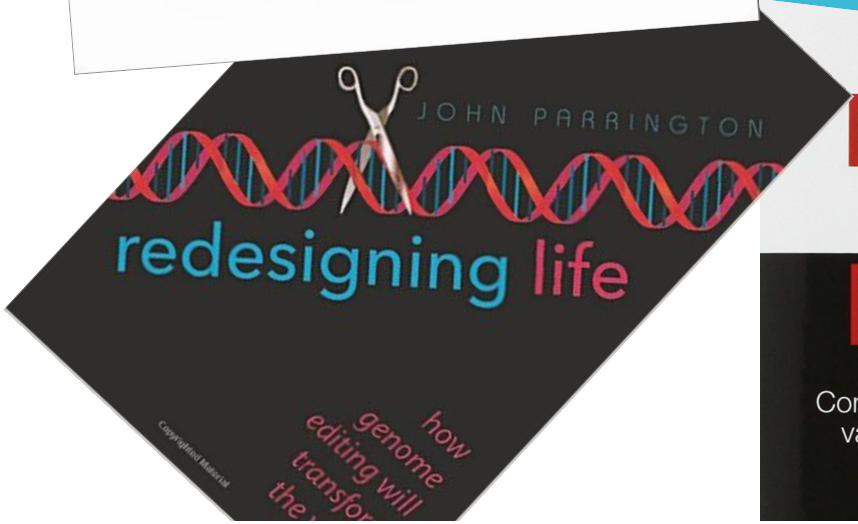


Jacques Vaucanson
1709-1782


The Economist
Corbyn and the Left's strange allure
No-go for NGOs in China
Islamic State's taste for slavery
Commodities: the binge, the hangover
India's poet-politicians
Editing humanity
The prospect of genetic enhancement
High IQ
No baldness
Low risk of Alzheimer's, breast cancer and strokes
Perfect pitch
Sprinter
20/20 vision


Science & santé
N°14 • MAI-JUIN 2013
Le magazine de l'Institut national de la santé et de la recherche médicale
Drépanocytose
30 ans de recherche
Cannabis
Un avenir thérapeutique ?
Biopôle de Lyon
Les clés d'une réussite
MÉDECINE PERSONNALISÉE
Les promesses du sur-mesure

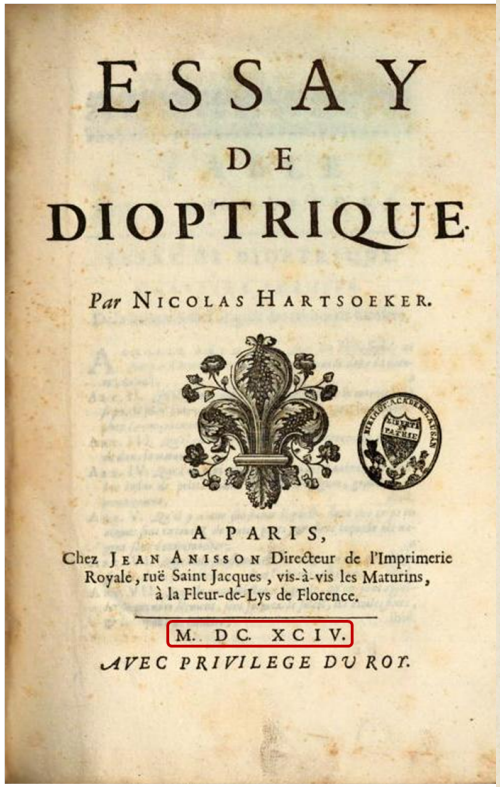

DRUG METABOLISM AND PERSONALIZED THERAPY
OFFICIAL JOURNAL OF THE EUROPEAN SOCIETY OF PHARMACOGENETICS AND PERSONALISED THERAPY
ESPT


JOHN PARRINGTON
redesigning life
how genome editing will transfor


LA MORT DE LA MORT
Comment la technomédecine va bouleverser l'humanité

Heredity is one of the clearest manifestations of the order in nature

Heredity is one of the clearest manifestations of the order in nature



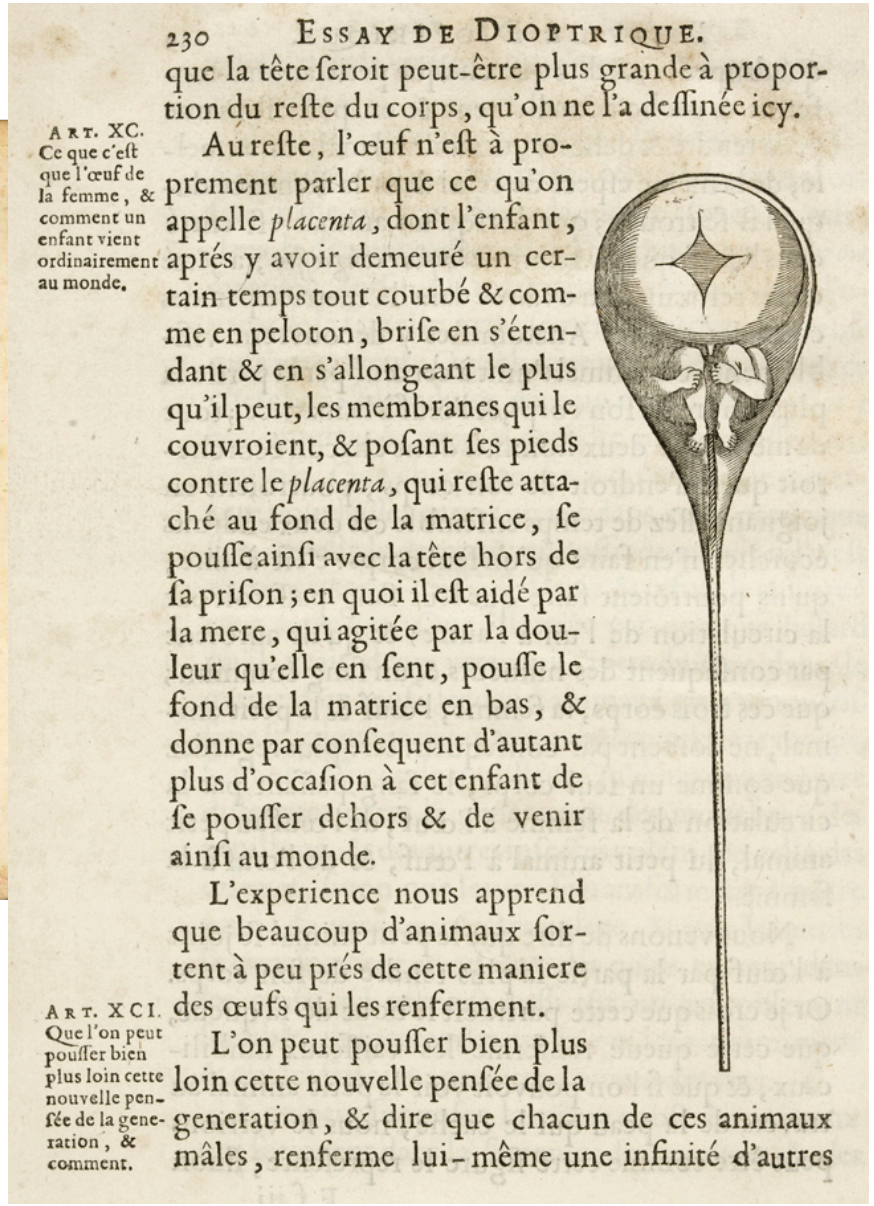
ESSAY
DE
DIOPTRIQUE.

Par NICOLAS HARTSOEKER.

A PARIS,
Chez JEAN ANISSON Directeur de l'Imprimerie Royale, ruë Saint Jacques, vis-à-vis les Maturins, à la Fleur-de-Lys de Florence.

M. DC. XCIV.

AVEC PRIVILEGE DU ROY.



que la tête seroit peut-être plus grande à proportion du reste du corps, qu'on ne l'a dessinée icy.

ART. XC. Ce que c'est que l'œuf de la femme, & comment un enfant vient ordinairement au monde.

Au reste, l'œuf n'est à proprement parler que ce qu'on appelle *placenta*, dont l'enfant, aprés y avoir demeuré un certain temps tout courbé & comme en peloton, brise en s'étendant & en s'allongeant le plus qu'il peut, les membranes qui le couvroient, & posant ses pieds contre le *placenta*, qui reste attaché au fond de la matrice, se pousse ainsi avec la tête hors de sa prison; en quoi il est aidé par la mere, qui agitée par la douleur qu'elle en sent, pousse le fond de la matrice en bas, & donne par consequent d'autant plus d'occasion à cet enfant de se pousser dehors & de venir ainsi au monde.

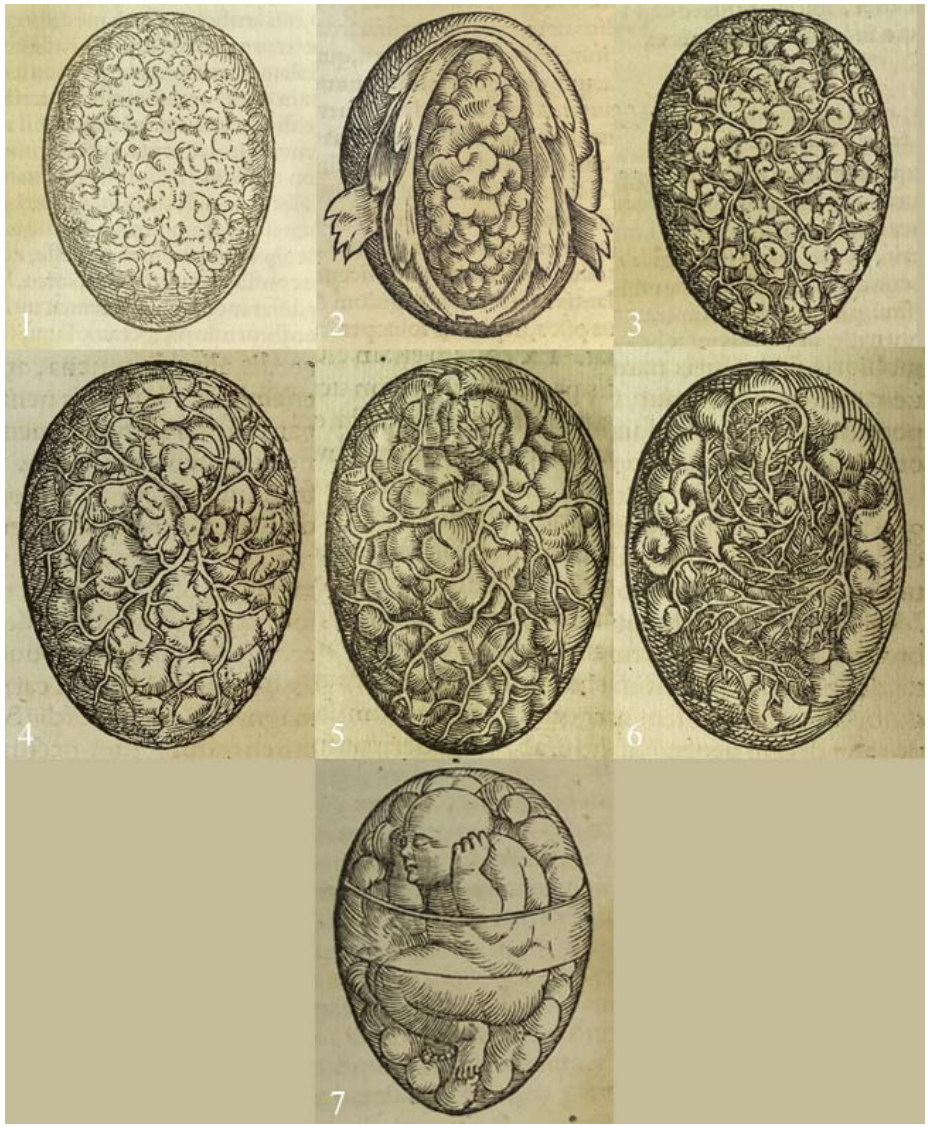
L'experience nous apprend que beaucoup d'animaux sortent à peu prés de cette maniere des œufs qui les renferment.

ART. XCI. Que l'on peut pousser bien plus loin cette nouvelle pensée de la generation, & comment.

L'on peut pousser bien plus loin cette nouvelle pensée de la generation, & dire que chacun de ces animaux mâles, renferme lui-même une infinité d'autres

Hartsoeker, N. (1694).

Epigénèse

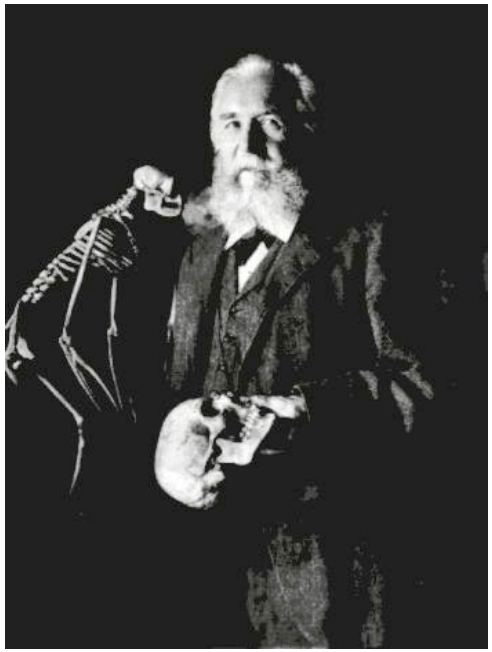


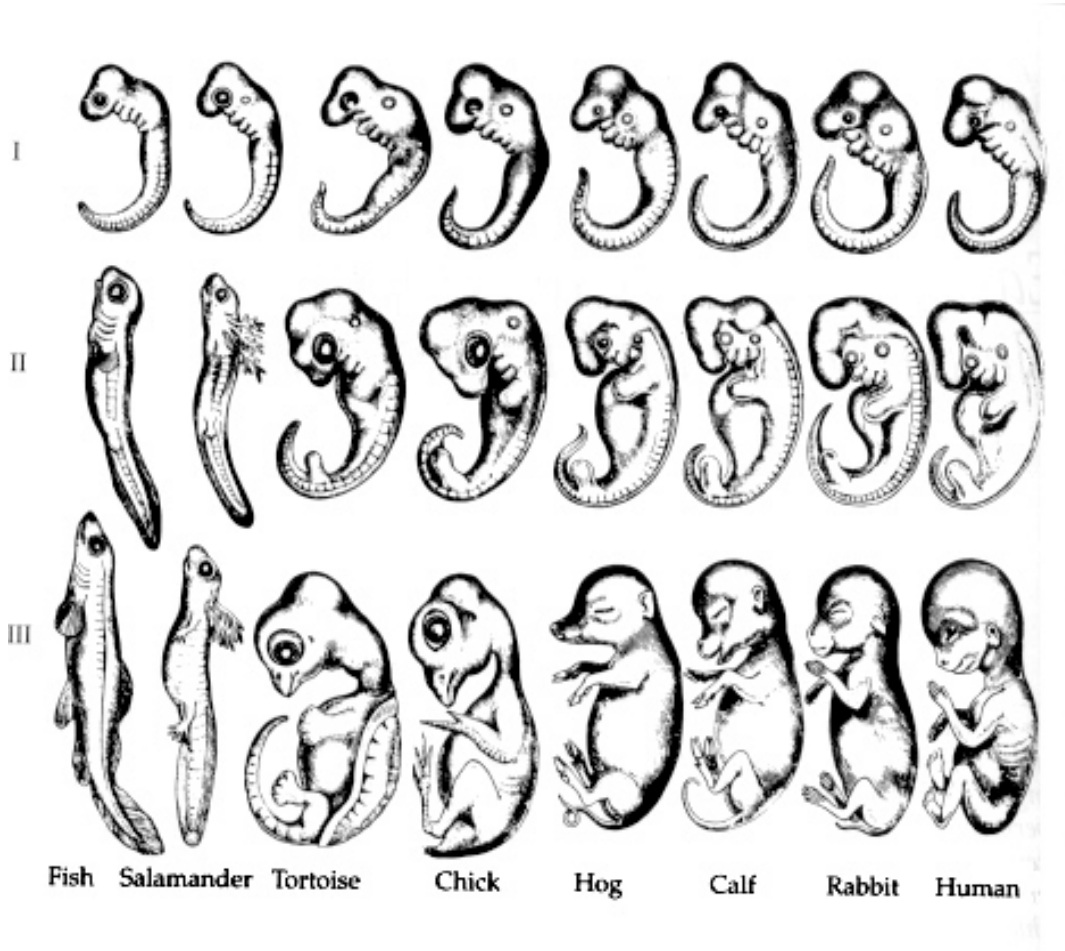
from Jacob Rueff, *De conceptu et generatione hominis …*
1554

Caspar Friedrich Wolff
1734-94

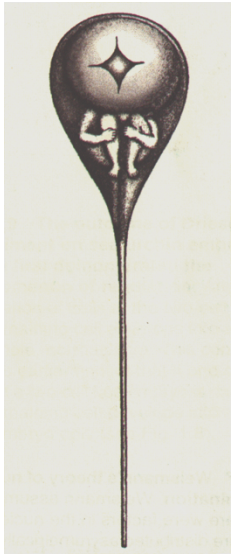
Willian Harvey
1578-1657

Ernest Haeckel, 1834-1919

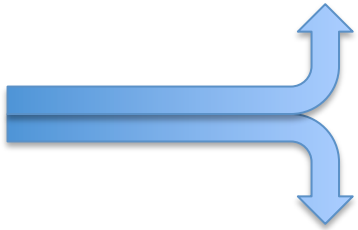




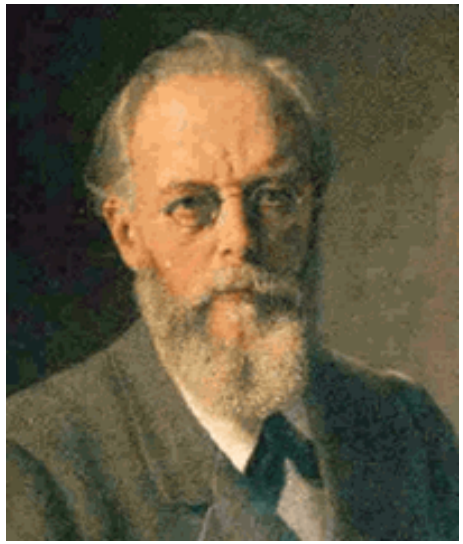
Modern genetics: dissociation of the two concepts



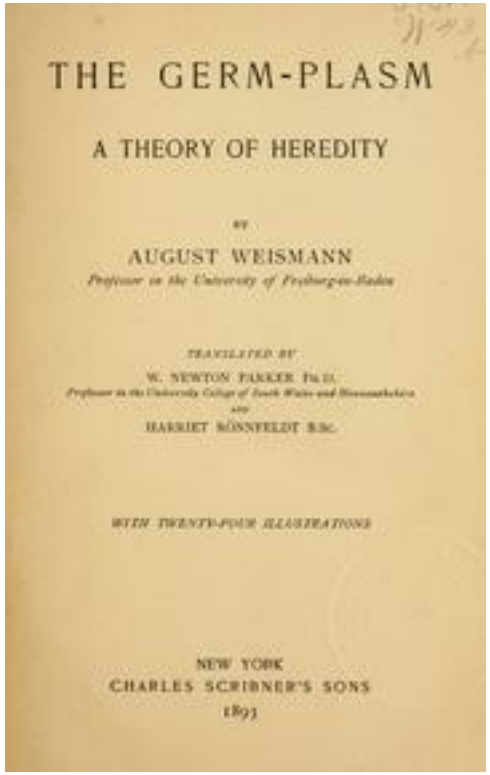
Genotype: **stable over generations**

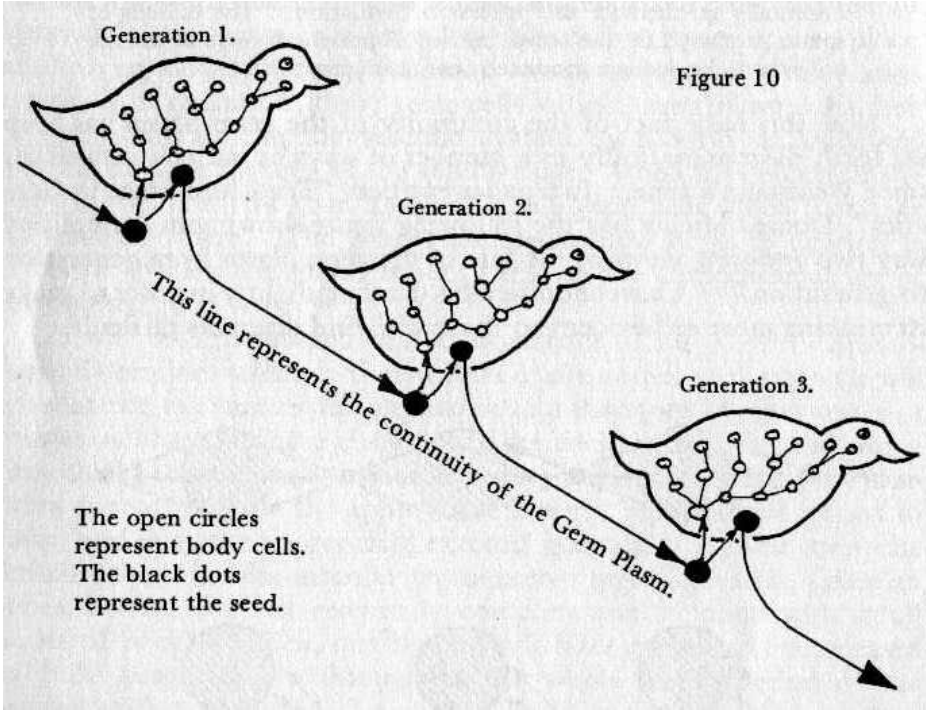


Phenotype: **variable**



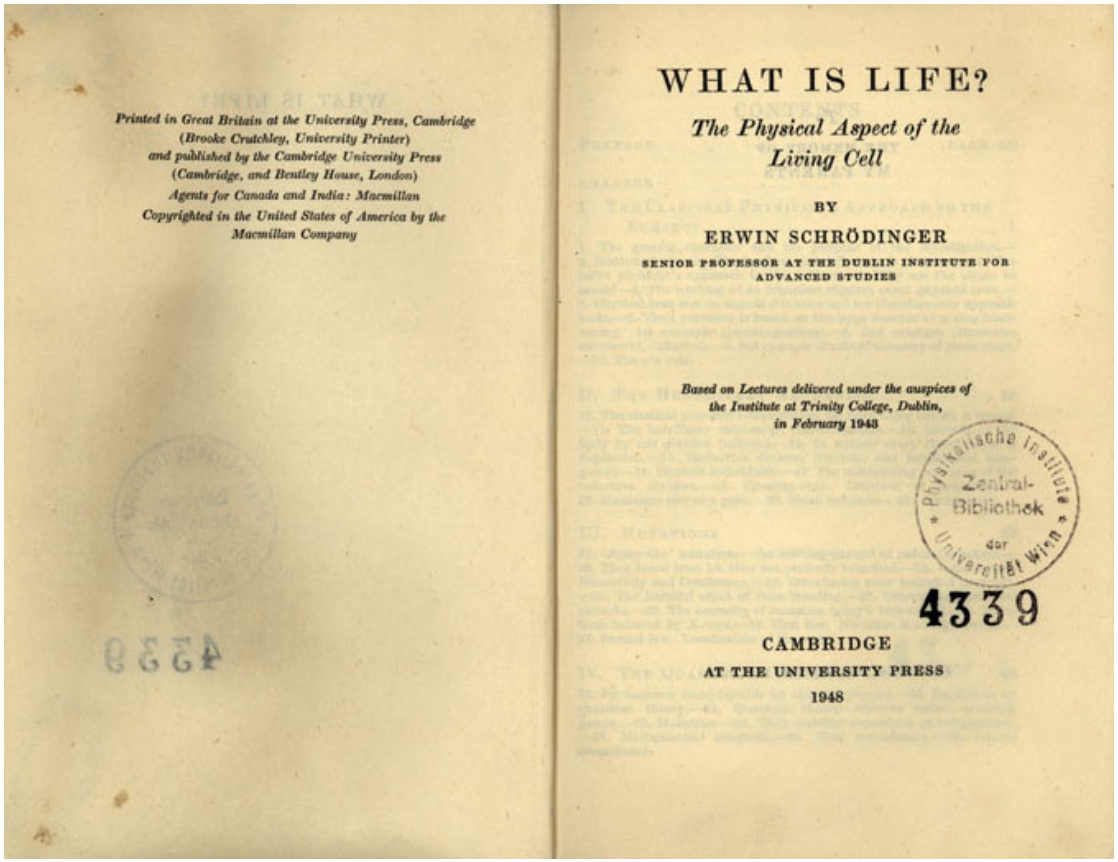
August Weisman
1834-1914





Hardy, Sir Alister, T*his Living Stream,* London, Collins, 1965, p.76.

Order from disorder ⟺ Order from order



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Agents for Canada and India: Macmillan

WHAT IS LIFE?

The Physical Aspect of the Living Cell

BY

ERWIN SCHRÖDINGER

SENIOR PROFESSOR AT THE DUBLIN INSTITUTE FOR ADVANCED STUDIES

Based on Lectures delivered under the auspices of the Institute at Trinity College, Dublin, in February 1943

CAMBRIDGE
AT THE UNIVERSITY PRESS
1948

Erwin Schrödinger 1944

Order from disorder  Order from order

“It appears that there are two different ‘mechanisms’ by which orderly events can be produced: the ‘statistical mechanism’ which produces ‘order from disorder’ and the new one, producing ‘order from order’.”

“From all we have learnt about the structure of living matter, we must be prepared to find it working in a manner that cannot be reduced to the ordinary laws of physics. And that not on the ground that there is any “new force” or what not, directing the behavior of the single atoms within a living organism, but because the construction is different from anything we have yet tested in the physical laboratory. ”

“…living matter, while not eluding the "laws of physics" as established up to date, is likely to involve "other laws of physics" hitherto unknown, which however, once they have been revealed, will form just as integral a part of science as the former. ”

“We must also be prepared to find a new type of physical law prevailing in it. Or are we to term it a non-physical, not to say a super-physical, law? ”

“It is these chromosomes … that contain in some kind of code-script the entire pattern of the individual's future development and of its functioning in the mature state. Every complete set of chromosomes contains the full code…”

« Chaque œuf contient donc, dans les chromosomes reçus de ses parents, tout son propre avenir, les étapes de son développement, la forme et les propriétés de l'être qui en émergera. L'organisme devient ainsi la réalisation d'un programme prescrit par l'hérédité. »

François Jacob 1970

"Life, we now know, is nothing but a vast array of coordinated chemical reactions. The 'secret' to that coordination is the breathtakingly complex set of instructions inscribed, again chemically, in our DNA. »

James Watson

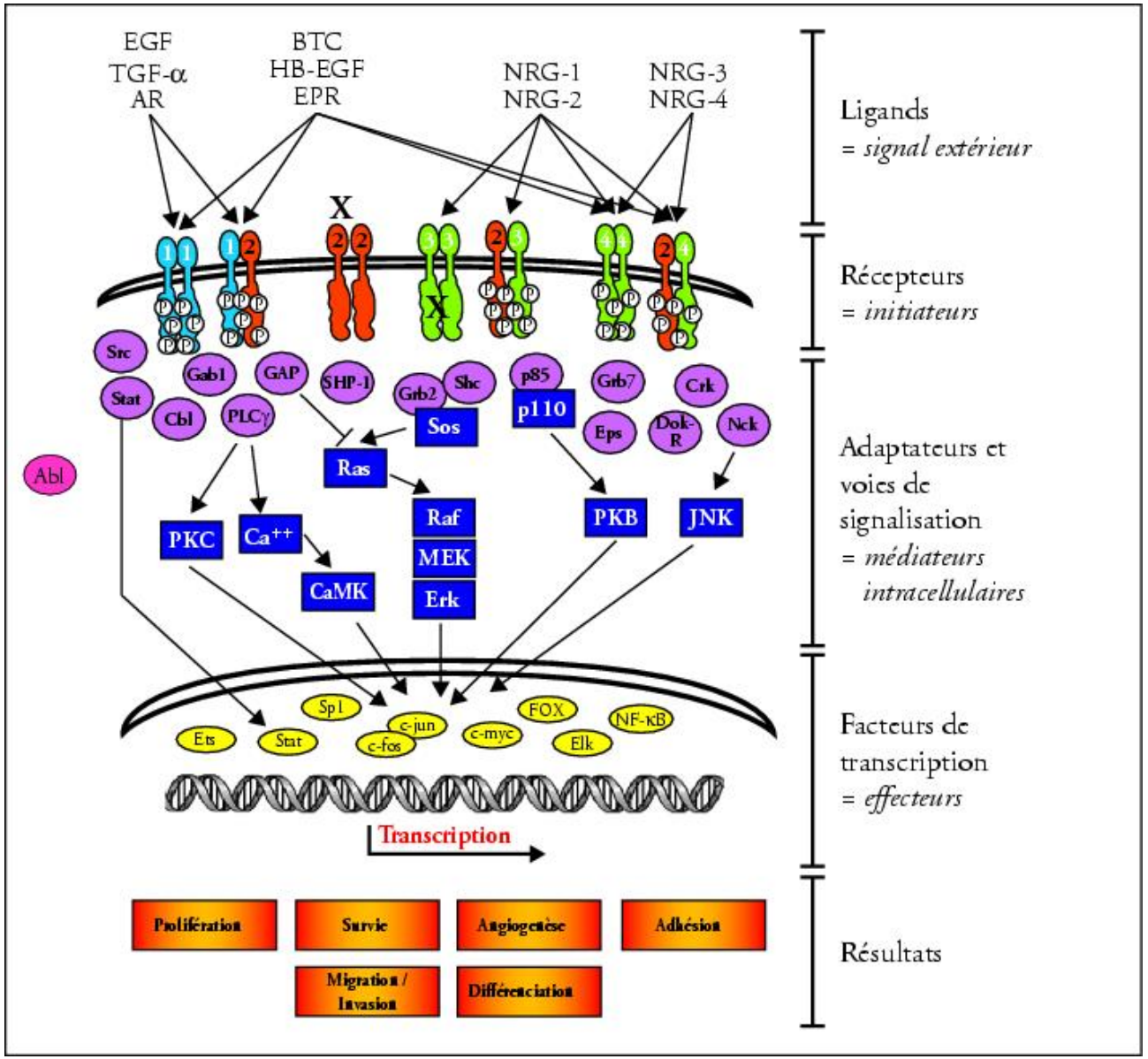
Order from order

Phenotype from genotype

Genotype → phenotype

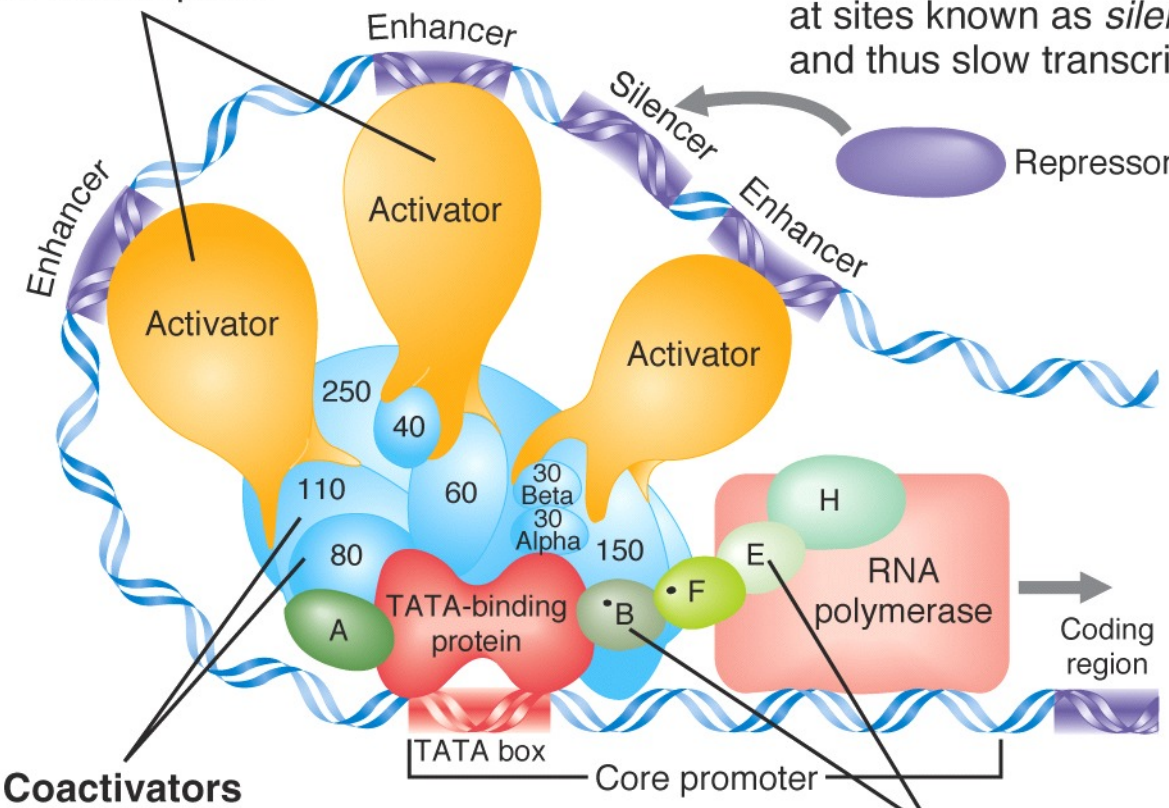
Information flow

René Magritte 1898-1968


EGF
TGF-α
AR
BTC
HB-EGF
EPR
NRG-1
NRG-2
NRG-3
NRG-4
X
X
Src
Stat
Gab1
Cbl
PLCγ
GAP
SHP-1
Grb2
Shc
Sos
p85
p110
Grb7
Eps
Crk
Dok-R
Nck
Abl
Ras
Raf
MEK
Erk
PKC
Ca++
CaMK
PKB
JNK
Sp1
Ets
Stat
c-jun
c-fos
c-myc
FOX
Elk
NF-κB
Transcription
Prolifération
Survie
Angiogenèse
Adhésion
Migration / Invasion
Différenciation
Ligands
= signal extérieur
Récepteurs
= initiateurs
Adaptateurs et voies de signalisation
= médiateurs intracellulaires
Facteurs de transcription
= effecteurs
Résultats

Activators
These proteins bind to genes at sites known as *enhancers* and speed the rate of transcription.

Repressors
These proteins bind to selected sets of genes at sites known as *silencers* and thus slow transcription.



Coactivators
These "adapter" molecules integrate signals from activators and perhaps repressors.

Basal transcription factors
In response to injunctions from activators, these factors position RNA polymerase at the start of

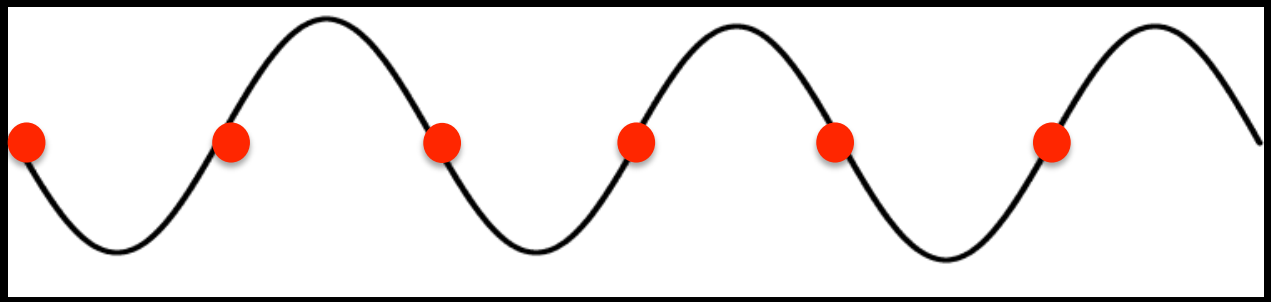
Stability  Change

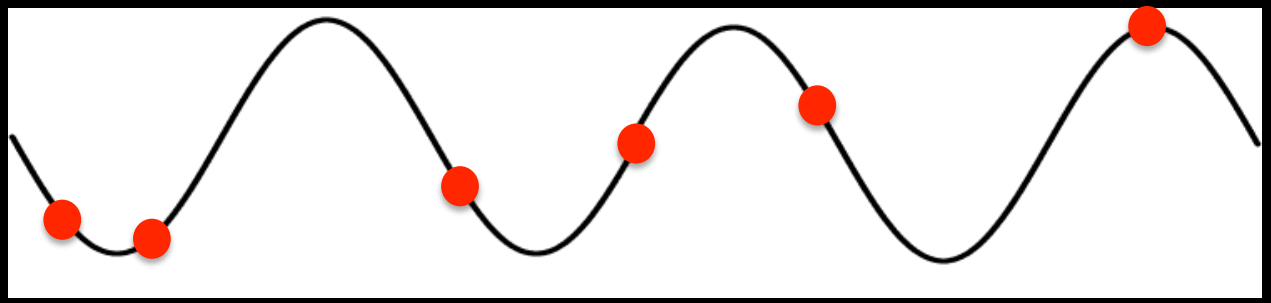
But how to detect change?

