Transcription coordinates histone amounts and genome content

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Abstract

Biochemical reactions typically depend on the concentrations of the molecules involved, and cell survival therefore critically depends on the concentration of proteins. To maintain constant protein concentrations during cell growth, global mRNA and protein synthesis rates are tightly linked to cell volume. While such regulation is appropriate for most proteins, certain cellular structures do not scale with cell volume. The most striking example of this is the genomic DNA, which doubles during the cell cycle and increases with ploidy, but is independent of cell volume.

Here, we show that the amount of histone proteins is coupled to the DNA content, even though mRNA and protein synthesis globally increase with cell volume. As a consequence, and in contrast to the global trend, histone concentrations (i.e. amounts per volume) decrease with cell volume but increase with ploidy. We find that this distinct coordination of histone homeostasis and genome content is already achieved at the transcript level, and is an intrinsic property of histone promoters that does not require direct feedback mechanisms. Mathematical modelling and histone promoter truncations reveal a simple and generalizable mechanism to control the cell volume- and ploidy-dependence of a given gene through the balance of the initiation and elongation rates.

Introduction

Maintaining accurate protein homeostasis despite cell growth and variability in cell volume is essential for cell function. Most proteins need to be kept at a constant, cell-volume-independent

25 concentration. Since the amount of ribosomes and transcriptional machinery increases in proportion
 26 to cell volume, constant protein concentrations can be achieved through machinery-limited protein
 27 biogenesis, where protein synthesis depends on the availability of limiting machinery components
 28 and thus increases in direct proportion to cell volume^{1,2}. While machinery-limited regulation can
 29 maintain constant concentrations of proteins, total mRNA, and individual transcripts³⁻⁶, it poses a
 30 conundrum for histones. As components of nucleosomes, histones are likely needed at a constant
 31 protein-to-DNA stoichiometry, implying that their amount should increase with ploidy but be
 32 independent of cell volume. In other words, histone concentration, *i.e.* amount per volume, should
 33 increase with ploidy but decrease with cell volume. Since accurate histone homeostasis is crucial for
 34 fundamental biological processes⁷⁻¹⁰ and to avoid toxic effects¹¹⁻¹³, cells use several layers of
 35 regulation by translation, transcription and degradation to tightly coordinate histone production with
 36 genome replication¹⁴⁻¹⁶. However, how cells produce histones in proportion to genome content, even
 37 though protein biogenesis is generally linked to cell volume remains unclear.

38 Here, we use budding yeast as a model to show that histone protein amounts are coupled to genome
 39 content, resulting in a decrease of histone concentration in inverse proportion with cell volume, and
 40 an increase in direct proportion with ploidy. We find that this specific regulation of histones is
 41 achieved at the transcript level and does not require direct feedback mechanisms. While our data
 42 suggest that 3'-to-5'-degradation by the nuclear exosome is necessary for the correct decrease of
 43 concentration with cell volume, we show that histone promoters alone are sufficient to couple
 44 transcript amounts to gene copy number rather than cell volume. Our results suggest that this
 45 differential regulation of histones can be achieved through template-limited transcription, where
 46 mRNA synthesis is limited by the gene itself and does therefore not increase with cell volume. This
 47 provides a general mechanism by which cells can couple the amount of a subset of proteins to
 48 genome content while most protein concentrations are maintained constant.

49

50 Results

51 Histone protein concentrations decrease with cell volume and increase with ploidy

52 Typically, total protein amounts as well as the amounts of individual types of protein increase
 53 roughly in direct proportion to cell volume to maintain constant concentrations. However, such
 54 regulation is inappropriate for histones, whose amount we predicted should be coupled to the cellular
 55 genome content instead. To test if this is the case, we chose the budding yeast histone *HTB2*, one of
 56 two genes encoding for the core histone H2B, as an example, because it can be fluorescently tagged
 57 without pronounced effects on cell growth. We endogenously tagged *HTB2* with the fluorescent
 58 protein *mCitrine* in a haploid strain, and measured cell volume and the amount of Htb2-mCitrine as a
 59 function of time in cycling cells by microfluidics-based live-cell fluorescence microscopy^{17,18}. To
 60 obtain a large range of cell volumes, we grew cells on synthetic complete media with 2% glycerol
 61 1% ethanol as a carbon source (SCGE). As expected¹⁴, we find that Htb2 amounts are constant
 62 during early G1, rapidly double during S-phase and reach a plateau before cytokinesis (**Fig. 1a**). We
 63 then quantified the Htb2-mCitrine amounts in new-born cells directly after cytokinesis and find that
 64 the amount of Htb2-mCitrine is largely constant, independent of cell volume (**Fig. 1b**). To further
 65 test whether histone amounts are coupled to genomic DNA content rather than cell volume, we next
 66 analyzed a diploid strain in which both alleles of *HTB2* are tagged with *mCitrine*. Indeed, Htb2-
 67 mCitrine amounts in diploid cells are approximately a factor of two higher than in haploid cells (**Fig.**
 68 **1b**). To more accurately compare Htb2 concentrations in haploids and diploids of similar volume, we
 69 sought to increase the overlapping range of observable volumes in both strains. For this purpose, we
 70 deleted the endogenous alleles of the G1/S inhibitor *WHI5* and integrated one copy of *WHI5*
 71 expressed from an artificial, β -estradiol-inducible promoter system¹⁹ (**Fig. 1c**). Using this system, we
 72 were able to increase the mean volume of steady-state exponentially growing populations by up to
 73 three-fold through overexpression of Whi5 (**Fig. 1d**) without drastically affecting doubling times,
 74 budding indices or cell cycle distributions (**Supplementary Fig. 1**). We repeated the microscopy

75 experiments described above with the inducible-Whi5 haploid and diploid strains in the presence or
 76 absence of β -estradiol. Again, we find that Htb2-mCitrine amounts are only very weakly dependent
 77 on cell volume, but show a roughly two-fold increase in diploid compared to haploid cells
 78 (**Supplementary Fig. 2a**). Consistently, we find that the concentration of Htb2-mCitrine at birth in
 79 both haploid and diploid cells decreases strongly with cell volume (**Fig. 1e**). To quantify this
 80 decrease, we performed a linear fit to the double-logarithmic data, and defined the slope as the
 81 ‘volume-dependence-parameter’ (VDP). The observed VDPs of -0.87 ± 0.04 (haploids) and
 82 -0.97 ± 0.03 (diploids), respectively, are close to the value of -1 expected for proteins that are
 83 maintained at constant amount, resulting in a decrease of concentration with $c \sim 1/V$. In contrast,
 84 proteins that are maintained at constant concentration would show a VDP of 0.

