

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

Phil Hodgkin

KITP Santa Barbera, 2012



Adaptive immunity: Complexity at multiple scales

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

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

P. M. DORNEY*

There are large entremt theoretical intergogains of millidoly production which, following "almange (1987) may be referred to an the direct template theory in which the antigen acres in a template means which the predicpattern of the antihody is protocolar, a million template theory which postators a scendary template incorporated into the genetic-systemize processes of the antihody post-for-systemize processes of the antihody genetic-systemize processes of the antihody genetic-systemize processes of the antihody genetic-systemize processes of the antihody section theory in which the antipon acts section they relevant production, of mitmal antibody molecules of corresponding type (Jeren, Biol).

The two Inffer theories were dealed primarily to account for two sets of phenomena for which the direct template theory seems quite irrevelant. The first is the absence of immunological response in 'self' constituents and the related phenomena of immunological tolerance; the second is the evidence that antibody production can continue in the absence of antipes. Some means for the recognition and differentiation of potentially antigonic compenents of the body from faceign argunic material must be provided in any acceptable formulation. In Burnet and Fenner's (1949) account a positive recognition of 'self' material was ascribed to the presence of 'scif-markers' in all potentially antiperic macromolocules, and envesponding recognition units in the stavenger cells of the body. At the time it was regarded as inconstituble that a mechanism could callst which would recognize in positive fashion all foreign material and no attempt was made to devise one, despite the fact that we have always recognized the clumay character of the solf-marker add-recognition scheme.

It is the great virtue of Jerne's hypothesis that it provides an approach to this afformative method of recognizing well' from not sell. There is no doubt about the presence in all hasmonium or arian zera of a wide range of reactive globalias which can begithmately be

"The Walter and Eliza Hall Institute of Medical Research, Mallourpe,

called 'natural antibodies'. Jerne assumed that mongst these globallin molecules were all the possible patterns mooded for specific immunoopical type reaction with any antigen, except for those patterns corresponding to body antigons which would be eliminated by is vice absorption. When a foreign antigen enters the blood it unites, according to Jerus's scheme with one of the corresponding natural antibody malicentes. The complex is taken up by a phagocytic cell in which the natigen plays no further part, but the antibady globalin provokes the production by the cell of a fresh error of similar molecules which are liberated as antibody. If this basis is accepted, most immune inginal physical can be well described in terms of the theory. Its major objection is the absence of any precedent for, and the intrinate unlikelihood of the suggestion, that a melecule of partially denatured antibody could stimulate

a series of replicas of the molecule. Talmage (1987) has suggested that Jonar's view is basically an extension of Ehrlich's side chain theory of antitexts production and that it would be more sufficientory if the replicating elements essential to any such theory were reibilar in character ob initio rather than cuivacellular protein which can replicate only when taken inte an appropriate cell. Talmage does not elaborate this point of view but clearly averpls it as the best basis for the future development of antibody theory. He streams the multiplicity of the globally types that can be present in the blood and is profoundly corptical of any approach which attempts too 'unitarian' an interpretation of antibody. In his view properdin has as much right to be called an antibody as any other globulin.

a cell, into which it had been taken, to produce

Before rewriting Talmage's review we had adopted uittaally the same approach but had developed it from what might be called a 'dean' point of view. This is simply a recopnition that the expendable colls of the body ran be regarded as beforeing to choose which have arises as a result of sematic matation or essoriship other interiviable changes. Each and choose will have none individual characteristic and in a special zone will be subject to an evolutionary process of selective meritan within the informal servicement of the body. It is believed that the advantages of Jerna's

theory can be related and the difficulties overomes if the recognition of foreign pattern is anythed to closes of lymptocytic calls and not

to circulating natural autibody. The resulting formulation map be stated as follows: The plasma reglebulles comprise a wide variety of individually patterned molecules and probably several types of physically distinct structure. Amongst them are molecules will practive siles which can correspond probably with varying degrees of precision to all, or virtually all, the antigente determinants the secur in hiological material other than that characteristic of the body itself. Each type a pattern is a specific product of a clone pesenchemal cells and it is the essence of th hypothesis that each cell automatically has available on its surface representative reactive sitze onvivalent to those of the globalis the produce. For the sake of ease of exposition these only will be referred to as lymphosytes it being understood that other measurelymal types may also be involved. Under appropriate conditions, colls of most clones can either liberate soluble antibody or give rise to

Antibody as receptor

determinants. The capacity of a circulating lymphocyte to pass to tissue sites and there to

eceptor driven activation

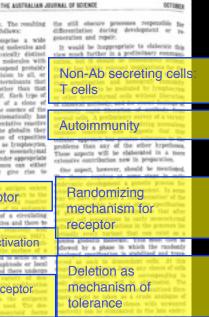
Proliferation and receptor secreted as Ab

capable of active liberation of seconds accused, and lymphorpic which can fulfil the same functions as the parential forms. The bell result will be a change in the composition of the globalis molecule population to give an excess of molecules capable of neutring with the actions, in other words the neutral will new

Memory as clones

same antigon will be extensive and supid, i.e. a secondary type immunological response will notur.

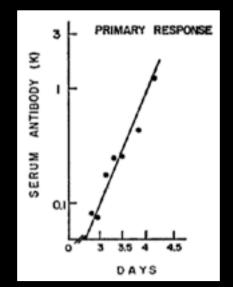
Such a point of view in basically an attempt in apply the source of populating protection to the choice of mesenchymal cells within the body. It is dear that the index and intermement involved is an exceedingly complex one and in all populatility many factors will implice an existence of antibady-producing cells from the environment. It is coupling version that indextable changes in the choice lower was interestable changes in the choice lower of the interval of a result of source in result.



of immune tolerance.

The hyperbesis has many of the sense implications as the Bartot-Future rad the Jerus theories. Its olds advantages ever the former is its relevance to the melver of neural andthe indeplies the red old isoagnithmin and the indeplies the red old isoagnithmin and the indeplication experienced in embryonic plantial axispan experienced in embryonic plantial axispan experienced in embryonic planting array is well as the production of classical antibady, and the diminist the very unlikely assumption that exity of a globellis molecule intu a coll will attimuties the edit in produce scatter replicas of that globellis.

Despite the speculative character of much of the detail of this modification of Jorn's there? —which might be exhed by the 'desail adoption' hypothesis'—it has no many implications entiting for experimental experiments of the has been thought problem to automit this periminary account for publication.



Macfarlane Burnet





"The principles of immunity are now known

Immunology is now just working out details"

Macfarlane Burnet

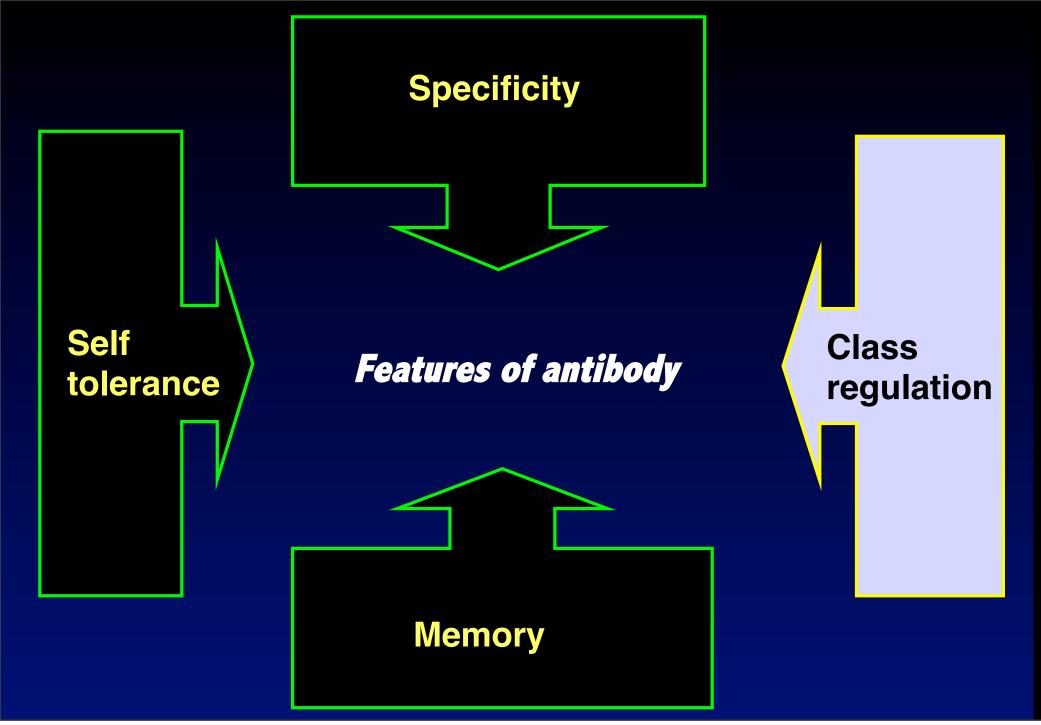




"The principles of immunity are now known

Immunology is now just working out details"

The details were hard!



