



HAL
open science

Elucidation of TFEB nuclear translocation dynamics in human cells by means of Quantitative Modelling and Microfluidics

Iacopo Ruolo, Gianpio Caringella, Sara Napolitano, Diego Di Bernardo

► **To cite this version:**

Iacopo Ruolo, Gianpio Caringella, Sara Napolitano, Diego Di Bernardo. Elucidation of TFEB nuclear translocation dynamics in human cells by means of Quantitative Modelling and Microfluidics. 9th IFAC Conference on Foundations of Systems Biology in Engineering FOSBE 2022, IFAC-PapersOnLine, 55 (23), Elsevier, pp.150-155, 2022, <10.1016/j.ifacol.2023.01.033>. <hal-04613586>

HAL Id: hal-04613586

<https://hal.science/hal-04613586v1>

Submitted on 16 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

Elucidation of TFEB nuclear translocation dynamics in human cells by means of Quantitative Modelling and Microfluidics

Iacopo Ruolo^{*,**,1} Gianpio Caringella^{***}
Sara Napolitano^{****,†} Diego di Bernardo^{*,**,2}

^{*} *Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Piazzale Tecchio 80, 80125 Naples, Italy.*

^{**} *Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, 80078 Pozzuoli, Italy.*

^{***} *Department of Electronics, Information and Bioengineering, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milan, Italy*

^{****} *Department of Computational Biology, Institut Pasteur, 28 rue du Docteur-Roux, 75015 Paris, France*

[†] *Inria Paris, 2 rue Simone Iff, 75012 Paris, France*

Abstract: The Transcription Factor EB (TFEB) plays a pivotal role in the transcriptional regulation of lysosomal biogenesis and autophagy in response to starvation. TFEB activity is regulated by the kinase mTOR. In nutrient-rich conditions, mTOR phosphorylates TFEB by sequestering it in the cytoplasm. During starvation, mTOR is inhibited and unphosphorylated TFEB translocates into the nucleus, where it regulates the expression of its target genes. We performed an experiment monitoring in real-time the nuclear localization of TFEB in individual cells growing in a microfluidics device following alternating pulses of starvation and refeeding. The results revealed the presence of an “overshoot” dynamics with TFEB translocating to the nucleus upon starvation but then partially retranslocating to the cytoplasm. To elucidate the mechanism behind its nuclear shuttling dynamics, we first developed a two-compartment dynamical model (nucleus and cytoplasm) with two different species (dephosphorylated and phosphorylated TFEB) for each of the two compartments. The transport and de/phosphorylation kinetics are described with first order kinetics whose parameters depend on the nutrient status, while the total TFEB is kept constant. This model was not capable to recapitulate the observed overshoot dynamics. Consequently, we investigated two alternative models, hypothesizing that upon starvation, a nuclear export channel is activated either by TFEB itself (negative feedback loop - NFL) or as a direct consequence of mTOR inhibition (incoherent feedforward loop - IFFL). The new models were both able to qualitatively recapitulate the observed dynamics. Finally, we propose an experiment able to distinguish between the two alternative models.

Copyright © 2022 The Authors. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Keywords: Systems Biology, Microfluidics, Single-Cell, TFEB, Compartmental Model, NFL, IFFL.

1. INTRODUCTION

Mammalian cells are dynamical systems able to sense, adapt and respond to time-varying inputs. Signalling pathways play a crucial role in the regulation of the cell state, sensing extracellular and intracellular environment and relaying this information to transcriptional regulatory networks, which drive the adaptation of cell homeostasis to environmental cues. Nucleo-cytoplasmic shuttling of transcription factors is used by the cell to control

gene expression programs in response to the environment (Puertollano et al. (2018)).

Recently, the Transcription Factor EB (TFEB) has been identified as a key player in the transcriptional regulation of lysosomal biogenesis and autophagy (Settembre et al. (2011)), a self-degradative process activated by cells to survive during nutrient deficiency (Glick et al. (2010)). TFEB activity is mainly controlled by the mammalian target of rapamycin (mTOR) (Settembre et al. (2012)). mTOR is central for regulation of the autophagic activity and senses the energy status by integrating signals from growth factors, amino acids, and glucose (Mizushima et al. (2011)).