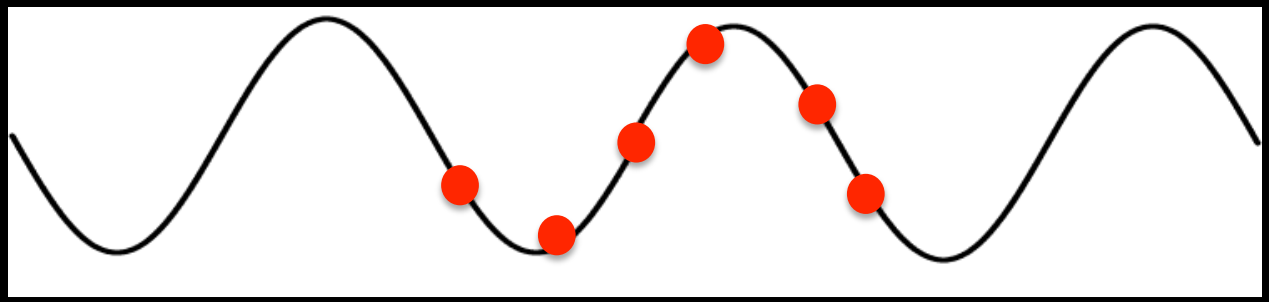
Stability  Change

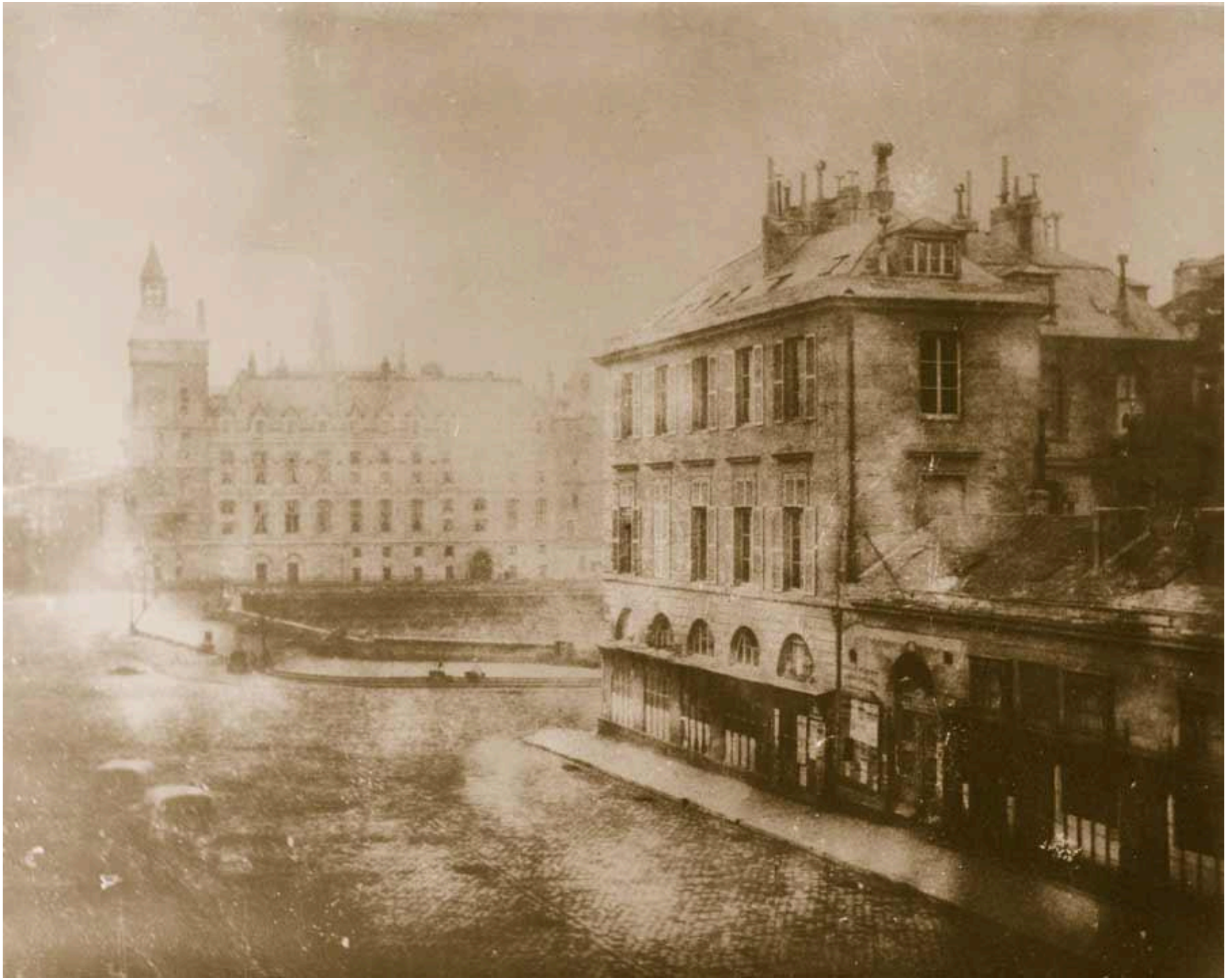
But how to detect change?

Complementary question: How create change with stable elements?









Stability  Change

But how to detect change?

Relativity of scales

1 Order and stability in living systems: a short (and superficial) historical background.

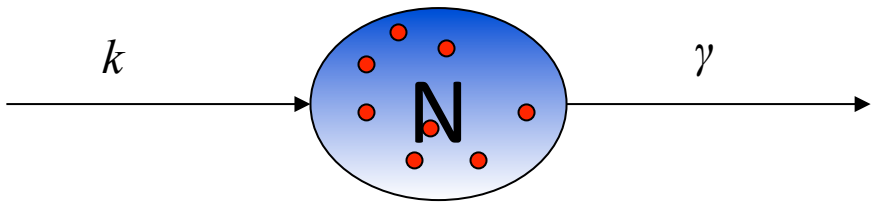
2 Stochasticity in living cells: noise or variation?

3 How cells function reliably with such an inherent variability in gene expression?

“And why could all this not be fulfilled in the case of an organism composed of a moderate number of atoms only and sensitive already to the impact of one or a few atoms only? Because we know all atoms to perform all the time a completely disorderly heat motion, which, so to speak, opposes itself to their orderly behaviour and does not allow the events that happen between a small number of atoms to enrol themselves according to any recognizable laws.”

Erwin Schrödinger, What is life?

Birth-death process: Protein synthesis and degradation



Deterministic solution:

$$\frac{dN}{dt} = k - \gamma N \Rightarrow N(t) = \frac{k}{\gamma}\left(1 - e^{-\gamma t}\right)$$

Stochastic solution:

$$\frac{dP_N}{dt} = kP_{N-1} + \gamma(N+1)P_{N+1} - (k+\gamma N)P_N; \quad N \neq 0,\ P_{-1} = 0$$

Master equation

$$\frac{d\langle N\rangle}{dt} = k - \gamma\langle N\rangle \Rightarrow \mu(t) = \frac{k}{\gamma}\left(1 - e^{-\gamma t}\right)$$

Solution: mean

$$\frac{d\langle N^2\rangle}{dt} = -2\gamma\langle N^2\rangle + (\gamma + 2k)\langle N\rangle + k \Rightarrow \sigma^2(t) = \frac{k}{\gamma}\left(1 - e^{-\gamma t}\right)$$

Solution: variance

$$CV = \frac{\sigma(t)}{\mu(t)} = \sqrt{\frac{\gamma}{k\left(1 - e^{-\gamma t}\right)}} = \frac{1}{\sqrt{\mu(t)}}$$

CV = Coefficient of Variation

Solution: noise

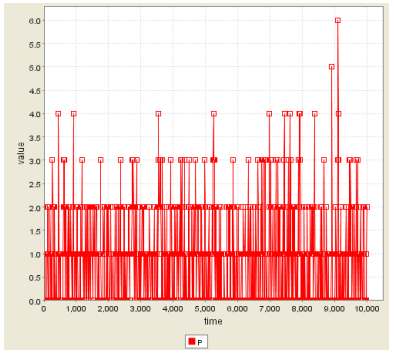
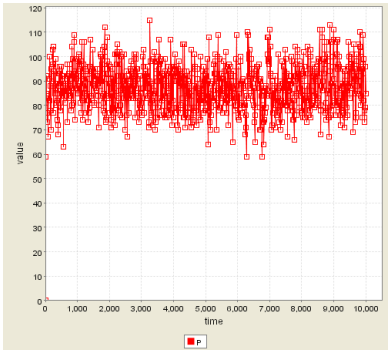
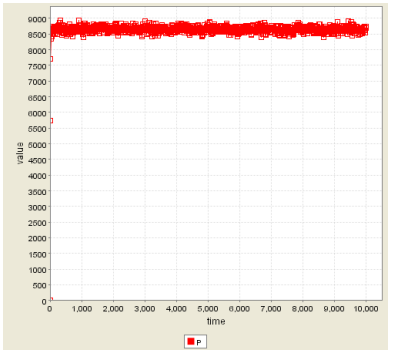
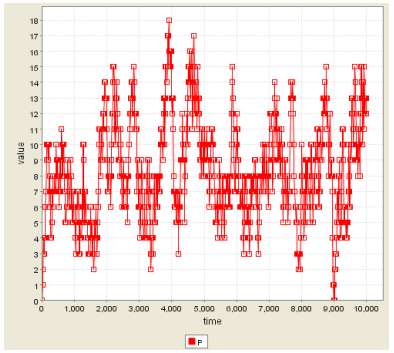
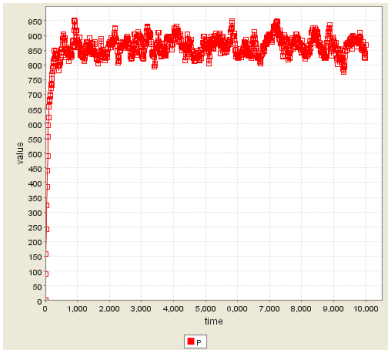
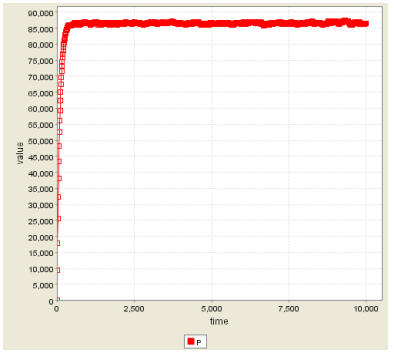
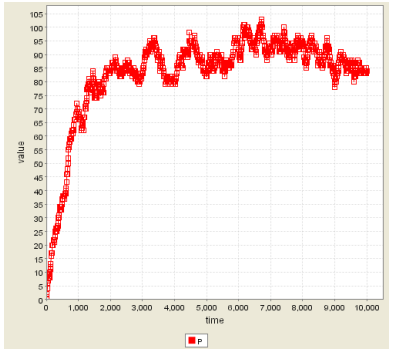
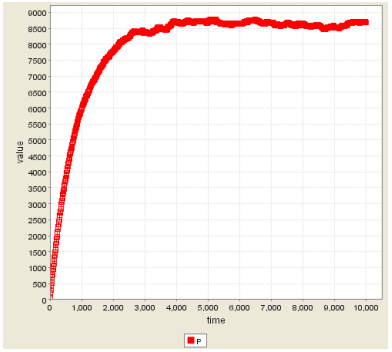
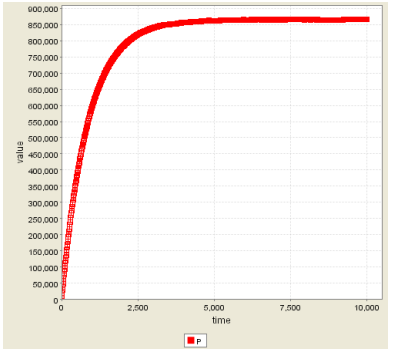
Stochastic Processes in Physics and Chemistry (North-Holland Personal Library) by N.G. Van Kampen

(by Gabor Balazsi)

Stochastic simulation: Protein synthesis and degradation

synthesis

degradation

	$k=0.1$	$k=10$	$k=1000$
$\gamma=ln(2)/6$			
$\gamma=ln(2)/60$			
$\gamma=ln(2)/600$			

D. T. Gillespie, *J. Comp. Phys.* **22**(4), 403-433 (1976); D. T. Gillespie, *J. Phys. Chem.* **81**, 2340-2360 (1977)

SnapShot: Key Numbers in Biology

Uri Moran,[1] Rob Phillips,[2] and Ron Milo[1]
[1]Weizmann Institute of Science, Rehovot, Israel; [2]California Institute of Technology, Pasadena, CA, USA

Cell

Cell size

Bacteria *(E. coli):* ≈0.7-1.4 μm diameter, ≈2-4 μm length, ≈0.5-5 μm^3 in volume; 10^8-10^9 cell/ml for culture with $OD_{600} \approx 1$

Yeast *(S. cerevisiae):* ≈3-6 μm diameter ≈20-160 μm^3 in volume

Mammalian cell volume: 100-10,000 μm^3; HeLa cell: 500-5000 μm^3 (adhering to slide ≈15-30 μm diameter)

Length scales inside cells

Nucleus volume: ≈10% of cell volume

Cell membrane thickness: ≈4-10 nm

"Average" protein diameter: ≈3-6 nm

Base pair: 2 nm (D) x 0.34 nm (H)

Water molecule diameter: ≈0.3 nm

Energetics

Membrane potential ≈70-200 mV ➞ 2-6 k_BT per electron ($k_BT \equiv$ thermal energy)

Free energy (ΔG) of ATP hydrolysis under physiological conditions
≈40-60 kJ/mol ➞ ≈20 k_BT/molecule ATP; ATP molecules required during an *E. coli* cell cycle ≈10-50 × 10^9

ΔG^0 resulting in order of magnitude ratio between product and reactant concentrations:
≈6 kJ/mol ≈60 meV ≈2 k_BT

Concentration

Concentration of 1 nM:
in *E. coli* ≈1 molecule/cell;
in HeLa cells ≈1000 molecules/cell

Characteristic concentration for a signaling protein: ≈10 nM-1 μM

Water content: ≈70% by mass; general elemental composition (dry weight) of *E. coli:* ≈$C_4H_7O_2N_1$; Yeast: ≈$C_6H_{10}O_3N_1$

Composition of *E. coli* (dry weight): ≈55% protein, 20% RNA, 10% lipids, 15% others

Protein concentration: ≈100 mg/ml = 3 mM. 10^6-10^7 per *E. coli* (depending on growth rate); Total metabolites (MW < 1 kDa) ≈300 mM

Division, replication, transcription, translation, and degradation rates

at 37°C with a temperature dependence (Q10) of ≈2-3

Cell cycle time (exponential growth in rich media): *E. coli* ≈20-40 min; budding yeast 70-140 min; HeLa human cell line: 15-30 hr

Rate of replication by DNA polymerase:
E. coli ≈200-1000 bases/s; human ≈40 bases/s. Transcription by RNA polymerase 10-100 bases/s

Translation rate by ribosome: 10-20 aa/s

Degradation rates (proliferating cells):
mRNA half life < cell cycle time;
protein half life ≳ cell cycle time

Diffusion and catalysis rate

Diffusion coefficient for an "average" protein: in cytoplasm D≈5-15 $\mu m^2/s$ ➞ ≈10 ms to traverse an *E. coli* ➞ ≈10 s to traverse a mammalian HeLa cell; small metabolite in water D≈500 $\mu m^2/s$

Diffusion-limited on-rate for a protein:
≈10^8-10^9 $s^{-1}M^{-1}$ ➞ for a protein substrate of concentration ≈1 μM the diffusion-limited on-rate is ≈100-1000 s^{-1} thus limiting the catalytic rate k_{cat}

Genome sizes and error rates

Genome size:
E. coli ≈5 Mbp
S. cerevisiae (yeast) ≈12 Mbp
C. elegans (nematode) ≈100 Mbp
D. melanogaster (fruit fly) ≈120 Mbp
A. thaliana (plant) ≈120 Mbp
M. musculus (mouse) ≈2.6 Gbp
H. sapiens (human) ≈3.2 Gbp
T. aestivum (wheat) ≈16 Gbp

Number of protein-coding genes:
E. coli = 4000; *S. cerevisiae* = 6000; *C. elegans, A. thaliana, M. musculus, H. sapiens* = 20,000

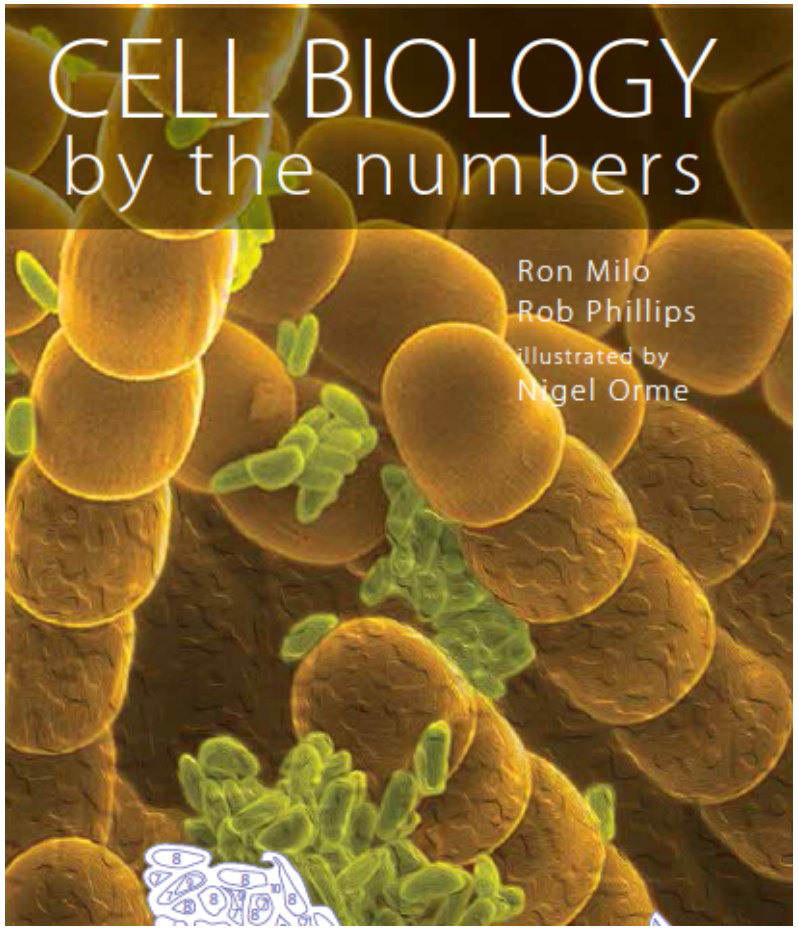
Mutation rate in DNA replication:
≈10^{-8}-10^{-10} per bp

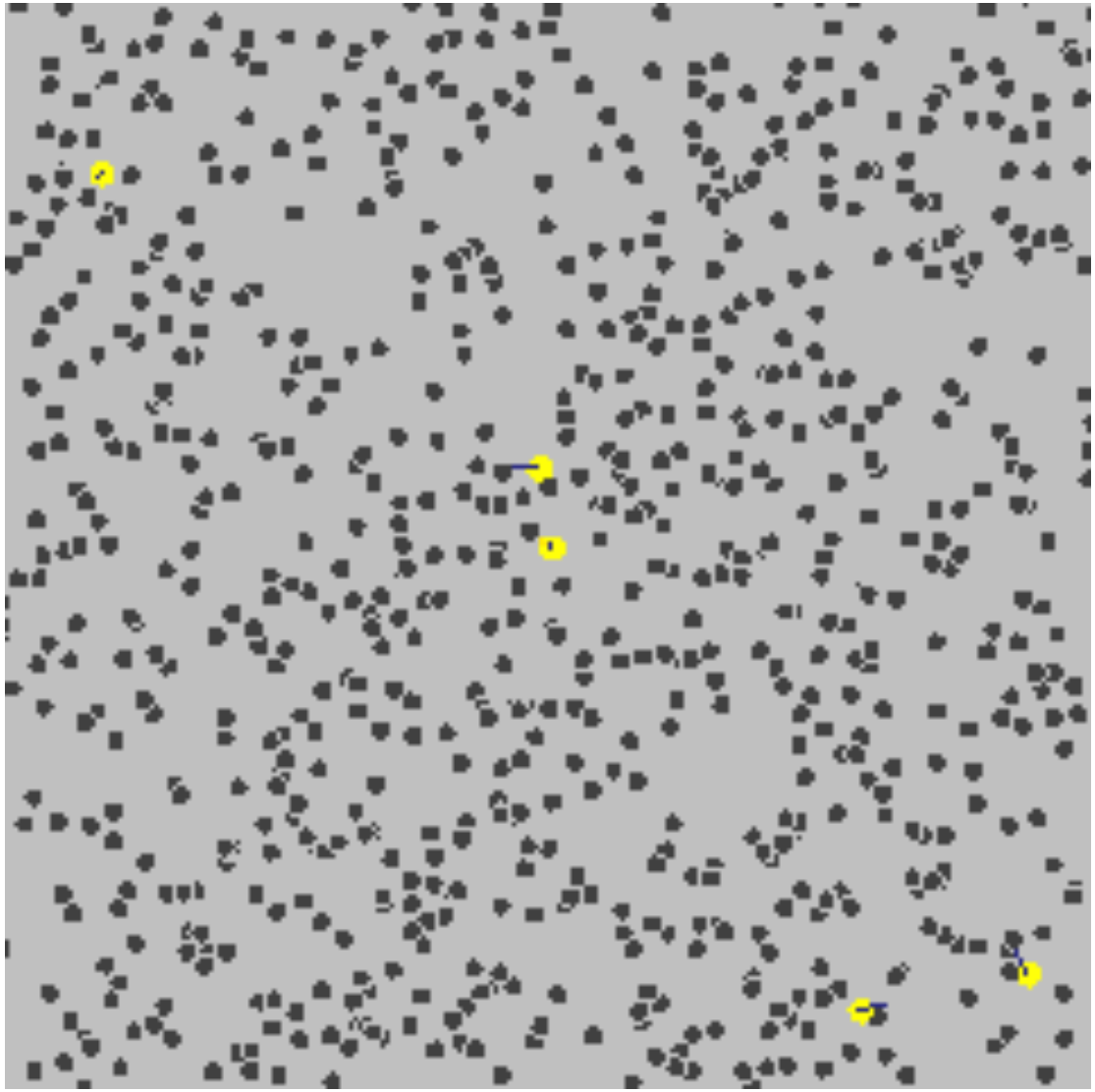
Misincorporation rate:
transcription ≈10^{-4}-10^{-5} per nucleotide
translation ≈10^{-3}-10^{-4} per amino acid

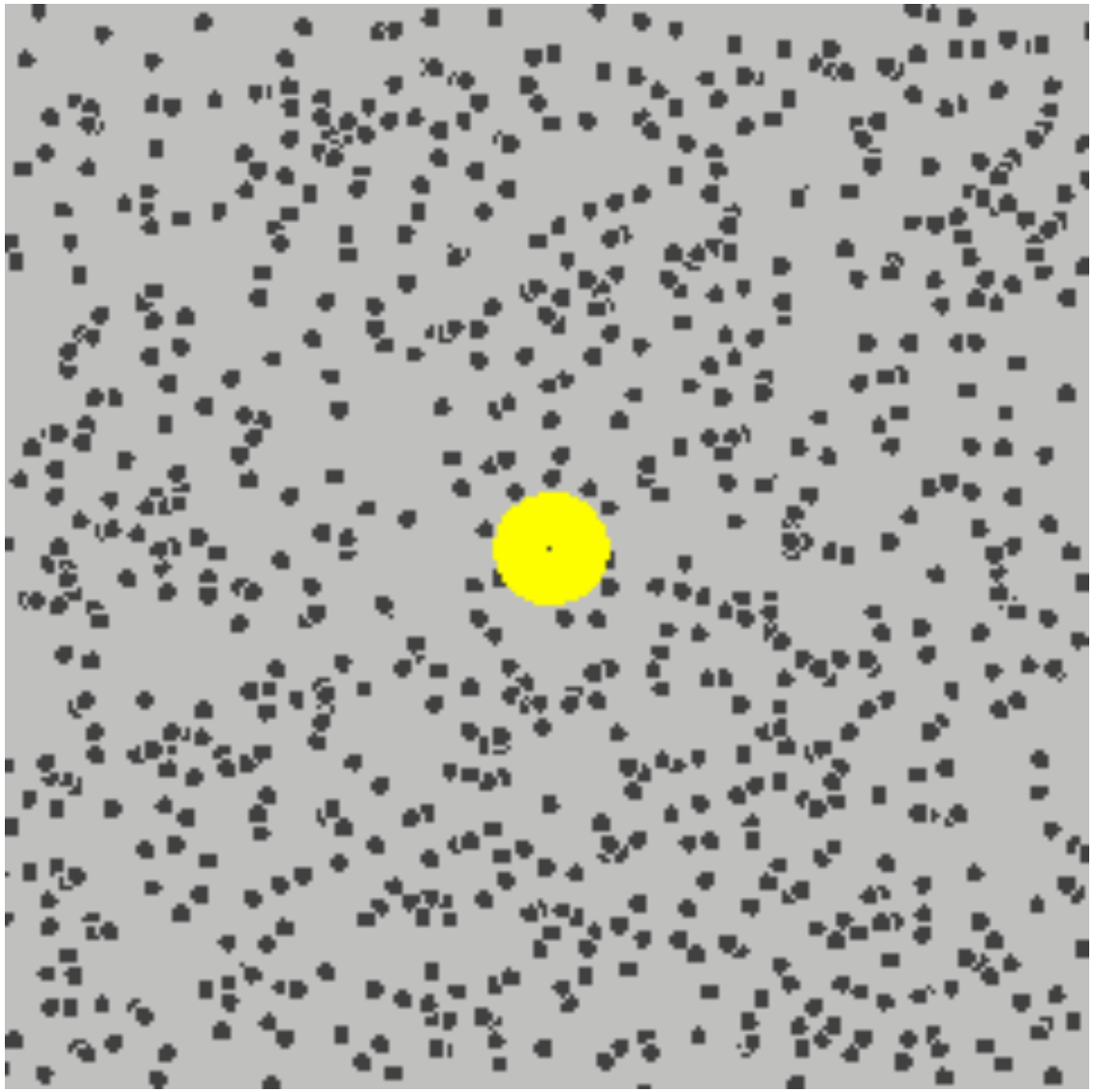
Organism	Transcription factor	Copies per cell order of magnitude	BNID
E. coli	LacI (carbon utilization)	10^1-10^2	100734
E. coli	AraC (carbon utilization)	10^2	105139
E. coli	ArcA (general aerobic respiration control)	10^4	102632
S. cerevisiae	Gal4 (carbon utilization)	10^2	109208
S. cerevisiae	Tfb3 (general transcription initiation factor)	10^3	109208
S. cerevisiae	Pho2 (phosphate metabolism)	10^4	109208
D. melanogaster, anterior blastoderm nuclei	Bicoid (development)	10^4	106843
D.melanogaster, Schneider cell line	GAGA zinc finger	10^6	106846
Mouse/Rat murine macrophage	Glucocorticoid, Thyroid and Androgen receptors associated zinc fingers	10^4	106899
Mouse/Rat murine macrophage	NF-kappaB p65	10^5	106901
H. sapiens cell lines	P53 (growth and apoptosis)	10^4-10^5	100420
H. sapiens cell lines	Glucocorticoid, Estrogen, Steroid receptors associated zinc fingers	10^4-10^5	106904, 106906, 106911
H. sapiens cell lines	STAT6	10^4-10^5	106914
H. sapiens cell lines	NF-kappaB p65	10^5	106909
H. sapiens cell lines	Myc (global chromatin structure regulation)	10^5	106907

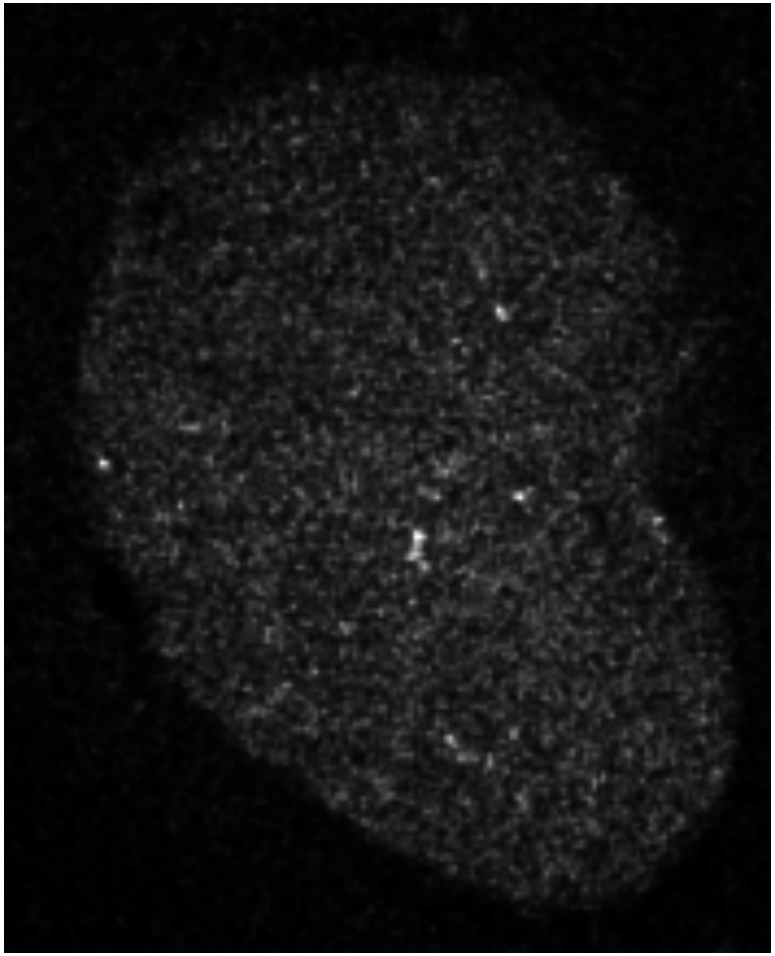
Protein	Name	Molecules/cell	Standard Error	Concentration (nM)
G protein-coupled receptor	Ste2	7,000	400	400
G-α	Gpa1	2,000	300	130
G-β	Ste4	2,000	100	110
PAK kinase	Ste20	4,000	500	200
Scaffold	Ste5	500	60	30
MAPKKK binding partner	Ste50	1,000	100	70
MAPKKK	Ste11	4,000	90	200
MAPKK	Ste7	900	70	50
MAPK	Fus3	20,000	3,000	1,100
MAPK	Kss1	20,000	2,000	1,200
MAPK	Hog1	6,000	400	300
Scaffold/MAPKK	Pbs2	2,000	200	140
MAPK phosphatase	Msg5	40	3	2
Cell cycle inhibitor	Far1	200	20	14
Transcriptional activator	Stw12	1,400	40	80
Transcriptional repressor	Dig1	5,000	500	300
Transcriptional repressor	Dig2	1,000	80	70

Table 1: Abundances of signaling molecules associated with the MAPK cascade in budding yeast before pheromone addition. Abundances are based on quantitative immunoblotting. Concentration was calculated assuming a cell volume of 29 fL. The standard error indicates the uncertainty on the number of molecules per cell as estimated in this specific experiment. Values were rounded to one significant digit. Adapted from Thomson et al, PNAS 2012 (BNID 107680).

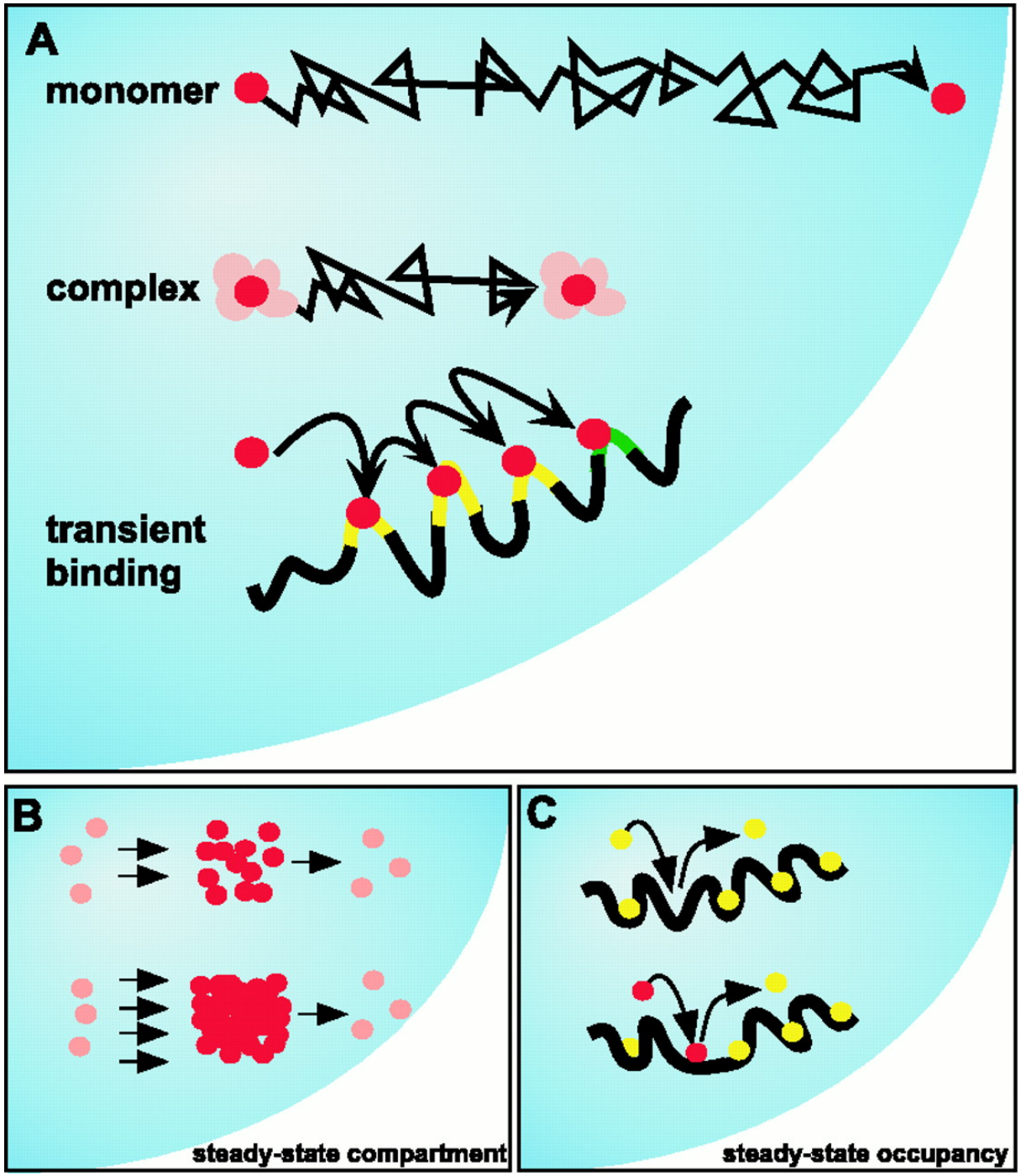

CELL BIOLOGY
by the numbers
Ron Milo
Rob Phillips
illustrated by
Nigel Orme

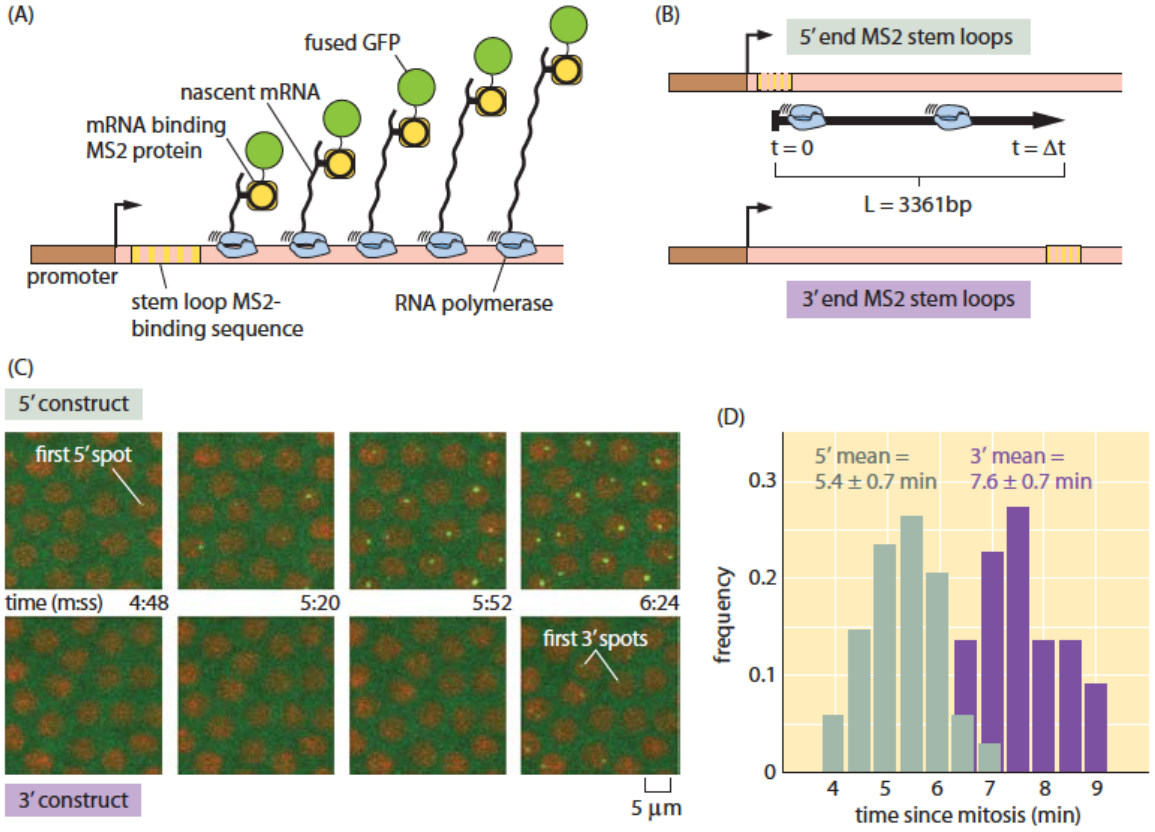






Biochemical reactions in the cells are limited by the diffusion of the molecules.


