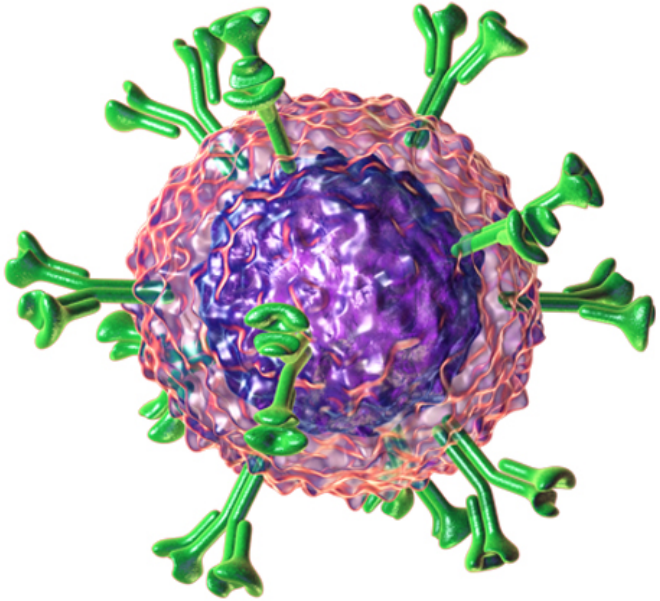
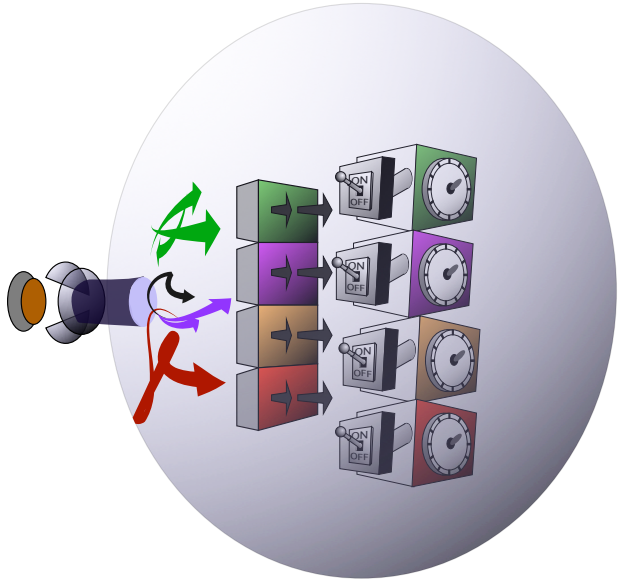




Controlling the lymphocyte - empirical rules for a calculus of signal integration

Phil Hodgkin

KITP Santa Barbera, 2012

FIAT LUX
Walter+Eliza Hall
Institute of Medical Research

Adaptive immunity: Complexity at multiple scales

Movies from WEHI-TV - see Fighting infection by Clonal Selection - http://www.youtube.com/watch?v=HUSDvSknIgI

Etsuko Uno & Drew Berry WEHI-TV

1957: Clonal Selection Theory

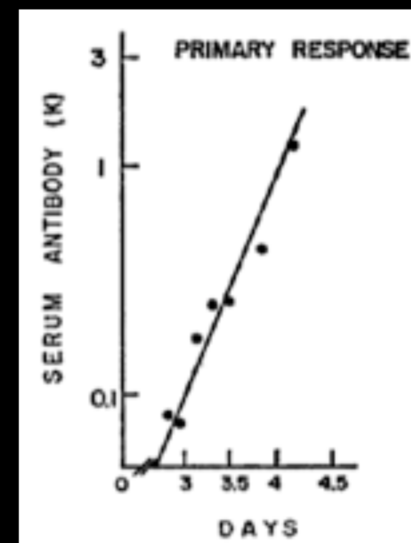
Burnet introduces the notions of clonal selection, deletional tolerance, expanded clones for memory - for the first time. Also boldly predicts a randomization of the genetic mechanism for coding antibody - all in only 2 pages!


The Australian Journal of Science

Volume 20 — 21 October 1957 — Number 3

A Modification of Jerne's Theory of Antibody Production using the Concept of Clonal Selection

F. M. Burnet*



Macfarlane Burnet

1965

"The principles of immunity are now known

Immunology is now just working out details"

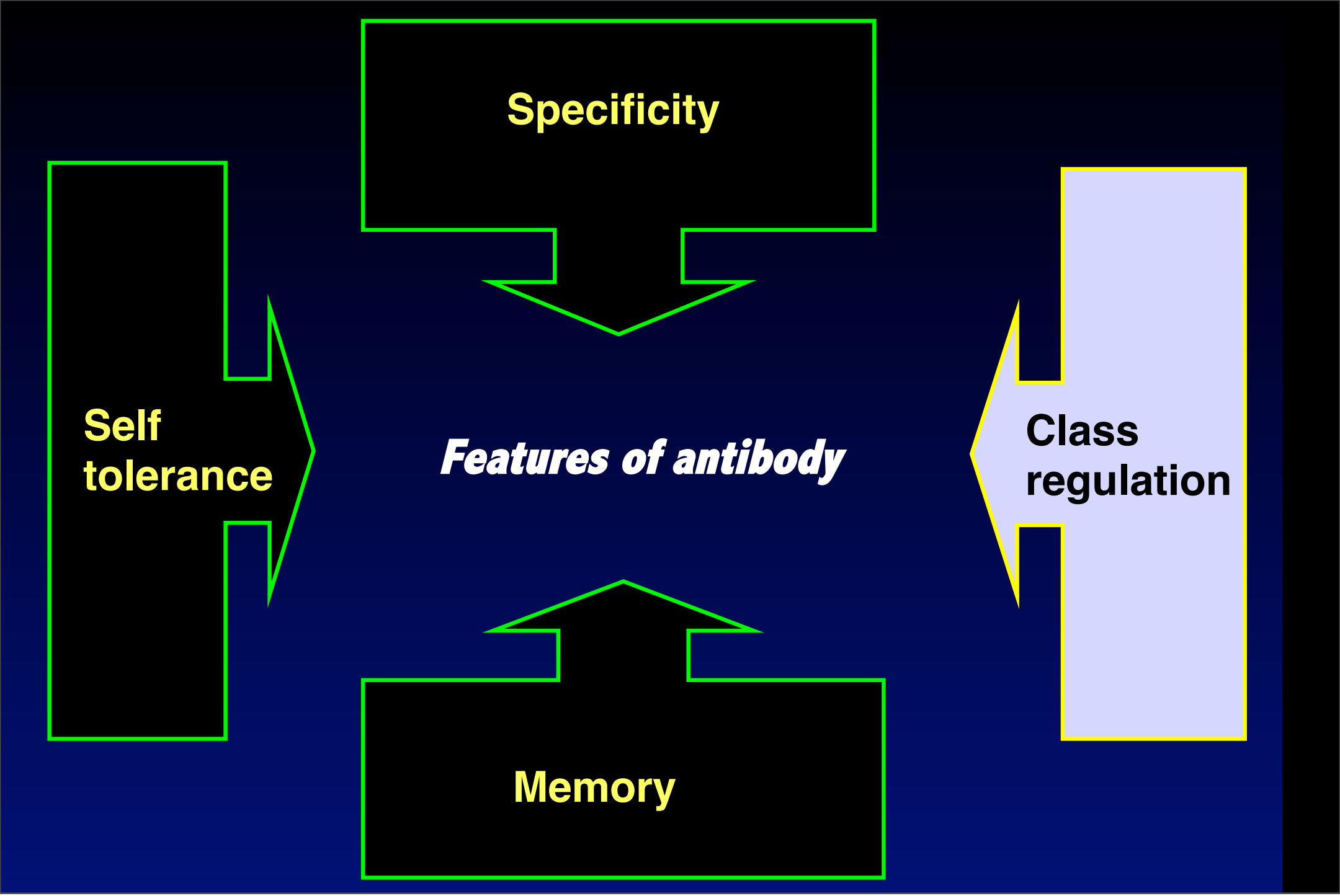
Macfarlane Burnet

1965

"The principles of immunity are now known

Immunology is now just working out details"

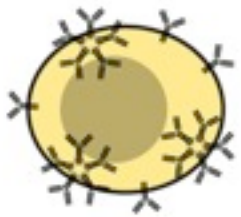
The details were hard!


Specificity
Self tolerance
Features of antibody
Class regulation
Memory

B cells play a key role in the humoral immune response

Antibody-producers

- Neutralisation
- Opsonisation
- Activation of complement
- Induction of phagocytosis



B cells play a key role in the humoral immune response

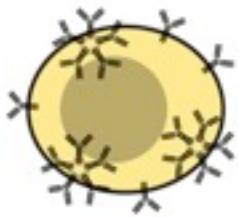
Antibody-producers

- Neutralisation
- Opsonisation
- Activation of complement
- Induction of phagocytosis

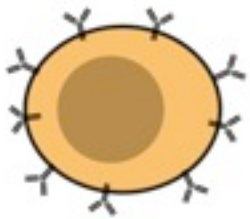
Differentiate by

isotype switching

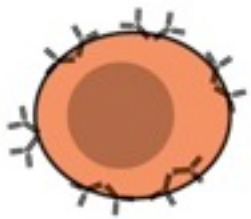
IgM, IgD



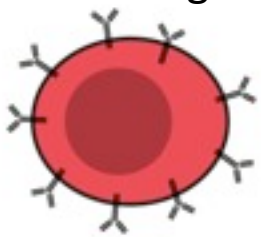
IgG



IgA



IgE



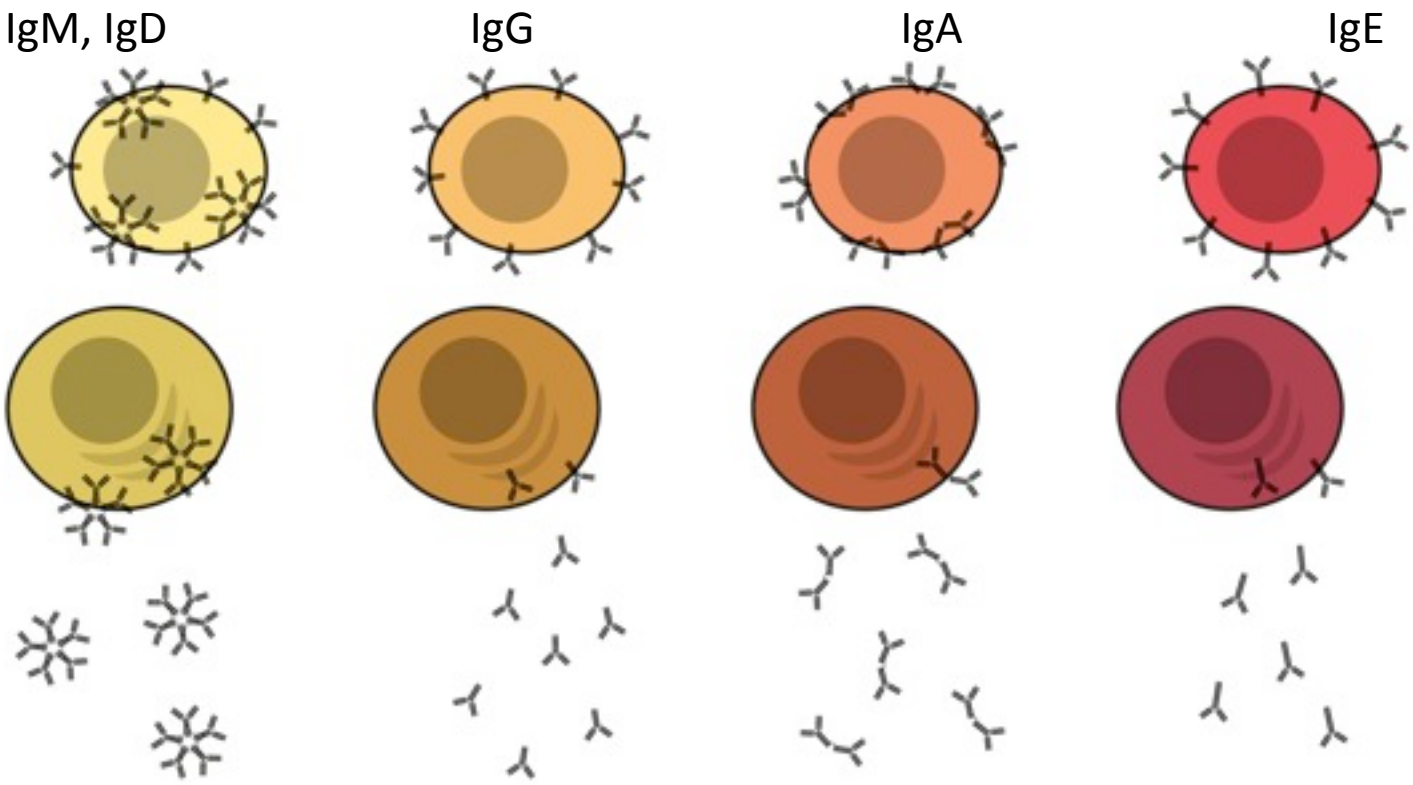
B cells play a key role in the humoral immune response

Antibody-producers

- Neutralisation
- Opsonisation
- Activation of complement
- Induction of phagocytosis

Differentiate by

isotype switching



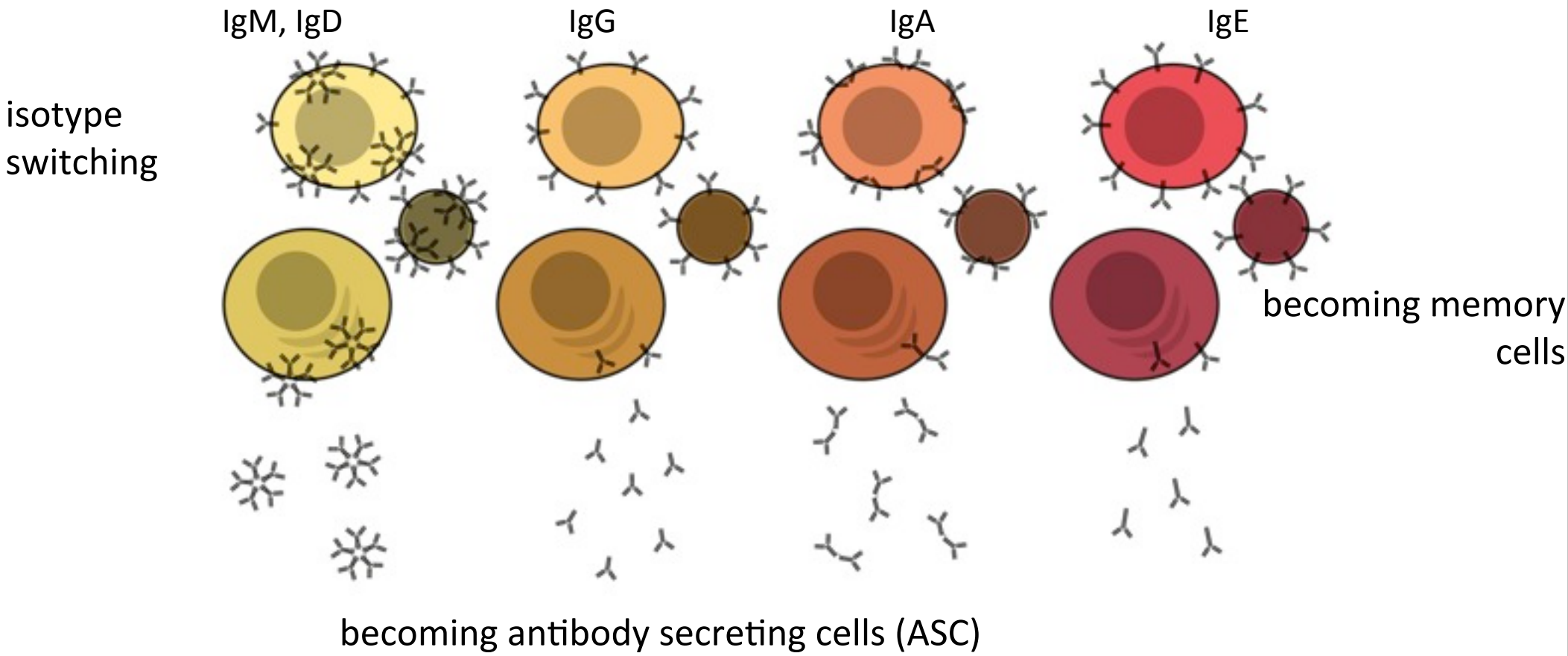
becoming antibody secreting cells (ASC)

B cells play a key role in the humoral immune response

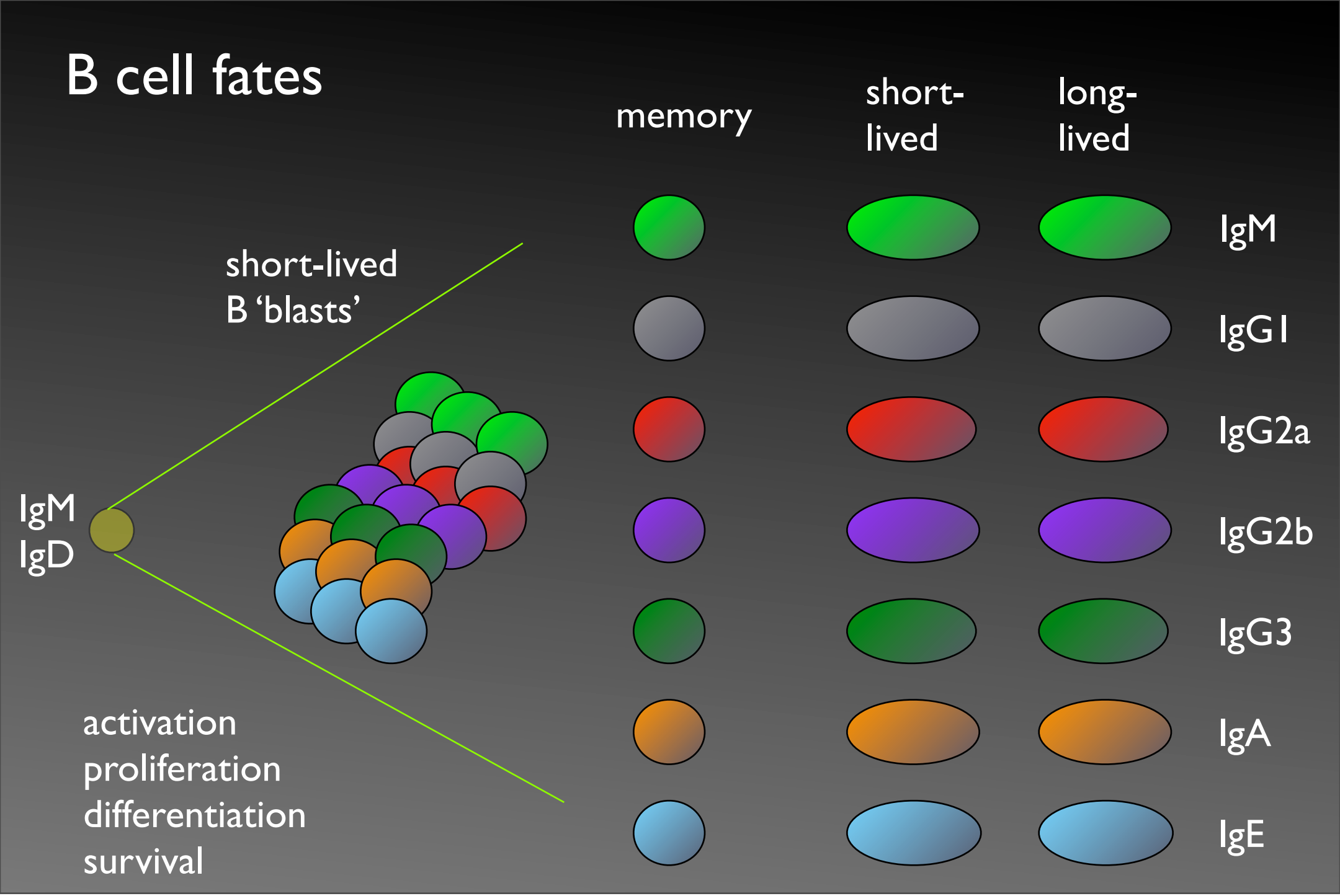
Antibody-producers

- Neutralisation
- Opsonisation
- Activation of complement
- Induction of phagocytosis

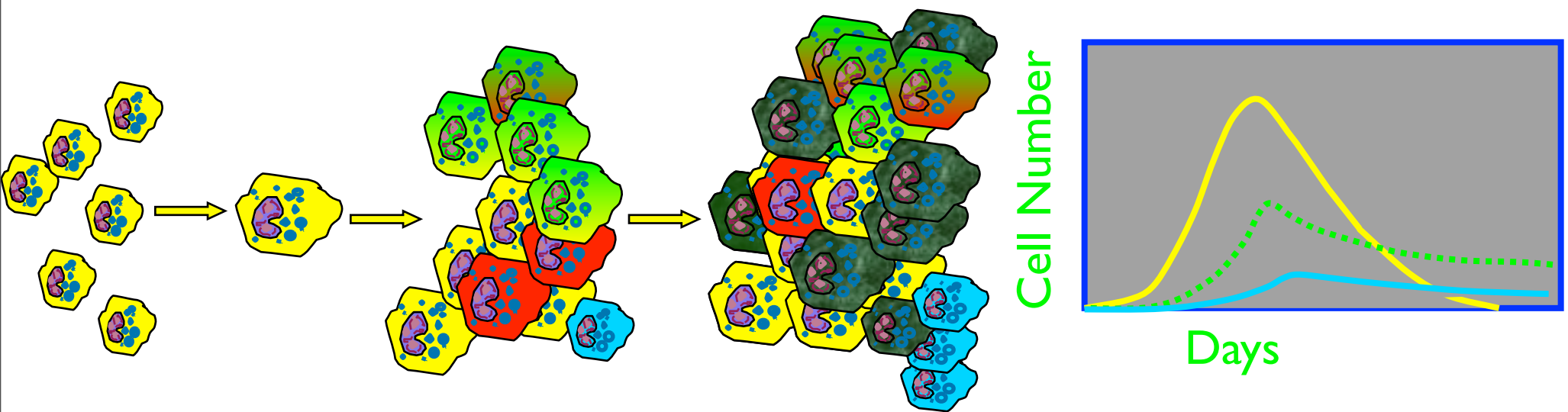
Differentiate by



B cell fates

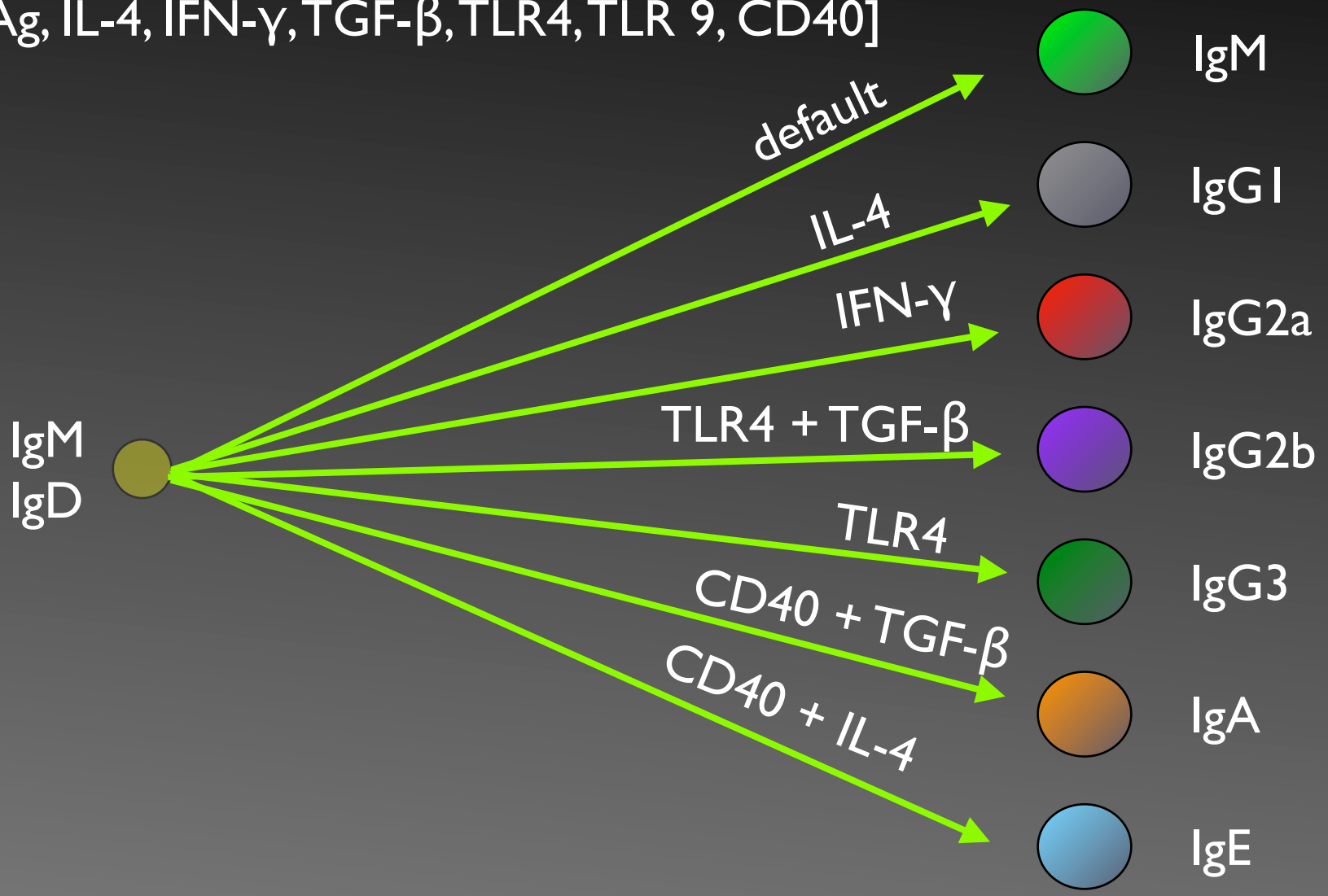

memory
short-lived
long-lived
short-lived
B 'blasts'
IgM
IgD
activation
proliferation
differentiation
survival
IgM
IgG1
IgG2a
IgG2b
IgG3
IgA
IgE