85 In budding yeast, histones are known to be tightly regulated at several layers. In particular, some
 86 histone genes – but not *HTB2* – exhibit dosage compensation at the transcript level^{20–22}. In addition,
 87 excess histones are known to be degraded¹⁶. In principle, a coupling of histone amounts to genomic
 88 DNA content could be achieved through such feedback mechanisms: For example, larger cells may
 89 produce histones in excess, and then degrade the surplus. Alternatively, direct feedback of histone
 90 protein concentration on transcription could ensure that histones are expressed only until the protein
 91 amount matches the genome content. To test whether direct feedback of histone amounts on
 92 transcription, translation, or degradation is necessary to couple histone production to genome
 93 content, we again focused on Htb2, because it was already shown to not exhibit dosage
 94 compensation at the transcript level²¹. We constructed an inducible-Whi5 diploid strain in which we
 95 deleted one of the two *HTB2* alleles, while the other allele is tagged with *mCitrine* (**Fig. 1f**). If
 96 feedback were responsible for the coupling of Htb2 amount to genome content, the remaining *HTB2*-
 97 *mCitrine* allele should at least partially compensate for the deleted allele. However, consistent with
 98 the absence of any feedback, we find that Htb2-mCitrine concentrations are reduced by factor of two
 99 in the hemizygous compared to the homozygous diploid (**Fig. 1g, Supplementary Fig. 2b**).

Moreover, at a characteristic volume of 60 fL, at which we find both haploid and diploid new-born cells, the concentration of Htb2-mCitrine in the hemizygous strain roughly equals the concentration in the haploid (**Fig. 1g**). While it is still possible that the reduced concentration of Htb2-mCitrine is compensated by an increased concentration of the other H2B copy Htb1, our results suggest that no direct feedback is required to couple Htb2 amounts to genome content. Instead, Htb2 amounts are intrinsically determined by the *HTB2* gene copy number, independent of ploidy and cell volume.

Histone mRNA concentrations decrease with cell volume

The fact that the decrease of histone protein concentrations with cell volume is not simply a consequence of feedback, for example through excess protein degradation, suggests that it might already be established at the transcript level. To test if this is the case, we again employed the Whi5-overexpression system to measure the cell-volume-dependence of transcript concentrations (**Fig. 2a**). Specifically, we grew wild-type haploid cells, as well as the inducible-Whi5 haploid cells at three different β -estradiol concentrations (0, 10 and 30 nM), on SCGE media, which lead to a roughly four-fold range in mean cell volumes ranging from 39 ± 4 fL to 143 ± 21 fL (**Supplementary Fig. 3a**). To ensure steady state conditions, we grew cells for at least 24 hours at the respective β -estradiol concentration, before then measuring cell volume distribution, extracting total RNA, and performing reverse-transcription-qPCR (RT-qPCR). First, we measured the concentration of the ribosomal RNA *RDNI8* relative to total RNA and found it to be constant (**Supplementary Fig. 4a**). This is consistent with the fact that ribosomal RNA constitutes the large majority of total RNA²³, which itself is expected to increase in direct proportion to cell volume²⁴ and allows us to now normalize other RT-qPCR measurements on *RDNI8*.

Next, we quantified the mRNA concentrations of *ACT1* and *ENO2*, two genes that we expect to be expressed in proportion to cell volume such that the mRNA concentration are maintained constant. Indeed, we find that the VDPs for both transcripts are not significantly different from 0 (**Fig. 2b & d**,

124 **Supplementary Fig. 4b**). Interestingly, as previously suggested²⁵ we observe a slight decrease in
 125 concentration for the transcripts of the RNA polymerase II subunits *RPB1* and *RPB3* with increasing
 126 cell volume (**Fig. 2d, Supplementary Fig. 4c**). We then quantified the concentrations of the
 127 transcripts of all core histone genes as well as the H1-like histone *HHO1*. In budding yeast, all core
 128 histone genes are present as two copies and expressed from bidirectional promoters controlling pairs
 129 of *H2A-H2B*²⁶ or *H3-H4*²⁷, respectively. Since the two copies of each core histone show high
 130 sequence similarity, we performed additional tests using deletion strains where possible to ensure
 131 qPCR primer specificity (**Supplementary Table 2**). We find that all histone transcripts show a
 132 significant decrease in concentration with cell volume, which is specific to the Whi5-dependent cell
 133 volume increase (**Supplementary Fig. 3b – d**). The histone mRNAs mostly exhibit VDPs close to -1
 134 (**Fig. 2c & d, Supplementary Fig. 4d**). Thus, histone mRNA concentrations decrease with cell
 135 volume to ensure constant amounts – in contrast to global transcription, which increases with cell
 136 volume.

137 **Hir1-dependent feedback is not necessary for cell-volume-dependence of histone mRNA** 138 **concentrations**

139 The observation that histone transcript concentrations decrease with $c \sim 1/V$ suggests that, similar to
 140 histone protein amounts (**Fig. 1e**), also histone transcript amounts are determined by gene copy
 141 number. We therefore measured the concentrations of representative histone transcripts in inducible-
 142 Whi5 diploids homozygous or hemizygous for *HTB2*. Again, we find that all histones analyzed
 143 exhibit a VDP close to -1 (**Supplementary Fig. 5a**), and as observed for Htb2 protein concentrations
 144 (**Fig. 1f**), the concentration of *HTB2* transcripts at a characteristic volume of 60 fL is clearly reduced
 145 in hemizygous compared to homozygous diploids (**Fig. 2e**). Moreover, we do not observe a
 146 significant overexpression of *HTB1* to compensate for the reduced *HTB2* transcript concentration
 147 (**Fig. 2e**).

148 So far, we have shown that in diploid cells with only one *HTB2* allele, the concentrations of *HTB2*
 149 transcript and protein are reduced compared to wild-type diploid cells. This highlights the absence of
 150 direct feedback mechanisms sensing and controlling the concentration of Htb2 with cell volume.
 151 However, extensive previous studies have shown that the eight budding yeast core histone genes
 152 show remarkably different modes of regulation. Specifically, only the gene pair *HTA1-HTB1* is
 153 known to exhibit dosage compensation, which is absent for *HTA2-HTB2*^{20–22}. Moreover, three out of
 154 four core histone gene pairs, not including *HTA2-HTB2*, show negative feedback regulation of
 155 transcript concentration upon replication stress^{14,28}. This feedback regulation is thought to be
 156 mediated by the HIR complex and to be dependent on *HIR1* and *RTT106*^{29–31}. Thus, to test if HIR-
 157 dependent sensing and feedback regulation of histone transcript concentration may also be
 158 responsible for the cell-volume-dependence of HIR-regulated histone genes, we measured the cell-
 159 volume-dependence of representative histone genes (*HTB1*, *HTB2*, *HHF1*, *HHO1*) in *hir1Δ* and
 160 *rtt106Δ* strains. Strikingly, we find that neither Hir1 nor Rtt106 are needed for the decrease of
 161 concentration with cell volume for any of the tested histone transcripts (**Fig. 2f, Supplementary Fig.**
 162 **5b**).