* This work was supported by the Italian Fondazione Telethon and the EU PROJECT - iPC Individualized Pediatric Cure.

¹ Email: i.ruolo@tigem.it

² Corresponding author. Email: dibernardo@tigem.it, Telephone: +3908119230619, FAX: +3908119230651.

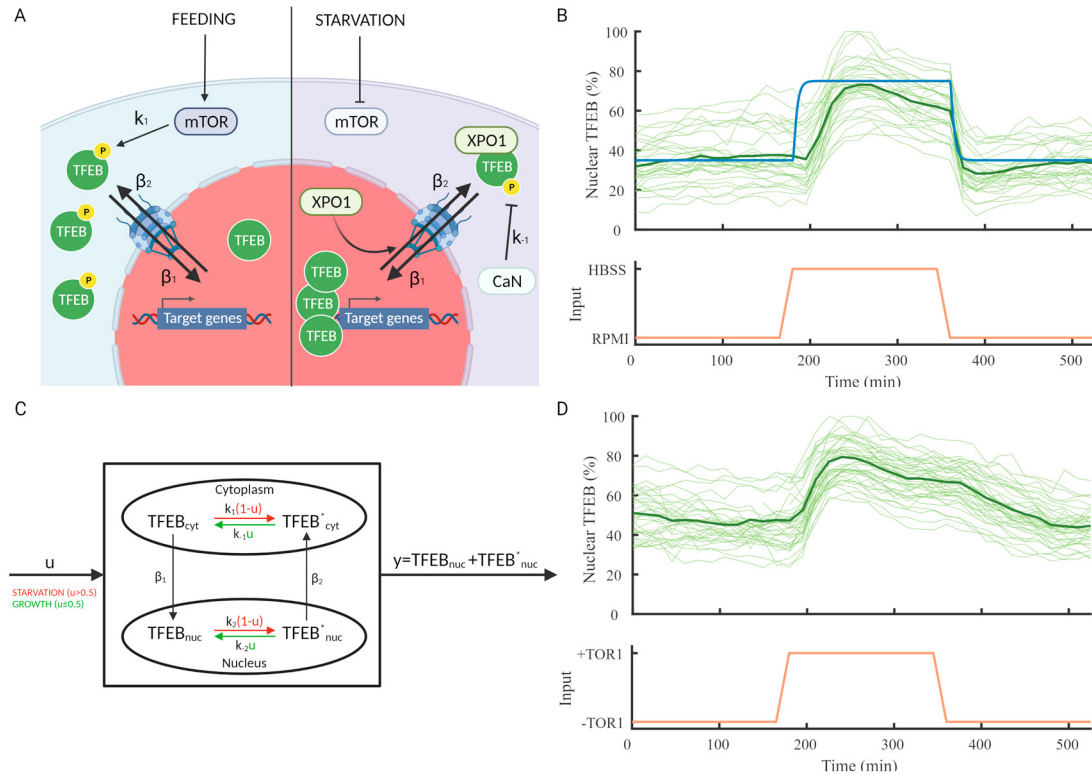


Fig. 1. TFEB nuclear translocation process and dynamics. (A) Scheme of the translocation process in the HeLa TFEB-GFP monoclonal cell line. In fed conditions, the fusion protein TFEB-GFP is phosphorylated by mTOR and trapped in the cytoplasm. In starvation condition, mTOR is inhibited, TFEB-GFP is dephosphorylated by Calcineurin, and it is able to translocate into the nucleus. TFEB-GFP nuclear export is driven by XPO1. (B) Comparison of experimental measurements and numerical simulations of TFEB translocation following a pulse of starvation. The figure shows single-cell traces of nuclear TFEB fluorescence following a pulse of starvation in light green. The average signal across the experimental single-cell traces is shown with a solid green line. The numerical simulation of nuclear TFEB fluorescence is shown with a blue line. (C) Block scheme of the model. $TFEB_{\text{cyt}}$ represents cytoplasmic TFEB; $TFEB_{\text{nuc}}$ the nuclear one; $TFEB_{\text{cyt}}^*$ and $TFEB_{\text{nuc}}^*$ are the respective phosphorylated species; k_i are the phosphorylation rates; k_{-i} the dephosphorylation ones; β_i the transport rates. The input $u \in [0, 1]$ to the system represents the level of starvation and affects the de/phosphorylation rates. The input represents the growth condition for $u \leq 0.5$, and the starvation condition for $u > 0.5$. The output of the system y represents the nuclear concentration of TFEB, given by the sum of the two nuclear species $TFEB_{\text{nuc}}$ and $TFEB_{\text{nuc}}^*$. (D) Experimental measurements of TFEB translocation following a pulse of growth medium (RPMI) supplied with the mTOR inhibitor Torin 1. The figure shows the single-cell traces of nuclear TFEB fluorescence. The average of the single-cell traces is highlighted with a solid line.