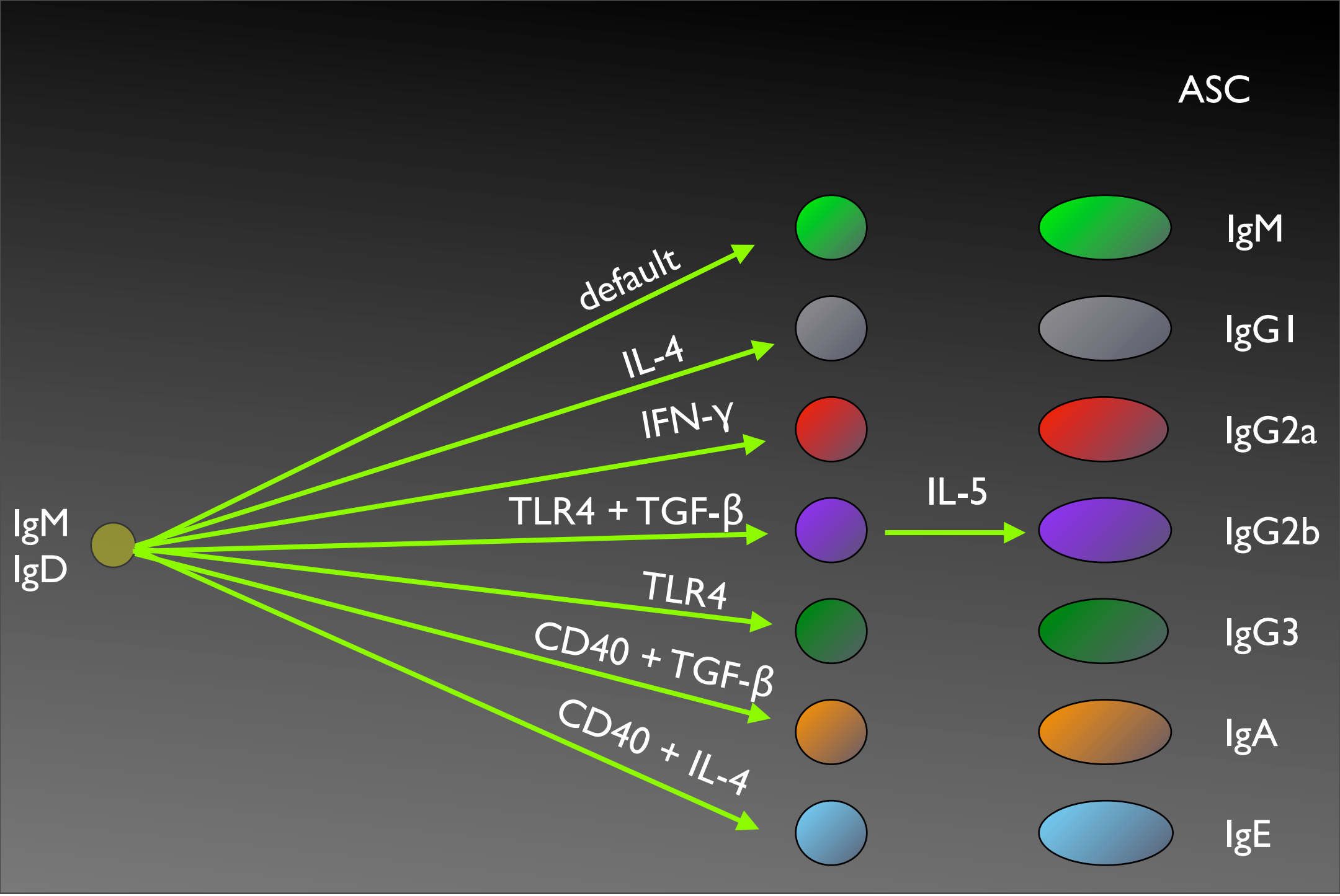
- Solve the logic puzzle - which signals and in what combinations lead to which outcomes?



Generation of diversity as a Cellular journey: A logic problem

activation [Ag, IL-4, IFN-γ, TGF-β, TLR4, TLR 9, CD40]




ASC
IgM
IgD
default
IL-4
IFN-γ
TLR4 + TGF-β
TLR4
CD40 + TGF-β
CD40 + IL-4
IL-5
IgM
IgG1
IgG2a
IgG2b
IgG3
IgA
IgE

Complex problem

Typically cytokines have varied effects on proliferation, survival and differentiation

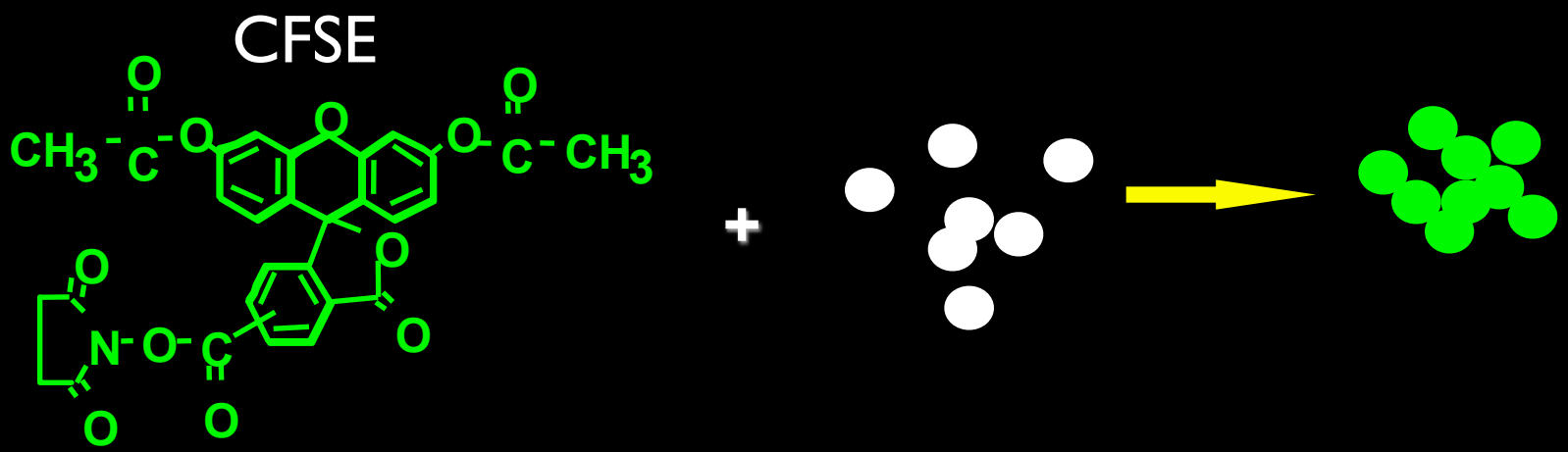
When exposed to combinations of different signals - how do cells calculate an outcome?

(search for principles of a 'Cellular Calculus' - governing signal 'integration' and cell 'differentiation') -

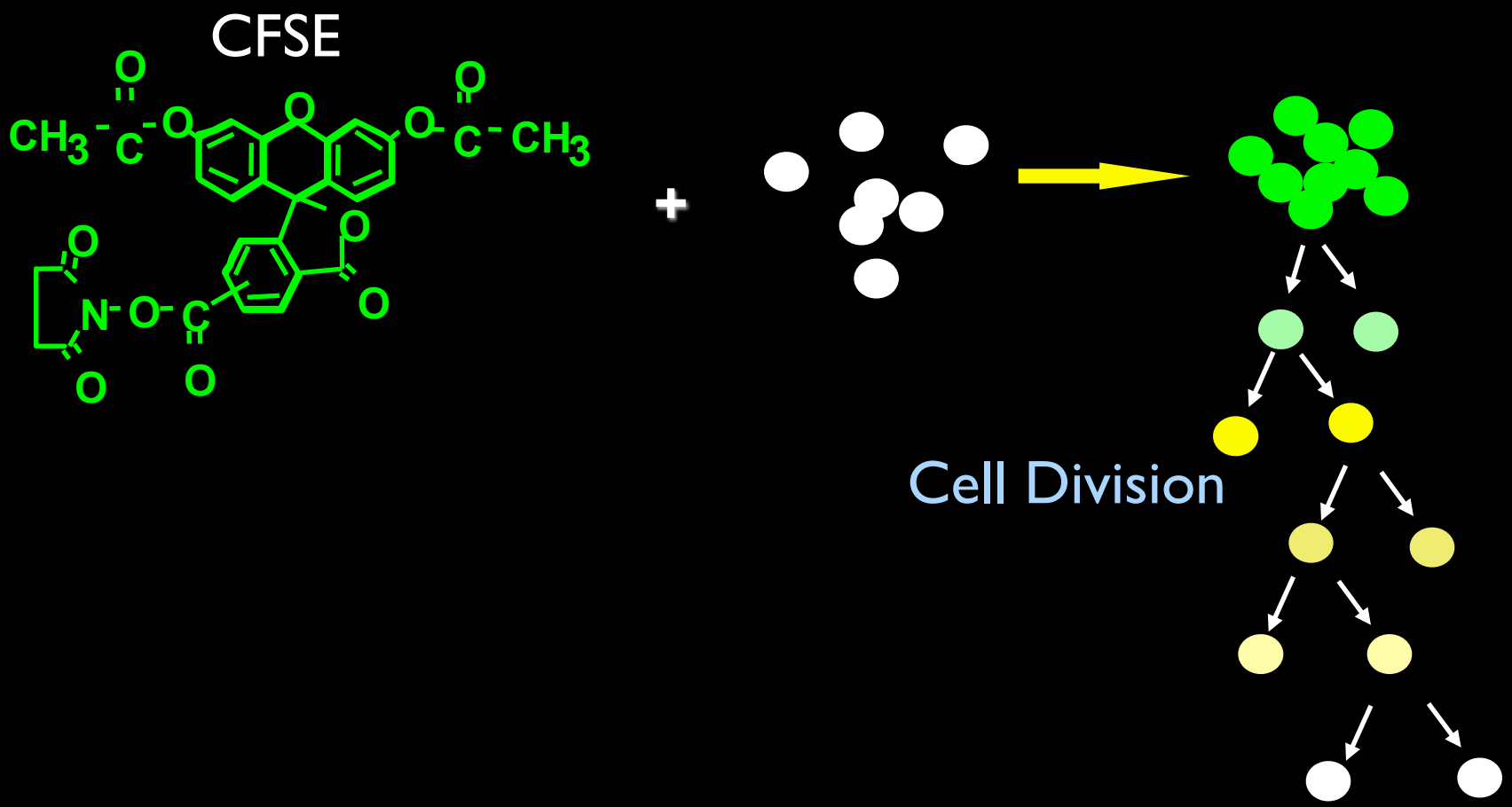
[note - strategy FIRST ORDER (no other interactions) first]

Cell Division number as a hidden variable in differentiation outcomes

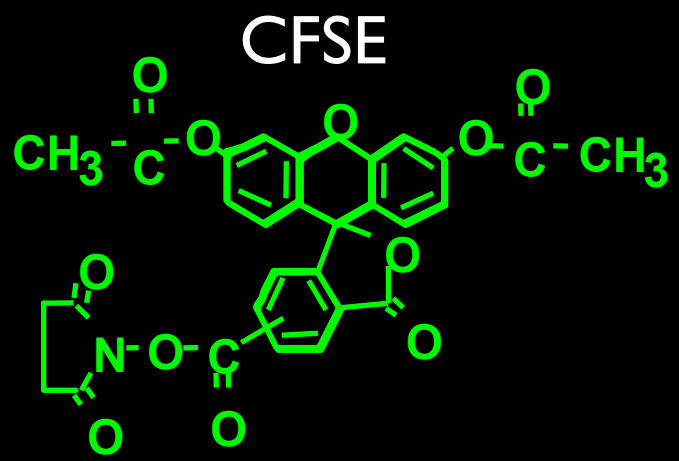
Division tracking with Carboxyfluorescein succinimidyl ester (CFSE) :Lyons and Parish

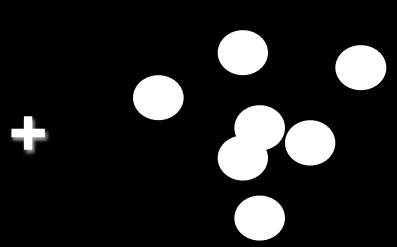


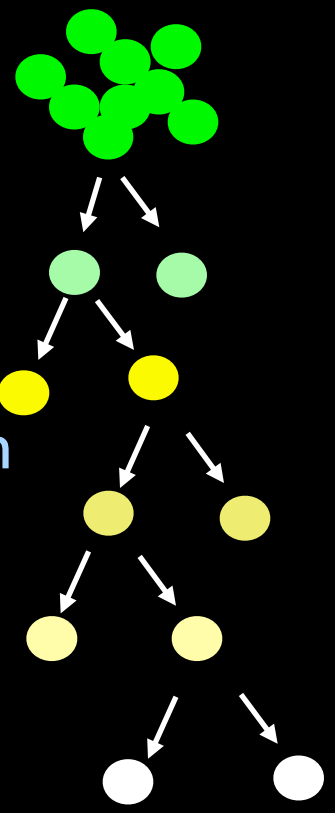
Division tracking with Carboxyfluorescein succinimidyl ester (CFSE) :Lyons and Parish

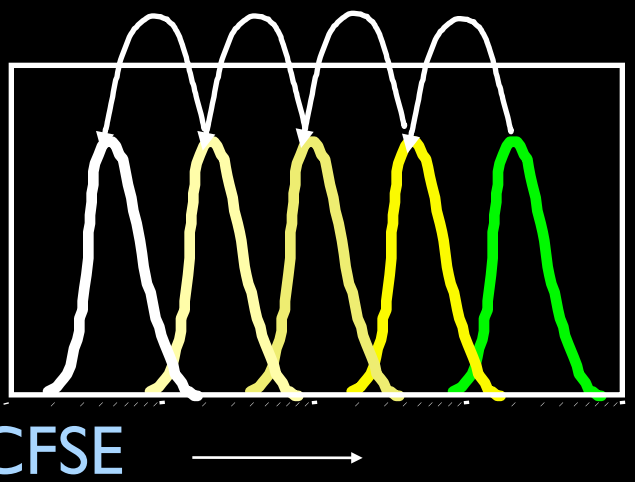


Division tracking with Carboxyfluorescein succinimidyl ester (CFSE) :Lyons and Parish



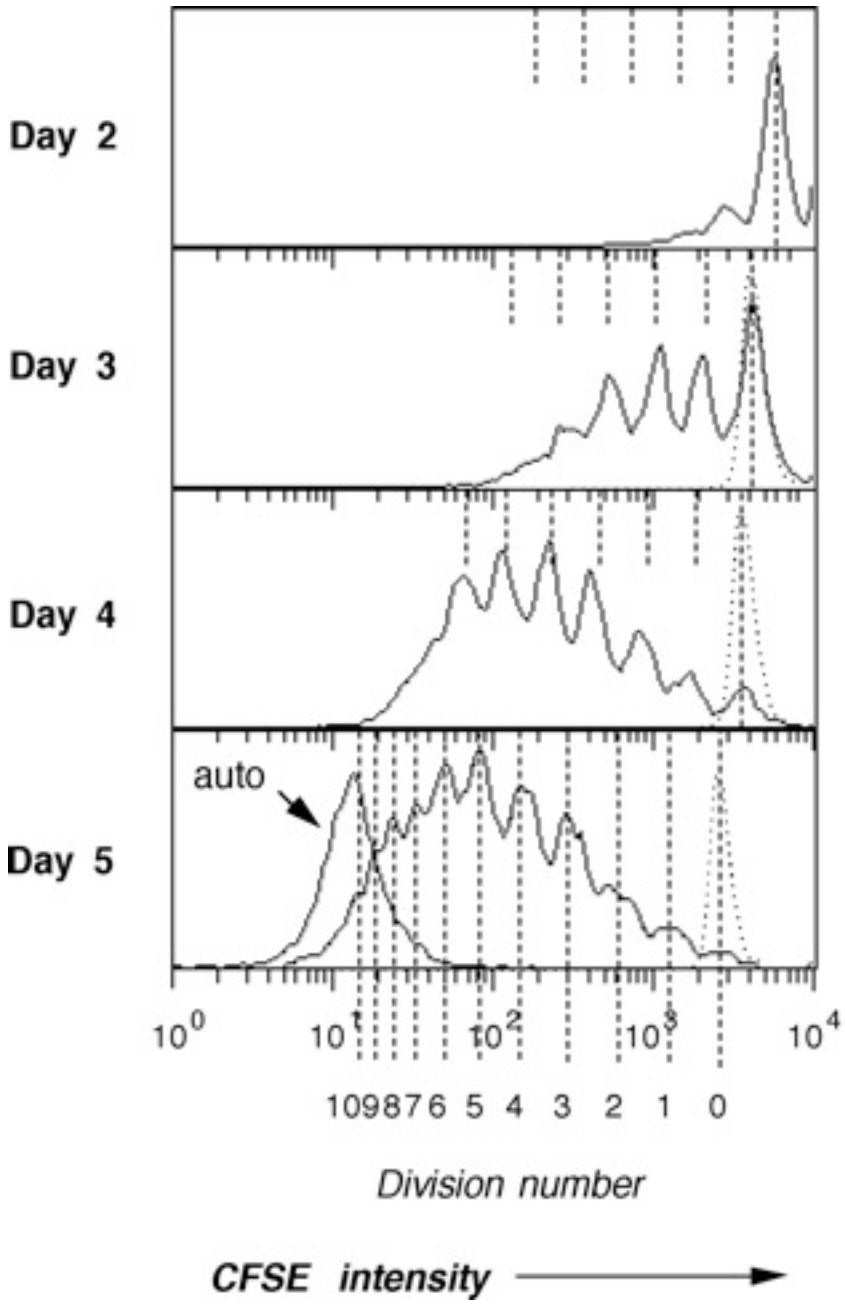






Flow cytometer

This method allows proliferation, survival & differentiation of 1000s of cells to be monitored