A
monomer
complex
transient
binding
B
steady-state compartment
C
steady-state occupancy



$$\Longrightarrow \text{transcription rate} = \frac{\text{length}}{\text{time difference}} = \frac{L}{\Delta t} = \frac{3361\,\text{bp}}{2.2\,\text{min}} \approx 1500\,\text{nt/min} \approx 25\,\text{nt/s}$$

Figure 4: Dynamics of transcription in the fly embryo. (A) Schematic of the

Global quantification of mammalian gene expression control

Björn Schwanhäusser[1], Dorothea Busse[1], Na Li[1], Gunnar Dittmar[1], Johannes Schuchhardt[2], Jana Wolf[1], Wei Chen[1] & Matthias Selbach[1]

Absolute mRNA and protein copy numbers

We calculated absolute cellular mRNA copy numbers based on the number of sequencing reads in the unfractionated sample in conjunction

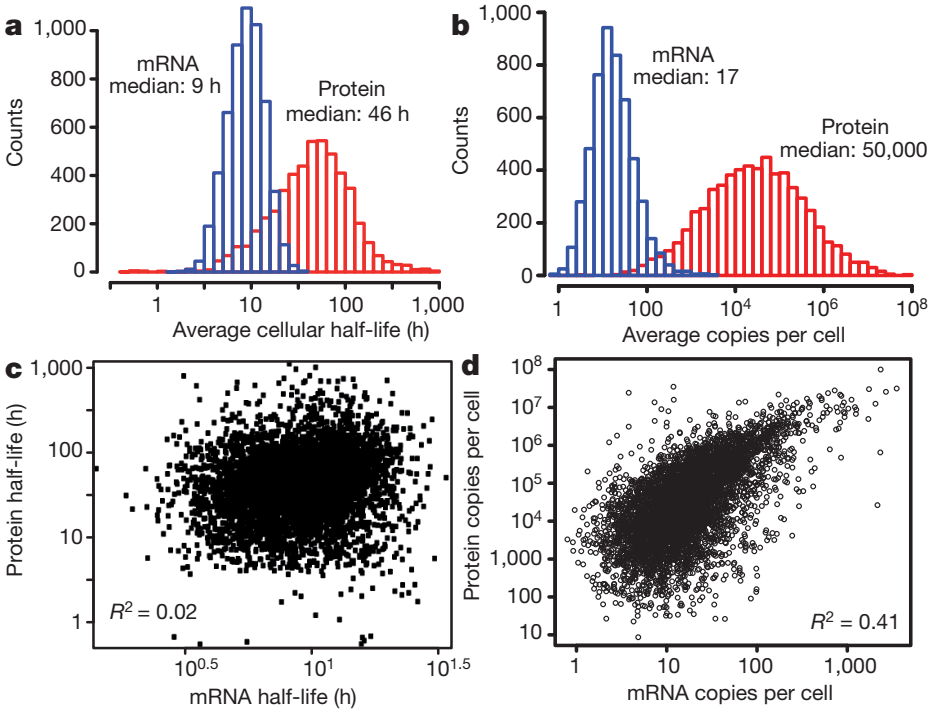


Figure 2 | mRNA and protein levels and half-lives. **a**, **b**, Histograms of mRNA (blue) and protein (red) half-lives (**a**) and levels (**b**). Proteins were on average 5 times more stable and 2,800 times more abundant than mRNAs and spanned a higher dynamic range. **c**, **d**, Although mRNA and protein levels correlated significantly, correlation of half-lives was virtually absent.

Chromosoma (2011) 120:533–545
DOI 10.1007/s00412-011-0340-y

REVIEW

Assembly of the transcription machinery: ordered and stable, random and dynamic, or both?

Timothy J. Stasevich · James G. McNally

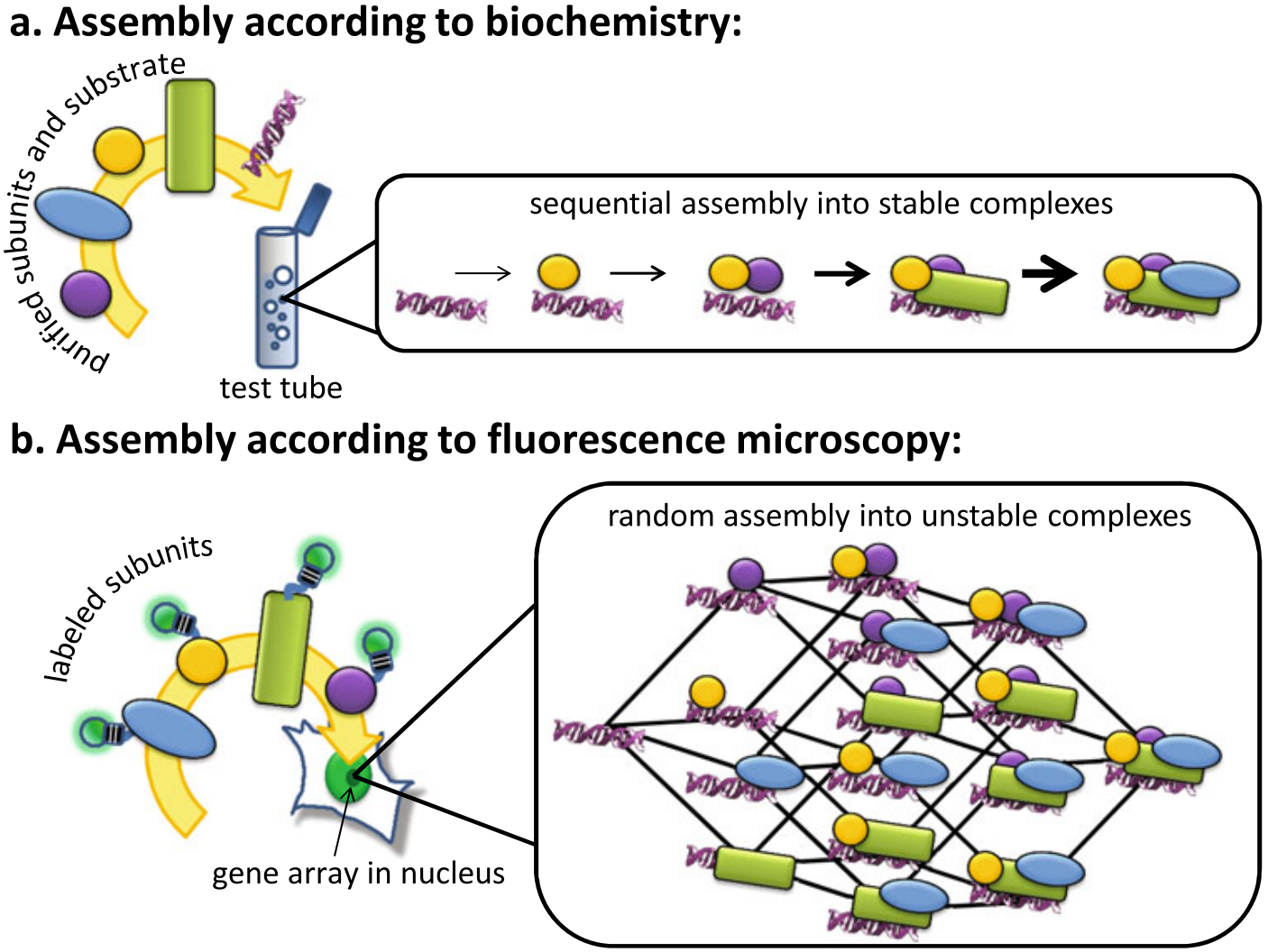


Table 1 Comparing biochemical measurements of transcription machinery binding times with live-cell microscopy measurements

Complex	Estimated binding times (min)			
	Biochemistry		Live cell microscopy	
TFIIA:DNA	*1-21*	*[t_{off}, TIRFM-array, yeast] (Bonham et al, 2009)*		
TFIIB:DNA	*4-260*	*[$t_{off,}$ TIRFM-array, yeast] (Bonham et al, 2009)*	*< 0.02*	*[$t_{1/2}$, FRAP, yeast] (Sprouse et al, 2008)*
TBP:DNA	*65-100*	*[$t_{1/2}$, TATA box, band shift, yeast] (Hoopes et al, 1992)*	*< 0.02*	*[$t_{1/2}$, FRAP, yeast] (Sprouse et al, 2008)*
	7-327	*[t_{off}, TIRFM-array, yeast] (Bonham et al, 2009)*	*~1*	*[$t_{1/2}$, FRAP, human] (Chen et al, 2002)*
	~37 / ~63 / ~80	*[t_{on+off}, Pol II / III / I DNA, ChIP, yeast] (van Werven et al, 2009)*	*1.7 - 2.8*	*[t_{off}, FRAP, human] (de Graaf et al, 2010)*
NF-KB:DNA	*3-7*	*[t_{off}, SPM, human] (Linnell et al, 2004)*	*0.02-0.03*	*[$t_{1/2}$, FRAP, mouse] (Sung et al, 2009)*
			< 0.3	*[t_{off}, p65, FLIP, human] (Bosisio et al, 2006)*
GR:DNA	*~1.5 / ~77*	*[t_{off}, monomer / dimer, band shift, rat] (Lieberman & Nordeen, 1997)*	*0.01 – 0.03*	*[t_{off}, FRAP/FCS, mouse] (Stasevich et al, 2010)*
	~ 151	*[t_{off}, DNAse footprint, rat] (Perlmann et al, 1990)*	*0.05*	*[t_{off}, FRAP, mouse] (Mueller et al, 2008)*
SWI/SNF : chromatin	*> 30*	*[t_{off}, comp. exp., yeast] (Hassan et al, 2002)*	*0.17*	*[$t_{1/2}$, FRAP, mouse] (Johnson et al, 2008)*
Gal4:DNA	*~ 250*	*[t_{off}, SPM, yeast] (Shumaker-Parry et al, 2004)*		
	~ 15	*[t_{on+off}, ChIP, yeast] (Nalley et al, 2009)*		
TFIIE:Pol II	*~ 4.8*	*[t_{off}, SPM, yeast] (Bushnell et al, 1996)*		
TFIIB:Pol II	*~ 44*	*[t_{off}, SPM, yeast] (Bushnell et al, 1996)*		
TFIIF:Pol II	*~ 3.7*	*[t_{off}, SPM, yeast] (Bushnell et al, 1996)*		

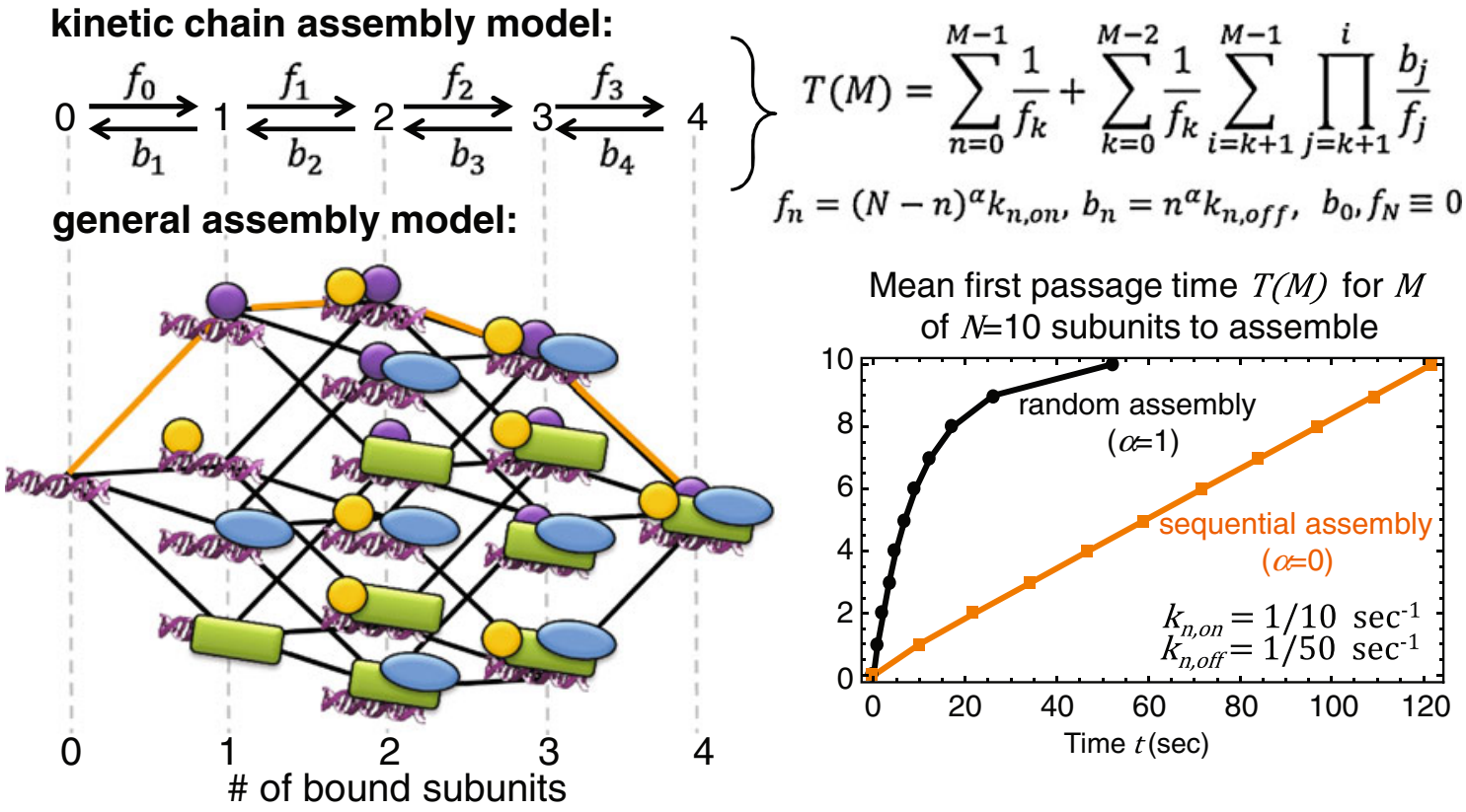


Fig. 3 Kinetic chain model for assembly. In the most general assembly model involving N components (either individual subunits or preformed subcomplexes), there are 2^N assembly states and $N2^{N-1}$ association constants. In the kinetic chain model of assembly, this is reduced to just $2N$ parameters representing forward (f_n) and backward (b_n) transitions between complexes with n bound subunits. In this simplified model, the mean time $T(M)$ to first form a complex with M of N subunits assembled, referred to as the MFPT, can be explicitly written in terms of f_n and b_n, which themselves depend on the subunit binding on and off rates, $k_{n,on}$ and $k_{n,off}$, respectively, with n ranging from 0 to N. In this model, when α=1 assembly is random and when α=0 assembly is sequential. The figure on the *left* illustrates the simplified kinetic chain model (*top of panel*) for the general assembly model (*bottom of panel*) for N=4 subunits. All paths are used in a random assembly strategy, but only one path (*thick orange*) is used in a sequential assembly strategy. The graph on the *right* compares the random (α=1) and sequential (α=0) MFPTs for a complex with N=10 subunits with $k_{n,\,on}$=1/10 s^{-1} and $k_{n,\,off}$=1/50 s

www.sciencemag.org SCIENCE VOL 291 2 FEBRUARY 2001

Protein Dynamics: Implications for Nuclear Architecture and Gene Expression

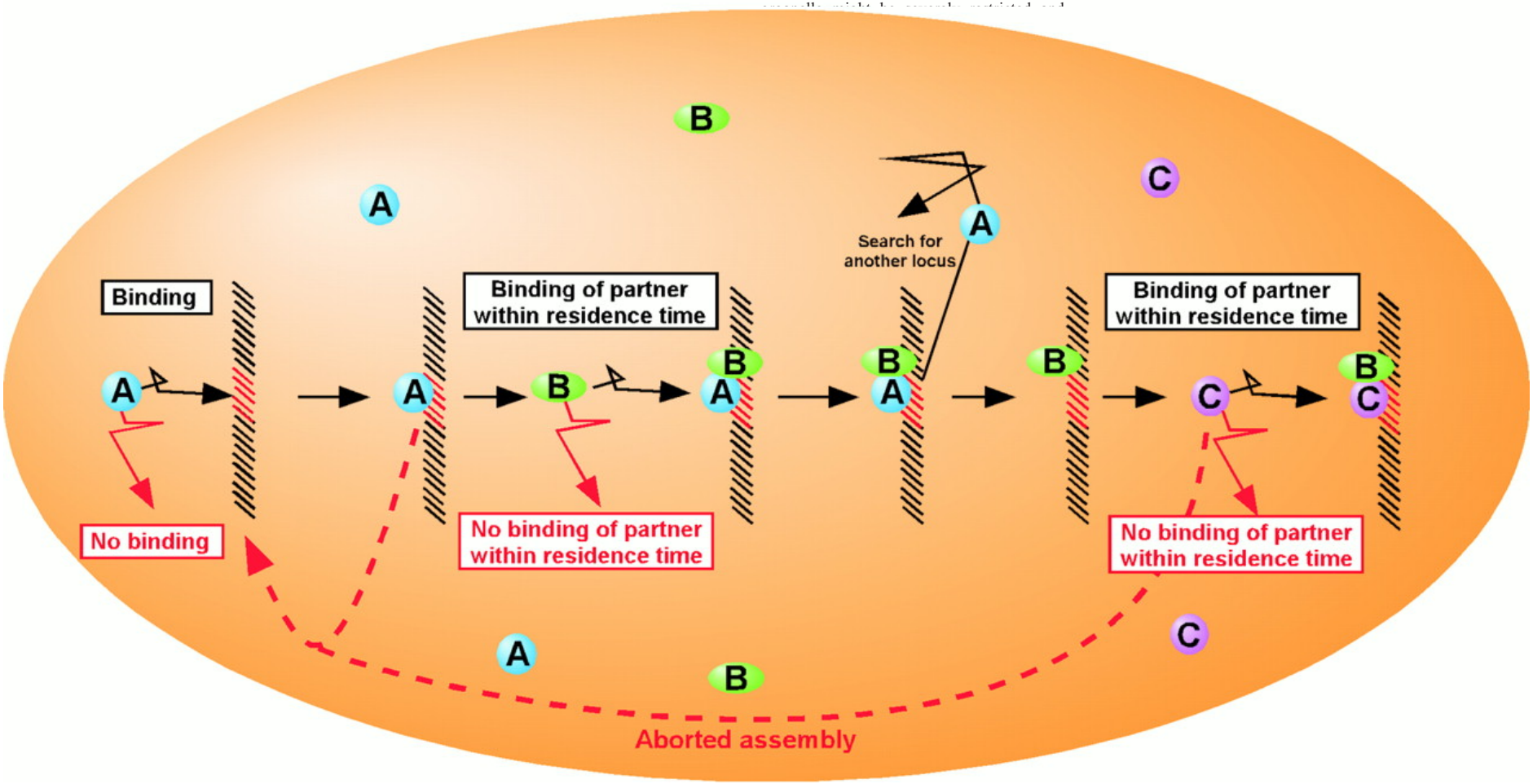
Tom Misteli

Studies of nuclear architecture reveal that the dynamic properties of proteins in the nucleus are critical for their function. The high mobility of proteins ensures their availability throughout the nucleus; their dynamic interplay generates an ever-changing, but overall stable, architectural framework, within which nuclear processes take place. As a consequence, overall nuclear morphology is determined by the functional interactions of nuclear components. The observed dynamic properties of nuclear proteins are consistent with a central role for stochastic mechanisms in gene expression and nuclear architecture.

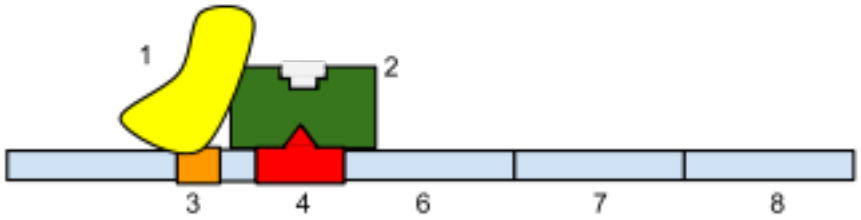
(Fig. 2). These experiments have given important new insights into nuclear architecture and function.

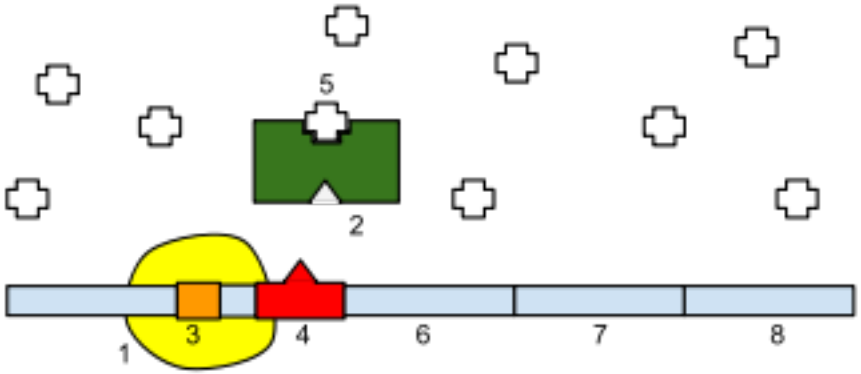
Proteins Roam the Cell Nucleus

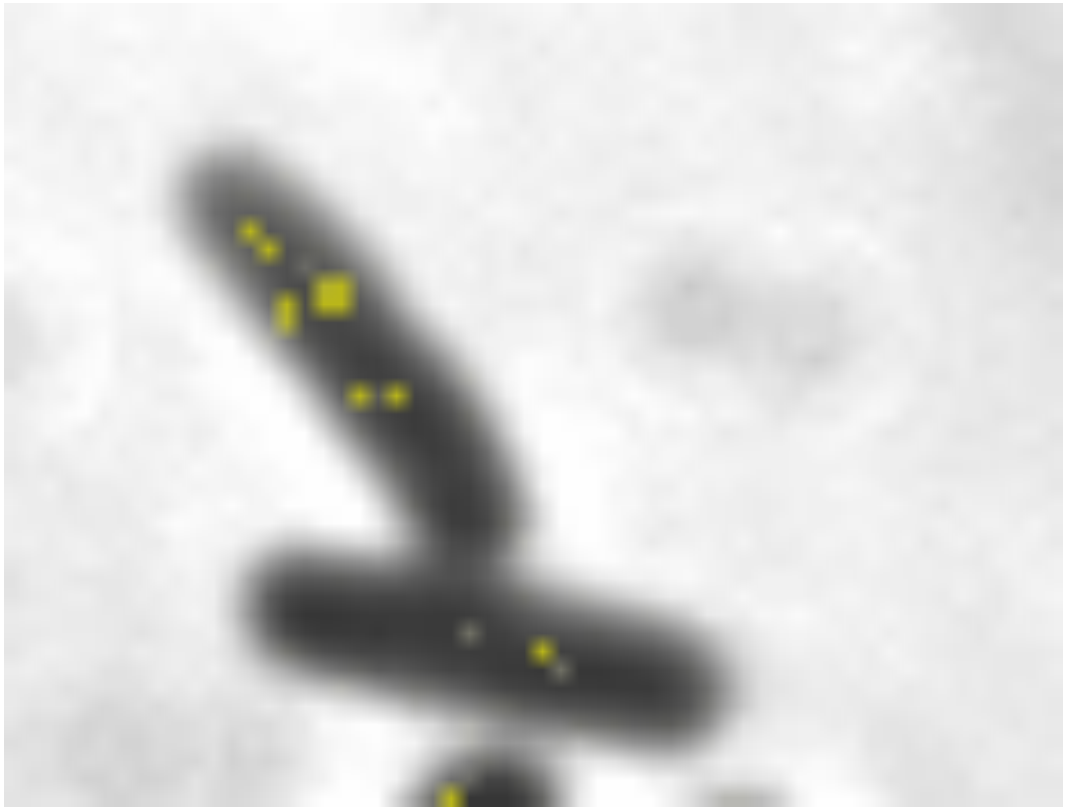
Considering the high DNA content and the large amounts of RNAs and proteins, one might intuitively think of the nucleus as a viscous, gel-like environment. If this were true, the movement of proteins within the



Lac operon







This time-lapse movie shows fluorescence overlay (yellow) on phase contrast images of a cell dividing into two genetically identical daughter cells. One daughter cell changes into a phenotype with very high fluorescence, while the other daughter cell does not. This change in phenotype is the result of the stochastic, full dissociation of the tetrameric repressor from all of its binding sites.

Choi Paul, Cai Long, Frieda Kirsten, Xie Xiaoliang Sunney(2008). A stochastic single-molecule event triggers phenotype switching of a bacterial cell. *Science* . **322** : 442-446.

Probing Transcription Factor Dynamics at the Single-Molecule Level in a Living Cell

Johan Elf,[1]* Gene-Wei Li,[2]* X. Sunney Xie[1]†

Transcription factors regulate gene expression through their binding to DNA. In a living *Escherichia coli* cell, we directly observed specific binding of a *lac* repressor, labeled with a fluorescent protein, to a chromosomal *lac* operator. Using single-molecule detection techniques, we measured the kinetics of binding and dissociation of the repressor in response to metabolic signals. Furthermore, we characterized the nonspecific binding to DNA, one-dimensional (1D) diffusion along DNA segments, and 3D translocation among segments through cytoplasm at the single-molecule level. In searching for the operator, a *lac* repressor spends ~90% of time nonspecifically bound to and diffusing along DNA with a residence time of <5 milliseconds. The methods and findings can be generalized to other nucleic acid binding proteins.

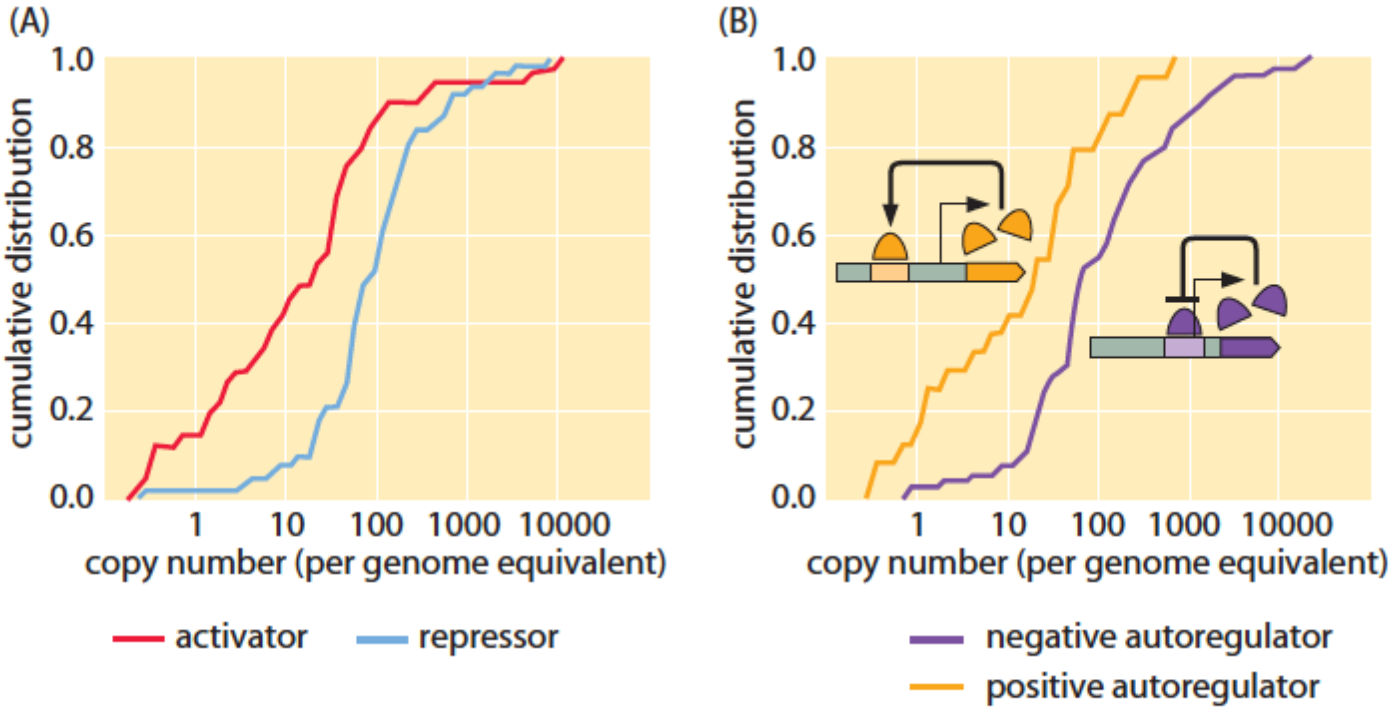
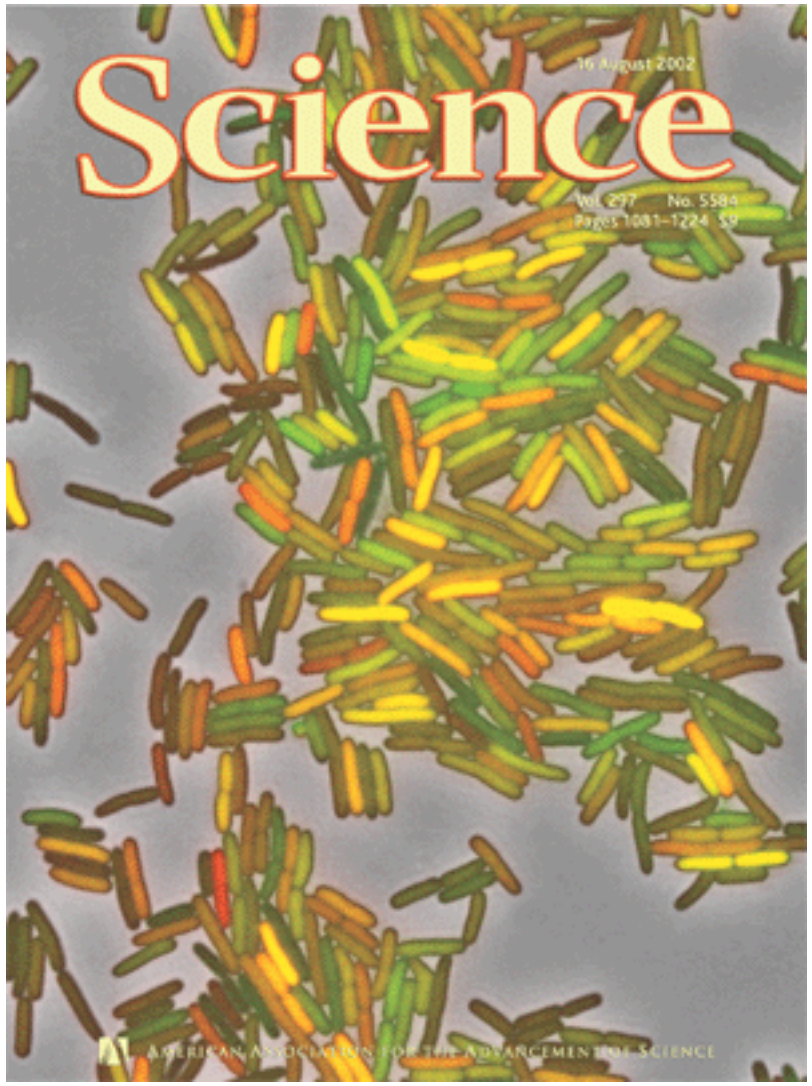


Figure 1: Measured copy numbers of transcription factors in *E. coli*. (A) Cumulative distributions for both activators and repressors showing that activators typically occur between 1 and 100 copies per cell whereas repressors generally occur between 10-1000 copies per cell. (B) Cumulative distributions for autoregulators. (adapted from G.-W. Li et al, Cell 157, 624–635, 2014)



Science 16 August 2002:
Vol. 297 no. 5584 pp. 1183-1186
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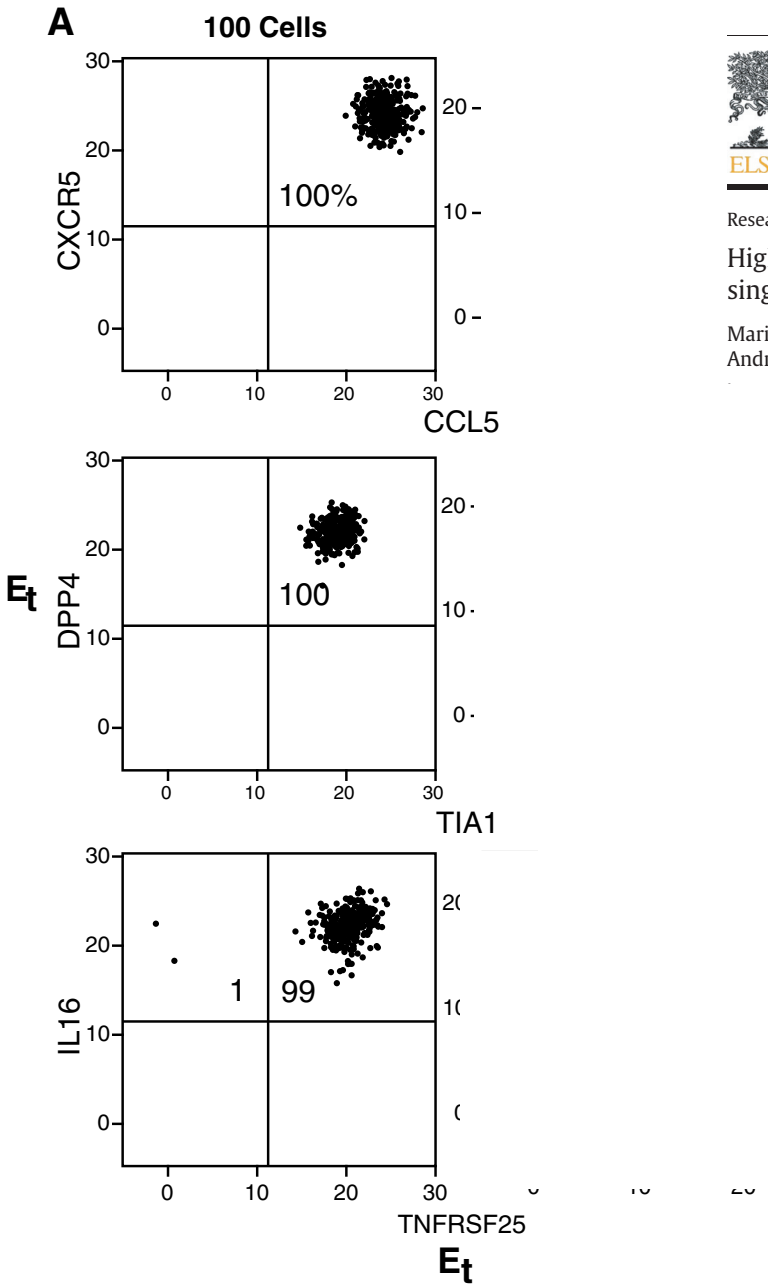
REPORT

Stochastic Gene Expression in a Single Cell

Michael B. Elowitz, Arnold J. Levine, Eric D. Siggia, Peter S. Swain



Journal of Immunological Methods 391 (2013) 133–145

Contents lists available at SciVerse ScienceDirect

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journal homepage: www.elsevier.com/locate/jim

Research paper

Highly multiplexed quantitation of gene expression on single cells

Maria H. Dominguez [a,1], Pratip K. Chattopadhyay [a,*,1], Steven Ma [b], Laurie Lamoreaux [b], Andrew McDavid [c], Greg Finak [c], Raphael Gottardo [c], Richard A. Koup [b], Mario Roederer [a]

Fig. 9. *Gene expression compared between single-cell and 100-cell samples.* A) 100-cell data suggests that genes are co-expressed; however, at the single-cell level the true, independent expression of these genes can be recognized (percent of cells expressing given marker combinations are shown at each quadrant). B) There is a strong correlation between single-cell and 100-cell gene expression data (normalized to single cells).

