Transcriptome-wide noise controls lineage choice in mammalian progenitor cells

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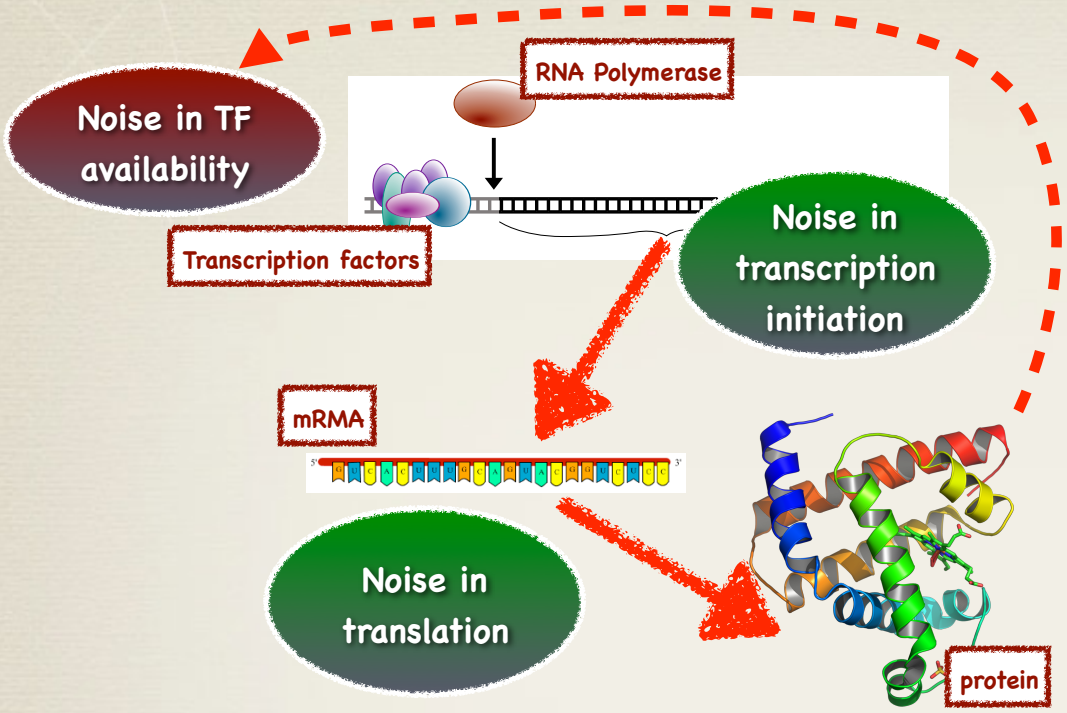
Nature 453, 2008.

Background on cellular noise



- in Eukariotic systems: dominated by **bursty transcription**
- phenotypic cell to cell variability
- beautifully measured, quantified, modelled in *E. coli* and *Yeast*

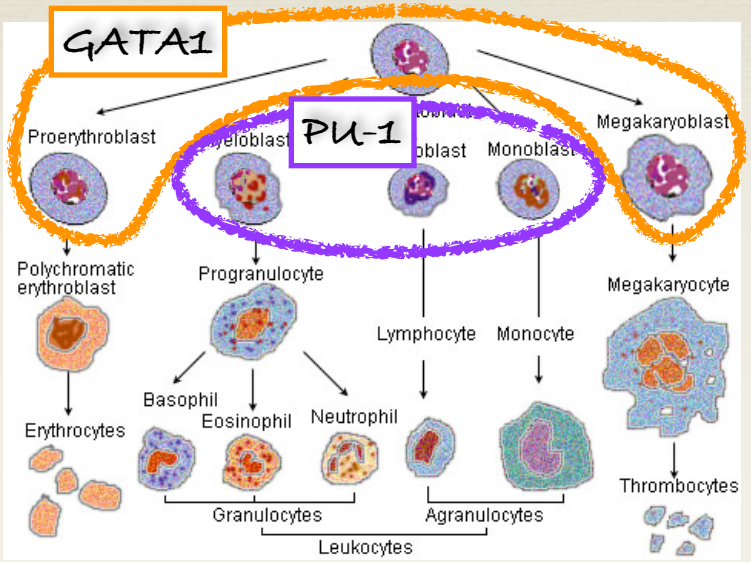
- **stable** phenotypic variants with the same genome (memory)
  - in human cells: some proteins fluctuate between the low and high range on the timescale of 2 cell cycles!
  - proteins in the same pathway **fluctuate in correlated fashion**
- **adaptive advantage** upon environmental challenge

(6) Kaern, M., Elston, T.C., Blake, W.J. and Collins, J.J. *Stochasticity in gene expression: from theories to phenotypes*. Nat. Rev. Genet, 6(6):451–464, 2005.

Problems in differentiation

Instructive

- external signals **impose** a genetic program
- **master regulators** of lineage
- tissue specific promoters



promiscuous expression of many lineage markers

Selective

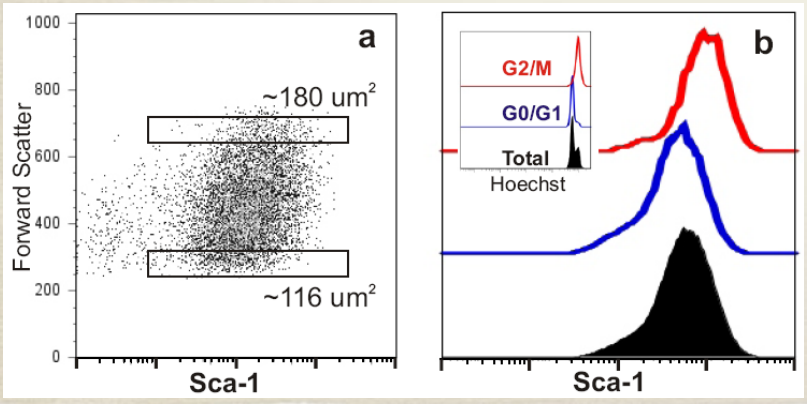
- spontaneously differentiated **subpopulations** "selected" by external factors

