Understanding translational control mechanisms of the mTOR pathway in CHO cells by polysome profiling

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The mammalian target of rapamycin (mTOR) pathway plays essential roles in the regulation of translational activity in many eukaryotes. Thus, from a bioprocessing point of view, understanding its molecular mechanisms may provide potential avenues for improving cell culture performance. Toward this end, the mTOR pathway of CHO cells in batch cultures was subjected to rapamycin treatment (inhibition) or nutrient supplementation (induction) and translational activities of CHO cells producing a monoclonal antibody (mAb) were evaluated with polysome profiling technology. Expectedly, rapamycin induced a shift of mRNAs from polysomes towards monosomes, thus reducing maximum cellular growth rate by 30%, while feeding additional nutrients extended mTOR pathway activity during the stationary growth phase in control batch culture, thereby contributing to an increase in global translation activity by up to 2-fold, and up to 5-fold higher specific translation of the heavy and light chains of the recombinant mAb. These increases in translation activity correlated with a 5-day extension in cellular growth and a 4-fold higher final product titer observed upon nutrient feeding. This first study of the relationship between the mTOR pathway and translational activity in CHO cultures provides key insights into the role of translational control in supporting greater productivity, which will lead to further enhancement of CHO cultures.

Introduction
Cellular organisms have evolved translational control mechanisms in order to adjust their translational activity, an energy-intensive process [1], with respect to resource availability. Hence by controlling translation, cells ensure an appropriate coupling of cellular growth and metabolism with their surrounding environmental conditions [2]. Translational control has been routinely studied in yeast and stem cells, as well as human cell lines via translomic profiling [3–6], which is based on the polysome profiling technique. Polysome profile analysis has been used for the past 40 years to investigate cellular translational status under various physiological conditions and environmental stresses [7], reveal defects in ribosome biogenesis [8], study functions of proteins involved in translation [9], and understand miRNA mediated translational repression [10]. The polysome profiling principle consists in assessing the degree of ribosome loading of each mRNA as an indication of translation efficiency [11]. Interestingly, our recent translomic profiling of CHO cells [12] showed that the degree of correlation between transcript level and translational efficiency is partly controlled by the mammalian target of rapamycin (mTOR) signaling pathway, considered to be one of the major conserved regulators of translational control from yeast to human cells [13].

Briefly, the mTOR pathway is divided into upstream and downstream phosphorylation cascades (Fig. 1), revolving around the

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TOR protein which is found in two different complexes, TOR complex 1 (TORc1) and TOR complex 2 (TORc2) [14]. TORc1 is inhibited by rapamycin and directly controls translational activity, while TORc2 is insensitive to rapamycin and has no direct implication on translation, although it was recently proposed to affect TORc1 activity through the AKT pathway [15]. The tumour suppressor complex (TSC) is one of the most well-established upstream regulators of TORc1, acting as a molecular switchboard that integrates several incoming environmental signals [16]. Environmental cues such as levels of amino acids [17] and growth factors [18] as well as energy [19] and oxygen levels [20] initiate the upstream phosphorylation cascade towards TORc1. Under favourable growth conditions, TORc1 activates the downstream cascade, which phosphorylates the main downstream substrates including 4E binding protein (4EBP) and ribosomal protein S6 kinase 1 (S6K1) [21]. Upon phosphorylation, 4EBP, the substrate with the most recognized impact on translation, becomes inhibited and ceases sequestering eukaryotic initiation factor 4E (eIF4E) [22], the release of which is required for translation initiation at the 5’CAP structure of mRNA, allowing translation to proceed [23]. Conversely, when TORc1 perceives adverse growth condition, such as nutrient limitation, TORc1 is inhibited and the consequent decrease in 4EBP phosphorylation reduces translation.

Recently, engineering of the mTOR pathway in CHO cells was shown to increase growth and productivity phenotypes [24], but the underlying cellular mechanisms supporting such change of phenotype remained unclear. The first investigation of mTOR pathway mechanisms in CHO cells was based on rapamycin treatment [25], a specific inhibitor of TORc1. Upon rapamycin target inhibition of the mTOR pathway, the growth of CHO cells appeared to be extended by autophagy mechanisms. However, no data was reported on the state of translation activity, otherwise widely known to be largely regulated by the mTOR pathway, and how it impacted the key phenotypes of growth and productivity. Hence, it is important to establish the missing relationship between translation activity and the mTOR pathway and its influence on the fate of CHO cell cultures.

