Complex genetic and epigenetic regulation deviates gene expression from a unifying global transcriptional program

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Abstract

Environmental or genetic perturbations lead to gene expression changes. While most analyses of these changes emphasize the presence of qualitative differences on just a few genes, we now know that changes are widespread. This large-scale variation has been linked to the exclusive influence of a global transcriptional program determined by the new physiological state of the cell. However, given the sophistication of eukaryotic regulation, we expect to have a complex architecture of specific control affecting this program. Here, we examine this architecture. Using data of *Saccharomyces cerevisiae* expression in different nutrient conditions, we first propose a five-sector genome partition, which integrates earlier models of resource allocation, as a framework to examine the deviations from the global control. In this scheme, we recognize invariant genes, whose regulation is dominated by physiology, specific genes, which substantially depart from it, and two additional classes that contain the frequently assumed growth-dependent genes. Whereas the invariant class shows a considerable absence of specific regulation, the rest is enriched by regulation at the level of transcription factors (TFs) and epigenetic modulators. We nevertheless find markedly different strategies in how these classes deviate. On the one hand, there are TFs that act in a unique way between partition constituents, and on the other, the action of chromatin modifiers is significantly diverse. The balance between regulatory strategies ultimately modulates the action of the general transcription machinery and therefore limits the possibility of establishing a unifying program of expression change at a genomic scale.

Introduction

The limited availability of the components of the cell expression machinery, for example, free RNA polymerases, cofactors, ribosomes, etc., creates a resource allocation problem that affects their activities. This differential allocation eventually represents a global program of regulation (1)(2)(3)(4), with some genes expressed at the cost of others. As the dosage of these components can be modulated by the growth rate at balanced growth, the influence of the global program has typically been studied by quantifying how the expression of genes responds to growth.

This led to the identification of laws that predict the expression of different cellular elements (3)(4)(5). Two broad models have been considered. In a first model (model 1, Fig. 1A, top), the impact of the global program is recognized by partitioning the genome in three minimal sectors (4)(5). One of them contains ribosomal genes whose expression increases with growth rate verifying its role in driving cell growth (6). Two other sectors comprise genes whose expression decreases or remains invariant with growth. The three-sector model describes therefore fundamental aspects of cellular economics (7) already advanced in the early work of bacterial physiologists (8), and acts as a basic constituent to investigate many subjects. For instance, modifications to this framework were advanced to explain the cost of unnecessary gene expression (4), the dependence of cellular composition with antibiotics (9), or the rationale behind some seemingly wasteful carbon utilization strategies (10).

In a second model, the emphasis is not so much in the trade-offs between ribosomal genes and the rest, but between genes that follow a common pattern of expression and a minimal subset that diverges (model 2 containing two sectors: nonspecific and specific, Fig. 1A, bottom) (11). Here, the common pattern of expression is explained by a single scaling factor, which incorporates the resources of the global program that are

not involved in the activation of the specific genes. This implies that most changes in expression result from a passive rather than active regulation what produces a unifying pattern that “simplifies” the expected complexities of genome-wide expression changes.

In this manuscript, we integrate both models (model 3, Fig. 1B). The new framework discriminates a fine-structure within the nonspecific sector of model 2 that corresponds to the three-sector partition representative of model 1. While the integration of both views is significant *per se*, models 1 and 2 might appear unrelated, we also show that this scheme is fundamental to help us expose how specific regulatory strategies modulate the impact of the global transcriptional program at the genome-wide level.

To this aim, we first reexamined the original data that led to model 2, i.e., promoter activity (PA) measurements of *Saccharomyces cerevisiae*’s genes obtained in different growing conditions (11), to present our new scheme. However, this data only includes a subgroup of ~900 yeast genes, and thus the quantification of the resource allocation can only be approximated. To obtain a more precise classification we considered genome-wide expression data of yeasts growing in chemostats (12) and developed a method to define the new model at a large scale. We validate our partition through functional analysis of the corresponding components.

Armed with this new framework, we focused on understanding how the global program becomes modulated by specific regulatory mechanisms, a question which is only beginning to be addressed. Indeed, within the framework of model 1 results are mixed. For instance, several recent reports in bacterial systems demonstrated the prevalence of the global expression program, while they have lowered the importance of transcription factors (TFs) controlling the assumed deviations from it. TFs seem only to fine-tune the action of the global regulation (13)(14) in combination with a few

metabolites (15). More recent work in yeast argued, in contrast, about the relevance of epigenetic factors (promoter nucleosomal stability) as modulators of the global program (16).

The function of the specific regulation might appear more straightforward *a priori* in the scheme of model 2. Specific genes are indeed enriched by specific regulation, generally coupled to the particular nutrient condition, whereas the behavior of those genes within the nonspecific sector does not seem connected to a particular transcription regulation strategy (11). We thus examine within our new framework to what extent we could observe differential genetic and epigenetic regulation acting on the genes constituting each sector. We discovered well-defined regulatory patterns.

More broadly, our results put forward an integrated view of the resource allocation connected to genome-wide expression and emphasize how the global program is eventually modified by the specific regulation. Active strategies of control are certainly at work in genes within the fine-structure of the “nonspecific” response and can eventually associate the metabolic status of the cell with gene expression. Overall, this complex hierarchy of regulation in eukaryotes enhances the adjustment of genome-wide expression patterns beyond the one that could be achieved through a passive unifying program of global transcriptional control.

Results

Integration of two preceding resource allocation models. We first examined how PA changes with the growth rate for a subset of *Saccharomyces cerevisiae* genes. Keren *et al*. (11) presented a binary partition to describe these changes, recognizing a common response in the absolute PA values of most genes and a specific one in a much smaller subgroup. To focus on resource reallocation, we studied here fractional

125 PA activities instead of absolute values, i.e., the fraction of PA of each gene in a given
126 nutrient condition out of the summed activity of all genes in the set (3), and quantified
127 their change for each pair of conditions (from low to high growth rate).
128
129 Figure 1C shows a descriptive case (glycerol to glucose conditions). The most extreme
130 deviations of the global program correspond to the specific genes (11). Note also that
131 the stronger allocation of "expression resources" to these genes the fewer resources to
132 biosynthesis (reduction of the nonspecific sector) affecting growth rate (Fig. 1A,
133 bottom). We revised the two-sector partition by further separating specific genes as
134 specifically activated genes (fractional PA becomes much larger between conditions),
135 and specifically repressed genes (fractional PA becomes much smaller), and also
136 delimiting three components within the nonspecific sector: genes whose fractional PA
137 remains approximately invariant (diagonal in Fig. 1C), positive genes, whose fractional
138 PA moderately increases between conditions, and negative genes (fractional PA
139 decreases to only a limited extent, see Methods for details). These three sectors relate
140 model 2 to model 1.
141
142 We validated this new partition in two ways. First, that a set of genes follows a precise
143 proportional response between conditions was suggested by (11) as an evidence that
144 they share a common functionality (e.g., being part of the same pathway, etc.). Here,
145 we find that invariant, positive and negative genes also follow a precise proportional
146 response (Fig.S1) suggesting that our analysis identifies a biologically relevant fine-
147 grained structure. Second, we similarly expect that this fine-grained structure would
148 parallel that proposed in model 1 (4) in terms of functional categories. This is the case.
149 The invariant class is enriched by transcription regulation and ribosomal proteins; the
150 latter being more extensively observed in the positive class. Indeed, positive genes are
151 enriched by ribosomal genes (~65% of genes code for small or large subunits of the

152 ribosome), while negative genes are enriched in ATP metabolic processes, e.g.,
153 oxidative phosphorylation or the TCA cycle (Table S1, Methods).
154
155 **The five-sector resource allocation model on a genomic scale.** To substantiate the
156 previous five-sector model based on ~900 genes, we examined a genome-wide DNA
157 microarray dataset of yeast cells exhibiting the same range of growth rates for six
158 limiting nutrients (12). We studied again changes of relative expression (Methods) and
159 applied singular value decomposition (SVD) to each nutrient separately. The first and
160 second SVD components (Fig. 2A) explain >90% of the variance in each condition (the
161 components exhibited an analogous trend in all nutrients, Fig. S2). As a result, the
162 fractional expression response to growth of each gene can be approximated by the
163 linear combination of these two components (Fig. 2B).
164
165 Furthermore, we can interpret the first element of the linear combination ($\vec{v}_1$) as the
166 baseline fractional expression of the gene, which does not change with growth rate,
167 and the second element ($\vec{v}_2$) as its monotonic response to growth (Fig. 2B). This
168 reading enables us to generalize the previous partition framework obtained with PA
169 data. More specifically, a gene that maintains the same baseline expression (similar
170 loading of $\vec{v}_1$ that we denoted a_i) between two conditions involves a nonspecific
171 response. When this response in observed in >8 pairwise changes then we reason that
172 the gene is nonspecific (recall that there are 6 different nutrients and consequently a
173 total of 15 pairwise nutrient changes). Genes are considered specific otherwise (Fig.
174 2C).
175
176 Beyond the classification above, the second component ($\vec{v}_2$) provides a quantitative
177 score (the second loading, b_i) to classify genes as invariant, positive or negative (b_i~0,
178 b_i>0, b_i<0, respectively; Fig. 2C. Methods). We labelled as invariant those nonspecific
179 genes which exhibit this behavior in >3 nutrient conditions (total of 6). Nonspecific and

