Non-genetic cell-to-cell variability: theory and experiments

Anne Schwabe
Members of the Doctoral Examination Committee:

dr.ir. Yves Bollen  
Vrije Universiteit Amsterdam

dr. Robert Planqué  
Vrije Universiteit Amsterdam

prof.dr. Peter Swain  
University of Edinburgh

prof.dr.ir. Sander Tans  
FOM-Institute for Atomic and Molecular Physics

prof.dr. Bas Teusink  
Vrije Universiteit Amsterdam
Non-genetic cell-to-cell variability: theory and experiments

Anne Schwabe

geboren te Siegburg/Duitsland
promotor: prof.dr. F.J. Bruggeman

copromotoren: prof.dr. H.V. Westerhoff
dr. P.J. Verschure
## Contents

1 General Introduction .......................................................... 1
  1.1 Variability in biological systems ...................................... 2
    1.1.1 Bacterial persistence to antibiotics .......................... 2
    1.1.2 Transcription factor variability and cell fate decisions in
        embryonic stem cells ............................................. 3
    1.1.3 Phenotypic bistability of lac expression in *E. coli* ........... 4
  1.2 Measuring cell-to-cell variability of transcript numbers .......... 6
  1.3 Stochastic models .......................................................... 7
    1.3.1 Explained and “stochastic” variability .......................... 7
    1.3.2 Flipping coins and simple burst models ........................ 8
    1.3.3 Waiting time distributions - exponential and non exponential 9
    1.3.4 Queueing Theory ................................................ 13
  1.4 Aim and outline of the thesis ......................................... 14

2 Origins of stochastic intracellular processes and consequences for
  cell-to-cell variability and cellular survival strategies .............. 17
  2.1 Cell-to-cell heterogeneity and measurement techniques ............ 18
  2.2 Theoretical insights and experimental evidence .................... 19
    2.2.1 Fluctuations in molecule numbers are inevitable consequences
        of the nature of molecular reactions .......................... 19
    2.2.2 Noise in mRNA numbers at steady state ........................ 20
    2.2.3 A switching-gene model that captures many experimental
        findings .............................................................. 23
    2.2.4 Eukaryotic translation bursts and eukaryotic protein noise .... 29
    2.2.5 Noise propagation in molecular networks ........................ 31
  2.3 Beneficial and detrimental effects of molecular noise .............. 33
    2.3.1 Changing and uncertain environments; stochastic phenotype
        switching by microorganisms ........................................ 33
    2.3.2 Bistable switches in cellular decision making .................... 34
    2.3.3 Eukaryotic signaling and cell-to-cell variability ............... 36
    2.3.4 Noisy decision making in eukaryotic development ............... 37
  2.4 Conclusion ................................................................. 38

3 Volume scaling of the exact mRNA concentration indicates
  homeostasis and explains cell-to-cell heterogeneity ................... 41
  3.1 Introduction ............................................................... 42
  3.2 Results ............................................................................ 44
    3.2.1 Single-cell transcript data indicates gene-location dependent
        mRNA expression ..................................................... 44
    3.2.2 Volume statistics of single cells ................................ 46
3.2.3 mRNA concentration statistics of single cells indicate mRNA concentration homeostasis ........................................ 46
3.2.4 The volume scaling of the mRNA concentration statistics explains the concentration variability ...................... 48
3.2.5 Discussion ........................................... 50
3.3 Supplemental Information .................................. 53
  3.3.1 Materials and Methods .................................. 53
  3.3.2 The law of total variance for the copy numbers and concentrations of mRNA ........................................ 57
  3.3.3 Average mRNA copy numbers correlate well with protein expression ........................................... 62
  3.3.4 Concentration homeostasis and proportionality of the mRNA copy numbers as function of volume ............... 62
  3.3.5 Summary of the distribution statistics ....................... 66
  3.3.6 Correlations All vs All .................................. 68
  3.3.7 Probe sequence ........................................ 72

4 Single yeast cells vary in transcription activity and not in delay time after a metabolic shift 73
  4.1 Introduction ........................................... 75
  4.2 Results ............................................. 77
    4.2.1 Nutrient downshift causes a 40 minute lag phase in growth ........................................... 77
    4.2.2 Single-molecule FISH of MET5 RNA shows that all cells respond to the nutrient shifts ....................... 78
    4.2.3 Transcription induction and repression displays large cell-to-cell variability ........................................ 80
    4.2.4 Cells respond with a homogeneous time delay in transcription induction ........................................... 81
    4.2.5 Direct activation of transcription reduces delay time fourfold ........................................... 83
  4.3 Discussion ........................................... 84
  4.4 Supplemental Information .................................. 86
  4.5 Experiments ........................................... 86
    4.5.1 Strains, media, growth conditions ........................................... 86
    4.5.2 Single molecule mRNA FISH ........................................... 87
    4.5.3 Determination of intracellular methionine levels ........................................... 87
    4.5.4 Oligos for mRNA FISH ........................................... 87
  4.6 Image analysis and statistical tests ........................................... 88
    4.6.1 Image acquisition ........................................... 88
    4.6.2 Image analysis ........................................... 88
    4.6.3 Statistical tests on RNA FISH data ........................................... 89
  4.7 Data for the cadmium addition experiments ........................................... 92
  4.8 Models and Data analysis .................................. 94
    4.8.1 Inference of delay time distribution ........................................... 94
4.8.2 Deconvolution of the average and variance profiles over time with the delay time distribution .................. 96
4.8.3 Calculating the instantaneous transcription rate ........ 96
4.8.4 Transient deviations from mRNA concentration homeostasis could indicate a dependence of delay time on cell volume ... 96
4.8.5 Stochastic simulations of dividing cell populations ....... 98
4.8.6 Pearson correlation between the number of RNA molecules in the nucleus and in the cytoplasm calculated for the subset of cells ................................................. 100

