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Myc coordinates transcription and translation to enhance transformation and suppress invasiveness

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 10 July 2015

Thank you for your submission to EMBO reports and please accept my apologies for the unusually long time it has taken us to contact you with a decision on your study. We have received reports from two of the three referees that agreed to evaluate your study (which can be found at the end of this email), and I am making a decision now to avoid any further loss of time. As you will see, although both referees find the topic of interest and in principle suitable for us, they both raise a number of issues.

Given that both referees provide constructive suggestions on how to strengthen the work, I would like to give you the opportunity to revise your manuscript. All issues are pertinent and should be addressed. If this is the case, we would be happy to consider your manuscript for publication. However, please note that it is EMBO reports policy to undergo one round of revision only and thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

Revised manuscripts must be submitted within three months of a request for revision unless previously discussed with the editor; they will otherwise be treated as new submissions.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision)
- a letter detailing your responses to the referee comments in Word format (.doc)

- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
- a separate PDF file of any Supplementary information (in its final format)

EMBO reports now accommodates the inclusion of extra figures (up to five) in the online version of the manuscript. These are presented in an expandable format inline in the main text so that readers who are interested can access them directly as they read the article. They are also provided for download in a separate typeset PDF to accompany the Article PDF. These should be those of particular value to specialist readers, but which are not required to follow the main thread of the paper (and not additional controls or reagent optimization). These should be labeled expanded view, and the rest supplementary.

We also encourage the publication of original source data -particularly for electrophoretic gels and blots, but also for graphs- with the aim of making primary data more accessible and transparent to the reader. If you agree, you would need to provide one PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figures and an Excel sheet or similar with the data behind the graphs. The files should be labeled with the appropriate figure/panel number, and the gels should have molecular weight markers; further annotation could be useful but is not essential. The source files will be published online with the article as supplementary "Source Data" files and should be uploaded when you submit your final version. If you have any questions regarding this please contact me.

REFEREE REPORTS

Referee #1:

The manuscript by Elkon and Puch et al. reports the first comprehensive analysis of gene expression responses to MYC activation at the RNA (RNAseq and GROseq) level and at the translational (Riboseq) level, providing insights into the coupling of transcription and translation. They found that MYC, as previously inferred, could stimulate the protein synthetic machinery for cell growth. Further, through studying the effects of TOR inhibition on MYC stimulated transcriptional and translational responses, they found that TOR is required for the translational of the protein synthesis machinery. Further, they demonstrate that TGFbeta modifies the cells' adhesion and extracellular matrix. Overall, this is an important study that directly investigated the translational consequences of MYC-induced gene expression; however, there are a number of caveats that should be addressed. The general interest for this work would be significantly increased if the authors could address point number 3 below.

1. While the authors acknowledge that the studies of Myc-inducible U2OS cells were performed at a time-frame after Myc induction well before cell stress and death, this ultimate death phenotype is likely to affect the cellular responses to Myc even in the time frame used in the study. That is, the trajectory of Myc-induce cellular responses ends up as cell stress and death. Hence, some transcriptional and translational responses could be specific for this experimental model and some caution should be exercised in the discussion about generalizability (in a study using only one cell line).

2. The instructive observations about the role of TOR in MYC-induced gene expression could be further highlighted in this paper, since this is an area that is not well-understood. Specifically, since phosphorylation of 4EBP1 by TOR affects translation of transcripts induced by MYC, the authors' comprehensive data set could be gleaned for further clarity and insights.

    a. For clarity, the authors should provide examples of genes affected by TOR inhibition and display histograms (as used in Figure 2A) for MYC and control cells. In this case, displaying GRO-seq, RNAseq, and Riboseq for selected genes would be extremely informative. It is expected that there are classes of genes that are transcriptionally induced by MYC (GRO-seq) and the mRNAs (RNAseq) are elevated, but the translation (Riboseq) is inhibited by Torin (TOR inhibitor). Other

genes, such as those responsive to TGFbeta, would show a different patter.

b. The presentation of Figure 4a, hence should be modified so that it provides more insights for biologists.

c. The authors should provide a clearer view on the categories (GO or GSEA) of genes that are induced by MYC transcriptionally but whose transcripts did not engage the ribosomes with Torin. The authors should explicitly report whether or not there is a category of MYC-induced genes (rather than TGFbeta) that were not inhibited by Torin as determined by Riboseq. Is there a set of genes that were induced by MYC, but the translation of their RNAs were not inhibited by Torin?