In CHO cell cultures, besides rapamycin treatment, more natural environmental stresses such as limiting levels of amino-acids [26] or low energy levels [27,28], are known to alter the activation of the mTOR pathway and its downstream effector 4EBP. It was therefore postulated that depletion of nutrients in batch cultures will deactivate the mTOR pathway, whereas maintenance of nutrient levels in fed-batch cultures will extend its activation and translation activity. Translation activity can be assessed by polysome profiling which measures the degree of association between mRNAs and ribosomes [29], where actively translated mRNAs are loaded with several ribosomes and referred to as polysomes. Polysome elongation along mRNAs is arrested by the elongation inhibitor cycloheximide and the resulting distribution of polysome loading is accounted for by translation initiation only, which is widely accepted as the limiting step controlling the rate of translation [11]. Since translation initiation is targeted by the TORc1-4EBP cascade, we opted for this approach to assess the effect of the mTOR pathway on translation activity in CHO cells cultures. Furthermore, polysome profiling allows access to specific mRNAs for the determination of targeted gene translation efficiency [11].

In this study, we attempted to evaluate the impact of the mTOR pathway’s translational control activity on the growth and productivity of CHO cells producing monoclonal antibodies cultures. It was initiated by specifically inhibiting TORc1 with rapamycin in order to establish a reference effect on translation activity as control (Fig. 2). Then batch and fed-batch cultures were compared to study how addition of supplementary nutrients to cells would affect the mTOR pathway and translational activity. Global translation and specific translation efficiencies of the heavy and light chain (HC, LC) of the recombinant monoclonal antibody were tracked in relation to the activity of the mTOR pathway.

**Materials and methods**

**CHO-mAb cell line and medium**

The DG44-CHO cell line stably expressing monoclonal immunoglobulin G (IgG) against a human rhesus-D antigen [30] was cultured in 300 mL of an inhouse proprietary protein-free and chemically defined medium, supplemented with 8 mM i-glutamine (Sigma–Aldrich, St. Louis, MO), 600 µg mL⁻¹ G418 (Sigma–Aldrich, St. Louis, MO), 250 nM MTX (Sigma–Aldrich, St. Louis, MO) and 0.1% (v/v) Pluronic® F68 (Invitrogen, Carlsbad, CA) in 1 L disposable Erlenmeyer flasks. For rapamycin treatment, 20 ng mL⁻¹ of rapamycin (Sigma–Aldrich, St. Louis, MO) was added on day 0 of culture. The cultures were seeded at 3 × 10⁶ cell mL⁻¹ and grown in suspension at 37°C, under an 8% CO₂ atmosphere and shaker platforms set at 110 rpm in a humidified incubator (Kühner, Basel, Switzerland). Fed-batch cultures were fed once a day with a proprietary feed-solution in order to maintain a glutamine concentration above a threshold of 0.3 mM. The volume of feed-solution to be added at time t was determined based on projected consumption of glutamine by the cell till next feeding at time t + 1. Projected consumption was calculated according to a constant glutamine consumption rate and projected cell density at t + 1. Projected cell density depended on growth rate and cell density at time t as well as duration to next feeding. Cell densities and viabilities were determined by the trypan blue exclusion method using a Cedex automated cell counter (Innovatis, Roche, Basel, Switzerland). Glutamine was quantified in technical triplicate by YSI-7100 analyser (Life Science, Yellow Springs, OH). Titers of secreted monoclonal antibody were measured by IMMAGE® 800 immunochemistry system (Beckman Coulter, Pasadena, CA) according to the manufacturer’s instructions.

