# Additional document 5. Transcriptional control of cell growth

## Gene ontologies and processes regulating cell growth at the transcriptional level

*Genes up-regulated with increasing growth rate*

The initiation of translation (i.e., formation and regulation of the eIF4E-cap complex) is tightly regulated during growth [1-3] and probably acts to integrate cell growth and cell division [4]. Thus, dysregulation of cap-dependent translation has been reported to confer malignant characteristics and to induce cancer [5, 6]. Our finding that all yeast genes involved in translational initiation are amongst those whose transcription is consistently and significantly up-regulated with increasing growth rate (Table S8) confirms the critical role of this process in the overall control of growth, with a relevant participation of post-transcriptional and post-translational mechanisms as well (i.e. association of the mRNA cap binding protein eIF4E with 4E-binding proteins, controlled by phosphorylation-dephosphorylation) [2-4, 7]. The integration of growth and division via translation initiation (at least, in response to nutrient supply) is thought to be exerted mainly through the rapamycin-sensitive TOR signal transduction pathway (TORC1 complex) [1, 2].

Ribosome biogenesis and assembly require a huge investment of the cell's energetic and material resources. Moreover, all ribosomal components have to be synthesised co-ordinately in exact stoichiometric proportions in order to sustain balanced growth [8-10]. The fact that many ribosomal protein genes can act as haploinsufficient tumour suppressors in zebrafish [11] reflects the importance of coordinate stoichiometric regulation of the ribosome biogenesis and assembly machinery in balanced growth [9, 10, 12]. Growth-rate dependent regulation of ribosomal-protein synthesis at the transcriptional level has been reported for batch cultures of yeast and

other organisms grown on different carbon sources [9, 13-15] although, translational controls have also be shown to be involved [10]. Our results (Figs. S5 and S17) confirm that ribosome biogenesis and assembly are central growth-related processes, in which a large number of genes are significantly up-regulated with increasing growth rates, irrespective of the growth-limiting nutrient (Fig. S17). Ribosome biogenesis has been reported to be regulated through the cAMP-dependent protein kinase (PKA) of the RAS/cAMP signal transduction pathway – which is, itself, subject to control by the TOR-signalling pathway [2, 10, 12, 16].

RNA polymerases are central to the control of growth via the regulation of mRNA synthesis, as well as being responsible for the RNA components of the translational apparatus, rRNAs and tRNAs. RNA polymerase III (pol III) occupies a unique position, in that it is responsible for the synthesis of two components of the translational machinery (tRNAs and 5S rRNA), as well as 7SL RNA. A wide range of transformed and tumour cell types have been shown to express elevated levels of pol III products [17]. The exact mechanisms involved in regulating the expression of genes encoding components of the three RNA polymerase complexes are the subject of intensive study (White, 2004b [18] and references therein). Our data show a clear coordinated up-regulation of the transcription of these genes with increasing growth rate (Fig. S18). Aminoacyl-tRNA synthetases are responsible for the charging of tRNAs with amino acids to sustain protein synthesis. Our results show that a majority of the 20 genes encoding these enzymes are up-regulated with increasing growth-rates (Fig. S19). This is also confirmed by proteomics data which showed that the levels of the aminoacyl-tRNA synthetases were also consistently up-regulated at the level of protein synthesis with increasing growth rates (see proteomic studies).

The proteasome is a supramolecular protein complex dedicated mainly to controlled proteolysis of ubiquitin conjugated proteins [19, 20], although new roles and participation in mechanisms of control of gene expression have also been reported (e.g. altering the SAGA coactivator and its interactions with transcriptional activators) [21]. It's a highly complex ATPase-dependent protease consisting of over 30 subunits, the majority of which are essential (SGD database; [22]). Subunit expression, protein composition and levels of individual proteolytic activities have been reported to vary under different growth states [23], which points to a relevant role of this complex in balanced growth, not always considered or being overlooked in the majority of cases. In our transcriptional studies eight specific protein subunits (mainly 20S proteasome subunits) were found consistently up-regulated with growth rate (Table S3) which points to a coordinate regulation of protein synthesis and degradation during balanced growth.