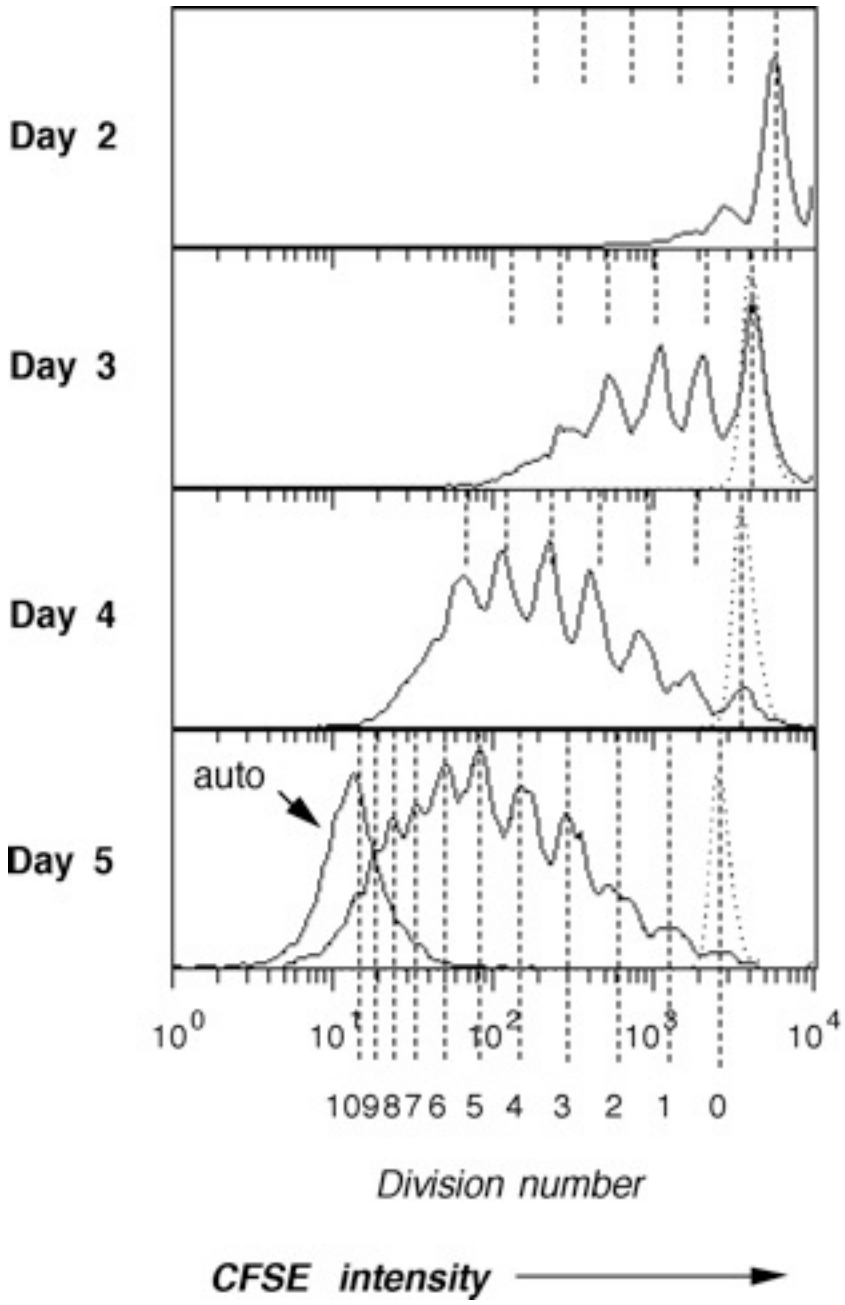
B cells stimulated in vitro

Purified mouse spleen resting B cells + CD40Ligand and Interleukin 4

•Division is asynchronous

•The peaks are limited to the autofluorescence by formula

((Start FL –AF)/2^div number)+AF



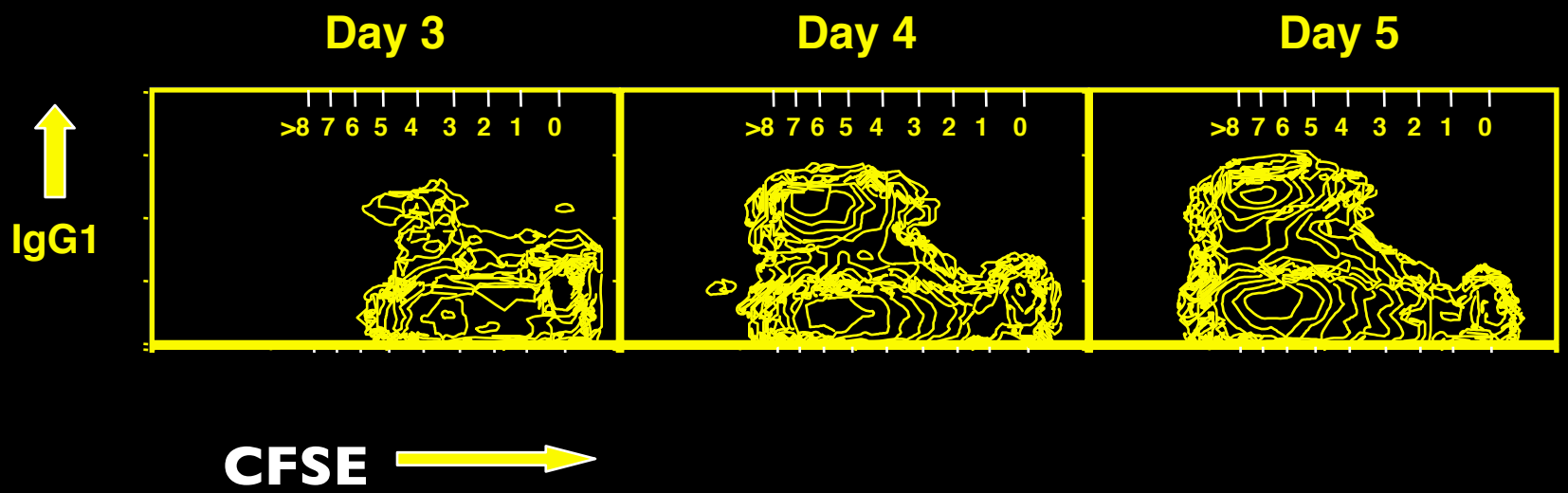
B cells stimulated in vitro

All B cells start as IgM+ and switch to IgG1 due to IL-4

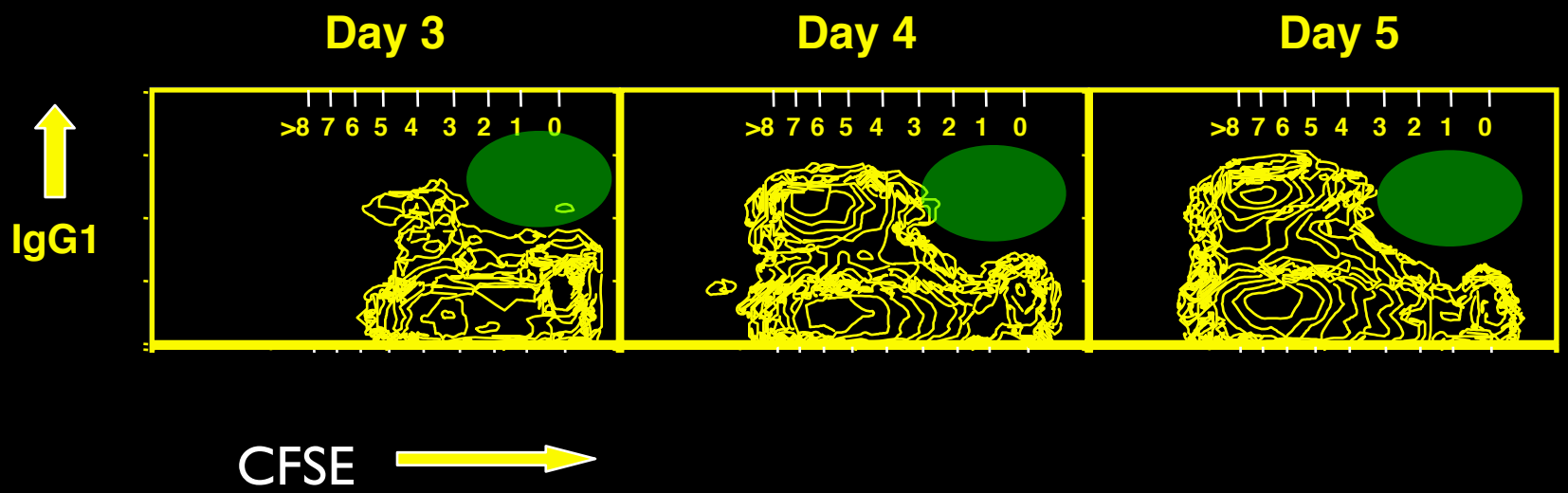
When do they switch?

(Work of J Hasbold)

Switch from IgM to IgG1 comes after 3 divisions

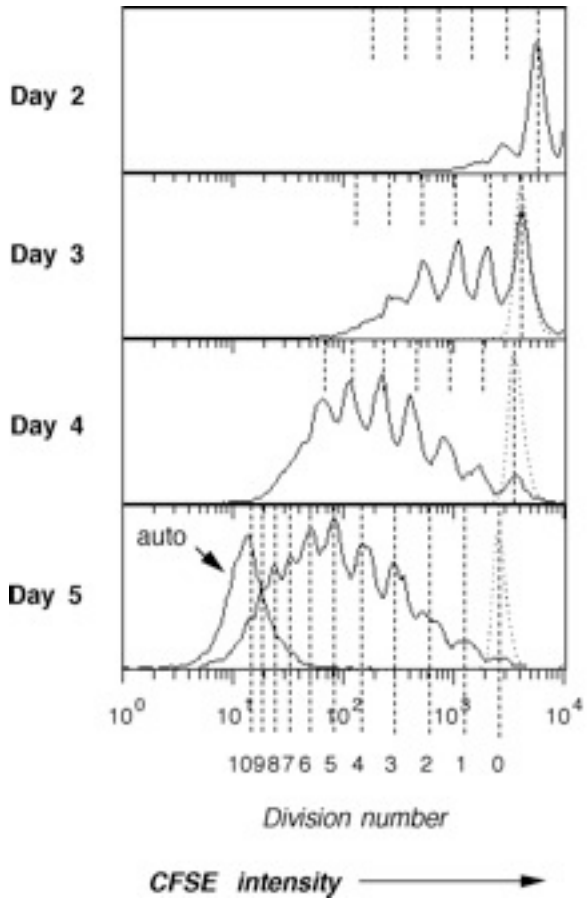


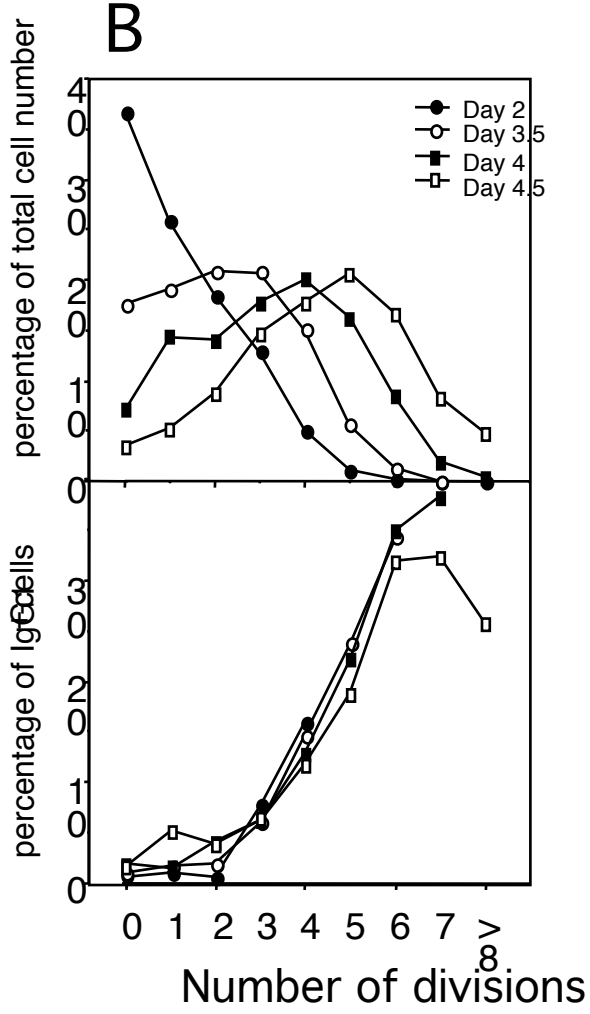
Switch from IgM to IgG1 comes after 3 divisions



No IgG1 cells appears before 3 divisions

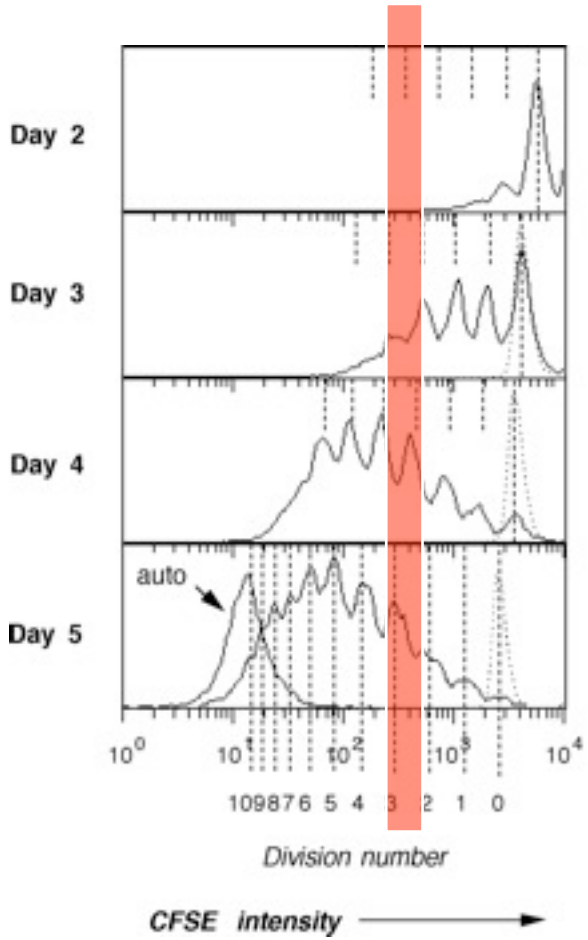
Isotype switching is 'Division-linked'

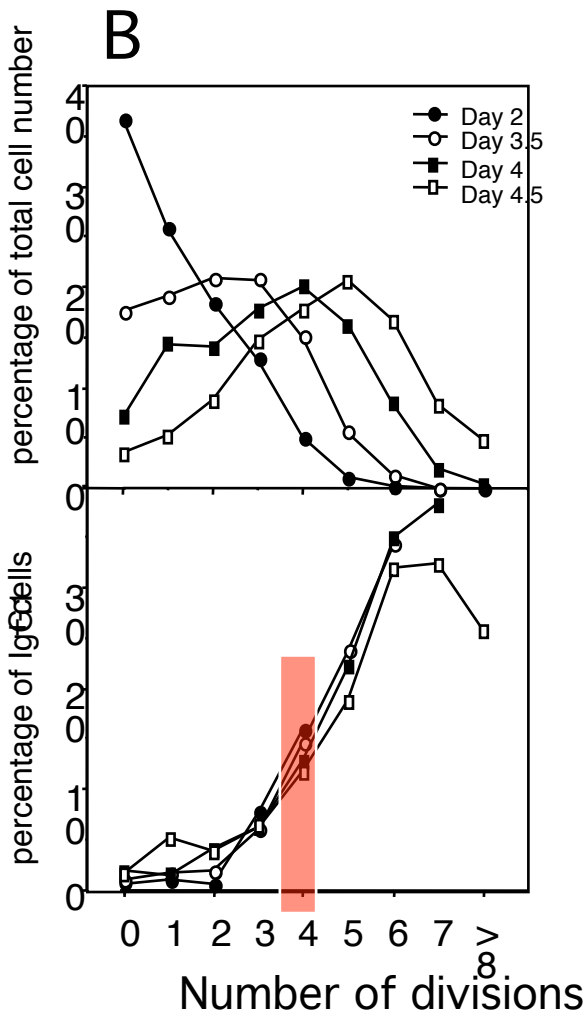




Not time-linked

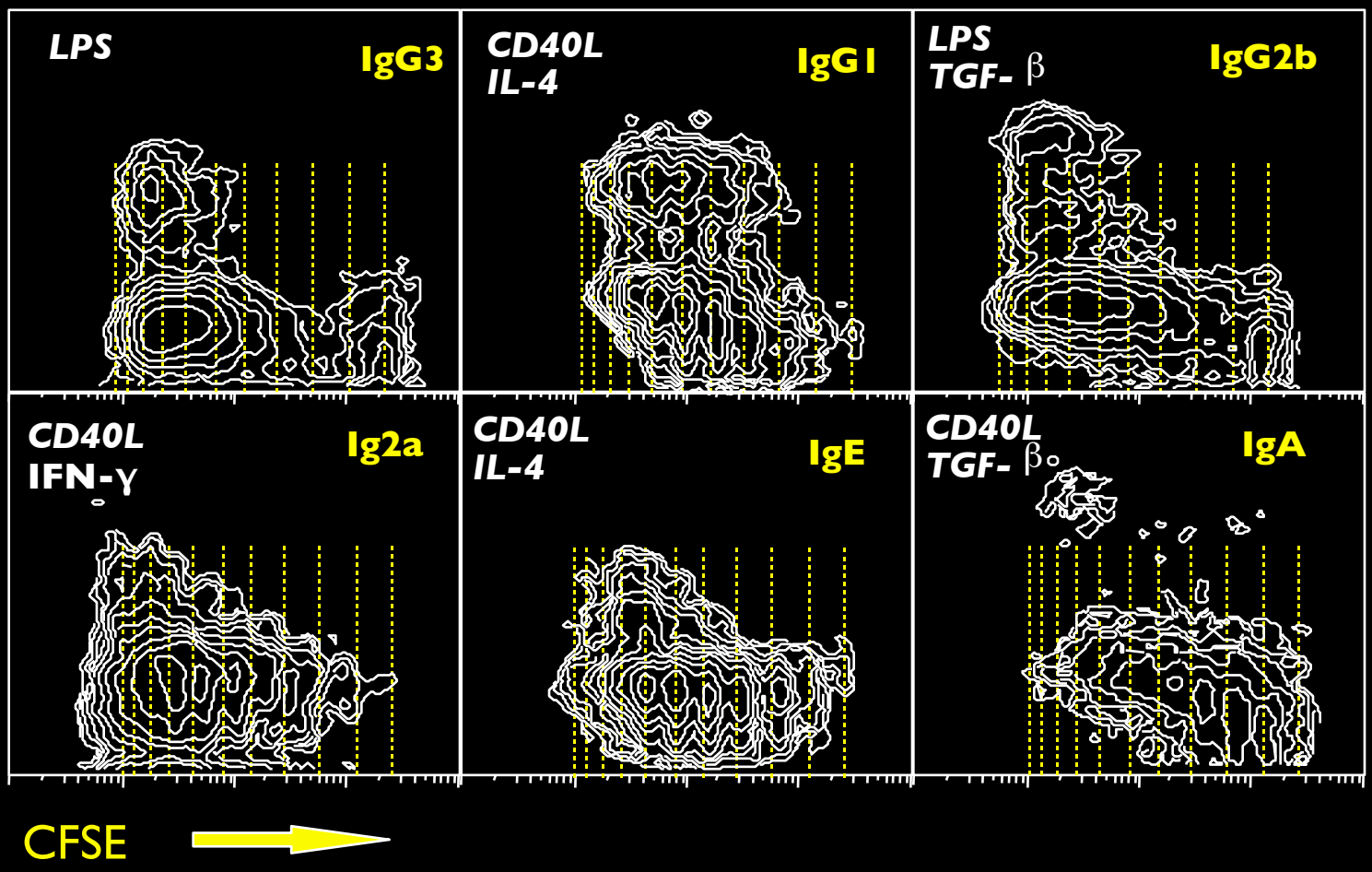
Isotype switching is 'Division-linked'



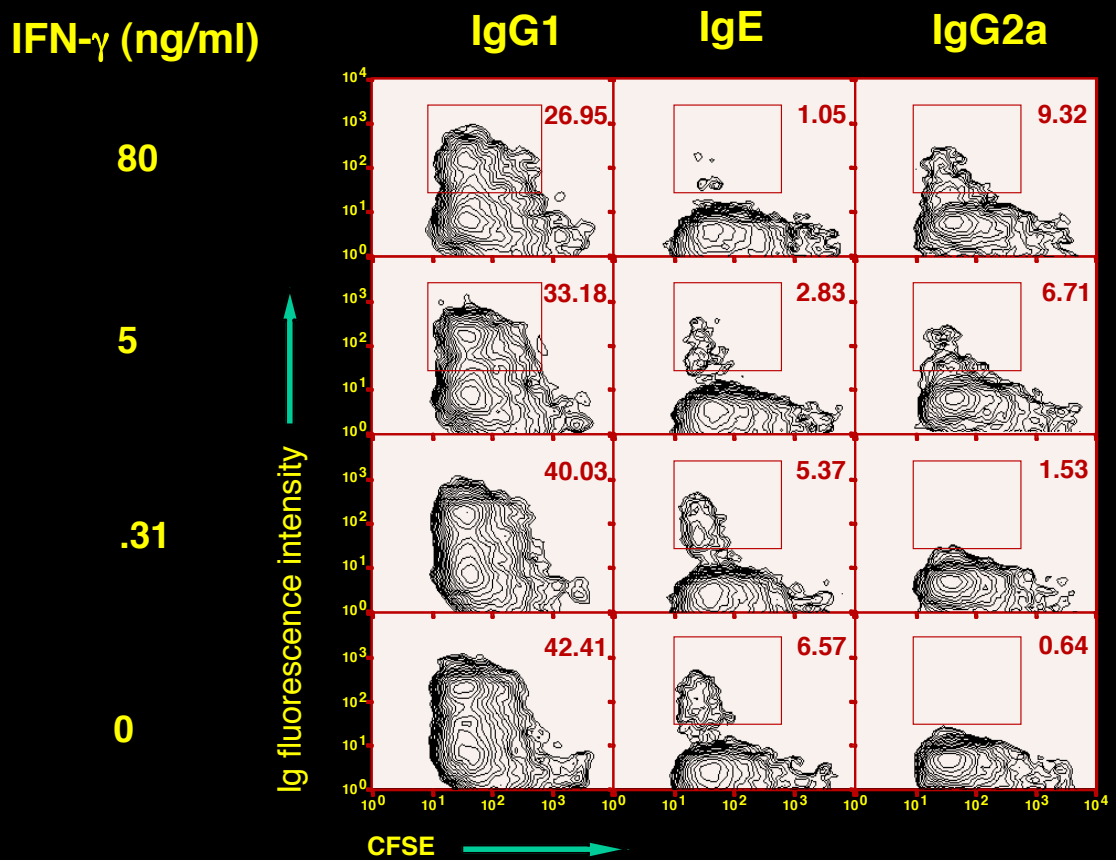


Not time-linked

All Isotypes show Division-linked switching



IFN-γ induces IgG2a expression, but down-regulates IgE



B cell rules:

Progression through division changes probability of switching

Cytokines/signals change relation with division

Rules can be found for combinations of signals - indicating cross talk or independence

2. Division linked-differentiation separate from regulation of -

Proliferation and survival
Model differentiation - combine

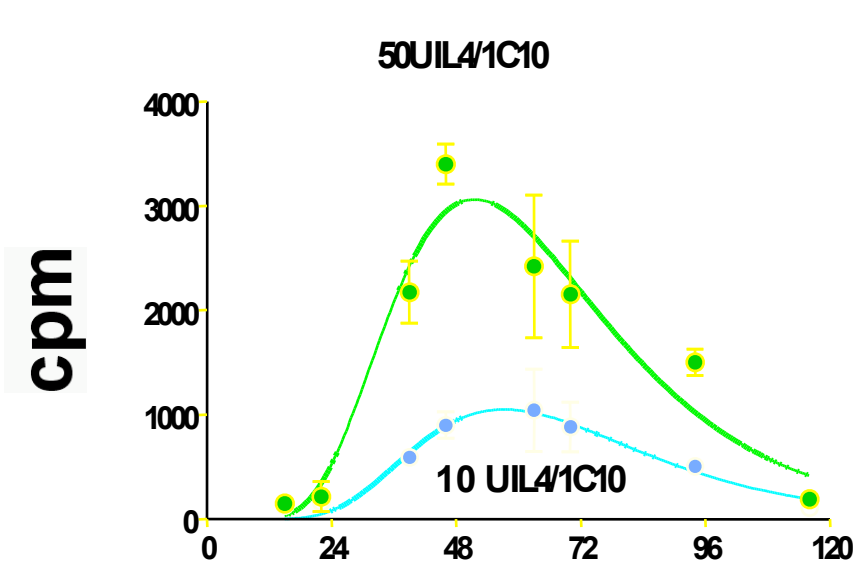
Models of CFSE proliferation patterns: insights into regulation of growth