163 **3'-to-5'-degradation by the nuclear exosome is not necessary for cell-volume-dependence of** 164 **histone mRNA concentrations**

165 The fact that the correct dependence of histone transcript concentration on cell volume does not
 166 require direct feedback suggests that instead it is an intrinsic property of either transcription rate or
 167 mRNA degradation. To test if degradation from the 3'-end by the nuclear exosome is required, we
 168 analyzed the cell-volume-dependence of histone transcript concentrations in strains where we deleted
 169 *RRP6*, a component of the nuclear exosome exonuclease^{32,33}. As shown in **Fig. 2g**, we find that also
 170 in *rrp6Δ* cells, histone transcript concentrations decrease with cell volume. Interestingly, due to
 171 increased transcript concentrations in small cells (**Fig. 2h, Supplementary Fig. 5c**), this decrease
 172 with a VDP close to -2 is significantly stronger than in wild-type cells, suggesting that the volume-

dependence of histone transcripts is modulated by Rrp6-dependent degradation. Thus, while degradation by the nuclear exosome is not needed for the volume-dependent decrease of histone transcript concentrations, it may contribute to achieve the correct VDP of -1.

Histone promoters are sufficient for cell-volume-dependence of transcript concentrations

Given that degradation from the 3'-end does not seem to be crucial for the cell-volume-dependent decrease of histone transcript concentration, we next asked whether the promoter alone is sufficient to establish this cell-volume-dependence. To address this, we created strains that carry additional copies of either the *ACT1* or the histone *HHF1* promoter driving expression of the fluorescent protein mCitrine, regulated by the identical *ADHI* terminator (**Fig. 3a**). Strikingly, we find that the dependence of *mCitrine* transcript concentration on cell volume is determined by the promoter: If driven by the *ACT1* promoter, the VDP of *mCitrine* resembles that of endogenous *ACT1*; if driven by the *HHF1* promoter, it resembles that of endogenous *HHF1* (**Fig. 3b**).

To test if this also holds true for other histone promoters, we made use of the fact that the fluorescent reporter mCitrine enables a faster experimental readout using flow cytometry (**Fig. 3a**). First, we analyzed the cell-volume-dependent fluorescence of mCitrine expressed from the *ACT1* or *HHF1* promoters, which revealed that flow cytometry can be used to qualitatively distinguish the distinct volume-dependences. Similarly, we find that also all other histone promoters tested show significantly negative VDPs in haploid and diploid cells (**Fig. 3c – e, Supplementary Fig. 6**).

Histones not only need to be maintained at cell-volume-independent amounts, leading to a decrease of concentration with $1/V$, but also need to increase in proportion to cell ploidy (**Fig. 1**). This is in contrast to most other genes, which are maintained at a ploidy-independent concentration³⁴. To test if the histone promoters are also sufficient to establish this distinct ploidy-dependence, we compared the expression level of the single *mCitrine* copy in diploid versus haploid cells. For *ACT1*, which needs to be maintained at a ploidy-independent concentration, we expect that a single gene allele in a

diploid should produce half of the protein compared to a homozygous diploid or haploid of similar volume². Indeed, for the *ACT1* promoter we find that at a given cell volume, the concentration of mCitrine expressed from a single additional promoter is 50% lower in diploids compared to haploids (**Fig. 3d & f**). In contrast, for each of the three histone promoters tested, we observe that the concentration in diploids is considerably higher than 50% of that in haploids of comparable volume, with a ratio close to 1 for the *HTB1* promoter (**Fig. 3e & f, Supplementary Fig. 6**). This demonstrates that in addition to setting the cell-volume-dependent decrease in concentration, regulation by the histone promoters also largely accounts for the fact that histones are needed in proportion to ploidy.

Different cell-volume and ploidy dependences can be explained by competition of promoters for limiting transcriptional machinery

To better understand how the transcription rate of one specific promoter depends on cell volume and ploidy context, we sought to build a minimal model (**Fig. 4a**). Briefly, we considered two classes of promoters, a specific promoter of interest, p , present as a single copy, and a general pool of promoters, g , which are present as $n_h = 6000$ in haploids or $n_d = 12000$ copies in diploids. We then assume that transcription can be described by a single component of the transcriptional machinery, whose concentration c_{TM} stays constant with cell volume. Each promoter is competing for the transcriptional machinery, and is modelled as a single binding site for the limiting machinery component. Initiation, *i.e.* binding of the limiting machinery, occurs at a rate k_{on}^p or k_{on}^g , respectively. Furthermore, we assume that all other steps of transcription can be summarized in a single rate-limiting step, occurring at a rate k_{off}^p or k_{off}^g , respectively. Each transcript is then degraded with the same rate $k_{deg} = 1$. Depending on the parameters chosen for the specific promoter, the model predicts qualitatively different dependences of transcript concentration on cell-volume and ploidy (**Fig. 4b & c**). For example, at a given k_{off}^p , a high on-rate k_{on}^p can result in

221 histone-promoter-like behavior, *i.e.* cell volume-dependent but ploidy-independent transcript
222 concentration. In contrast, at lower k_{on}^p we observe actin-promoter-like behavior, *i.e.* cell volume-
223 independent but ploidy-dependent transcript concentration. Interestingly, due to the competition with
224 general promoters, the transcript concentration can even increase with cell volume if k_{on}^p is much
225 smaller than k_{on}^g .

226 One key prediction of this model is that if all other parameters are fixed, reducing k_{on}^p for a histone-
227 like promoter should eventually shift its behavior to that of an actin-like promoter (**Fig. 4d**). To
228 experimentally test this prediction, we aimed to decrease the initiation rate k_{on}^p of the *HHF1* and
229 *HTB1* promoters by creating series of haploid and diploid strains with increasingly shorter fragments
230 of the promoters, each truncated from the 5'-end (**Fig 5a**). Again, we used flow cytometry to analyze
231 mCitrine expression driven by these additional, endogenously integrated promoter fragments. For
232 both promoters we observe a decrease of mCitrine expression once part of the known upstream
233 activating sequences (UASs)³⁵ are truncated (**Fig 5b, Supplementary Fig. 7a**). Fully consistent with
234 the model, for both promoters, and for haploids and diploids, this drop in expression coincides with a
235 change of the VDP towards 0 (**Fig 5b & c, Supplementary Fig. 7b & c**). At the same time and also
236 consistent with the model, the ratio of the mCitrine concentration at a given volume in diploid
237 compared to haploid cells decreases from close to 1 towards 0.5 (**Fig. 5c**). Thus, our analysis shows
238 that for both the *HHF1* and *HTB1* promoter truncation series, a transition from histone-like to actin-
239 like behavior occurs between the 450 bp to 300 bp truncations.

240 While we consistently observe the same qualitative trend in flow cytometry measurements, we note
241 that the exact VDP depends on the forward scatter settings, which determine the observed cell-
242 volume range. Thus, to quantitatively confirm our results, we repeated the experiment for the 450 bp
243 and 300 bp truncations of the *HTB1* promoter using RT-qPCR. Again, we observe a change in the
244 VDP towards 0, and a decrease of the ratio of the mCitrine concentration between diploid and

haploid cells from close to 1 to close to 0.5 (**Fig. 5d**). In summary, our analysis of the histone promoter truncations demonstrates that decreasing promoter strength can shift the volume- and ploidy-dependence of the histone promoters to an actin-like behavior, as predicted by our minimal model.

