Noise in gene expression is coupled to growth rate

Supplementary material

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Supplementary notes

Supplementary note 1: Predictions for how CV^2 should change in different growth conditions

High-throughput single-cell studies in *S. cerevisiae* (Newman et al. 2006; Bar-Even et al. 2006) and *E. coli* (Taniguchi et al. 2010) have shown that expression variability is tightly coupled to the mean expression of the population. Generally, noise decreases as mean expression increases as depicted schematically in **Fig. 1A**. At high expression levels, there is no longer a dependence on the mean, as global, extrinsic factors set a lower bound (extrinsic limit) for the overall variability (Bar-Even et al. 2006; Newman et al. 2006; Taniguchi et al. 2010). Here we speculate whether this relationship might change and how when cells are moved to slower-growth conditions.

In slower growth conditions there is a general reduction in the expression of most genes (Keren et al. 2013). Thus, it may be expected that genes will shift left along the mean-noise curve. This will result in higher noise for most genes in the slow growth condition. However, similar mean expression levels will yield similar noise values in both conditions **(Fig. 1B, line 2)**. Alternatively, it is also possible that similar mean expression values in both conditions will be coupled to higher noise levels in the slow-growth condition. This coupling can be unique to the extrinsic component (for example, due to growth-related population dynamics (Volfson et al. 2006), **Fig. 1B, line 3**), the intrinsic component (for example, due to noise propagated from RNA polymerase and ribosomes, expressed at lower levels in slow-growth conditions) or both (**Fig. 1B, line 4**). Finally, slow growth may also result in reduced levels of noise for similar values of mean expression (for example, reduced levels of extrinsic noise as cells integrate protein levels over a longer cell-cycle period (Bar-Even et al. 2006; Raser and O'Shea 2004); or reduced levels of intrinsic noise due to reduced burst sizes in slow growth, a process that has been shown to reduce noise in the population (Dadiani et al. 2013; Sanchez and Golding 2013). **Fig. 1B, line 5**). In general, current models of gene expression noise do not make clear predictions regarding the existence of global noise changes between conditions, their direction or degree as several opposing forces play a role in this process.

Supplementary note 2: Both extrinsic and intrinsic noise increase at slow growth conditions

Here, we examine whether the observed increase in the extrinsic noise limit in slow growth conditions is sufficient to account for the increase in noise for both low and high expressing genes. Extrinsic noise is an additive component (Taniguchi et al. 2010). Since we graph mean and noise on a log-log scale, a constant extrinsic noise component is observed as a noise "floor" and is only visible for low noise (high expression) genes. We note that extrinsic noise is present at the low expression (high noise) genes, however since it is additive, it is an insignificant part of the total

noise and therefore won't "shift" the noise in this regime. (For example if the extrinsic noise limit is 10^{-2} and the intrinsic noise for a low-expressing gene is 1, then the total noise of this gene will be $1+10^{-2}$=~1, regardless of whether extrinsic noise increases/decreases 3-fold). For the same reason intrinsic noise is negligible for high-expressing genes. The model and data presented by Taniguchi et al. nicely demonstrate this (see their Fig.2B, and their supplementary equations S14-17 (Taniguchi et al. 2010)).

To show what happens when the extrinsic noise level shifts we have sampled mean and noise levels for varying levels of extrinsic noise. We assume the gamma model of gene expression (Friedman et al. 2006) and randomly sample burst frequency and burst size values uniformly that translate to mean and noise (cv^2 = 1 / burst frequency * burst size, mean = $1/cv^2$). Total noise is then computed as cv^2 + c, where c is a constant extrinsic noise component. Figures S7A-D demonstrate that when extrinsic noise is increased, its elevation will only be visible for the high-expression/low-noise genes. Although in our simulation extrinsic noise has changed for all genes, its change is negligible for the low-expression/high-noise genes, and is not enough to cause a global shift in the intrinsic-noise regime. Therefore, global changes in extrinsic noise are not sufficient in order to see a shift in the low-expression/high-noise regime, as we see in the data.

Furthermore, we have analyzed our own dataset to examine the effect of changes in extrinsic noise on low-expression and high-expression genes. To this this end we generated a dataset in which mean expression levels for all conditions, as well as noise measurements for glucose remained as measured. For all other conditions noise values were assigned the noise in glucose plus the increase in extrinsic noise, derived from the real data (e.g. for every gene $Noise_{gene(i)\_galactose}$ = $Noise_{gene(i)\_glucose}$+ ($Extrinsic\_Noise\_Lim_{galactose}$-$Extrinsic\_Noise\_Lim_{glucose}$). This artificial dataset was then subjected to independent linear fitting, as done for the original data (methods). We find that while this perturbed dataset nicely recapitulates the changes in noise for the regime of high-expression/low-noise, it largely underestimates the changes in noise for the regime of low-expression/high-noise (**Fig. S7E-F**). This shows that also for our data, the global changes in extrinsic noise are not sufficient in order to see a shift in the low-expression/high-noise regime, as we see in the data. Consequently, we conclude that in slow growth conditions the global increase in extrinsic noise is accompanied by a global increase in intrinsic noise.