5 Transcription stochasticity of complex gene regulation models 101
5.1 Introduction ...................................................... 102
5.2 Results ........................................................... 104
  5.2.1 Complex transcription regulation mechanisms .......... 104
  5.2.2 Burst-size probability distributions for different transcription mechanisms ............................................. 106
  5.2.3 The effective burst-size distribution: consideration of mRNA degradation during the burst phase ............ 108
  5.2.4 Noise in mRNA copy numbers for genes with short on periods 109
  5.2.5 Noise in mRNA copy numbers for a gene with deterministic switch times ........................................ 111
  5.2.6 Time-resolved single-molecule mRNA counting allows for model discrimination ................................. 112
5.3 Discussion ........................................................ 114
5.4 Supplemental Information ..................................... 117
  5.4.1 Molecular ratchet: reconstruction from the literature ... 117
  5.4.2 Waiting Time Distributions .................................. 117
  5.4.3 Burst-Size Distributions ..................................... 121
  5.4.4 mRNA noise for the ratchet model .......................... 122
  5.4.5 Comparison to Experimental Data .......................... 131

6 Inference of transcription dynamics from snapshot multi-color single molecule mRNA FISH 135
6.1 Introduction ...................................................... 136
6.2 Results ........................................................... 138
  6.2.1 A gene switch model with non-exponential waiting times ... 138
  6.2.2 An algorithm for fitting multi-color FISH data to the gene switch model ............................................. 139
  6.2.3 Simulated example and comparison to single-color FISH data from literature ........................................... 141
  6.2.4 Sensitivity to model assumptions ........................... 143
  6.2.5 The effects of extrinsic noise on the interpretation of fitting results ..................................................... 146
6.3 Discussion and future directions ................................ 147
6.4 Supplemental Information ........................................ 149
  6.4.1 Analytical solutions for the gene switch model .......... 149
  6.4.2 Preliminary experimental results with the MET5 gene .... 154
  6.4.3 Materials and Methods .................................... 162
  6.4.4 Image analysis in matlab ................................. 164

7 Contributions of cell growth and biochemical reactions to non-
genetic variability of cells ........................................ 181
  7.1 Introduction .................................................. 182
  7.2 Results ....................................................... 183
    7.2.1 Decomposition of molecule copy number variance into
         biochemical reaction and cell growth contributions .... 183
    7.2.2 Cell-division variance .................................... 184
    7.2.3 Mean and variance of the copy number at a particular cell age 187
    7.2.4 Variance due to the distribution of cell ages ............ 188
    7.2.5 Variance due to the biochemical reactions and (extrinsic) noise
         propagation ................................................... 189
    7.2.6 The full copy number distribution for zero-order synthesis and
         first-order degradation ....................................... 190
    7.2.7 Variance decomposition for concentrations ............... 193
  7.3 Discussion .................................................... 194
  7.4 Supplemental Information ...................................... 197
    7.4.1 Decomposition of the variance in molecule copy numbers .. 197
    7.4.2 Variance from cell division ................................ 198
    7.4.3 Variance due to partitioning for a stable molecule ....... 198
    7.4.4 Population level variance decomposition for a simple burst
         model .......................................................... 199
    7.4.5 Using generating functions to calculate the cell age dependent
         copy number distribution ..................................... 200
    7.4.6 Noise in interdivision times ............................... 203

8 General Discussion ............................................. 211
  8.1 Conclusions from this thesis .................................. 212
    8.1.1 The mRNA concentration of our reporter construct was
         homeostatic with volume .................................... 212
    8.1.2 The response time of single yeast cells to a switch in sulphur
         source proved very precise .................................. 212
    8.1.3 Coarse-grained reaction modules can often be modeled with
         gamma distributed waiting times ............................ 212
    8.1.4 Steady-state copy number distributions contain little
         information about the underlying molecular mechanisms .. 213
    8.1.5 Inference of burst statistics could be improved with FISH
         experiments using multiple probe sets in different colors .. 213
    8.1.6 Cell growth contributes to cell-to-cell heterogeneity .... 213
<table>
<thead>
<tr>
<th>Contents</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2   Outlook</td>
<td>214</td>
</tr>
<tr>
<td>8.2.1 Time-resolved measurements</td>
<td>214</td>
</tr>
<tr>
<td>8.2.2 Queueing theory</td>
<td>215</td>
</tr>
<tr>
<td>8.2.3 Reflections</td>
<td>217</td>
</tr>
<tr>
<td>Bibliography</td>
<td>219</td>
</tr>
<tr>
<td>Summary</td>
<td>250</td>
</tr>
<tr>
<td>Samenvatting</td>
<td>252</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>255</td>
</tr>
<tr>
<td>List of publications</td>
<td>257</td>
</tr>
<tr>
<td>Curriculum Vitae</td>
<td>259</td>
</tr>
</tbody>
</table>