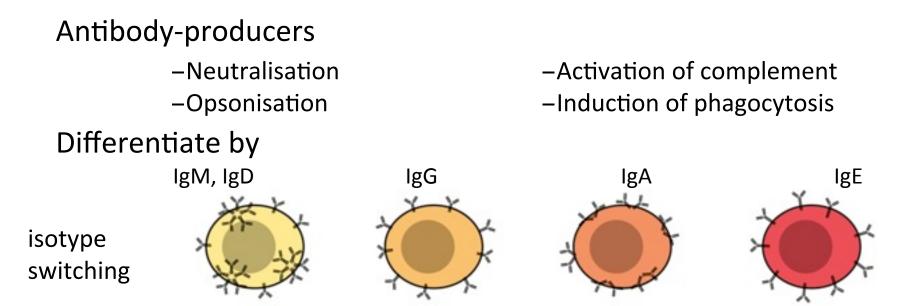
Wednesday, December 19, 2012

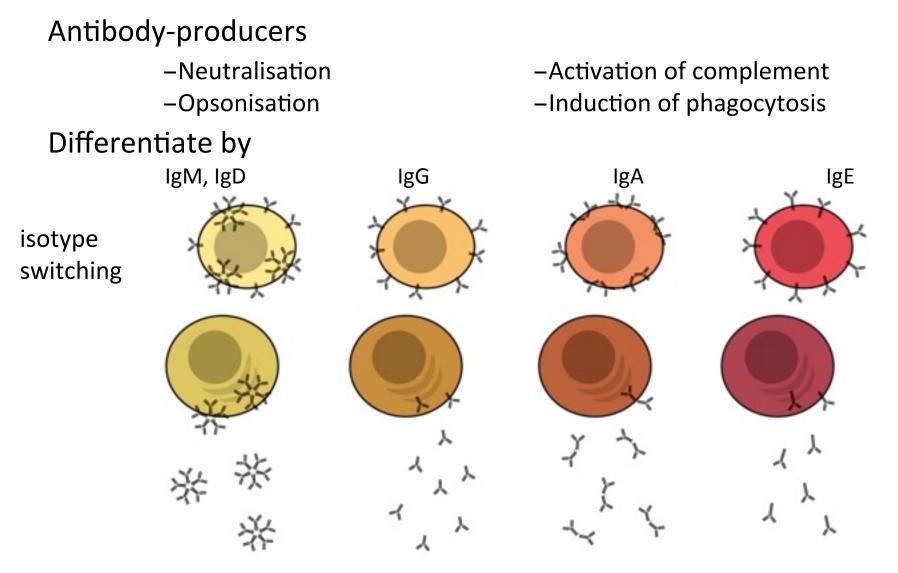
Antibody-producers

- -Neutralisation
- -Opsonisation

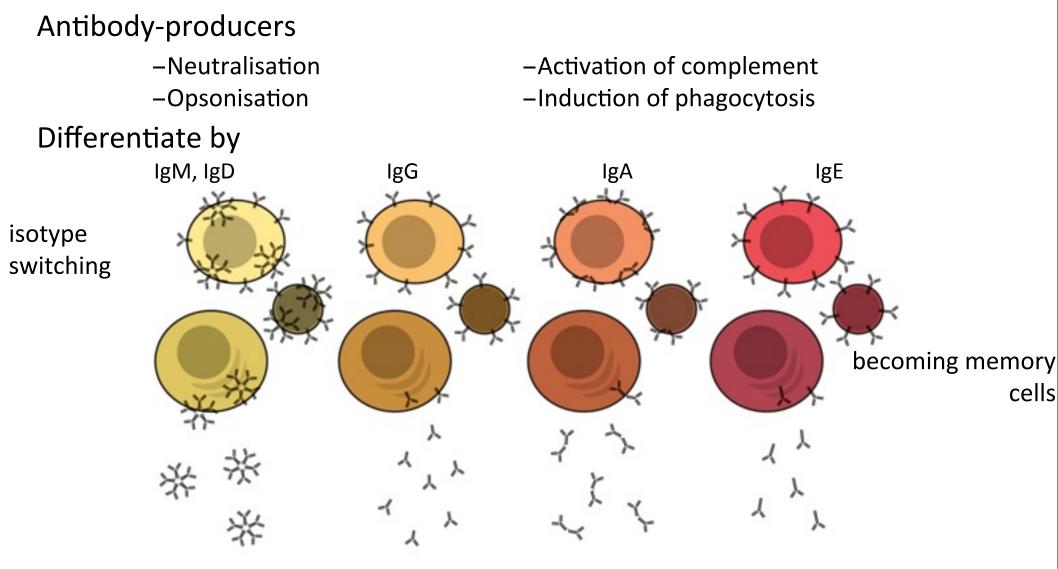
Activation of complementInduction of phagocytosis



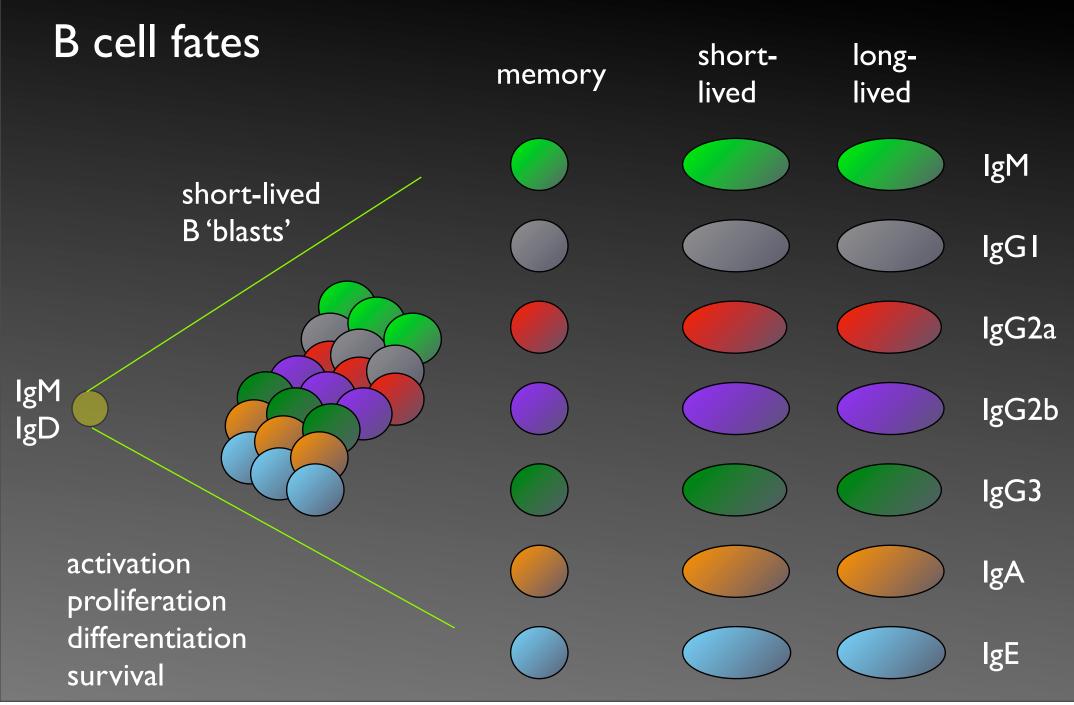




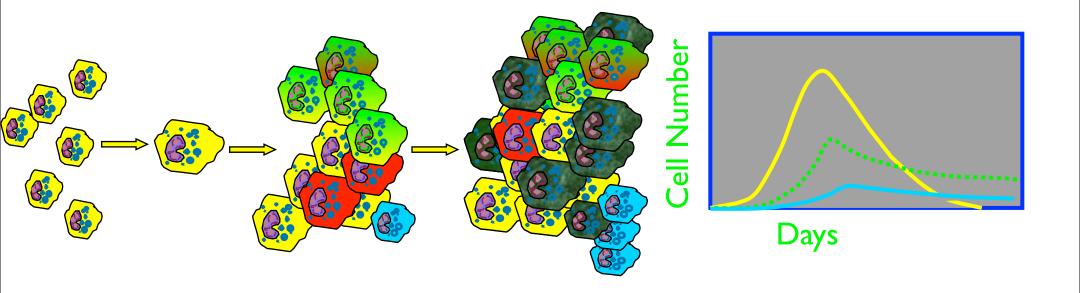
becoming antibody secreting cells (ASC)

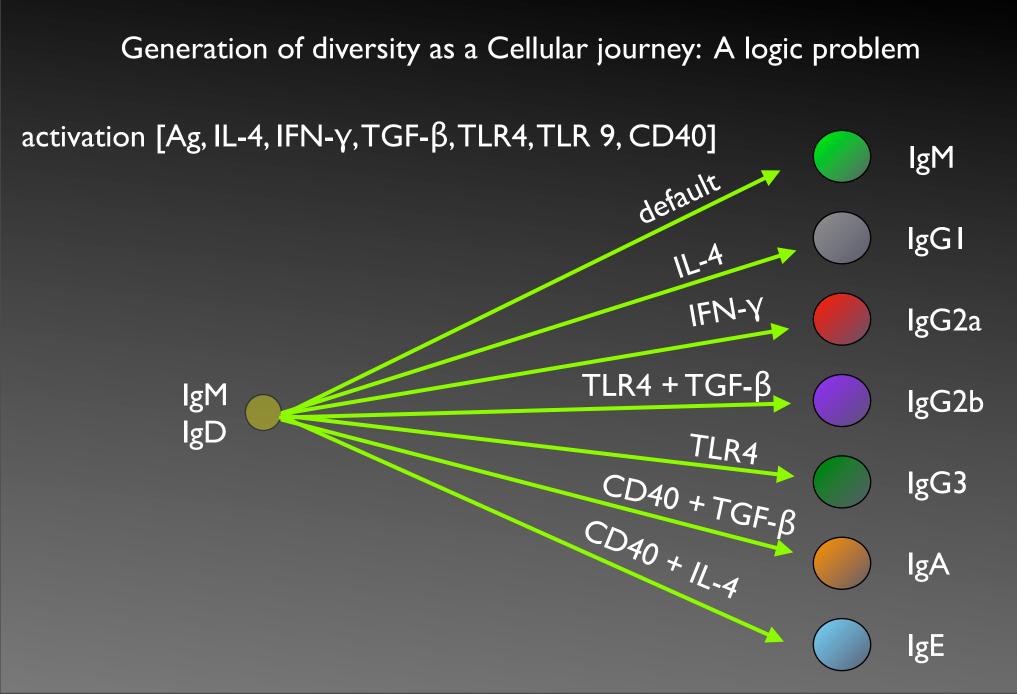


becoming antibody secreting cells (ASC)

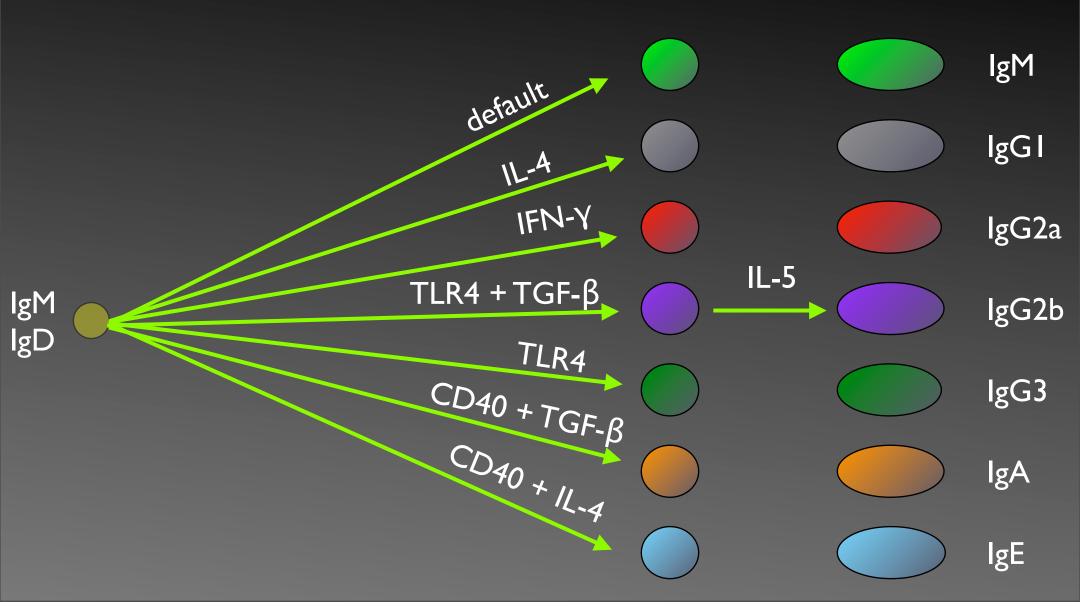


• Solve the logic puzzle - which signals and in what combinations lead to which outcomes?