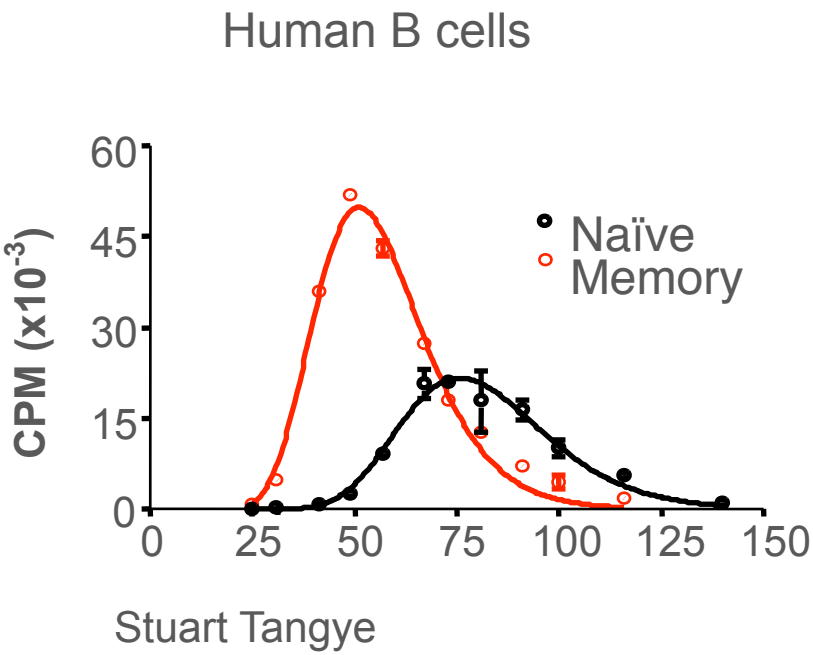
- *Separation of differentiation/proliferation*

- 4-parameter model of proliferation
  - (Amanda Gett: Excel models) 2000
- 6-parameter model of proliferation and survival – (Elissa Deenick: Excel and Cellular Calculator) 2002
- 13-parameter model: Cyton model – (Edwin Hawkins, Carel van Gend: java and matlab) 2007
- Multi-parameter Cyton model: Branching process formulation (Vijay Subramanian, Ken Duffy) 2008

Proliferation empirical law -

Lognormal variation in time to first division





Variables - Mean time to divide, variance and area

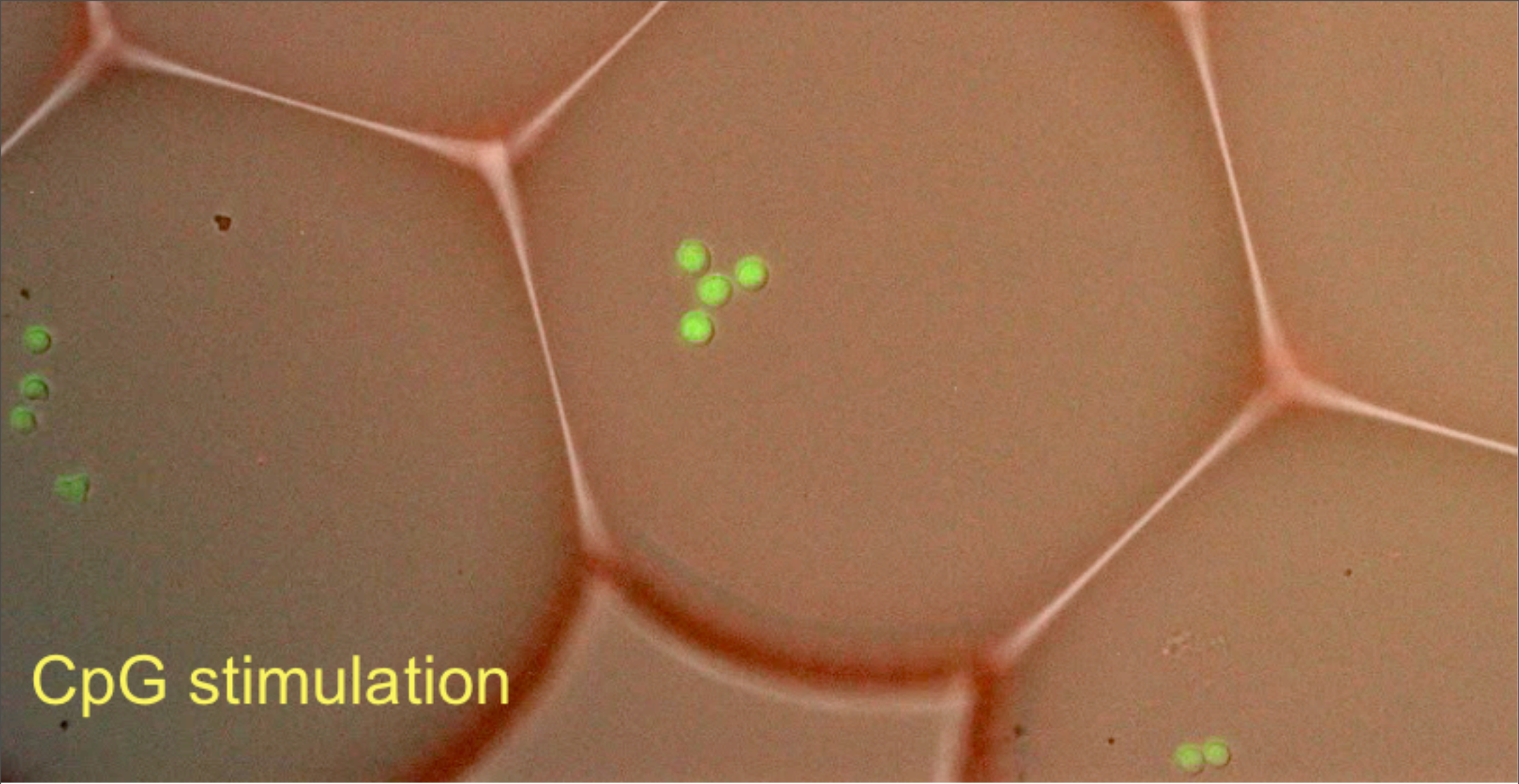
This is major source of division heterogeneity

All examples - mouse T and B - human T and B - all stimuli - one model fits all!

Subsequent divisions and inheritance of times?

Hypothesis

****Filming***



A single-cell pedigree analysis of alternative stochastic lymphocyte fates

E. D. Hawkins[a], J. F. Markham[a,b], L. P. McGuinness[a], and P. D. Hodgkin[a,1]

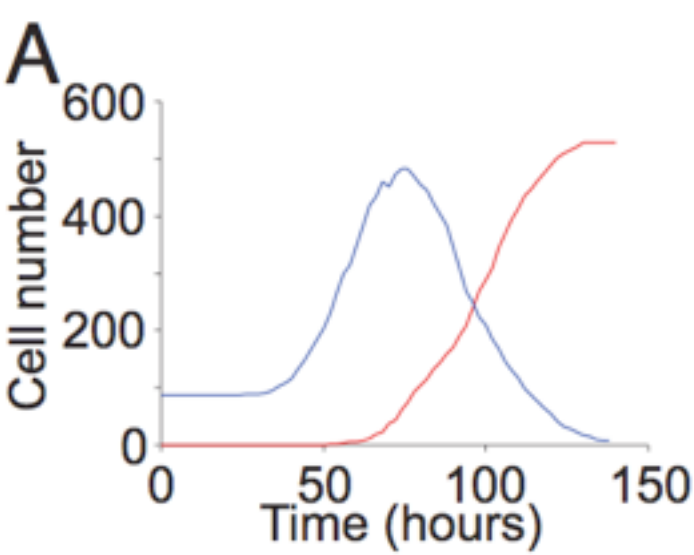
[a]Immunology Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Victoria 3050, Australia; and [b]National Information and Communications Technology Australia, and Department of Electrical Engineering, University of Melbourne, Lvl2/Building 193, Victoria 3010, Australia

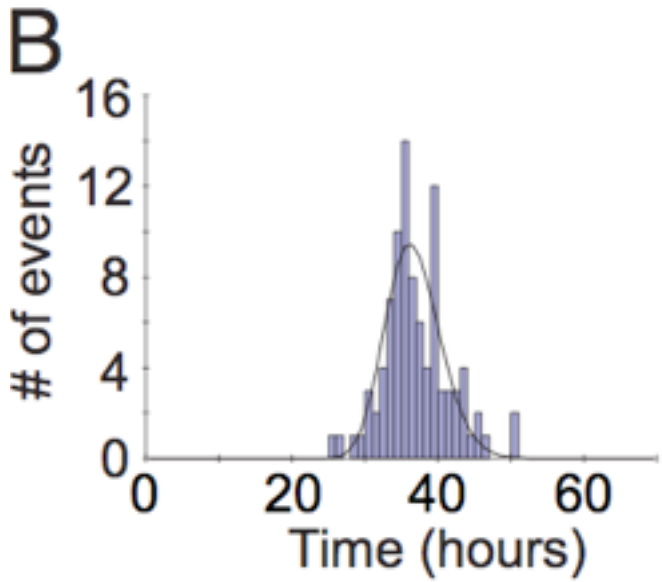
Communicated by Gustav J. Nossal, University of Melbourne, Victoria, Australia, June 8, 2009 (received for review February 2, 2009)

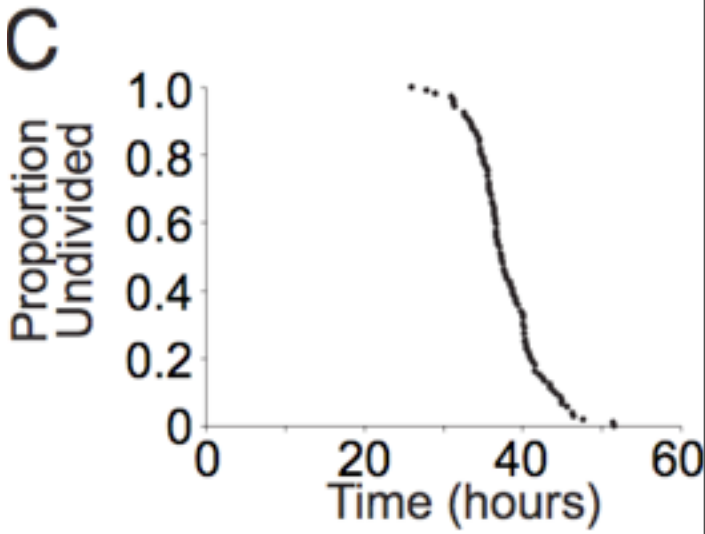
In contrast to most stimulated lymphocytes, B cells exposed to Toll-like receptor 9 ligands are nonself-adherent, allowing individ-

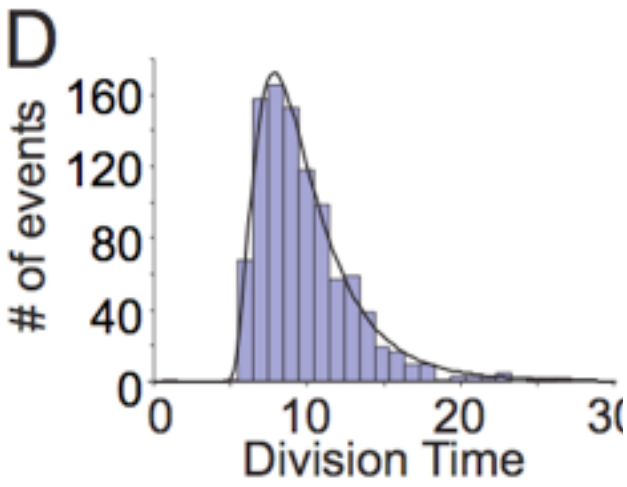
of lymphocyte responses, and to date there is no quantitative information on how lymphocyte survival is regulated and altered

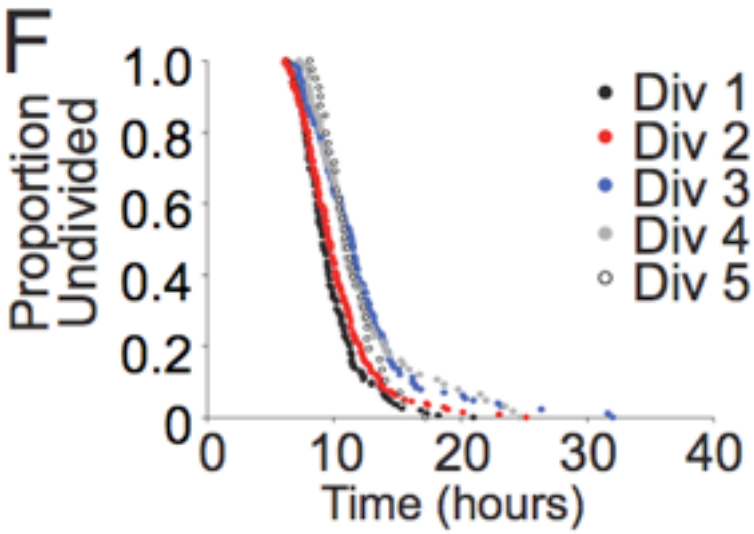
Proliferation, cessation and death



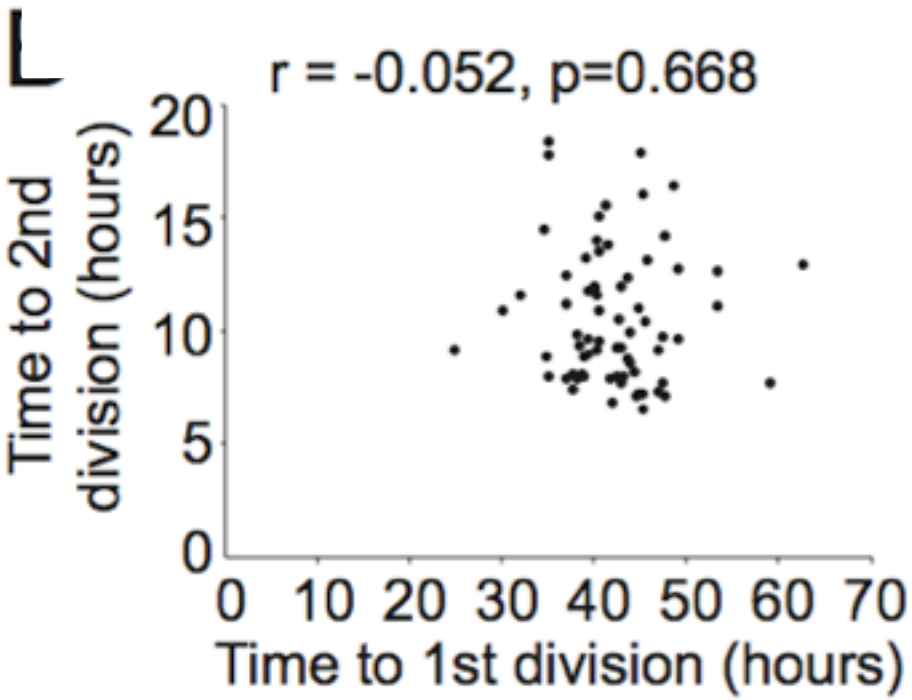


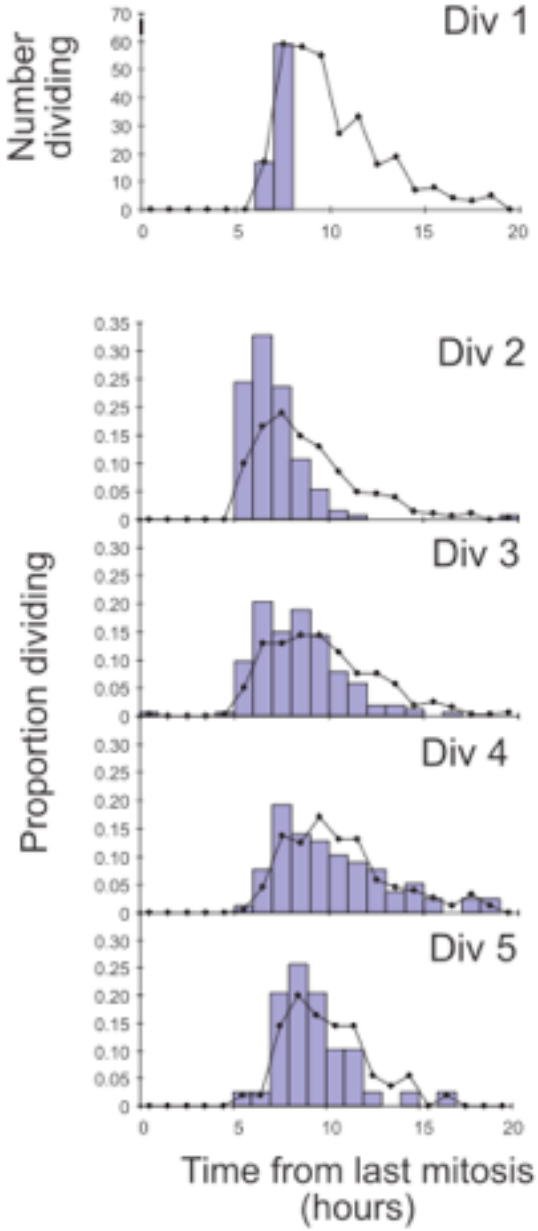




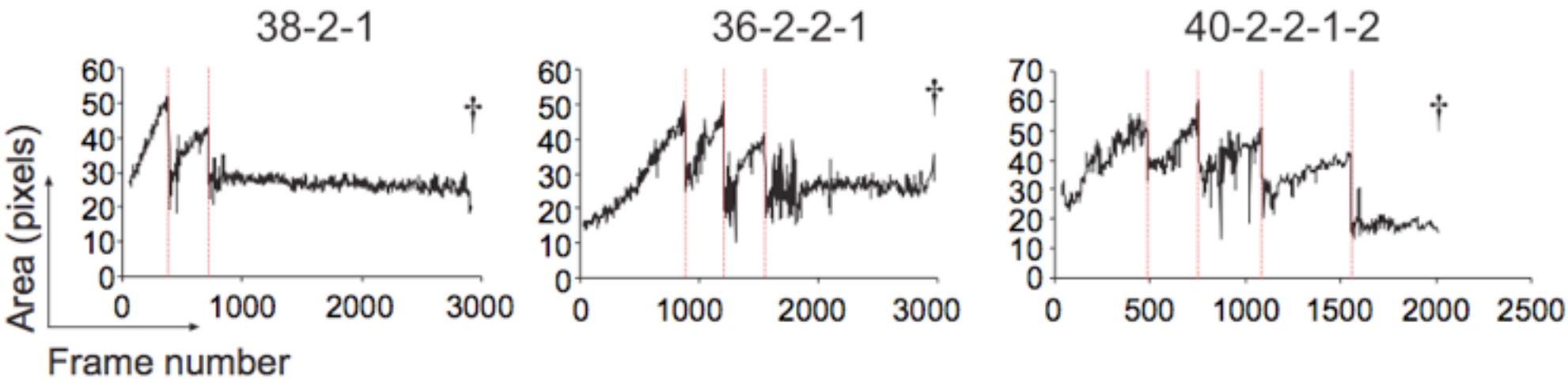


Fast does not beget fast...

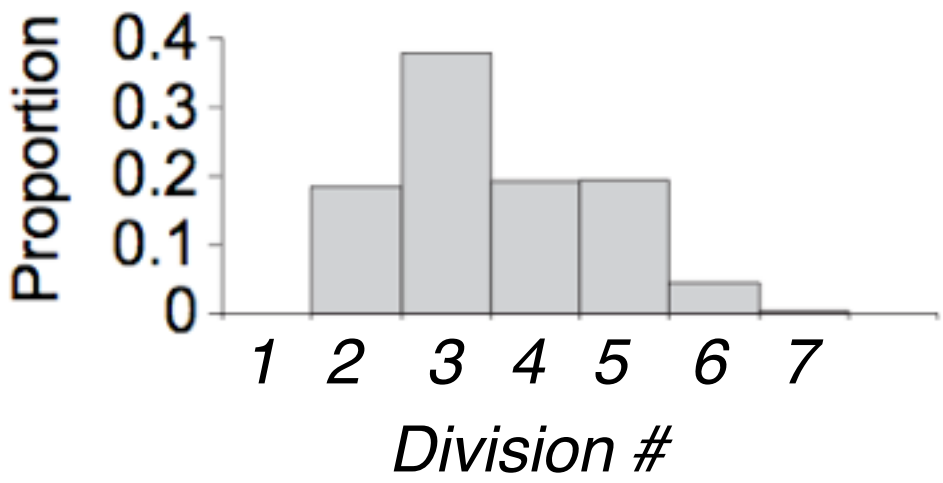




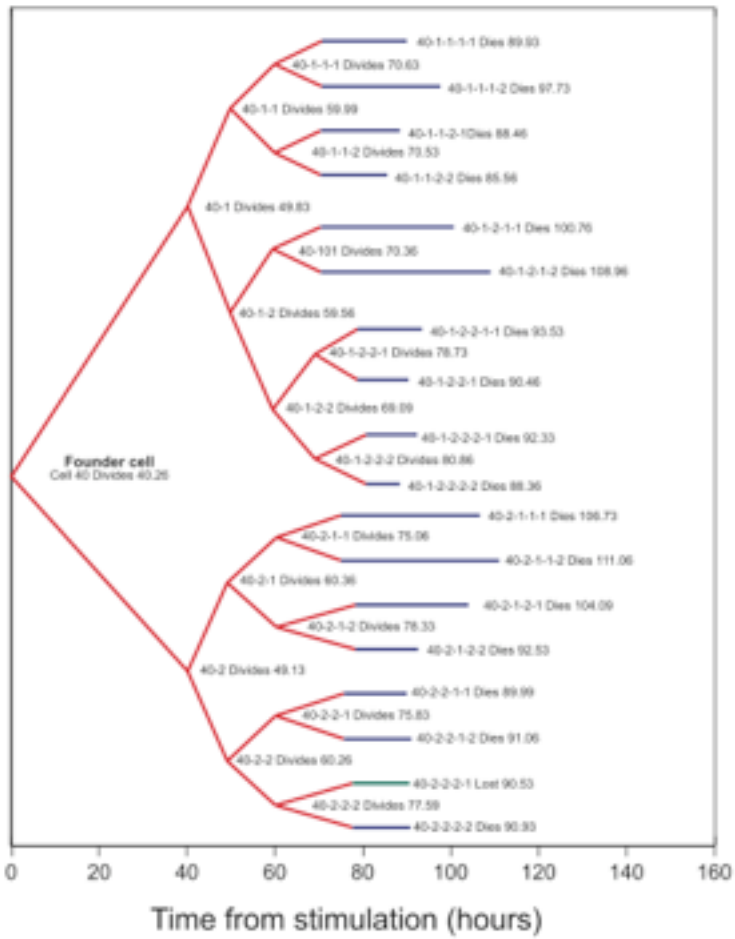
Growth, division, cessation and death



Founder effects on division 'destiny'



4 symmetric divisions



Key experimental observations -

1. Lognormal times to divide
2. Resetting of times after division - lack of inheritance
3- Division 'counting' can alter parameters
   eg. division progression

4 - Independent regulation of division and death

A model of immune regulation as a consequence of randomized lymphocyte division and death times