Discussion

Taken together, we identified a mechanism that allows cells to deal with a fundamental challenge – how to quantitatively couple histone production to DNA content even though total biosynthetic capacity is linked to cell volume instead. We found that this coordination is already achieved at the transcript level. While mRNA degradation and feedback mechanisms contribute to histone homeostasis, we find that competition for potentially limiting transcriptional machinery is sufficient to achieve differential regulation of histone and other transcript concentrations with cell volume and ploidy. Specifically, if transcription is limited by the availability of limiting machinery, larger cells with more machinery will produce proportionally more mRNA, maintaining constant transcript concentrations, which do not depend on ploidy. If transcription is instead limited by the gene itself, transcript concentrations will decrease with cell volume but will be proportional to ploidy. In addition to histones, other proteins will require differential regulation. For example, the G1/S inhibitors Whi5 in yeast¹⁸ and Rb in mammalian cells³⁶ have recently been shown to decrease in concentration with cell volume, enabling cells to sense and control their size. Along those lines, a recent study suggested that many cell cycle regulators show differential transcriptional regulation with cell volume³⁷. The simplicity of template-limited transcription therefore suggests that it may be broadly employed across species to differentially regulate the concentrations of larger subsets of proteins, in particular to couple the amount of DNA binding proteins to DNA content. Moreover, in addition to the ideal template- or machinery-limited regimes, cells can achieve a large variety of cell volume- and ploidy-dependences, which importantly can be decoupled from the expression level of a given gene by independently tuning its initiation and elongation rates. Specific regulation of mRNA

and protein degradation provides yet another level of control that cells can employ to tune the dependence of protein concentrations on cell volume and ploidy. In fact, our observation that the cell-volume-dependence of histone transcripts is even stronger in *rrp6* deletion cells, suggests that such additional regulation contributes to cell-volume-dependent histone homeostasis in budding yeast. To quantitatively understand the cell volume- and ploidy-dependence of protein homeostasis on a genome wide level, it will therefore be crucial to identify the rate-limiting steps of transcription and mRNA degradation as well as the corresponding rate-limiting molecules.

Materials and methods

Yeast strains

All yeast strains used in this work are based on W303 and were constructed using standard methods. Full genotypes of all strains are listed in **Supplementary Table 1**.

Inducible-Whi5 strain

In order to increase the range of observable cell volumes, we used strains with β -estradiol inducible *WHI5*, similarly described in previous works^{18,38}. For this purpose, we deleted the endogenous alleles of the G1/S inhibitor *WHI5* and integrated one copy of *WHI5* expressed from an artificial, β -estradiol-inducible promoter system¹⁹. Specifically, this inducible promoter system consists of a β -estradiol-dependent, artificial transcription factor, which can bind an artificial promoter. This promoter is then used to induce *WHI5* expression.

To ensure that β -estradiol addition itself has no effect on cell growth, we grew cell cultures of a non-inducible *WHI5* haploid strain and cell cultures of a *whi5 Δ* haploid strain, containing the β -estradiol-dependent, artificial transcription factor, but no copy of *WHI5*. We then added β -estradiol to those cultures and quantified the mean cell volumes after 24 h of growth in the presence of β -estradiol, by measuring the cell volume distributions using a Coulter Counter (Beckman Coulter, Z2 Particle Counter). Finally, we compared the mean cell volumes to the mean cell volumes obtained from cell

populations without β -estradiol addition (**Supplementary Fig. 3a**). In addition, we performed reverse-transcription-qPCR (RT-qPCR) on cell populations with and without β -estradiol addition and compared the obtained mean values for several genes (**Supplementary Fig. 3b & c**). For the non-inducible *WHI5* haploid strain, we could not identify a significant deviation of the population means between the cell populations with and without β -estradiol addition. For the *whi5Δ* haploid strain, containing only the β -estradiol-dependent, artificial transcription factor, we observed a slight but significant reduction of the relative mean mRNA concentrations of *HTA2*, *HHF2* and *HHO1* at 30 nM compared to 0 nM β -estradiol, which was consistent with a slightly increased mean cell volumes at 30 nM β -estradiol. In contrast, performing the same experimental procedure on cell cultures of an inducible *WHI5* haploid strain, leads to much stronger changes of mean cell volumes and relative mean mRNA concentrations for all histone genes, demonstrating that the observed decrease of histone mRNA concentrations is specific to the Whi5-dependent cell volume increase (**Supplementary Fig. 3a & d**). Significances were tested using two-tailed two-sample t-tests, after checking for normal distribution and equal variance distributions using a Shapiro-Wilk test and a Bartlett test, respectively.

Live-cell fluorescence microscopy

Cultures (3 mL) were grown at 30°C in synthetic complete media containing 2% glycerol and 1% ethanol (SCGE) for at least 6 h in a shaking incubator at 250 rpm (Infors, Ecotron). Appropriate β -estradiol concentrations were then added to inducible cells (0 nM and 30 nM for haploids or 50 nM for diploids) and the cultures grown for at least 24 h to ensure steady-state conditions. Optical densities were measured using a spectrophotometer (Perkin Elmer, Lambda Bio+) and $OD_{600} < 1.0$ was maintained through appropriate dilutions during culture growth. For imaging, 1 mL of cells ($OD_{600} < 1.0$) was spun down at 10k g-force for 1 min (Thermo Fisher Scientific, Pico 17), resuspended in 200 μ L SCGE and sonicated for 5 s (Bandelin electronics, HD2070 & UW2070).

318 100 μ L of this cell suspension was then introduced in a Cellasic microfluidics Y04C (haploids and
319 non-induced diploids) or Y04D (induced diploids) plate.

320 Live-cell fluorescence microscopy experiments were performed on a Zeiss LSM 800 microscope
321 with additional epifluorescence setup using a Cellasic microfluidics device to ensure constant media
322 (SCGE) flow in the microfluidics plate throughout the experiment. Experiments ran for 12 h with
323 images being taken every 3 min using an automated stage (WSB Piezo Drive Can), a plan-
324 apochromat 40x/1.3 oil immersion objective and an axiocam 506 camera. Phase-contrast images
325 were taken at an illumination voltage of 4.5 V and an exposure time of 30 ms. *mCitrine* images were
326 taken using the Colibri 511 LED module at 25% power and an exposure time of 10 ms. For each
327 condition, at least two independent biological replicates were measured on different days.

328 To correct for inaccuracies of the x-y-stage between time points, movies were first aligned using a
329 custom Fiji script. Then, cell segmentation and quantification of the fluorescent signal as well as
330 subtraction of background fluorescence and cell-volume-dependent autofluorescence (determined
331 from control strains not expressing a fluorescent protein), and determination of time points of cell
332 birth, bud emergence, and cytokinesis were performed with MATLAB 2017b using previously
333 described methods^{17,18,39}. For our analyses, we only included cells born during the experiment. Total
334 fluorescence intensity after background- and autofluorescence correction was used as a proxy for
335 total protein amount.