3. The authors has a opportunity to address an interesting question of whether MYC-overexpressing as compared to control cells display non-linear changes in gene expression. For example, if the authors compare a selected list of ribosomal protein genes with and without MYC-overexpression using GROseq, RNAseq, and Riboseq, could the authors uncover whether MYC induction led to a quantitative alteration in the ratio of signals between the ribosomal protein genes as measured by GROseq, RNAseq, and Riboseq? Was there an imbalance induced by MYC-overexpression such that some ribosomal subunits were non-linearly expressed more highly than others? Could general imbalances induced by MYC led to the ultimate death of these U2OS cells?

Referee #2:

In this manuscript, Elkon et al. utilize genome-wide technologies to demonstrate a role for Myc in the differential combinations of both transcriptional and translational regulation of gene networks. While the translational influence of genes/gene programs by Myc have been previously invoked, this work for the first time dissects the varying contributions of specific aspects of transcription and translation to given networks using a combination of genome-wide GRO-seq, RNA-seq, ribosomal profiling and proteomic analysis. Thus, the authors specifically identify clusters of both up- and down-regulated genes that are regulated at the level of protein translation. As well, the authors use these data to further investigate several ontological findings that may be functionally relevant for better understanding Myc-mediated tumorigenesis. These include the coordinated role of mTOR translational regulation and the opposing influence of the TGFβ pathway in malignant cell migration.

While this work lacks mechanism for understanding how Myc mediates translational regulation (direct and indirect), it does for the first time provide the Myc, and more broadly, cancer research fields with a precise snapshot, across several cell types, of the relative contributions via Myc stimulation to both gene transcription and protein translation.

However, this reviewer's concerns surround the functional exploration of the impact of Myc on the observed translation efficiencies. Firstly, the use of Torin-1, an ATP-binding dual inhibitor of the PI3K/mTOR, to examine the role of mTOR on the observed effects of Myc on TE is intriguing. While this is an important result, several questions remain. It has been published that in a system of granulocyte differentiation, use of the mTORC1 inhibitor, rapamycin (an allosteric mTORC1 inhibitor) resulted in reduction of the amount of c-Myc mRNA associated with polysomes while total cellular c-Myc mRNA and c-MYC protein stability remained unchanged (Wall et al., Blood, 2008, PMID:18621930). Thus, the authors need to confirm that the abundance of c-Myc mRNA itself in polysome fractions is unaffected by Torin-1 in this system (the authors may have this data already). Alternatively, this was not the case in the EuMyc model when confronted with everolimus, as well an mTORC1 inhibitor, where mTORC1-dependent evasion of senescence is critical for cellular transformation by MYC (Wall et al., Cancer Discovery, 2013, PMID: 23242809). Thus it is important that the authors incorporate this cell-type specific complexity into their discussion and acknowledge the point of differentiation between Torin-1 and other commonly employed inhibitors of mTORC1 only.

Finally, further discussion of the complexity of TGFβ signaling in the context of Myc oncogenicity is warranted, specifically around cell type and cancer stage-specific and tumours/stromal cell-

dependent effects of TGFβ signaling that may be relevant but which are not detected in vitro. For example, the study by Reimann et al. (Cancer Cell 2010, PMID: 20227040), which highlights the role of TGFβ in precipitating senescence in Myc-driven lymphoma.
In conclusion, this study is of excellent scientific quality and offers important insights towards understanding Myc oncogene-mediated global regulation of the malignant cell's net "expressome" via its contributions to both transcription and translation.

Minor points:

- Results section, first paragraph: clarify upfront that the 36 hour time point is beyond the point at which one would be looking at direct mechanistic effects of MYC on transcription (and presumedly translation, e.g., cap-methylation). Alternatively make this clear in the Discussion.

- Results section, p.6, second paragraph, clarification is needed for the following sentence: "Interestingly, this result indicates that upon Myc activation subunits of both the transcription and translation cellular machineries are coordinately induced at their mRNA levels and translation efficiencies." Are the authors concluding that the genes induced upon Myc activation in cluster #1, i.e. ribosome biogenesis and transcription factors, NOT components of the transcription/translation cellular machineries but rather more regulatory components? A better delineation of what the authors are referring to as "machinery" is required here.