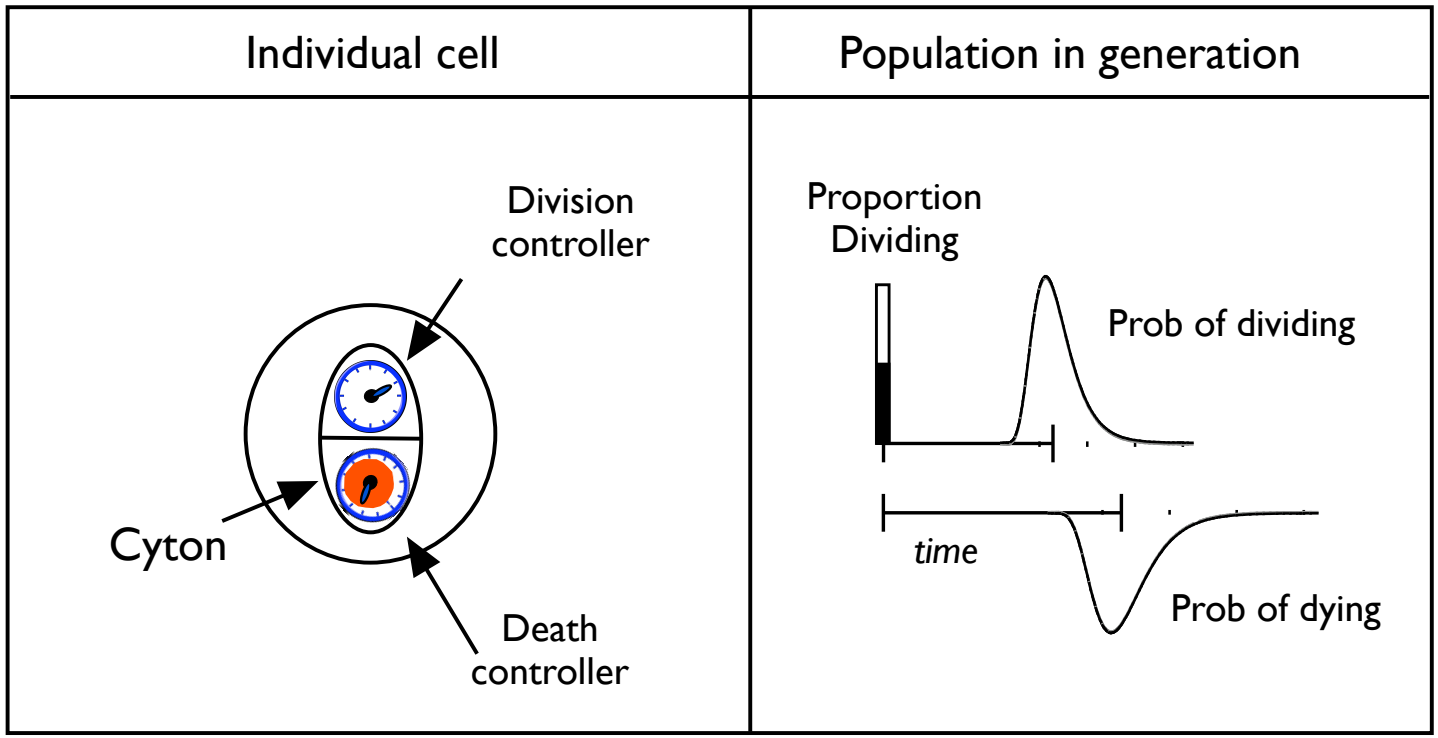
E. D. Hawkins*†, M. L. Turner*†, M. R. Dowling*‡, C. van Gend*, and P. D. Hodgkin*§

*Immunology Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia; †Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia; and ‡School of Physical Sciences, University of Queensland, Queensland 4072, Australia

Communicated by Gustav J. Nossal, University of Melbourne, Victoria, Australia, January 6, 2007 (received for review September 19, 2006)

The magnitude of an adaptive immune response is controlled by the interplay of lymphocyte quiescence, proliferation, and apoptosis. How lymphocytes integrate receptor-mediated signals influencing

When first examined, cell loss seemed to follow an exponential decay function consistent with a constant probability of dying over time (Fig. 1*A* and refs. 7, 9, and 15). However, we noted deviations



Use to measure genetic effects

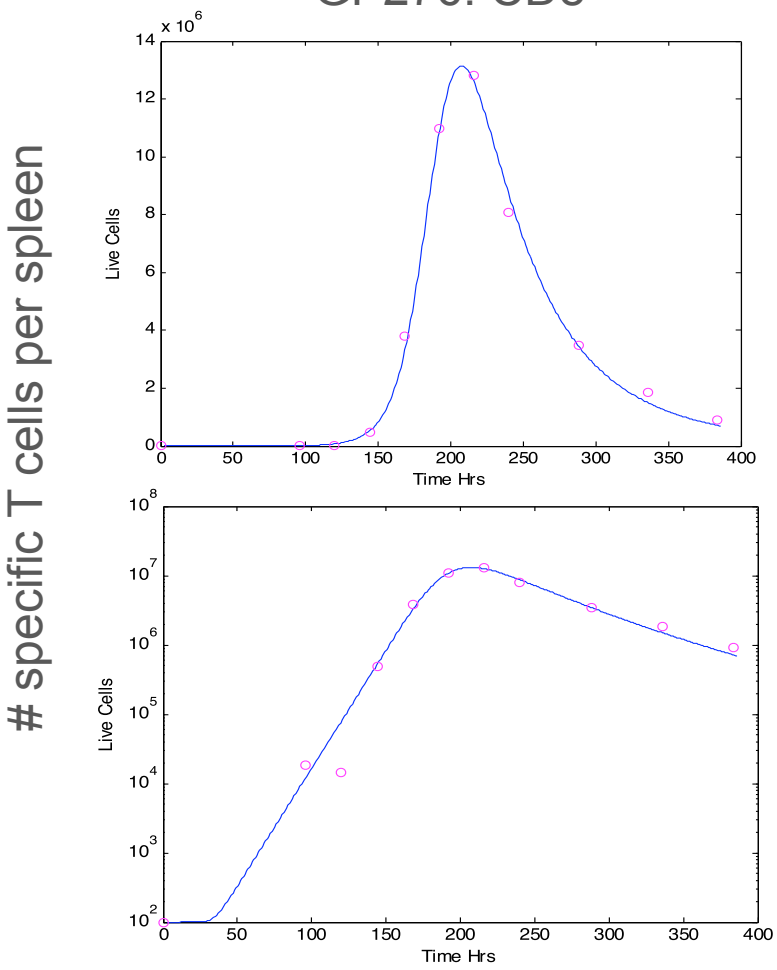
and ‘Calculation of signal integration’

Fits T and B cells - allows sensitive regulation
relies on the randomising features for control

The model can provide a quantitative description of *in vivo* cellular immune responses

Proliferation of LCMV-specific T cells post infection

GP276: CD8



Starting cell number
GP61: 100
GP276: 100

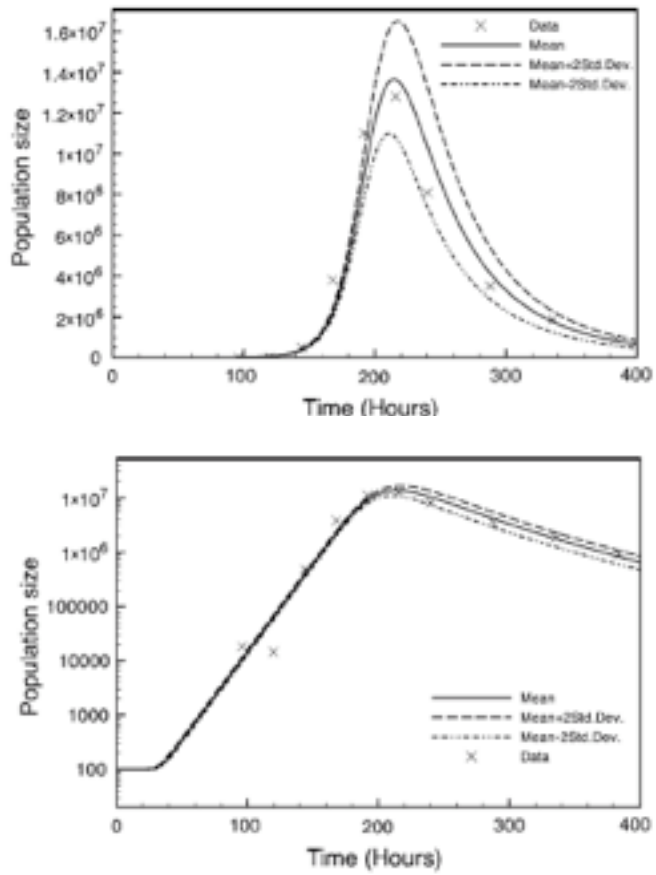
Subsequent division time
GP61: med = 9.9 h, s = 0.09
GP276: med = 9.4 h, s = 0.06

Division destiny
GP61: med = 19.3, s = 0.9
GP 276: med = 17.5, s = 1.1

Subsequent division death rat
GP61: med = 46.1 h, s = 1.3
GP276: med = 56.7 h, = 0.8

Branch process -

V. Subramanian, K Duffy



Data provided by Dirk Homann, (Homann et al., Nature Medicine (2003))

Cyton Model

Exploits evidence for heritable stochastic processes governing division and death times

Individual cells exhibit extreme heterogeneity

Population highly predictable

External signals alter parameters of stochastic process

Differentiation

How do division, death, differentiation all interleave?

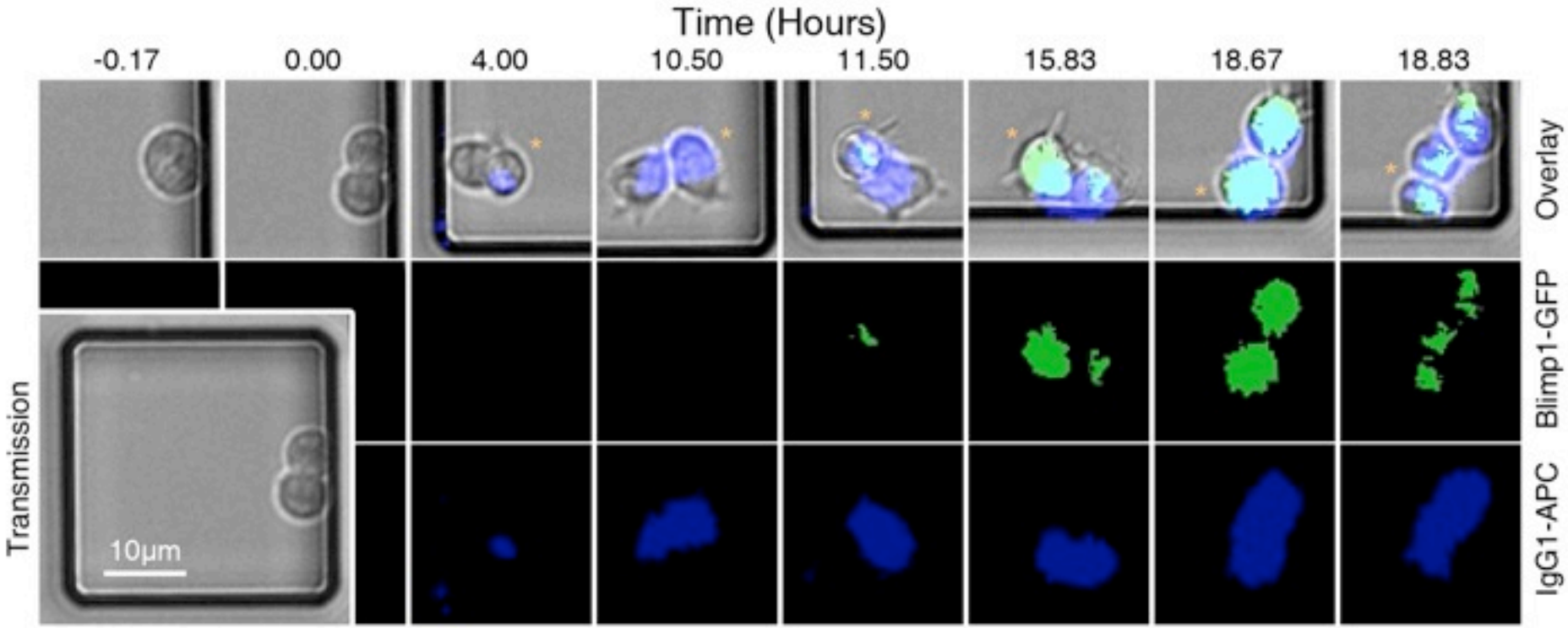
B cells - Switch antibody type, develop to ASC

Can we extend internal competition in cells to other fates?

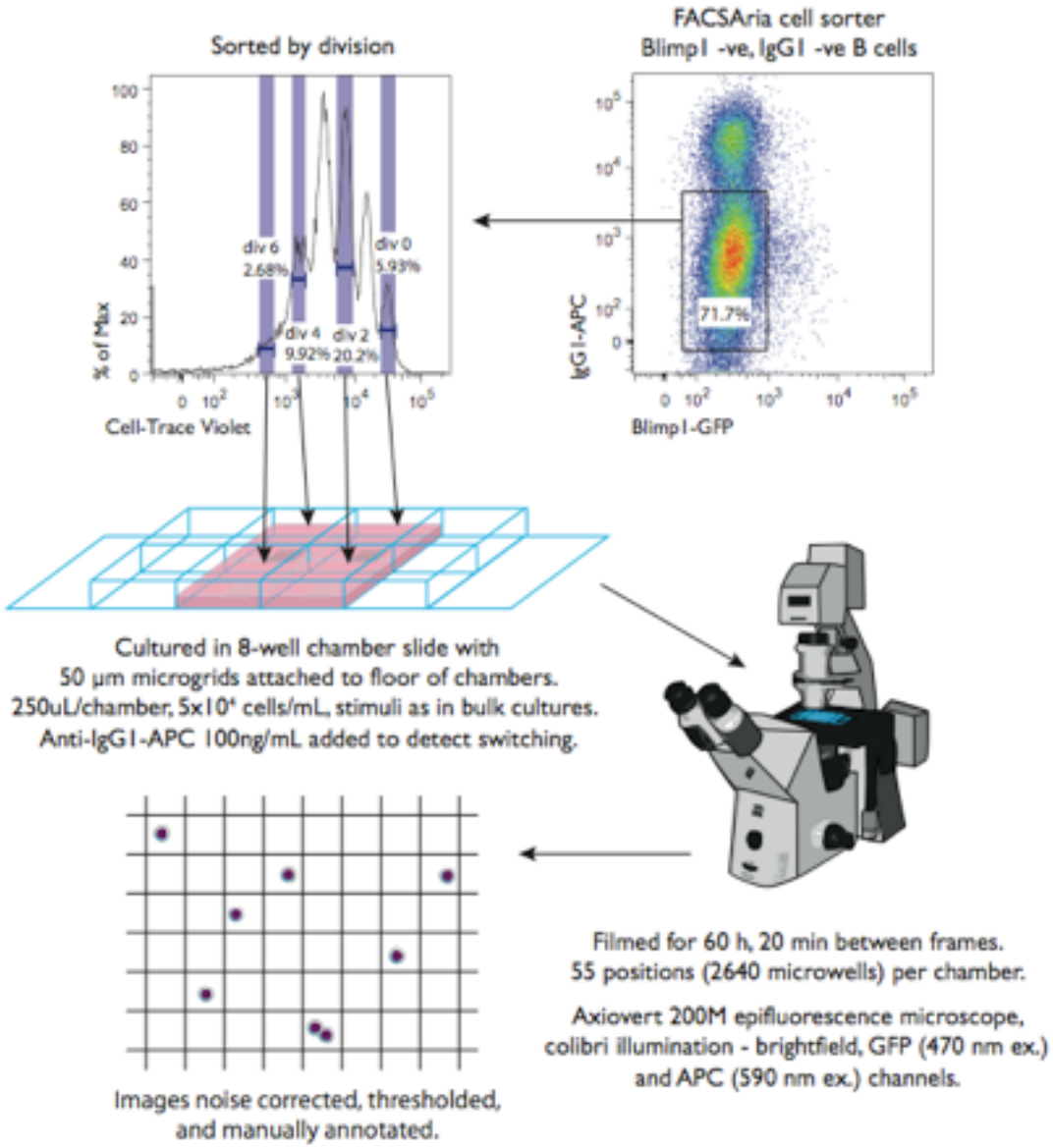
Microscopy allows the study of individual cell fates over time

(Hawkins, E.D *et al.*, *PNAS* 2009;
Duffy, K.R. *et al.*, *Science* 2012)

- Blimp-1-GFP reporter
- IgG1-APC stain



Generation filming

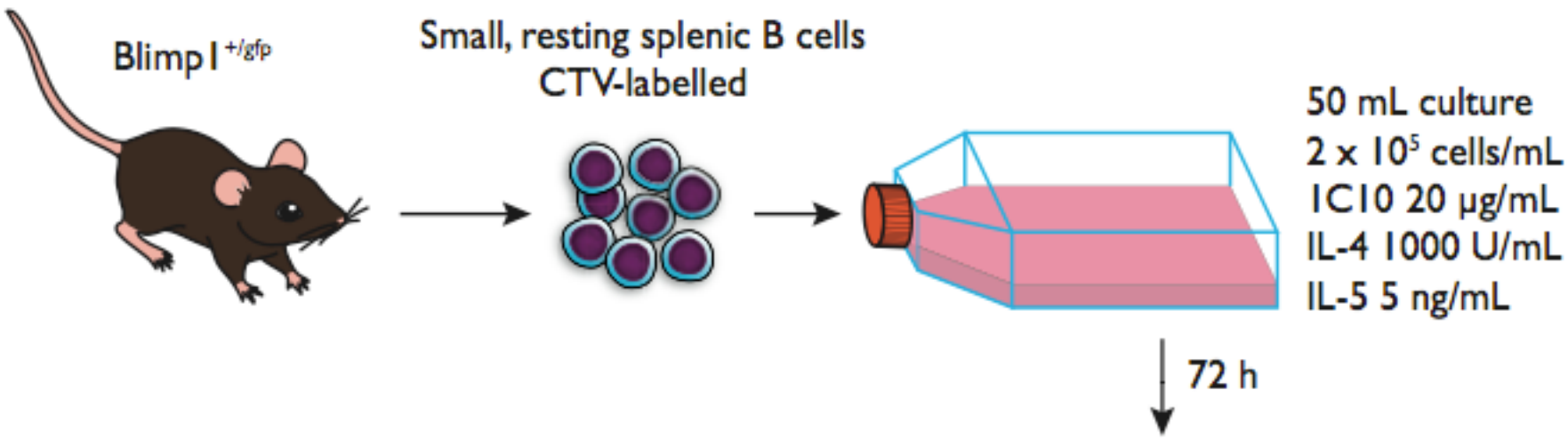


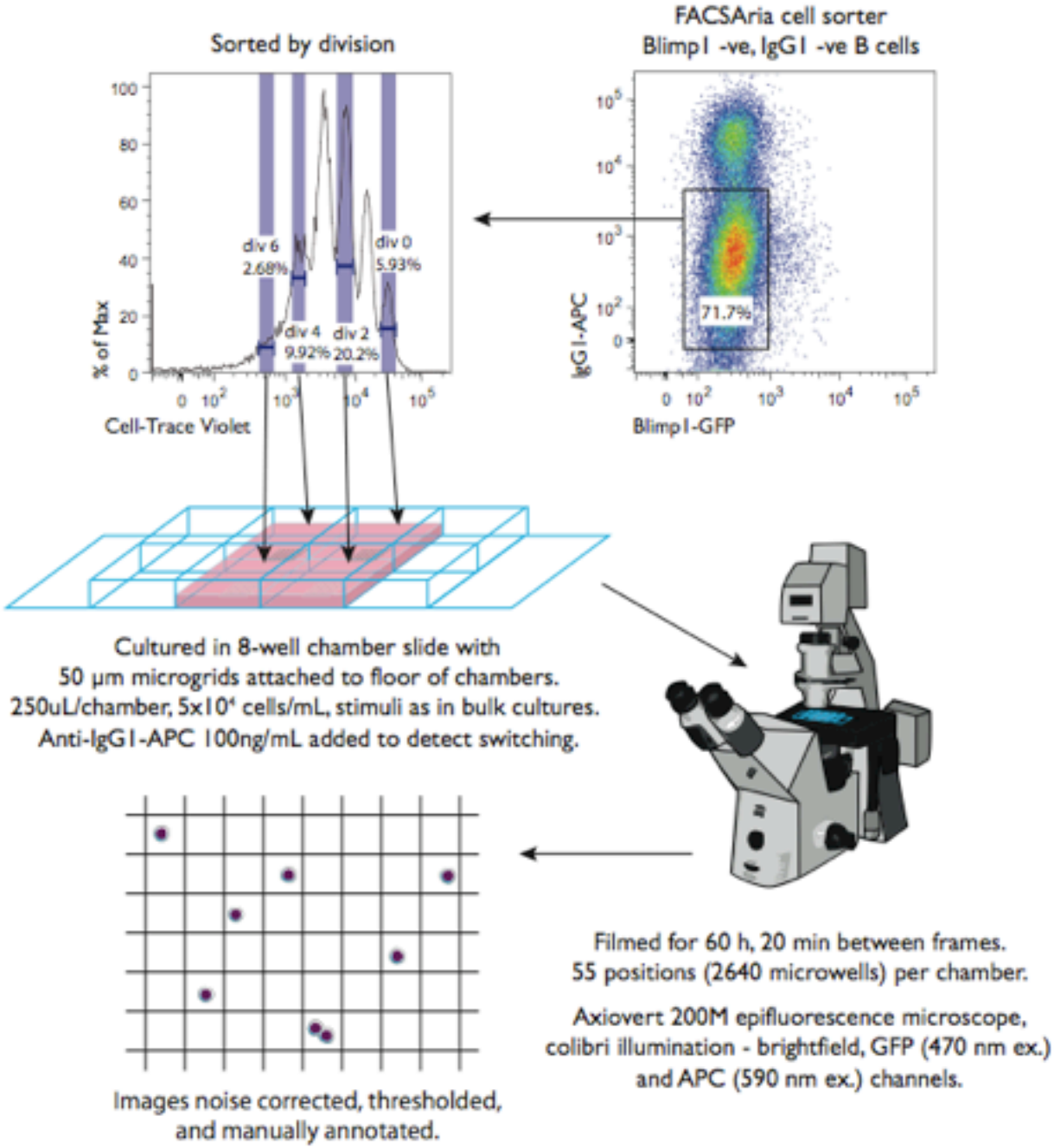
Experiment - Observe 4 fates operating simultaneously Test for competition

Mark Dowling, John Markham, Hasbold,
Ross Holmberg, Jie Zhou, Cam Wellard, Ken Duffy

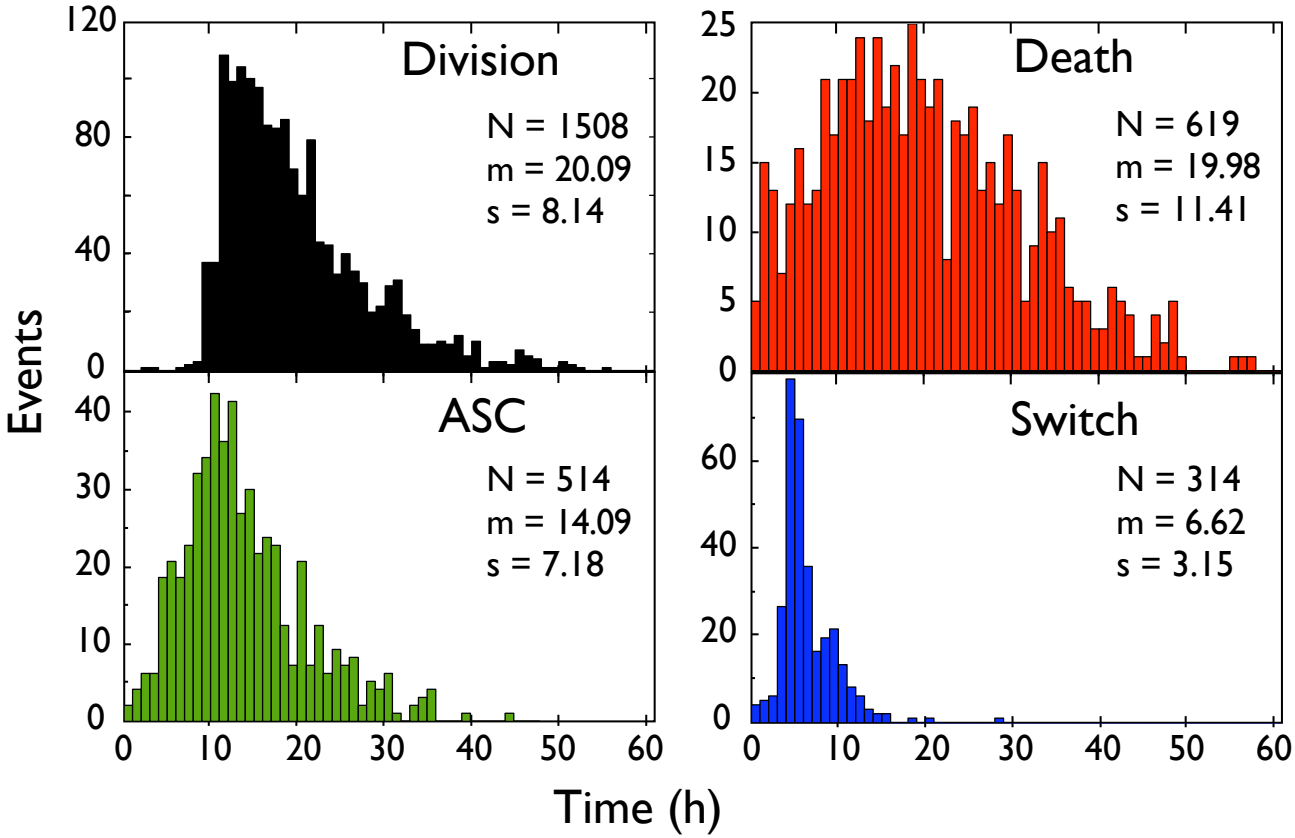
Conditions that induce division, death, switch to IgG1 and development of ASC

Examine for statistical hallmarks of competition*




FACSAria cell sorter
Blimp1 -ve, IgG1 -ve B cells
Sorted by division
% of Max
Cell-Trace Violet
div 6
2.68%
div 4
9.92%
div 2
20.2%
div 0
5.93%
IgG1-APC
Blimp1-GFP
71.7%
Cultured in 8-well chamber slide with
50 µm microgrids attached to floor of chambers.
250uL/chamber, 5x10⁴ cells/mL, stimuli as in bulk cultures.
Anti-IgG1-APC 100ng/mL added to detect switching.
Filmed for 60 h, 20 min between frames.
55 positions (2640 microwells) per chamber.
Axiovert 200M epifluorescence microscope,
colibri illumination - brightfield, GFP (470 nm ex.)
and APC (590 nm ex.) channels.
Images noise corrected, thresholded,
and manually annotated.