Amino acid accumulation in lysosomal lumen represents the stimulus for mTOR activation. As illustrated in Fig. 1A, in normal fed condition, mTOR is active and phosphorylates TFEB. Phosphorylated TFEB exposes a nuclear export signal and it is actively exported from the nucleus by the exportin XPO1, and thus accumulates in the cytoplasm, preventing transcription of its target genes (Martina et al. (2012); Napolitano et al. (2018)).

During starvation, mTOR becomes inactive and TFEB is dephosphorylated by Calcineurin (Medina et al. (2015)). In this way, dephosphorylated TFEB can freely translocate into the nucleus where it transcriptionally activates the genes involved in the lysosomal/autophagic pathway (Medina et al. (2015)).

Here, we investigated the dynamics of TFEB nuclear shuttling following starvation. In particular, we performed a preliminary experiment monitoring the nuclear localiza-

tion of TFEB in real-time following alternating stimulation either with nutrient rich medium or starvation medium. We then developed alternative quantitative dynamical models to explain the observed dynamics and performed experiments to exclude some of the models previously proposed in the literature (Napolitano et al. (2019)).

2. METHODS

Models and simulations were developed, analyzed and simulated in *Mathworks Matlab R2019a*. The experiments were performed on a human HeLa cell line overexpressing the fusion protein TFEB-GFP. The fusion protein TFEB-GFP enables to follow the protein localisation of TFEB in response to the external stimulus. This cell line also expresses a nuclear mCherry protein to facilitate image processing (Postiglione et al. (2018)). The technological platform we used for experiments is constituted by a microfluidic device (Kolnik et al. (2012)), a time-lapse

microscopy apparatus (Postiglione et al. (2018, 2016)) and a set of automated syringes all controlled by a computer (Fracassi et al. (2016)); the platform has been previously described (Postiglione et al. (2018); Fracassi et al. (2016)). The microfluidic device ensures the precise regulation of the cellular micro-environment and allows to track the single cells over time. The fluorescence microscopy followed by image analysis enabled quantitative measurement of TFEB cytoplasmic and nuclear localization in the single cells. The nuclei fluorescence was normalized with respect to the cells fluorescence and the percentage of nuclear TFEB was then measured.

3. MICROFLUIDICS EXPERIMENT TO OBSERVE TFEB DYNAMICS

We performed an experiment to analyze the translocation dynamics of TFEB following starvation and growth stimuli. In particular, starvation response was induced by growing cells in HBSS, a saline solution, while the feeding condition was induced using RPMI, a nutrient rich medium.

The experiment was realized by alternating starvation and growth media following a pulse of starvation (Fig. 1B). The cells were treated with RPMI for 180 minutes, then with HBSS for 180 minutes and finally again with RPMI for other 180 minutes. Cells were captured for a total of 540 minutes in the microfluidics device at 15 minutes sampling interval, while the input was dynamically changed by changing the relative heights of two syringes connected to the microfluidics device, as previously described (Fracassi et al. (2016)).