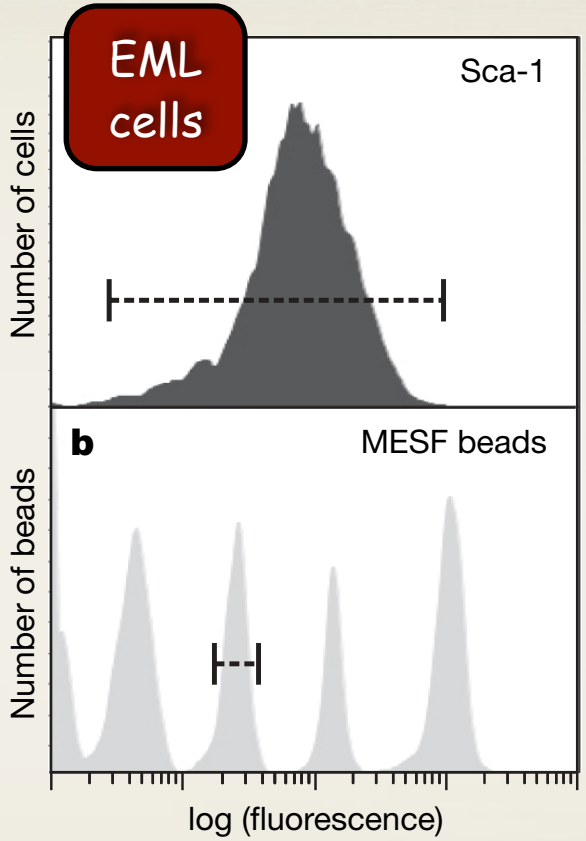
- complicated interplay between many regulators
- directing differentiation is very difficult... (<50% efficiency)

- mouse embryonic stem cells: remove stem cell medium => spontaneous differentiation to many lineages
- single cell plating of hematopoietic stem cells => macrophage, erythrocyte, platelet

Does cellular noise play a role in differentication?

- Lets find this noise...
  - immunofluorescence flow cytometry of **cell surface Sca-1 protein**
  - clonal population
  - 1000-fold range!
- stable over time
- much larger than measurement noise
- NOT just cell size or cycle





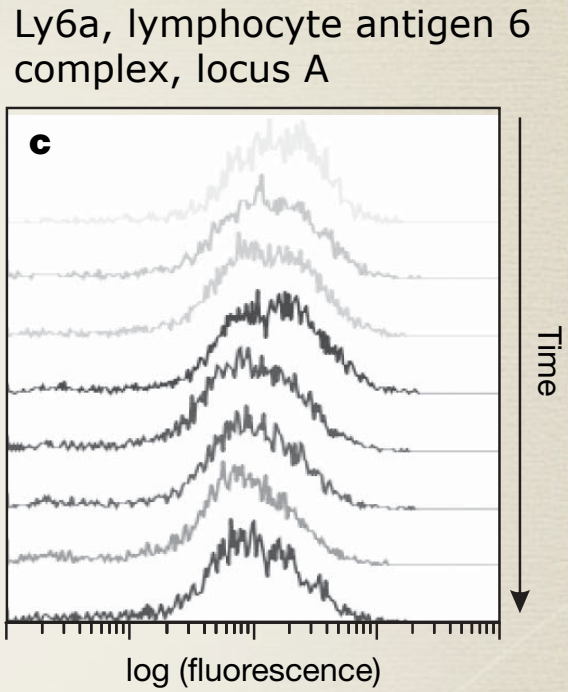
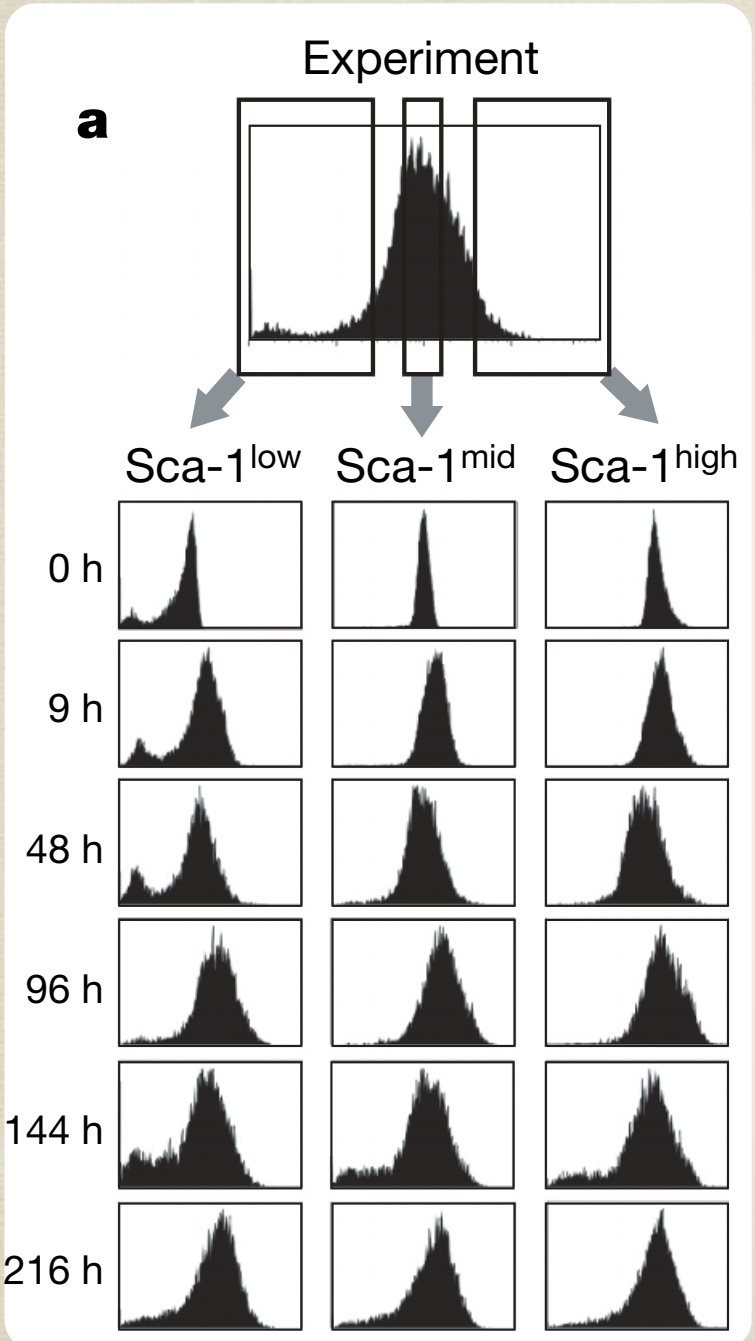