**Activity of the mTOR pathway**

The activity of the mTOR pathway was determined by the degree of phosphorylation of eukaryotic initiation factor 4E binding protein (4EBP) [31]. Immunoblotting on total proteins was used to assess the phosphorylation degree of 4EBP. Total proteins were extracted from 10⁶ cells for 45 min with ice-cold RIPA buffer (Thermo Scientific, Walthman, MA) supplemented with 1X Halt™ phosphatase inhibitor cocktail (Thermo Scientific, Walthman, MA) and 1X Halt™ protease inhibitor (Thermo Scientific, Walthman, MA). Supernatants were collected and stored at −20°C after centrifugation at 18,000 rpm for 15 min at 4°C (Beckman Coulter, Pasadena, CA). The total protein concentration in the supernatant was quantified using BCA assay (Thermo Scientific, Walthman, MA) according to manufacturer’s instructions. For
immunoblotting, 25 μg of protein extract from each sample was separated in a 12% Bis–Tris gel (Invitrogen, Carlsbad, CA) and transferred to a PVDF membrane using the iBlot® dry blotting system (Invitrogen, Carlsbad, CA). The membrane was blocked in bovine serum albumin (BSA) blocking solution (Sigma–Aldrich, St. Louis, MO). Total 4EBP was probed with an antibody raised in rabbit against total-4EBP #9452, dilution 1:1000 (Cell Signaling Technology, Beverly, MA) and detected by using an anti-rabbit HRP conjugated secondary antibody #7074, 1:2000 (Cell Signaling Technology, Beverly, MA). Antigen bands were visualised using the ECL or ECL plus kits (Amersham Bioscience, Piscataway, NJ) according to the manufacturer’s instructions and recorded onto chemiluminescent film (Roche Diagnostics, Pleasanton, CA). Consequently, the membrane was restored using western blot stripping buffer (Thermo Scientific, Walthman, MA) and reprobed with an anti-actin antibody ab8226, dilution 1:5000 (Abcam, Cambridge, England) and secondary antibody ab6728, dilution 1:8000 (Abcam) as a loading control.

Polysome profiling, fractionation and pooling and RNA extraction and purification

All the polysome profiles and subsequent RNA preparation steps were essentially performed according to the translational platform, which we recently developed [12]. In brief, total RNA was extracted from cells treated with 100 μg mL⁻¹ cycloheximide (Sigma–Aldrich, St. Louis, MO) and separated on 10–50% sucrose gradient. Thirteen fractions from the 80S pick were pooled together in pool A (polysome enriched) and pool B (monosome enriched). Pooled RNAs were purified via several phenol–chloroform (Sigma–Aldrich, St. Louis, MO) extraction steps.

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Translation efficiency of heavy and light chain mRNA of mAb

RNA extracted from each pool was reversed transcribed into cDNA using oligo dT primer (Promega, Madison, WI). For pool A (polysome enriched) and pool B (monosome enriched) pairs, cDNA synthesis was based on equal volume of input RNA which was determined based on the highest RNA concentration among the 2 pools. cDNA synthesis was performed using the Improm II reverse transcription system (Promega, Madison, WI) according to manufacturer instruction in 20 μL reaction volume. HC, LC and thrB (spike-in control) mRNA levels were determined using quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) with the primers shown in Table 1. All primer pairs were tested for ampli-