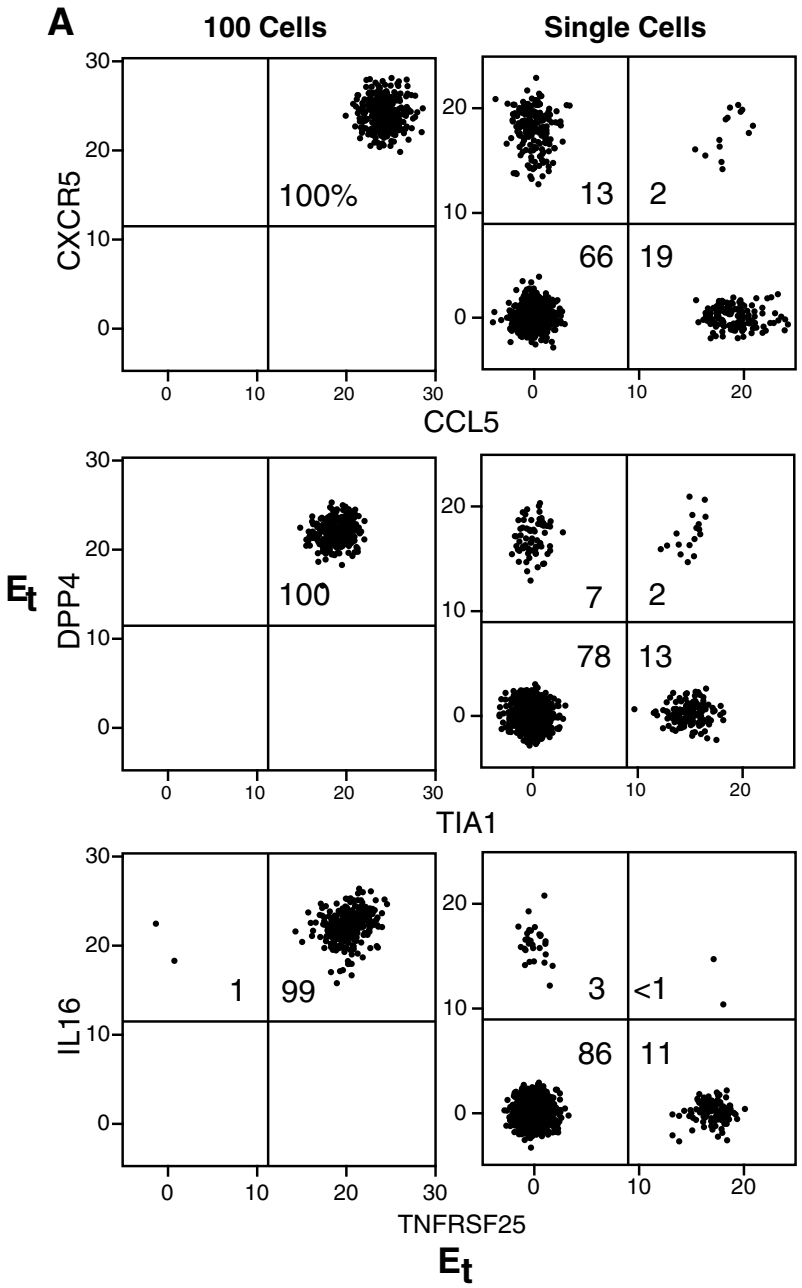
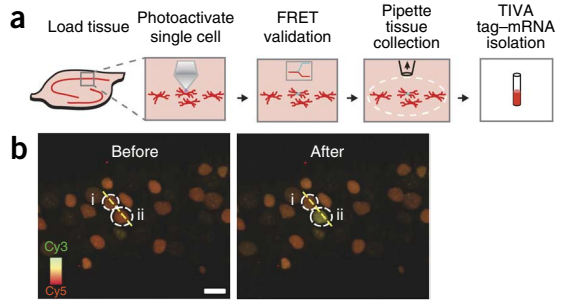


Fig. 9. *Gene expression compared between single-cell and 100-cell samples.* A) 100-cell data suggests t
the true, independent expression of these genes can be recognized (percent of cells expressing given
is a strong correlation between single-cell and 100-cell gene expression data (normalized to single

Transcriptome *in vivo* analysis (TIVA) of spatially defined single cells in live tissue

Ditte Lovatt[1,6], Brittani K Ruble[2,6], Jaehee Lee[1], Hannah Dueck[3], Tae Kyung Kim[1], Stephen Fisher[3], Chantal Francis[3], Jennifer M Spaethling[1], John A Wolf[4], M Sean Grady[4], Alexandra V Ulyanova[4], Sean B Yeldell[2], Julianne C Griepenburg[2], Peter T Buckley[1], Junhyong Kim[3,5], Jai-Yoon Sul[1], Ivan J Dmochowski[2,7] & James Eberwine[1,5,7]

| VOL.11 NO.2 | FEBRUARY 2014 | NATURE METHODS



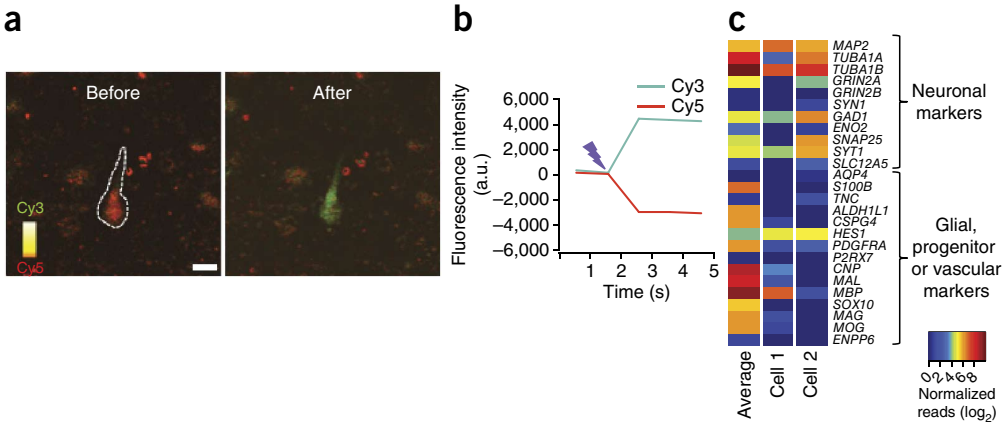


Figure 5 | TIVA tag capture of mRNA from cells in human live brain tissue specimen obtained from biopsy of the right frontal cortex from a subject undergoing surgery for communicating hydrocephalus. (**a**) Micrographs of TIVA tag–loaded cells identified by FRET signal, before and after uncaging, which was performed using the same parameters as in mouse. Scale bar, 10 µm. (**b**) FRET signal upon TIVA-tag activation (lightning bolt). (**c**) Heatmap comparing expression of common cell type–specific markers in an average pool of 13 TIVA tag captured cells and in two TIVA tag captured individual cells.

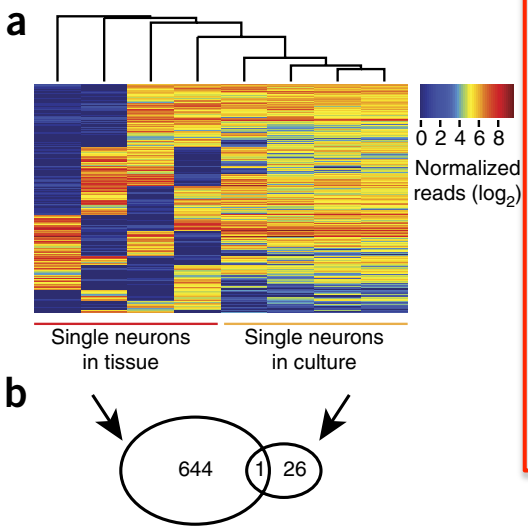
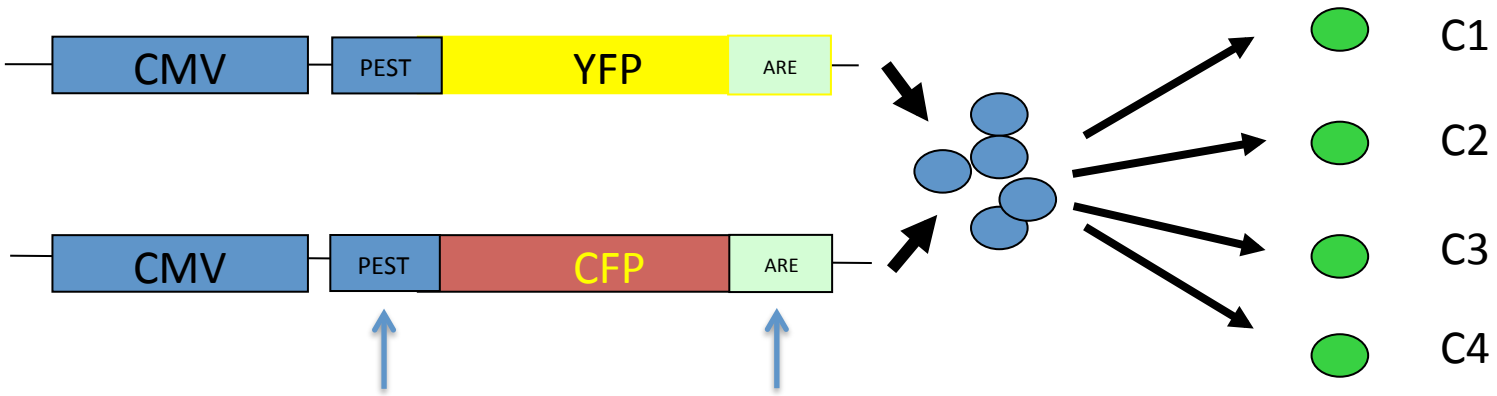


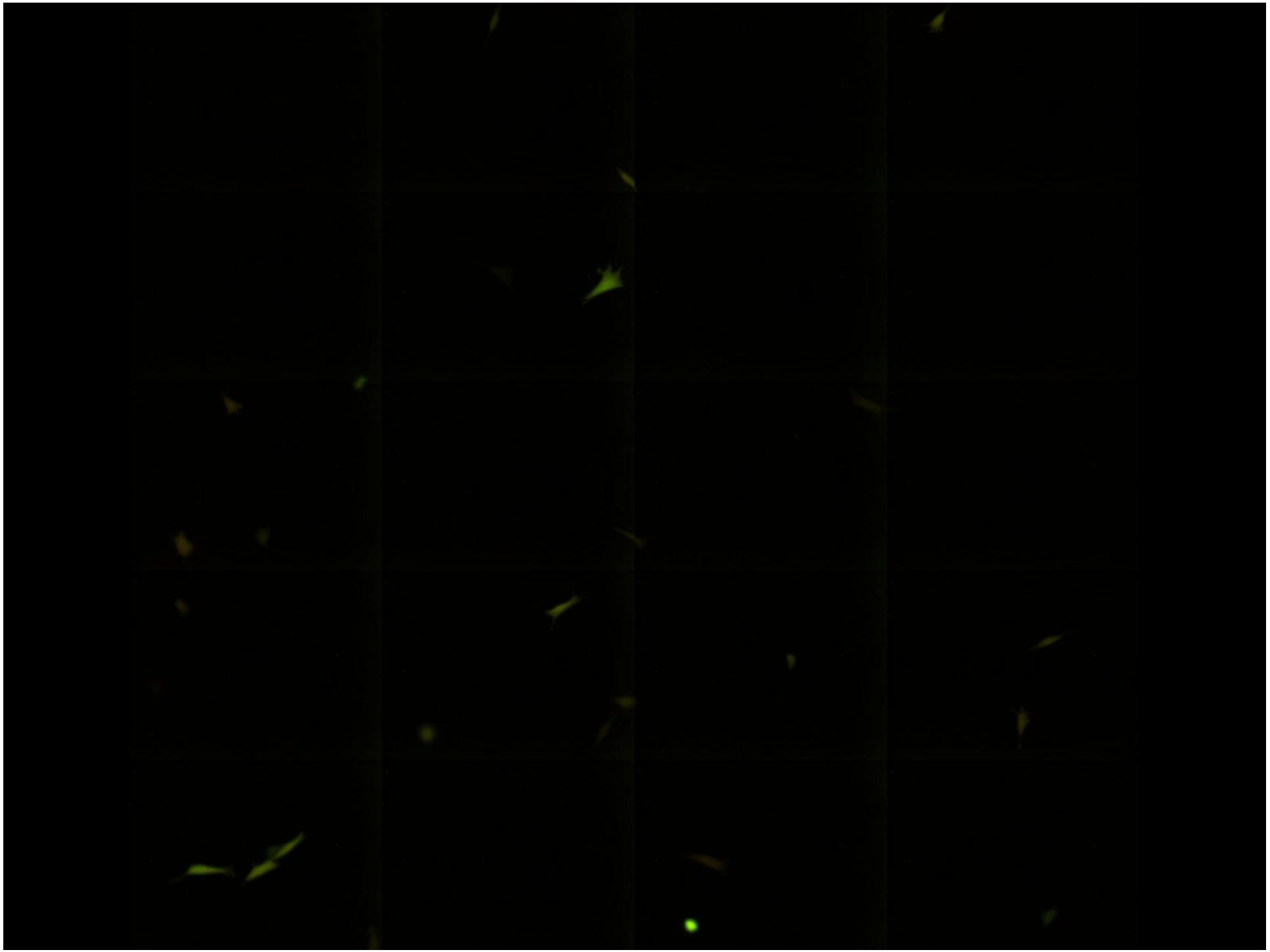
Figure 6 | Bimodal transcripts in single hippocampal neurons in tissue and in culture. (**a**) Heatmaps shows clustering of 645 bimodally expressed genes (horizontal lines) in four single cells from hippocampal tissue compared to four single hippocampal neurons in culture. Bimodally expressed genes were defined as having a gap in expression of at least four log units in two samples. To be especially stringent in this analysis, two samples were required to have expression values on either side of this gap. The cells with low expression were required to have fewer than ten normalized counts for the biomodally expressed transcript. (**b**) Overlap between bimodal genes in single neurons from tissue and from culture (4 cells in each group).

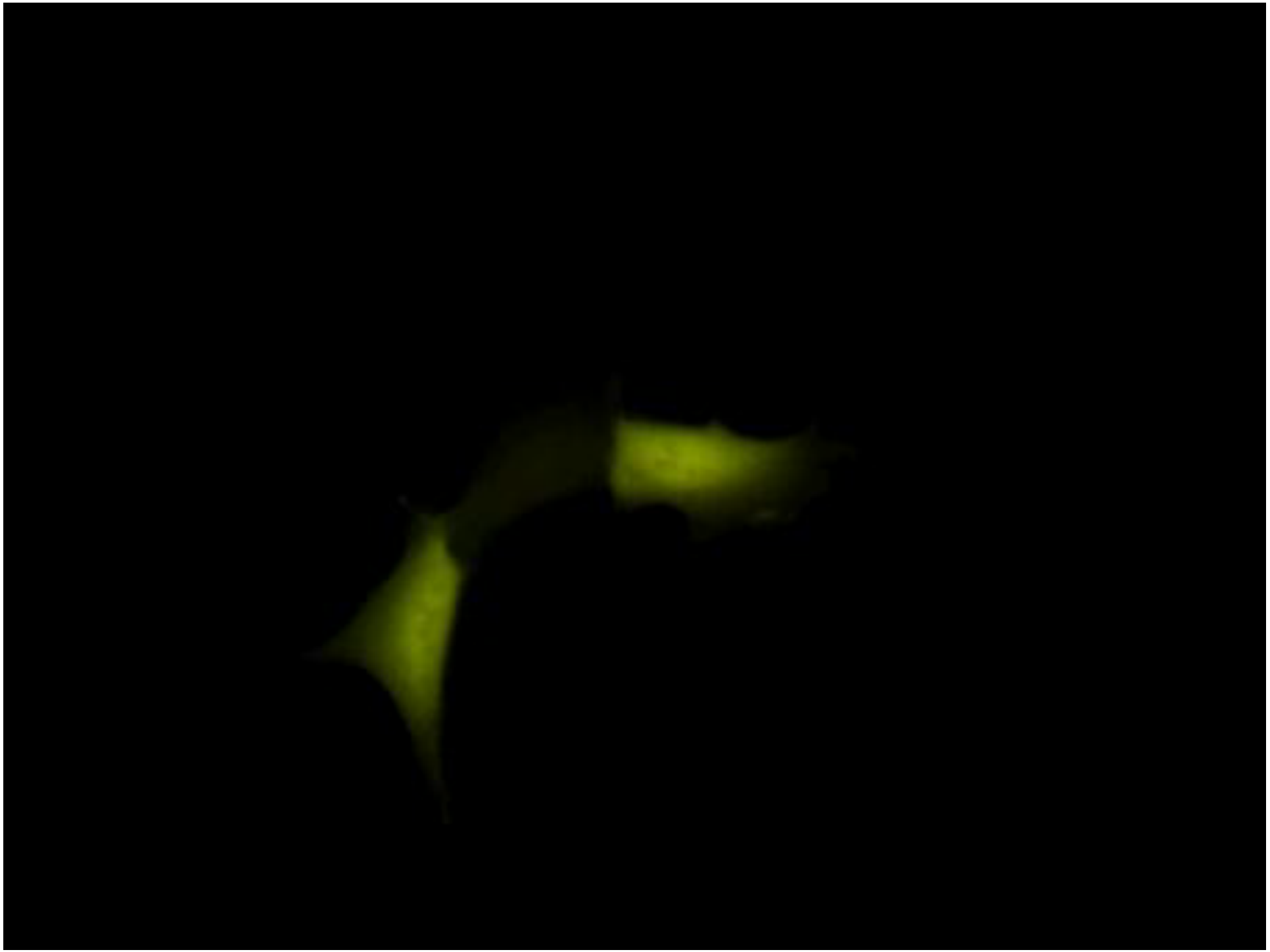
Single-cell heterogeneity is a well-accepted phenomenon but has remained understudied in complex tissues owing to technical limitations of mRNA isolation. We showed using the TIVA tag that CA1 hippocampal neurons in live hippocampal tissue expressed fewer genes overall but had more bimodally expressed genes than hippocampal neurons in culture (**Fig. 6**), suggesting an important role for the microenviroment in modulating gene expression in cells. Recently, there have been suggestions that variation in expression is in part stochastic, arising from both intrinsic noise (stochastic nature of biochemical reactions) and extrinsic noise (changes in cellular regulatory proteins)[4]. Our data suggest that the ~30% difference in number of genes expressed may be attributable to extrinsic noise. When a neuron is removed

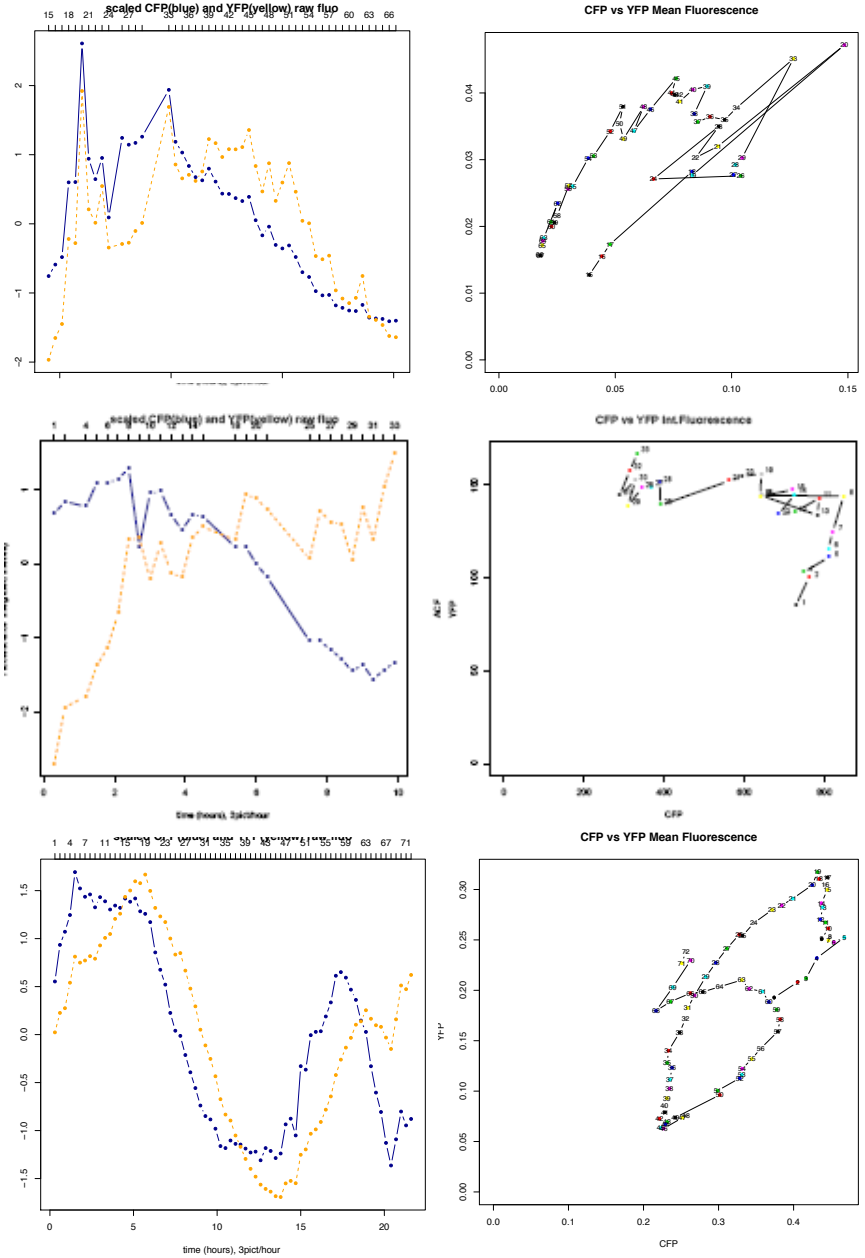


reduced mRNA and protein stability

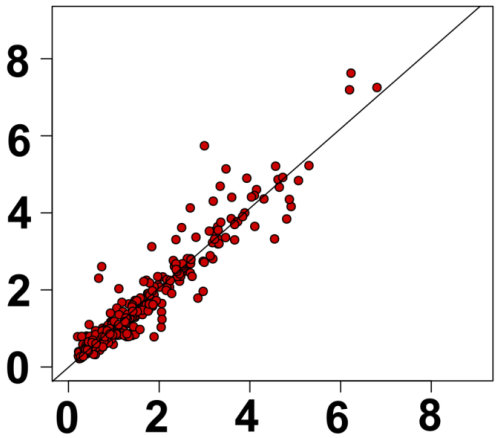
Corre et al. PlosONE







Correlation of the half-life of the YFP and CFP fluorescent proteins.



hours

Fundamental limits on the suppression of molecular fluctuations

Ioannis Lestas[1], Glenn Vinnicombe[1] & Johan Paulsson[2]

Life in the cell is a complex battle between randomizing and correcting statistical forces: births and deaths of individual molecules create spontaneous fluctuations in abundances[1–4]—noise—and many control circuits have evolved to eliminate, tolerate or exploit the noise[5–8]. The net outcome is difficult to predict because each control circuit in turn consists of probabilistic chemical reaction

In such systems, the minimal error decreases with the quartic root of the integer number of signalling events, making a decent job 16 times harder than a half-decent job. This perhaps explains why there is so much biochemical noise—correcting it would be too costly—but also constrains other aspects of life in the cell. For example, the noise levels may increase or decrease along signalling cascades, depending on the kinetic details at each step, but information about upstream states is always progressively and irreversibly lost. Although it is tempting to believe that large reaction networks are capable of almost anything if the rates are suitably nonlinear, the opposite perspective may thus be more appropriate: having more steps where one component affects the rates of another creates more opportunities for information to be lost and fundamentally prevents more types of behaviour.

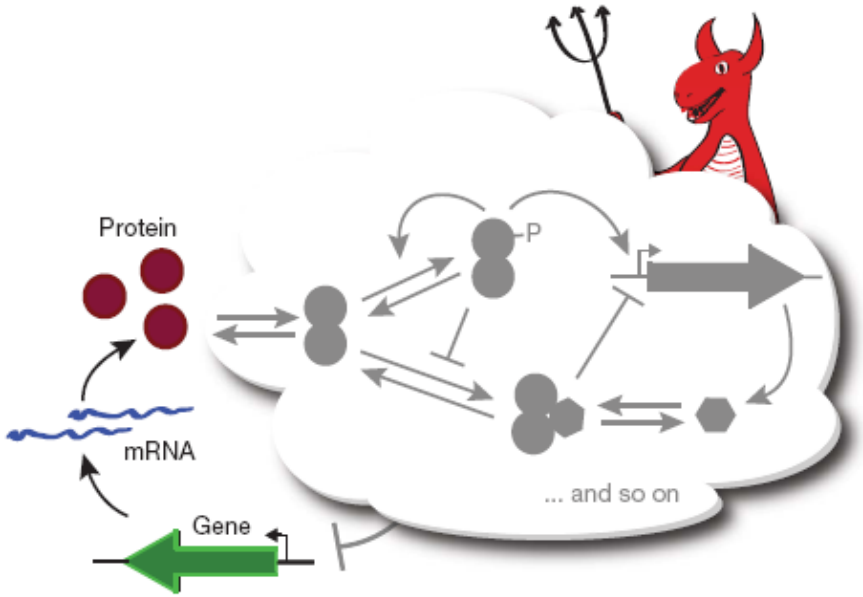
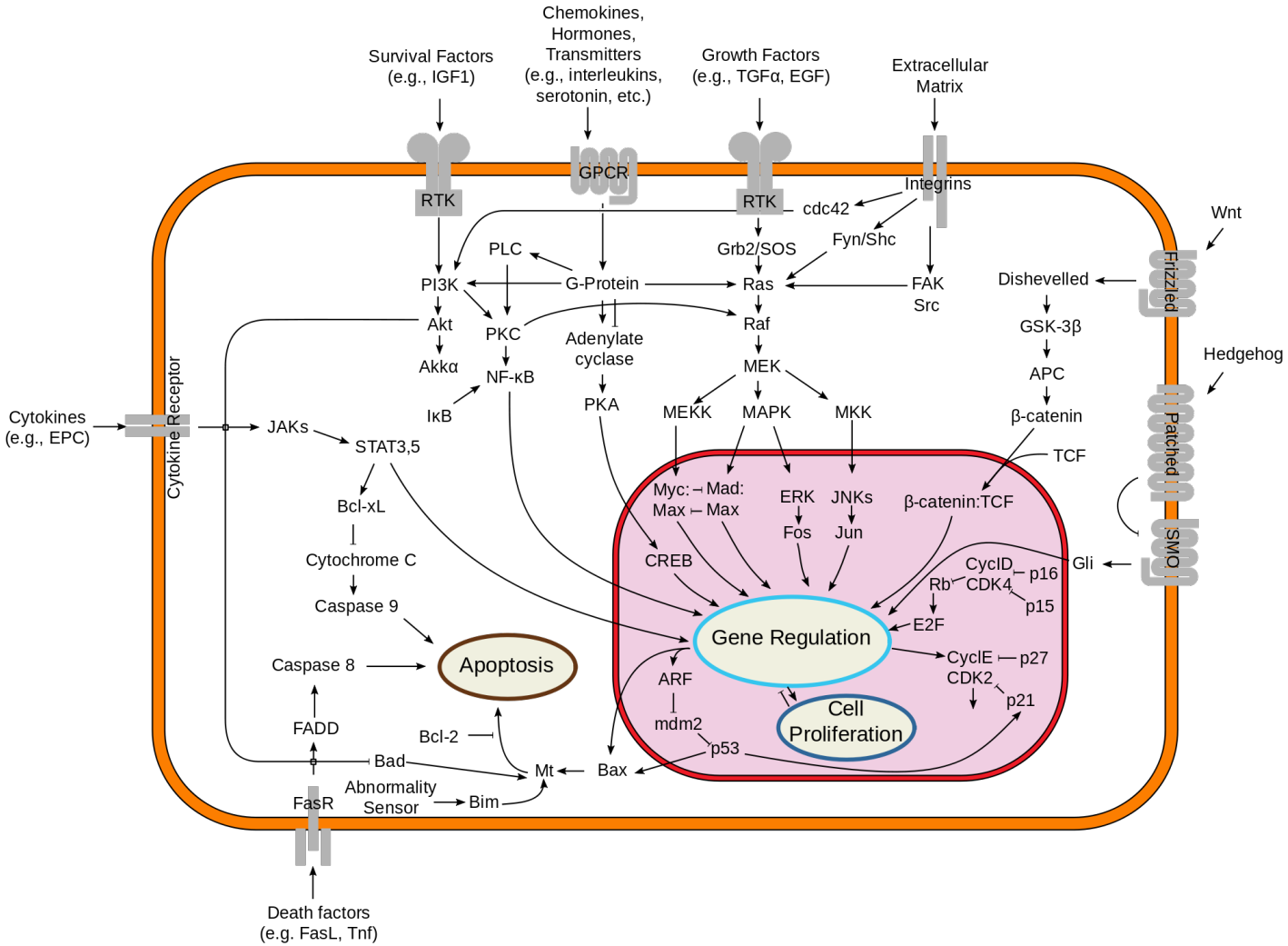


Figure 1 | Schematic of optimal control networks and information loss. Biological networks can be overwhelmingly complex, with numerous feedback loops and signalling steps. Predictions about noise then rely on quantitative estimates for how every probabilistic reaction rate responds to every type of perturbation. To investigate bounds on behaviour, most of the network is here replaced by a 'control demon' representing a controller that is optimized over all possible network topologies, rates and mechanisms. The bounds are then calculated in terms of the few specified features.

Vol 467|9 September 2010|doi:10.1038/nature09333


Survival Factors
(e.g., IGF1)
Chemokines,
Hormones,
Transmitters
(e.g., interleukins,
serotonin, etc.)
Growth Factors
(e.g., TGFα, EGF)
Extracellular
Matrix
RTK
GPCR
RTK
Integrins
cdc42
Fyn/Shc
Grb2/SOS
PLC
PI3K
G-Protein
Ras
FAK
Src
Wnt
Dishevelled
Frizzled
Akt
PKC
Adenylate
cyclase
Raf
GSK-3β
Akkα
NF-κB
MEK
APC
Hedgehog
IκB
PKA
MEKK
MAPK
MKK
β-catenin
Cytokines
(e.g., EPC)
Cytokine Receptor
JAKs
STAT3,5
TCF
Patched
Bcl-xL
Myc:
Max
Mad:
Max
ERK
JNKs
β-catenin:TCF
Fos
Jun
Cytochrome C
CREB
CyclD
p16
Gli
Rb
CDK4
p15
Caspase 9
E2F
Gene Regulation
SMO
Caspase 8
Apoptosis
CyclE
p27
ARF
CDK2
p21
FADD
Cell
Proliferation
mdm2
Bcl-2
p53
Bad
Mt
Bax
Abnormality
Sensor
FasR
Bim
Death factors
(e.g. FasL, Tnf)

TATA INSTITUTE OF FUNDAMENTAL RESEARCH

Living matter April 16 to April 26

Random walk across the epigenetic landscape
Part 2

András Páldi

andras.paldi@ephe.sorbonne.fr

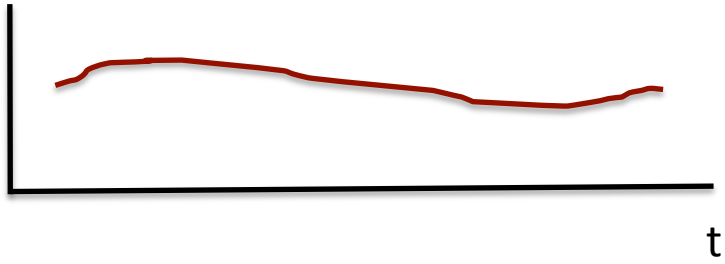
1 Order and stability in living systems: a short (and superficial) historical background.

2 Stochasticity in living cells: noise or variation?

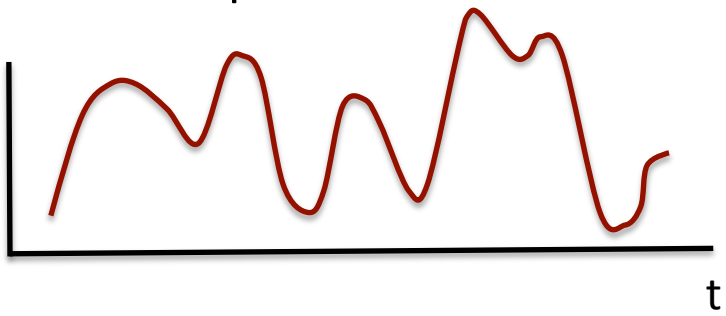
3 How cells function reliably with such an inherent variability in gene expression?

STABILITY/VARIATION

Postulate of stability



Observed phenomenon: variation



Stochasticity and Cell Fate

Richard Losick[1] and Claude Desplan[2]

Fundamental to living cells is the capacity to differentiate into subtypes with specialized attributes. Understanding the way cells acquire their fates is a major challenge in developmental biology. How cells adopt a particular fate is usually thought of as being deterministic, and in the large majority of cases it is. That is, cells acquire their fate by virtue of their lineage or their proximity to an inductive signal from another cell. In some cases, however, and in organisms ranging from bacteria to humans, cells choose one or another pathway of differentiation stochastically, without apparent regard to environment or history. Stochasticity has important mechanistic requirements. We speculate on why stochasticity is advantageous—and even critical in some circumstances—to the individual, the colony, or the species.

"*I, at any rate, am convinced that He does not play dice.*"
Albert Einstein, 1926

SCIENCE VOL 320 4 APRIL 2008

Stochastic switching as a survival strategy in fluctuating environments

Murat Acar[1,2], Jerome T Mettetal[1,2] & Alexander van Oudenaarden[1]

A classic problem in population and evolutionary biology is to understand how a population optimizes its fitness in fluctuating environments[1–4]. A population might enhance its fitness by allowing individual cells to stochastically transition among multiple phenotypes, thus ensuring that some cells are always prepared for an unforeseen environmental fluctuation. Here we experimentally explore how switching affects population growth by using the galactose utilization network of *Saccharomyces cerevisiae*. We engineered a strain that

and concentrations of nutrients and toxins. Early theoretical work on this topic often focused on understanding the connection between environmental fluctuations and genetic diversity[1–4]. However, recent studies demonstrating the importance of phenotypic heterogeneity in genetically identical cells have renewed an interest in studying this problem from the perspective of an isogenic population that is able to express multiple distinct phenotypes[10–17]. Without the need to sense the environment, cells could 'blindly' anticipate and survive environ-

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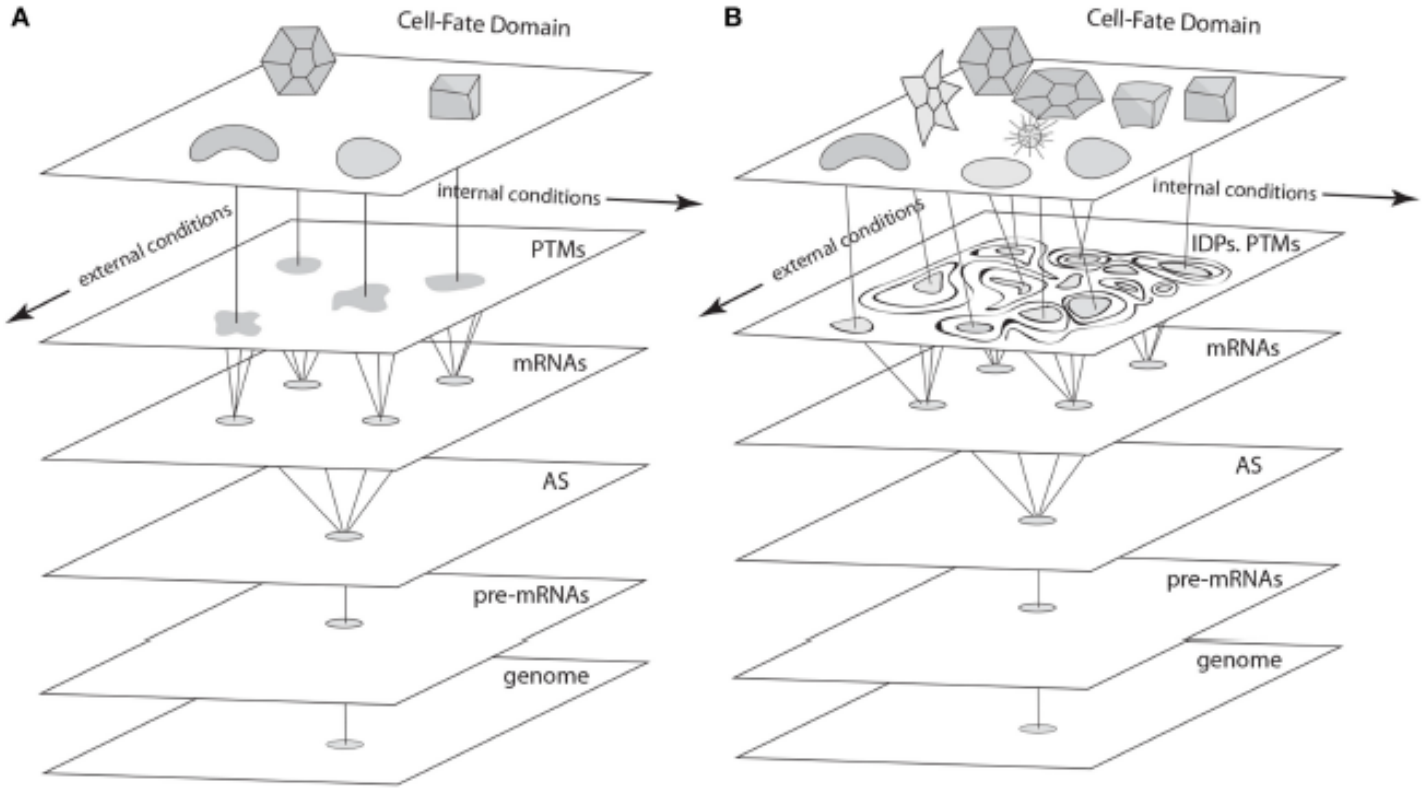


FIGURE 4 | Schematics of cell fate specification viewed from the standard deterministic GRN perspective (A) and the non-deterministic GRN perspective described in the text (B). (A) In the standard view, pre-mRNAs undergo alternative splicing (AS), and transcription factors specified by the variant mRNAs undergo post-translational modifications (PTMs) to form a cadre of proteins involved in cell-fate specification networks (GRNs, represented as irregular shapes) via their cis-acting targets. Discrete cell types result from the deterministic properties of these GRNs. **(B)** In the proposed non-deterministic view, transcription factors are generated by AS and PTM operating in the context of intrinsically disordered protein (IDP) domains. Cell-fate determination in this case (represented by interactions among components of variable, context-dependent identity and specificity), is a consequence of the time- and spatial-context dependency of each of the levels shown in this schematic, which depend on internal and external cellular conditions in a fashion that eludes deterministic description at the level of GRNs.

frontiers in
CELL AND DEVELOPMENTAL BIOLOGY

HYPOTHESIS AND THEORY ARTICLE
published: 26 February 2015
doi: 10.3389/fcell.2015.00008

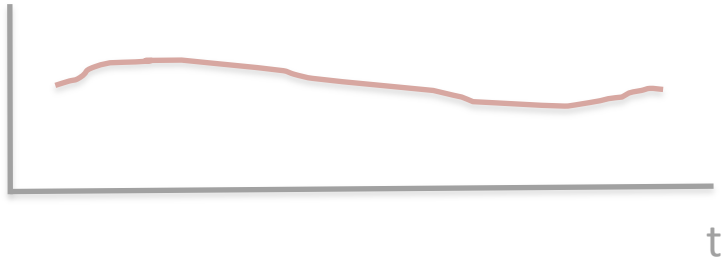
Rethinking gene regulatory networks in light of alternative splicing, intrinsically disordered protein domains, and post-translational modifications

Karl J. Niklas[1], Sarah E. Bondos[2], A. Keith Dunker[3] and Stuart A. Newman[4]*

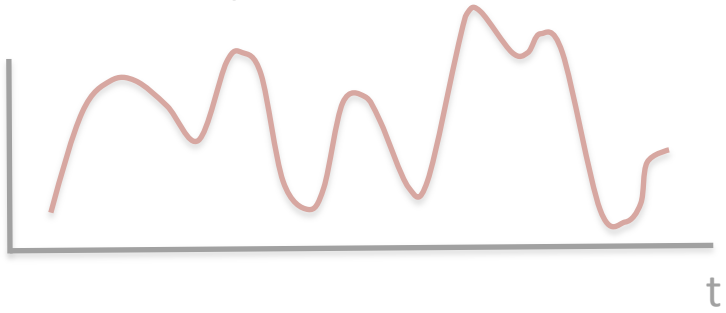
These are particular examples that explain only a small subset of observed cases!