Figure 1 | Robust clonal heterogeneity. **a**, **b**, Heterogeneity among clonal cells in Sca-1 protein expression, detected by immunofluorescence flow cytometry (**a**), was significantly larger than the resolution limit of flow cytometry approximated by measurement of reference fluorescent MESF[24] beads (**b**). The dashed lines show the difference in spread of the distributions as explained in the text. **c**, Stability of clonal heterogeneity in Sca-1 over three weeks.

How does the heterogeneity arise?



- Lets FACS sort the subpopulations!
  - Low, Med, High Sca-1
  - culture the fractions
  - slow restoration of full heterogeneity (> 12 cell doublings) !
  - also works with single-cell clones (very slowly...):

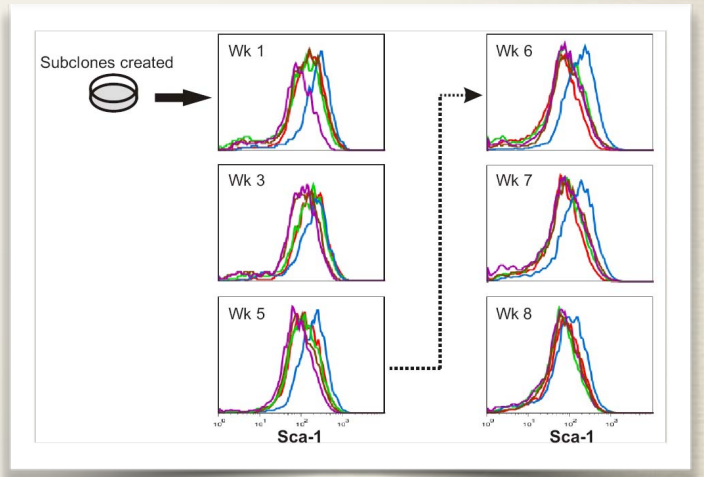
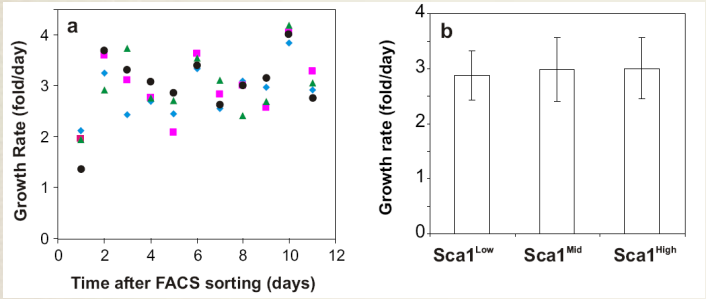


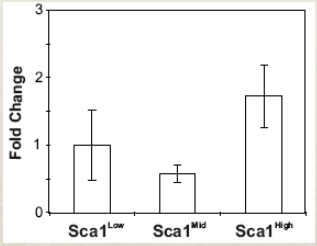
Figure 2 | Restoration of heterogeneity from sorted cell fractions. a, Clonal cells with the highest (Sca-1^{high}), middle (Sca-1^{mid}) and lowest (Sca-1^{low}) 15% Sca-1 expression independently re-established the parental extent of clonal heterogeneity after 216 h in separate culture. As an example, each cell in the Sca-1^{high} experiment was theoretically partitioned into one of two GMM subpopulations (blue and red, right).

What drives the heterogeneity?

- A few things to exclude:
  - NO differential growth of Sca-1 subpopulations

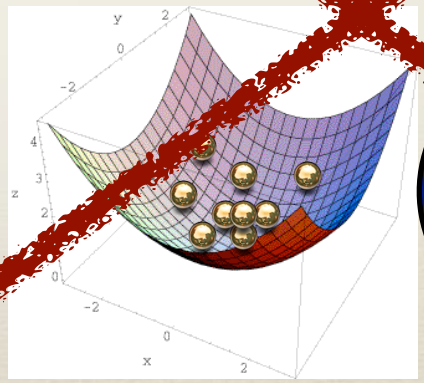


  - mutations are too slow (9 days, 12 cell divisions)
  - widening of distribution too fast for uneven partitioning of Sca-1 protein during cell division
  - Not much difference in transcription



- What governs Sca-1 expression?
  - circuitry not known
  - explicit modeling unfeasible
- Phenomenological approach
  - find **class of stochastic processes** that can explain the data