180 *not* invariant genes appearing more times as positive than as negative are categorized
181 as positive, and likewise for negative. Finally, specific genes which appear more times
182 as positive than as negative (again in all 6 conditions) are categorized as activated,
183 and analogously for repressed (Fig. 2C).
184
185 Finally, the functional analysis of genes within each sector agrees with that obtained
186 with the PA data and previous reports, what substantiates the biological significance of
187 the partition (Table S2; see also Table S1 showing how this classification maps onto
188 the subset of genes with PA data). In this way, we have generalized at a large scale
189 the new resource allocation model, a genome-wide classification that we use
190 throughout the next sections.
191
192 **TFs are typically nonspecific genes.** We inspect next the influence of the most direct
193 elements related to specific regulation, i.e., TFs. But before examining their impact on
194 the activity of the global transcription program, we asked first how TFs themselves are
195 framed in the previous partition.
196
197 We observed that most constituent TFs of the transcriptional regulatory network (122 of
198 a total of 133 composing the network, Methods) are nonspecific genes, i.e., they
199 present similar basal fractional expression (a_i loadings) across all pairwise condition
200 changes. Within this set, 31% presents a dominant invariant response (b_i~0 in >3
201 nutrient conditions, of a total of 6), with five genes acting as invariant in all six
202 conditions (*rsc1*, *mbp1*, *pho2*, *rgr1*, and *swi6*). Two of these (*mbp1*, *swi6*) are at the top
203 of the network hierarchy (being involved in the mitotic cell cycle), and two are elements
204 of relevant complexes that interact with RNA polymerase II (*rsc1* of the RSC chromatin
205 complex, and *rgr1*/*med14* of the mediator complex); they can be considered as
206 elements of a general transcriptional machinery, for which maintaining its concentration
207 invariant across conditions could be essential. Moreover, 32% of nonspecific TFs are

208 dominantly negative, and only 4% positive. Of note, some of the TFs whose expression
209 decreases with growth (b_i<0) are positive regulators of transcription in response to
210 stress (e.g., *bur6*, *gcn4*, *rpn4*) what justifies their overexpression at low growth rates.
211
212 **Genes within each partition sector present different degree of transcriptional**
213 **regulation.** Is the extent of regulation of target genes dependent on which sector they
214 belong to? We computed the mean number of regulators acting on genes within each
215 sector. Nonspecific genes are less regulated, on average, by TFs than specific ones as
216 expected [by 3.09 TFs *vs.* 5.06 TFs, $p = 1.20\ 10^{-4}$, two-sample Kolmogorov-Smirnov
217 (KS) test]. Within nonspecific genes, invariant genes are less regulated than
218 nonspecific and *not* invariant ones (by 2.56 TFs *vs.* 3.3 TFs, $p = 8.16\ 10^{-13}$, two-sample
219 KS test). Finally, nonspecific and positive genes are slightly more regulated than
220 nonspecific and negative genes (by 3.33 TFs *vs.* 3. 27 TFs, p = 0.0018, two-sample KS
221 test). Overall, specific genes are subjected to more regulation (larger number of TFs)
222 (Fig. 3A), while nonspecific and invariant ones show the least.
223
224 **TFs and their regulated genes frequently exhibit the same response to growth.**
225 Although Fig. 3A shows how the number of transcriptional interactions is reflected
226 differentially in the sectors of the partition it does not assure us whether these
227 interactions are functioning, e.g., regulation by TFs have been shown to be “inactive” in
228 bacteria (13). For this, we examined several features.
229
230 We initially inspected if target genes presenting a particular growth response are
231 enriched by TFs showing the very same response, as the similarity of the responses
232 could indicate that these TFs do influence the expression of the target. We thus
233 computed –for each target gene– the fraction of its regulators that behave as negative,
234 invariant, or positive with the growth rate in a given condition (TF_{neg}, TF_{inv}, TF_{pos},

235 respectively). Figure 3B shows the mean of these fractions for target genes which
236 themselves show a negative, invariant, or positive response, respectively. Negative
237 TFs are more likely to be found acting on target genes that are also negative (higher
238 mean TF_{neg} on negative genes), while invariant (TF_{inv}) and positive (TF_{pos}) TFs regulate
239 more often invariant and positive target genes, respectively (the latter signal is weaker
240 and depends on the particular condition, Fig. S3). Thus, TFs that exhibit the same
241 behavior as their cognate target gene tend to appear, on average, dominant on its
242 regulation; this indicates that part of the regulatory structure is functional.
243
244 **Specific TFs regulate genes fitting to each partition sector.** To further estimate the
245 active regulatory character of TFs, we measured the correlation of the response to
246 growth rate between any particular gene and all its cognate TFs ("regulatory
247 coherence", Methods). Specific genes showed stronger regulatory coherence than
248 nonspecific ones (Fig. S4A), and remain coherent in more nutrient conditions (Fig.
249 S4B), both results implying an existing contribution of TFs to deviate gene expression
250 from the global program.
251
252 Moreover, Fig. 3C shows those TFs whose operation is particularly coherent per
253 partition sector (Methods). One can identify here that TFs controlling specific genes are
254 normally not acting on nonspecific ones [this is supported by earlier reports(17)].
255 Moreover, some of the TFs that work coherently on positive genes (gray color, Fig. 3C)
256 are particularly absent in negative ones (pink color, Fig. 3C) and *vice versa*. In addition,
257 those that control nonspecific genes are higher up in the network hierarchy (Methods).
258 We also noted that some of these (significantly coherent) TFs are involved in chromatin
259 remodeling (Cyc8, Ume6, Spt6, Msn4, Abf1, Msn2, Nhp6A, acting on nonspecific
260 ones), or chromatin organization (Spt3, Spt2, Pho4, FKh2, Sin3, Spt20, Wtm2, Wtm1,
261 Hif1, acting on specific genes). We examine epigenetic aspects next.
262

263 **The resource allocation model also reveals distinctive epigenetic regulation.** To
264 inspect the function of epigenetic control mechanisms, we first quantified the proportion
265 of general transcription factors (GTFs) found within the set of TFs acting on a given
266 gene (18). GTFs (Rap1, Abf1, Reb1, Cbf1, and Mcm1) usually have little intrinsic
267 regulatory activity and together with the presence of chromatin remodelers (in
268 particular, RSC –Remodels Structure of Chromatin) control an alleged general
269 machinery of expression. We observed that GTFs constitute a larger and significant
270 fraction in the regulation of positive genes, while the opposite is observed for negative
271 ones (Fig. 4A).
272
273 GTFs are also associated with particularly fragile nucleosome promoter architectures
274 (19), a connection recently examined (16). Using this data, we computed the
275 nucleosome landscape for the different gene classes (Methods). Promoters of positive
276 genes are indeed enriched in fragile nucleosomes (Fig 4.B) while both negative and
277 invariant genes lack these structures. This indicates that positive genes are more
278 sensitive to adjust the global program of expression by means of chromatin modulation.
279 Enrichment of other promoter features contribute to this observation (Fig 4.C), like the
280 absence of TATA boxes, the action of TFIID over SAGA [but this precise grouping has
281 been recently reexamined (20)], the presence of nucleosomal free regions closer to the
282 transcriptional starting site (shNFRs) (partially associated to the previous score of
283 fragile nucleosomes), and the dominant effect of *trans* variability (Fig. S5) (21).
284
285 **Chromatin modifiers further modulate the global program.** Finally, we examined
286 the effects of mutating different types of *trans*-acting chromatin regulators on the genes
287 constituting the sectors using a previously assembled compendium (22) (Methods). We
288 considered first the magnitude of change of expression (i.e., the *absolute* values of
289 expression) before/after the mutation of several types of modifiers. With the exception
290 of histone acetyltransferases (HATs) and TATA-binding protein related factors (TAFs),

291 the influence of most chromatin modifiers is dominant in specific genes as compared to
292 nonspecific ones (Fig. S6, Methods).
293
294 Within nonspecific genes, we also quantified the type of expression change
295 (experienced after mutation of the modifiers) and found three broad relationships (Fig.
296 5): 1) Epigenetic regulators acting as part of a general machinery (HATs –including
297 SAGA–, TAFs and methyltransferases) whose mutation causes a general decrease in
298 expression, very particularly in invariant and positive classes. Indeed, work by (20) and
299 (23) demonstrated that SAGA and TFIID are recruited to pol II promoters genome-wide
300 and that each complex is generally required for pol II transcription, i.e., its mutation
301 would lead to a genome-wide decrease of gene expression. 2) Regulators (e.g.,
302 histones, etc.) acting in a dual manner: increasing the expression of negative genes
303 after mutation (remodeler as a repressor) or reducing their expression in positives
304 (remodeler as an activator). This underlines the enrichment of negative and positive
305 classes by stress and ribosomal genes, respectively, which are largely regulated in an
306 opposite manner (24); a dual role of remodelers as activators and repressors have
307 been previously reported (25)(26). And 3) regulators as broad repressors (mutation
308 increases expression), e.g., those that represent regulation by gene silencing.
309