STABILITY/VARIATION

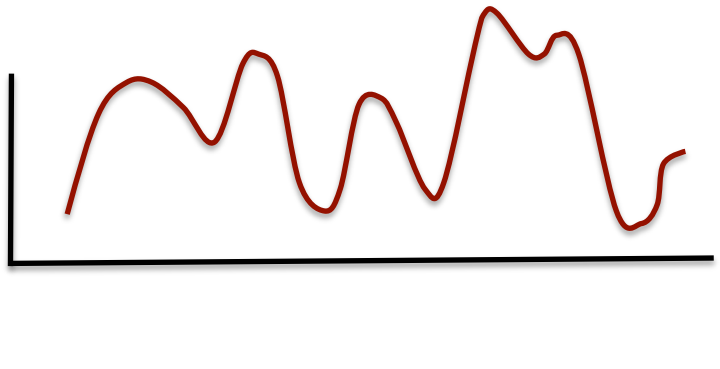
Postulate of stability



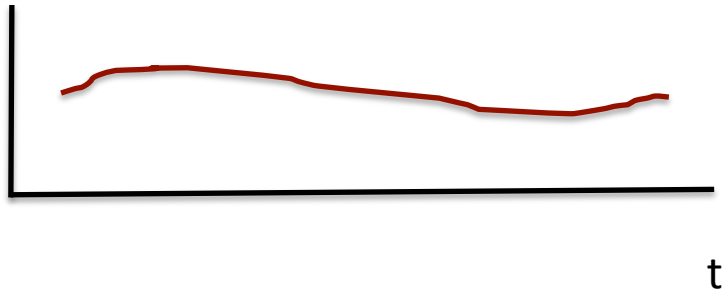
Observed phenomenon: variation



Postulate of variation



Observed phenomenon: stability



How cells function reliably with such an inherent variability of biochemical reactions (i.e. gene expression)?

How cells function reliably with such an inherent variability of biochemical reactions (i.e. gene expression)?

Spatio-temporal averaging

How cells function reliably with such an inherent variability of biochemical reactions (i.e. gene expression)?

‘‘All [the] epistemological value of the theory of probability is based on this:
large scale random phenomena in their collective action create strict, non random regularity’’.
(From: B.V. Gnedenko and A.N. Kolmogorov,
Limit Distributions for Sums of Independent Random Variables,
Reading, Ma: Addison-Wesley, 1954).

How cells function reliably with such an inherent variability of biochemical reactions (i.e. gene expression)?

“The non-physicist finds it hard to believe that really the ordinary laws of physics, which he regards as the prototype of inviolable precision, should be based on the statistical tendency of matter to go over into disorder.”

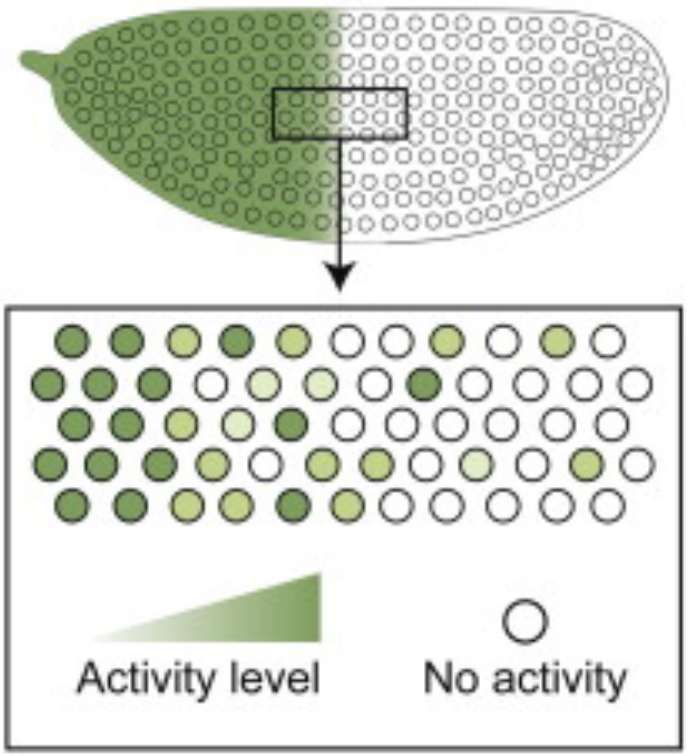
Erwin Schrödinger, What is life?

Quantitative Imaging of Transcription in Living *Drosophila* Embryos Links Polymerase Activity to Patterning

Hernan G. Garcia,[1] Mikhail Tikhonov,[1,2] Albert Lin,[1] and Thomas Gregor[1,2,*]
[1]Joseph Henry Laboratories of Physics, Princeton University, Princeton, NJ 08544, USA
[2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA

Current Biology *23*, 2140–2145, November 4, 2013

of the dynamic range of the expression boundary. This amplification is accomplished by nuclei randomly adopting active or inactive states of transcription, leading to a collective effect where the fraction of active nuclei is modulated in space. Thus, developmental patterns are not just the consequence of reproducible transcriptional dynamics in individual nuclei, but are the result of averaging expression over space and time.



Precise Developmental Gene Expression Arises from Globally Stochastic Transcriptional Activity

Shawn C. Little,[1,4] Mikhail Tikhonov,[2,3,4] and Thomas Gregor[2,3,*]
[1]Department of Molecular Biology, Howard Hughes Medical Institute

These regions thereby reveal the greatest degree of precision achievable by the system. We show that in these regions, the earliest expressed genes share common expression characteristics: despite their expression in spatially distinct territories, their rates of production are identical, and all display intrinsically stochastic transcriptional activity. These similarities suggest that expression rate and variability result from fundamental, global features of transcriptional regulation that limit the attainable degree of precision. Nevertheless, the stochastic expression results in precise and nearly uniform transcript accumulation, achieved by straightforward spatiotemporal averaging.

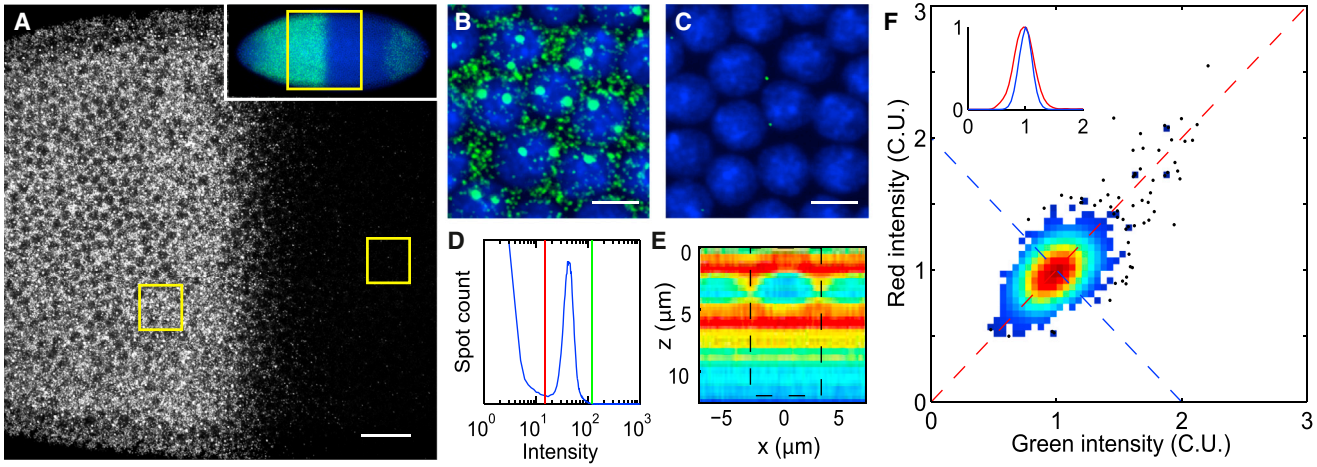


Figure 1. Counting of Absolute Transcript Number in *Drosophila* Embryos

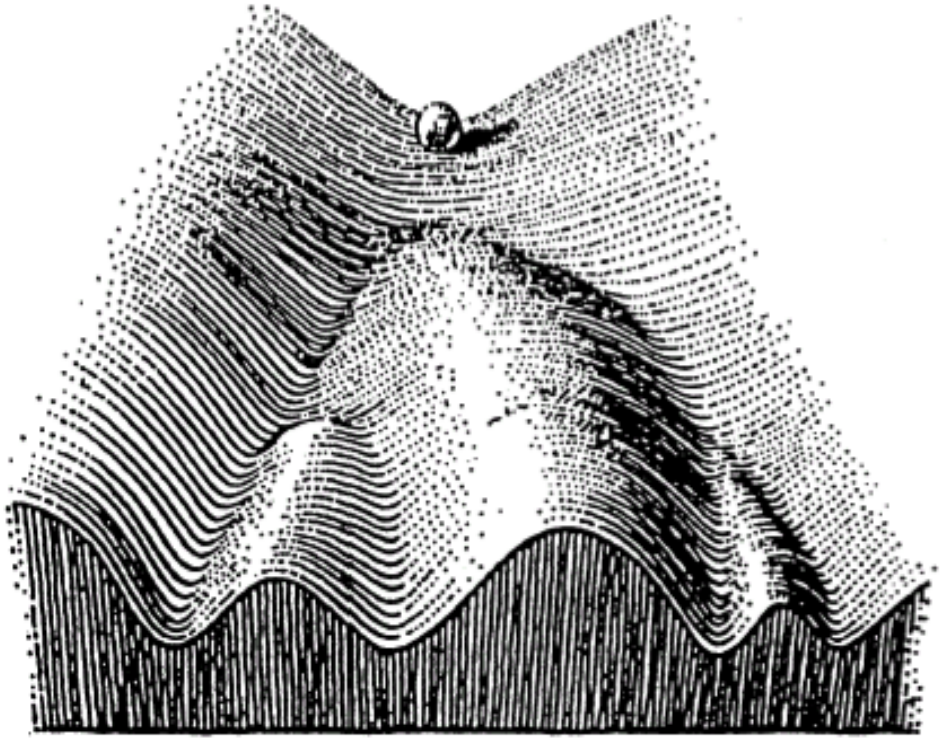
How cells function reliably with such an inherent variability of biochemical reactions (i.e. gene expression)?

Epigenetics: the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being.

Epigenesys	+	**Genetics**
Process of change		Stability
Development		Heredity

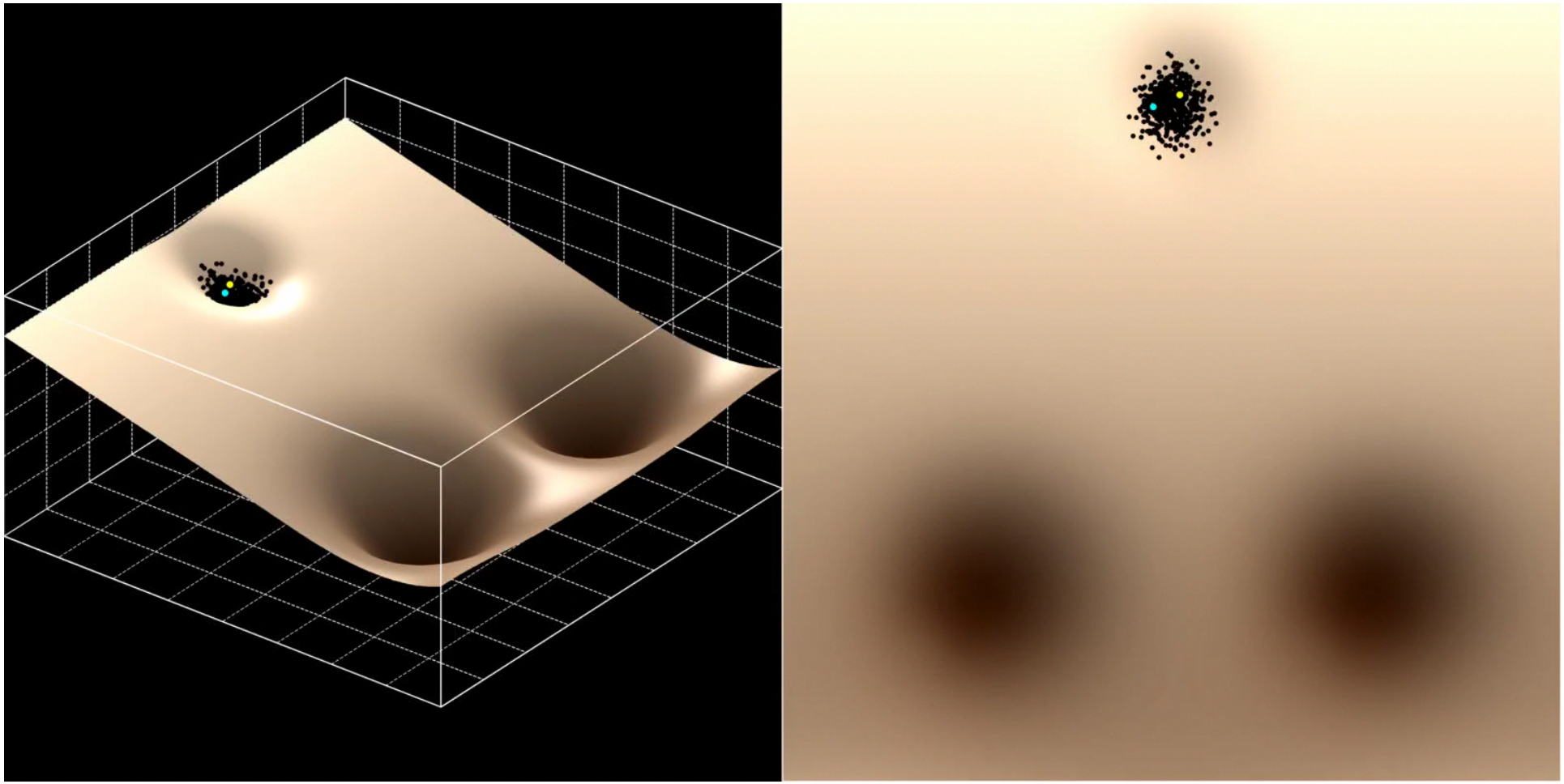
C. Waddington (1942)

Epigenetics: the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being.



C. Waddington (1942)

Rebhahn et al (2014). Eur. J. Immunol. 44: 2216–2229



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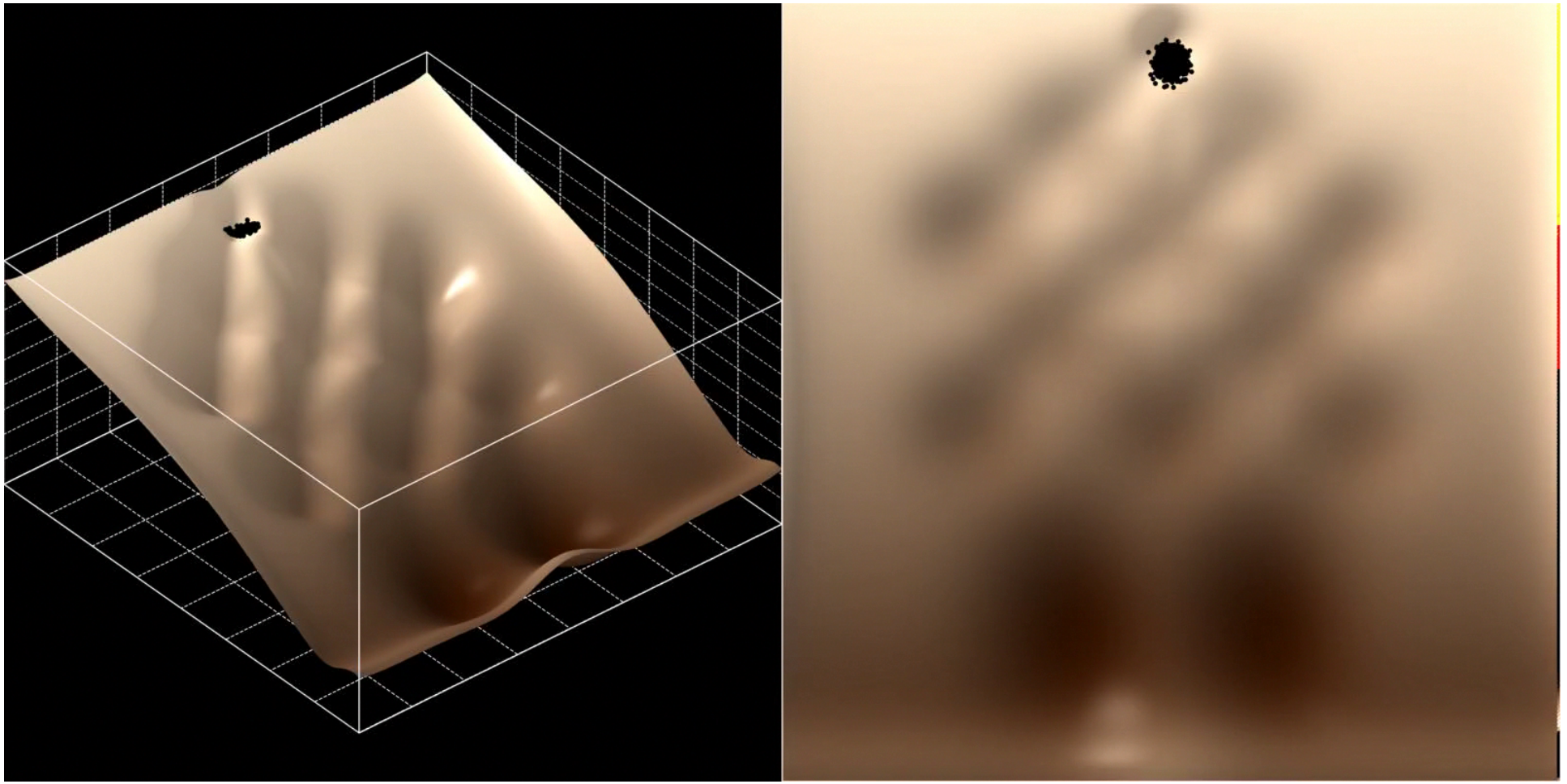
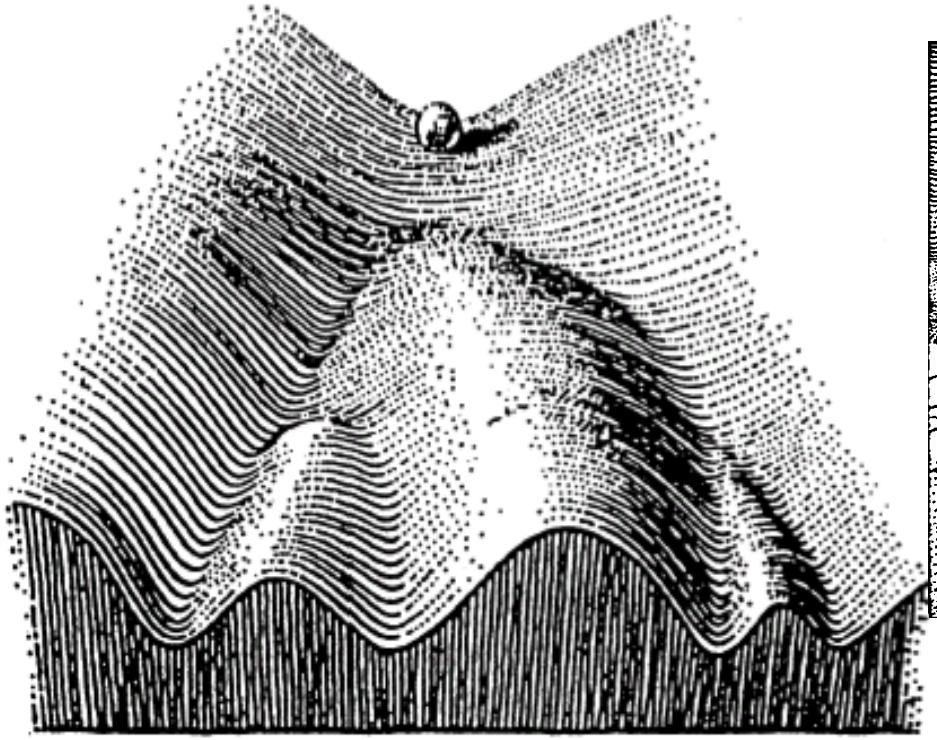
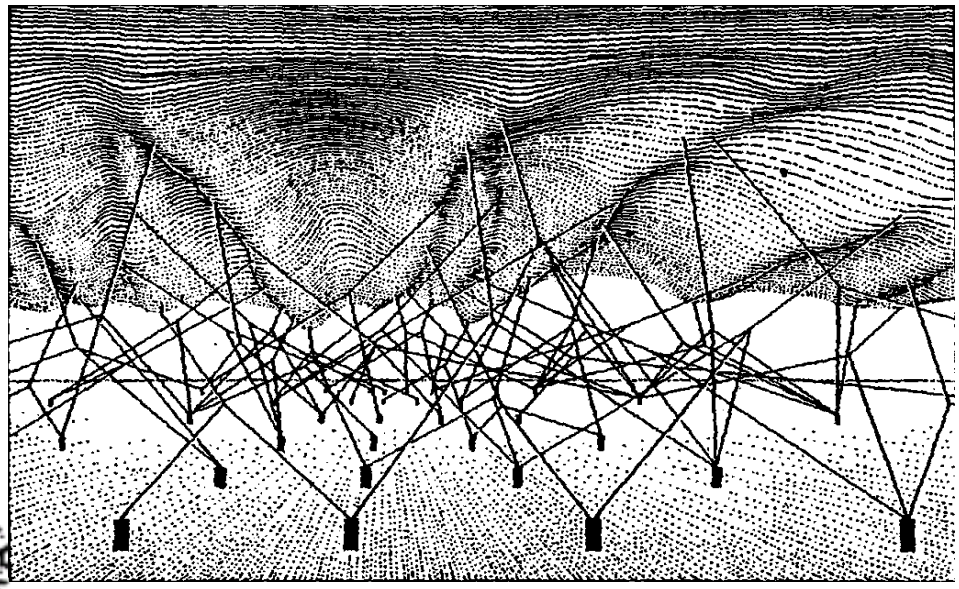


Epigenetics

C. Waddington (1942)





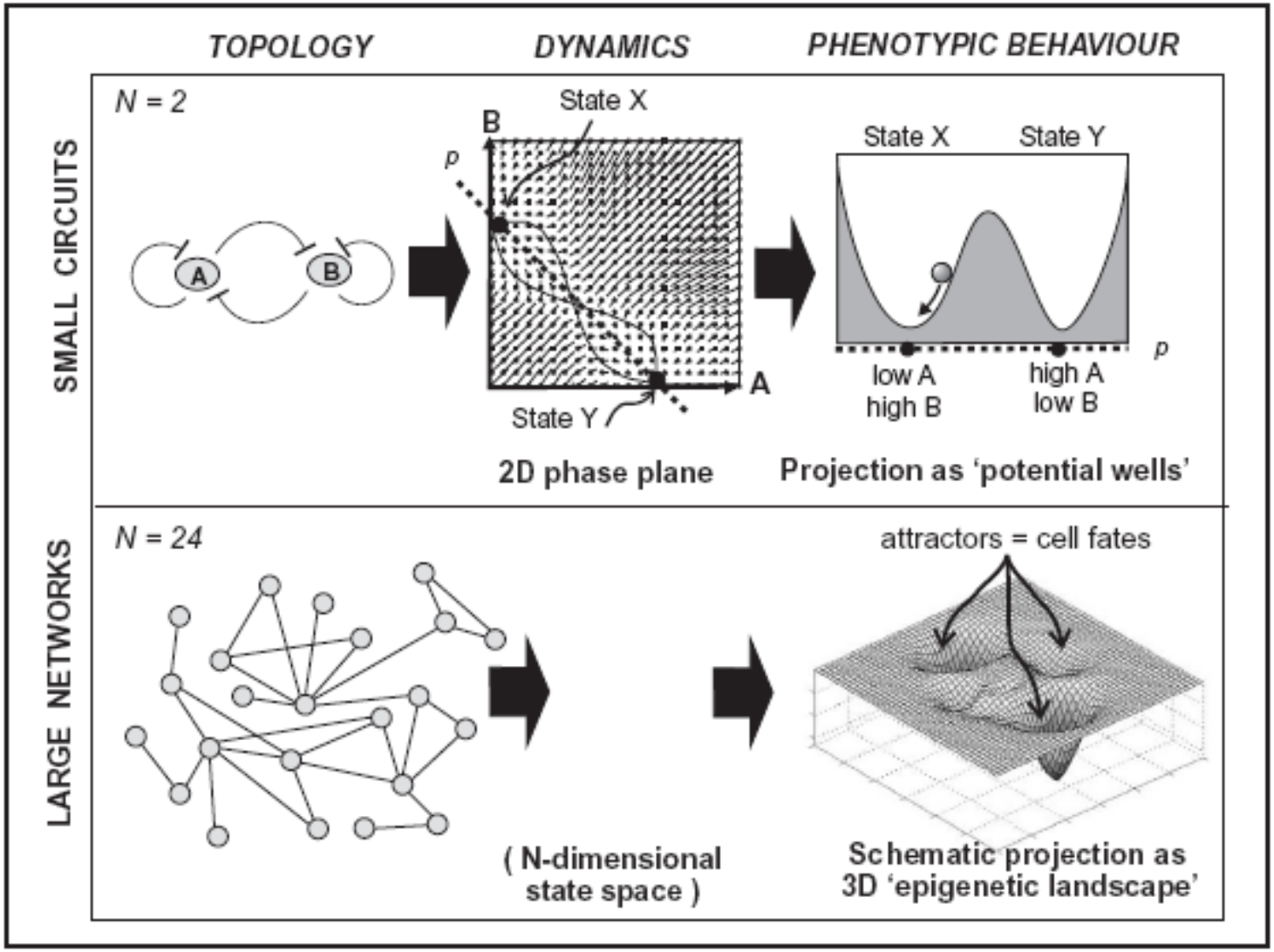
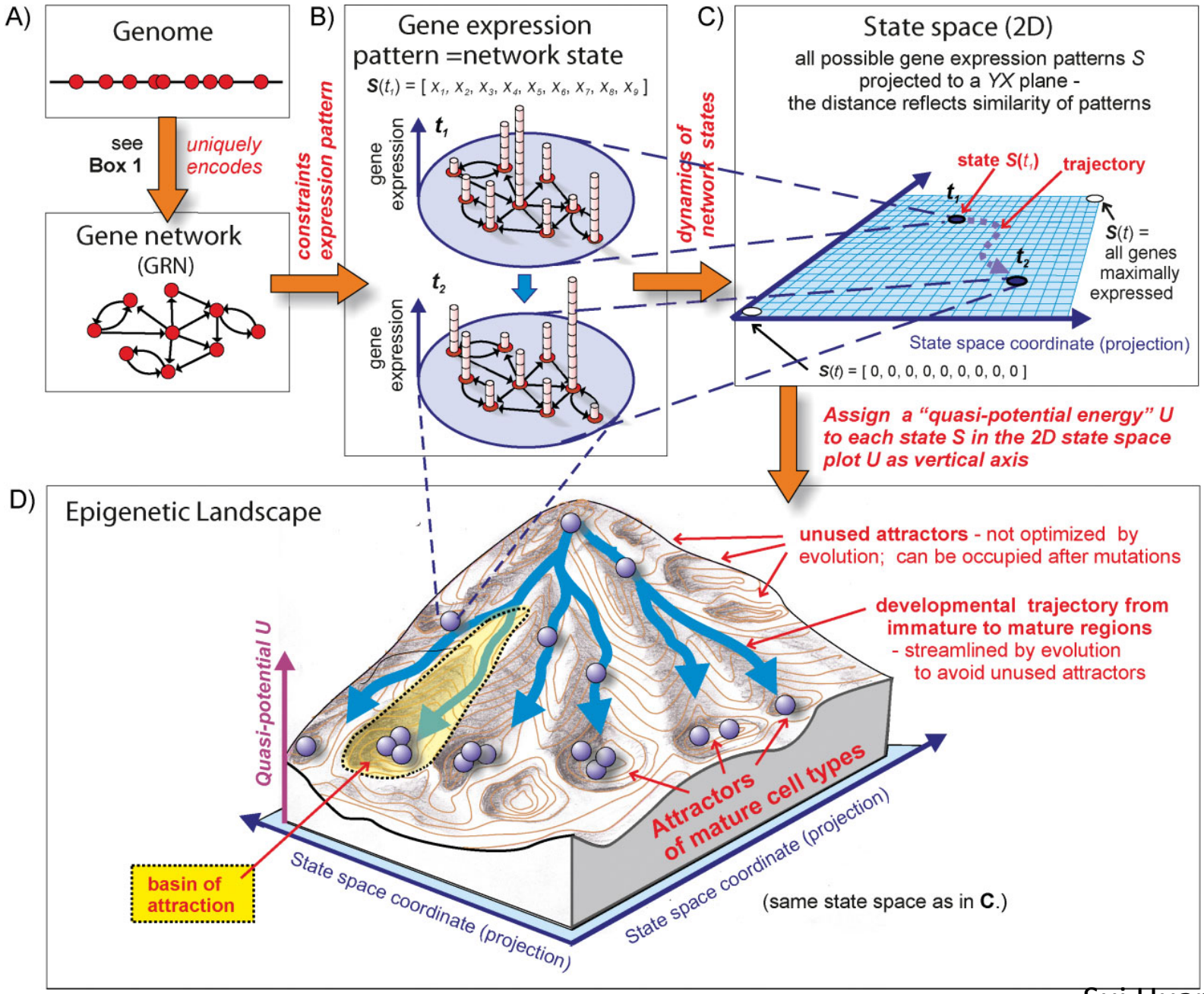


Figure 3: From topology to dynamics; illustrated for a small circuit and a large network. *Top panel*: Small circuit (signalling module) consisting of two proteins *A* and *B*, which are mutually inhibitory, and promote their own decay. For a wide range of kinetic parameters, this topology gives rise to bistability. The middle diagram shows a phase plane (two-dimensional state space)

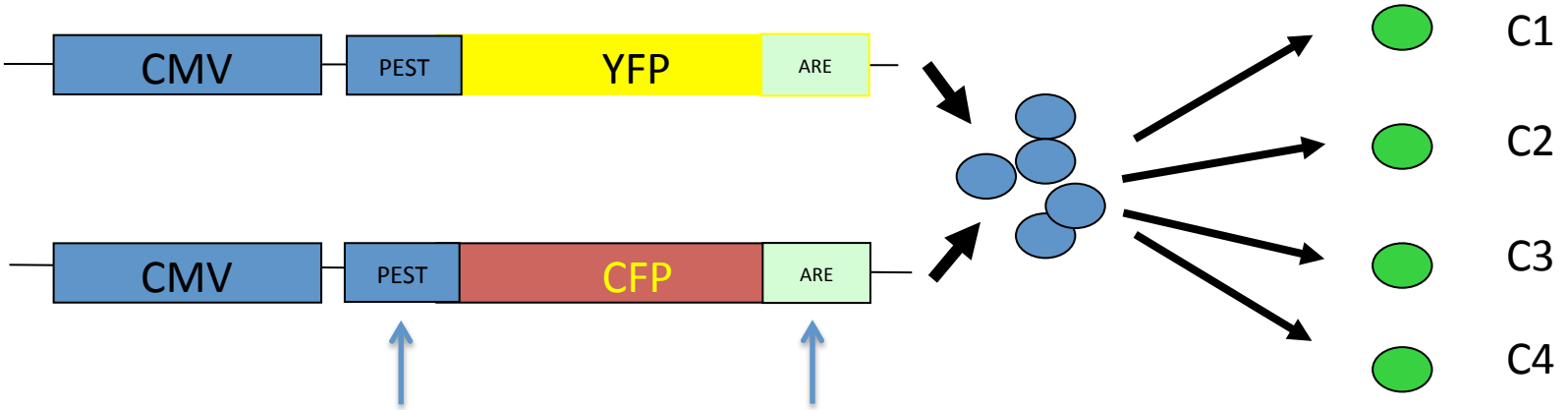
Sui Huang


A)
Genome
see Box 1
uniquely encodes
Gene network (GRN)
B)
Gene expression pattern =network state
S(t1) = [x1, x2, x3, x4, x5, x6, x7, x8, x9]
gene expression
t1
t2
constraints expression pattern
dynamics of network states
C)
State space (2D)
all possible gene expression patterns S projected to a YX plane - the distance reflects similarity of patterns
state S(t1)
trajectory
S(t) = all genes maximally expressed
State space coordinate (projection)
S(t) = [0, 0, 0, 0, 0, 0, 0, 0, 0]
Assign a "quasi-potential energy" U to each state S in the 2D state space plot U as vertical axis
D)
Epigenetic Landscape
unused attractors - not optimized by evolution; can be occupied after mutations
developmental trajectory from immature to mature regions - streamlined by evolution to avoid unused attractors
Quasi-potential U
Attractors of mature cell types
basin of attraction
State space coordinate (projection)
State space coordinate (projection)
(same state space as in C.)

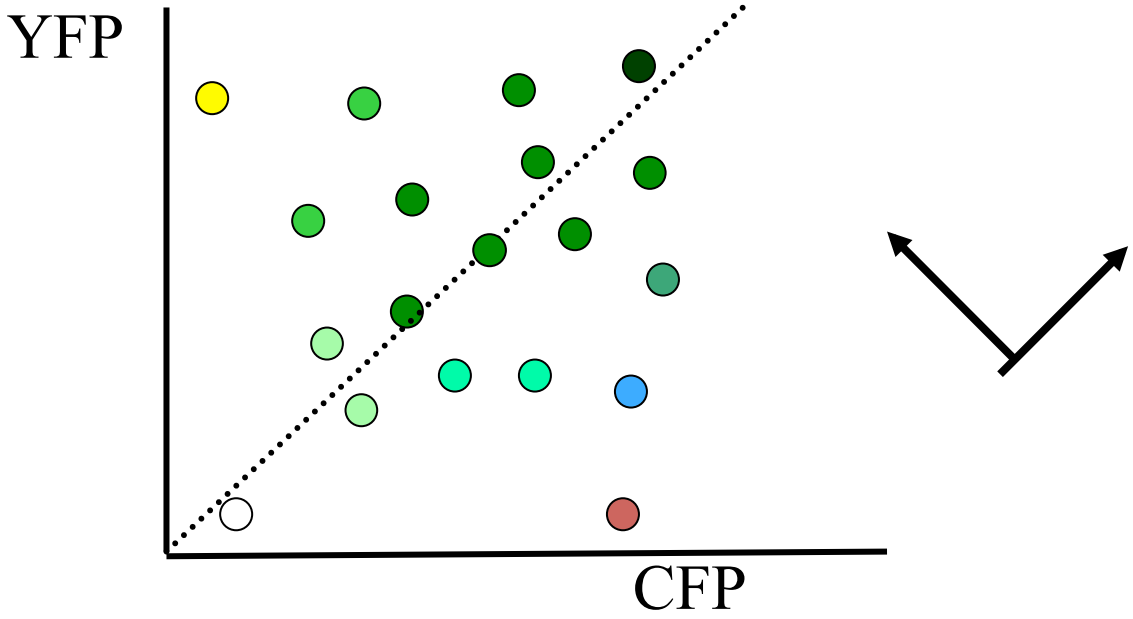
Sui Huang

How interactions in the network state can stabilize the fluctuations?