ASC



Complex problem

Typically cytokines have varied effects on proliferation, survival and differentiation

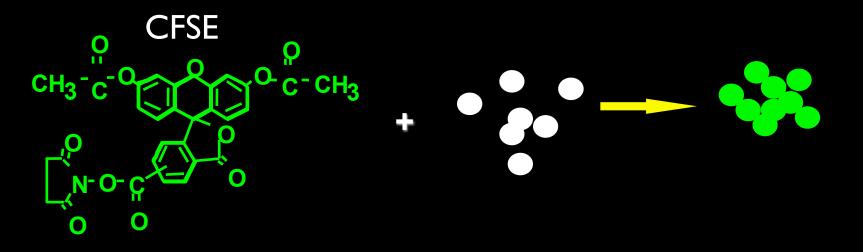
When exposed to combinations of different signals - how do cells calculate an outcome?

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

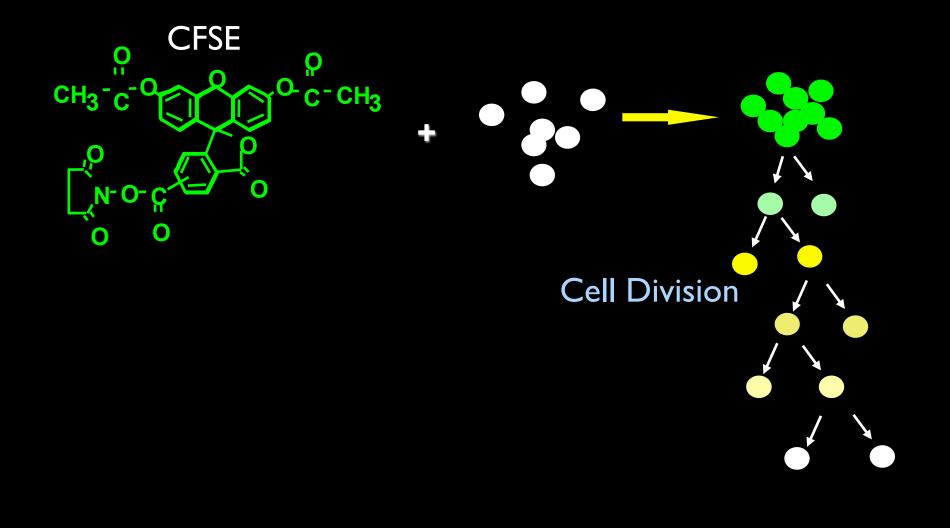
[note - strategy FIRST ORDER (no other interactions) first]

Cell Division number as a hidden variable in differentiation outcomes

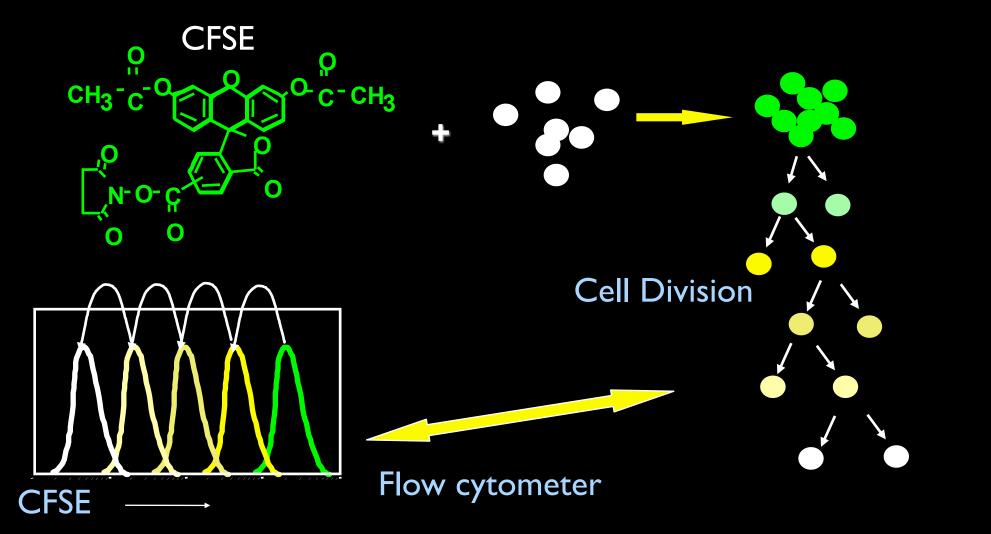
Division tracking with Carboxyfluorescein succinimidyl ester (CFSE) :Lyons and Parish



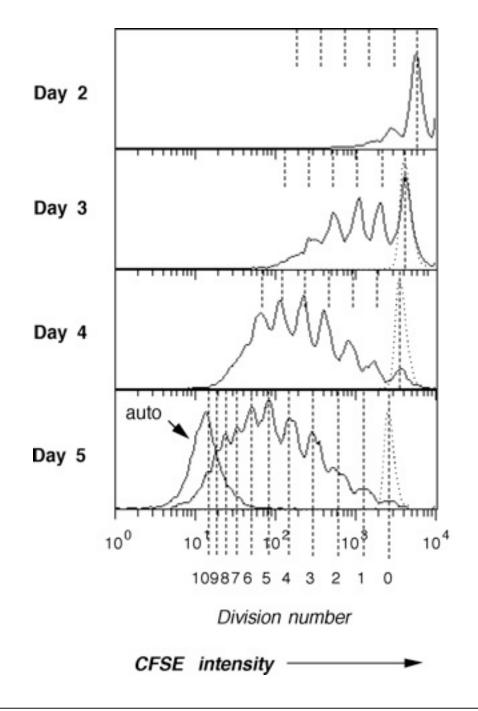
Division tracking with Carboxyfluorescein succinimidyl ester (CFSE) :Lyons and Parish



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



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



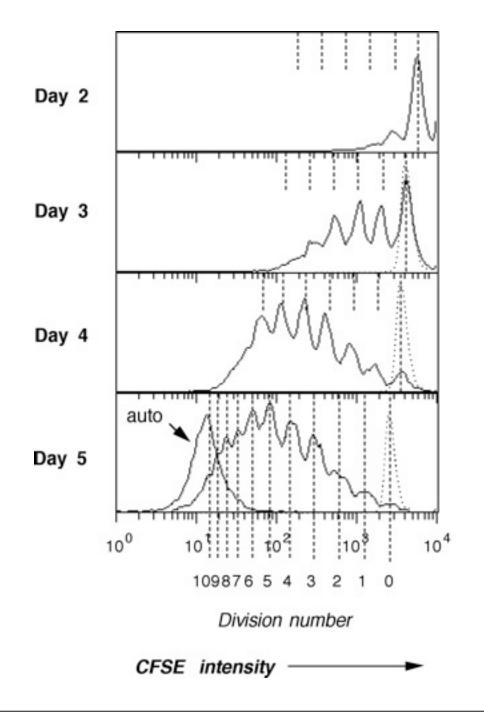
B cells stimulated in vitro

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



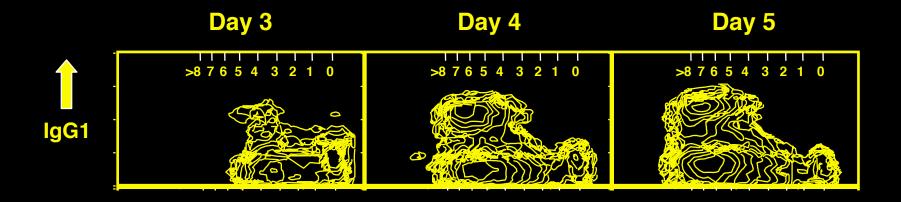
B cells stimulated in vitro

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

When do they switch?