<table>
<thead>
<tr>
<th>Targeted gene</th>
<th>Forward primer (5′–3′)</th>
<th>Reverse primer (5′–3′)</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>AAGGGCCGATTCACCATCTC</td>
<td>CGGCCTCTCGCACAGTAATAC</td>
<td>1.00</td>
</tr>
<tr>
<td>LC</td>
<td>CTTCCACCTGTCTGCATCTG</td>
<td>GATCCTTGATGGGACCCC</td>
<td>1.05</td>
</tr>
<tr>
<td>thrB</td>
<td>CTCgccTCAAGCTGTCATGTAC</td>
<td>CGGTTGAATTCTCACAGATGG</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Figure 2
Overview organigram of the experimental design to study translational control by the mTOR pathway in CHO cells. A preliminary rapamycin treatment of control batch cultures specifically inhibited TORC1, thereby providing the reference effect of mTOR pathway inhibition on global translation activity. Thereafter, batch cultures were fed with additional nutrients, to induce the mTOR pathway while alleviating nutrient limitation as cultures progressed. Under batch and fed-batch comparison, the translatomics platform that we previously developed [12] was utilised to further measure the specific translation efficiency of the heavy and light chains of the monoclonal antibody.
FIGURE 3
Rapamycin treatment of batch cultures: (a) Inhibition of the mTOR pathway. The activity level of the mTOR pathway was assessed through the degree of phosphorylation of the downstream protein eukaryotic initiation factor 4E-binding protein (4EBP). The most phosphorylated the 4EBP, the higher the activity of the mTOR pathway. The gel shift approach utilized in this study resolved the three phosphorylated isoforms α, β and γ of 4EBP around 20 kDa on immunoblotting. The α band was the least phosphorylated, while the γ band was the most phosphorylated according to Ikenoue et al. (2009) [31]. Rapamycin treated and control cultures were compared from day 1 to day 5 with β-actin as loading control. (b) Cellular growth performance. (c) Product accumulation of recombinant monoclonal antibody. (d) Residual concentration of glucose and glutamine metabolites. Rapamycin-treated cells (red) and control batch cultures (blue). Error bars show ± standard deviation of three biological replicates. (e) Effect of mTOR pathway on global translation activity upon rapamycin treatment. For each polysome profile, the first three peaks detected before the dashed vertical line represented non-actively translating ribosomes referred as the monosome [11] whereas the subsequent series of peaks after the vertical line, represented the polysomes with increasing numbers of ribosomes attached to the mRNA. Arrows locate 80S peak increase.
FIGURE 4

Nutrient feeding of batch cultures. (a) Cellular growth performance. (b) Product accumulation of recombinant monoclonal antibody. (c) Residual concentration of glutamine. Fed-batch cultures (green) and control batch cultures (blue). Error bars show ± standard deviation of three biological replicates. (d) Extension of mTOR pathway activation by nutrient supplementation. Gel shift analysis of 4EBP phosphorylation degree for batch (day 1–day 8) and for fed-batch (day 1–day 12) cultures. Results are shown only up to day 8 for the batch culture, when the culture ended. β-Actin served as a loading control. Lysate of cells treated with rapamycin, served as a negative control. (e) Effect of mTOR pathway on global translation activity upon nutrient supplementation. Daily comparison of fed-batch.
fication efficiency and specificity in the particular experimental conditions used in this study. Each qRT-PCR reaction was performed in technical duplicates in a 96-well IQ real-time PCR plate (Bio-rad, Hercules, CA) for 40 cycles at 95°C for 30 s and 60°C for 10 s. For each technical duplicate of 10 μL reaction mixtures were prepared as master mix with 8 μL cDNA solution, 10 μL SsoFastTM EvaGreen® supermix (Bio-rad, Hercules, CA) and 2 μL 10 μM primer pair. Raw CT values of pool A and B were first normalised to their respective thrB gene CT values in order to account for possible loss of RNA during the extraction and purification from the polysome fractions. Once normalised, all values were processed with the standard Δ(CT) method [32]. Thereafter, translation efficiency of HC or LC was calculated as the ratio of quantified mRNA in pool A over pool B as following $\frac{2^{\Delta CT}}{ct\text{r}}$ where CTa and CTb represent the thrB normalized CT values of gene X in pool A and pool B respectively.

**Results and discussion**

**Targeted inhibition of the mTOR pathway by rapamycin reduced global translation and cellular growth**

Twenty ng mL⁻¹ of rapamycin was added on day 0 of batch cultures to specifically inhibit the mTOR pathway in CHO cells. The inhibitory effect was monitored over the five days following rapamycin treatment by assessing the degree of 4EBP phosphorylation by the gel shift approach that resolved three phosphorylated forms (γ, β and α) of 4EBP on immunoblot. The γ band is the most phosphorylated 4EBP while the α band is the least phosphorylated [31]. As can be seen on the immunoblot results in Fig. 3a, there was a strong shift of total 4EBP in its α-form upon rapamycin treatment, confirming inhibition of the mTOR pathway as compared to the control culture where there was higher phosphorylation degree in the γ and β bands. However, unlike Choo et al. (2008) [33] who reported that 4EBP phosphorylation increased over time after rapamycin treatment in HEK-293 and HeLa cells, the least phosphorylated α-form appeared to be persistent in this experiment, which could be due to the cell type and treatment conditions.