336 In order to determine total protein concentrations as total protein amounts divided by cell volume, we
337 calculated cell volumes based on phase-contrast images. Briefly, after segmentation, cell areas were
338 aligned along their major axis. We then divided the cells into slices perpendicular to their major axis,
339 each 1 pixel in width. To estimate cell volume, we then assumed rotational symmetry of each slice
340 around its middle axis parallel to the cell's major axis, and summed the volumes of each slice to

obtain total cell volume. This allowed us to analyze protein amounts and protein concentrations as a function of cell volume.

Estimation of cell cycle phases and histone production period using live-cell microscopy

To test whether the decrease of histone concentrations with cell volume could be explained by a decrease in the S-phase duration, and thus a shorter time period during which histones are produced, we aimed to estimate the duration of the *histone production period* (H-period) from the Htb2-mCitrine fluorescent intensity traces. For each single cell, we first performed a constant linear fit in each of the two plateaus of the fluorescence intensity, linked to G1- or G2/M-phase, respectively, and denoted them as P_1 and P_2 . P_1 was obtained by performing the linear fit through the data points of the fluorescent intensity trace from cell birth to first bud emergence, P_2 was obtained by performing the linear fit through the last 30 minutes of the fluorescent intensity trace. We then set a threshold of 5%, determined the last time point for which $I_{Htb2-mCitrine} < P_1 + 0.05 \cdot P_1$, and defined this time point as the beginning of the H-period. Similarly, we defined the first time point for which $I_{Htb2-mCitrine} > P_2 - 0.05 \cdot P_2$ as the end of the H-period. Finally, the duration of the H-period was calculated as the difference between those two time points. We defined G1-phase duration as the time from cell birth to first bud emergence, and G2/M duration as the time between the end of the H-period and cytokinesis.

RNA extraction and RT-qPCR

Cultures (25 mL) were grown at 30°C in yeast peptone media containing 2% glucose (YPD) for at least 6 h in a shaking incubator at 250 rpm, before being washed and transferred to SCGE. The cultures were grown for at least 16 h before appropriate β -estradiol concentrations were added to inducible cells (0 nM, 10 nM and 30 nM). The cultures (final volume of 50 mL) were then grown for at least 24 h in order to ensure steady-state conditions. During culture growth, $OD_{600} < 1.0$ was

364 maintained through appropriate dilutions. Cell volume distributions of the cultures were measured
365 with a Coulter counter after sonication for 5 s.

366 Remaining cell cultures were spun down at 4000 rpm for 5 min and the cell pellet resuspended in 50
367 μ L nuclease-free water (Qiagen). Total RNA was extracted using a hot acidic phenol (Sigma-
368 Aldrich) and chloroform (Thermo Fisher Scientific) extraction method adapted from an established
369 protocol⁴⁰. Yield of RNA was increased by precipitation in 100% ethanol (Merck Millipore) at -20°C
370 overnight, followed by a second precipitation in 100% ethanol at -80°C for 2-4 h. As a quality check
371 for total RNA extraction, agarose gel electrophoresis (1% agarose gel, run 30 min at 100 V) was
372 performed to check for the presence of the 25s, 18s and 5.8s ribosomal RNA bands. Concentration
373 and purity of the RNA samples were measured with a spectrophotometer (Thermo Fisher Scientific,
374 NanoDrop 2000) at 260 nm and 280 nm. cDNA was then obtained from 800 ng total RNA in a PCR
375 cyclor (Applied Biosystems, ProFlex PCR system 3x32-well) using random primers and a high-
376 capacity cDNA reverse transcription kit following the included protocol (Thermo Fisher Scientific).

377 Quantitative PCR (qPCR) measurements were carried out on a LightCycler 480 Multiwell Plate 96
378 (Roche) using a DNA-binding fluorescent dye (BioRad, SsoAdvanced Universal SYBR Green
379 Supermix) and mRNA sequence specific primers (Sigma-Aldrich). The qPCR was performed with 2
380 μ L of a 1:10 dilution of the cDNA for the genes *ACT1*, *HHO1*, *HTB2* and *mCitrine*, or a 1:100
381 dilution for all other genes. Melting curve data were analyzed to verify primer specificity. Each
382 sample was measured in technical duplicates and the mean value C_p^{Gene} was used for further analyses
383 if $\sigma_{C_p^{Gene}} < 0.5$. Relative concentrations, normalized on the reference gene *RDN18* were calculated
384 using the equation:

$$\log_2(\text{relative concentration}) = -(C_p^{Gene} - C_p^{RDN18}) \# (1)$$

385 In order to analyze relative concentrations as a function of cell volume, the mean cell volumes were
386 determined from the measured cell volume distributions.

387 **Test for qPCR primer specificity**

388 To test the specificity of the qPCR primer used to quantify histone mRNA concentrations, we
 389 analyzed deletion strains, where possible, for their respective deleted gene to check for unspecific
 390 primer binding. For example, we performed a qPCR measurement with the *HHO1* primers on a
 391 *hho1Δ* strain and compared the obtained C_p values with the C_p values obtained in the reference strain
 392 MS63-1 (**Supplementary Table 1**). We constructed deletion strains for the genes *HHO1*, *HTB2*,
 393 *HHF1*, *HHF2*, *HHT1* and *HHT2*, for which we obtained viable colonies without dramatic growth
 394 defects. RNA was extracted as described above, and 1 μg of total RNA was reverse-transcribed using
 395 the above mentioned high capacity cDNA synthesis kit. The qPCR was performed with 2 μL of a
 396 1:10 dilution of each cDNA sample, and measured in 3 or 6 technical replicates. C_p values and
 397 melting curve data were analyzed to verify primer specificity. Results are shown in **Supplementary**
 398 **Table 2**, deletion strains used are listed in **Supplementary Table 1**, a list of all qPCR primers used
 399 can be found in **Supplementary Table 3**.

400 **Flow Cytometry**

401 Cultures (2 mL – 5 mL) were grown in YPD for at least 6 h in a shaking incubator (30°C, 250 rpm)
 402 before being washed and transferred to SCGE and grown for at least 16 h. Appropriate β-estradiol
 403 concentrations were then added to inducible cells (0 nM and 30 nM for haploids or 50 nM for
 404 diploids), and the cultures grown for at least 24 h in a final volume of 3 mL – 5 mL. During cell
 405 growth, $OD_{600} < 1.3$ was maintained through appropriate dilutions.

406 Cell volume distributions of cultures were measured with a Coulter counter after sonication for 5 s.
 407 Cells were fixed using a 37% formaldehyde solution (Sigma-Aldrich) by pipetting 100 μL of
 408 formaldehyde into 900 μL of cell cultures in order to achieve a final formaldehyde concentration of
 409 3.7%. Cultures were incubated at room temperature on a rotator (VWR International, Tube Rotator)
 410 for 15 min, spun down at 10k g-force for 3 min and subsequently washed and resuspended in 100 μL

411 - 1000 μ L 100mM potassium phosphate (pH 7.5). Samples were then stored on ice until being used
412 for flow cytometry.