1. Mean-reverting process (Ornstein–Uhlenbeck process)
   - noisy relaxation process towards an equilibrium



Gaussian distribution

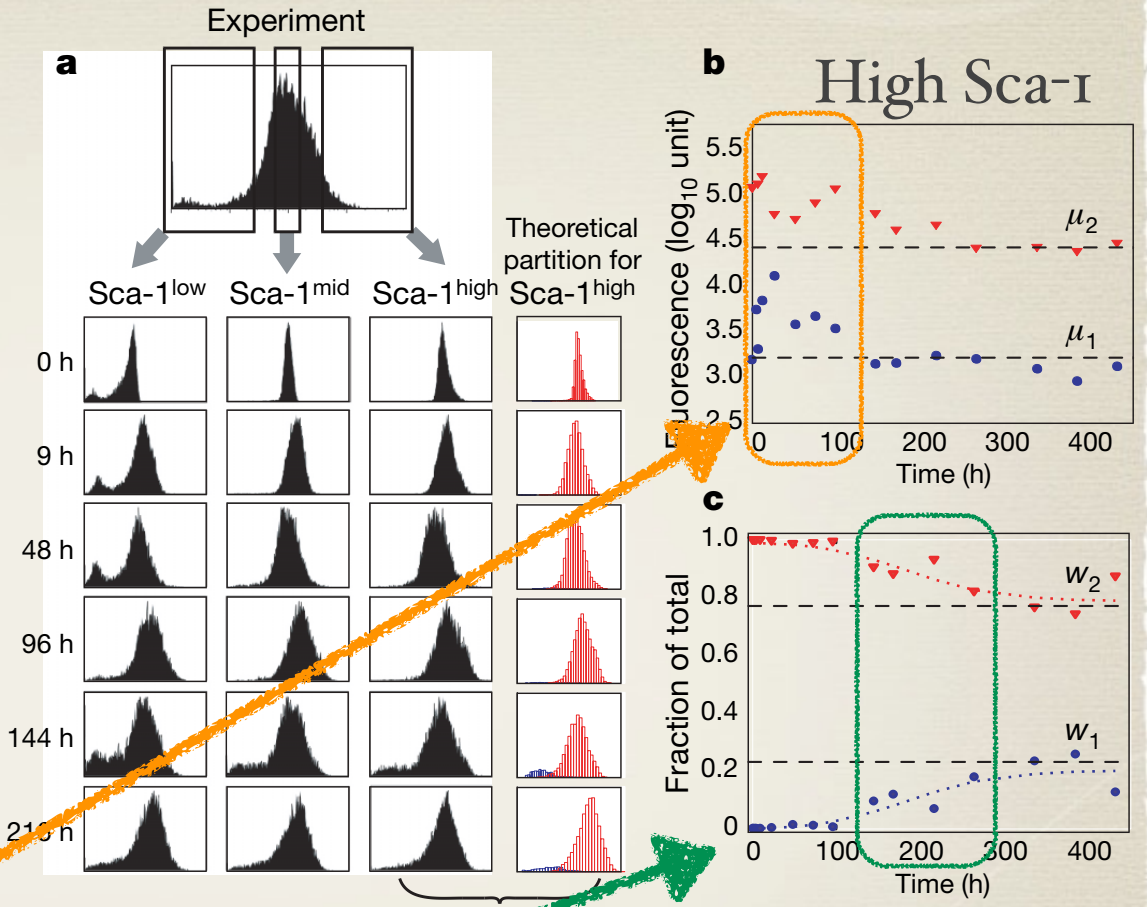
What drives the heterogeneity?

- How about this one?

2. Gaussian mixing model

- rugged landscape
- multiple meta-stable states
- relaxation within basins (sub-populations!)
- stochastic transition between states

Overlap of 2 Gaussians!



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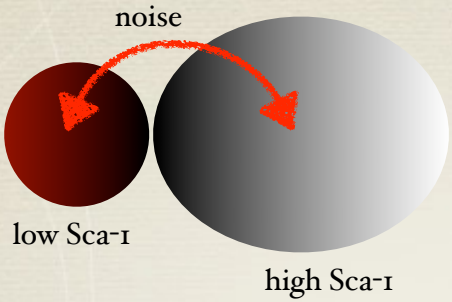
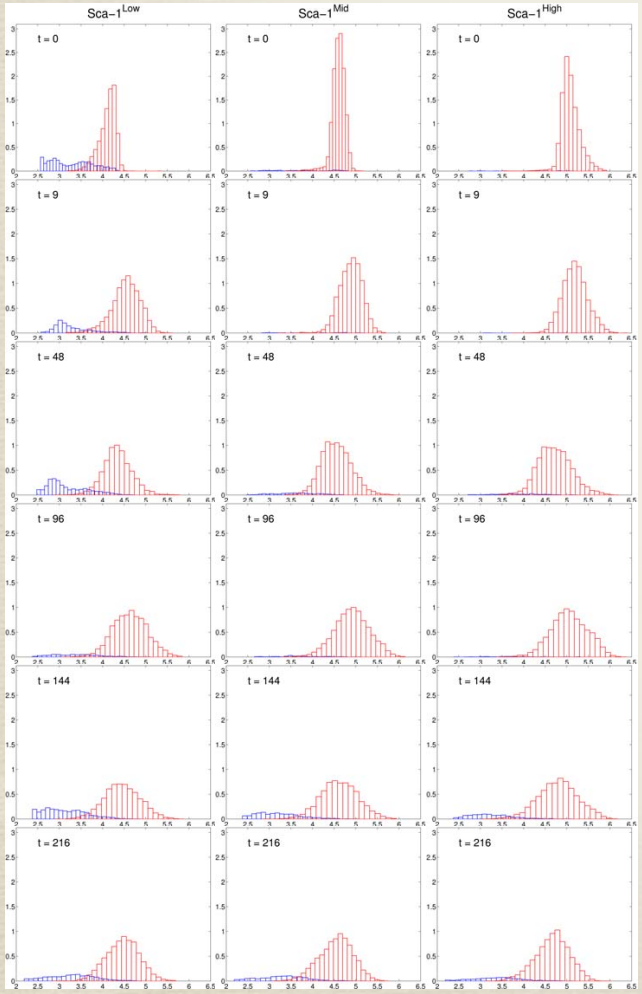
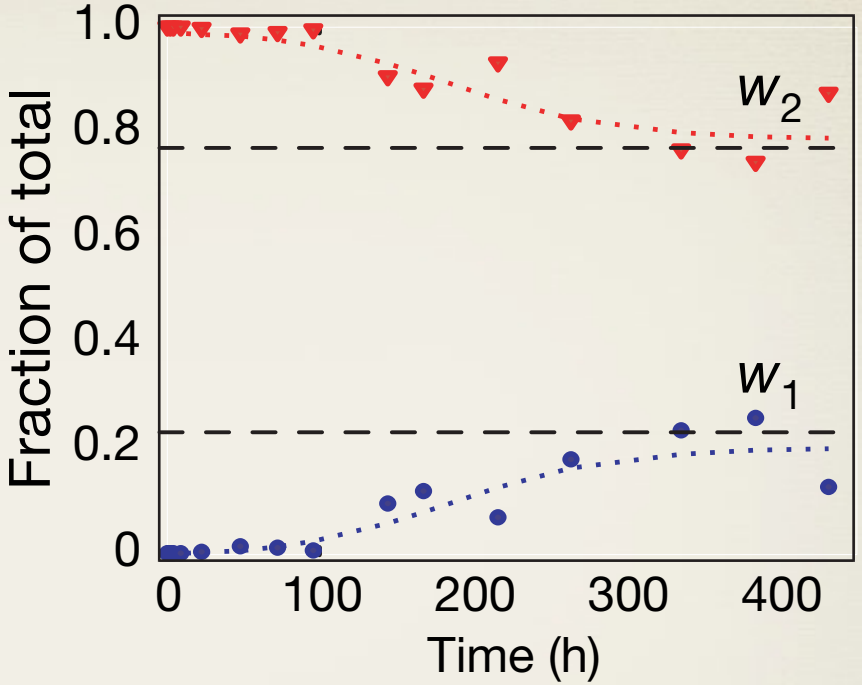
Hidden surprise in Supplementary Data!