The inhibition of the mTOR pathway resulted in a significant diminution of cellular growth (Fig. 3b) with a 30% decrease in the maximal growth rate $\mu_{max}$ from 0.648 ± 0.007 to 0.457 ± 0.001 day⁻¹ (means ± SD, n = 3; p < 0.05) in the rapamycin treated cultures. Moreover, there was a 1.5-fold decrease of the maximal viable cell density from $6.15 \times 10^6$ to $4.26 \times 10^6$ cells mL⁻¹. Interestingly, the culture was extended for 3 additional days compared to the control culture and the viability remained high up to day 9 (87% vs. 47% for control culture). This prolonged culture led to a 10% increase of final mAb titer from 357.52 ± 0.71 to 415.06 ± 11.31 mg L⁻¹ (means ± SD, n = 3; Fig. 3c). In parallel, upon rapamycin treatment, consumption rates of glutamine and glucose (slopes of curves) were also markedly slower on average from day 0 to day 4 and day 5 respectively (Fig. 3d). However, glutamine and glucose reached depletion on the same days for both conditions (day 6 for glutamine and day 9 for glucose) since control cultures progressively entered death phase ($\mu < 0$) after day 5 while rapamycin treated cells continued to grow. The viability extension with rapamycin treatment was previously reported in hybridoma cells [34] and could be related to autophagy [25]. Autophagy could recycle unnecessary cellular components to support growth and extend cellular viability towards the end of cultures (not investigated in this study), when nutrients such as glutamine became limiting (Fig. 3d), but other molecular mechanisms of the mTOR pathway were responsible for the decrease in growth rate at the earlier stage of the culture (day 1–day 5) when there was no nutrient limitation.

Since the mTOR pathway is known to regulate cellular growth principally by leveraging translation activity [13], the interplay between mTOR pathway inhibition and cellular growth decrease was studied from a translational perspective. Figure 3e compares the polysome profiles from day 1 to day 5 of both the control and rapamycin treated cultures. Rapamycin inhibition of the mTOR pathway resulted in a shift in proportion of polysomes towards monosomes, with a clear increase of the 80S peak. This resulted in a 23% decrease in the ratio of polysome over monosome area under the curve from 2.15 ± 0.03 to 1.64 ± 0.09 units (means ± SD, n = 3; p < 0.05) on day 1. Similar changes of ribosome distribution were also observed in other cell lines treated with rapamycin [35,36]. This polysome run-off concomitant to an increase in the amount of monosomes indicated a slowdown in translation initiation rate [11] upon rapamycin treatment, which was a likely element causing the decrease of growth rate. However, on day 5, the 80S peak content of the control culture exceeded the rapamycin treated culture, indicating a drop of translation activity as well. It was postulated that such a sudden increase of the 80S peak on day 5 of control culture could arise after inhibition of the mTOR pathway due to a nutrient limitation such as glutamine (Fig. 3D).

**Supply of nutrients to batch culture extended the activation of the mTOR pathway**

We investigated the response of the mTOR pathway to nutrient supplementation by comparing control batch to fed-batch cultures. Batch cultures were characterised by a fixed initial amount of nutrients which would deplete as the culture progressed, whereas additional nutrients were supplemented daily in fed-batch cultures. These cultures were performed in shake-flasks because the limiting nutrient availability in the batch cultures has a greater impact compared to minor fluctuations of pH and oxygen, which could have been overcome using bioreactor control. Fed-batch cultures exhibited a 1.5-fold higher maximum viable cell density and a 5 day extension of culture viability (above 50%) compared to batch cultures (Fig. 4a). Peak cell densities of 6.4 and 9.4 × 10^6 cell mL⁻¹ were achieved on days 5 and 8 for the batch and fed-batch cultures respectively. In addition, the final recombinant

**Fitter assessment of global translation was derived from the ratio of polysome to monosome area under the curve of polysome profiles. Error bars show ± standard deviation on three biological replicates.**