Supplementary note 3: G2 fraction in the population at stationary state

Several works have measured G1/G2 fractions in yeast under different batch growth conditions (Saldanha et al. 2004; O'Duibhir et al. 2014; Tyson et al. 1979). Curiously, reported G2 fractions were always above 0.3. In an attempt to reach a very low G2 fraction we measured our library in early stationary phase, following batch growth. Although we anticipated that the vast majority of cells will arrest in G1, we actually found our G2 fraction to be ~40% (**Fig. S10B**). In agreement

with our results and with previous results (Guido et al. 2007) we found that stationary cells exhibit higher levels of noise than all other conditions (**Fig. S10A**).

Supplementary note 4: Expansion of the model for CV^2 as a function of cell cycle composition of the population to incorporate intrinsic variability

We extend our model to incorporate the fact that when at G2, cells have 2 copies of their DNA. We consider each promoter as having some mean expression (E) and a variance (V). Each gene may have a unique expression expectation and variance, according to its unique properties. We assume that the two copies of DNA produce transcripts independently. Thus a cell in G1 samples once from the distribution of possible values, whereas a cell in G2 samples twice from the same distribution. According to the central limit theorem the variance of the sample sum decreases with n, so: $V_{g2} = V_{g1}/2$. Reduced noise with increased ploidy has also been shown experimentally (Di Talia et al. 2007).

We use our previous model, only this time cells do not assume only two expression values (namely, E_{g1} and E_{g2}), but rather they draw from the following bimodal distribution:

$p = (1 - f) \cdot g_1(x) + f \cdot g_2(x)$

Where

- f is the fraction of cells in G2
- $g_i(x)$ is the distribution of expression values for cells in Gi.
- $g_i(x)$ is distributed with mean E_{gi} and variance V_{gi}

Under this model

Mean expression is:

$$\mu_E = (1 - f) \cdot E_{g1} + f \cdot E_{g2}$$

Variance is:

$$\sigma^2 = (1 - f)(V_{g1} + (E_{g1} - \mu_E)^2) + f(V_{g2} + (E_{g2} - \mu_E)^2)$$

We next assume:

$$E_{g2} = 2 \cdot E_{g1}$$

$$V_{g2} = 0.5 \cdot V_{g1}$$

We get that

1) $CV^2 = \frac{Variance}{Mean^2} = \frac{V_{g1}}{{E_{g1}}^2} \cdot \frac{2-f}{2 \cdot (f+1)^2} + \frac{f(1-f)}{(f+1)^2}$

CV^2 has two additive components. The first includes the properties of the specific promoter (its variance and mean). The second is only dependent on the fraction of cells in G2

We can define:

$$\eta_{g1}^2 = \frac{V_{g1}}{{E_{g1}}^2}$$

With the parameter η_{g1}^2 summarizing the properties of the promoter. We note that:

1. The actual expression of the promoter is not important in this equation, just how variable it is.
2. By definition η_{g1}^2>0.
3. For a highly variable promoter, η_{g1}^2 is very large. For a very non-noisy promoter $\eta_{g1}^2 \to 0$ and we obtain the expression for CV^2 from part A.

In figure S15A we plot CV^2 as a function of the fraction of the population in G2 for different promoter types (different values of η_{g1}^2). We find that for genes with higher values of η_{g1}^2, the decrease in CV^2 with increasing G2 fraction (i.e., faster growth rate) is more dramatic.

Supplementary note 5: Model for CV^2 as a function of cell cycle composition of the population predicts cell-cycle regulated genes will have elevated levels of noise

Here, we develop the model presented in the main text, without the assumption that expression in G2 is twice as high as the expression in G1. This model predicts that genes whose expression cycles with cell cycle will have higher noise.

Assumptions:

1) The population of growing cells is asynchronous: at any given time some cells are in G1 and the rest in G2 (we ignore S for simplicity)
2) Differences in growth rate lead to different compositions of the population at different conditions. Growth rate mainly affects the time spent in G1, such that f, the fraction of the population in G2, increases with increasing growth rate.(Tyson et al. 1979)
3) Gene expression in G2 is higher than G1.

Now we ask how will CV^2 be affected by changes in growth rate?