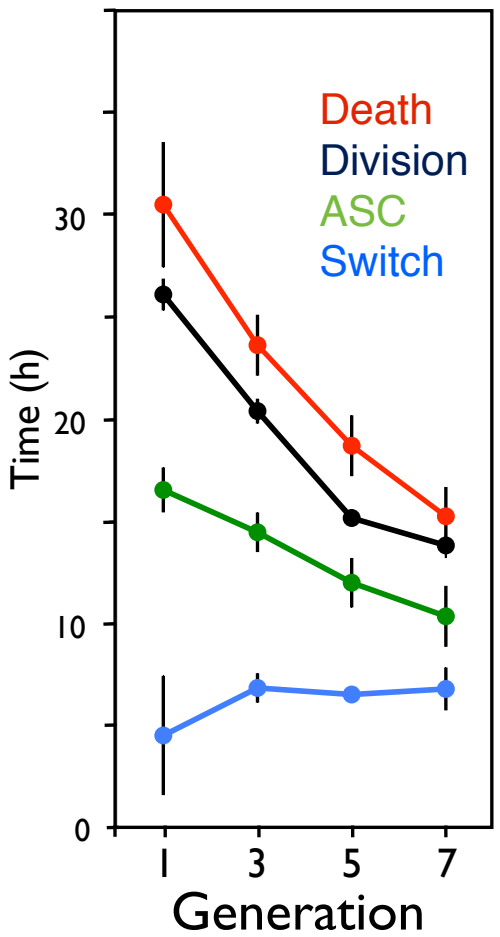
All Divisions - summary



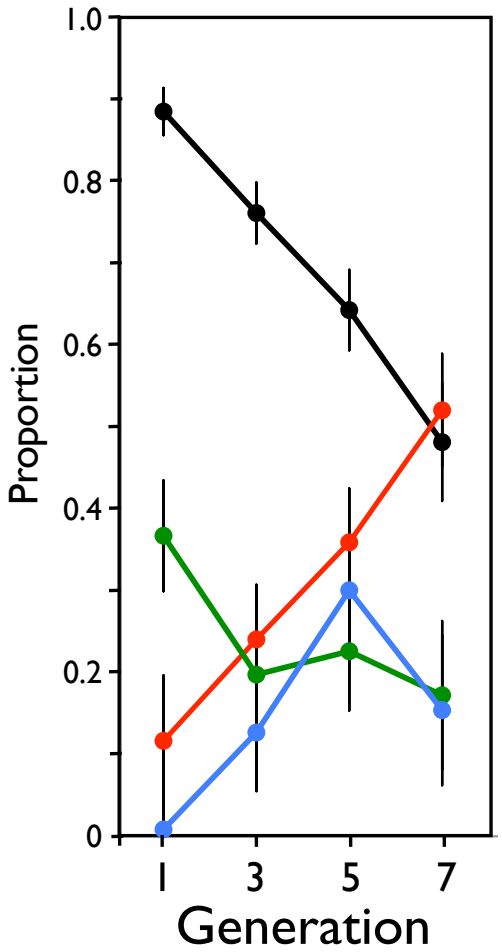
Times - long tailed distributions

Different means/variances/frequencies

Average time

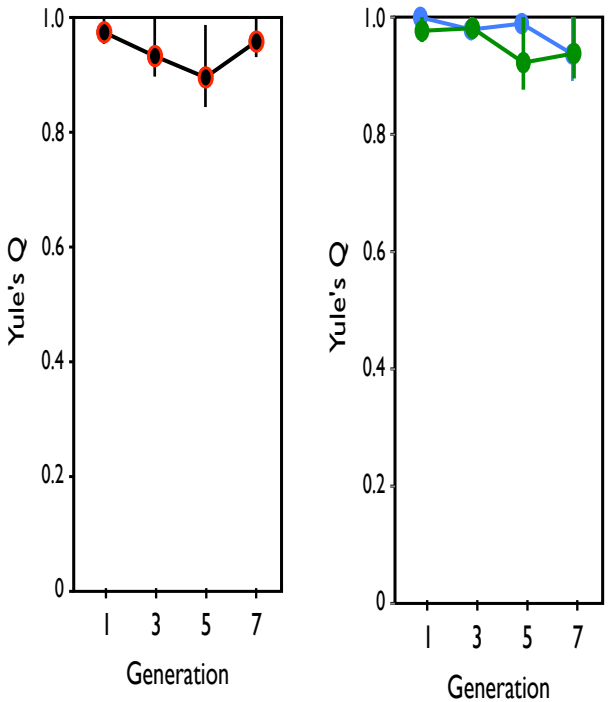


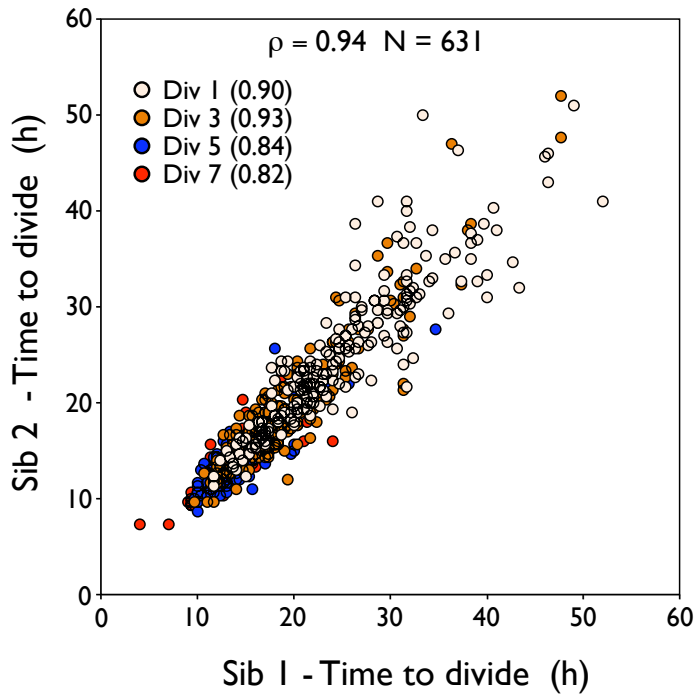
Frequency

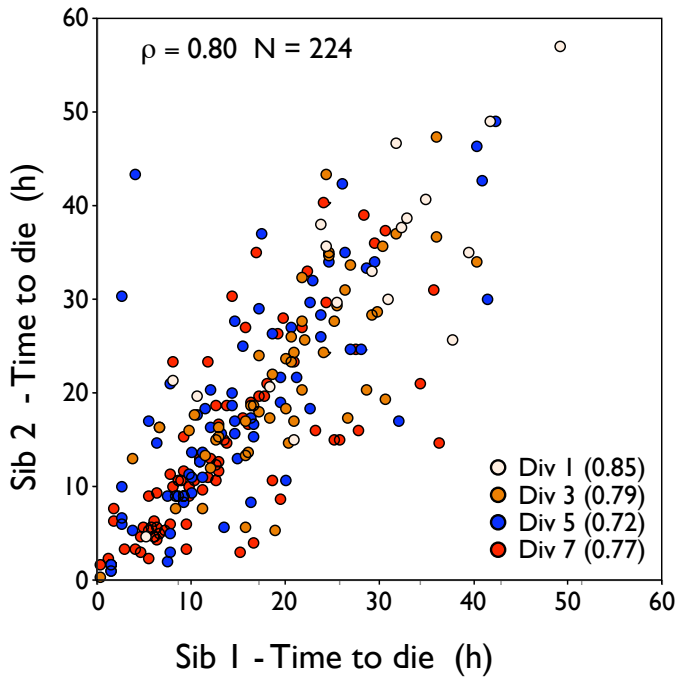


Sibling correlations

Intercellular concordant fates

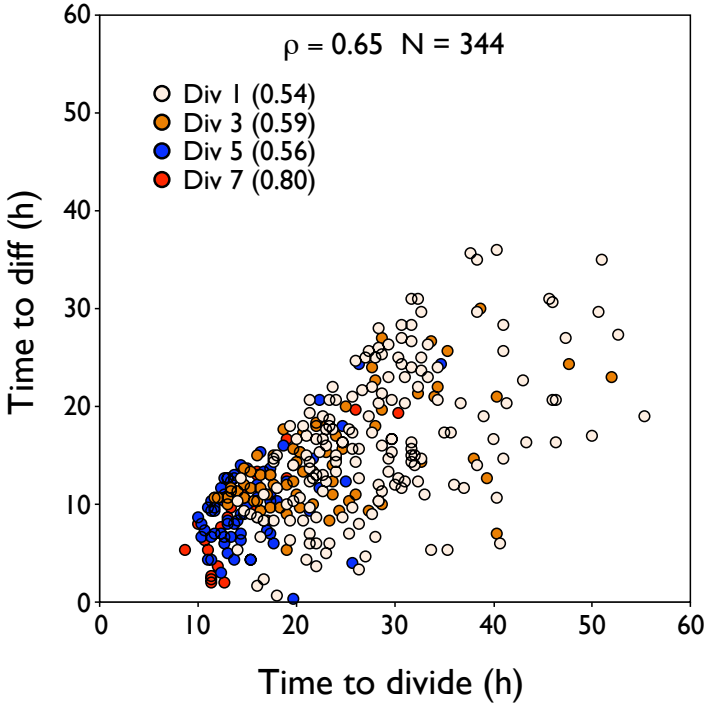


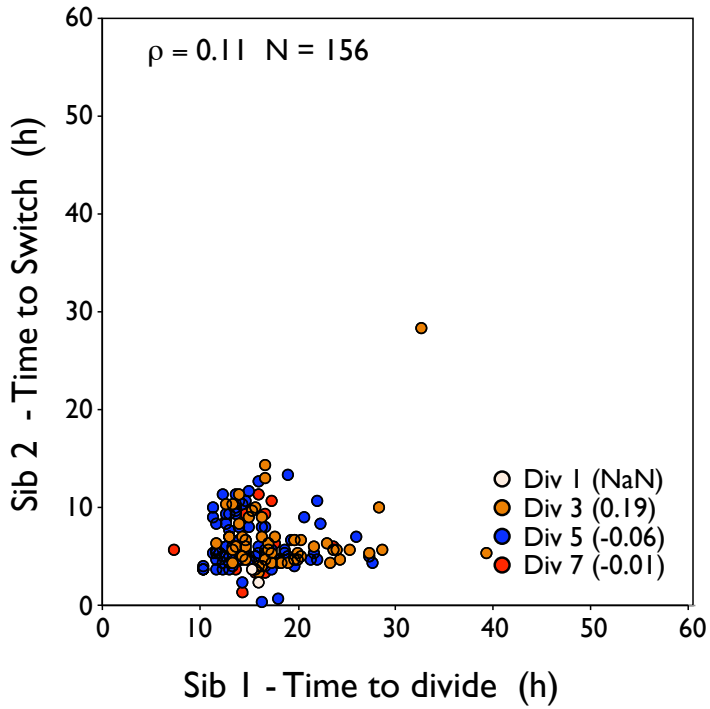


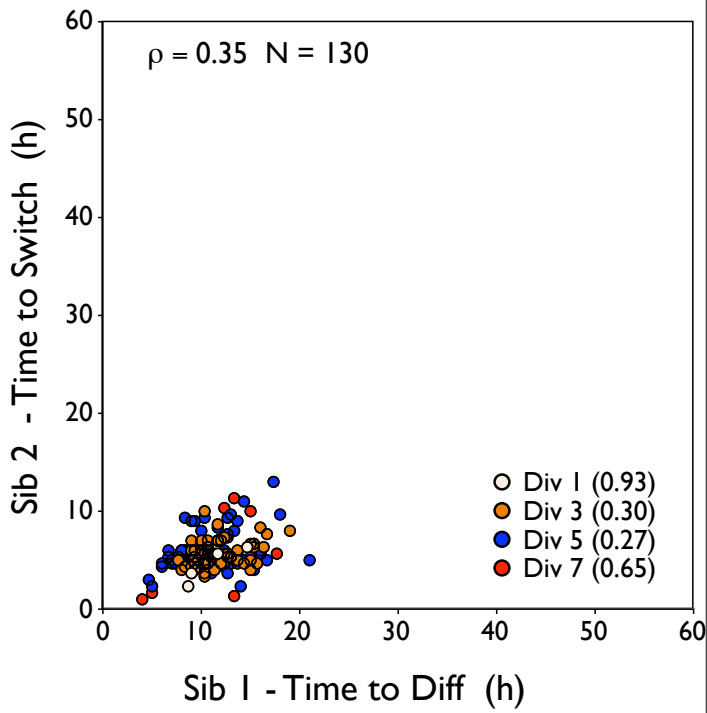


Intracellular correlations

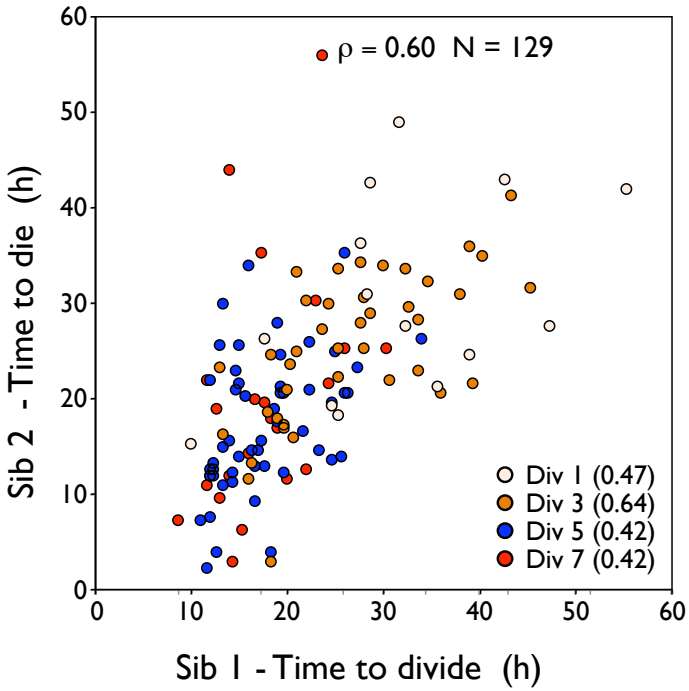
Intracellular fates

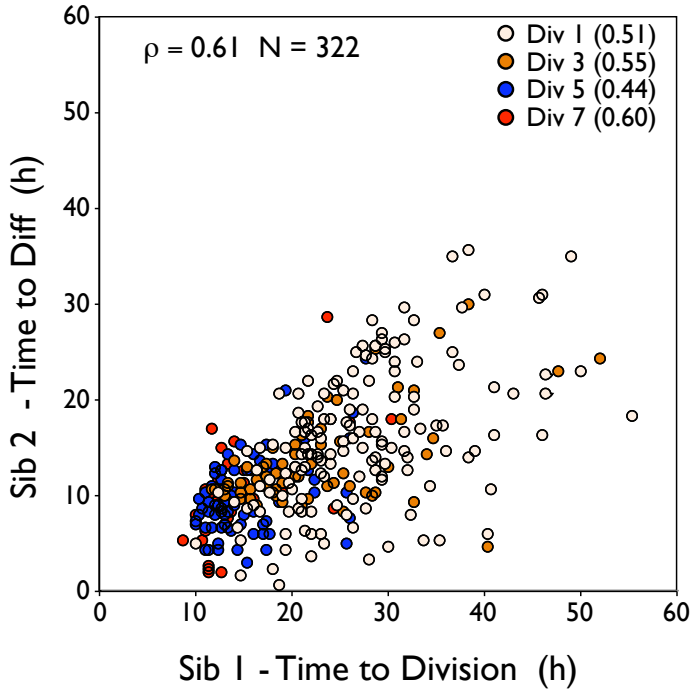


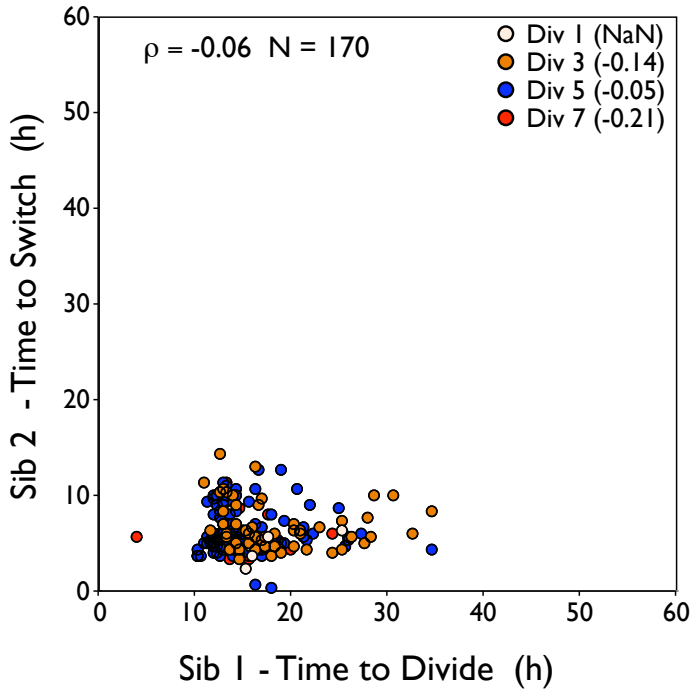




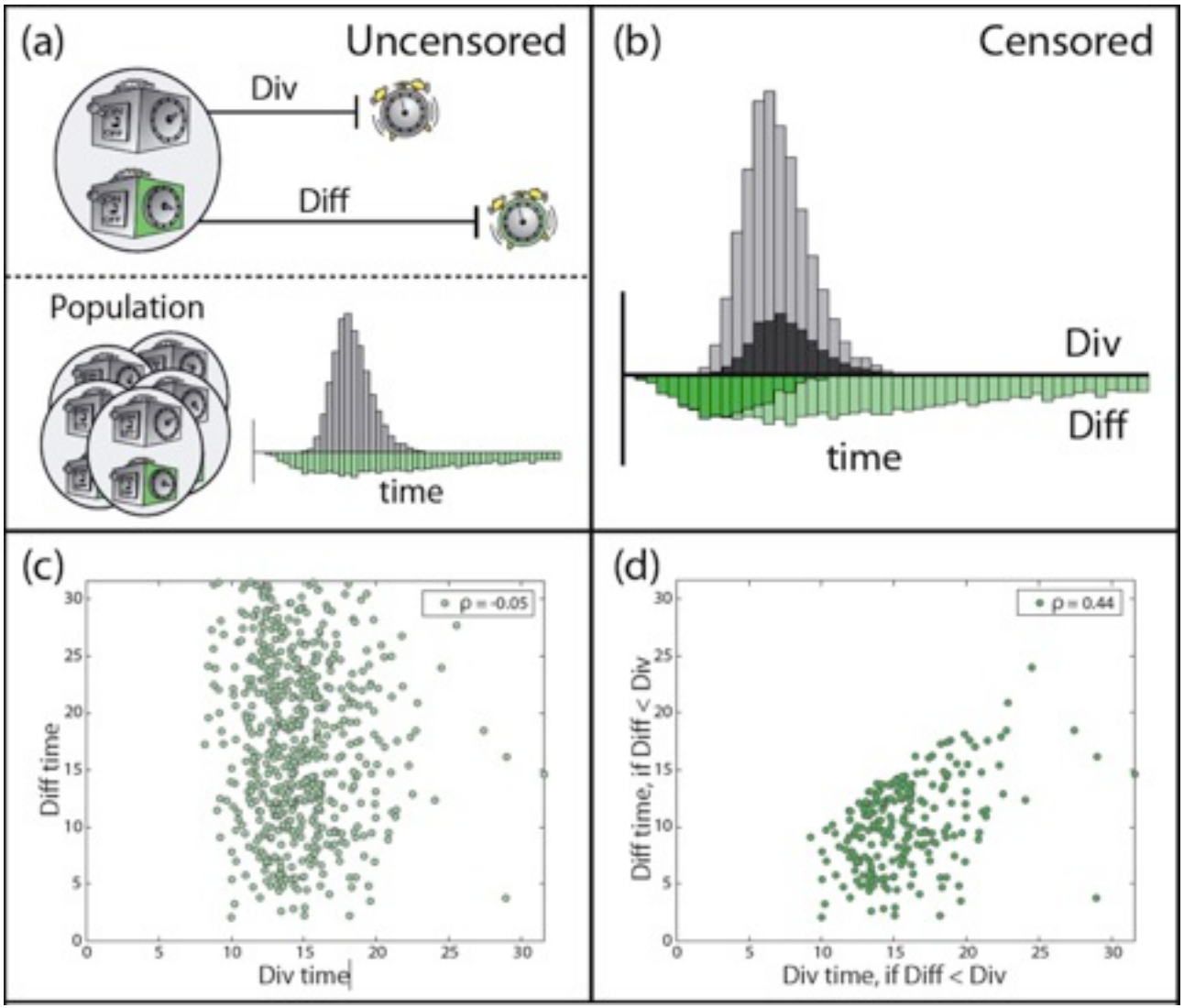
Intercellular correlations
non-concordants