Figure 1B shows the TFEB nuclear percentage of the single-cell signals as thin green lines while their average is shown as a bold green one. By looking at the signals, it is possible to observe that at the beginning of the experiment, when the growth medium is supplied, TFEB is mostly cytoplasmic, with about 30% of total TFEB being nuclear. Then, upon switching from nutrient rich medium to starvation medium, TFEB accumulates in the nucleus in about 30 minutes, reaching a peak of about the 80% nuclear. Afterwards, it slowly decreases with a clear overshoot, causing a cytoplasmic relocalisation during the starvation phase. Finally, it quickly drops when switching back from starvation to nutrient rich medium. A similar dynamics, an "undershoot", is present also when switching from starvation to nutrient rich medium. This behavior is shared by all the single cells traces.

In summary, a prolonged starvation treatment of at least three hours highlighted the presence of a novel TFEB translocation dynamic: the transcription factor exhibits adaptation by settling to equilibrium with an overshoot dynamic. A similar phenomenon was recently observed in Zapata et al. (2016). The authors suggested that the rapid rheostatic response, mediated by mTOR, allows the cell to quickly adapt to metabolic changes, while the long-term, mTOR-independent homeostatic response controls the magnitude and duration of TFEB activation, and presumably limits excessive autophagy. However, no mechanism was put forward to explain the origin of this dynamic behaviour.

4. RESULTS

We previously developed a model to describe TFEB nuclear shuttling dynamics, as reported in Fig. 1C (Napolitano et al. (2019)). The model considers the presence of two compartments (Atkins (1969)), the nucleus and the cytoplasm, through which TFEB shuttles (Napolitano et al. (2018)). Within each compartment, de/phosphorylation reactions occur, in addition to two transport reactions to model TFEB transport. We distinguished TFEB in four different species: (i) nuclear dephosphorylated ($TFEB_{nuc}$), (ii) nuclear phosphorylated ($TFEB_{nuc}^*$), (iii) cytoplasmic dephosphorylated ($TFEB_{cyt}$), (iv) cytoplasmic phosphorylated ($TFEB_{cyt}^*$). We assumed first order kinetics for the de/phosphorylation and transport reactions (Salazar et al. (2003)). Furthermore, we considered the dependence of de/phosphorylation on the nutrients (amino acids) concentration by assuming a linear dependence of the reaction rates on the input u . Let $u \in [0, 1]$ be the external input to the system with $u > 0.5$ corresponding to starvation and $u \leq 0.5$ to growth. Let also x_C and x_N be respectively cytoplasmic and nuclear TFEB concentrations and x_C^* and x_N^* their phosphorylated counterparts. We further assumed that the total amount of TFEB protein is constant adding a conservation rule: $x_C + x_N + x_C^* + x_N^* = 1$. Moreover, we considered symmetric both the transport rates and the reaction rates, thus reducing the system parameters to two: (i) k that is the de/phosphorylation rate and (ii) β that is the transport rate. This simplification is justified by the observation that nuclear import of dephosphorylated TFEB and nuclear export of phosphorylated TFEB have similar kinetics (Napolitano et al. (2018)). The two parameters were inferred from literature: $k = 1.5 \cdot 10^{-2}$, $\beta = 5 \cdot 10^{-3}$ (Napolitano et al. (2018)). The equations of the model are:

$$\begin{aligned}\dot{x}_C^* &= k(1-u)(1-x_C^*-x_N-x_N^*)-kux_C^*+\beta x_N^* \\ \dot{x}_N &= kux_N^*-k(1-u)x_N+\beta(1-x_C^*-x_N-x_N^*) \\ \dot{x}_N^* &= k(1-u)x_N-kux_N^*-\beta x_N^*\end{aligned}$$

Further details were previously discussed in Napolitano et al. (2019). To check whether the model could recapitulate the observed experimental data, we performed a numerical simulation, reported in Fig. 1B, by applying the same experimental input to the model. The simulations were performed in *Mathworks Matlab R2019a* by using the *ode45* solver. For a better comparison between the experimental and numerical data, the simulation was performed by considering an input $u = 0.35$ for the fed condition and $u = 0.75$ for the starvation and adapting the initial conditions to the experimental data ($x_0 = [0.65, 0.35, 0]^T$). It is possible to appreciate that the model can replicate the fast dynamics observed in the experiments during medium switches. Conversely, it is not able to recapitulate the slower overshoot dynamic.