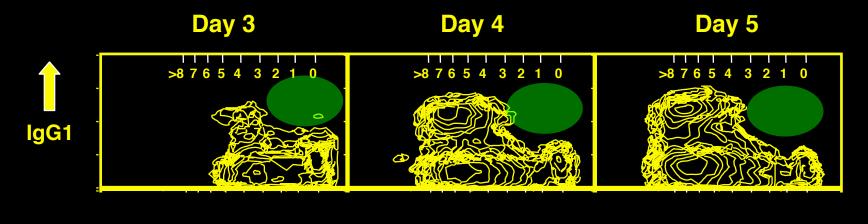
(Work of J Hasbold)

Switch from IgM to IgGI comes after 3 divisions





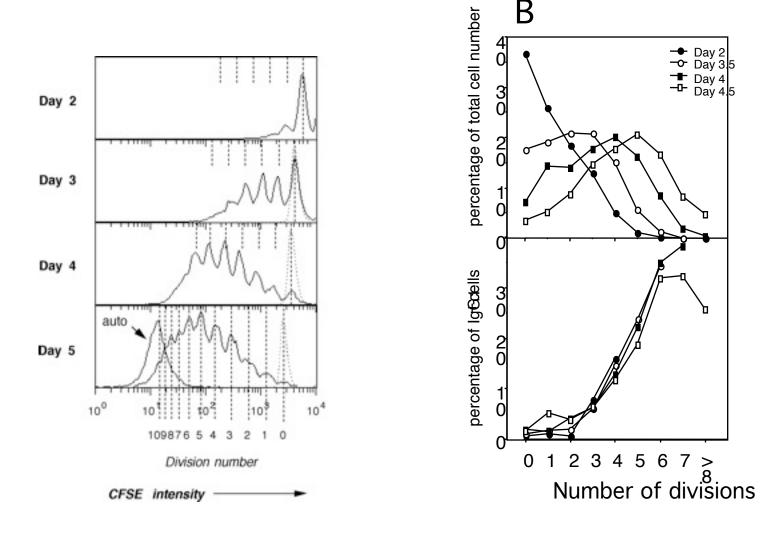
Switch from IgM to IgG1 comes after 3 divisions





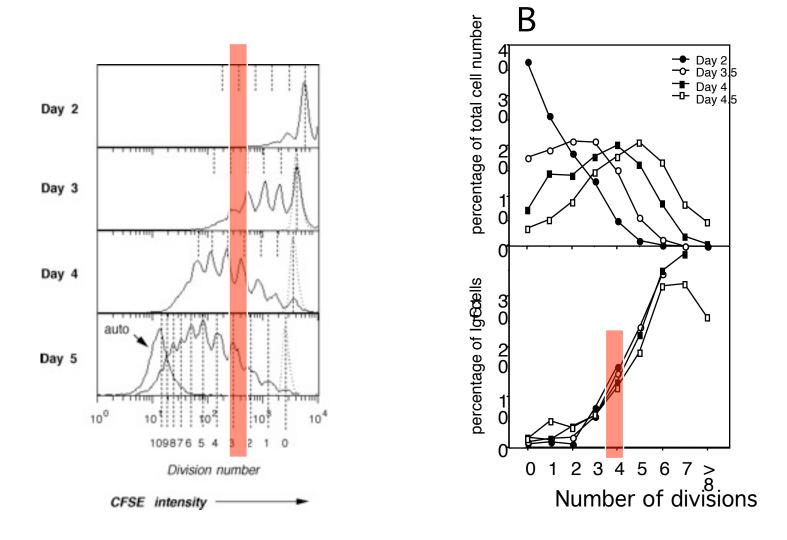
No IgGI cells appears before 3 divisions

Isotype switching is 'Division-linked'



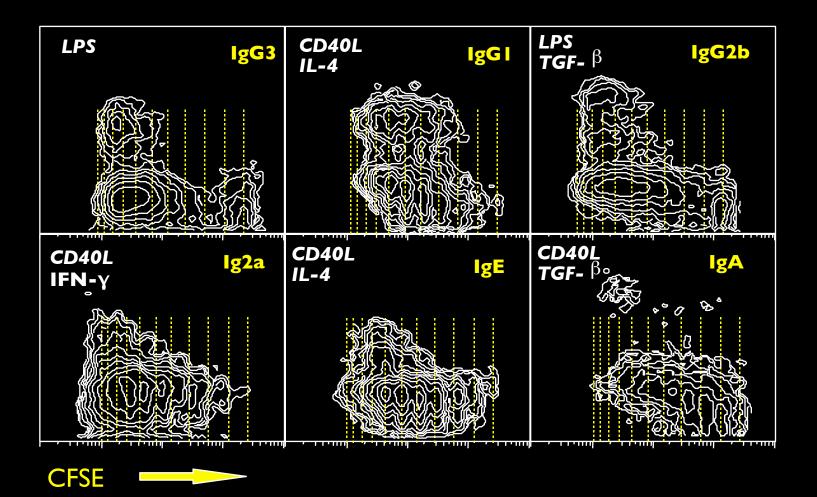
Not time-linked

Isotype switching is 'Division-linked'

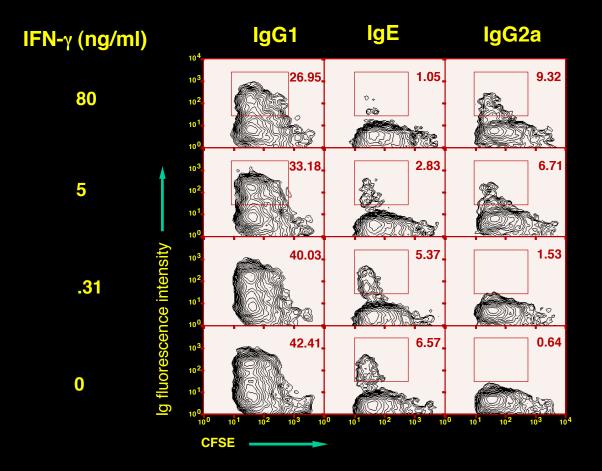


Not time-linked

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

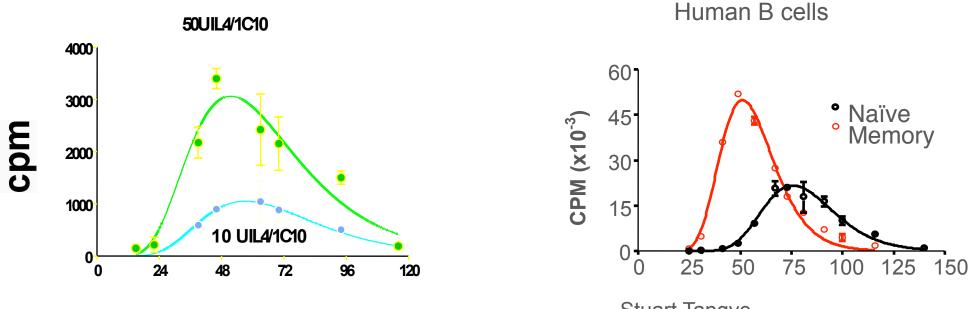
2. Division linked-differentiation separate from regulation of -

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



Stuart Tangye

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

CpG stimulation

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}

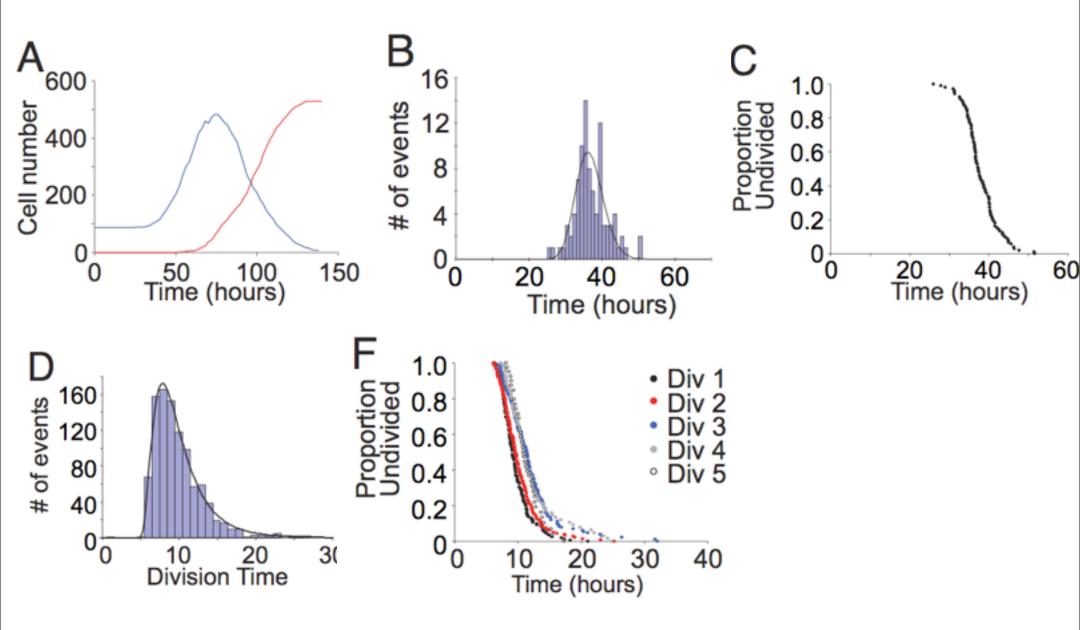
Immunology Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Victoria 3050, Australia; and INational Information and Communications Technology Australia, and Department of Electrical Engineering, University of Melbourne, LvI2/Building 193, Victoria 3010, Australia

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

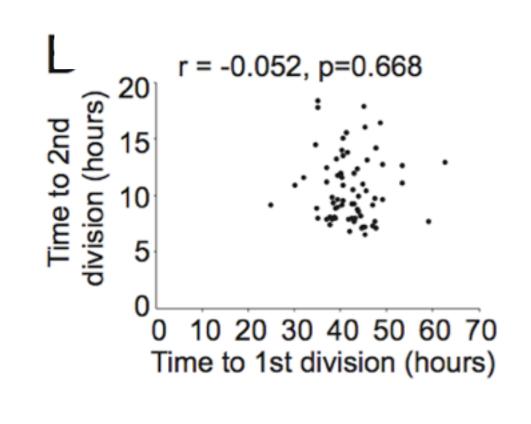
In contrast to most stimulated lymphocytes, 8 cells exposed to Toll-like recentor 9 lipands are nonself-adherent, allowing individinformation on how lymphocyte survival is regulated and altered

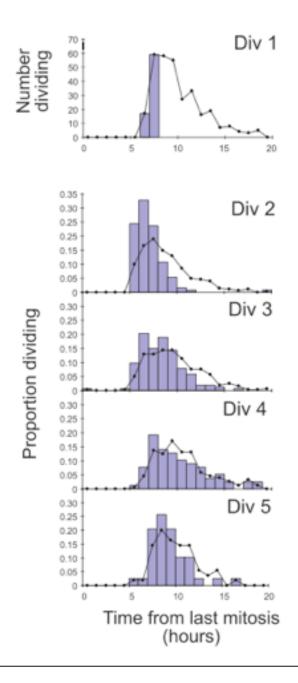
SANG

Proliferation, cessation and death

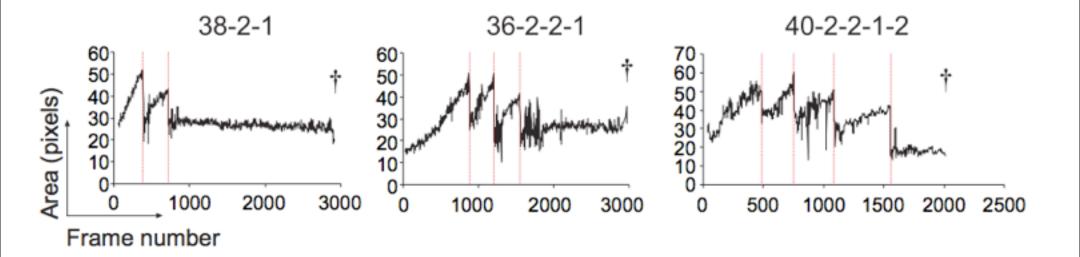


Fast does not beget fast...