310 ## Discussion

311 Recent studies emphasized how most genome-wide expression changes might be the
312 consequence of a unifying global program of transcription control. This program is
313 determined by the availability of the general components of the expression machinery
314 and inevitably refers to a problem of resource reallocation, in which different parts of
315 the genome are to use the limited expression resources.
316

317 We followed here this interpretation to present a new partition of the yeast genome
318 through the integration of two earlier models, not connected until now (6)(11). Essential
319 to the framework is the faculty to discriminate what it was contemplated before as a
320 common nonspecific proportional response –subjected to the same global
321 regulation(11)– into three subclasses: invariant genes, that best follow the global
322 program, and positive and negative genes, which were broadly defined in other studies
323 as growth-related genes (12)(27)(28). The analysis of relative expression values is
324 important here as it allows us to appreciate expression reallocation among partition
325 sectors (3)(4).
326
327 We then focused on unravelling how global and specific regulation combine within this
328 new scheme. This is largely an open question, with only a few preliminary studies in
329 minimal bacterial systems suggesting a (somehow unexpected) minor role of TFs in the
330 control of expression (13)(14)(15). We studied here the problem at a genomic scale
331 using all available regulatory data to first identify invariant genes as the ones that are
332 least regulated by TFs. The amount of regulation increases among the rest of
333 nonspecific classes, and between these and the specific ones. Moreover, specific
334 genes also show a stronger regulatory coherence than nonspecific genes. Coherence
335 quantifies the similarity of expression response (to growth rate) between genes and the
336 TFs acting on them and a strong signal suggests an operative role of the latter. In
337 addition, among those TFs whose action is particularly coherent, we identify groups
338 that almost exclusively regulate specific or nonspecific genes, or positive and negative
339 genes, i.e., the functioning of the TF network is somehow segregated.
340
341 Beyond TF regulation, we discriminated two general promoter architectures. Those that
342 are TATA enriched and shNFR/TFIID depleted (nonspecific negative and specific
343 genes), and those that are TATA depleted and shNFR/TFIID enriched (nonspecific
344 invariant and positive genes); features that are similarly observed in metazoans

345 promoters (29) (Type I promoters, genes expressed in a tissue-specific manner, and
346 Type II promoters, ubiquitously expressed genes, respectively).
347
348 That (nonspecific) positive genes are moderately controlled by TFs (like negative) but
349 depleted in TATA box (unlike negative) could suggest certain expression features (e.g.,
350 high level of transcription, Fig. S7) and alternative modes of epigenetic regulation.
351 Indeed, positive genes are enriched in fragile nucleosomes, which highlights the
352 regulatory role of nucleosomal stability (16). This is supported by the particular action
353 of GTFs on these genes [as GTFs fine-tune nucleosomal stability (19)]. In addition, we
354 find the expression of nonspecific genes being adjusted in distinctive manner by
355 epigenetic modifiers, with three main configurations: 1/ general activators of invariant
356 and positive genes, 2/ remodelers working in a dual manner; repressors of negative
357 genes and activators of positive ones, and 3/ elements acting as general repressors of
358 negative genes.
359
360 **Global and specific regulation in a broader context.**
361 Some of the previous features match those observations related to environmental
362 stress response genes (ESR) (30), so it is interesting to examine how this set fits into
363 our partition. ESR genes included two complementary subsets, which are enriched in
364 nonspecific negative and repressed genes (induced ESR genes), or nonspecific
365 positive genes (repressed ESR genes, Methods). This confirms the suggestion of
366 previous studies that stress response genes were not responding directly to stress but
367 rather to the associated decrease in growth rate [but see (31)].
368
369 More generally, two models to coordinate gene expression to the available nutrients
370 can be imagined: a *feed-forward* regulation by signaling pathways that predict growth
371 rate in a certain environmental condition, or a *feedback* mechanism, which senses
372 growth rate, or other related internal cell variable, and then modifies expression (32). In

373 this context, a passive resource allocation model could explain that the global program
374 is responding always to the environment, although indirectly (as it can only use those
375 resources that were not consumed in the mounting of the specific response). This
376 validates, for instance, that ribosomal genes follow the feed-forward model (33). The
377 fine-grained structure of the nonspecific class (invariant/positive/negative) could
378 nevertheless monitor growth rate, at least partially, with the feedback being mediated
379 by epigenetic mechanisms (see below).
380
381 If, as suggested by Hansen and O'Shea (34), TFs can mostly transmit qualitative
382 (presence/absence of a particular nutrient) rather than quantitative (amount of nutrient)
383 information, how can we then explain the monotonic variation of fractional expression
384 (with nutrient dilution in the chemostats) of the genes in the negative and positive
385 partition components? One way is that metabolism, which is highly sensitive to the
386 limiting nutrient (35) acts as a regulator of the epigenetic factors discussed above.
387 Indeed, several metabolites (e.g., GlcNAc, NAD+, acetyl-CoA, alpha KG, ATP) are
388 known to regulate transcription through interactions with enzymes involved in
389 epigenetic modifications (36). For example, acetyl-CoA induces cell growth and
390 proliferation by promoting the acetylation of histones at growth genes (37) (histone
391 acetylation affects rather similarly specific and nonspecific genes, Fig. S6, what
392 supports its potential role as a widespread mechanism). Another explanation is that the
393 monotonic variation observed is the result of cell population shifts with growth rate,
394 instead of changes in single-cell resource allocations. Note that these shifts cannot be
395 attributed to the fact that slow-growing cells enlarged their G1 cell cycle phase as
396 neither (12) nor us observed a bias in positive/negative genes with any particular phase
397 of cell cycled genes.
398
399 In this work we have studied changes in fractional expression but not in mRNA
400 abundances. It is known that the global program dictates that the faster a population of

401 cells growths, the higher the promoter activity (rate of RNA synthesis) (11) or total
402 mRNA abundance (rate of RNA synthesis and degradation) (38). We expect most (if
403 not all) gene products to follow this (absolute) global program, with potential additional
404 layers of regulation (which are nutrient and gene dependent) that increment or
405 decrement mRNA levels. Expression of the genes within the invariant group best
406 follows the absolute global program, with positive genes being slightly above -and
407 negative genes slightly below- this response (but all of them incrementing mRNA levels
408 or promoter activities) (e.g., Fig. S1B). On the other hand, it would be interesting to
409 quantify the degree to which single cells can present a distribution of resources that is
410 separated from the model here discussed (39), as well as to understand the
411 mechanisms that lead to such divergence.
412
413 In summary, although one could argue that cellular physiology can indeed determine a
414 global transcriptional program of gene expression control, our work highlights that this
415 program is adjusted by the integration of effective genetic and epigenetic modes of
416 regulation. This modulation limits the prospect of "simplifying" our understanding of
417 genome-wide expression change.
418

419 Materials and Methods

420 **Promoter activity (PA) data.** Keren *et al.* (11) measured the activities of ~900 *S.*
421 *cerevisiae* promoters in 10 different growing conditions using a library of fluorescent
422 reporters. For each strain in every growth condition, PA was obtained as the YFP
423 production rate per OD per second in the window of maximal growth.
424 **Five-sector partition based on PA data.** We computed fractional PA (f_{PA}) for each
425 growth condition as the ratio of the PA of each gene to the summed PA of all promoters
426 (for a gene *i*, $f_{PA,i} = PA_i / \Sigma PA_i$). Ratios of f_{PA}s for each pair of conditions (with
427 increasing growth rate) were also calculated. We then computed the absolute distance

428 of these ratios to ratio 1 (i.e., same f_{PA} in both conditions), and defined as invariant
429 genes the top 350 genes (distance closest to 0) and as activated (repressed) the
430 bottom 50 with ratio >0 (< 0). The rest of genes with ratio >0 (<0), and both f_{PA}s > 10^{-4},
431 were designated as nonspecific positive (negative). We used the "typical" class of a
432 gene (the most frequently occurring category that a gene presents in all pairwise
433 growth rate changes) to functionally characterize the sectors (Table S1) what
434 confirmed their biological significance. Minor modifications on the threshold values
435 defining these sectors did not alter the conclusions.
436 **Microarray data.** Brauer *et al.* (12) grew yeast cultures in chemostats under different
437 continuous culture conditions (six different limiting nutrients each at six dilution rates)
438 and measured mRNA abundance with two-color microarrays. Since the original
439 reference channel for all samples corresponded to a particular glucose condition, which
440 mixes the response of different nutrients, we reanalyzed the data without this reference
441 by considering the red processed signal as independent channel (40), and normalizing
442 by the corresponding sum for each case to obtain a fractional score; more specifically,
443 the fractional expression value of gene *i* is given by $f_i = \log_{10}[10^6(g_i/\Sigma g_i)]$, with g_i being
444 the corresponding red-processed signal. SVDs were computed on this processed data.
445 **Five-sector partition based on microarray data.** We defined as nonspecific genes
446 those whose difference on the loadings of the 1st component (a_i's) between two
447 conditions is less, or equal, than three standard deviations of all gene differences (in
448 absolute values). Genes are otherwise considered specific (activated or repressed if
449 the difference of a_i's is positive or negative, respectively). Moreover, absolute values of
450 the loadings of the 2nd component (b_i's) were sorted to define those with smallest
451 values (top 2500) as invariant genes, with the rest being positive or negative
452 (determined by the sign of b_i). To define the partition, we classify as nonspecific those
453 genes that act as nonspecific in >8 pairwise conditions (out of 15). Nonspecific genes
454 acting as invariant in > 3 conditions (recall that the total number is 6) are labeled as
455 invariant. Nonspecific and not invariant genes appearing more times as positive than