- p.7, second paragraph: the word "top" is presumably referencing the previously described highest ranking MYC target genes according to the strength of MYC binding to their promoters. Given the recent advent of the transcriptional amplifier model, this is useful strategy to triage and incorporate a category of direct MYC target genes. However, should this be more precisely defined (i.e., a cutoff defined) and should they be described as "high affinity" if this is what the authors mean?

- In the same paragraph: are the MYC repressed genes enriched for other transcription factor binding motifs (SP1)? Alternatively, given the data is available from the Walz et al. dataset, it is unclear if are the promoters/genes that were co-bound and repressed by MYC and MIZ1 enriched in this cluster representing repressed genes? This should be indicated given the wealth of data available from the indicated dataset.

- spelling/gramatical errors:

    1. Abstract, second last sentence: "to TGFβ pathway" should be "to the TGFβ pathway"

    2. Same sentence: should it read "...we detected a genome-wide coordination..."?

    3. Discussion, p.11, second paragraph: "...that was reporter to suppress Myc expression..." should read "...that was reported to suppress Myc expression

1st Revision - authors' response 14 August 2015

We first wish to thank you and the referees. We highly appreciate the referees' time and effort in reviewing our manuscript. We were very happy to see that both referees valued the novelty and significance of our results. We modified the text according to the referees' comments and feel that their constructive suggestions have indeed help us to improve our study.

Below please find our detailed point-by-point response to the comments raised by the referees. (Please note that in the revised manuscript that we submit, any new text is marked in blue).

Referee #1:

The manuscript by Elkon and Puch et al. reports the first comprehensive analysis of gene expression responses to MYC activation at the RNA (RNAseq and GROseq) level and at the translational

(Riboseq) level, providing insights into the coupling of transcription and translation. They found that MYC, as previously inferred, could stimulate the protein synthetic machinery for cell growth. Further, through studying the effects of TOR inhibition on MYC stimulated transcriptional and translational responses, they found that TOR is required for the translational of the protein synthesis machinery. Further, they demonstrate that TGFbeta modifies the cells' adhesion and extracellular matrix. Overall, this is an important study that directly investigated the translational consequences of MYC-induced gene expression; however, there are a number of caveats that should be addressed. The general interest for this work would be significantly increased if the authors could address point number 3 below.

1. While the authors acknowledge that the studies of Myc-inducible U2OS cells were performed at a time-frame after Myc induction well before cell stress and death, this ultimate death phenotype is likely to affect the cellular responses to Myc even in the time frame used in the study. That is, the trajectory of Myc-induce cellular responses ends up as cell stress and death. Hence, some transcriptional and translational responses could be specific for this experimental model and some caution should be exercised in the discussion about generalizability (in a study using only one cell line).

   We certainly agree with the referee that it is possible that some of the Myc-induced responses that we observed might be cell specific. We added a comment on this issue as the concluding remark of our Discussion.

2. The instructive observations about the role of TOR in MYC-induced gene expression could be further highlighted in this paper, since this is an area that is not well-understood. Specifically, since phosphorylation of 4EBP1 by TOR affects translation of transcripts induced by MYC, the authors' comprehensive data set could be gleaned for further clarity and insights.

   We thank the referee for this comment and now clarify better the effect of mTOR inhibition by Torin on the Myc-induced response. We make it clearer, in both the main text and in new supplementary figures (Fig. S5A-C), that this effect is very specific. Fig. S5C shows in a global manner that Torin treatment specifically abolished the induction of the ribosomal-protein genes, while having no significant effect on other components (induced or repressed) of the myc response. Fig. S5A and S5B respectively give specific examples of Myc-induced genes that were and were not affected by Torin.

   a. For clarity, the authors should provide examples of genes affected by TOR inhibition and display histograms (as used in Figure 2A) for MYC and control cells. In this case, displaying GRO-seq, RNAseq, and Riboseq for selected genes would be extremely informative. It is expected that there are classes of genes that are transcriptionally induced by MYC (GRO-seq) and the mRNAs (RNAseq) are elevated, but the translation (Riboseq) is inhibited by Torin (TOR inhibitor). Other genes, such as those responsive to TGFbeta, would show a different patter.