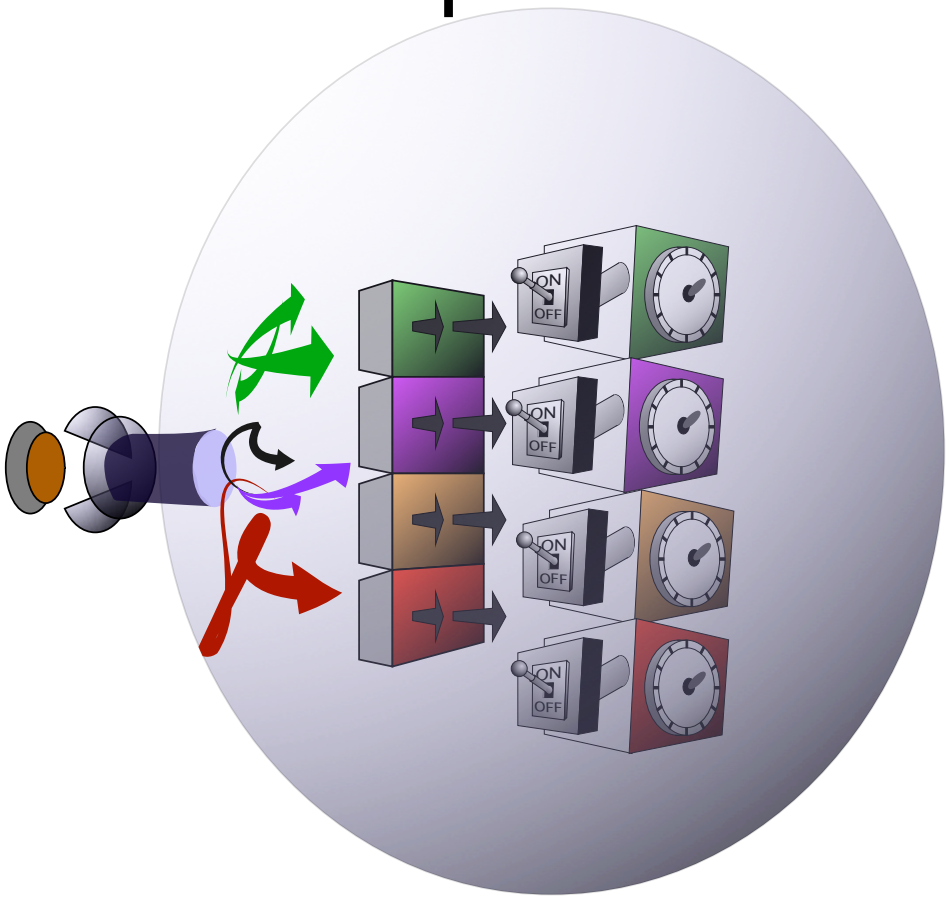


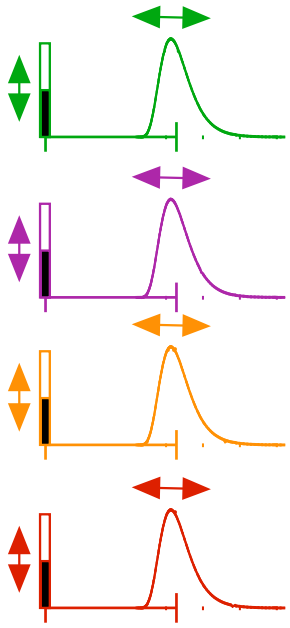
Autonomous Intracellular Competition: Hallmarks of censorship



Duffy and Hodgkin, 2012 Trends in Cell Biology

Four way Cyton - Autonomous Intracellular Competition - Rules of censorship





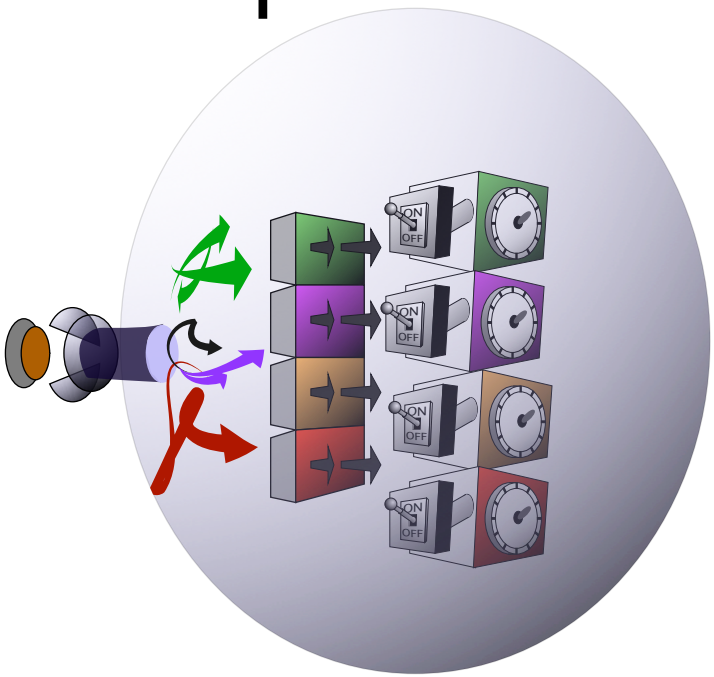
Division (restart)

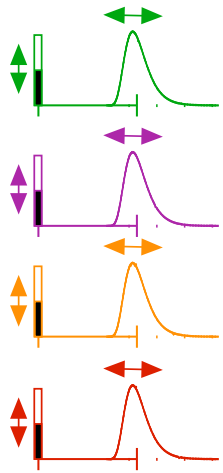
Death (always wins)

Switch (no affect)

ASC (stops switch)

Autonomous Intracellular Competition - Model parameters





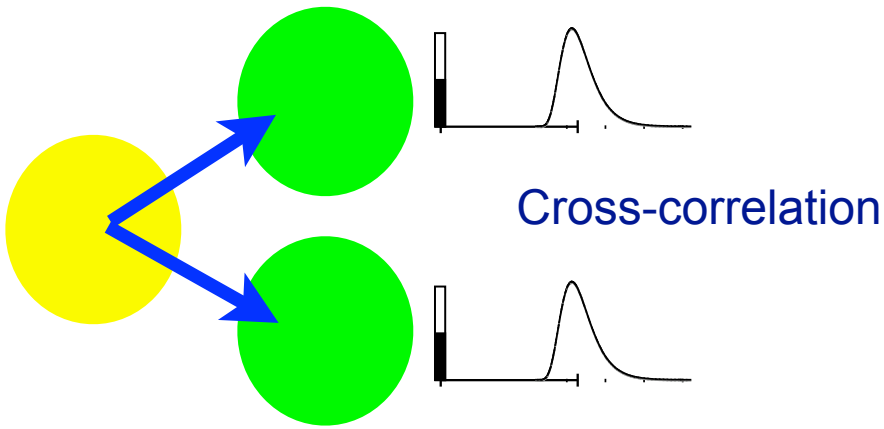
Division (freq, m, s, correlation)

Death (m, s, correlation)

Switch (freq, m, s, correlation)

ASC (freq, m, s, correlation)

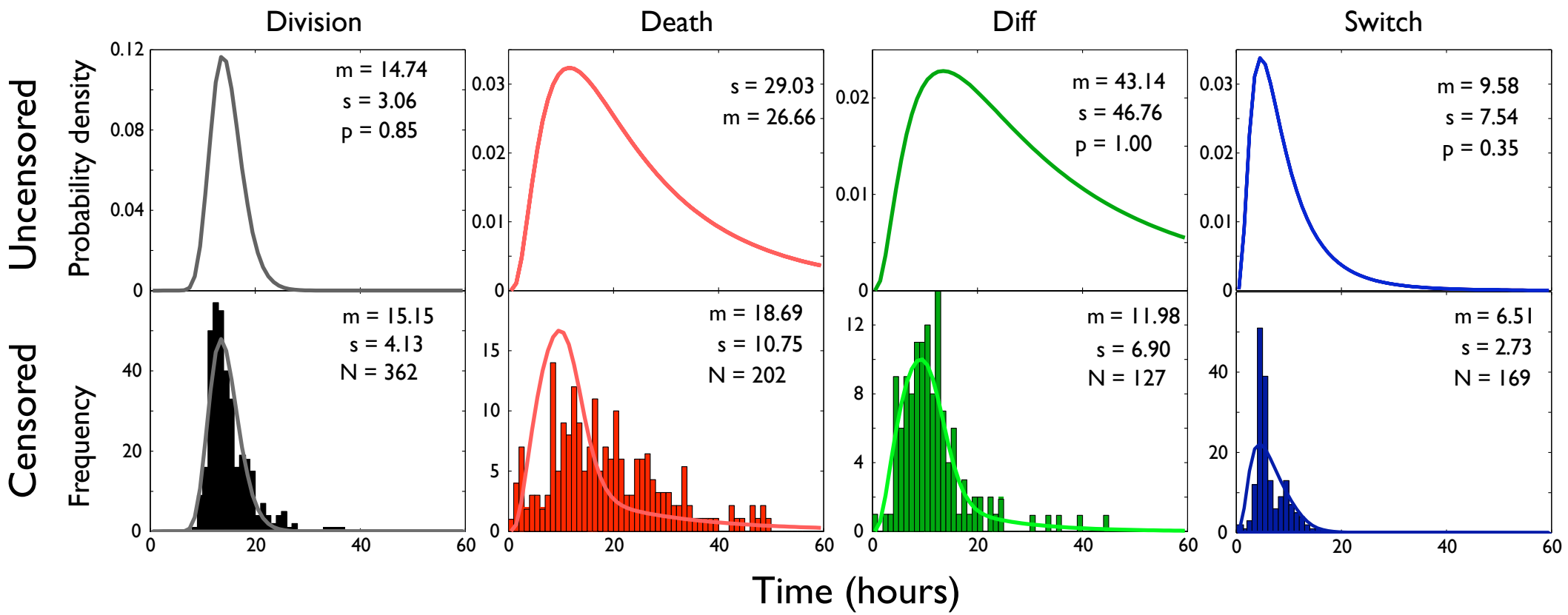
= 15 - parameters!



Cross-correlation

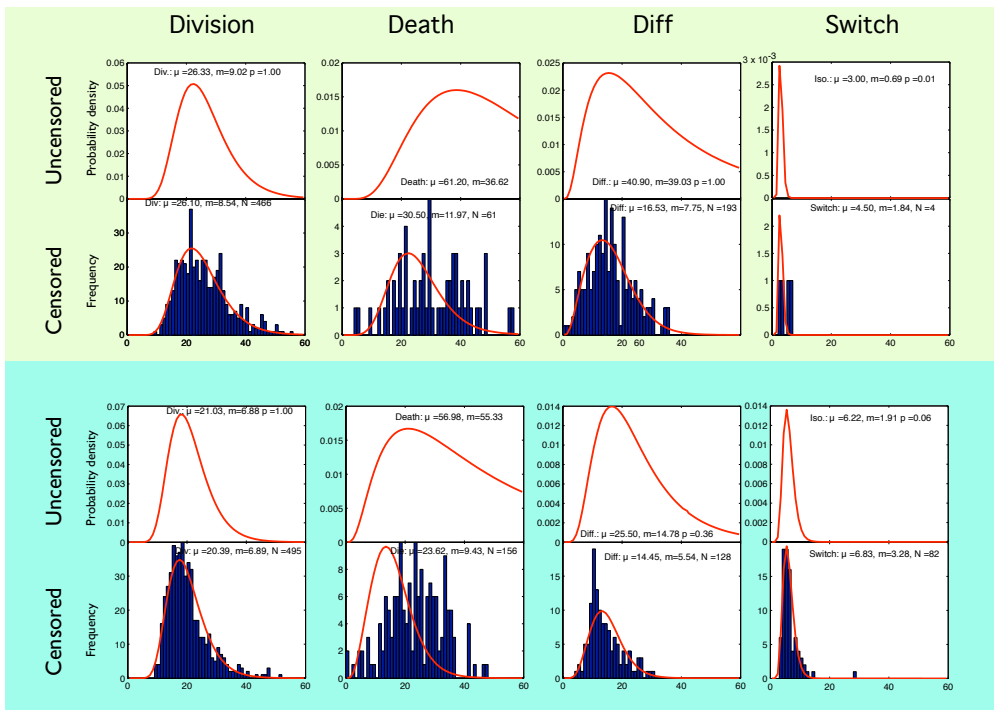
Statistical model (Duffy, Wellard)

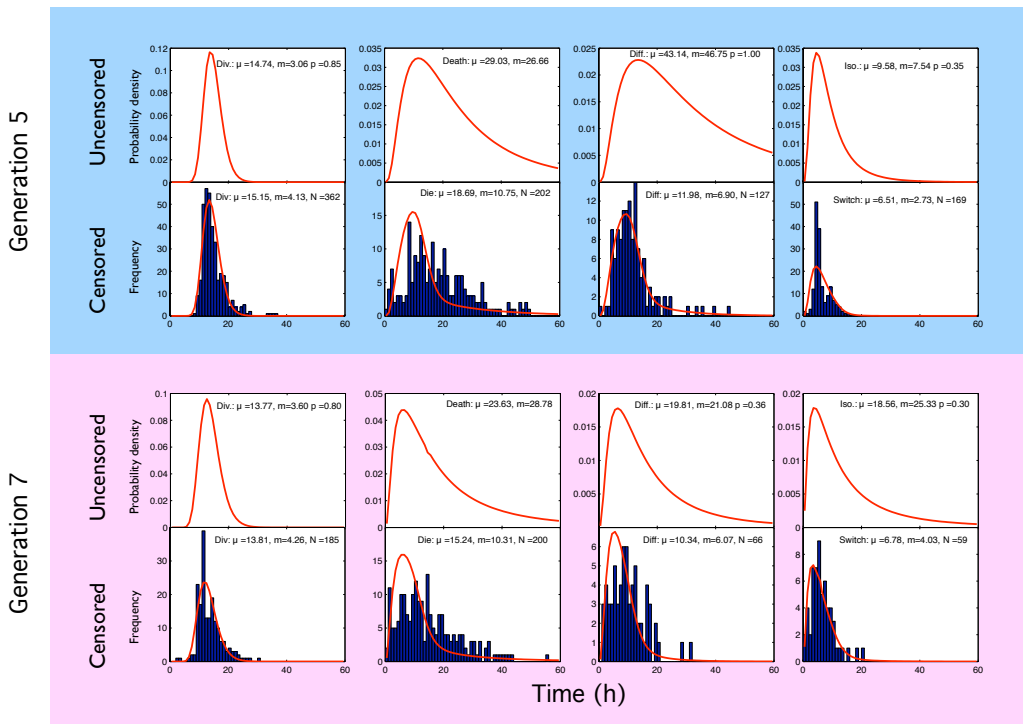
lognormal time to each event (m, s, f)
Independent with Censorship (death, div)

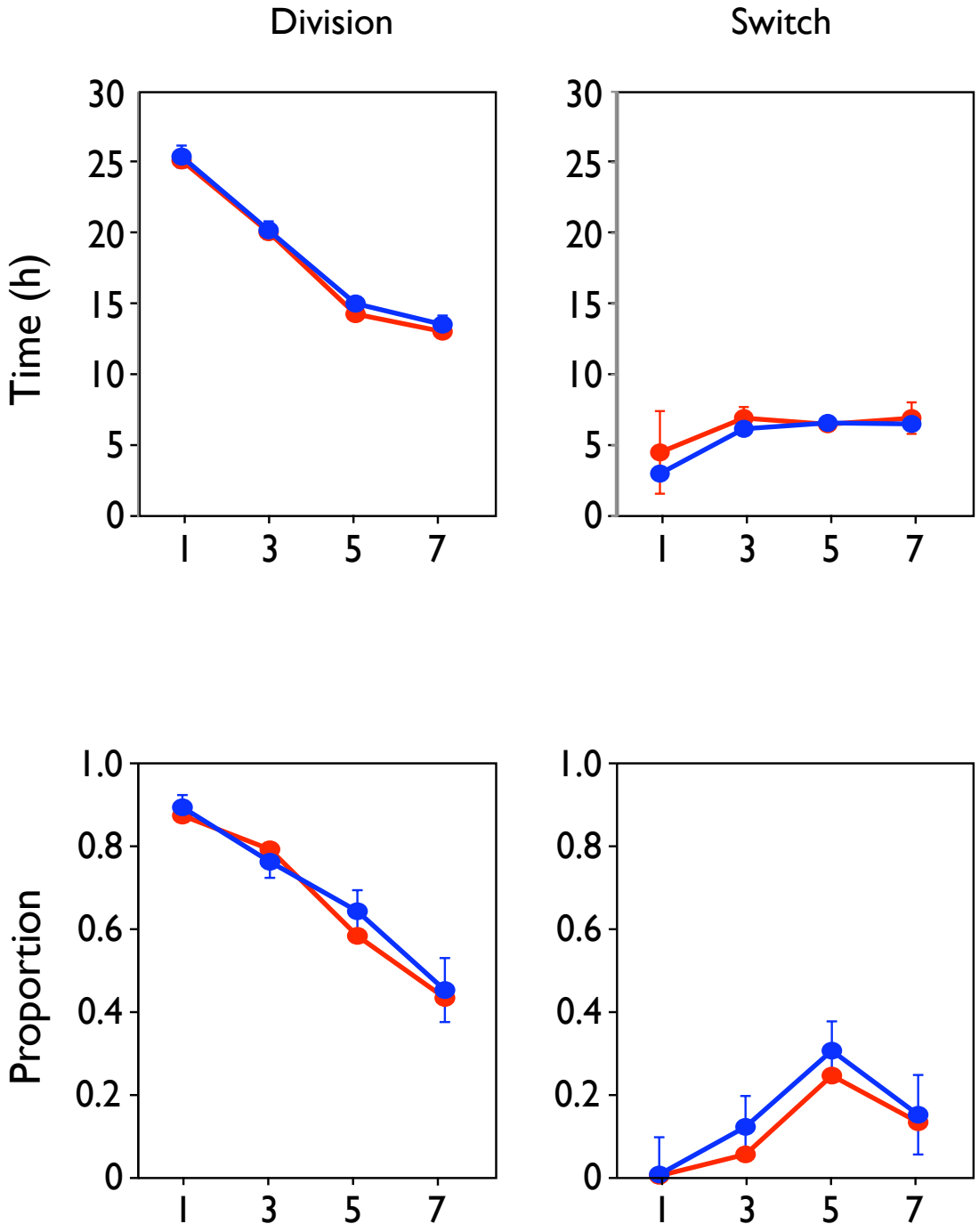


Generation 5

All generations







Fitted features

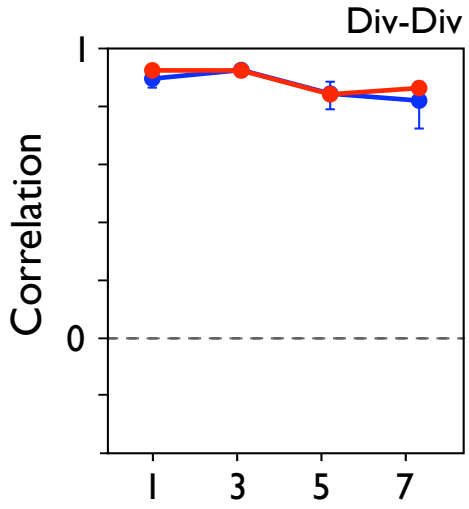
Time/Division

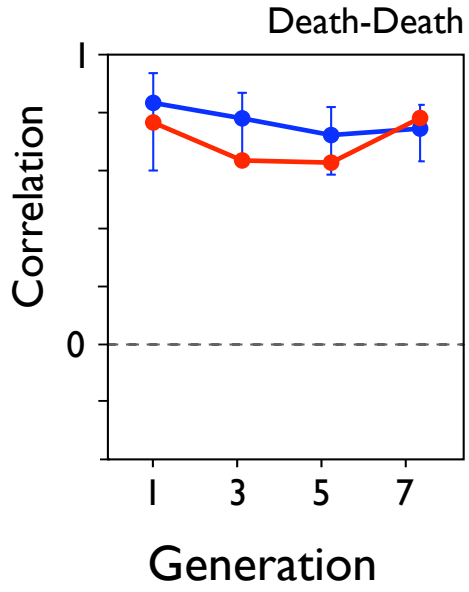
and

Proportion/division

Blue - measured
Red - model

Intercellular concordant





Fitted features

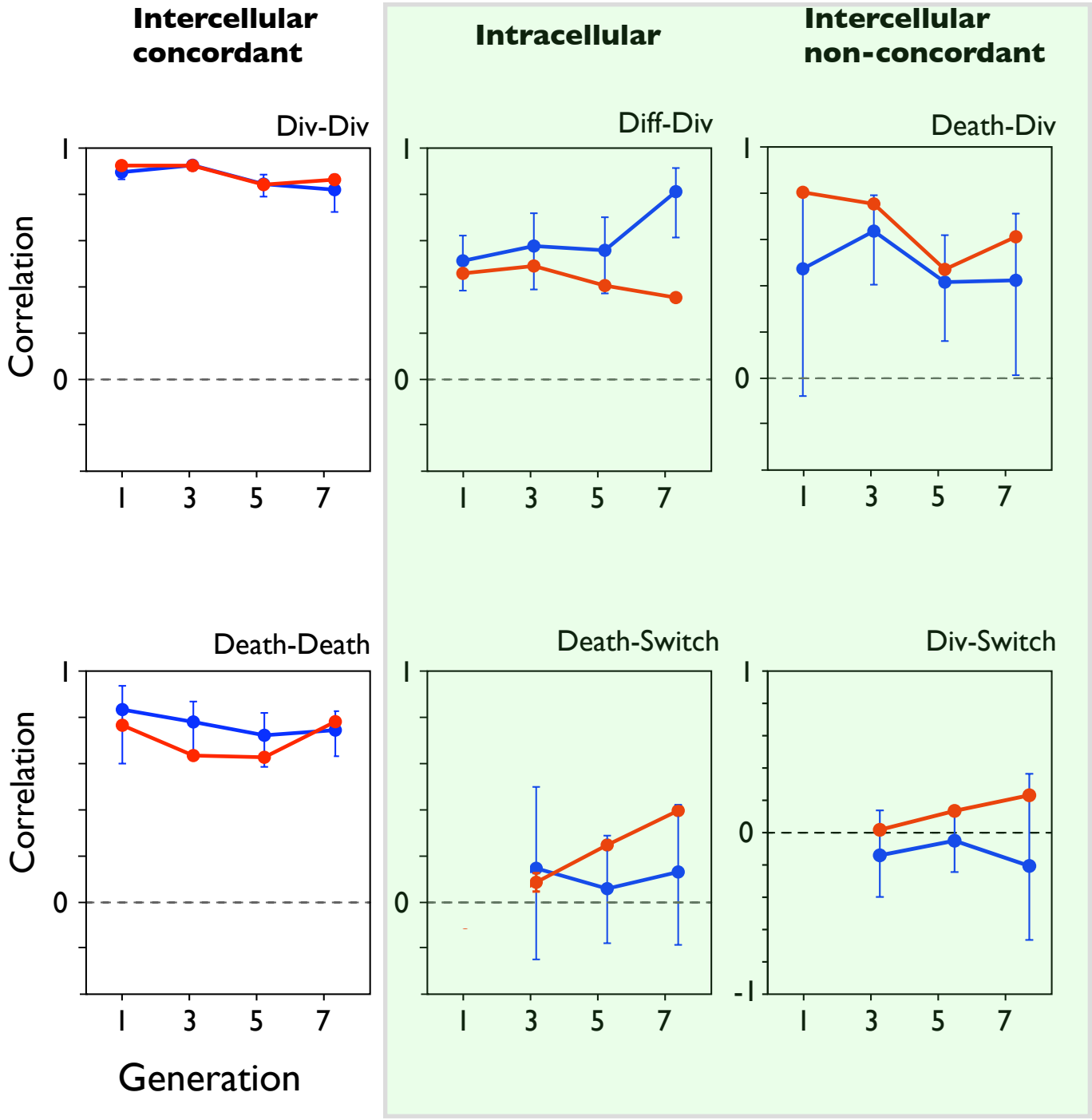
Inter-cellular Correlations

Blue - measured
Red - model

Passed test of getting back what you put in..