456 as negative (in all 6 conditions) are categorized as positive, and likewise for negative.
457 Specific genes which appear more times as positive than as negative (again in all 6
458 conditions) are categorized as activated, and analogously for repressed. This partition
459 is the one used for the all the regulatory analysis (Table S2 shows the connection
460 between these sectors and those obtained with PA data). Finally, note that minor
461 modifications on the threshold values defining these sectors did not alter the
462 conclusions, and that the biological significance of the sectors are validated by the
463 regulatory and functional signals observed.
464 **Regulatory network.** We obtained regulatory data from
465 http://yeastmine.yeastgenome.org. No microarray data is considered for the TF info;
466 only data from different manuscripts using chromatin immunoprecipitation, chromatin
467 immunoprecipitation-chip, chromatin immunoprecipitation-seq, combinatorial evidence,
468 and computational combinatorial evidence for a total of 20,673 interactions with 133
469 TFs involved (12). We also calculated the hierarchical organization of the network (41).
470 Bas1, Mbp1, Med6, Spt7, and Swi6 appear at the top of the hierarchy.
471 **Regulatory coherence.** We identified the set of TFs regulating each gene and
472 quantified the Pearson's correlation coefficient between the expression vector (as a
473 function of growth rate) of each TF within the set and the target gene to then take the
474 mean. This is the (mean) regulatory coherence in a given nutrient condition.
475 Randomizing expression vectors for each gene, 1000 times, we obtained a score of
476 significance for each gene's regulatory coherence. With this, we identified a list of
477 genes displaying significant regulatory coherence. Identification of TFs acting more
478 significantly on each partition component is computed by first measuring how often it
479 acts on significantly coherent genes, within the five-sector partition, and then estimating
480 a null value by randomization of the partition classes.
481 **Fragile nucleosome data.** Nucleosome occupancy and position have been measured
482 by analysis of MNase-digested chromatin. Recent work noted that certain nucleosomes

483 were extremely sensitive to this digestion, and thus obtained a quantitative score of
484 nucleosome fragility that we used for our analysis, Table S6 in (19).
485 **Chromatin compendium.** This set includes 170 gene expression profiles for
486 chromatin-regulation related mutations (expressed in $\log_2$ ratios) taken from 26 different
487 publications (22). It covers more than 60 potential interacting chromatin modifiers such
488 as histone acetyltransferases (HATs; the NuA4, HAT1 and SAGA complexes), histone
489 deacetylases (HDACs; the RPD3, HDA1 and SET3 complexes), histone
490 methyltransferases (the COMPASS complex), ATP-dependent chromatin remodelers
491 (the SWI/SNF, SWR1, INO80, ISWI and RSC complexes), and other chromatin-
492 affecting genes and cofactors such as Spt10, Sir proteins and the TATA-binding protein
493 (TBP). We normalized each dataset to unit variance (21). For Fig. S6, we took absolute
494 values to estimate the strength of the chromatin regulator effect. Note here that growth
495 rate reduction can be connected to the impact associated to many of these deletions,
496 so we controlled for the possible contribution of cell cycle population shifts as described
497 (next section). This enables us to better identify expression changes due to regulation
498 (42).
499 **Removal of the slow growth signature.** We took the full data in (43) to obtain the
500 slow growth profile and remove the slow growth signature in the epigenetic data
501 following (42). In brief, the slow growth profile is obtained as the first-mode
502 approximation of the data after SVD decomposition. To compare with the epigenetic
503 compendium data, we chose the column of this approximation with the largest norm as
504 the slow growth signature. The correlation with the slow growth signature is removed
505 by transforming the epigenetic data in Gram-Schmidt fashion by subtracting from their
506 projection onto the basis vector, given by the normalized slow growth profile.
507 **Statistical analysis.** Null models associated to most results are obtained by randomly
508 assigning each gene to one of the sectors, to then compute the precise statistic (e.g.,
509 mean number or regulators in Fig. 3A). We typically considered 10000 randomizations
510 unless it is stated otherwise and show the mean and +/- 2 std deviations of the

511 corresponding statistic (e.g., gray shading in Fig. 3A). Moreover, all P values shown in
512 enrichment analyses (Table S1-2) are calculated using the Hypergeometric distribution
513 with Holm-Bonferroni correction for multiple testing.
514 **ESR genes.** There are 281 stress-induced and 585 stress-repressed genes –as
515 defined in (30)– within the set of genes delineating the five-sector partition. A subset of
516 nonspecific negative genes and specific repressed genes corresponds to stress-
517 induced (232 out of 2053, and 10 out of 70, respectively), while a subset of nonspecific
518 positive genes corresponds to stress-repressed (485 out of 1914). Note that most of
519 the features discussed in the main text associated with the five-sector partition remain
520 when controlling for ESR genes.
521