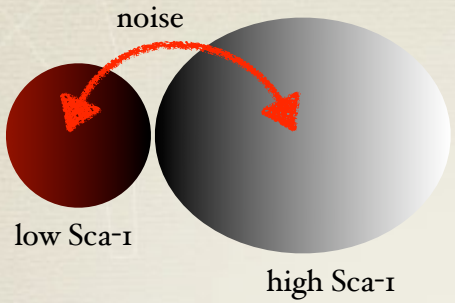


- does the fitted line mean anything?

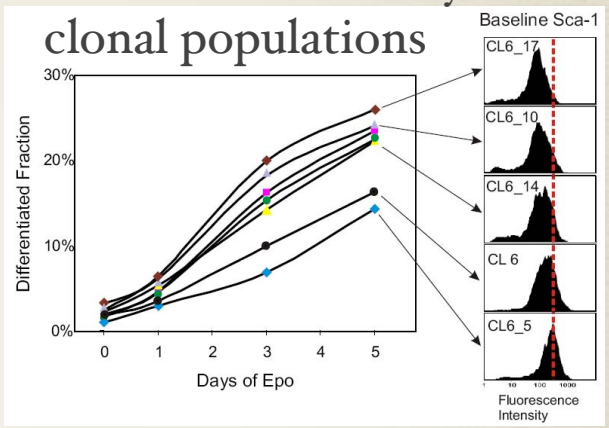
Best fit: relative population size AFFECTS transition rates!