<ref>Figure 3e</ref> compares the polysome profiles from day 4 to day 8. The vertical dashed line marked the separation between monosomes and polysomes. Fitter assessment of global translation was derived from the ratio of polysome to monosome area under the curve of polysome profiles. Error bars show ± standard deviation on three biological replicates.
mAb titer was increased 4-fold in the fed-batch as compared to the batch culture from 243.36 to 975.75 mg L\(^{-1}\) (means; \(n = 3\); Fig. 4b). In parallel, residual glutamine, the key metabolite observed to be depleted in the rapamycin experiment (Fig. 3d), was similarly depleted and dropped below the 0.044 g L\(^{-1}\) (0.3 mM) threshold from day 5 onwards in batch, while it was steadily maintained above 0.044 g L\(^{-1}\) through feeding in fed-batch cultures (Fig. 4c). This threshold defined the glutamine limitation state in our feeding strategy.

Both increases of maximal viable cell density (1.5-fold) and productivity (4-fold) were much greater than those observed in similar comparison of batch and fed-batch cultures by Han et al. (2011) [37] who reported a 1.2 (viable cell concentration) and 1.5 (final recombinant protein titer) fold increase after addition of feed solutions. The amplitude of change could be cell and process specific. However, the authors also found that there was a glutamine consumption phase (day 1–5) followed by a starvation phase (day 5–8) in batch cultures and that supplementation of glutamine in feed solution allowed extended growth. Glutamine, a key source of energy in CHO cultures [38], was the controlling nutrient in our cell culture platform, which dictated the feeding strategy of fed-batch cultures. The continuous supply of nutrient in fed-batch cultures was expected to stimulate the phosphorylation cascade upstream of the mTOR pathway towards an enhancement of translation activity after day 5.

Phosphorylation events on 4EBP served to assess the mTOR pathway response to nutrient supplementation. Immunoblots in Fig. 4d show that the phosphorylation state of 4EBP was maintained after day 5 of the fed-batch, with the presence of the three phospho-isofoms of 4EBP as compared to batch cultures, where 4EBP largely accumulated in the least phosphorylated α-band during the same period of time. It was noted that while the distribution of 4EBP among the three bands is the direct indicator of mTOR pathway activation, 4EBP appeared to be in overall higher quantity on the last days of batch culture concurrent with cellular death. This phenomenon may reflect some cellular mechanisms possibly leading to the increased expression level of 4EBP during apoptosis, to enforce further inhibition of translation activity as nutrients are limiting. 4EBP phosphorylation patterns indicated that supplementation of glutamine, among other nutrients in the feed solution, was able to extend the activation of the mTOR pathway in fed-batch cultures relative to batch cultures. The extension of mTOR pathway activation in fed-batch culture kept 4EBP inactivated by phosphorylation [39,40], which would allow the binding between IF4G and IF4E required for efficient translation initiation activity [41]. The effect of the mTOR pathway on translation activity was assessed via polysome profiling on day 4–day 8 of cultures because it was during this time period that the enhancement of the mTOR pathway activity in fed-batch cultures was most obvious compared to batch cultures.

**Control of translation activity by the mTOR pathway in CHO cultures**

*mTOR pathway and global translation activity*

Figure 4e compares polysome profiles from day 4 to day 8 for both the batch and fed-batch cultures. There was an increase of polysome content upon nutrient supplementation while the monosome content was lower than in batch cultures. This shift of mRNA from monosomes towards polysomes indicated a relative increase of translation initiation rate in fed-batch cultures compared with batch cultures. The ratio of area under the curve of the polysomes and monosomes was used as a semi-quantitative indicator of translation activity as reported in the literature [42]. Although the overall trend indicated a slowdown of global translation activity in both batch and fed-batch cultures (Fig. 4e), there was nonetheless a consistent 1.2- to 2-fold higher translation activity upon feeding as compared to batch cultures, with the greatest difference on day 5.