From Fig. 1B it is possible to observe that the overshoot dynamic is much slower than the TFEB response to starvation. In particular, TFEB enters in the nucleus in about 30 minutes, while the overshoot takes about 110 minutes. Consequently, we hypothesized that another

slower biological mechanism could be responsible for this behavior.

The negative feedback loops (NFLs) and incoherent feedforward loops (IFFLs) are the only two gene network motifs that can give rise to adaptation (Rahi et al. (2017)). This observation led us to conjecture the presence of a non-identified species that could regulate TFEB nuclear amount through either a NFL or an IFFL regulatory motif. Considering the biological process represented in Fig. 1A and the block scheme proposed in Fig. 1C, we reasoned that if present, the species could influence either the phosphorylation state of TFEB, or alternatively its nuclear transport.

We previously investigated the hypothesis that the TFEB overshoot is caused by a negative feedback on its phosphorylation state caused by a partial reactivation of mTOR as recently proposed in literature (Napolitano et al. (2018); Li et al. (2018)). Under starvation TFEB is dephosphorylated and enters inside the nucleus activating autophagic genes. Autophagy promotes the generation of a pool of amino acids used by the cell to survive in this situation of stress. The hypothesis is that the cellular nutrient sensing machinery detects the presence of these extra-nutrients and partially reactivates mTOR, which in turn phosphorylates TFEB causing its partial nuclear export.

This hypothesis was already characterized in Napolitano et al. (2019). The model proposed was able to qualitatively recapitulate the TFEB overshoot dynamics, despite the real dynamics being slower, suggesting that the hypothesis formulated could be plausible.

We then experimentally tested *in vitro* the reliability of this mTOR reactivation hypothesis. To this end, we performed an experiment by keeping cells in nutrient rich medium and by treating them with the small molecule Torin 1, a highly potent and specific mTOR inhibitor. As shown in Fig. 1D, the overshoot dynamic is still present despite mTOR being fully inhibited, and it is comparable with the dynamics already observed in Fig. 1B, suggesting that this hypothesis is not supported by experimental evidence.

We thus hypothesized that an additional unknown species may affect TFEB nuclear transport rates through either a negative feedback loop, or an incoherent feedforward loop, as schematically depicted in Fig. 2A, B. In order to test the new hypotheses, we developed two alternative models by adding a species z , which acts as an additional nuclear exporter able to export nuclear TFEB with a rate γ , independently of its phosphorylation state. The NFL equations are reported below:

$$\begin{aligned}\dot{x}_C^* &= k(1-u)(1-x_C^*-x_N-x_N^*)-kux_C^*+\beta_2x_N^* \\ &\quad +\gamma zx_N^* \\ \dot{x}_N &= kux_N^*-k(1-u)x_N+\beta_1(1-x_C^*-x_N-x_N^*) \\ &\quad -\gamma zx_N \\ \dot{x}_N^* &= k(1-u)x_N-kux_N^*-\beta_2x_N^*-\gamma zx_N^* \\ \dot{z} &= -az+b(x_N+x_N^*)\end{aligned}$$

Since there is a difference in the dynamics between the overshoot observed during the starvation phase and the

undershoot observed during the growth phase (Fig. 1B), we decided to break the symmetry on the transport rates to better fit the experimental dynamics. Consequently, we performed an optimization process and found $\beta_1 = 0.22 \cdot \beta$ and $\beta_2 = 0.5 \cdot \beta$ as best fitting parameters for the transport rates. Moreover, we added a new equation modelling the dynamics of the new species z . Here, a and b are respectively the degradation and the production rates of z . Parameter a was set according to the experimental slow overshoot dynamics (of the order of three hours). Parameter b was set according to the experimental equilibrium reached by the system (60% of nuclear TFEB, as shown in Fig. 1B). Therefore, the parameters were set to: $a = 1.5 \cdot 10^{-4}$, $b = 8.8 \cdot 10^{-5}$. The transport rate γ was set equal to $0.44 \cdot \beta$ as result of the optimization process.