413 Flow Cytometry measurements were carried out on a benchtop flow cytometer with octagon and
414 trigon detector arrays (BD Biosciences, LSR II). Strains expressing the fluorescent protein *mCitrine*
415 were excited with a 488 nm coherent sapphire solid-state laser paired with a 530/30 nm filter set.
416 Side-scatter voltage was set to 220 V for all measurements, voltages for forward-scatter and
417 photomultiplier tubes were adjusted depending on whether haploid or diploid cells or both were
418 being measured. However, identical settings were used for replicate experiments. After removing
419 obvious outliers or potential doublets through standard gating strategies, at least 10.000 cells were
420 imaged in the final stopping gate. For each experiment, cells not expressing *mCitrine* were measured
421 to determine the cell-volume-dependent autofluorescence background which was subtracted from the
422 mean fluorescence intensity of each sample measured in the same experiment. In order to calculate
423 fluorescence concentrations, mean cell volumes were determined from the cell volume distributions
424 measured with the Coulter counter. Mean fluorescence concentrations were then calculated by
425 dividing the mean fluorescence intensity of each sample by its mean cell volume, allowing us to
426 analyze mCitrine fluorescence concentrations as a function of cell volume.

427 **Cell cycle analysis using flow cytometry**

428 To get insights into the distributions of cell cycle phases in cell populations of non-inducible and
429 inducible *WHI5* haploid and diploid strains, we performed cell cycle analysis using flow cytometry.
430 For this purpose, cell cultures (5 mL) were grown in YPD for at least 6 h in a shaking incubator
431 (30°C, 250 rpm), before being washed and transferred to SCGE; where appropriate β -estradiol
432 concentrations were added (10 nM or 30 nM for haploid cells, 50 nM for diploid cells). The cultures
433 were then grown for at least 24 h, assuring $OD_{600} < 1.3$ during culture growth through appropriate
434 dilutions. Cell volume distributions of cultures were measured with a Coulter counter after sonication

for 5 s. To fixate the cells and subsequently stain the DNA, we followed an already established protocol⁴¹. Specifically, 1 mL of each cell culture was pipetted into 9 mL of cold 80% ethanol and incubated at 4°C on a rotator overnight. The cultures were then spun down at 4000 rpm for 2 min and washed twice in 50 mM Tris-HCl (pH = 8.0). Cells were then successively treated with a 1 mg/mL RNase A (Thermo Fisher Scientific) solution for 40 min at 37°C, a 20 mg/mL Proteinase K (Promega) solution for 1 h at 37°C and a 10x SYBR Green I (Sigma-Aldrich) solution for 1 h at room temperature. Between each treatment, cells were washed twice with 50 mM Tris-HCl and resuspended in 50 mM Tris-HCl. After the last treatment, cells were sonicated for 5 s. Flow Cytometry measurements were carried out on the benchtop flow cytometer described above, using the same laser, filter sets and side-scatter voltage. Settings for forward-scatter and photomultiplier tubes were adjusted depending on the condition measured. To estimate cell-cycle fractions, imaged DNA content frequency histograms were analyzed using Watson modelling. However, we noticed that for cell populations with large cell volumes (*i.e.* high β -estradiol concentrations), the DNA content distributions showed pronounced tails at large cell volumes that were not fit by the model. We speculate that this tail represents an increased mitochondrial DNA content in large cells⁴², which suggests that a fraction of G1 cells would be wrongly identified as S phase. Thus, we decided to limit our analysis to classifying cells as either G1/S-phase or G2/M-phase (**Supplementary Fig. 1c**). Using this approach, we did not find a drastic influence of the β -estradiol concentration used for Whi5 induction on the cell cycle distributions.

Volume-dependence parameter

Analyzing protein and mRNA concentrations as a function of cell volume reveals a decrease of concentration with increasing cell volume for histones. In order to quantify this decrease, we performed a linear regression on the double logarithmic data and define the slope of the fit as the *volume-dependence parameter* (VDP):

$$\log_2(c) = \log_2(c_0) + \text{VDP} \cdot \log_2(V) \#(2)$$

459 The VDP gives us a quantitative measure for the relation of protein and mRNA concentrations with
 460 cell volume: A negative VDP indicates a decrease of concentration with increasing cell volume. The
 461 special case of $\text{VDP} = -1$ corresponds to a decrease of concentration with $c \sim 1/V$, and therefore
 462 signifies a constant amount of protein or mRNA with increasing cell volume. A positive VDP
 463 indicates an increase of concentration with increasing cell volume, and $\text{VDP} = 0$ corresponds to a
 464 constant concentration c_0 .

465 Statistical analyses

466 Significance of VDPs

467 To test for a significant deviation of the VDP from 0, we performed two-tailed one-sample t-tests on
 468 the regression coefficients of the linear fit at a confidence level of $\alpha = 0.05$. Our null hypothesis H_0
 469 assumes the respective coefficient to be equal to 0. In order to test for the significance of the VDP,
 470 we are interested in the slope of the linear fit: for a p-value smaller than α , we reject H_0 and consider
 471 the slope, *i.e.* the VDP, to be significantly different from 0.

472 To test whether the VDPs of two different conditions significantly deviate from each other, we used
 473 a general linear regression model with a categorical variable, *Type*, to differentiate between the two
 474 conditions analysed:

$$\log_2(c) = \log_2(c_0) + \text{VDP}_0 \cdot \log_2(V) + \delta_1 \cdot \text{Type} + \delta_2 \cdot \text{Type} \cdot \log_2(V) \#(3)$$

475 with c_0 and VDP_0 corresponding to the reference condition ($\text{Type} = 0$), δ_1 describing the average
 476 difference in the intercepts of the linear fits between the two conditions, and δ_2 describing the
 477 change in the slopes (VDPs) between the two conditions. In order to test for a significant difference
 478 between the two VDPs, we perform a two-tailed one-sample t-test on δ_2 , with the null hypothesis H_0
 479 assuming $\delta_2 = 0$, at a confidence level of $\alpha = 0.05$. For a p-value smaller than α , we reject H_0 and

480 consider the change between the two slopes to be significant, *i.e.* we consider the two VDPs to be
481 significantly different from each other.

482 *Error estimation of concentrations at 60 fL*

483 To calculate concentrations at a characteristic cell volume of 60 fL with respective error estimates,
484 we evaluated the linear fits to the double logarithmic data at 60 fL and estimated the 95 %
485 confidence intervals of the fit at 60 fL. When normalizing the concentration to a chosen value x ,
486 errors were calculated using error propagation:

$$\Delta y = y \cdot \sqrt{\left(\frac{\Delta c^2}{c^2}\right)^2 + \left(\frac{\Delta x^2}{x^2}\right)^2} \quad \#(4)$$

487 with y being the new normalized concentration and c the previously calculated concentration.