It is to be noted that there was no strict correlation between mTOR pathway activity and global translation trends. For example, the deactivation of the mTOR pathway activity in batch cultures seemed to occur gradually over days 5–8 (Fig. 4d) while most of the shift in polysomes to monosomes took place on day 5 (Fig. 4e). This could be due to the semi-quantitative nature of the gel shift assay used to assess mTOR pathway activation levels. Furthermore, other parameters than the mTOR pathway may have contributed slightly to the overall control of translation activity, such as the initiation factor IF2 [43] or miRNAs [44], which were reported to potentially affect translation activity under nutrient depletion stress. Nonetheless, the relative increase of global translation in fed-batch as compared to batch cultures corresponded with the observed extended activation of the mTOR pathway (Fig. 4d), thus supporting the involvement of the mTOR pathway in the overall translational control mechanisms. Furthermore, it seemed clear that the mTOR pathway response to nutrient depletion/supplementation signals as well as its corresponding impact on translation activity was analogous to that of rapamycin inhibition as both led to a shift of mRNA between 80S peak and polysomes altering cellular growth. To the best of our knowledge, this is the first evidence on the role of the mTOR pathway on translation activity in CHO cell cultures. In such bioprocessing oriented cultures, the two most desirable phenotypes are high cellular growth and specific productivity, which are primarily the consequence of protein biosynthesis via translation [45]. The following sections investigate the impact of mTOR translational control on cellular growth and productivity.

**mTOR pathway and cellular growth**

Extension of cellular growth after day 5 in fed-batch as compared to batch cultures appeared to be driven by the sustained translation activity supported by mTOR pathway activation. Day 5 of batch cultures (Fig. 4a) was a transition from active cellular growth to cell death, where the death constant \(k_d\) was greater than the growth rate \(\mu\). This shift happened on the same day as the drop of translation activity following mTOR pathway deactivation by glutamine starvation. Since feeding fresh nutrients sustained cells growth (maintenance of \(\mu\)) at high viability (Fig. 4a) it indicated that cell death in batch cultures could be prevented at the condition where the mTOR pathway was activated. As a result, higher global translation driven by the mTOR pathway appeared to be a determinant factor of cellular growth.

**mTOR pathway and productivity**

We then investigated how the translational control affected the mAb productivity of CHO cells, as highly desirable new knowledge...
to further understand the expression regulation of the gene of interest. While the production of cell mass (growth) encompassed the translation of all mRNA (global translation), the production of the mAb involved the mRNA of its heavy (HC) and light chains (LC). Targeted quantification of these two mRNAs from fractionated polysome profiles with qRT-PCR enabled determination of specific translation efficiencies, which were measured as the ratio of intensity quantified in pool A (polysome enriched) over pool B (monosome enriched) [12]. Both HC and LC (Fig. 4f) mRNA translational efficiencies showed similar trends being maintained at relatively stable and high levels in fed-batch while decreasing in batch cultures. These greater specific translational efficiencies of the HC and LC in fed-batch cultures coincided with the global translation activity, thus validating the higher polysome content observed on the polysomes profiles (Fig. 4e). Furthermore, HC and LC specific translational efficiency trends were in agreement with product accumulation profiles (Fig. 4b); therefore translation activity seemed to support the increase in final titer upon nutrient supplementation. These results represent the first evidence that higher translation activity in fed-batch cultures relative to batch cultures supported the greater production of recombinant protein in CHO cells.

Conclusion

In this study, we provide the first comprehensive evaluation of the mTOR pathway’s role on translation activity and its impact on growth and productivity in CHO-mAb cultures. Feeding additional nutrients was shown to be an effective strategy to maintain mTOR pathway activation and to increase translation activity as compared to batch cultures. The current data represent information complementary to the work reported by Dreesen and co-authors [24], by providing evidence that the TORC1-4EBP cascade affected both growth (global translation) and productivity (specific translation) phenotypes through its translational control activity. Consequently, these findings fully support cell-engineering applications aimed at maintaining the activation level of the mTOR pathway in order to enhance CHO cell cultures. A potential application could be to knock down TSC or over-express AKT [46], upstream of the mTOR pathway, in order to induce constitutive activation of the mTOR pathway regardless of the environmental conditions. Alternatively, cell-engineering strategies for maintaining 4EBP in its hyperphosphorylated state, independently of mTOR pathway activation level, could extend translational activity for improved growth and productivity. In addition, these first data of HC and LC specific translational efficiency allow us to enhance understanding of the biosynthesis of recombinant protein of interest under conditions that offer a productivity advantage, such as sodium butyrate treatment or temperature shift.

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References

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