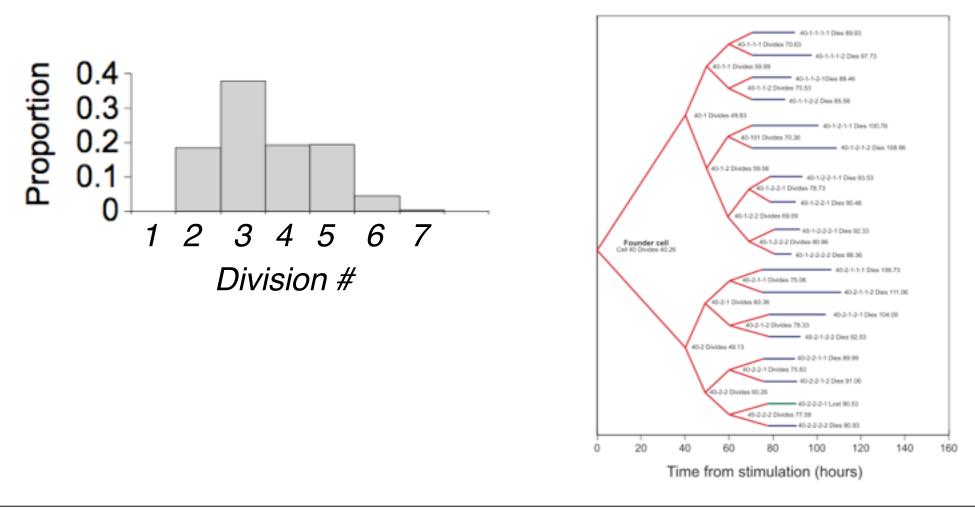


Growth, division, cessation and death



Founder effects on division 'destiny'

4 symmetric divisions



Key experimental observations -

- Lognormal times to divide
 Resetting of times after division lack of inheritance
 Division 'counting' can alter parameters

 eg. division progression
- 4 Independent regulation of division and death

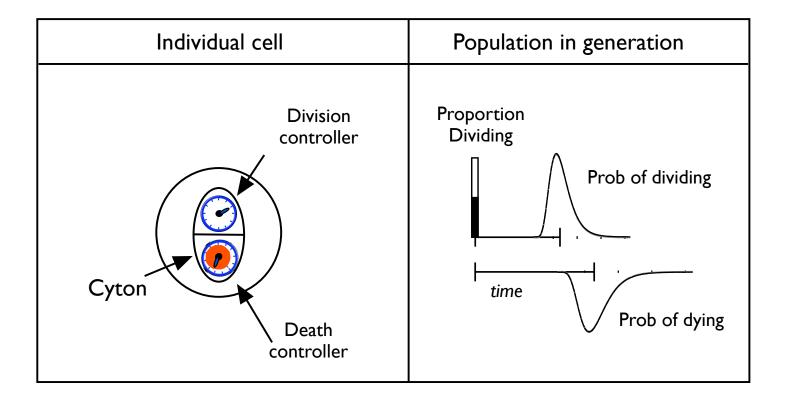
A model of immune regulation as a consequence of randomized lymphocyte division and death times

E. D. Hawkins*[†], M. L. Turner*[†], M. R. Dowling*[‡], C. van Gend*, and P. D. Hodgkin*⁵

*Immunology Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia; ¹Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia; and ¹School 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. 1A and refs. 7, 9, and 15). However, we noted deviations



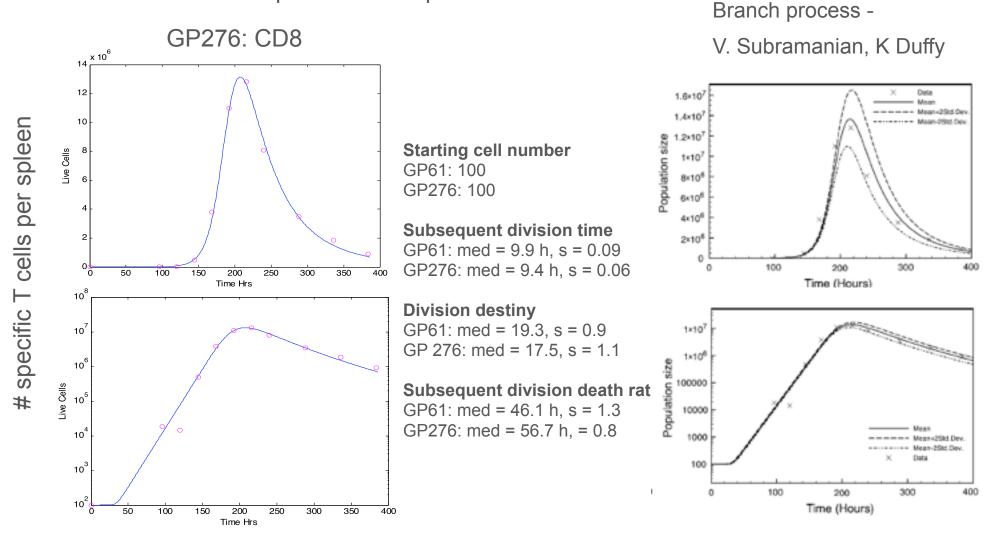
Use to measure genetic effects

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



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

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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?

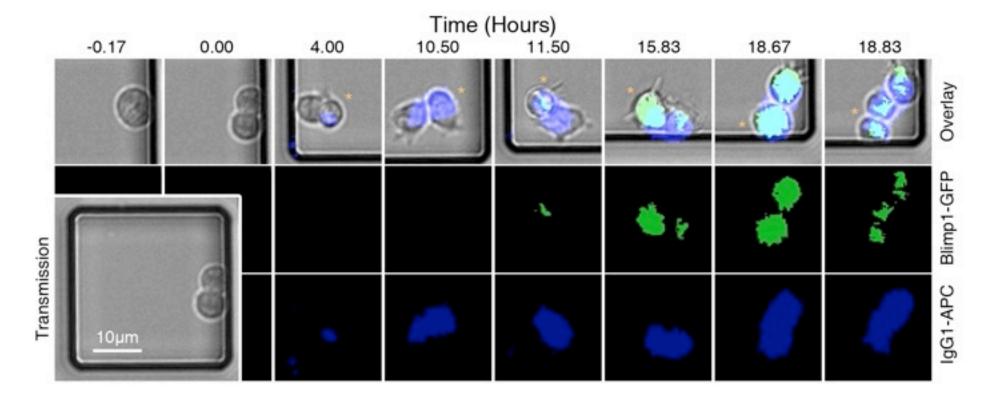
B cells - Switch antibody type, develop to ASC

Can we extend internal competition in cells to other fates?

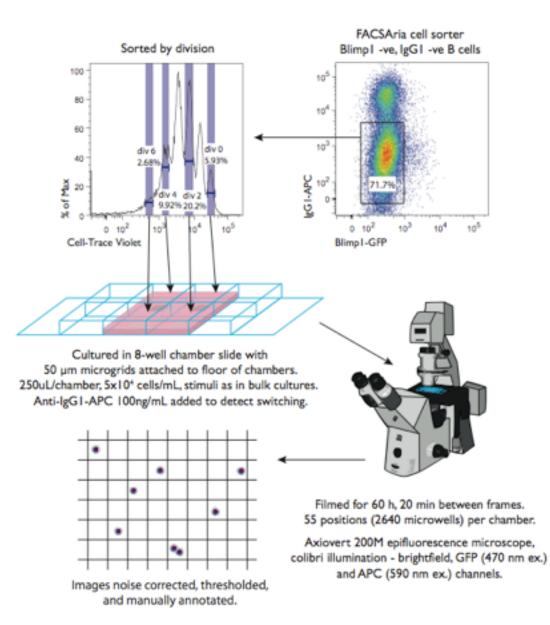
Microscopy allows the study of individual cell fates over time

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

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



Generation filming

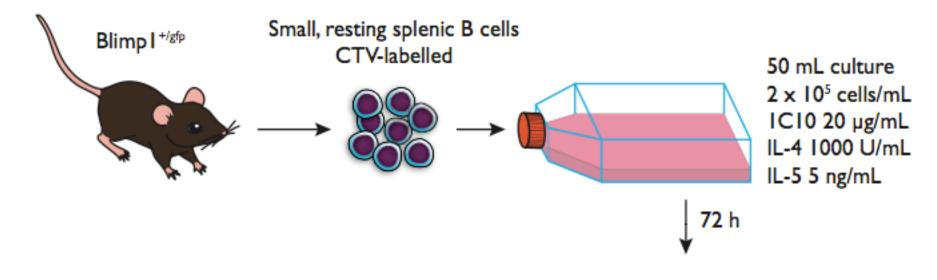


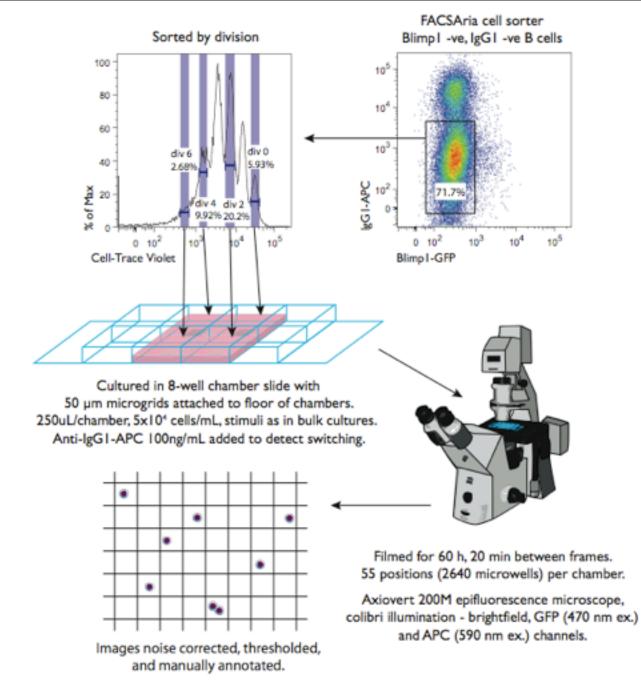
Experiment - Observe 4 fates operating simultaneously Test for competition