488 To estimate the error associated with the ratio between the concentrations at 60 fL in haploids and
489 diploids, we used bootstrap analysis. Specifically, we treated the measurements of protein or mRNA
490 concentration and corresponding cell volume as a set of linked variables, both for haploid and diploid
491 cells. We then resampled $n = 10000$ populations of same size by random sampling with replacement
492 from this experimental two-dimensional population. Next, we performed a linear regression on the
493 double logarithmic data for each of the resampled populations and estimated the concentration at 60
494 fL, giving us a distribution of $n = 10000$ concentrations at 60 fL for both haploid and diploid cells.
495 Finally, we randomly selected a concentration in each of those distributions, and divide the
496 concentration for diploids by the concentration for haploids. We repeated this process 10000 times
497 with replacement to obtain a distribution of $n = 10000$ concentration ratios, for which we calculate
498 the median and the 2.5- and 97.5-percentiles.

499 **Minimal model**

500 To obtain mechanistic insight on how the transcription rate of one specific promoter depends on cell

501 volume and ploidy context, we sought to build a minimal model. For this, we consider transcription
 502 being limited by one component of the transcriptional machinery, potentially a subunit of the RNA
 503 polymerase. In addition, we assume transcript degradation to be the same for all transcripts, and set
 504 the corresponding degradation rate $k_{deg} = 1$, *i.e.*, all other rates are normalized with respect to k_{deg} .
 505 Note that in the case of stable transcripts, k_{deg} also describes dilution of transcripts by cell growth.
 506 To account for the competition of different promoters for a finite number of the limiting component
 507 of the transcriptional machinery (TM), our model distinguishes two classes of promoters - a *general*
 508 *pool* of promoters, g , with $n_h \approx 6000$ (haploids) or $n_d \approx 12000$ (diploids), and a single *promoter of*
 509 *interest*, p , present as a single copy. We describe each promoter as one single binding site for TM
 510 and denote the number of TM bound to general promoters as R^g . Binding of TM at the single
 511 promoter of interest is described by R^p , which can assume values between 0 (not bound) and 1
 512 (bound). Moreover, R^f denotes the number of free TM . We assume that the total number of TM (free
 513 and bound) scales proportionally to cell volume V and is given by

$$R^g + R^p + R^f = c_{TM}V \#(5)$$

514 with c_{TM} being the total TM concentration.

515 Assuming that the arrival of TM at promoters is proportional to the concentration of free TM , the
 516 change in number of bound general promoters over time is given by following equation:

$$\frac{dR^g}{dt} = k_{on}^g(n_{h/d} - R^g)\frac{R^f}{V} - k_{off}^g R^g \#(6)$$

517 where k_{on}^g is the rate at which transcription is being initiated at each general promoter, $n_{h/d} - R^g$ are
 518 the number of general promoters not bound to TM in haploids or diploids, respectively, and k_{off}^g
 519 models the rate at which bound TM complete transcriptional elongation.

520 Similarly, the change in binding of TM to the single promoter of interest over time is given by:

$$\frac{dR^p}{dt} = k_{on}^p(1 - R^p) \frac{R^f}{V} - k_{off}^p R^p \#(7)$$

521 with parameters k_{on}^p and k_{off}^p representing transcriptional initiation and elongation, respectively, at
522 the promoter of interest.

523 Solving (6) and (7) at steady-state ($\frac{dR^g}{dt} = \frac{dR^p}{dt} = 0$), constraints the number of bound *TM*s via the
524 following nonlinear equations

$$k_{on}^g(n_{h/d} - R^g) \frac{R^f}{V} = k_{off}^g R^g \#(8)$$

$$k_{on}^p(1 - R^p) \frac{R^f}{V} = k_{off}^p R^p \#(9)$$

525 Finally, the steady-state concentration of transcripts produced from the single promoter of interest is
526 equal to $k_{off}^p R^p / V$.

527 Given a set of parameters $c_{TM}, k_{on}^g, k_{off}^g, k_{on}^p, k_{off}^p$, numerically solving equations (5), (8) and (9)
528 allows to calculate the transcript concentration, generated by the single promoter of interest as a
529 function of cell volume V . We set $c_{TM} = 2000, k_{on}^g = 1, k_{off}^g = k_{off}^p = 3$ and calculate the steady-
530 state concentration in haploids and diploids over cell volume for $k_{on}^p = [0.01, 100]$.

531 In order to determine the VDP as a function of k_{on}^p , we calculated the concentration for each value of
532 k_{on}^p over a cell volume range of $V = \left[\frac{1}{3}, 3\right]$ and performed a linear regression fit on the logarithm of
533 the concentration as a function of the logarithm of the cell volume, with cell volumes being equally
534 spaced on the log scale. The VDP is then determined as the slope of the linear fit.

535 **Data availability statement**

536 Yeast strains and raw data are available upon reasonable request.

537 **Code availability statement**

538 Additional information on image analysis approaches described in the methods and previous
539 publications is available upon reasonable request.

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650 **Competing interests**

651 The authors declare no competing interests.

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Figures

Figure 1. Htb2-mCitrine protein concentrations measured by live-cell fluorescence microscopy decrease with cell volume and increase with ploidy. (a) Htb2-mCitrine amounts during the first cell cycle of new-born cells. Red dashed trace highlights data corresponding to cells shown in the microscopy images (new-born cell: red outline, its bud: blue outline), blue traces show additional randomly selected example curves, black line the mean of $n = 185$ cells. All traces are aligned at the time of first bud emergence ($t = 0$). (b) Htb2-mCitrine amounts at birth for haploid (blue) and diploid (green) cells as a function of cell volume. Lines connect binned means, error bars indicate standard errors. (c) Whi5 controls cell volume in a dose-dependent manner. To manipulate cell volume, the endogenous allele is replaced by a copy of *WHI5* expressed from an artificial, β -estradiol-inducible promoter. Adding higher β -estradiol concentrations results in cells with bigger mean cell volumes. (d) Mean cell volumes for non-inducible (WT) and inducible haploids (blue) and diploids (green) measured in *HTB2-mCitrine* single cells with live-cell fluorescence microscopy (■), or in bulk populations of cells with untagged *HTB2* with a Coulter counter (○). Error bars indicate standard

deviations of the mean between single cells for single cell measurements
 $(n_{haploid}^{WT} = 185, n_{haploid}^{not\ ind.} = 120, n_{haploid}^{ind.} = 108, n_{diploid}^{WT} = 170, n_{diploid}^{not\ ind.} = 99, n_{diploid}^{ind.} = 243)$
or the standard deviation of the population means across 5 biological replicates for bulk
measurements. Haploid cells were induced with 30 nM β -estradiol, diploid cells with 50 nM. Note
that no β -estradiol was used in the microfluidic device during the microscopy experiments, resulting
in a gradual decrease of cell volume of induced cells after the start of the experiment. (e) Htb2-
mCitrine concentrations of non-inducible and inducible haploids and diploids as a function of cell
volume are shown in a double logarithmic plot. Individual data points for the different conditions ($\blacktriangledown$
0 nM, $\bullet$ WT, $\star$ 30 nM, for haploids and $\blacktriangleleft$ 0 nM, $\blacksquare$ WT, $\star$ 50 nM, for diploids) are highlighted in
blue (haploids) and green (diploids). Lines show linear fits to the double logarithmic data. (f)
Illustration of the impact of potential feedback mechanisms on the concentration of Htb2-mCitrine
concentration in a *HTB2-mCitrine/htb2 Δ* hemizygous diploid compared to a *HTB2-mCitrine*
homozygous diploid. (g) Htb2-mCitrine concentrations at 60 fL for haploids (blue), *HTB2-mCitrine*
homozygous diploids (green) and *HTB2-mCitrine/htb2 Δ* hemizygous diploids (teal) normalized on
concentration at 60 fL in haploids. Error bars are derived by error propagation of the 95% confidence
interval of the linear fit at 60 fL.