522 ## Acknowledgments

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526 microarray data.
527

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Figures

Fig. 1

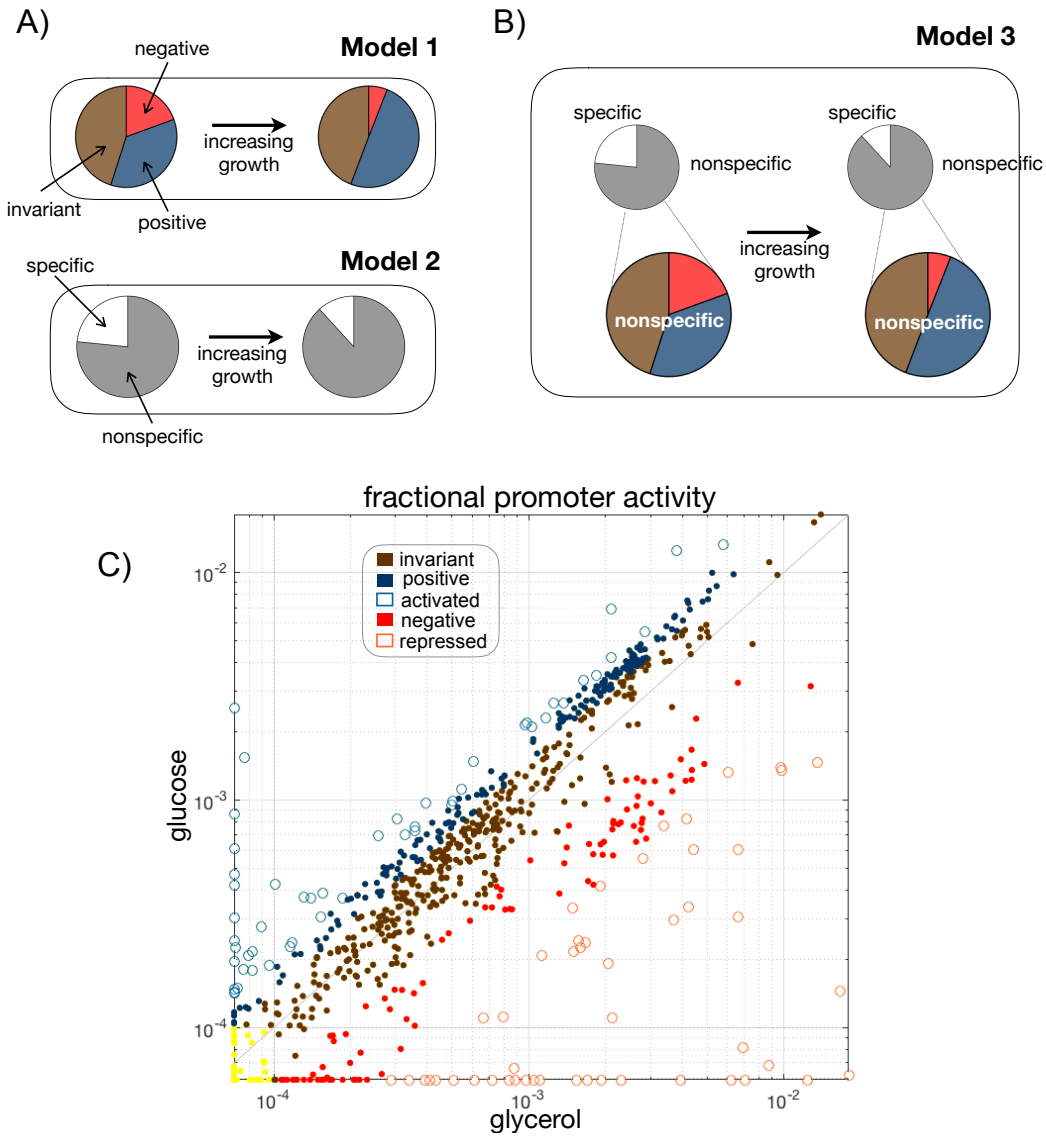
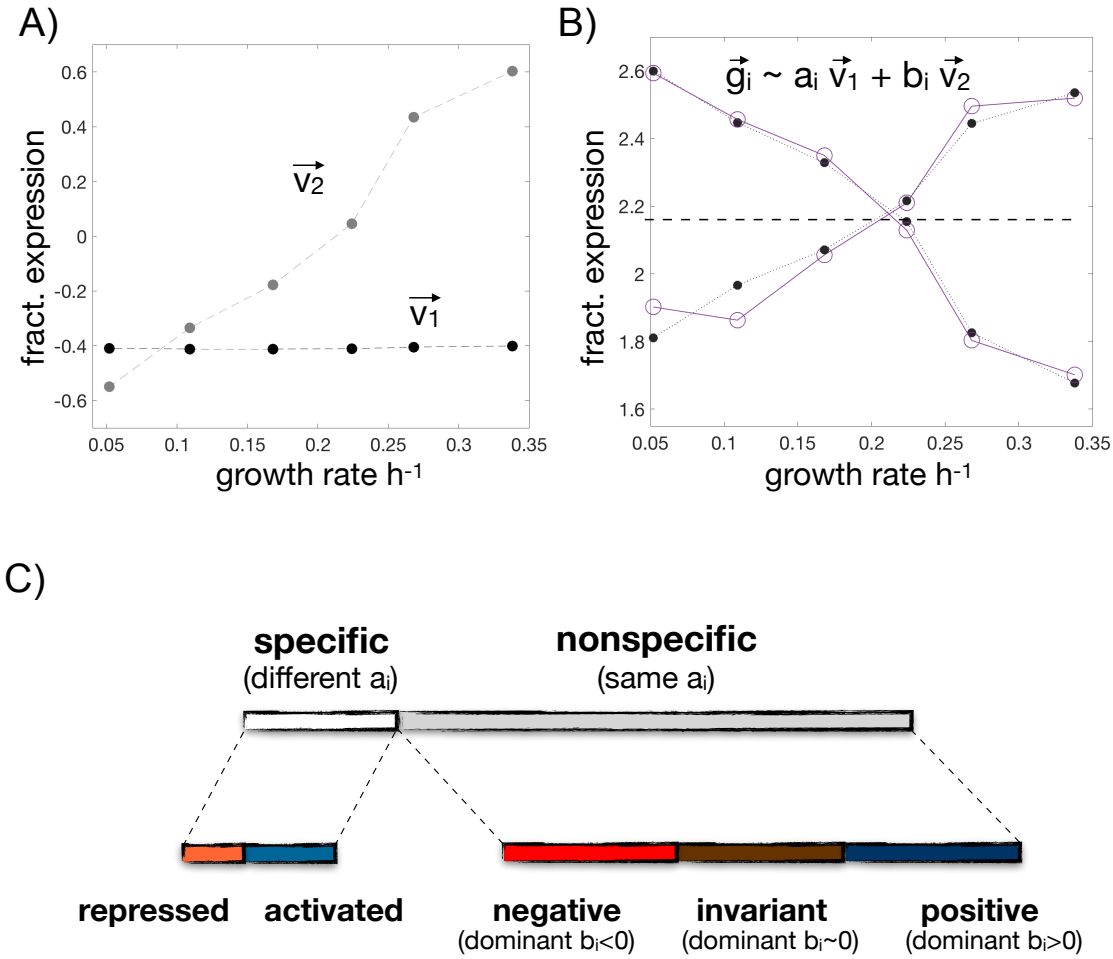


Figure 1. A new resource allocation model partitions the genome in five expression sectors. A) Top. Resource allocation model (Model 1) based on three sectors in which the expression of their constituent genes increases (positive genes, blue), decreases (negative genes, red), or remains constant (invariant genes, brown) with increasing growth rate. Bottom. Resource allocation model (Model 2) based on a specific and nonspecific sectors in which the expression of their constituent genes increases (nonspecific, white) or decreases (specific, grey) with increasing growth rate. **B)** Partition of genome expression into five sectors that combine the two previous models. A model-1-like partition appears as a fine structure within the nonspecific sector. We labelled this scheme as Model 3. **C)** Fractional promoter activity (fPA) for a transition between two example conditions increasing growth (glycerol to glucose). Promoters can be classified into the five sectors of model 3 depending on how their fPA changes (yellow dots indicate those with very low activity in both conditions). Repressed and activated promoters constitute the specific sector. Nonspecific promoters are constituted by one invariant type and two other subclasses whose fPA depends on the growing condition.

664 **Fig. 2**



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667 **Figure 2. Genome-wide partition of gene expression in five sectors based on SVD. A)**
668 SVD components $\vec{v}_1$ and $\vec{v}_2$ describe baseline fractional expression and dependence with
669 growth, respectively, and together explain most of the expression variance. **B)** The
670 fractional expression of every gene $\vec{g}_i$ as a function of growth rate can be approximated by
671 a linear combination of these two components, with loadings a_i and b_i. We show two
672 examples (purple circles denote the expression vector, while the black dots correspond to
673 the two-component approximation; lines added to help visualization) with the same
674 baseline (dashed line; same a_i) but whose expression increases (b_i>0) or decreases (b_i<0)
675 with growth. Data in A) and B) corresponds to growth in limiting glucose conditions. **C)** The
676 baseline expression of a gene between two conditions can change (top left) or not change
677 (top right). A gene is nonspecific if its baseline expression remains the same (similar a_i
678 loadings) in >8 pairwise change of conditions (total of 15), being specific otherwise. Within
679 nonspecific genes the second loadings b_i's on each nutrient condition (total of 6) enable us
680 to determine if the gene is positive (b_i>0), invariant (b_i~0) or negative (b_i<0). Specific genes
681 can similarly be divided between repressed or activated. In this way, we can partition the
682 genome into five sectors what generalizes the scheme discussed with PA data in a subset
683 of yeast genes (Figure 1). See main text for details.

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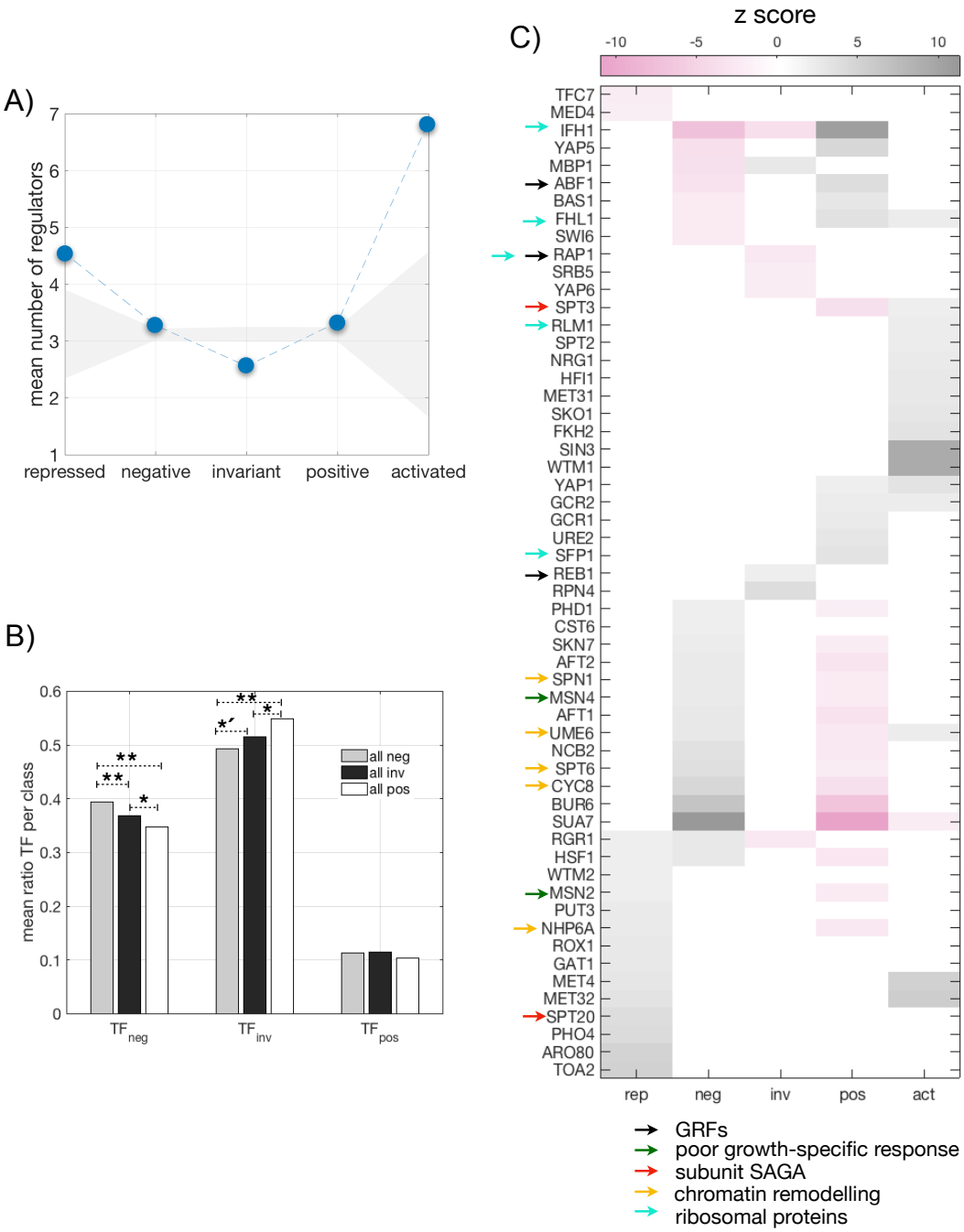
686 **Fig. 3**