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

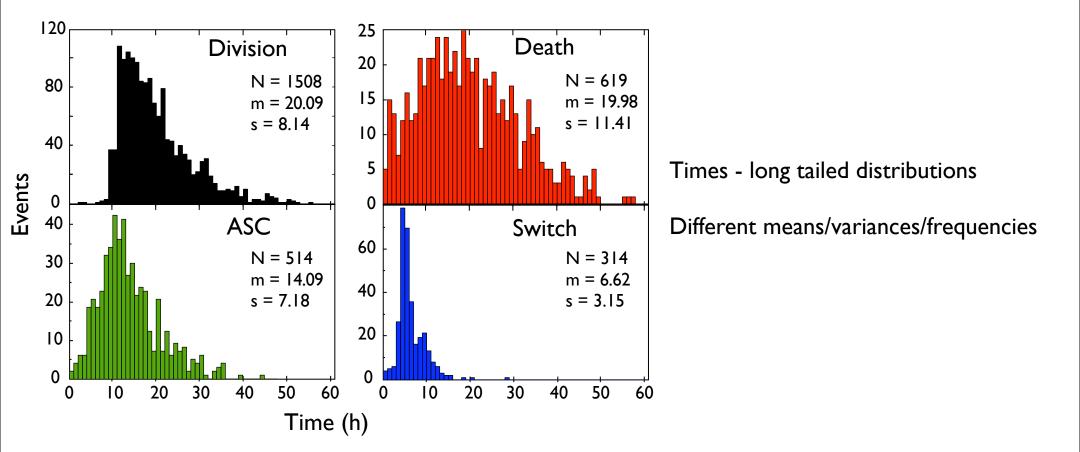
Conditions that induce division, death, switch to IgGI and development of ASC

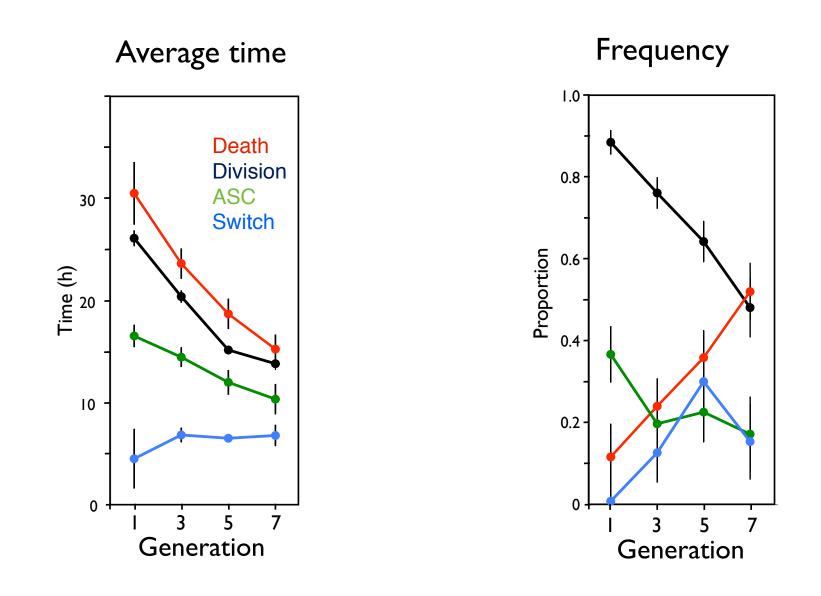
Examine for statistical hallmarks of competition*





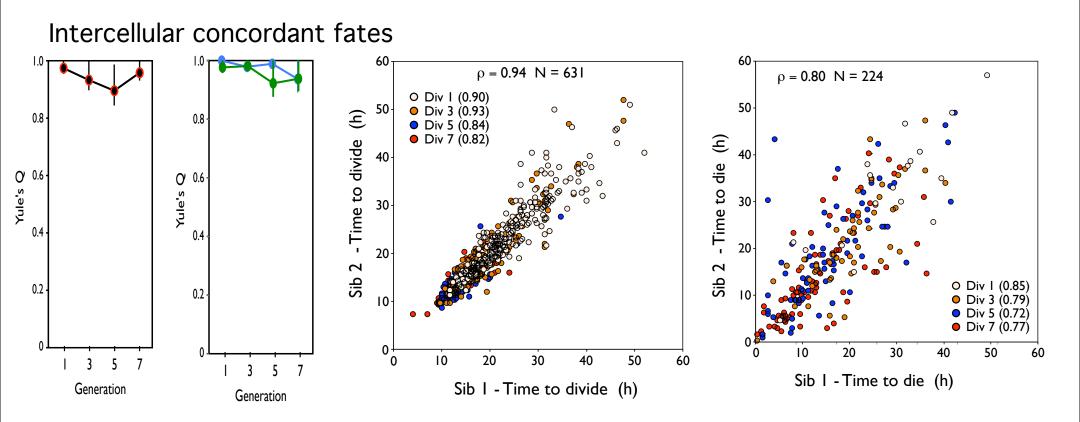
All Divisions - summary



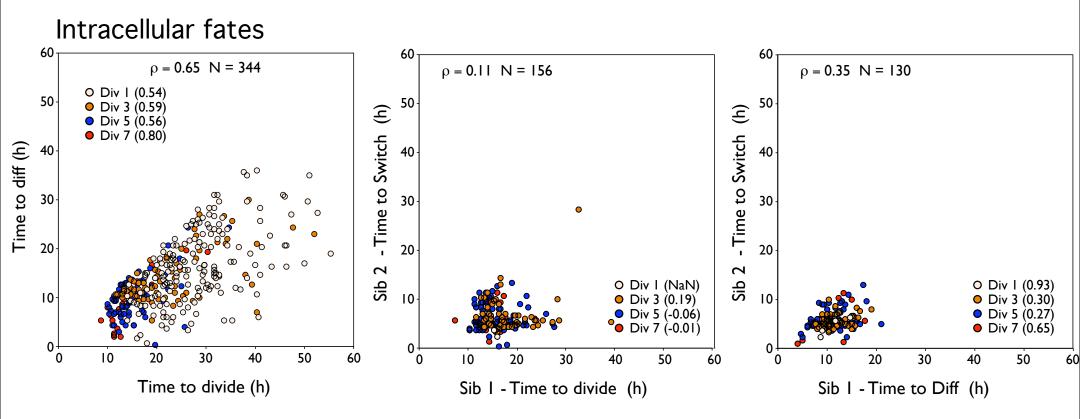


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Sibling correlations

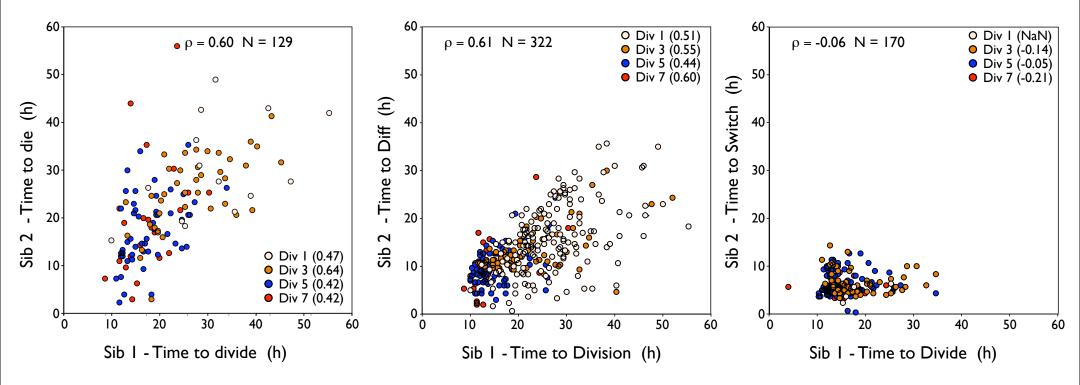


Intracellular correlations

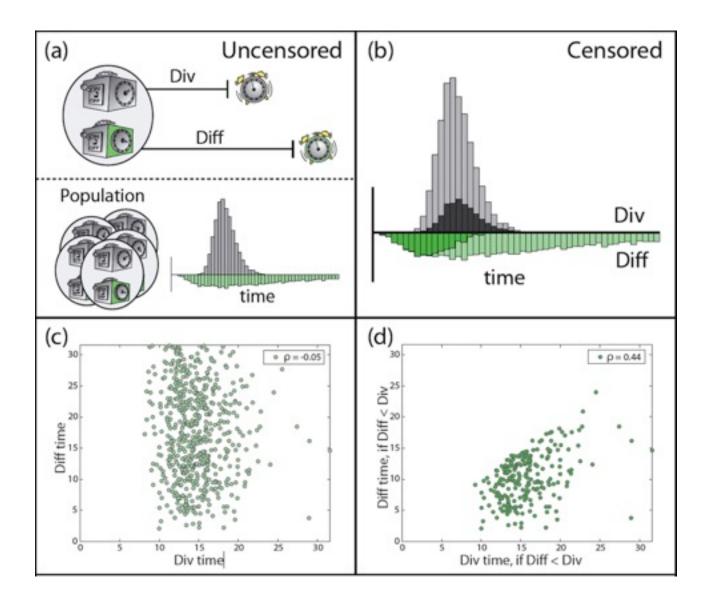


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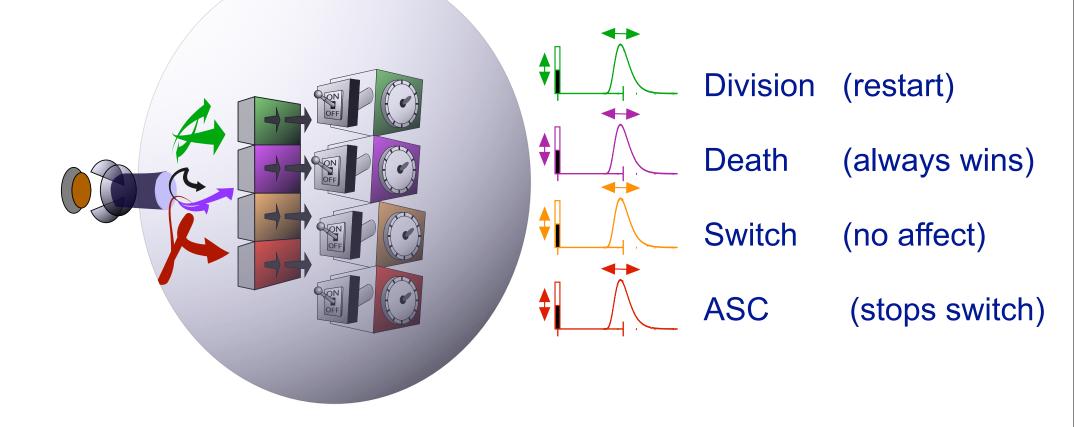
Intercellular correlations non-concordants