      We now provide specific examples of genes (encoding ribosomal proteins) whose induction by Myc is completely blocked (and, in fact, reversed) upon Torin treatment (Fig. S5A), as well as, few examples of genes whose activation was not affected by Torin (Fig. S5B). (Please note that as we examined Torin effect using RNA-seq and Ribo-seq, but not GRO-seq, we used in these new figures data only from the first two techniques.)

   b. The presentation of Figure 4a, hence should be modified so that it provides more insights for biologists.

      The plot shown in Fig. 4a has become a standard one for presenting results of GSEA analysis. So we opt to leave it, but we made its legend more elaborated and added examples of two specific genes next to it. We hope that with these modifications, the figure is now clearer.

   c. The authors should provide a clearer view on the categories (GO or GSEA) of genes that are induced by MYC transcriptionally but whose transcripts did not engage the ribosomes

with Torin. The authors should explicitly report whether or not there is a category of MYC-induced genes (rather than TGFbeta) that were not inhibited by Torin as determined by Riboseq. Is there a set of genes that were induced by MYC, but the translation of their RNAs were not inhibited by Torin?

As we now make clearer in the text, Torin1 specifically affected ribosomal proteins. Other components of the Myc response network were not significantly affected by Torin (specific examples are given in Fig. S5B; a global analysis is shown in Fig. S5C). (We particularly emphasized in the text the fact that the TGFb pathway is not affected by Torin1 because of the potential therapeutic importance of this observation, as discussed in our Discussion section).

3. The authors has an opportunity to address an interesting question of whether MYC-overexpressing as compared to control cells display non-linear changes in gene expression. For example, if the authors compare a selected list of ribosomal protein genes with and without MYC-overexpression using GROseq, RNAseq, and Riboseq, could the authors uncover whether MYC induction led to a quantitative alteration in the ratio of signals between the ribosomal protein genes as measured by GROseq, RNAseq, and Riboseq? Was there an imbalance induced by MYC-overexpression such that some ribosomal subunits were non-linearly expressed more highly than others? Could general imbalances induced by MYC led to the ultimate death of these U2OS cells?

This is a good point and in fact, the DELTA-TE (ΔTE) measure that we use in our analyses quantifies alterations in ratio of signals between expression of genes as measured by RNA-seq and Ribo-seq. For clarity, we added to Fig. 4A two examples of ribosomal-protein genes that, upon Myc activation, showed no (or very minimal) change in GRO-seq or RNA-seq signals, but showed strong increase in Ribo-seq signal.

Indeed, different RP genes showed different levels of such TE modulation in response to Myc. This can be seen in Fig. 4B (middle box). The functional implications of such imbalances are very interesting to explore empirically. But we felt that this aspect is out of the scope of our current study and therefore do not discuss it in the manuscript.

Referee #2:

In this manuscript, Elkon et al. utilize genome-wide technologies to demonstrate a role for Myc in the differential combinations of both transcriptional and translational regulation of gene networks. While the translational influence of genes/gene programs by Myc have been previously invoked, this work for the first time dissects the varying contributions of specific aspects of transcription and translation to given networks using a combination of genome-wide GRO-seq, RNA-seq, ribosomal profiling and proteomic analysis. Thus, the authors specifically identify clusters of both up- and down-regulated genes that are regulated at the level of protein translation. As well, the authors use these data to further investigate several ontological findings that may be functionally relevant for better understanding Myc-mediated tumorigenesis. These include the coordinated role of mTOR translational regulation and the opposing influence of the TGFβ pathway in malignant cell migration.
While this work lacks mechanism for understanding how Myc mediates translational regulation (direct and indirect), it does for the first time provide the Myc, and more broadly, cancer research fields with a precise snapshot, across several cell types, of the relative contributions via Myc stimulation to both gene transcription and protein translation.

1. However, this reviewer's concerns surround the functional exploration of the impact of Myc on the observed translation efficiencies. Firstly, the use of Torin-1, an ATP-binding dual inhibitor of the PI3K/mTOR, to examine the role of mTOR on the observed effects of Myc on TE is intriguing. While this is an important result, several questions remain. It has been published that in a system of granulocyte differentiation, use of the mTORC1 inhibitor, rapamycin (an allosteric mTORC1 inhibitor) resulted in reduction of the amount of c-Myc mRNA associated with polysomes while total cellular c-Myc mRNA and c-MYC protein stability remained

unchanged (Wall et al., Blood, 2008, PMID:18621930). Thus, the authors need to confirm that the abundance of c-Myc mRNA itself in polysome fractions is unaffected by Torin-1 in this system (the authors may have this data already). Alternatively, this was not the case in the EuMyc model when confronted with everolimus, as well an mTORC1 inhibitor, where mTORC1-dependent evasion of senescence is critical for cellular transformation by MYC (Wall et al., Cancer Discovery, 2013, PMID: 23242809). Thus it is important that the authors incorporate this cell-type specific complexity into their discussion and acknowledge the point of differentiation between Torin-1 and other commonly employed inhibitors of mTORC1 only.