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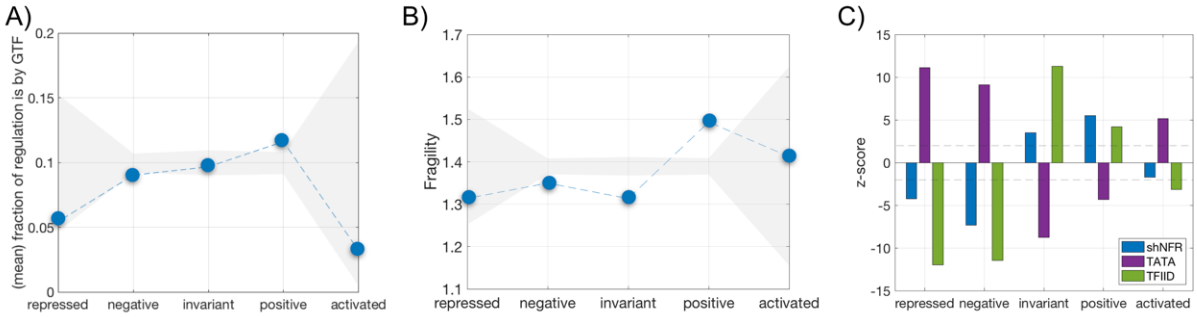
688 **Figure 3. Differential integration of transcriptional regulation and the five-sector**
689 **resource allocation model. A)** Mean number of regulators acting on genes as a function
690 of their response to growth (blue dots; dashed line to help visualization). Grey shading
691 denotes the average null values +/- 2 standard deviations obtained by randomization. **B)**
692 Mean ratio of the fraction of TFs of a given class with respect to growth (e.g., TF_{neg} denotes
693 TFs which are negative genes) for each group of target genes (also for a given class; here
694 we do not distinguish between nonspecific and specific). Histogram obtained in glucose
695 conditions, see also Fig. S5 for other nutrients (** $p < 0.001$, *´ $p < 0.01$, * $p < 0.05$, two-
696 sided KS test). **C)** Regulators that act dominantly, or secondarily, in genes showing a
697 significant regulatory coherence. Color denotes z-score with respect to a null obtained by
698 randomization (positive values denoting enrichment; negative values denoting lack of it).
699 Properties of some regulators are also included (arrows). See main text for details.

700 **Fig. 4**



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703 **Figure 4. Differential integration of epigenetic regulation and the five-sector resource**
704 **allocation model. A)** Mean fraction of global transcriptional regulators (Rap1, Reb1, Cbf1
705 and Mcm1) within the full set of regulators acting on each gene. Grey shading denotes the
706 average null values +/- 2 standard deviations obtained by randomization. Dashed lines to
707 help visualization. **B)** Mean nucleosomal fragility. Shading/lines as before. **C)** Enrichment
708 of nucleosomal free regions (shNFR, blue), presence of TATA boxes (purple), or action of
709 TFIID global factor (green) as function of response class (measured as z-score with respect
710 to a null by randomization; dashed line indicates z-score = +/- 2).

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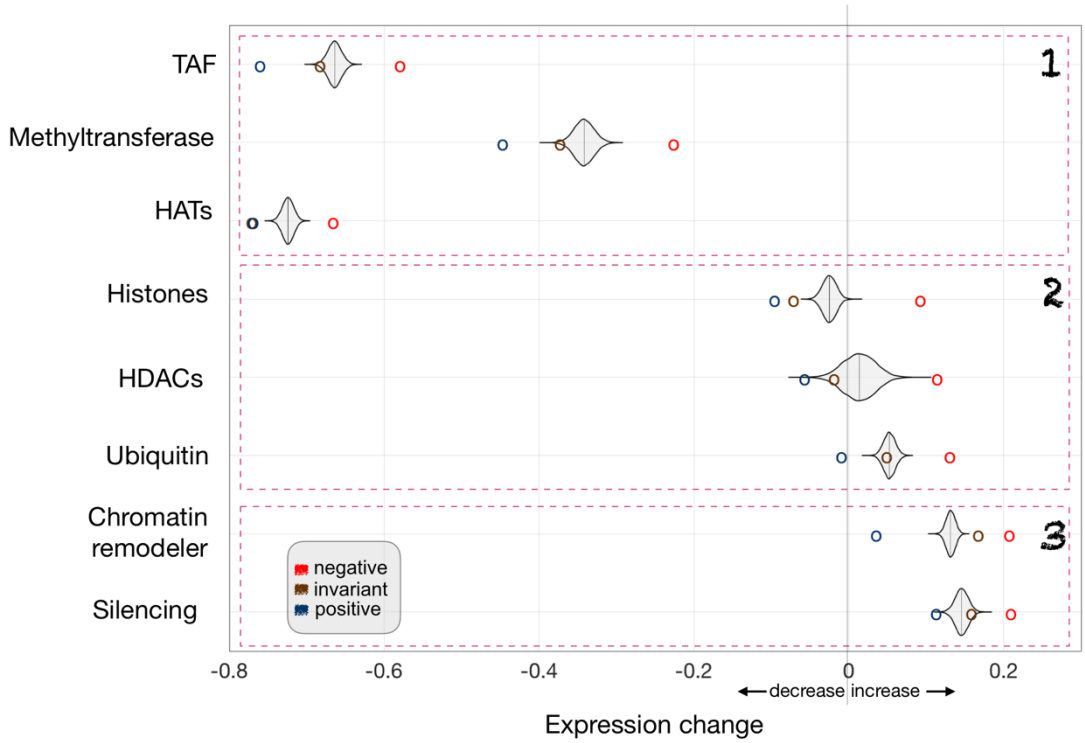
733 **Fig. 5**



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736 **Figure 5. Chromatin modifiers act differentially on nonspecific genes.** Mutations in
737 chromatin modifiers reveal their diverse regulatory role. For each modifier, we plotted its
738 mean effect, after mutation, on the genes constituting the negative (red circle), invariant
739 (brown circle), or positive (blue circle) sectors. A kernel density plot corresponding to a null
740 distribution obtained by permutation is also shown (Methods). We observed three broad
741 categories: 1/ modifiers acting as activators (mutation decreases significantly the
742 expression of invariant/positive genes), 2/ dual activator/repressor (mutation
743 decreasing/increasing significantly expression of positive/invariant or negative genes,
744 respectively), and 3/ repressors (mutation increasing significantly expression of negative
745 genes). TAF: TATA-binding protein related factors; HATs: histone acetyltransferases;
746 HDACs: histone deacetylases.