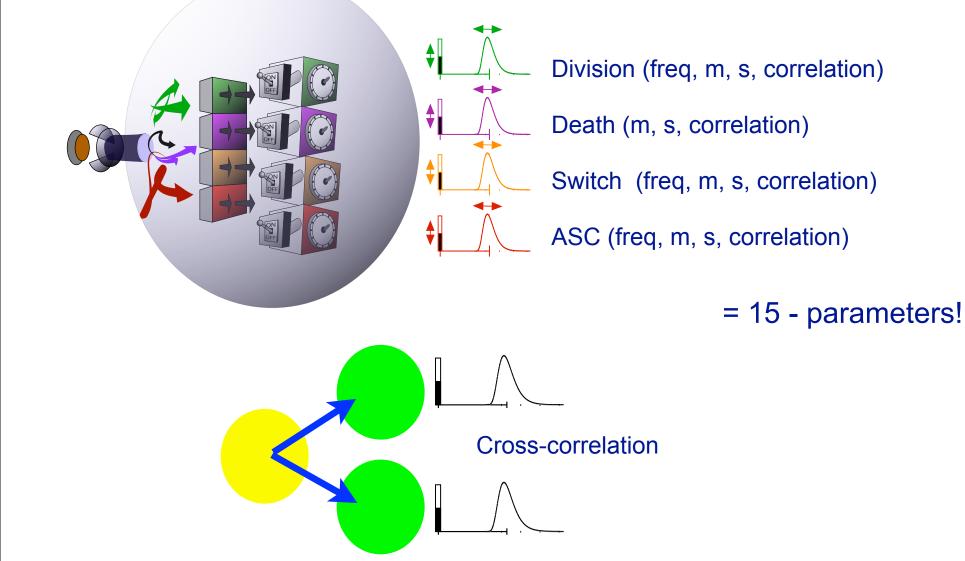
Autonomous Intracellular Competition: Hallmarks of censorship



Four way Cyton - Autonomous Intracellular Competition - Rules of censorship

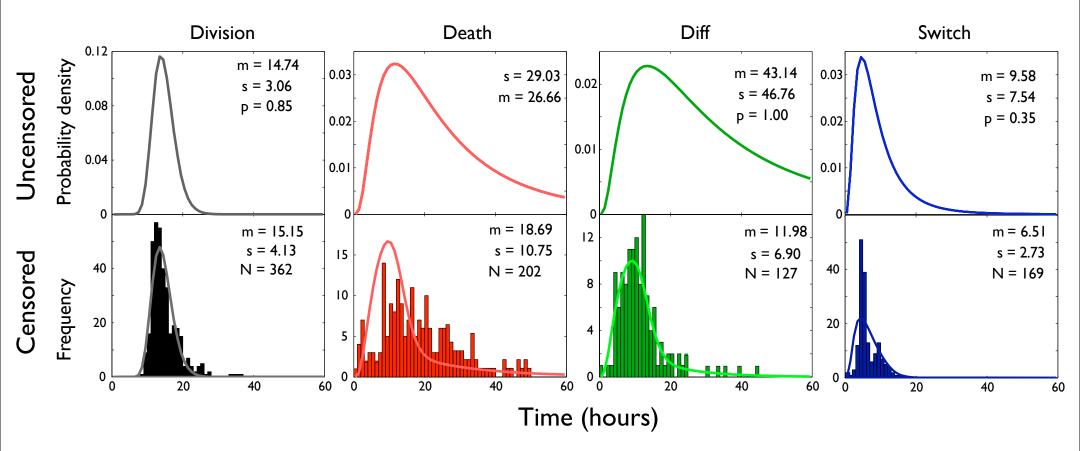


Autonomous Intracellular Competition - Model parameters



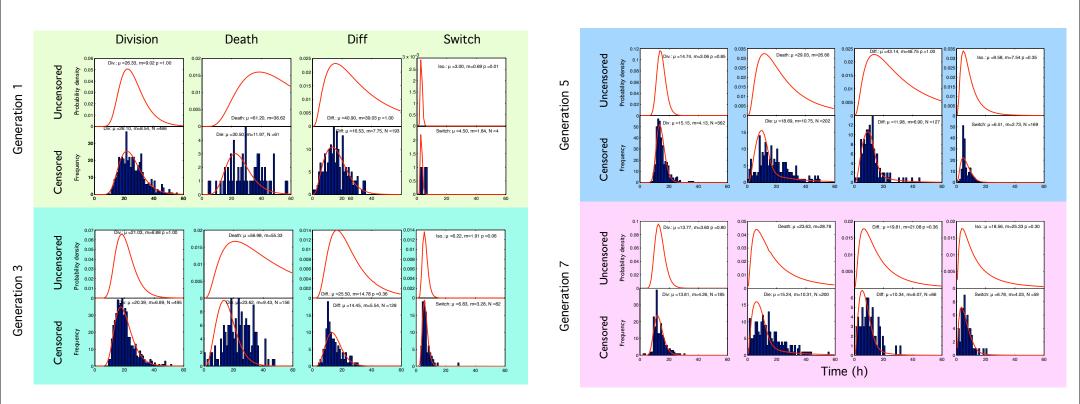
Statistical model (Duffy, Wellard)

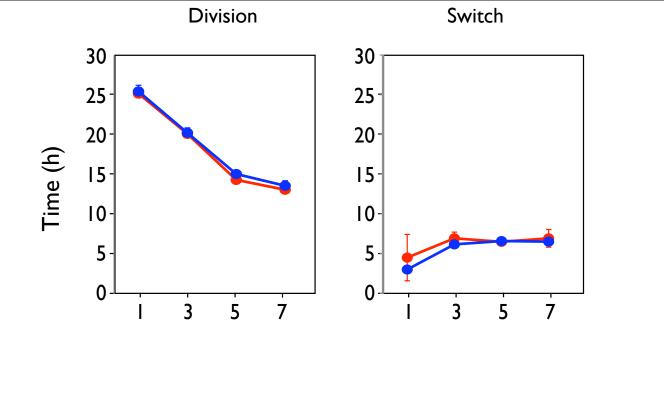
lognormal time to each event (m, s, f) Independent with Censorship (death, div)