We define:

E_{g1} is the expression in G1

E_{g2} is the expression in G2

μ_E is the mean population expression

f is the fraction of cells in G2

Mean expression is: $\mu_E = f \cdot E_{g2} + (1-f)E_{g1}$

Variance is: $$\sigma^2 = f(E_{g2} - \mu_E)^2 + (1-f)(E_{g1} - \mu_E)^2$$

We get that:

1) $$CV^2 = \frac{\sigma^2}{\mu_E{}^2} = \frac{f(E_{g2}-\mu_E)^2 + (1-f)(E_{g1}-\mu_E)^2}{(f \cdot E_{g2} + (1-f)E_{g1})^2}$$

We can now define

$$N = E_{g2}/E_{g1}$$

In figure 2A in the main text we assume that $= 2$. We can now plot the CV^2 as a function of f for different values of N, as is depicted in figure S8. We see that the qualitative behavior of the curve is maintained, however larger N results in increased noise.

Genes whose expression cycles across the cell cycle can be regarded in the framework of our model as having larger N. In the most extreme case, a gene which is only expressed in G2 and not at all in G1, $N \to \infty$. Therefore, for the same growth condition (i.e. a specific value of f), cell-cycle related genes (with higher values of N) are expected to have higher extrinsic noise.

Supplementary note 6: Chemostat experiments with the promoter-reporter of *RPL28*

The promoter-reporter strain for RPL28 was grown in the DasBox Bacterial mini fermentation system (DASGIP, Eppendorf) by inoculating a 1ml overnight starter to 100 ml of minimal defined (MD) media as described previously (Saldanha et al. 2004). Briefly, the media contained 0.1 g/l CaCl2:2H2O, 0.1g/l NaCl, 0.5g/l MgSO4:7H2O, 1.0g/l KH2(PO4)2, 5.0g/l (NH4)2SO4, 10gr/l Glucose supplemented with 1x vitamins mix, 1x trace elements mix, Methionine (20mg/l), Leucine (200 mg/l), & Uracil (40mg/l), and a limiting concentration of Histidine (6mg/l; this concentration was found to be the minimal concentration that elicited slowed growth, without causing detrimental effect on the cells, not shown) .

The culture was grown at 30C, 100 rpm, supplied with 100 ml filtered air/min in batch mode to mid log phase (10.5 hours), and were then switched to chemostat mode, by feeding with the above fresh MD media at the following dilution rates and order: 0.3 h-1 0.25 h-1, 0.2 h-1, 0.15 h-1, 0.1 h-1, & 0.05 h-1. Culture was let to reach steady state level and was further kept at same dilution & growth rate for at least 24 hours (typically 3-4 days) before switching to a slower dilution rate (steady state level was defined as the stage at which the levels of cell density, dissolved oxygen, pH, and glucose concentration in the media (measured by Accu-Check sticks, Roche Diagnostics) remained steady for at least a day). For each dilution rate 1.5 ml samples from the chemostats were harvested, sonicated for 5 seconds at 80pmt and analyzed by flow cytometry with stringent gating as described in methods. Sub-G1 cells (5-20% of the cells) were discarded from further analysis as described in methods.

Supplementary note 7: Model for CV^2 as a function of cell size does not predict elevated noise in conditions in which cells are smaller

Yeast vary ~2-fold in size in the experimental conditions we examined (Tyson et al. 1979). Here, we model whether a ~2-fold reduction in size is by itself predictive of higher noise levels.

We define:

Fast growing cells are of volume v. Expression, X_{fast}, is Poisson distributed with mean expression $E(x_{fast}) = \mu$ and variance $V(x_{fast}) = \sigma^2$. Slow growing cells are of volume $v/2$, and can therefore be assumed to have expression values which are twice lower. Thus, in the slow condition expression, X_{slow}, is Poisson distributed with mean expression $E\left(\frac{1}{2} \cdot x_{fast}\right) = \frac{1}{2}E(x_{fast}) = \frac{1}{2}\mu$ and variance $V\left(\frac{1}{2} \cdot x_{fast}\right) = \frac{1}{4}V(x_{fast}) = \frac{1}{4}\sigma^2$.

The expected noise for the two conditions is:

$$CV^2{}_{large} = \frac{\sigma^2}{\mu^2}$$

$$CV^2{}_{small} = \frac{\sigma^2/4}{\mu^2/4} = \frac{\sigma^2}{\mu^2}$$

Supplementary Tables

Table S2: G2 fractions at different growth rates from literature

Condition	**Fraction in G2**	**Ref.**
Glucose+AA	0.7	Slater et al., 1977 (Slater et al. 1977)
Glucose+AA	0.7	Tyson et al., 1979 (Tyson et al. 1979)
Glucose-AA	0.65	Johnston et al., 1980 (Johnston et al. 1979)
Glucose-AA	0.63	Tyson et al., 1979 (Tyson et al. 1979)
Galactose	0.51	Tyson et al., 1979 (Tyson et al. 1979)
Galactose	0.53	Tyson et al., 1979 (Tyson et al. 1979)
Ethanol	0.3	Slater et al., 1977 (Slater et al. 1977)

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