Figure 2. Histone mRNA concentrations decrease with cell volume and increase with ploidy. (a) Experimental procedure for RT-qPCR measurements. Cells were grown for at least 24 h at the respective β -estradiol concentration before extracting total RNA and performing RT-qPCR. (b & c) Relative *ACT1* (b) or *HTB2* (c) mRNA concentrations (normalized on *RDN18*) for non-inducible and inducible haploid cells over mean cell volume are shown in a double logarithmic plot. Individual data points for the different conditions (∇ 0 nM, $\bullet$ non-inducible, $\blacklozenge$ 10 nM, $\blacktriangle$ 30 nM) are highlighted in grey. Red (b) or blue (c) symbols indicate the mean of the different conditions. Error bars indicate standard deviations for $n \geq 7$ biological replicates. Lines show linear fits to the double logarithmic data, with volume-dependence parameters (VDPs) determined as the slope of the fit. (d) Summary of the VDPs for all measured genes. Error bars indicate the standard error of the slope;

significances that the VDP is different from 0: ** $p < 0.01$, *** $p < 0.001$. (e) Median mRNA concentrations at 60 fL of *HTB2* (left) and *HTB1* (right) in diploid *HTB2* homozygous (green) and *HTB2/htb2Δ* hemizygous (teal) strains, normalized on the respective median concentration of the *HTB2*-homozygote. Error bars indicate the 2.5- and 97.5-percentiles determined from 10000 bootstrap samples. (f & g) Summary of VDPs for *hir1Δ* and *rtt106Δ* (f) as well as *rrp6Δ* (g) deletion strains. Error bars indicate the standard error. Significant VDP deviation from the wild-type VDP (carrying no deletion) was tested using linear regressions; * $p < 0.05$, *** $p < 0.001$. (h) Relative *HTB2* mRNA concentrations (normalized on *RDN18*) for inducible and non-inducible haploid cells over mean cell volume, shown in a double logarithmic plot. Data corresponding to the *rrp6Δ* cells are highlighted in blue. Light blue symbols highlight the different conditions (◆ non-inducible, ◀ 0 nM, ▲ 10 nM, ▶ 30 nM). Dark blue symbols (■) indicate the mean for each condition. Grey symbols (●) indicate the mean for each condition of the wild-type (carrying no deletion). Lines show the linear fits to the double logarithmic data.

Figure 3. Histone promoters are sufficient for cell-volume- and ploidy-dependence of transcript concentrations. (a) Illustration of haploid (1N) or diploid (2N) strains carrying a single additional copy of a promoter of interest, driving the expression of the fluorescent reporter *mCitrine* regulated by the *ADH1* terminator. RT-qPCR or flow cytometry were used to analyze expression of the fluorescent reporter. (b) Summary of VDPs determined with RT-qPCR for the genes *ACT1*, *HHF1* and *mCitrine* in a wild-type strain (black ●), a strain carrying an additional *ACT1* promoter (red ▼), or a strain carrying an additional *HHF1* promoter (blue ▲). Error bars indicate the standard error. Significant VDP deviation between two genes was tested using linear regressions; ***p<0.001. (c) Summary of VDPs determined with flow cytometry for different strains in haploid (●) and diploid (□) cells. Error bars indicate the standard error. (d – e) *mCitrine* concentration, driven by an additional copy of the *ACT1* (d) or *HTB1* (e) promoter in haploid (●) and diploid (□) cells, shown as a function of cell volume in a double logarithmic plot. Lines show linear fits to the double logarithmic data with volume-dependence parameters (VDPs) determined as the slope of the fit, with respective standard error. (f) Median concentration of *mCitrine* in diploid cells compared to the

755 median concentration in haploid cells at 60 fL. Error bars indicate the 2.5- and 97.5-percentiles
756 determined from 10000 bootstrap samples.

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769 **Figure 4.** Minimal model for the dependence of transcription rate of one specific promoter of interest
770 on cell volume and ploidy. (a) The model includes two classes of promoters: the general pool of
771 promoters g and the specific promoter of interest p with their respective initiation rates k_{on}^p or k_{on}^g ,
772 describing the binding of the limiting machinery and off-rates k_{off}^p or k_{off}^g , summarizing all other
773 steps of transcription. (b - d) The model predicts that tuning k_{on}^p while keeping all other parameters
774 fixed ($c_{TM} = 2000, k_{\text{on}}^g = 1, k_{\text{off}}^g = k_{\text{off}}^p = 3$) results in a qualitative change of the cell volume-
775 dependence of transcript concentration obtained from the specific promoter (b), as well as a change
776 in the ratio between the concentration in diploid cells and the concentration in haploid cells (c). (d)
777 Model prediction for the VDP (right, black) and the ratio between the concentration in diploid cells
778 and the concentration in haploid cells at a characteristic volume $V_0 = 1$ (left, orange) as a function of
779 k_{on}^p .

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782 **Figure 5.** Reducing the strength of a histone promoter shifts its behavior from histone-like to actin-
783 like. (a) Illustration of a series of haploid and diploid strains carrying a single additional copy of
784 increasingly shorter fragments of promoters driving *mCitrine* expression, each truncated from the 5'-
785 end. (b) mCitrine concentration at 60 fL normalized on maximum concentration of the respective
786 promoter (upper panel) and VDP of mCitrine (bottom panel) determined by flow cytometry for the
787 respective promoter truncations of the *HTB1* promoter (dark blue ●) and the *HHF1* promoter (light
788 blue ■) driving *mCitrine* expression, integrated in haploid cells. Error bars in the upper panel are
789 derived by error propagation of the 95% confidence interval of the linear fit at 60 fL. In the bottom
790 panel, error bars show the standard error. (c) VDP of mCitrine in haploid (blue ●) and diploid (green
791 □) cells (upper panel) and mCitrine concentration at 60 fL in diploids compared to the concentration
792 in haploids (bottom panel) determined by flow cytometry. Left shows results for the *HTB1* promoter

truncations, right shows results for the *HHF1* promoter truncations. Error bars in the upper panels show the standard error. In the bottom panel, error bars indicate the 2.5- and 97.5-percentiles determined from 10000 bootstrap samples. (d) VDP of *mCitrine* in haploid (blue ●) and diploid (green □) cells (upper panel) and *mCitrine* mRNA concentration at 60 fL in diploids compared to the concentration in haploids (bottom panel) determined by RT-qPCR for *HTB1* promoter truncations driving *mCitrine* expression. Error bars in the upper panel show the standard error. Error bars in the bottom panel indicate the 2.5- and 97.5-percentiles determined from 10000 bootstrap samples.