*Genes down-regulated with increasing growth rate*

Cell growth entails the coordinated up- and down-regulation of a variety of processes. Genes that are down-regulated with increasing growth rate are probably involved in maximising the efficient utilisation of cellular resources at each different growth rate and culture condition, particularly when nutrients are scarce. Our data indicate that this is a poorly understood aspect of the cell's economy since a significant number of these genes (140/398) are of as yet undetermined function (Fig. 2). This is despite the fact that nutrient scarcity is likely to be a common circumstance in the organism's natural environment [24]. Among the genes of known function that are up-regulated at low growth rates are those involved in mobilisation and storage of available resources at the level of the vacuole (Fig. S20). Another interesting example

of genes that are up-regulated at low growth rates are those involved in autophagy (Fig. S21). Autophagy is a major system of bulk degradation of cellular components. It participates in the coordinate degradation of cytoplasmic components, including proteins, large complexes and organelles whose turnover is important in the control of cell growth. Autophagy mediates the shrinkage of the ribosome pool, thus slowing cell growth when nutrients are limiting [25]. Autophagy in yeast has been reported to be a TOR-mediated response to nutrient starvation [26], and we have demonstrated previously the induction of autophagy genes in stationary phase [27]. Autophagy genes are well conserved from yeast to mammals, suggesting that it is a fundamental activity of eukaryotic cells, being implicated in processes such as homeostasis, development and differentiation [25]. Other genes that are up-regulated at low growth rates are those encoding specific transcriptional repressors whose action results in the activation of alternative routes for the assimilation of substrates and/or as an adaptation to the environment.

In all, the data on the down-regulated genes present a picture of the yeast cell at low growth rates, activating pathways involved in the response to external stimuli, maintenance of homeostasis, vacuolar transport and storage, and autophagy; the whole being directed towards a more efficient use of scarce resources.

*Protein-protein interactions*

Remarkably, whereas proteins encoded by the up-regulated genes participate in a large number of interactions with each other (876; expected by chance 287), the ones encoded by the down-regulated genes rarely interact with one another (89; expected by chance 193) (Tables S12 and S13). The existence of a high number of interactions in the first list can be explained since the up-regulated genes include many protein

complexes and components of the translational machinery. The existence of a low number of interactions in the group of down-regulated genes points to a more relevant contribution of other mechanisms, with participation of kinases, phosphotransferases, ATPases and oxidoreductases among others (Table S10). Moreover, an important number of pair-interactions were encountered between the up- and down-regulated lists (233; predicted by chance 472) (Table S14), which points to the existence of direct, close interrelationships between the two sets.

*Coordination of cell growth and division. Transcriptional studies*

The most accepted, generally occurring model is that one in which cell growth precedes cell division [4] where the cell must be able to monitor its actual 'cell size', either as mass, volume or biosynthetic fluxes. The present perspective points to the existence of two different mechanisms: (1) Mechanisms controlling the actual cell size, and (2) Mechanisms controlling the critical size, maximum threshold attainable by the cell under each specific condition before being committed to cell division at 'Start' [10, 28-31]. As far as the control of actual cell size is concerned, Jorgensen and coworkers (2002) [28] tested the entire yeast gene knockout set for genes affecting cell size, finding 451 candidate genes (221 small mutants and 230 large mutants) with translation initiation factors, ribosomal protein genes, aminoacyl-tRNA synthetases, RNA pol II transcription complexes and proteasome subunits among the most relevant families and functional categories underlying cell size control, in good agreement with our results, particularly on genes and functions transcriptionally up-regulated with increasing growth rates (Fig S5 and S6; Table S6). We found a group of 94 genes with mutants affected in cell size significantly growth-rate regulated (76 up-regulated genes, 18 in the down regulated set) (Tables S3 and S4), whereas other

groups of genes with mutants affected in cell size do not appear in our lists. The global results show good congruence and agree with the present perspective that control of actual cell size is complex and can be exerted by different mechanisms at different regulatory levels (i.e. transcriptional and post-transcriptional level), with the role of many individual genes still to be elucidated [30].

As far as the control of the critical cell size, same relevant study by Jorgensen and coworkers (2002) [28] resulted in the discovery of a 'regulon' of 29 genes underlying the mechanisms controlling 'critical cell size' and the coupling between growth and cell division [28, 29]. In this study, two relevant genes were found situated upstream this 'regulon', Ptk2, a reported putative serine/threonine kinase, and the transcriptional activator Sfp1 controlling 15 ribosomal protein genes [28, 29]. In our studies Ptk2 was found significantly down-regulated (Table S4) with increasing growth rates. We didn't find Sfp1 significantly regulated at the transcriptional level, which agrees with studies that refer its predominant role at the subcellular localization (nucleus:cytosolic ratio) level, as reported for other ribosomal protein genes regulators (e.g. Sch9; Ifh1, Fhl1) [32-36]. With independence of this, we could identify eight of the ribosomal protein genes controlled by Sfp1 as exhibiting significantly up-regulation with growth at the transcriptional level (Table S3). Apart from this, the initiation of translation has also been reported as a critical point which can mediate the coupling between cell growth and cell division [4], which is also regulated by TOR [3, 37]. More comprehensive studies at the transcriptional, translational, post-translational and metabolic level are necessary to advance our knowledge of the mechanisms coordinating cell growth and cell division.

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