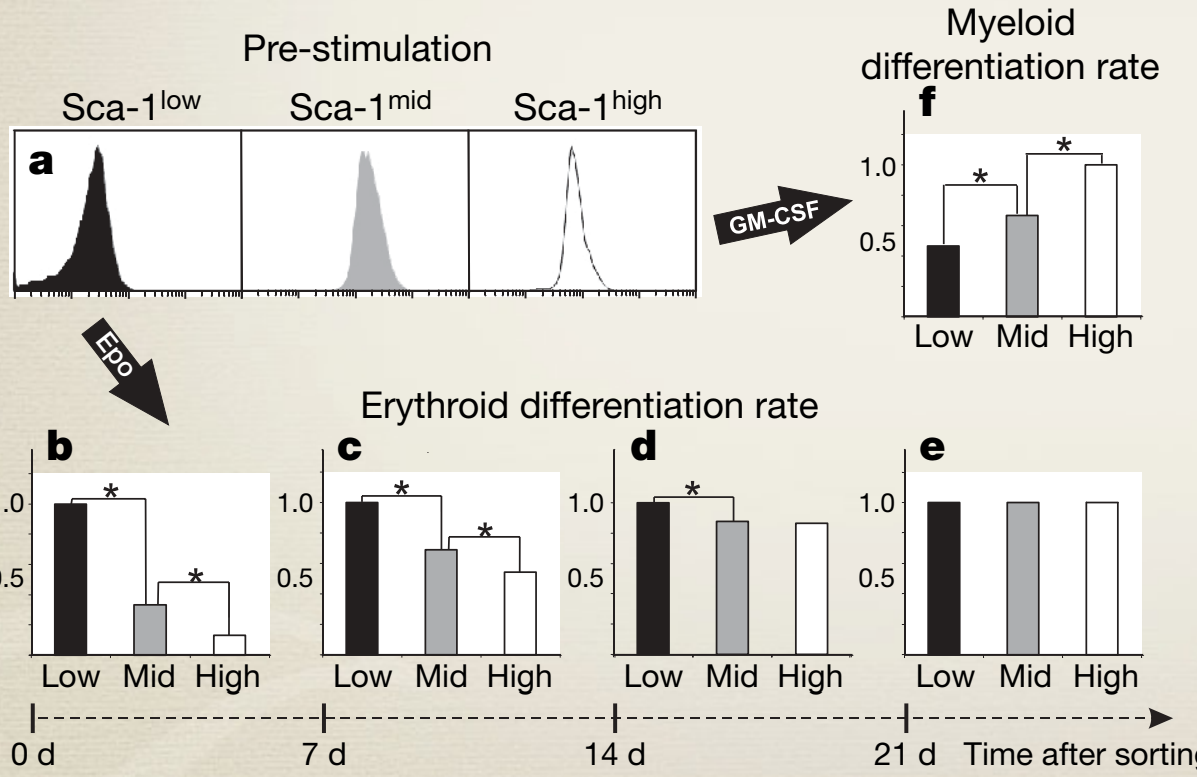
Autocrine signaling

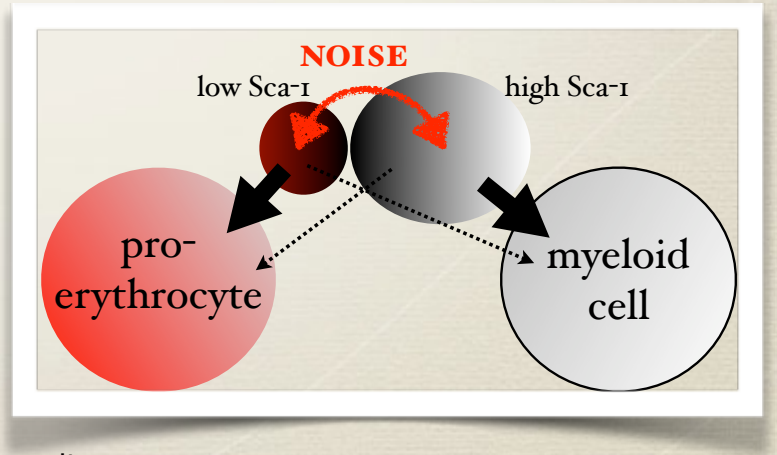
A cell population with two states!



- Is it biologically relevant?
  - how about differentiation potential?
- Similar in secondary clonal populations

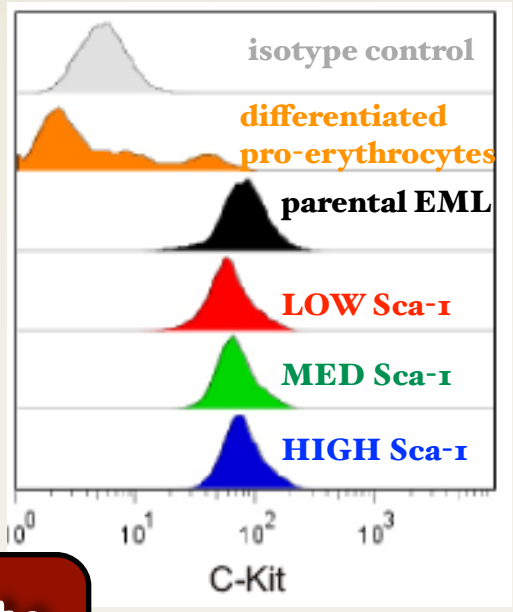


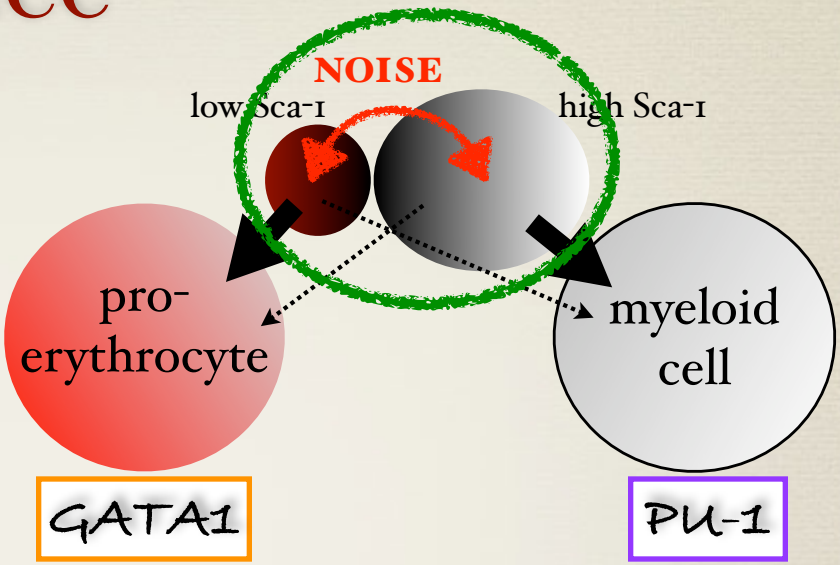




Persistent but reversible lineage preference

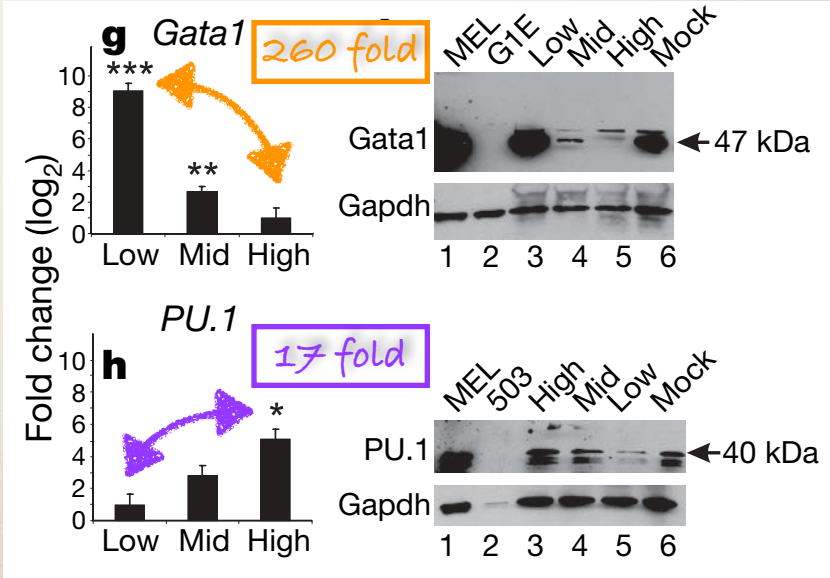
- The low Sca-1 subpopulation is not pre-comitted!
- preference lost in 3 weeks (distributions are no longer distinguishable at day 7!)