We thank the reviewer for this comment. In response, we have examined the effect of Torin-1 on Myc translation efficiency using polysome factions, and confirmed that in our system there is not effect. This new result was added to the manuscript and presented in Fig. S5D.

In addition, we added to the Discussion part a comment about the possible difference in biological effects exerted by ATP-competitive and allosteric inhibitors of mTOR. We also added a comment on cell-specific effects as the concluding remark of our Discussion.

2. Finally, further discussion of the complexity of TGFβ signaling in the context of Myc oncogenicity is warranted, specifically around cell type and cancer stage-specific and tumours/stromal cell-dependent effects of TGFβ signaling that may be relevant but which are not detected in vitro. For example, the study by Reimann et al. (Cancer Cell 2010, PMID: 20227040), which highlights the role of TGFβ in precipitating senescence in Myc-driven lymphoma.

   We now elaborate more in our Discussion section on the complexity of the interlinks between Myc and TGFβ signaling.

In conclusion, this study is of excellent scientific quality and offers important insights towards understanding Myc oncogene-mediated global regulation of the malignant cell's net "expressome" via its contributions to both transcription and translation.

Thank you. We were delighted to see that the referee has appreciated the potential contribution of our study to the cancer research field.

Minor points:

- Results section, first paragraph: clarify upfront that the 36 hour time point is beyond the point at which one would be looking at direct mechanistic effects of MYC on transcription (and presumedly translation, e.g., cap-methylation). Alternatively make this clear in the Discussion.

We added a comment on this upfront.

- Results section, p.6, second paragraph, clarification is needed for the following sentence:

  "Interestingly, this result indicates that upon Myc activation subunits of both the transcription and translation cellular machineries are coordinately induced at their mRNA levels and translation efficiencies."

  Are the authors concluding that the genes induced upon Myc activation in cluster #1, i.e. ribosome biogenesis and transcription factors, NOT components of the transcription/translation cellular machineries but rather more regulatory components? A better delineation of what the authors are referring to as "machinery" is required here.

Cluster Up 1 is enriched for TFs and genes that encode for proteins assigned to the GO category 'ribosome biogenesis' (mainly proteins that function in the processing and maturation of rRNAs), while cluster up #2 is enriched for subunits of Pol-II and for ribosomal-protein genes. To make our conclusion clearer we now use the term "core machinery" and indicate that by this term we refer to the RNA-Pol-II complex (for the core transcriptional machinery) and to the ribosome itself (for the core translational machinery).

- p.7, second paragraph: the word "top" is presumably referencing the previously described highest ranking MYC target genes according to the strength of MYC binding to their promoters. Given the recent advent of the transcriptional amplifier model, this is useful strategy to triage and incorporate a category of direct MYC target genes. However, should this be more precisely defined (i.e., a cutoff defined) and should they be described as "high affinity" if this is what the authors mean?

Indeed, by 'top Myc target genes' we referred to those with strongest Myc binding. In line with the referee suggestion, we changed this term and now use 'high affinity Myc target genes' instead.
As using any specific cutoff value is in a way arbitrary, we show in Tab 2 that our findings are robust and are not sensitive to specific cutoff levels.

- In the same paragraph: are the MYC repressed genes enriched for other transcription factor binding motifs (SP1)? Alternatively, given the data is available from the Walz et al. dataset, it is unclear if are the promoters/genes that were co-bound and repressed by MYC and MIZ1 enriched in this cluster representing repressed genes? This should be indicated given the wealth of data available from the indicated dataset.

Following the referee's comment, we carried out a motif enrichment analysis which indeed detected that the top scoring signature in the promoters of genes repressed upon Myc activation is the SP1 binding motif. In addition, we examine intersection with MIZ1 targets (based on ChIP-seq data from Walz et al.) and found that the repressed genes were (mildly) enriched for putative MIZ1 target genes. We added these two results to the manuscript.