But can it explain the unexpected correlations?

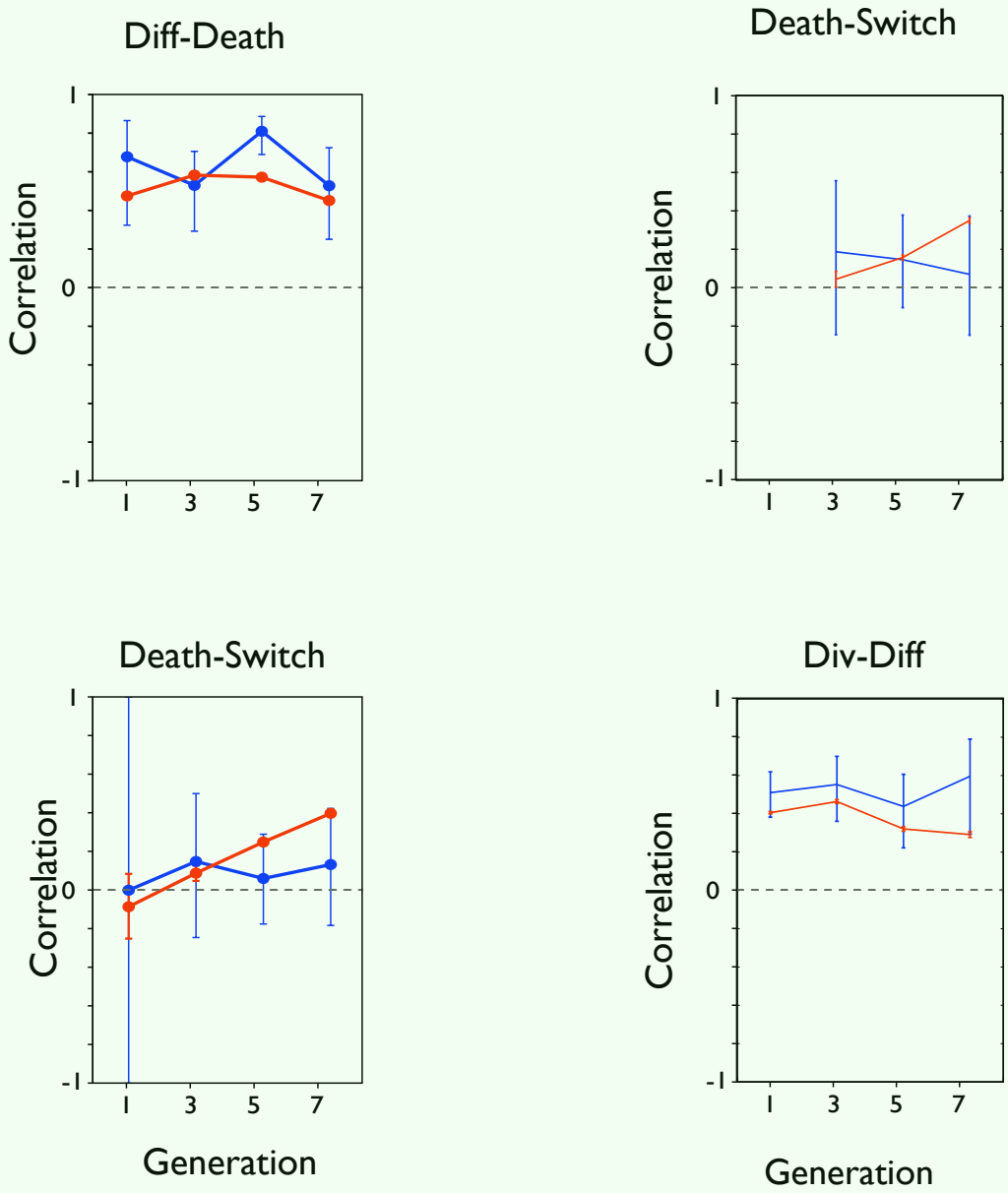
Intra cellular correlations
intercellular correlations of different fates of sibs



Blue - measured
Red - model

Intracellular

Intercellular non-concordant



Blue - measured
Red - model

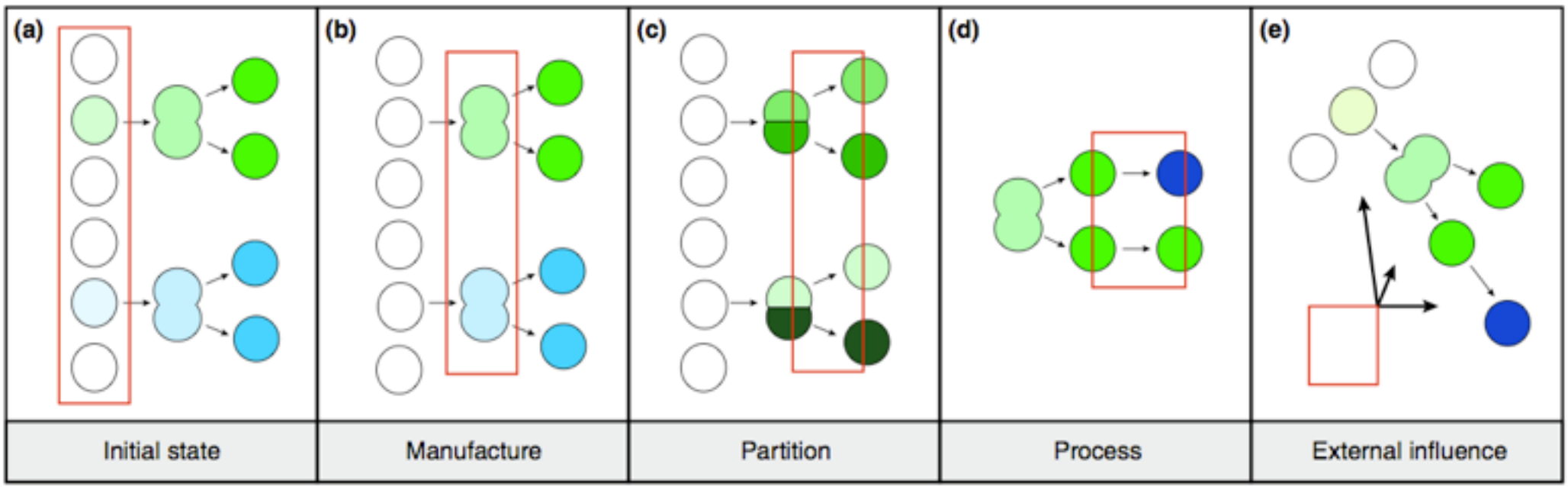
- Hallmarks of competition for fates apparent
- Asymmetric fates observed but conform to statistical likelihood

Activation-Induced B Cell Fates Are Selected by Intracellular Stochastic Competition

Ken R. Duffy,[1] Cameron J. Wellard,[2,3] John F. Markham,[4] Jie H. S. Zhou,[2,3] Ross Holmberg,[2] Edwin D. Hawkins,[5] Jhagvaral Hasbold,[2,3] Mark R. Dowling,[2,3]* Philip D. Hodgkin[2,3]*†

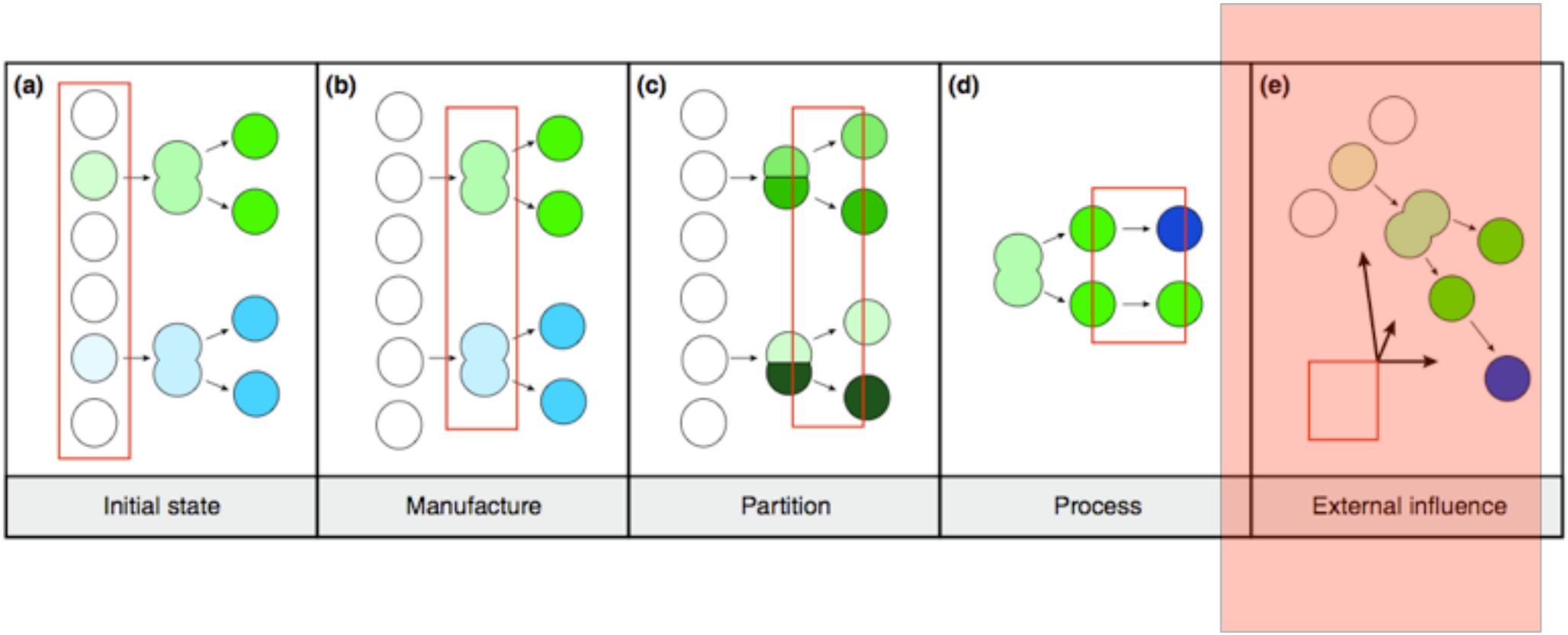
20 JANUARY 2012 VOL 335 SCIENCE

Homing in on the source of cellular heterogeneity



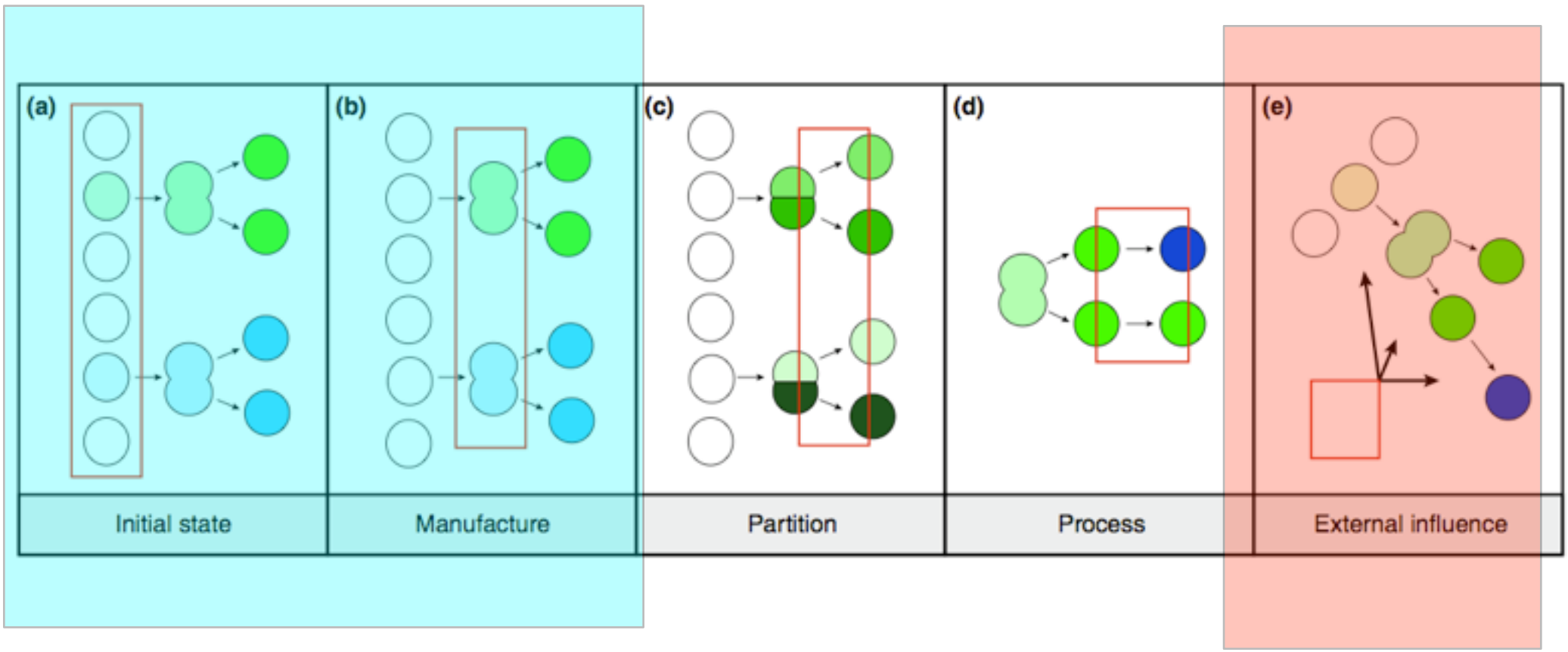
Duffy and Hodgkin, 2012 Trends in Cell Biology

Homing in on the source of cellular heterogeneity



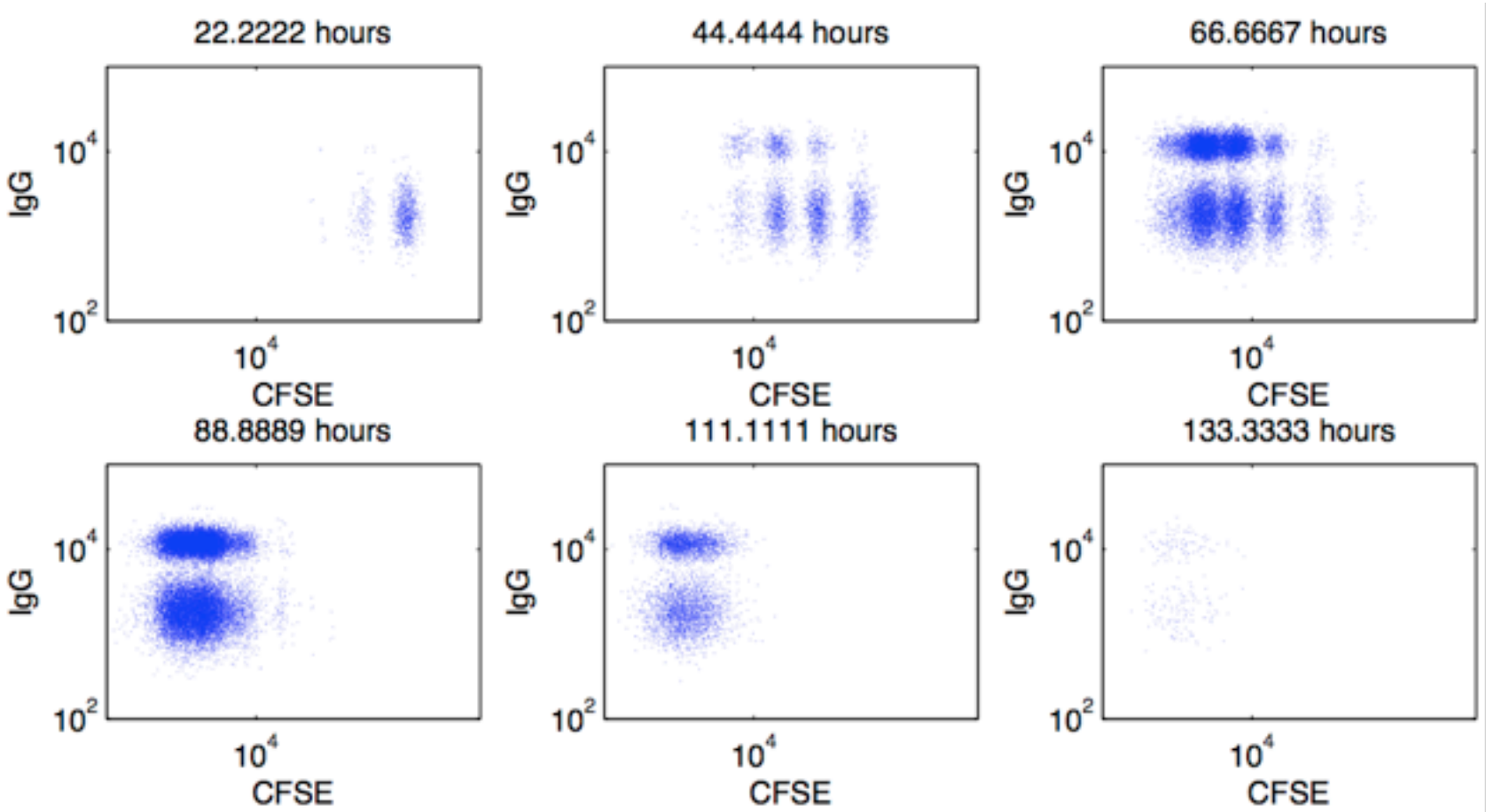
Duffy and Hodgkin, 2012 Trends in Cell Biology

Homing in on the source of cellular heterogeneity



Duffy and Hodgkin, 2012 Trends in Cell Biology

Computer flow cytometry!

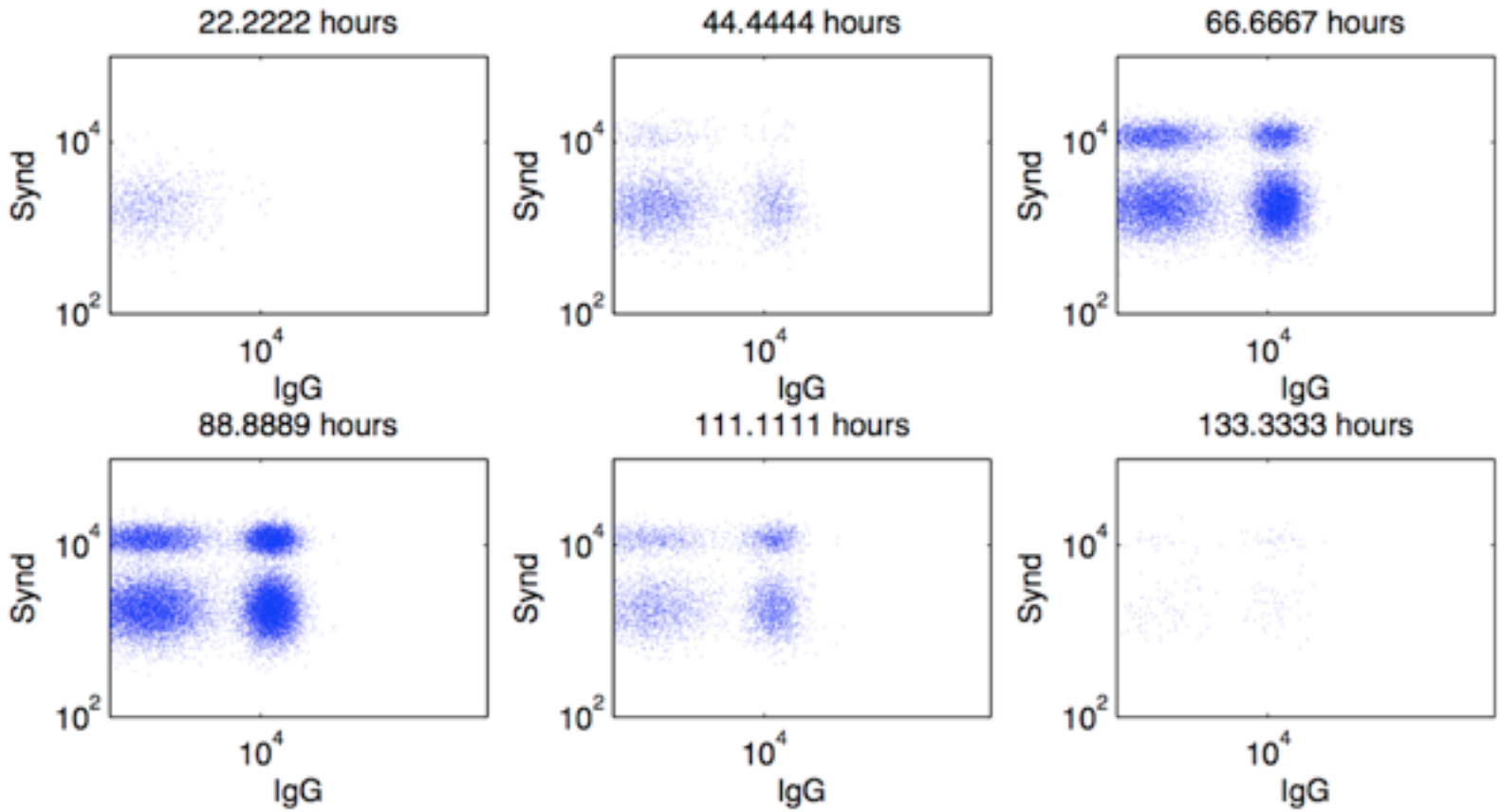


Run simulator for thousands of cells

Record features at every time for all cells

'Plot as if real cells'

Computer flow cytometry!



Run simulator for thousands of cells

Record features at every time for all cells

'Plot as if real cells'

Conclusions

Complex features of lymphocyte control result from a form of modulated 'randomness' of times to different fates set in competition in each cell

By manipulating the frequencies and times to change, by signals and cell division, a robust system for allocating different cells to large number of different fates is created

Combinatorially for example - just 20 independent surface marker 'machines' - gives one million possible 'phenotypes'

Thanks to..

B cell proliferation/ diffn/death

*Mark Dowling
*Has Hasbold
*Edwin Hawkins
*Jie Zhou
Marian Turner
Nadine Taubenheim

Differentiation simulator
Image analysis/model development

*Cam Wellard

*Ken Duffy [Hamilton - Maynooth]
Vijay Subramanian [Hamilton - Maynooth]

Systems
B cell Program WEHI

Dave Tarlinton
Lynn Corcoran
*Steve Nutt

Axel Kallies

Imaging & microwells

*John Markham [NICTA]
*Mark Dowling

*Ed Hawkins
Liam McGuinness
Ross Holmberg
**Jie Zhou

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N H M R C

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