As previously discussed, the NFL architecture is not the only plausible architecture able to biologically explain this phenomenon. Consequently, we hypothesized an alternative model acting via an incoherent feedforward loop (IFFL) model, as shown in Fig. 2A, B (in blue). In the IFFL model the production of the z species directly depends on the input and not on the TFEB nuclear concentration. The IFFL model equations are:

$$\begin{aligned}\dot{x}_C^* &= k(1-u)(1-x_C^*-x_N-x_N^*)-kux_C^*+\beta_2x_N^* \\ &\quad +\gamma zx_N^* \\ \dot{x}_N &= kux_N^*-k(1-u)x_N+\beta_1(1-x_C^*-x_N-x_N^*) \\ &\quad -\gamma zx_N \\ \dot{x}_N^* &= k(1-u)x_N-kux_N^*-\beta_2x_N^*-\gamma zx_N^* \\ \dot{z} &= -az+bu\end{aligned}$$

We characterized *in silico* the two models performing new simulations. We thus set $u = 0.45$ for the feeding condition, $u = 1$ for starvation. Then, we set $x_0 = [0.44, 0.13, 0.11, 0.14]^T$ as initial condition for the NFL model and $x_0 = [0.45, 0.12, 0.10, 0.26]^T$ as initial condition for the IFFL model. The two simulations were reported in Fig. 2C (the NFL in red, the IFFL in blue). As expected from theoretical consideration, with this choice of parameters both the models are able to qualitatively recapitulate the experimentally observed dynamics. Both the simulations clearly show the fast rise, the overshoot, the new equilibrium, and the fast decrease. A delay in TFEB response to the input in experimental data is present if compared with the simulations. This delay is probably due to the fact that the microfluidic device takes 10 minutes to completely change the media in the cell chambers (Postiglione et al. (2018)). Moreover, it is possible to appreciate how the new models are also able to recapitulate the asymmetry between the overshoot dynamics occurring during starvation, and the undershoot dynamics occurring during growth. This behavior was not caught by the previous NFL model, where the overshoot and the undershoot dynamics were symmetric (Napolitano et al. (2019)). In summary, both the models are able to qualitatively replicate the observed experimental dynamics.

To experimentally distinguish between the two alternative models, we used *in silico* simulations to identify the best dynamic stimulus able to distinguish between the two. The main difference between the two models lies in the