- spelling/gramatical errors:

    1. Abstract, second last sentence: "to TGFβ pathway" should be "to the TGFβ pathway"

    2. Same sentence: should it read "...we detected a genome-wide coordination..."?

    3. Discussion, p.11, second paragraph: "...that was reporter to suppress Myc expression..." should read "...that was reported to suppress Myc expression

Many thanks. We have corrected the errors.

2nd Editorial Decision 14 September 2015

Thank you for the submission of your revised manuscript to our offices. We have now received the enclosed reports from the referees that were asked to assess it. As you will see, both referees are very positive about the study and request only minor changes to the text that I would like you to incorporate before we can proceed with the official acceptance of your manuscript. In particular, as referee 2 points out, Figure S5D was missing and has to be supplied in the revised version.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

- As a standard procedure, we edit the title and abstract of manuscripts, which was a bit too long. Please find my suggested changes below my signature and see if you agree with them.

- As a standard procedure all manuscripts are cross-checked for textually similarity with published manuscripts. This triggered a high similarity of two sentences in the second paragraph of the introduction to Lin C. Y. et al, Cell 2012.
For the avoidance of doubt I suggest to modify the text a bit, see below. Please let me know if you agree with these changes.

"Myc is a basic helix loop helix (bHLH) transcription factor that forms a heterodimer with Max and binds to E-box sequences (canonical consensus 5'-CACGTG-3') near the promoter elements of actively transcribed genes (Blackwood & Eisenman, 1991). Many gene expression profiling studies have identified hundreds of Myc target genes in tumor cells (Dang et al, 2006; Kim et al, 2006; Schlosser et al, 2005; Schuhmacher et al, 2001; Zeller et al, 2003) "

- Regarding data quantification, can you please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD)? This information is currently incomplete in Fig S5D and must be provided in all figure legends.

- Regarding the authors checklist you sent, could you please also indicate the page numbers of the manuscript, where the relevant information can be found, e.g., in which figures was the Wilcoxon test used, where are the GRO-seq and RNA-seq experiments described?

- The Expanded View format has replaced the Supplementary information. A maximum of 5 EV figures can be supplied for a manuscript. At the moment you have 6 Supplementary Figures, but Fig. S3 and S4 could be merged into one Figure. Please also merge Fig. S1 and Fig. S5 in a file that fits on one page. Please follow the nomenclature Figure EV1 and Table EV1 for the Supplement. The legends for these figures and tables is included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. For the Figures that are not part of the Expanded View format a separate pdf file with legends has to be provided (Appendix Figure S1).

Shortened abstract

c-Myc is one of the major human proto-oncogenes and is often associated with tumor aggression and poor clinical outcome. Paradoxically, Myc was also reported as a suppressor of cell motility, invasiveness and metastasis. Among the direct targets of Myc are many components of the protein synthesis machinery whose induction results in an overall increase in protein synthesis that empowers tumor cell growth. At present, it is largely unknown whether beyond the global enhancement of protein synthesis, Myc activation results in translation modulation of specific genes. Here, we measured Myc-induced global changes in gene expression at the transcription, translation and protein levels, and uncovered extensive transcript-specific regulation of protein translation. Particularly, we detected a broad coordination between regulation of transcription and translation upon modulation of Myc activity, and show the connection of these responses to mTOR signaling to enhance oncogenic transformation and to the TGFβ pathway to modulate cell migration and invasiveness. Our results elucidate novel facets of Myc-induced cellular responses and provide a more comprehensive view of the consequences of its activation in cancer cells.

REFEREE REPORTS

Referee #1:

The authors have clarified a number of issues raised previously. A clear declaration of data submission to repositories was made.

Referee #2:

Comments for revised manuscript submitted to EMBO Reports by Elkon and Puch et al.:

1. p. 6, newly added "Ribosome" should not be capitalized.

2. p.7, the sentence: "In line with previous reports and using MIZ1 ChIP-seq data from (Walz et al, 2014), we observed..."
either remove the word "from" or the brackets ().

In this same section, there is an additional, superfluous bracket] following: (Janky et al, 2014)].

3. Figure S5D is missing in the pdf provided (but is referenced in the text and the legend is provided in the Supplementary Figure Legends). Please provide the corresponding Figure for Supp Fig4D.

2nd Revision - authors' response 15 September 2015

(Minor editorial changes were made.)

3rd Editorial Decision 21 September 2015

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