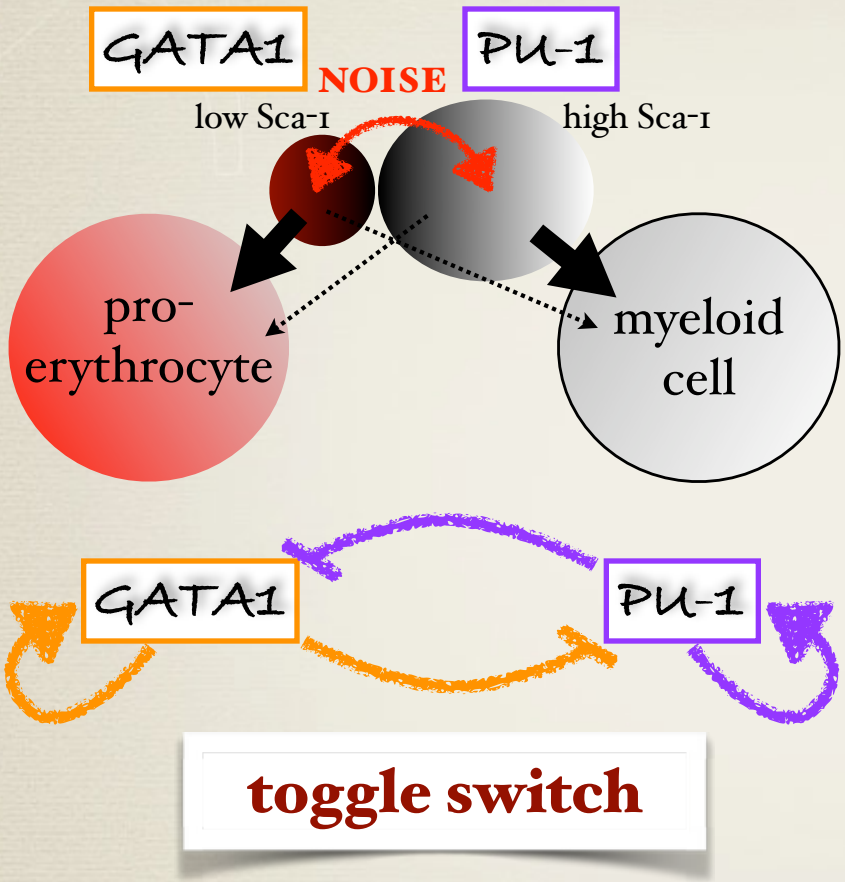


more than Sca-1 to the difference!

- how about **master regulators** of lineage ?



Lineage-specific markers drive a broad expression program!



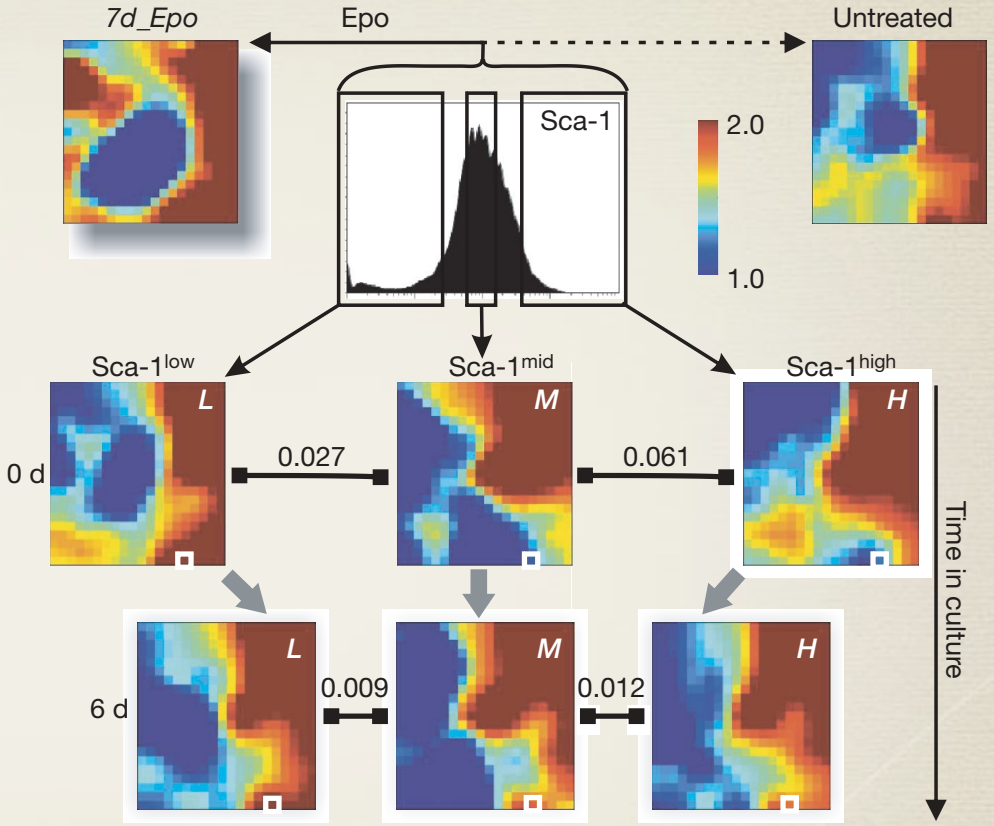


Figure 4 | Clonal heterogeneity of Sca-1 expression reflects transcriptome-wide noise. Self-organizing maps of global gene expression for a subset of 2,997 genes ... -1mid (*M*), Sca-1hi ... a differentiate ... treated control sam ... ontain the same mi ... troid value of gen ... ignal. Dissimilarit ... tal distance symbols. The Gata1-containing pixel is boxed in white.