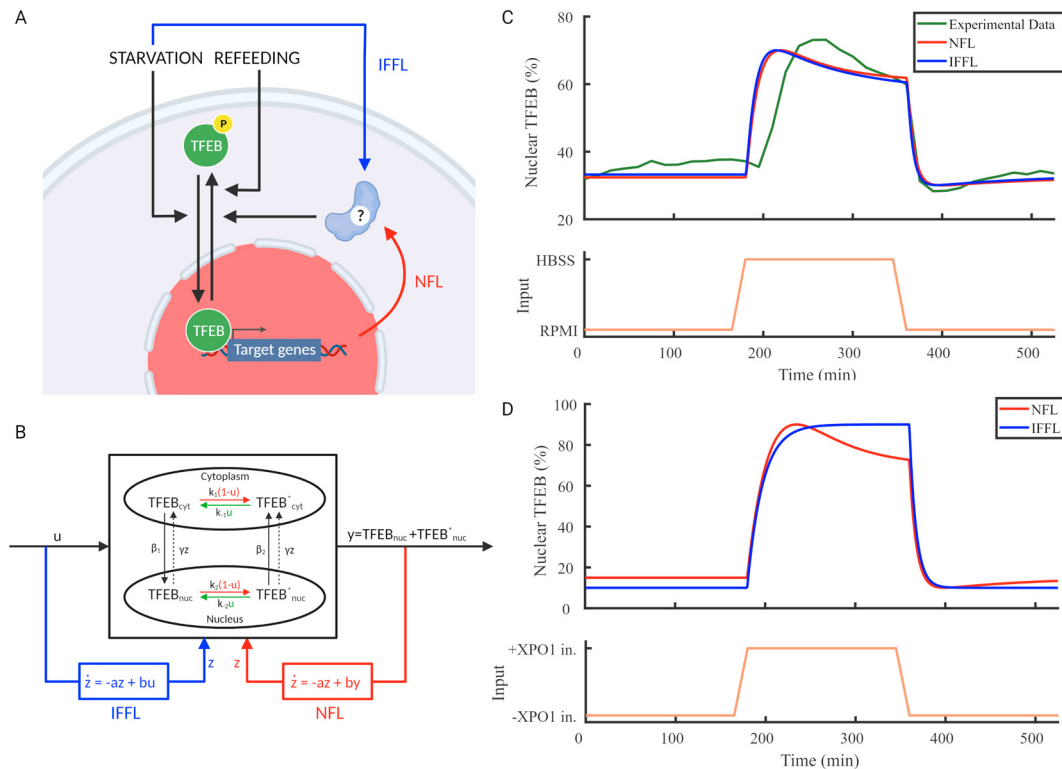


Fig. 2. Two alternative models of TFEB dynamics. (A) Biological hypothesis: an unknown species is produced or activated following TFEB nuclear translocation (NFL, in red) or following starvation (IFFL, in blue), influencing TFEB cellular localization through a negative feedback or a feedforward action. (B) Block scheme of the negative feedback regulation hypothesised for the overshoot dynamics is represented in red. The production of the new species z depends on the total amount of nuclear TFEB y . Block scheme of the incoherent feedforward regulation hypothesised for the overshoot dynamics is represented in blue. The production of the new species z depends on the input u . In both cases, the z species acts as a transporter able to export nuclear TFEB. (C) Comparison of experimental measurements and numerical simulations of TFEB translocation following a pulse of starvation. The numerical simulations of nuclear TFEB fluorescence are shown with a red line for NFL and with a blue line for IFFL. The average signal across the experimental single-cell traces is shown with a green line. (D) Numerical simulations of TFEB translocation following a pulse of XPO1 inhibition. The numerical simulations of nuclear TFEB fluorescence are shown with a red line for NFL and with a blue line for IFFL.

adaptation mechanism. In the IFFL model adaptation is directly driven by starvation, while in the NFL model, adaptation is caused by TFEB accumulating in the nucleus (Fig. 2A). Experimental discrimination between the two models is not trivial because of the correlation between the nutrients concentration in the cellular environment and the TFEB amount in the nucleus. We thus reasoned that inhibition of nuclear export in cells grown in nutrient-rich medium can be used to decouple TFEB nuclear accumulation from starvation, and thus distinguish between the two models. Indeed, a small amount of TFEB is normally present in the nucleus (Fig. 1A, B). By impairing the balance between the nuclear input flux and nuclear export flux, there should be an increase of the nuclear TFEB concentration. This increase of TFEB nuclear concentration will result in a different outcome according to the model considered: in the IFFL, the overshoot should not be present, since the nutrients concentration remains unaltered; on the other hand, in the NFL model, TFEB nuclear accumulation will lead to the activation of the new transporter and consequently to the appearance of the overshoot. We tested this idea *in silico*: we kept the

system in a growth condition for 180 minutes and then we inhibited nuclear export for 180 minutes. Finally, the stimulus was switched back to growth medium for the remaining 180 minutes. The switching between the normal condition and the condition with impaired nuclear export is achieved by switching β_2 between its physiological value and zero. From a biological point of view, the inhibition of the nuclear export corresponds to the inhibition of the exportin XPO1 (Napolitano et al. (2018)). The numerical simulation of the described experiment is shown in Fig. 2D. The simulation shows that the proposed experiment is able to highlight the differences between the two models. As expected, the NFL shows adaptation, while IFFL does not. Experimental validation is required to evaluate which model is the most plausible and what the exact biological mechanism might be.

5. CONCLUSION

TFEB is one of the main players in the transcriptional regulation of starvation-induced autophagy, linking lysosomal biogenesis to autophagy (Settembre et al. (2011)). TFEB activity is fundamental for cell function and homeostasis,

and its disfunction leads to severe disorders such as neurodegenerative disorders and cancer (Puertollano et al. (2018); Napolitano et al. (2016)). It is therefore evident the need to fully understand TFEB regulation and the key players influencing its activity and localization in the cell.