Generation 5

All generations

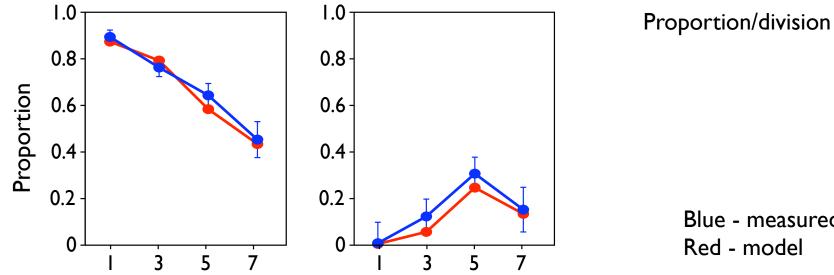




Fitted features

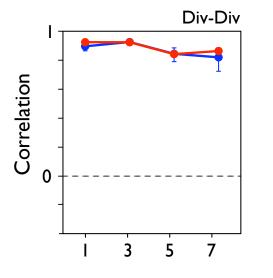
Time/Division

and



Blue - measured Red - model

Intercellular concordant



Death-Death Outer Outer

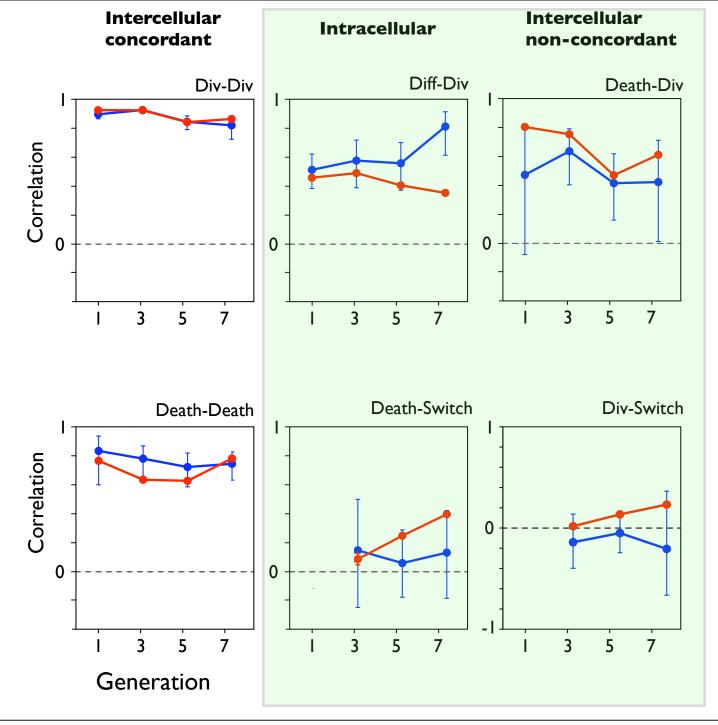
Fitted features

Inter-cellular Correlations

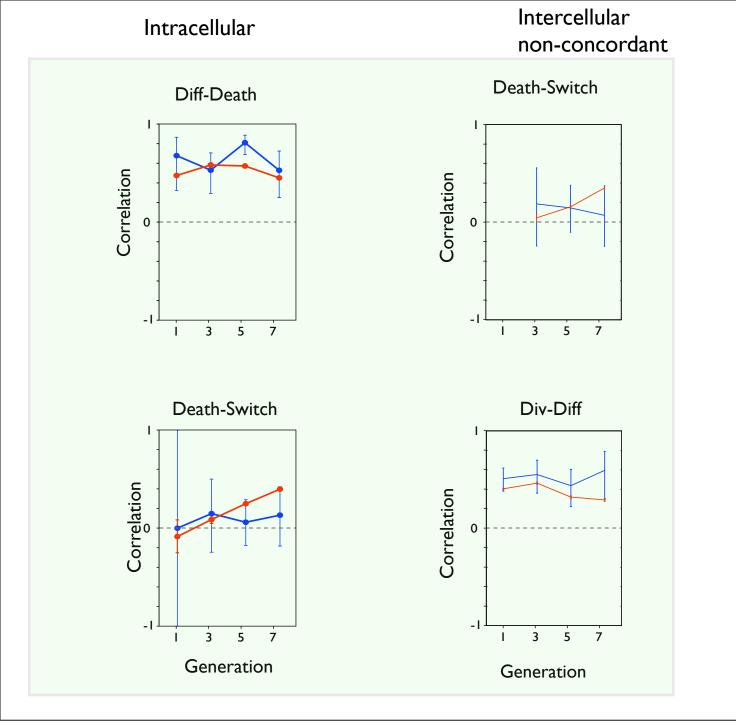
Blue - measured Red - model Passed test of getting back what you put in..

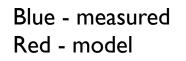
But can it explain the unexpected correlations?

Intra cellular correlations intercellular correlations of different fates of sibs



Blue - measured Red - model





Hallmarks of competition for fates apparent

Asymmetric fates observed but conform to statistical likelihood

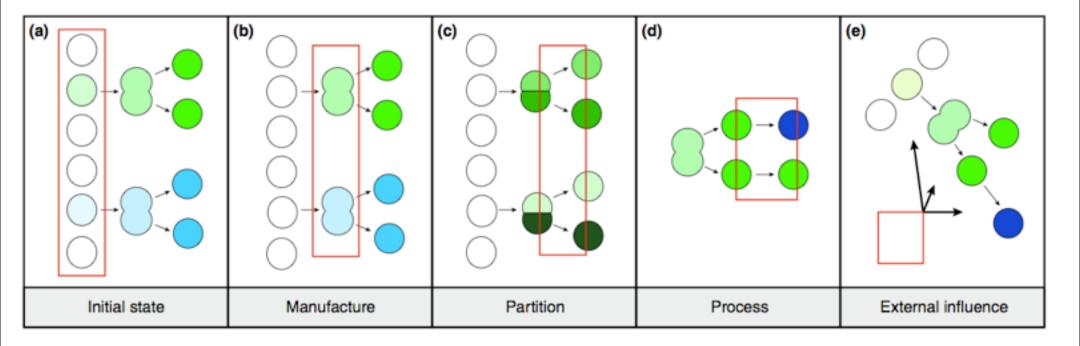
Activation-Induced B Cell Fates Are Selected by Intracellular Stochastic Competition

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}*†

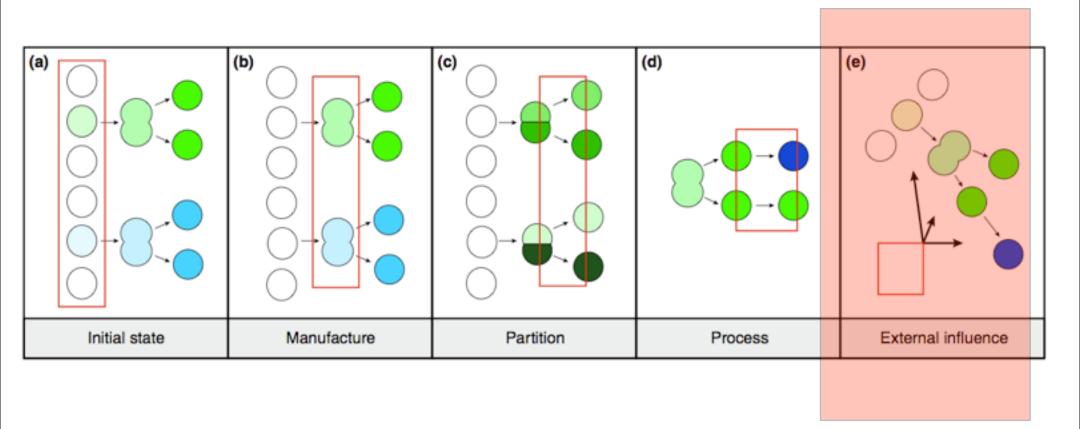
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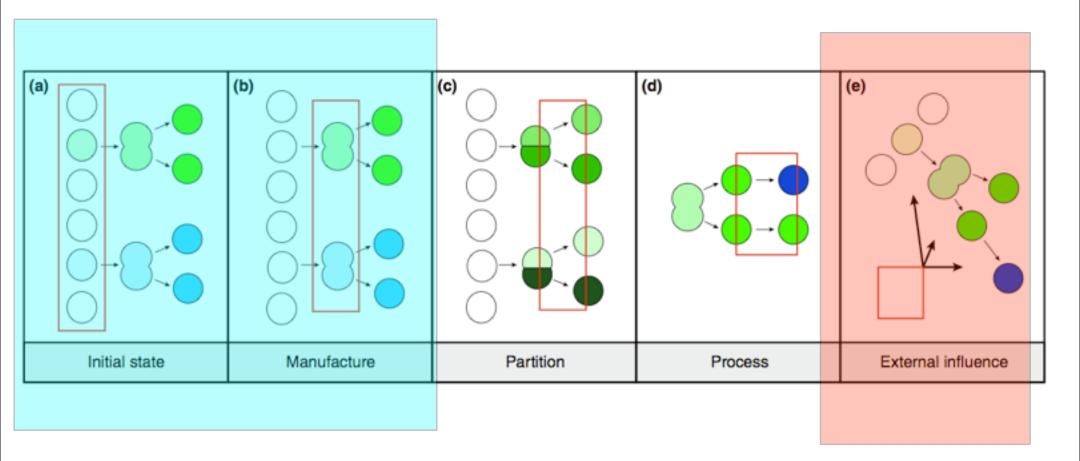
Homing in on the source of cellular heterogeneity



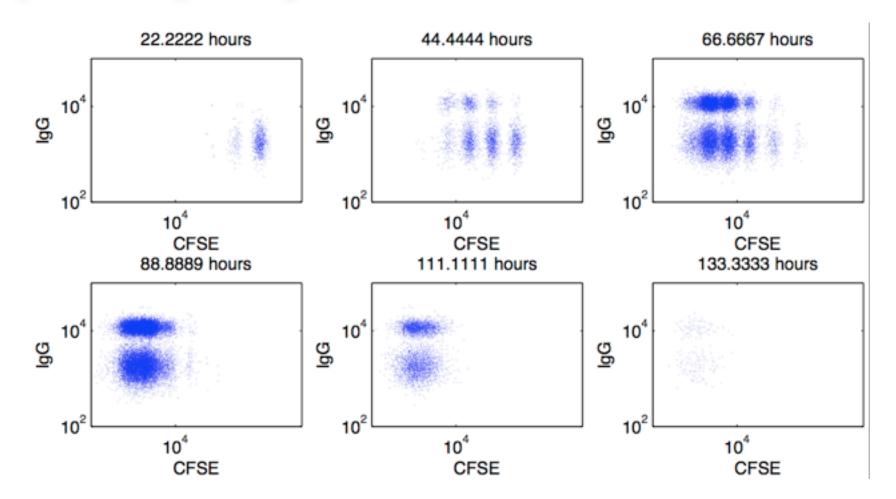
Homing in on the source of cellular heterogeneity



Homing in on the source of cellular heterogeneity



Computer flow cytometry!



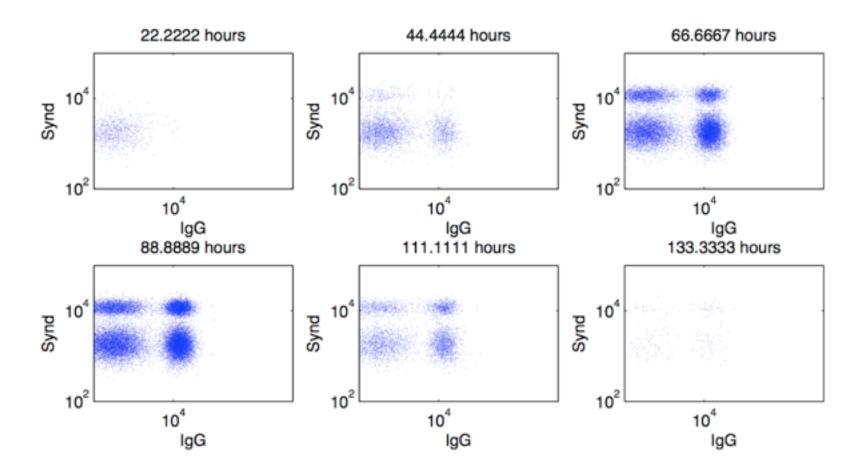
Run simulator for thousands of cells

Record features at every time for all cells

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'Plot as if real cells'
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Wednesday, December 19, 2012

Computer flow cytometry!



Run simulator for thousands of cells

Record features at every time for all cells

'Plot as if real cells'

Wednesday, December 19, 2012



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

Systems B cell Program WEHI

Dave Tarlinton Lynn Corcoran *Steve Nutt

Axel Kallies

Image analysis/model development

*Cam Wellard

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



Imaging & microwells

*John Markham [NICTA] *Mark Dowling

*Ed Hawkins Liam McGuinness **Ross Holmberg** **Jie Zhou

Daniel Day (Swin) Sarah Russell (PMac)

Funded by