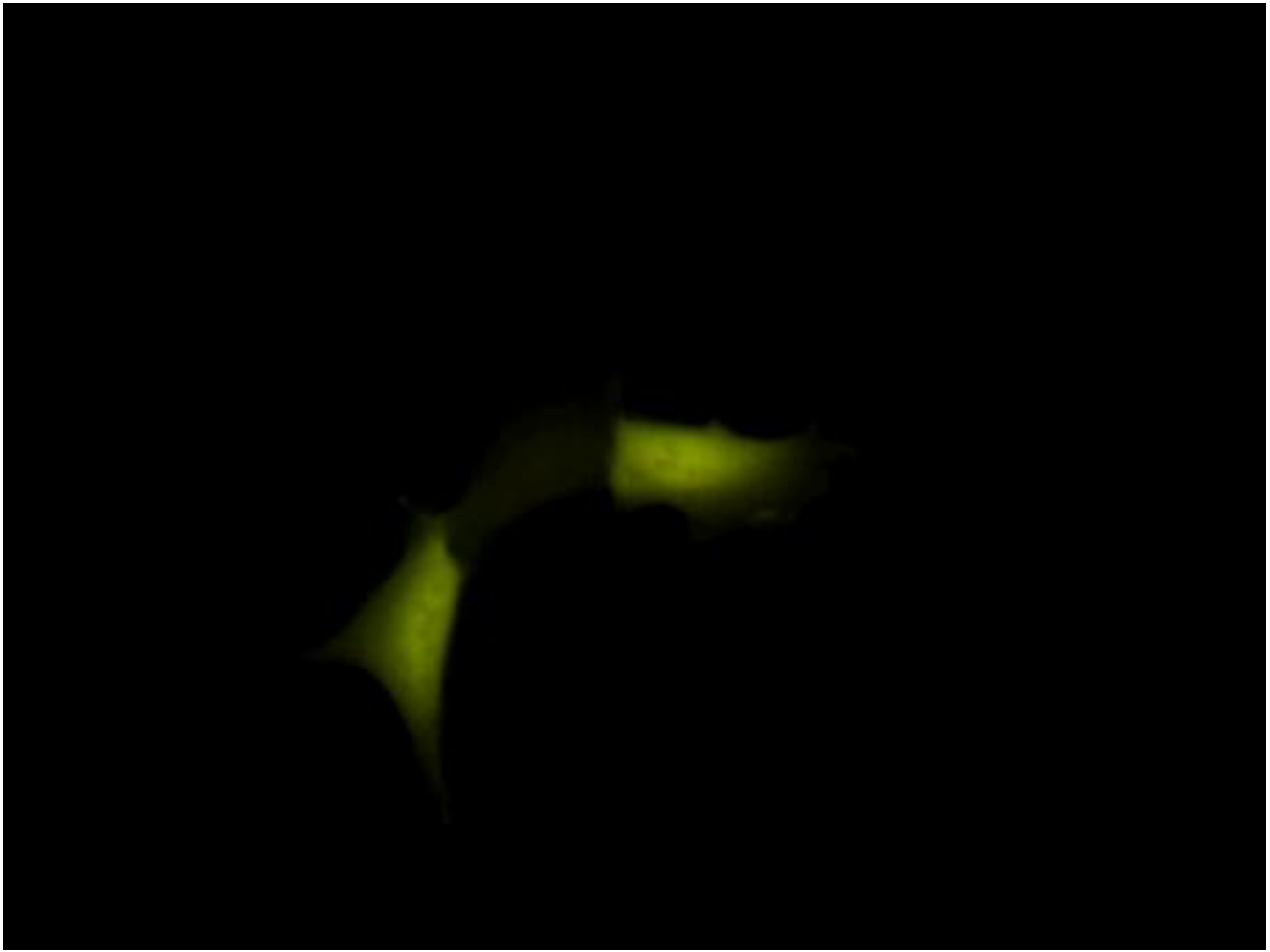
Example:

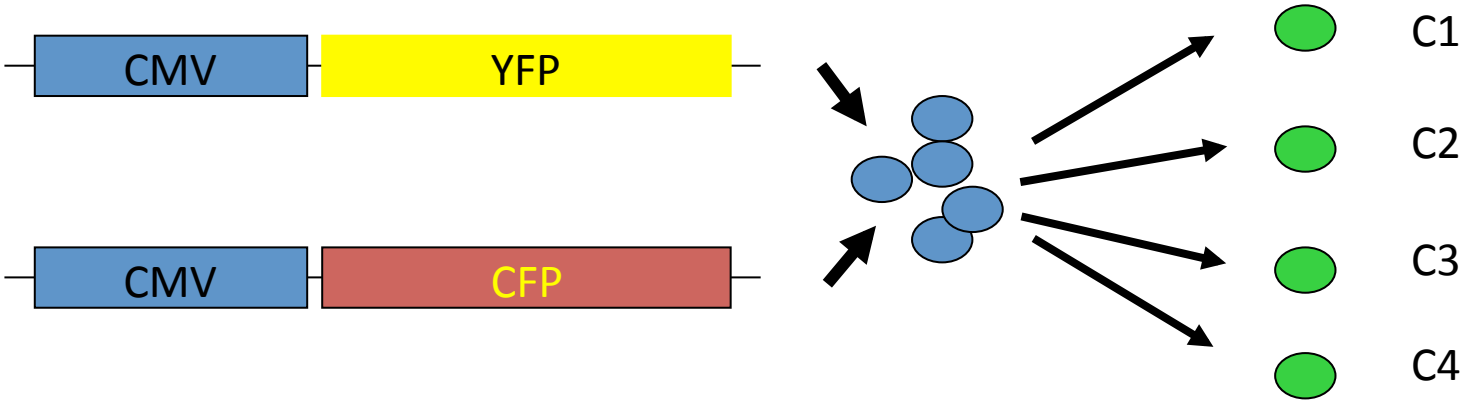


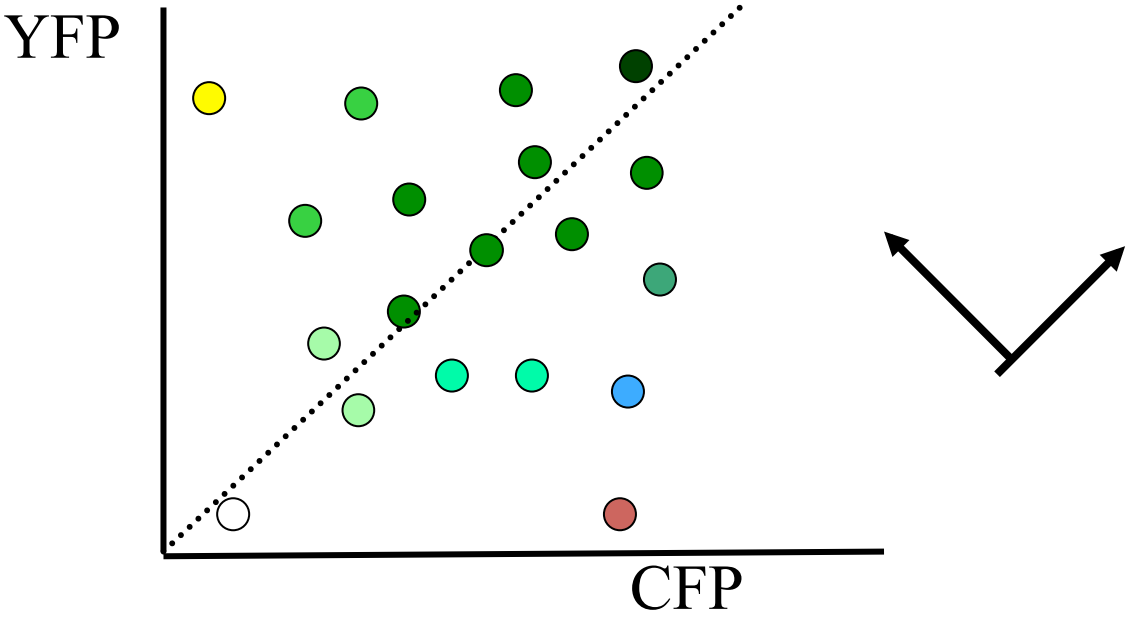
reduced mRNA and protein stability



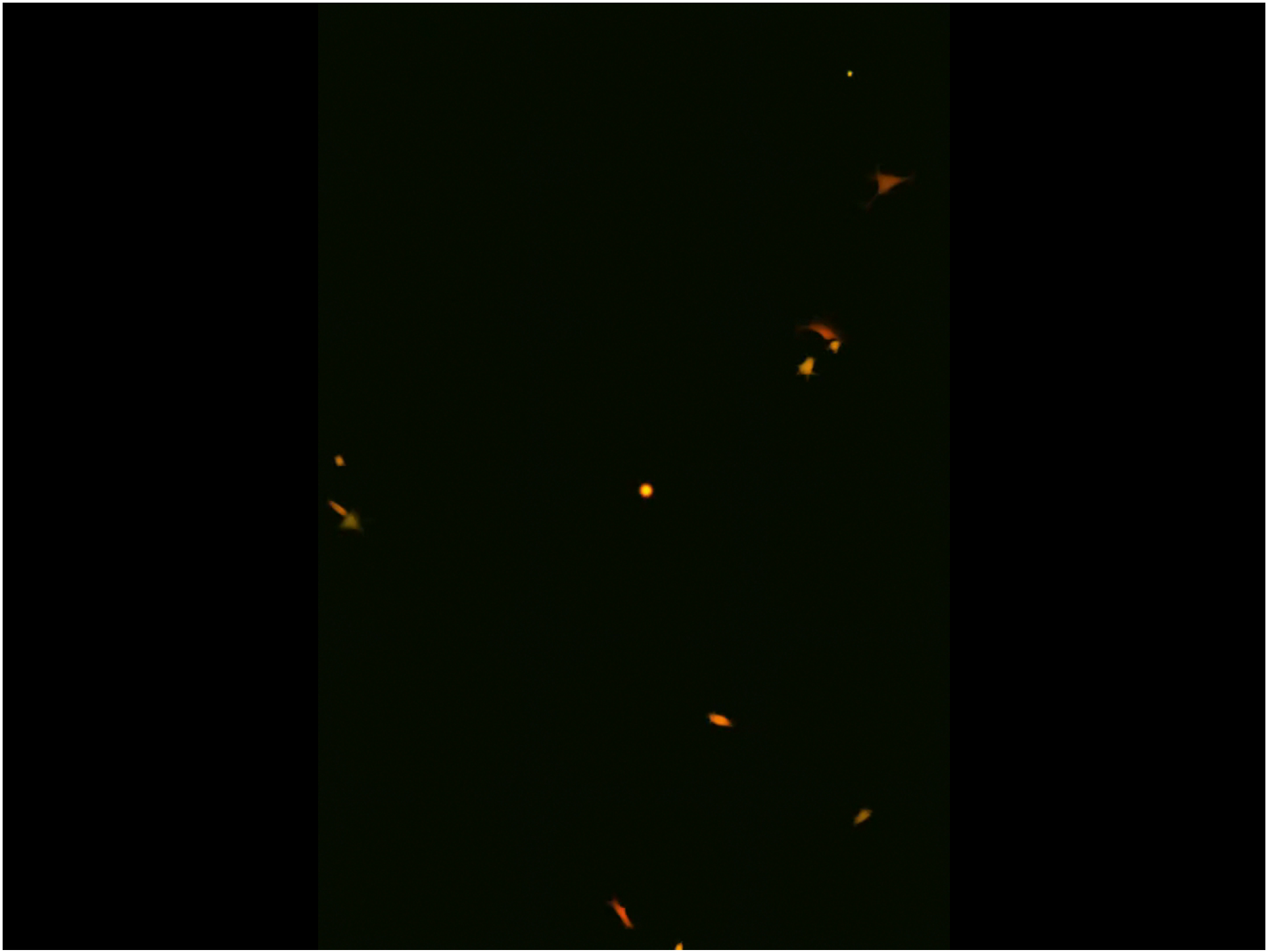
Corre et al. 2014 Dec 22;9(12):e115574. doi: 10.1371/journal.pone.0115574

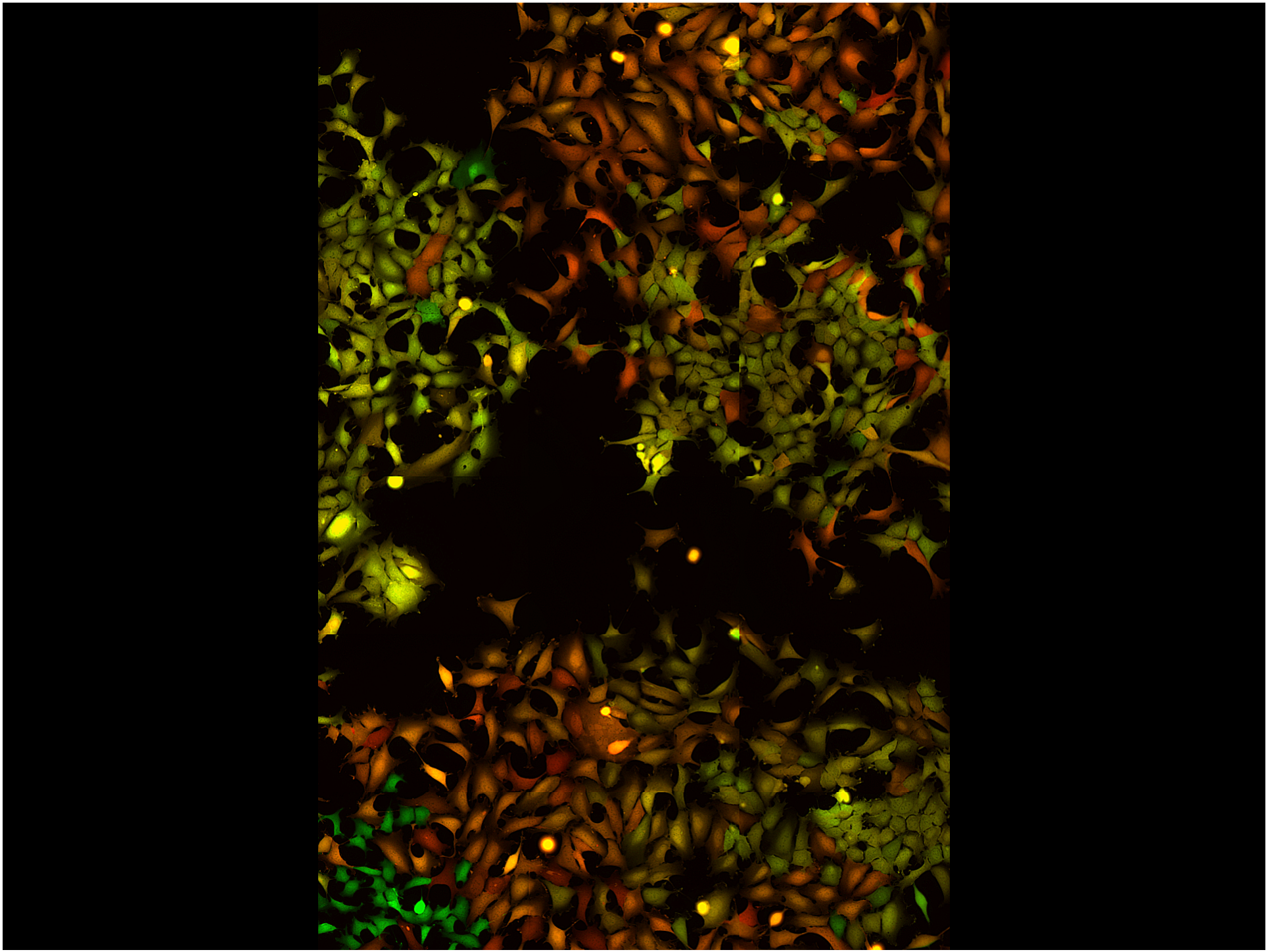


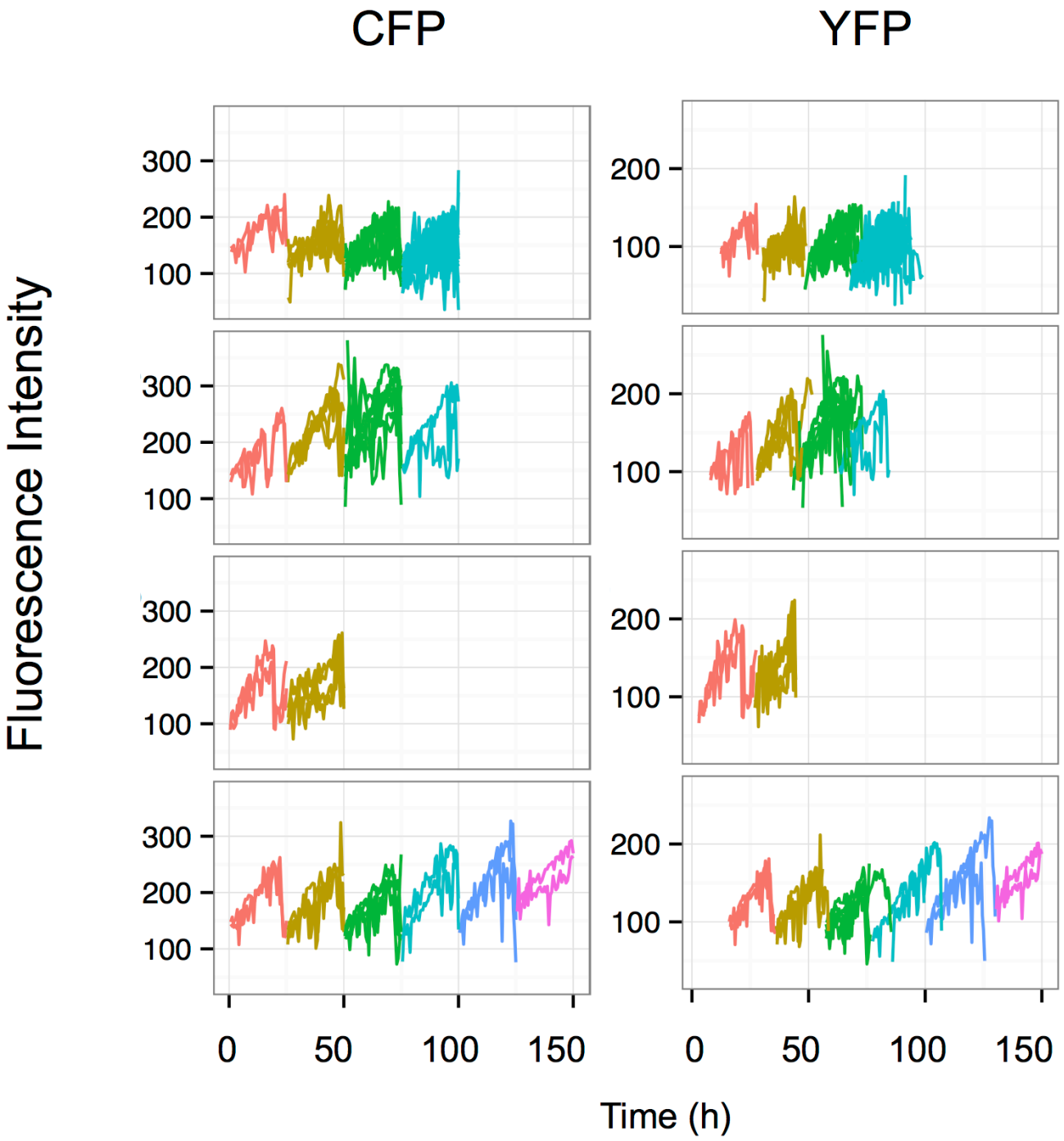


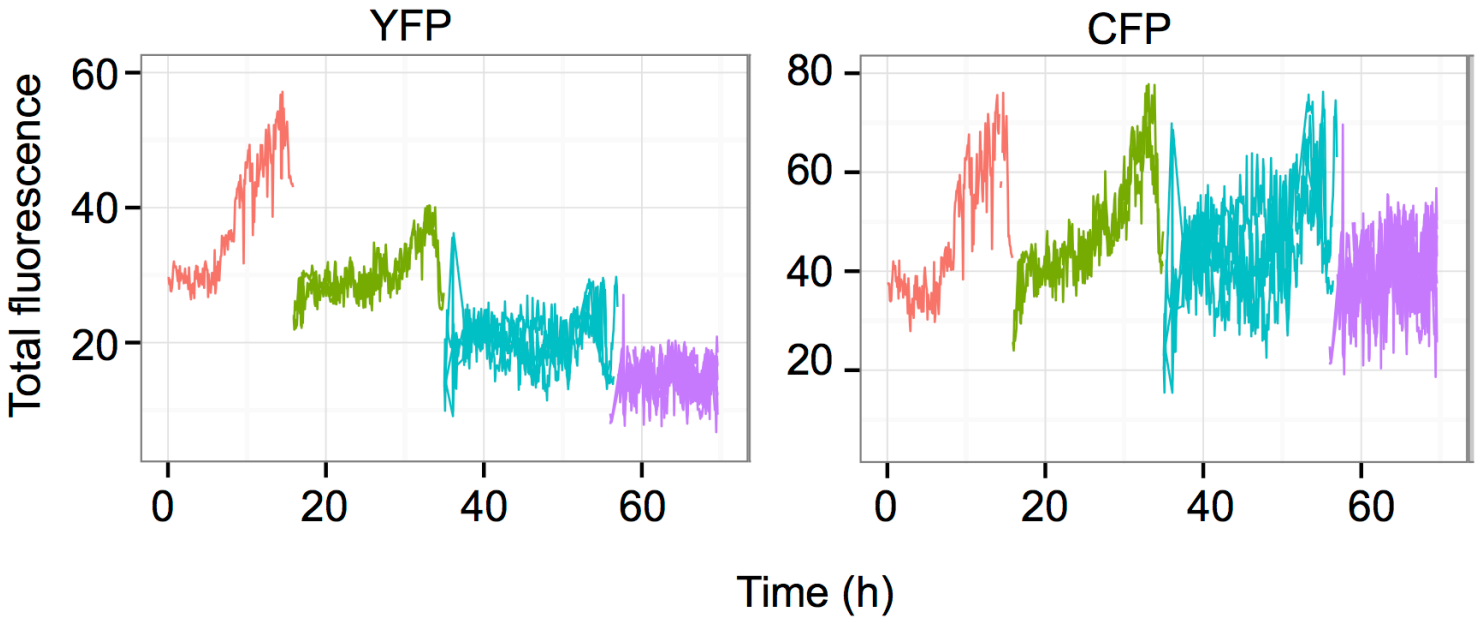


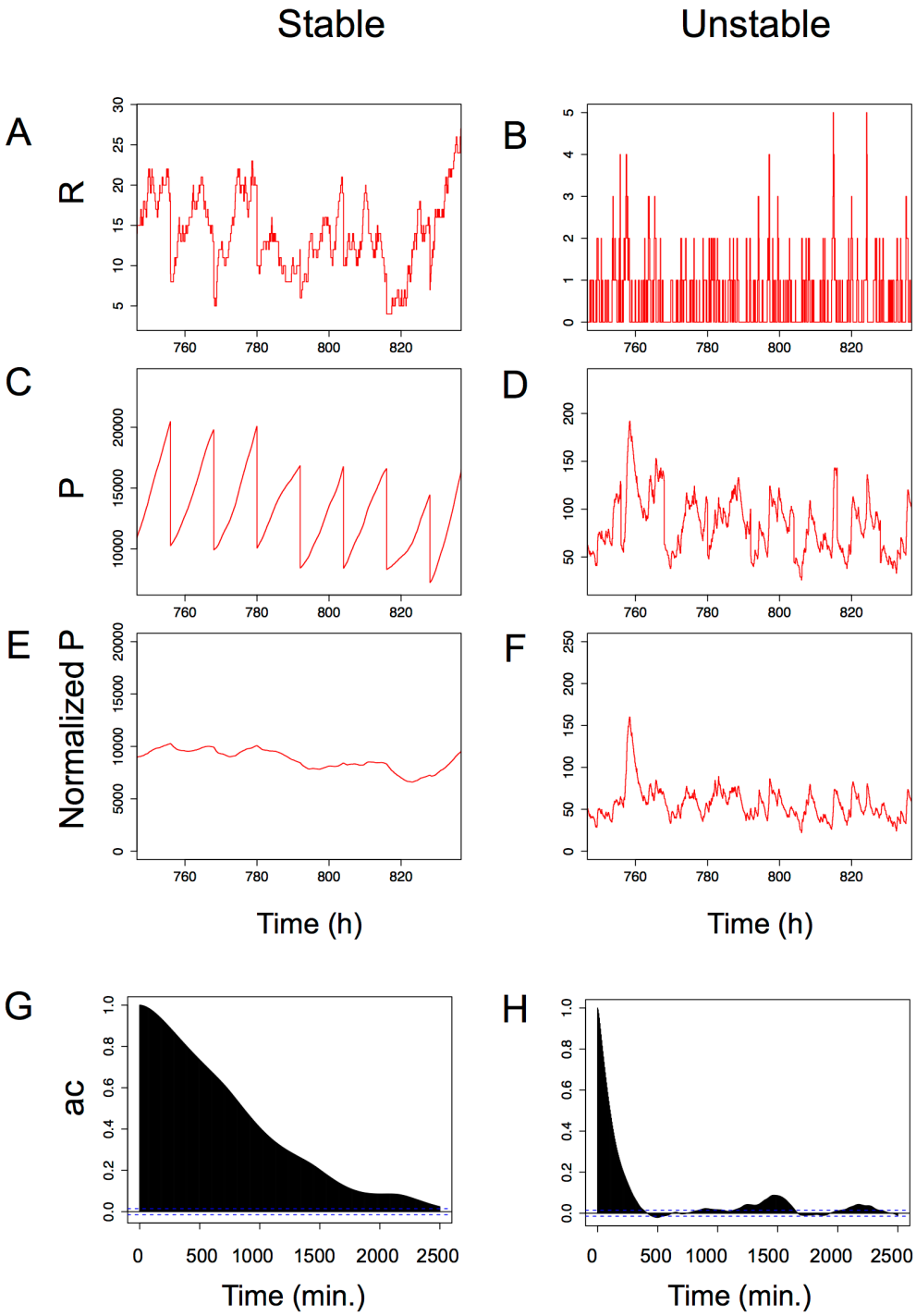
Corre et al. 2014 Dec 22;9(12):e115574. doi: 10.1371/journal.pone.0115574

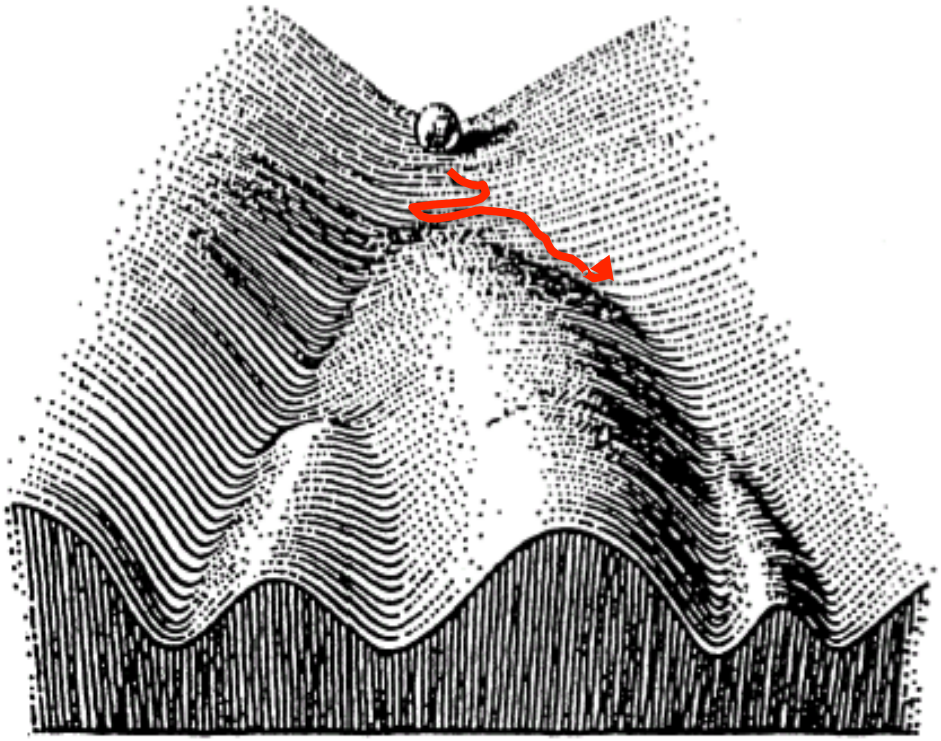


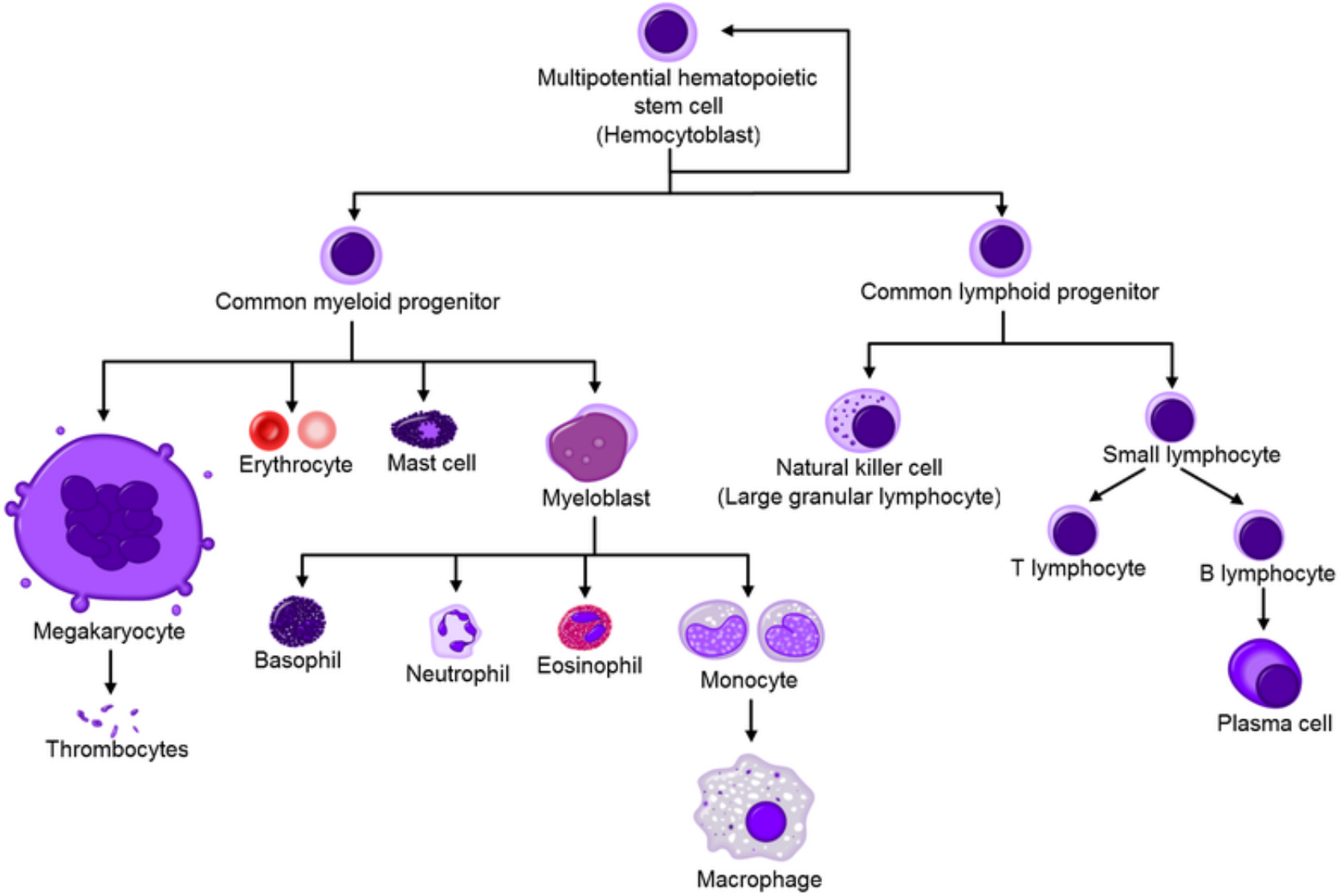


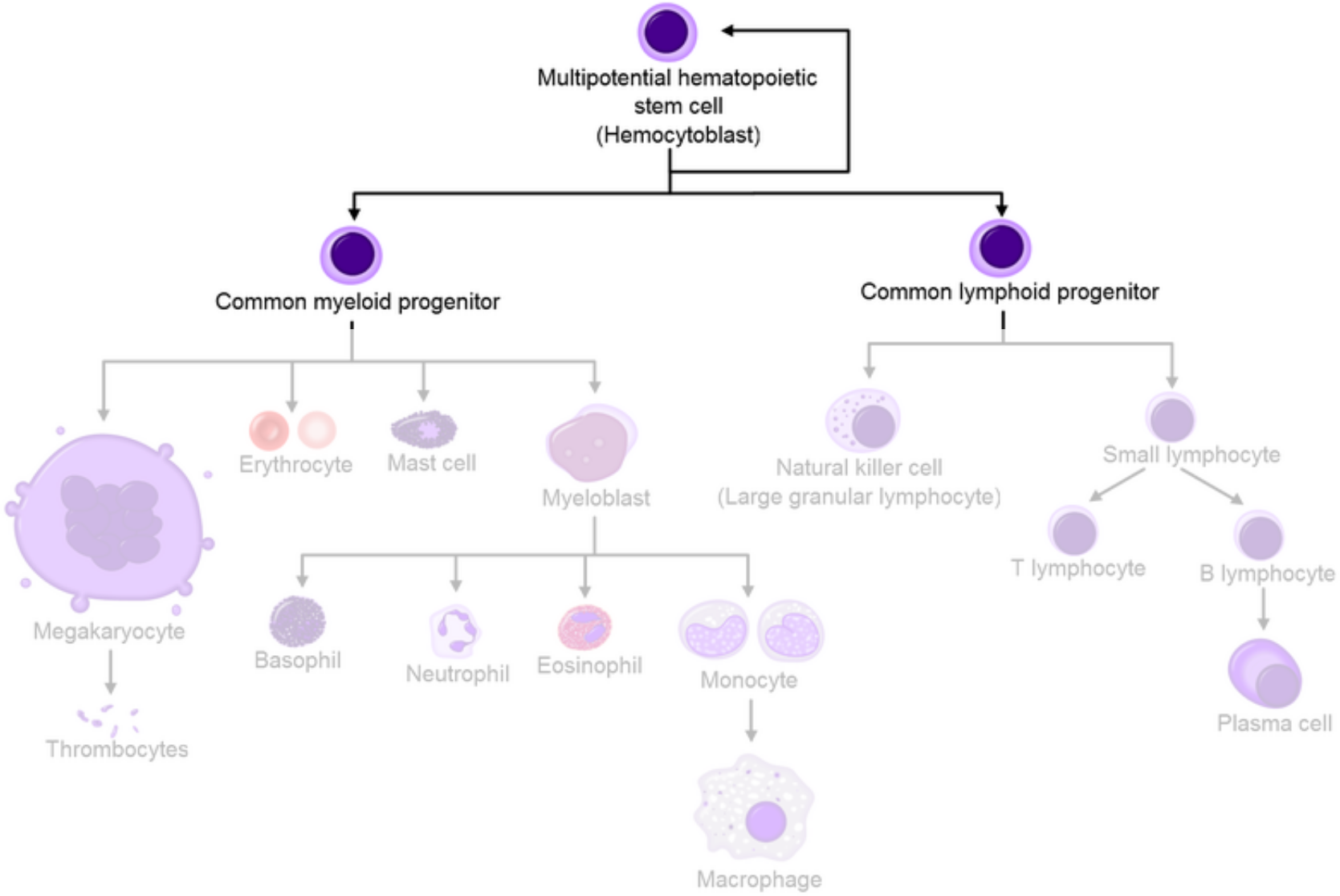

CFP
YFP
Fluorescence Intensity
300
200
100
0
50
100
150
Time (h)


YFP
CFP
Total fluorescence
60
40
20
80
60
40
20
0
20
40
60
0
20
40
60
Time (h)


Stable
Unstable
A
B
C
D
E
F
G
H
R
P
Normalized P
ac
Time (h)
Time (h)
Time (min.)
Time (min.)




Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell


Multipotential hematopoietic stem cell (Hemocytoblast)
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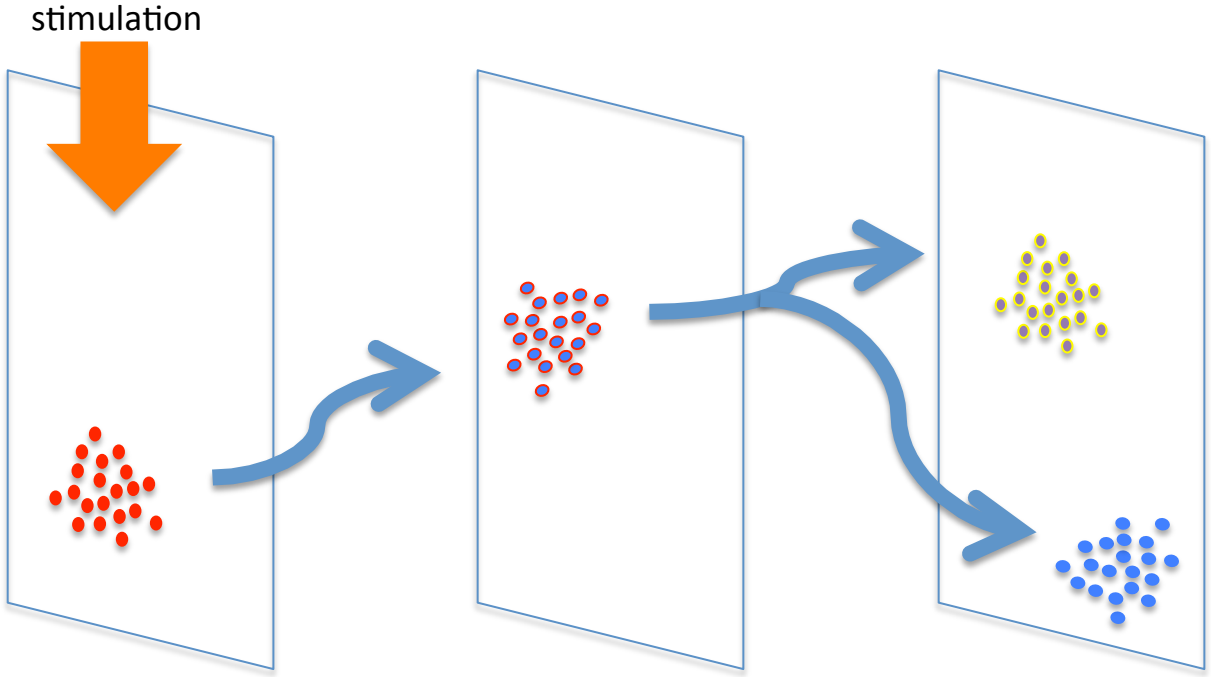
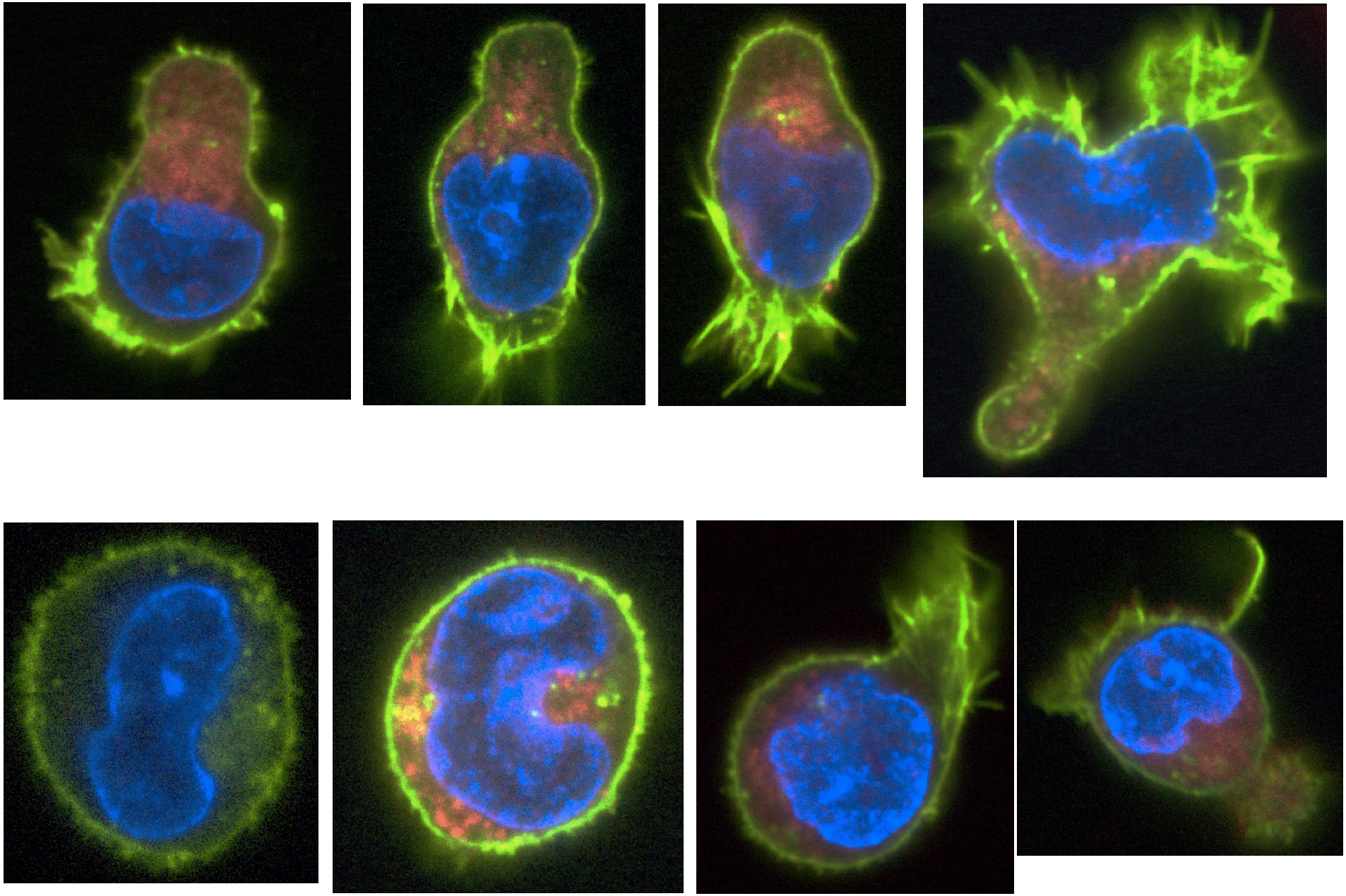
Myeloblast
Megakaryocyte
Thrombocytes
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

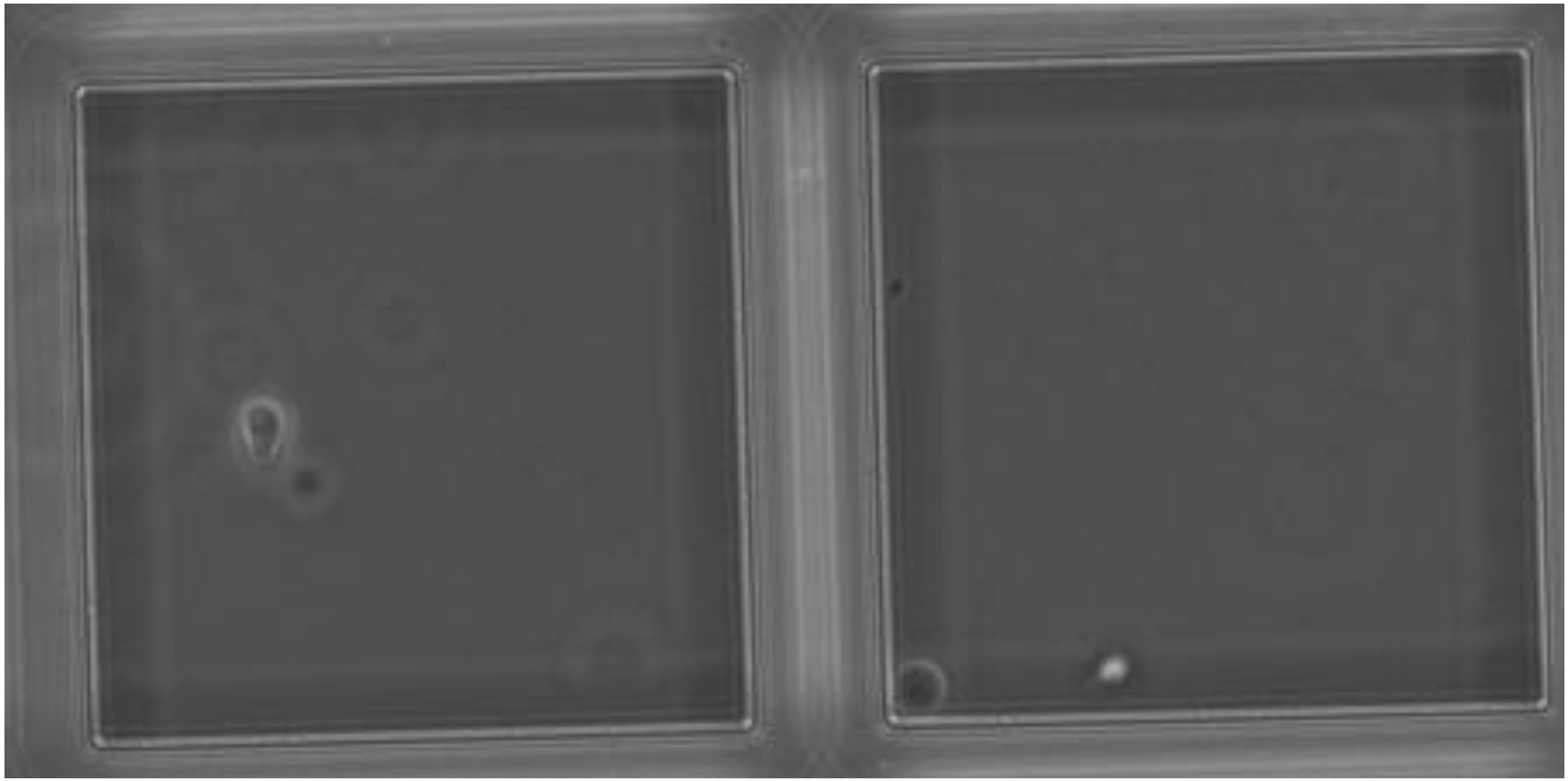
Evolution of single cell gene expression patterns

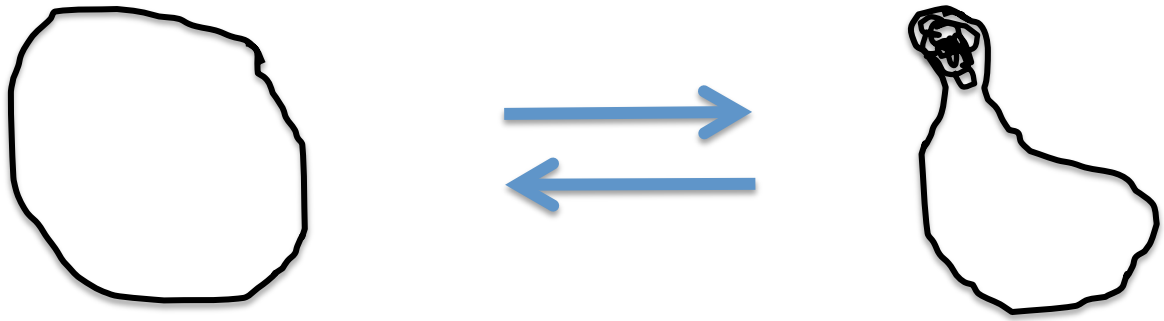


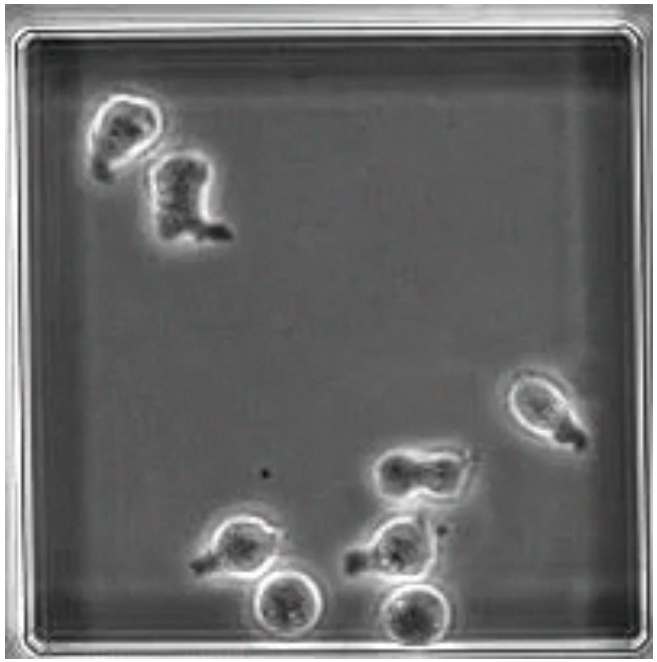
Multiprimed stage: simultaneous expression of lineage-associated genes

Exit from multiprimed and transition to two distinct profiles

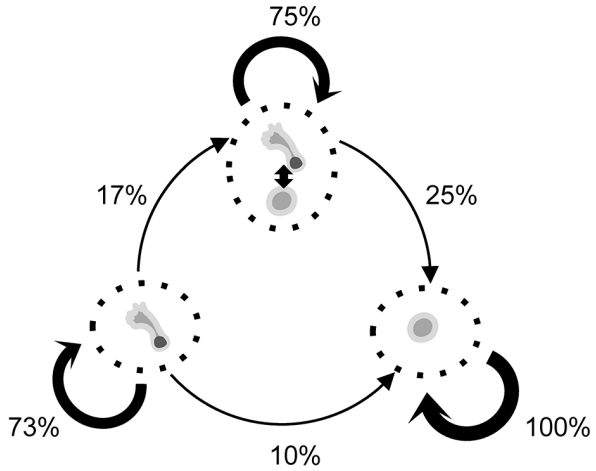


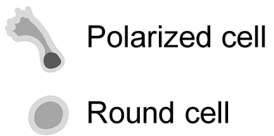






Upon cell division:





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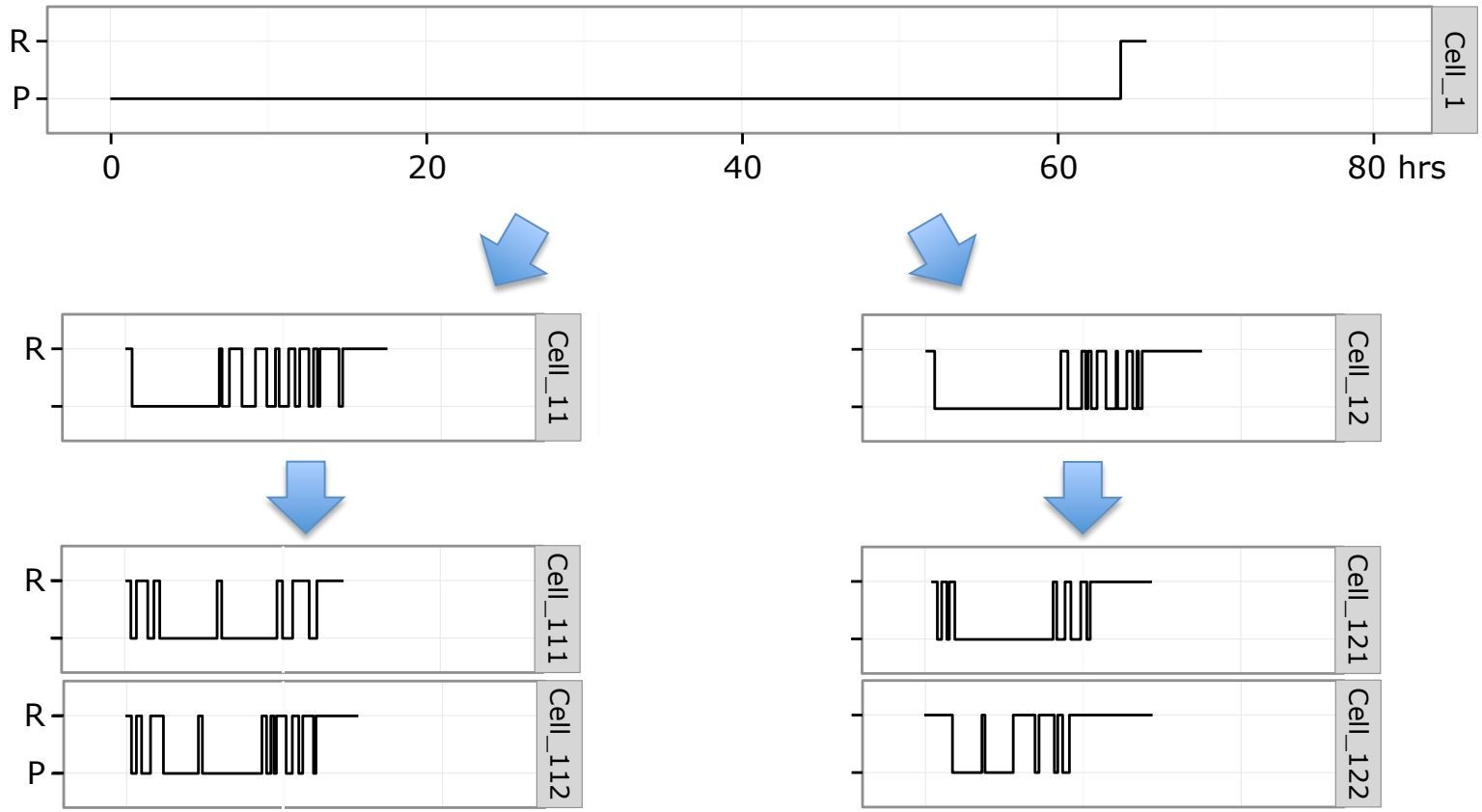
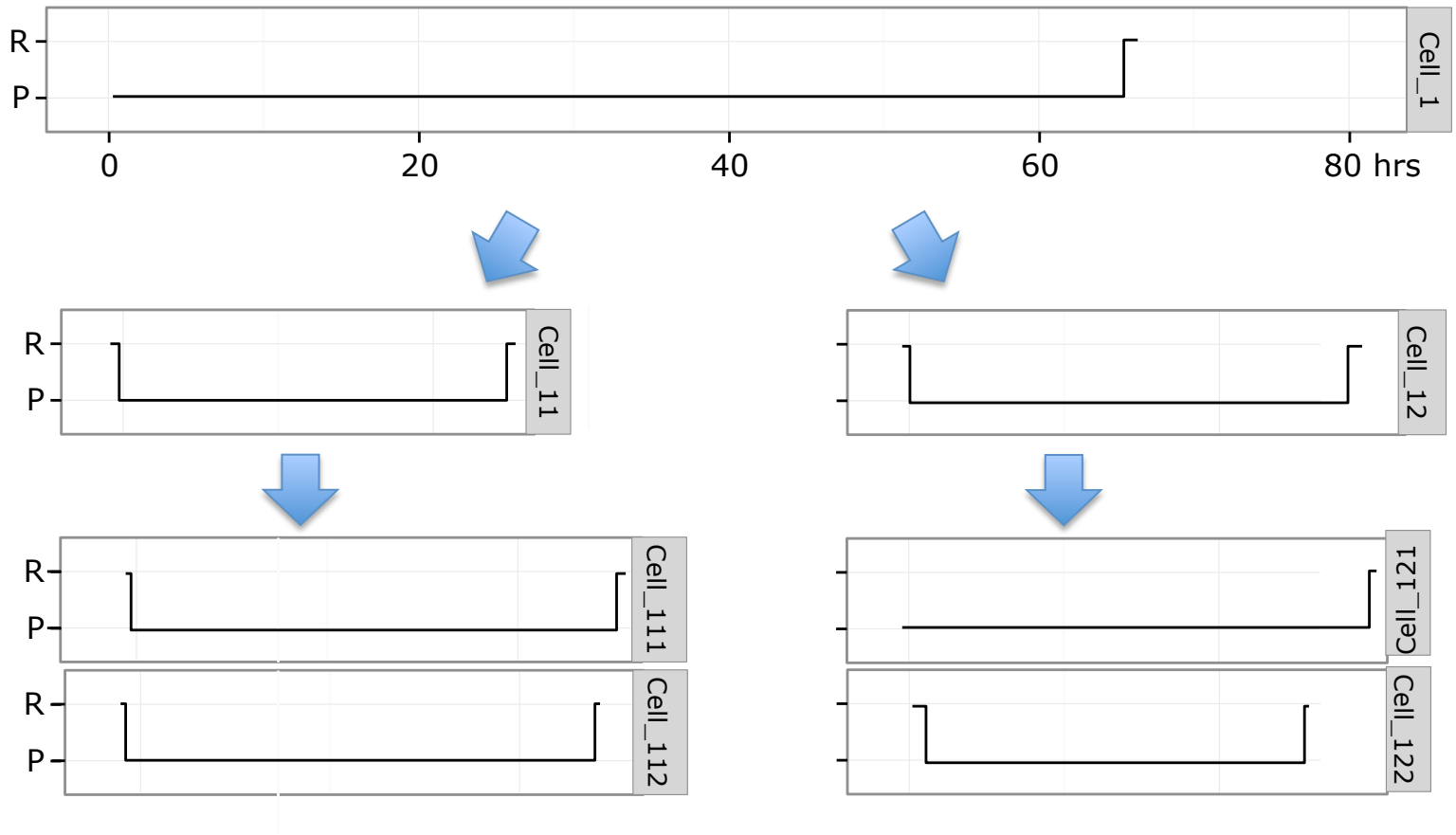
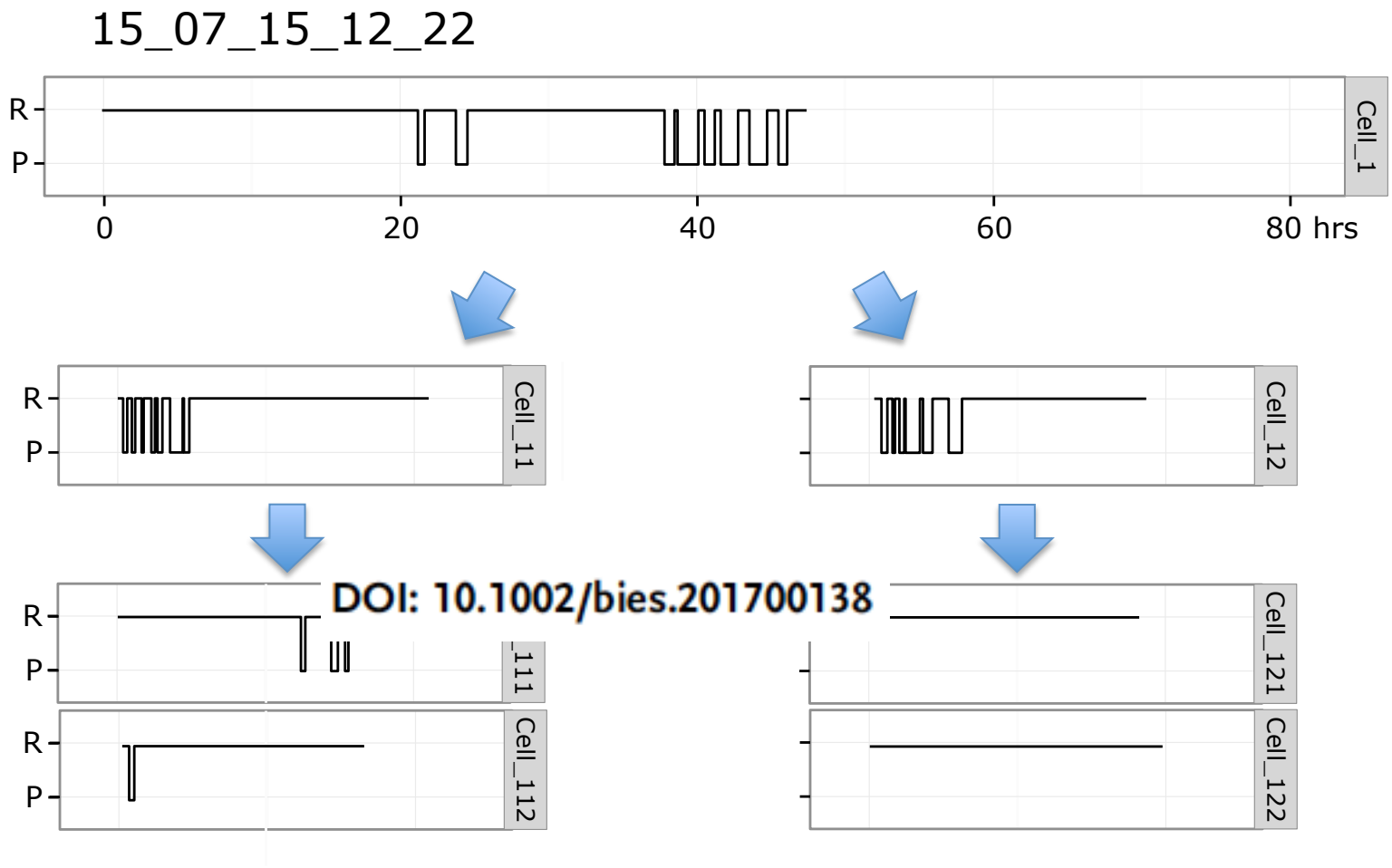

R
P
Cell_1
0
20
40
60
80 hrs
R
Cell_11
Cell_12
R
Cell_111
Cell_121
R
P
Cell_112
Cell_122

15_02_17_05_12


R
P
Cell_1
0
20
40
60
80 hrs
R
P
Cell_11
Cell_12
R
P
Cell_111
Cell_121
R
P
Cell_112
Cell_122



DOI: 10.1002/bies.201700138

PROBLEMS AND PARADIGMS

Prospects & Overviews

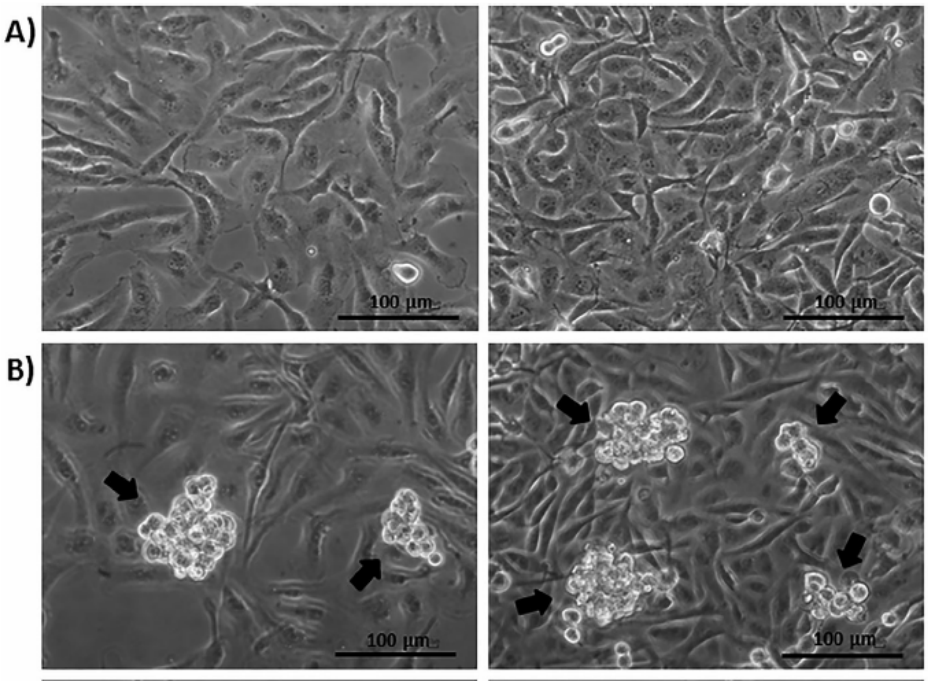
BioEssays
www.bioessays-journal.com

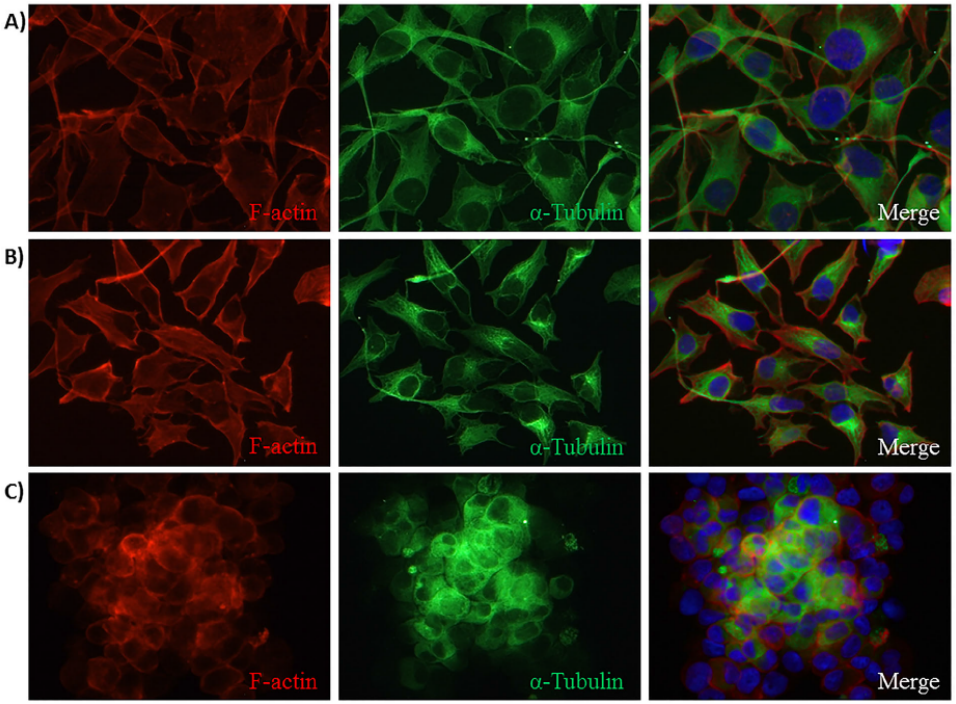
Gravity Constraints Drive Biological Systems Toward Specific Organization Patterns

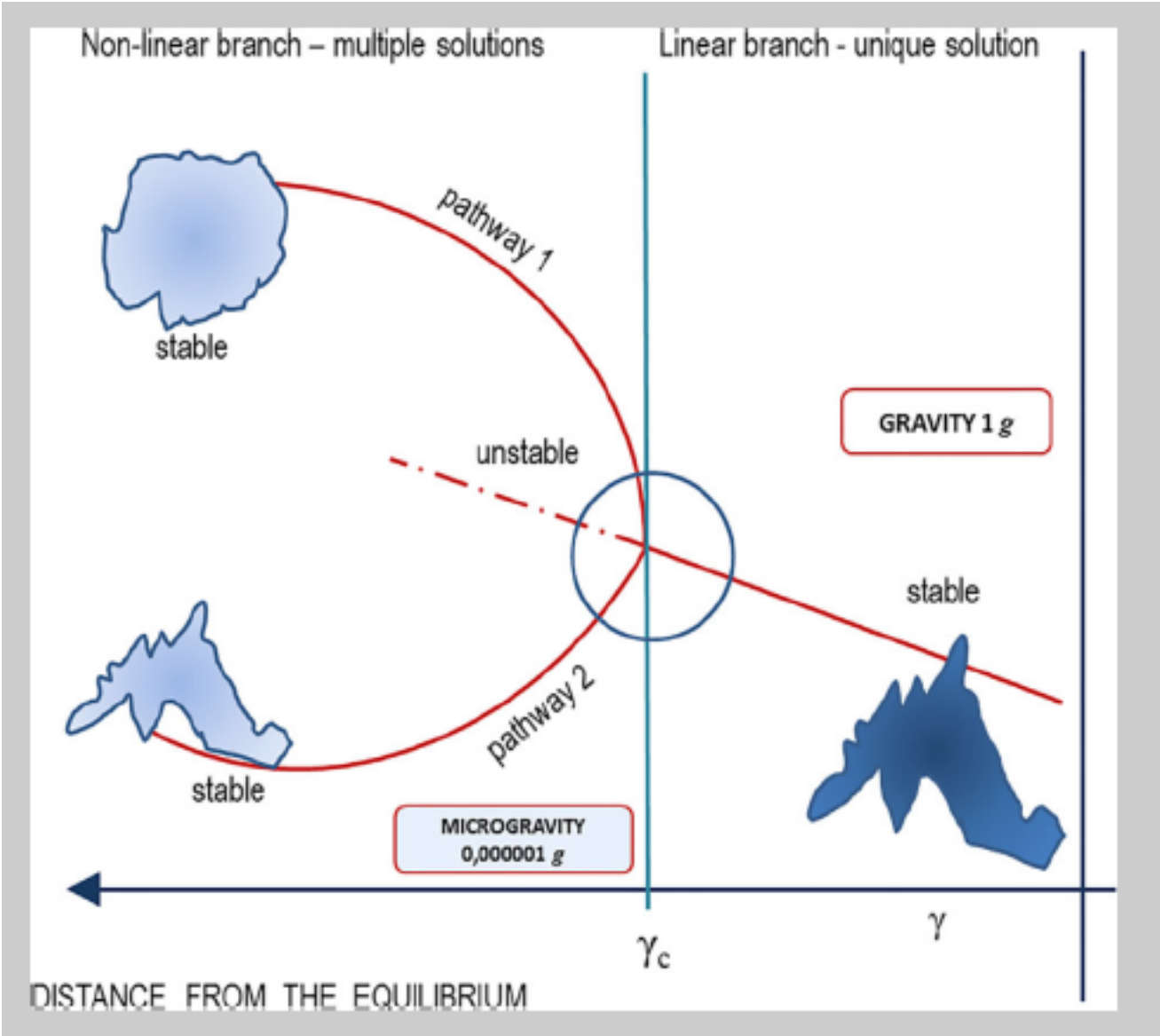
Commitment of cell specification is constrained by physical cues

Mariano Bizzarri, Maria Grazia Masiello, Alessandro Giuliani, and Alessandra Cucina*

BioEssays **2017**, **DOI: 10.1002/bies.201700138**






Non-linear branch – multiple solutions
Linear branch - unique solution
pathway 1
stable
GRAVITY 1 g
unstable
stable
pathway 2
stable
MICROGRAVITY
0,000001 g
γc
γ
DISTANCE FROM THE EQUILIBRIUM