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Supplementary Information

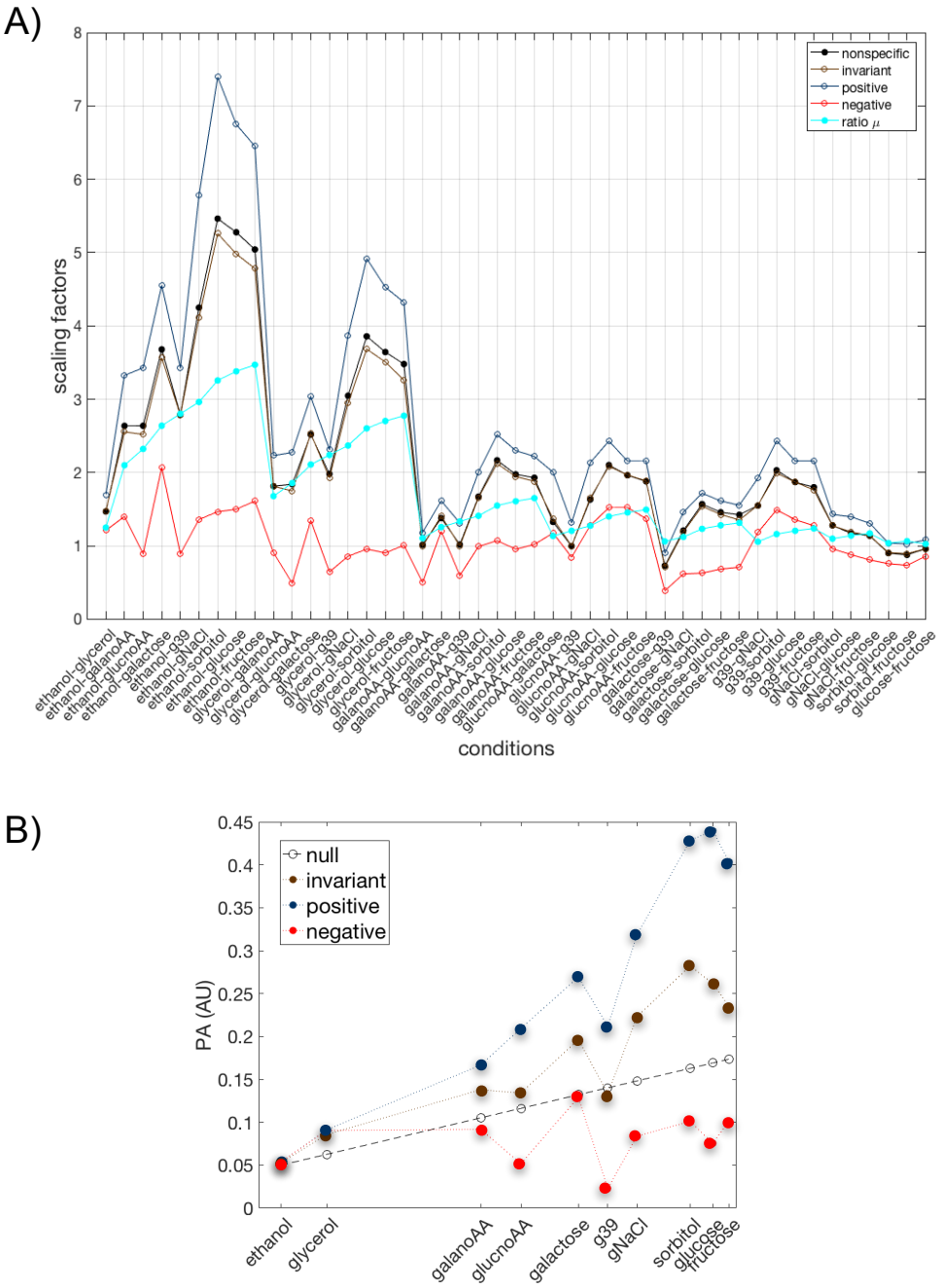

Figure S1. Scaling factors between pairs of conditions. A) Genes that follow a single proportional scaling may serve a definite cellular function according to *(11)*. We find a single scaling that describes the change of promoter activity (PA) for different subsets of promoters according to the five-sector partition. The three classes within the nonspecific promoters (invariant, negative, positive) clearly show singular scaling. Shown also a null that corresponds to the ratio of growth rates between conditions (cyan curve)**. B)** PA response of a typical invariant, positive and negative gene that corresponds to the *mrs11*, *rps6A* and *atp5*, respectively (conditions sorted by increasing growth rate; this is absolute PA not fractional PA). A null model of the dependence of PA with growth rate is given by the ratio of growth rates (empty circles). Gene categories within the global group clearly separate from the null.

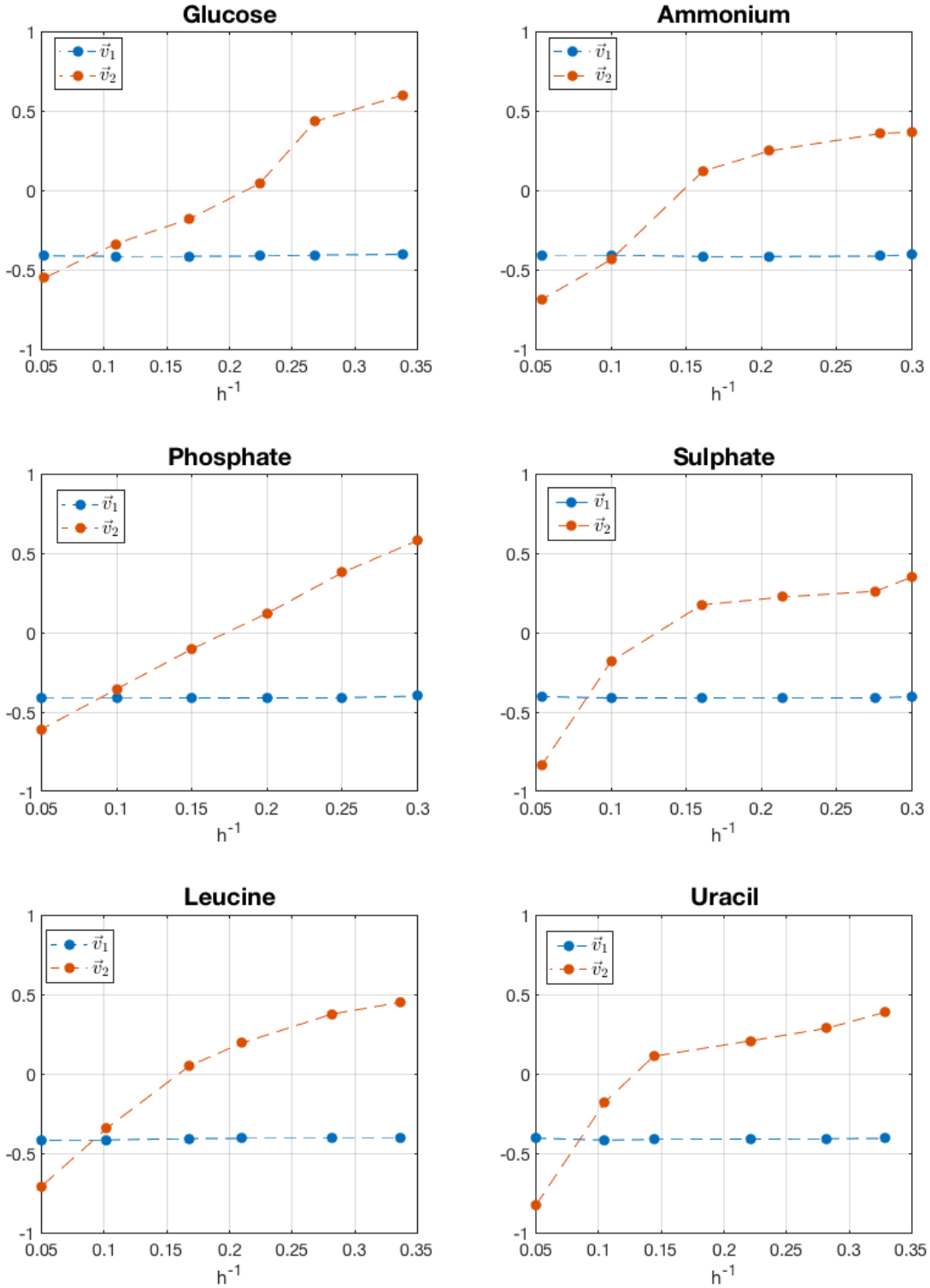

Figure S2. SVD components. First and second SVD components exhibited an analogous trend in all conditions what underlines a core response. As a result, expression of each gene can be approximated by the linear combination of these two components on each nutrient.

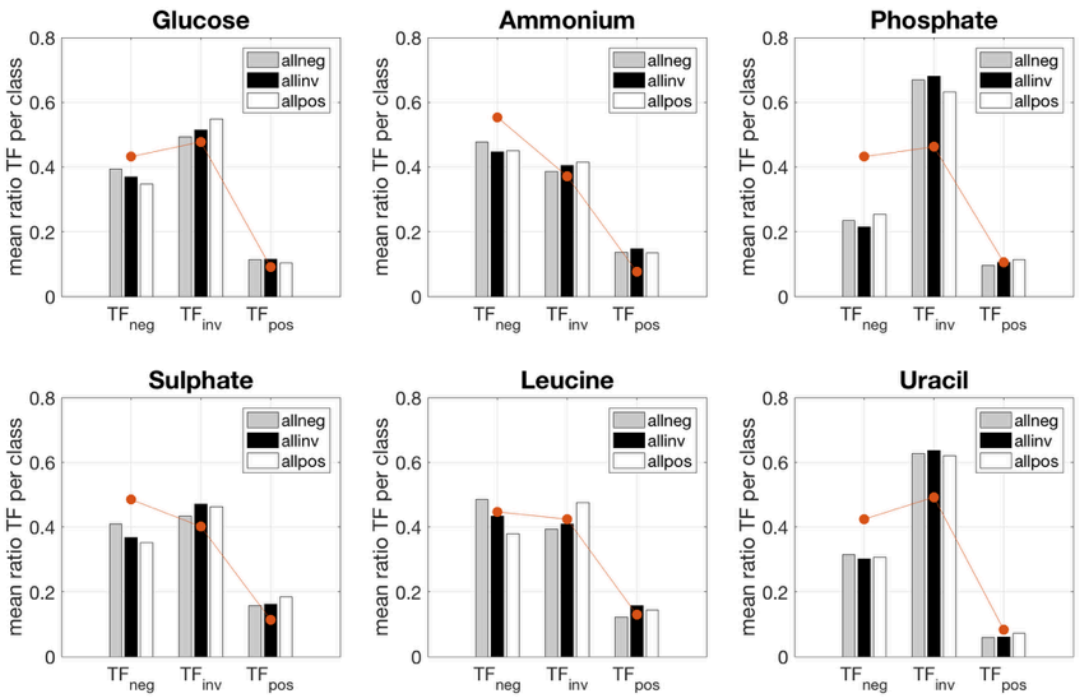

Figure S3. Association between class of TF and class of cognate target gene for all nutrients. Fraction of TF class (negative/invariant/positive) acting on target genes divided also with respect to growth response (negative/invariant/positive; nonspecific and specific genes were included that we denoted as allneg, etc.). Mean values of each grouping are shown in bars, while the orange curves show the distribution of each class of TF on each condition.