Our experiments highlight a complex nuclear translocation dynamics. We previously developed an NFL model that recapitulated the experimental dynamics (Napolitano et al. (2019)) by assuming a role of mTOR reactivation in starvation. Here we show that this hypothesis is not supported by our new experiments. Consequently, we developed two alternative models hypothesizing that a novel species regulates TFEB nuclear shuttling either through an NFL or an IFFL regulatory motif acting on its transport rates. The new models were both able to qualitatively recapitulate the entire dynamics observed experimentally. Moreover, they were also able to recapitulate the asymmetry between the overshoot and the undershoot dynamics, that was not captured by the previous model. We demonstrated *in silico* that, by inhibiting the nuclear export, it is possible to distinguish between these two alternative explanations. In future work, we will pharmacologically inhibit XPO1 by using our microfluidics platform to test which of the two models is more likely to describe the biological reality.

REFERENCES

- Atkins, G.L. (1969). Multicompartment models for biological systems.
- Fracassi, C. et al. (2016). Automatic control of gene expression in mammalian cells. *ACS synthetic biology*, 5(4), 296–302.
- Glick, D. et al. (2010). Autophagy: cellular and molecular mechanisms. *The Journal of pathology*, 221(1), 3–12.
- Kolnik, M. et al. (2012). Vacuum-assisted cell loading enables shear-free mammalian microfluidic culture. *Lab on a chip*, 12(22), 4732–4737.
- Li, L. et al. (2018). A tfeb nuclear export signal integrates amino acid supply and glucose availability. *Nature communications*, 9(1), 1–15.
- Martina, J.A. et al. (2012). Mtorc1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of tfeb. *Autophagy*, 8(6), 903–914.
- Medina, D.L. et al. (2015). Lysosomal calcium signalling regulates autophagy through calcineurin and tfeb. *Nature cell biology*, 17(3), 288–299.
- Mizushima, N. et al. (2011). Autophagy: renovation of cells and tissues. *Cell*, 147(4), 728–741.
- Napolitano, G. et al. (2016). Tfeb at a glance. *Journal of cell science*, 129(13), 2475–2481.
- Napolitano, G. et al. (2018). mtor-dependent phosphorylation controls tfeb nuclear export. *Nature communications*, 9(1), 1–10.
- Napolitano, S. et al. (2019). Tfeb dynamical model reveals a novel feedback loop biological mechanism. *IFAC-PapersOnLine*, 52(26), 213–218.
- Postiglione, L. et al. (2016). From a discrete to continuous actuation for improved real-time control of gene expression in mammalian cells. *IFAC-PapersOnLine*, 49(26), 14–19.
- Postiglione, L. et al. (2018). Regulation of gene expression and signaling pathway activity in mammalian cells by automated microfluidics feedback control. *ACS synthetic biology*, 7(11), 2558–2565.
- Puertollano, R. et al. (2018). The complex relationship between tfeb transcription factor phosphorylation and subcellular localization. *The EMBO journal*, 37(11), e98804.
- Rahi, S.J. et al. (2017). Oscillatory stimuli differentiate adapting circuit topologies. *Nature methods*, 14(10), 1010–1016.
- Salazar, C. et al. (2003). Allosteric regulation of the transcription factor nfat1 by multiple phosphorylation sites: a mathematical analysis. *Journal of molecular biology*, 327(1), 31–45.
- Settembre, C. et al. (2011). Tfeb regulates autophagy: an integrated coordination of cellular degradation and recycling processes. *Autophagy*, 7(11), 1379–1381.
- Settembre, C. et al. (2012). A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mtor and tfeb. *The EMBO journal*, 31(5), 1095–1108.
- Zapata, P.A.M. et al. (2016). Time course decomposition of cell heterogeneity in tfeb signaling states reveals homeostatic mechanisms restricting the magnitude and duration of tfeb responses to mtor activity modulation. *BMC cancer*, 16(1), 1–19.