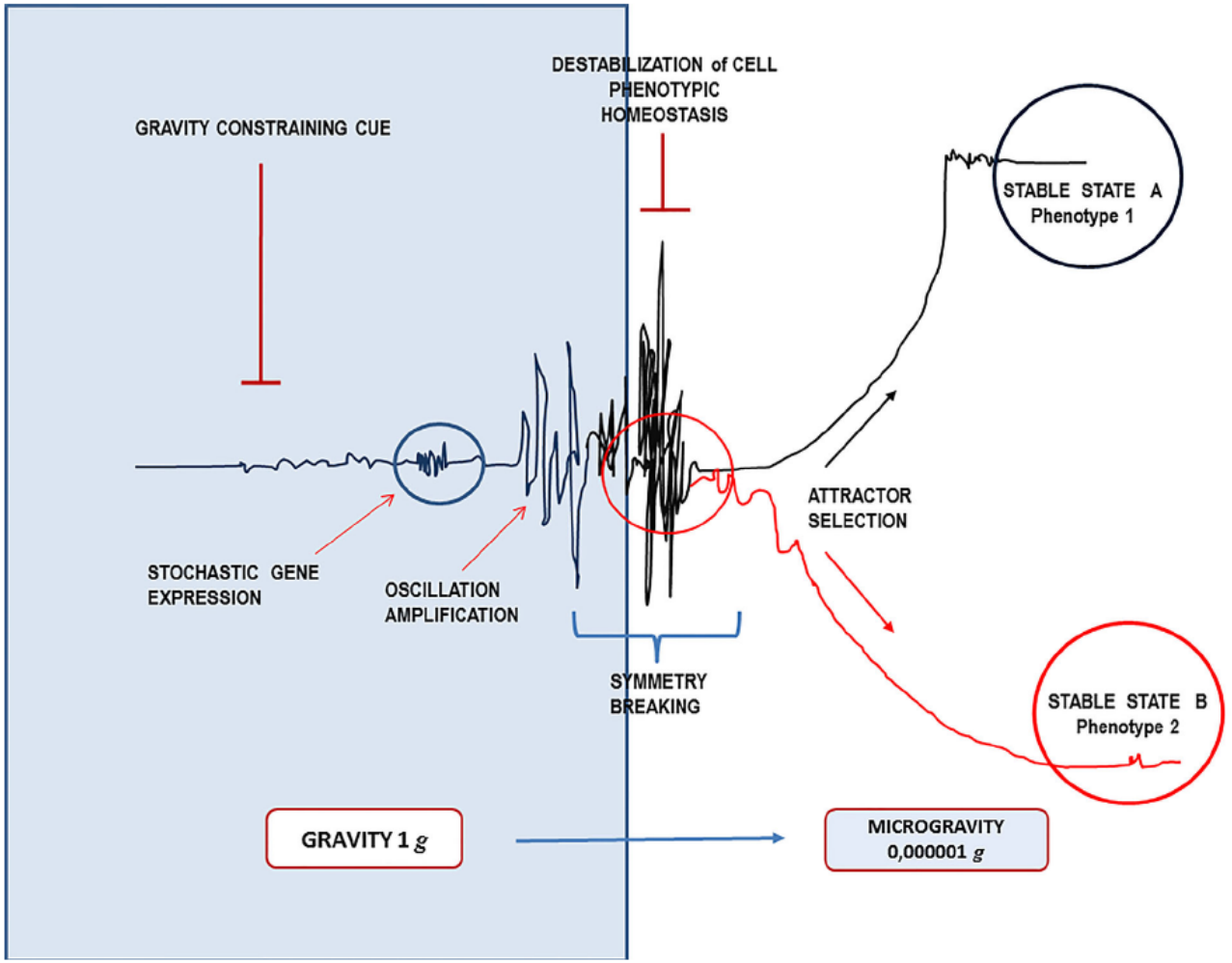
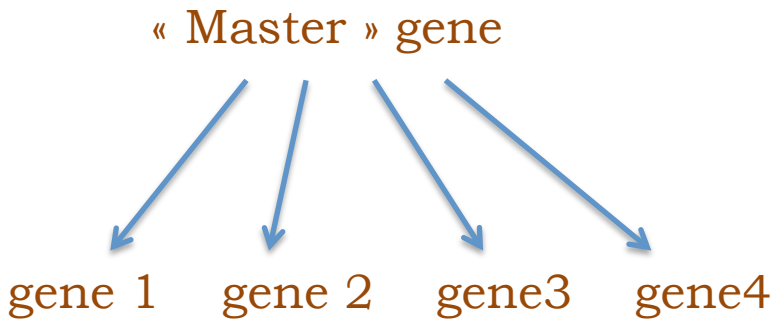
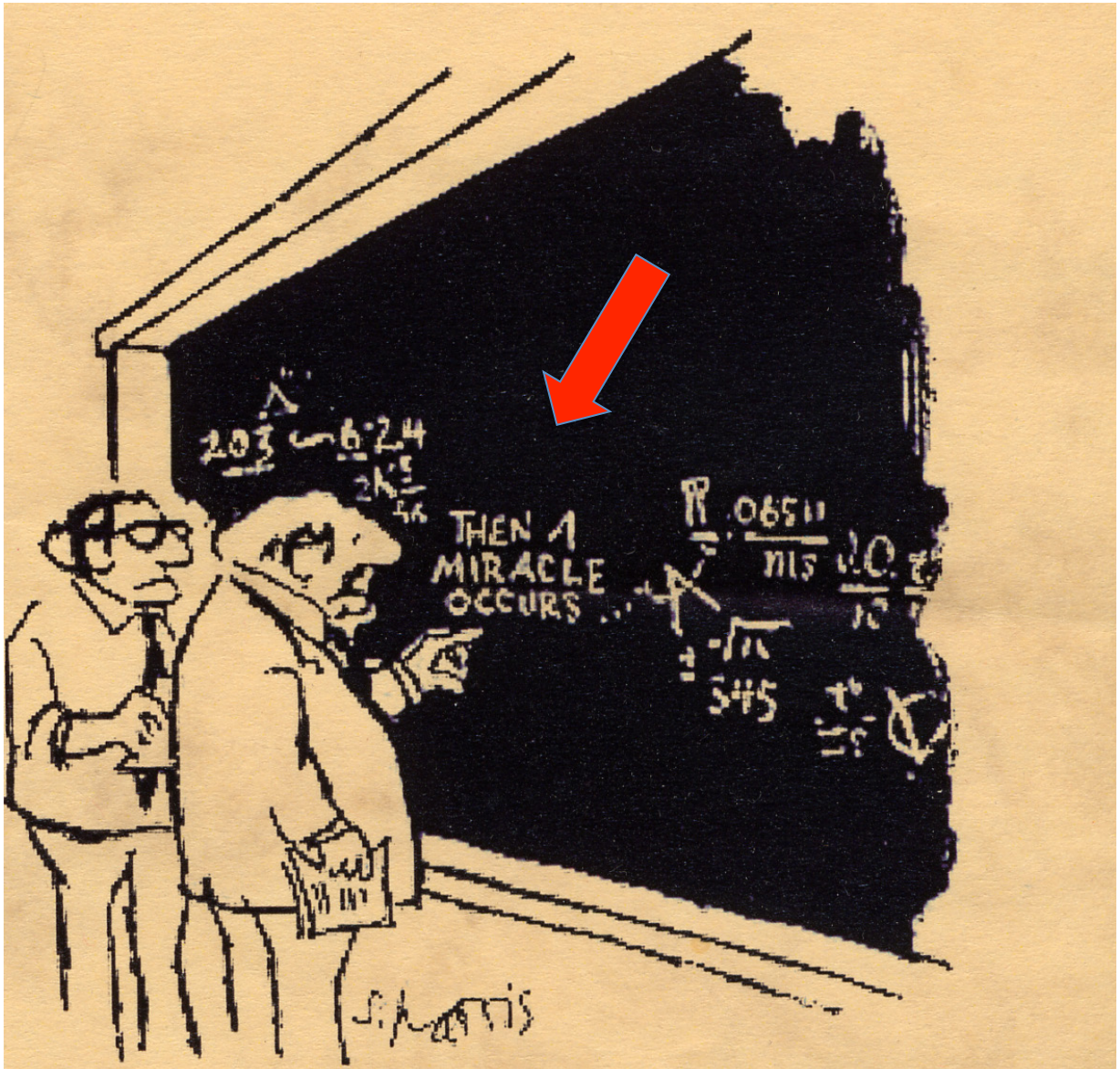


Figure 3. Phase transition toward cell fate commitment in complex systems in a non-equilibrium thermodynamics model. In dissipative systems, there are conditions far from the equilibrium in which even small external influences can produce dramatic effects, namely when the system approaches phase transitions, as those occurring during mitosis or cell differentiating processes. In the presence of normal gravity, despite the presence of oscillations in gene activity and/or availability of transcription factors, cooperativity among system's elements leads the external energy field to overcome the intrinsic fluctuation. In this condition, gene-regulatory networks and transcription signaling behave like "instructive factors," driving the system – seemingly in a "deterministic" way – toward a single, specific fate. However, when gravity disappears, fluctuation in many order parameters (gene expression patterns, transcription factors, enzymatic pathways, and cytoskeleton dynamics) steadily increases, leading to a symmetry breaking at the bifurcation point. Removal of gravity constraints leads thus to a widely destabilizing effect. As a result, the systems can freely explore new gene-regulatory networks, likely enacting a wide remodeling of the architecture of the transcription network. This will lead the systems to explore new phenotypic configurations, ultimately driving the systems into two new, stable attractors, in which fluctuation of order parameters can be more efficiently damped. Indeed, the new phenotypic configurations demonstrated themselves stable and reproducible, even for longer periods of observation (>7 days). Conversely, when the two population are seeded again on ground conditions, the superposition of the gravity field may break the system's symmetry, giving the system a directional preference, which will make it evolve into a specific state. In other words, the "weak" gravity force dramatically influences the system to favor one among different, potential configurations.

How (gene expression) change is initiated?

Deterministic view:



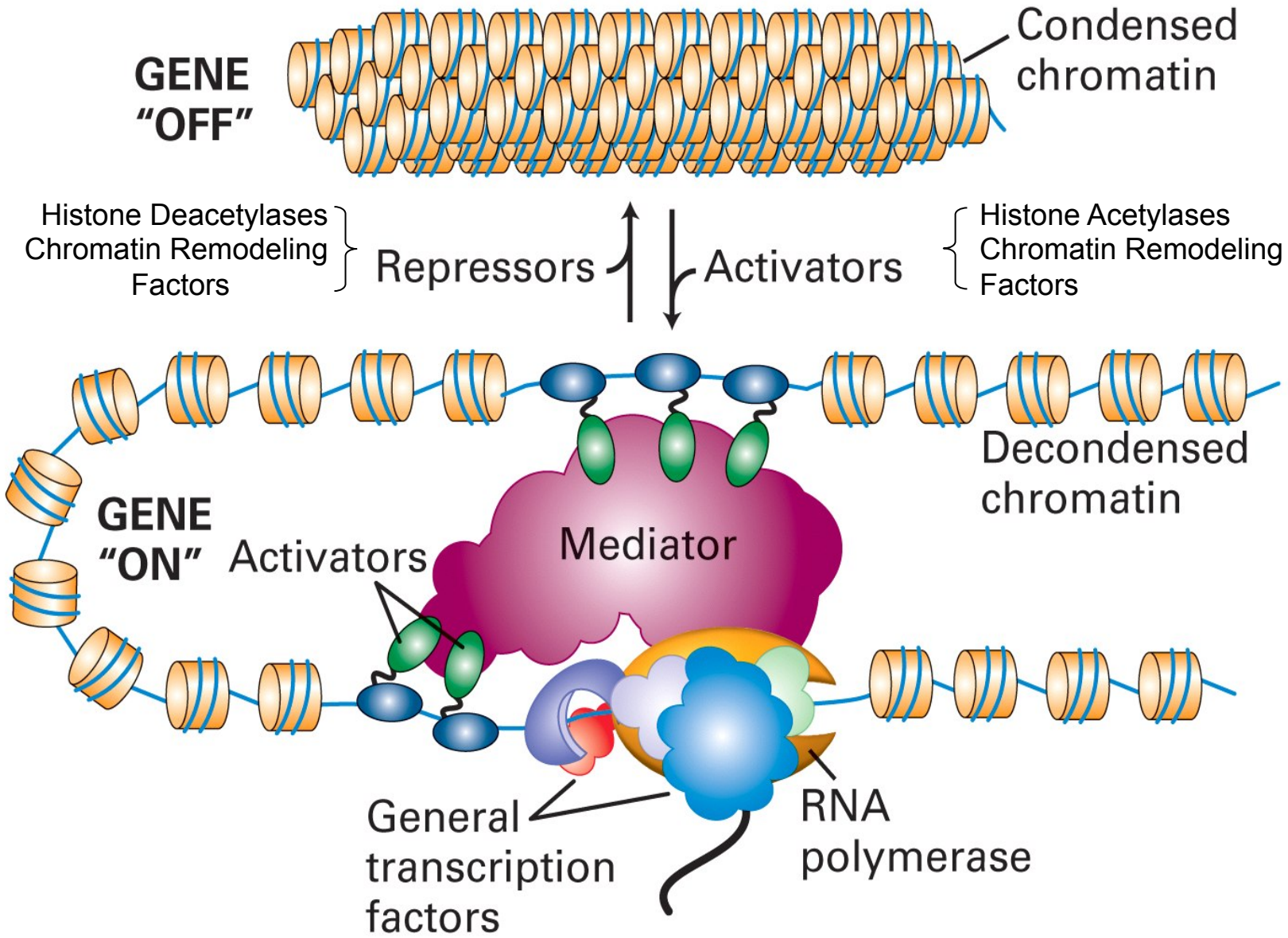


"I think you should be more explicit here in step one"

How gene expression change is initiated?

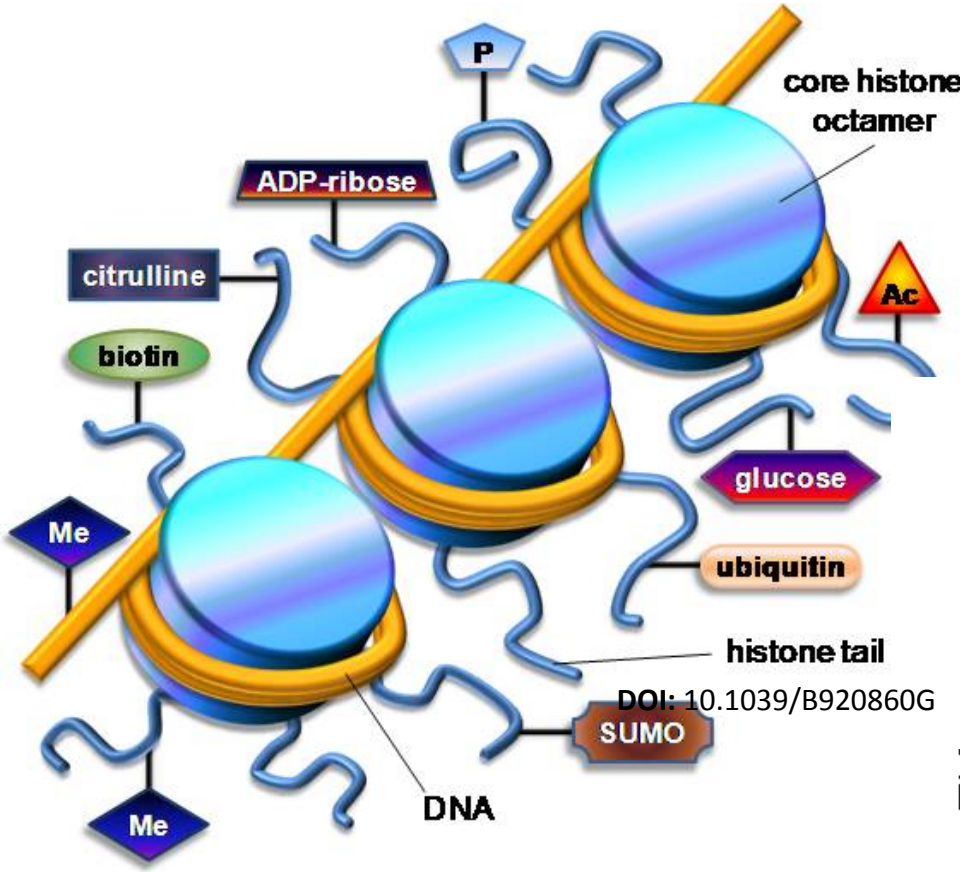
Deterministic view: causality is linear

Stochastic view: no need to explain, because change is the ground state.
What we need to explain is the stability


GENE
"OFF"
Condensed
chromatin
Histone Deacetylases
Chromatin Remodeling
Factors
Repressors
Activators
Histone Acetylases
Chromatin Remodeling
Factors
Decondensed
chromatin
GENE
"ON"
Activators
Mediator
General
transcription
factors
RNA
polymerase

Genomic DNA is not accessible to regulatory factors. Accessibility depends on chromatin.

Chromatin is a highly dynamic structure due to reversible post-translational modifications and cycles of association-dissociation.

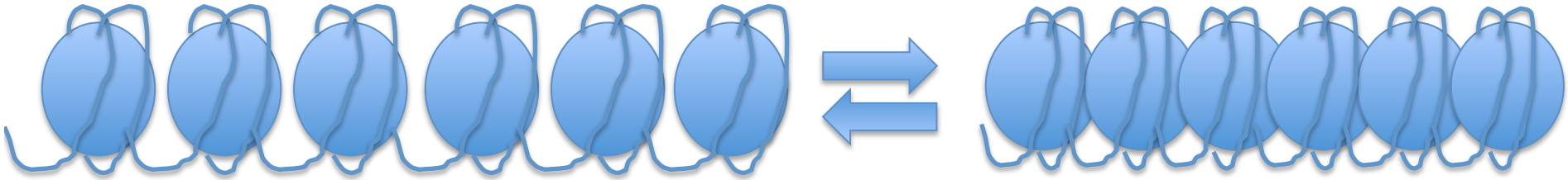


DOI: 10.1039/B920860G

Chromatin & Epigenetics

« open » « euchromatin »

« closed » « heterochromatin »



Determined by post-translational « epigenetic » changes

DNA is Hypomethylated
Hyperacetylation of Histones H3 and H4
Histone Methylation of H3Kme2, H3K4me3, H3K9me1

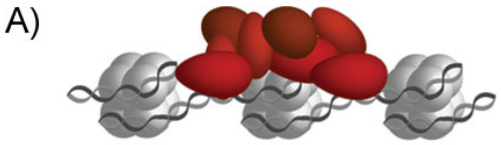
DNA that is Hypermethylated
Hypoacetylation of Histones H3 and H4
Histone Methylation of H3K27me2, H3K27me3, H3K9me2, H3K9me3

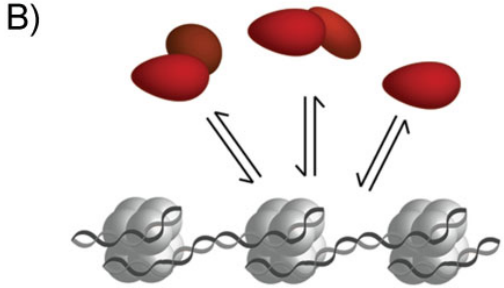
Epigenetic changes can be both reversible and heritable.

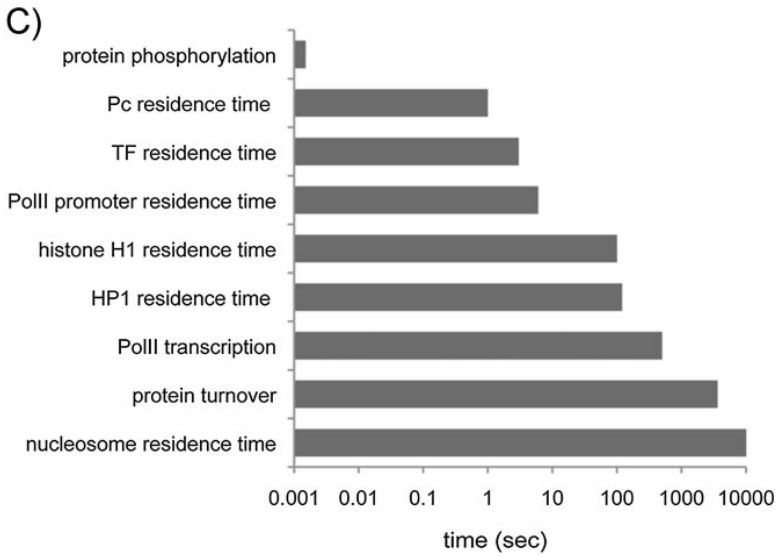
Epigenetics meets mathematics: Towards a quantitative understanding of chromatin biology

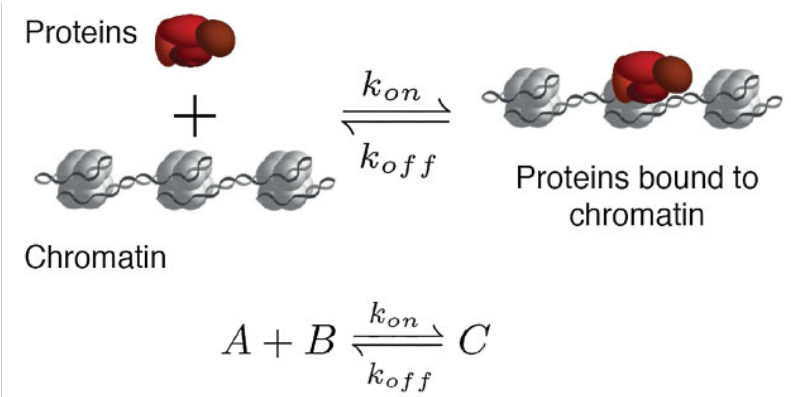
*Philipp A. Steffen†, João P. Fonseca† and Leonie Ringrose**

Bioessays 34: 901–913,© 2012





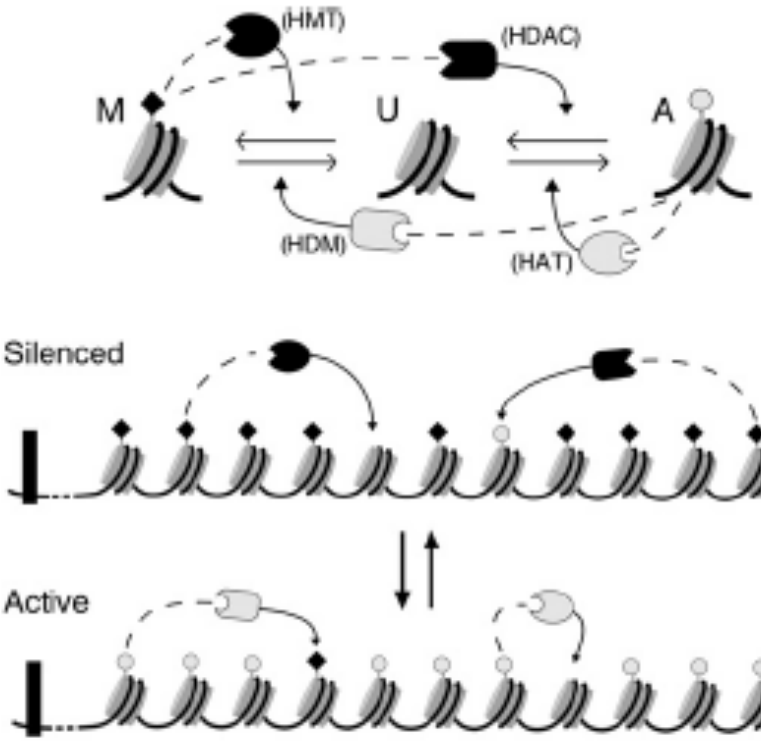




$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C$$

Theoretical Analysis of Epigenetic Cell Memory by Nucleosome Modification

Ian B. Dodd,[1,2] Mille A. Micheelsen,[1] Kim Sneppen,[1,*] and Geneviève Thon[3]



M – repressed chromatin
U – unmodified chromatin
A- Active chromatine
HMT, HDAC, HDM, HAT – enzymes

Cooperativity !!!

Figure 2. Bistability Is a Function of Noise

Cell 129, 813–822, May 18, 2007

Theoretical Analysis of Epigenetic Cell Memory by Nucleosome Modification

Ian B. Dodd,[1,2] Mille A. Micheelsen,[1] Kim Sneppen,[1,*] and Geneviève Thon[3]

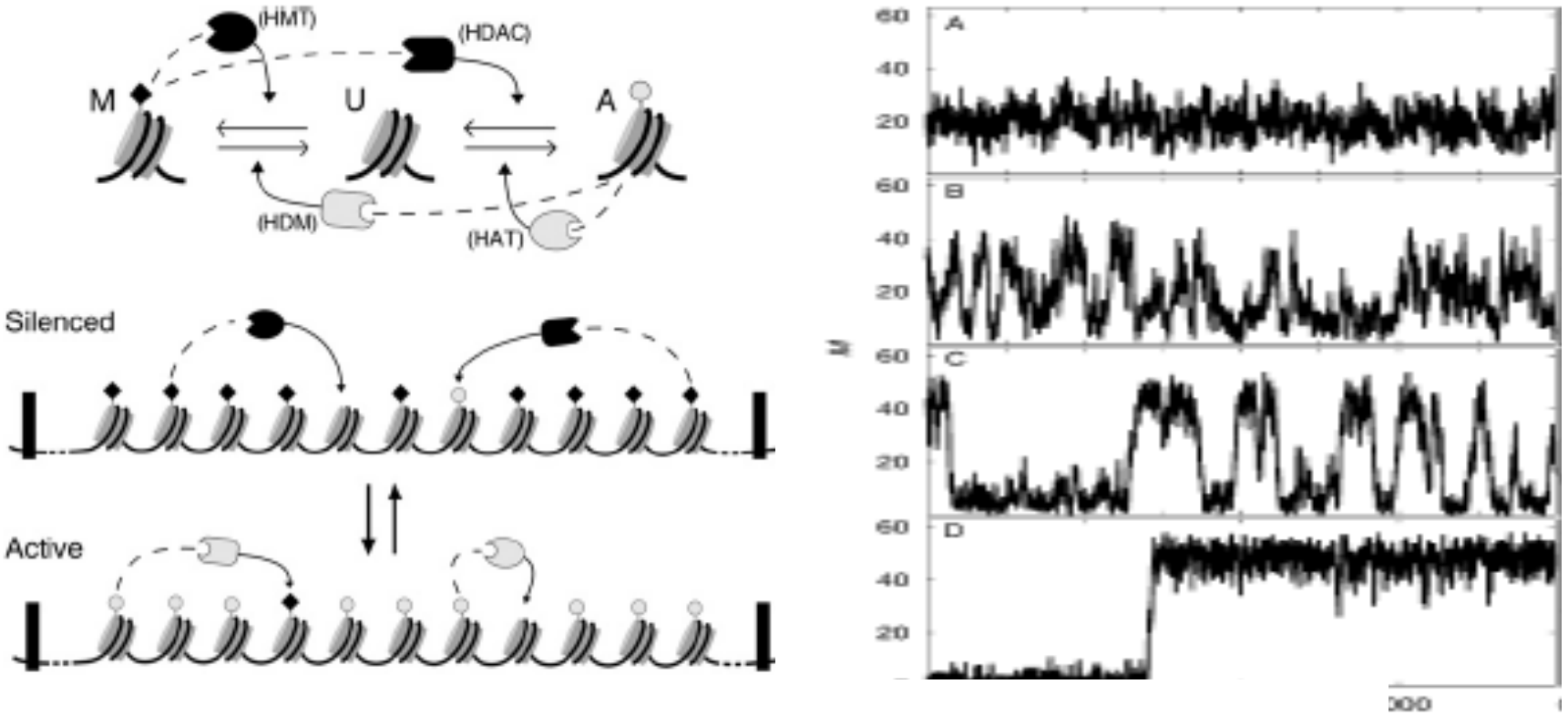
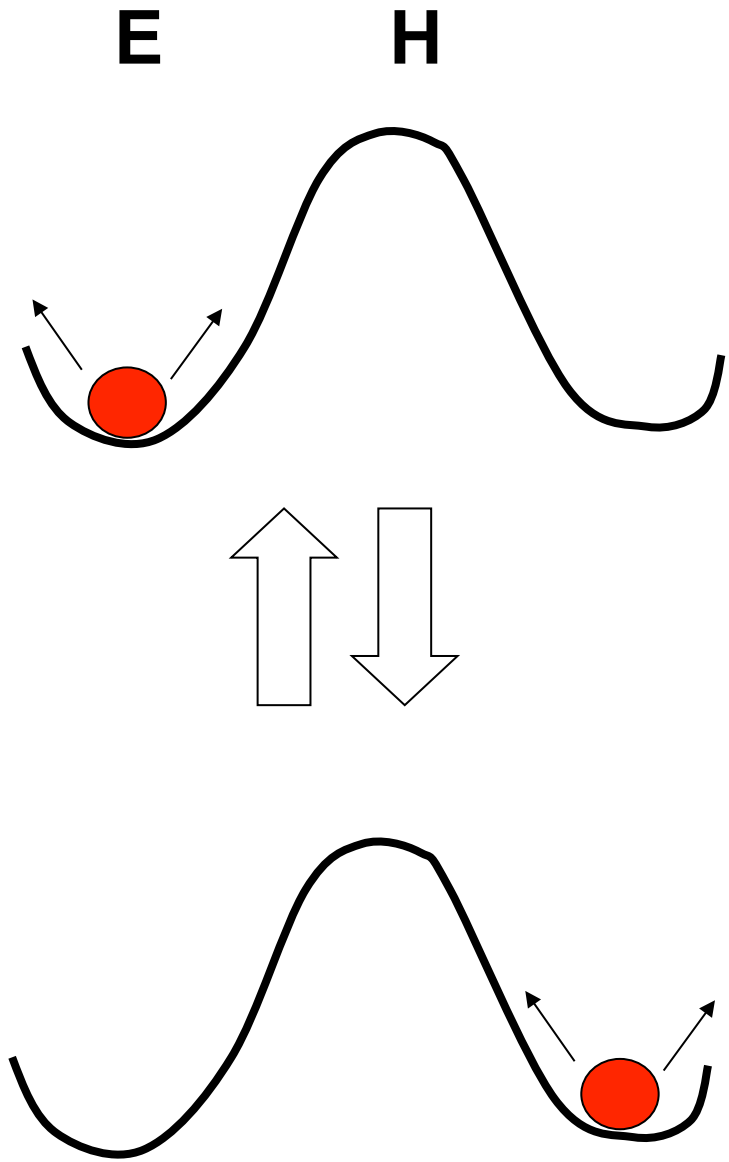


Figure 2. Bistability Is a Function of Noise

Cell 129, 813–822, May 18, 2007


E
H

Metabolic Regulation of Epigenetics

Chao Lu[1,2] and Craig B. Thompson[1,*]
[1]Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[2]Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: thompsonc@mskcc.org
http://dx.doi.org/10.1016/j.cmet.2012.06.001

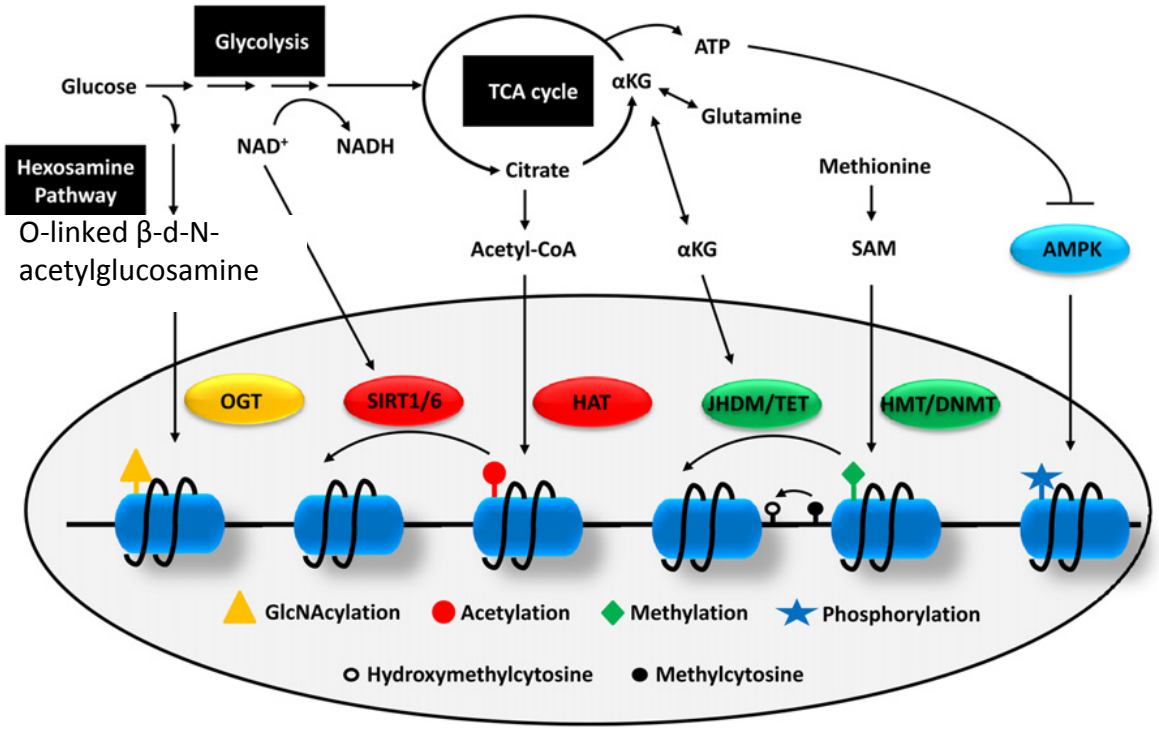


Figure 3. Crosstalk between Metabolism and Epigenetics

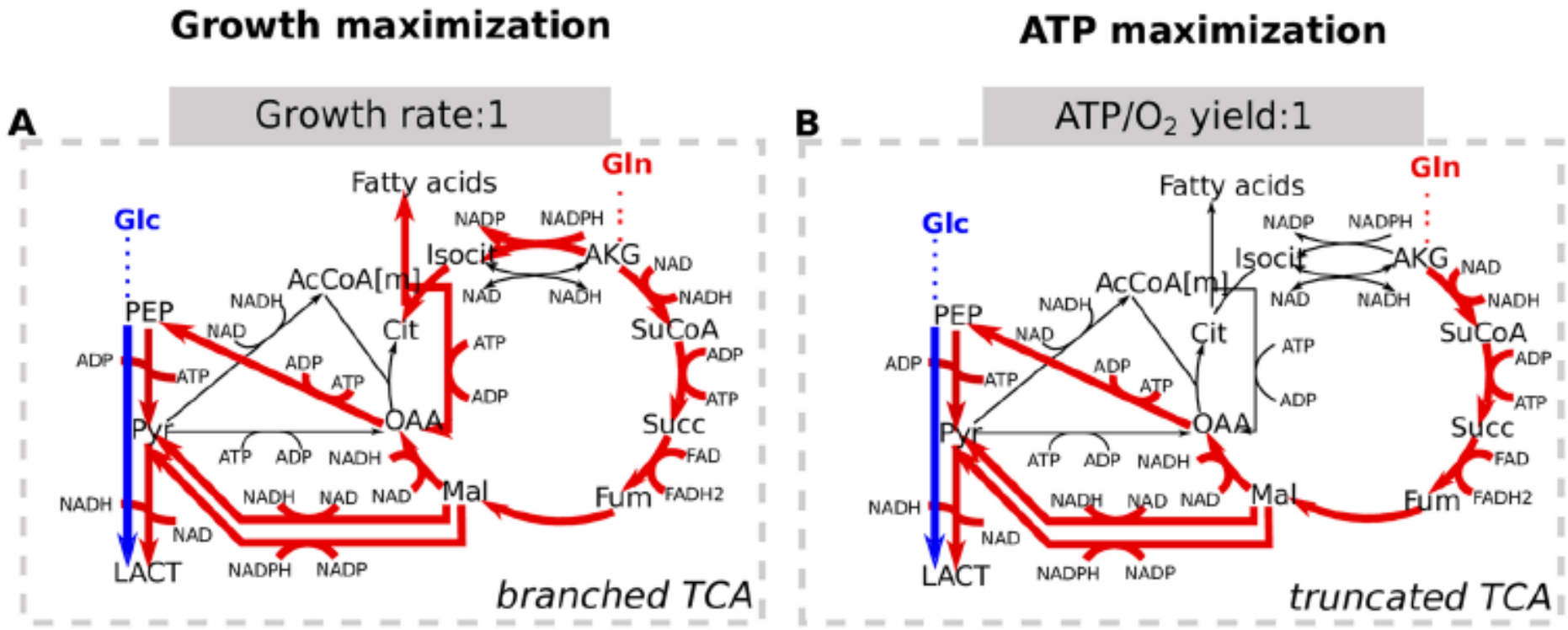
As glucose enters the glycolytic pathway, a minor portion is branched to hexosamine biosynthetic pathway to produce GlcNAc which can be used as substrate for histone GlcNAcylation by OGT. Flux through glycolysis determines the NAD^+/NADH ratio which is important for the activities of sirtuin histone deacetylases. Several TCA cycle intermediates can be exported out of mitochondria including citrate and αKG. Cytosolic citrate is converted to acetyl-CoA which is used as a donor for HAT-mediated histone acetylation. αKG is used as cofactor for histone and DNA demethylation reactions by JHDM and TET, respectively. The substrate for HMT and DNMT is SAM, which is synthesized from essential amino acid methionine. Finally, a low ATP/AMP ratio can activate AMPK, a kinase that phosphorylates histones.

RESEARCH ARTICLE

A metabolic core model elucidates how enhanced utilization of glucose and glutamine, with enhanced glutamine-dependent lactate production, promotes cancer cell growth: The WarburQ effect

Chiara Damiani[1,2], Riccardo Colombo[1,2], Daniela Gaglio[1,3], Fabrizia Mastroianni[1,4], Dario Pescini[1,5], Hans Victor Westerhoff[6,7,8], Giancarlo Mauri[1,2], Marco Vanoni[1,4]*, Lilia Alberghina[1,4]*

1 SYSBIO Centre of Systems Biology, Milano, Italy, **2** Dept of Informatics, Systems and Communication,



3. Summary

How cells function reliably with such an inherent variability in gene expression?

Intrinsic constraints: generated by the system itself
Extrinsic constraints: environment (what is environment?)

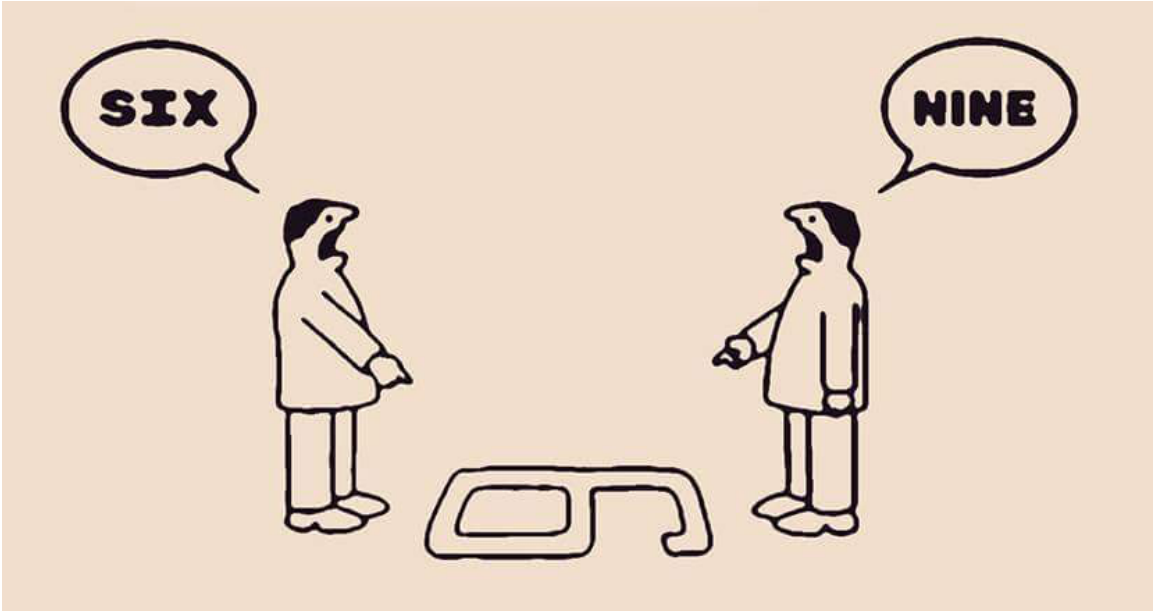
3. Summary

How cells function reliably with such an inherent variability in gene expression?

Intrinsic constraints: generated by the cells themselves
Extrinsic constraints: environment (what is environment?)

⇨ define a state space with many different attractors.
But not every attractor is accessible.

spatial (diffusion, steric etc.)
mechanic
thermodynamic (red-ox, “fuel” type)
historical contingency (heredity)



Thank you !

…and wait a moment please