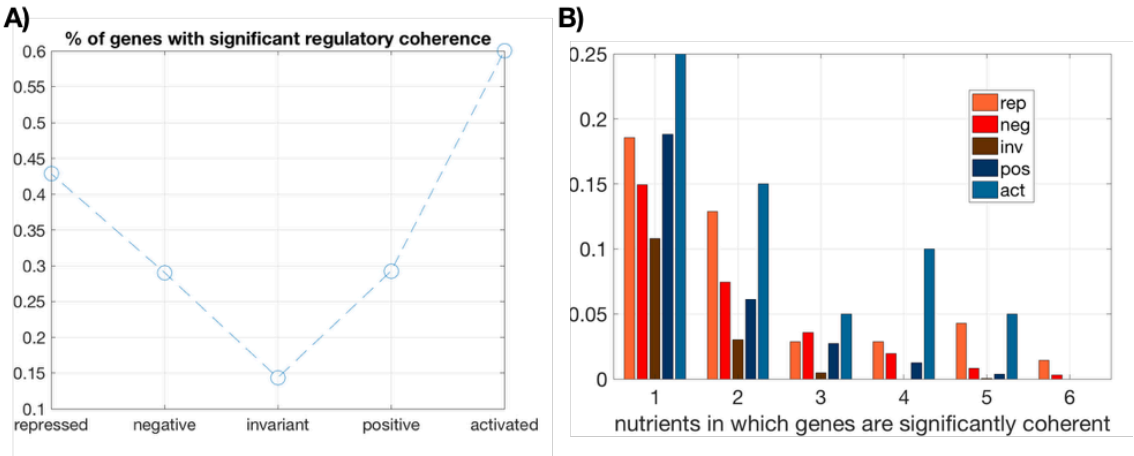

Figure S4. Regulatory coherence and five-sector resource allocation model. A) Percentage of genes within each class that exhibit a *significant* regulatory coherence with respect to a null in which classes were assigned randomly (1000 randomizations; significance implies z-scores > 2). Note that invariant genes show minimal coherence. **B)** Distribution of genes exhibiting *significant* regulatory coherence in 1 to 6 different nutrient conditions. Specific genes show more cases of genes significantly coherent in more different conditions, while invariant genes showed the opposite.

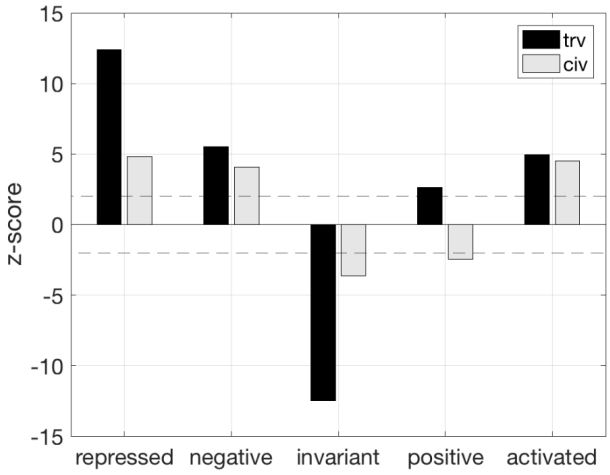

Figure S5. *Cis* and *trans* variability with respect to the five-sector resource allocation model. A cross between a standard laboratory yeast strain and a wild isolate allowed the computation of *cis* and *trans* effects on transcriptional variance *(21)*. For each partition, we quantified the mean of these measures and showed the associated z-score with respect to a null by randomization; dashed line indicates z-score = +/- 2. Positive genes show dominant effects associated with *trans* variability (trv and civ denote *trans* and *cis* variability, respectively).

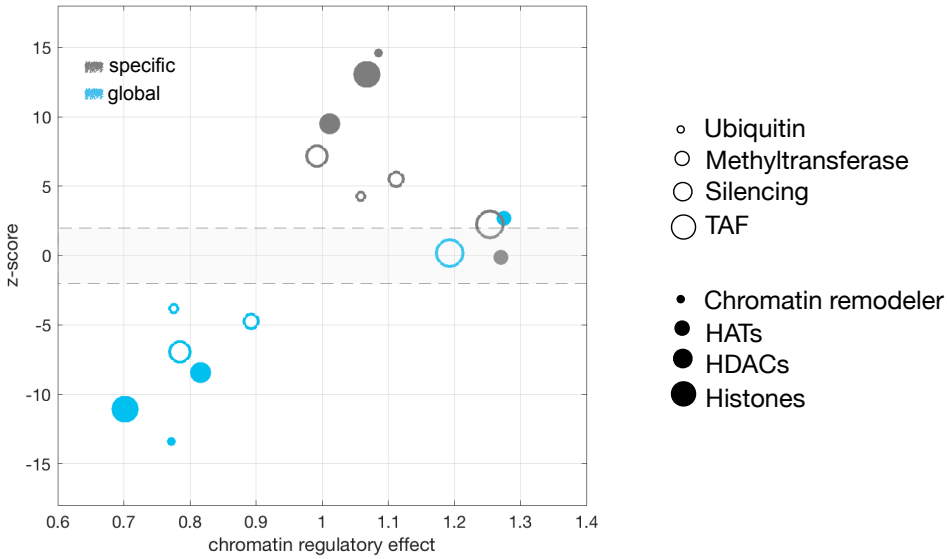

Figure S6. Chromatin modifiers act differentially on specific and nonspecific genes. The chromatin regulatory effect (CRE; *x* axis) quantifies change in gene expression (absolute value) due to mutations in chromatin modifiers. CRE values larger than expected by a null (z-scores > 2, obtained by randomization) are observed for most modifiers on specific genes. Each circle type corresponds to a class of epigenetic modifier (TAF: TATA-binding protein related factors; HATs: histone acetyltransferases; HDACs: histone deacetylases); *y* axis denotes z-scores and dashed lines emphasizes z-scores within +/-2 values.

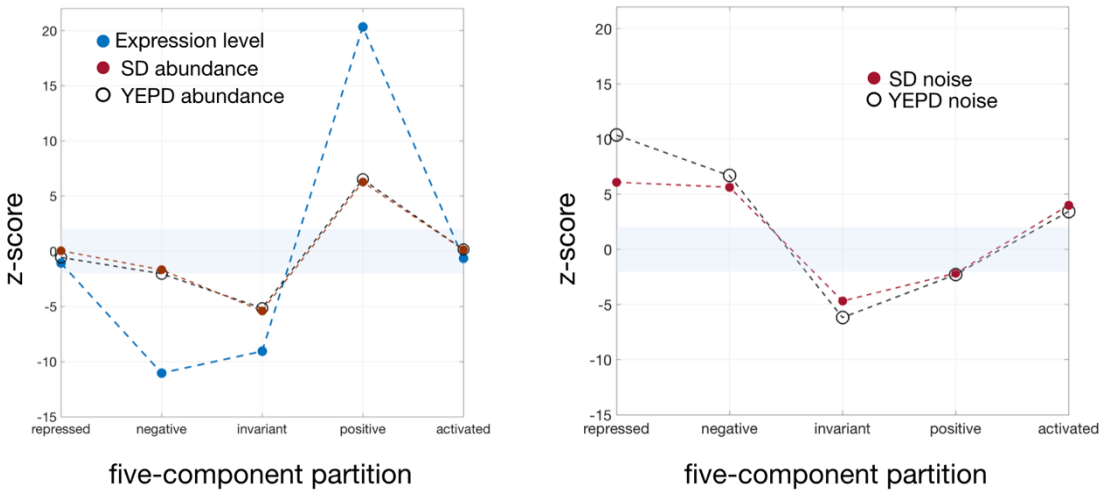

Figure S7. Expression abundance and noise with respect to the five-sector resource allocation model. Mean expression and protein abundance (A) and protein noise (B) with respect to the five-sector partition as compared to a null in which classes were assigned randomly (10000 randomizations; y-axis is plotting the associated z-score, shading corresponds to z-score values within a range of -/+ 2; SD/YEPD denote poor/rich growing conditions). Nonspecific and positive genes showed high expression and low noise, a signal that was associated to the presence of fragile nucleosomes in the promoter and the action of general transcription factors [both enriched in nonspecific positive genes, see main text and *(24)* for details on data].