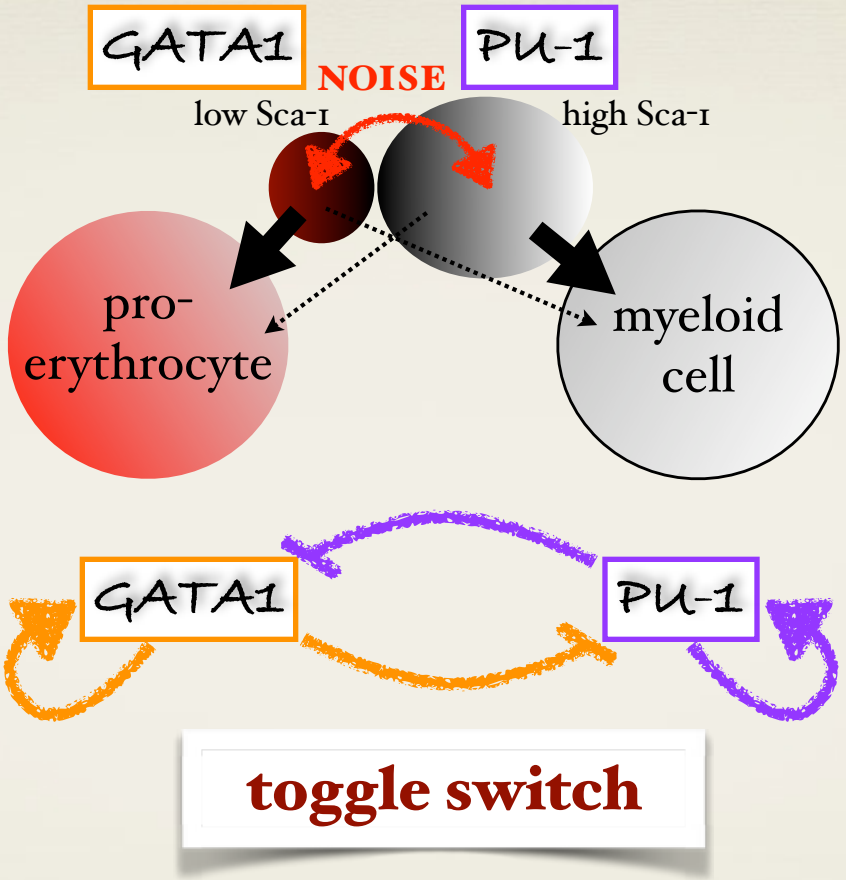
Large, transcriptome-wide differences!
>3,900 genes L <-> H

- these two master regulators do not DECIDE lineage!!!

Instructive AND Selective

Instructive

- terminal differentiation only happens upon stimulation
- master regulator expression is part of the comitment process



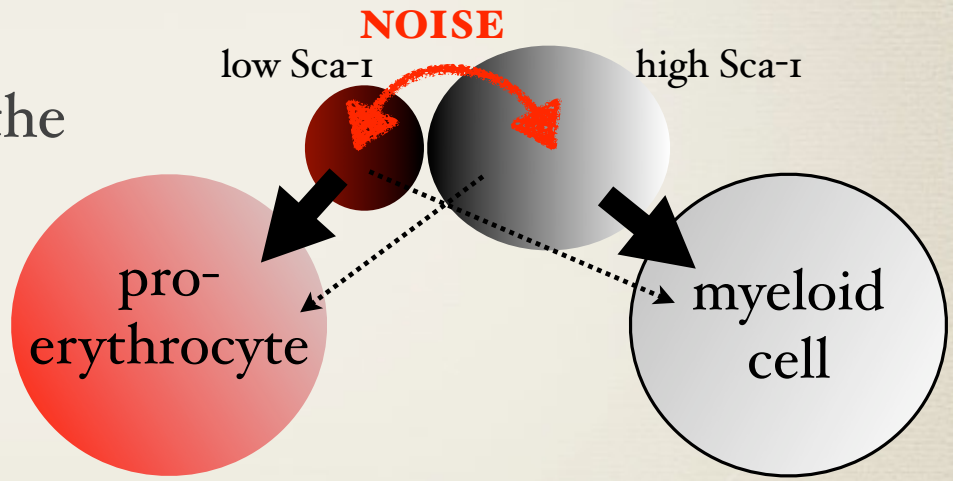
Selective

- noise can switch cells between functional states with different differentiation potentials
- master regulators do not always determine lineage choice

Noise drives preference but not pre-commitment of lineage

Outlook

- Strengths:
  - I admit I am biased: it's beautiful (inspiring is a more accepted way of putting it...)
  - it really asks us to keep in mind the complexity and non-linear nature of the regulatory network
  - supports the idea of cell states as stable attractors
- Weaknesses:
  - ??? (I have no expertise to judge the experimental techniques)



- More coming ...
  - Siu Huang's lab: working on switching cancer cell lines back to normal
  - Jim Collins's lab (bioengineer, leader in cellular noise control) with first author Hanna Chang: noise-assisted embryonic stem cell differentiation

Thank you!