Translation on Demand as a post-transcriptional regulation mechanism of ES cell differentiation

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submitted by Elias Schwall

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Reviewers:

Prof. Dr. Andreas Beyer

Prof. Dr. Achim Tresch

Supervised by:

Luke Brandwood

Declaration of originality

I hereby declare that this thesis is my own work and that no other sources and tools than stated were used.

Cologne, May 14, 2025 Elias Schwall

Acknowledgement

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Abstract

Introduction

Mouse embryonic stem cells

Mouse embryonic stem cells (mESCs) are defined by their self-renewal capabilities and pluripotency, allowing them to differentiate into any cell type from the three germ layers. While this differentiation process is limited in vivo, pluripotent stem cells (PSCs) cultivated in vitro can self-renew indefinitely under specific culture conditions (Nichols and Smith 2009). One such condition is the 2i+LIF (two inhibitors with leukemia inhibitory factor) medium, which maintains the naive pluripotent state of ESCs, closely mimicking the inner cell mass of pre-implantation mouse embryos.

The continuum of pluripotency delineates a progression from the naive state to a primed state, characterized by an increased propensity for differentiation (Nichols and Smith 2009). Naive mESCs serve as a model for the inner cell mass of the pre-implantation embryo, exhibiting minimal lineage commitment. In contrast, epiblast stem cells (EpiSCs), which are isolated from later developmental stages, correspond to the post-implantation epiblast and are primed for differentiation (Boroviak et al. 2014).

During the transition from the naive to the primed states, sequential changes occur at multiple molecular levels, encompassing alterations in signaling pathways, epigenetic markers, and gene expression (Dejosez and Zwaka 2012). Specifically, early modifications in the phosphoproteome serve as indicators of these state transitions, initiating a cascade of changes that ultimately impact the transcriptome and proteome (Needham et al. 2019). This progression underscores the dynamic nature of pluripotency and highlights the intricate molecular mechanisms governing cell fate decisions in early development.

It is worth noting that early mESCs development differs from human embryonic stem cells (hESCs). While the molecular mechanisms controlling developmental processes are evolutionarily conserved, the speed at which these processes occur can vary significantly between species. For instance, the differentiation of motor neurons from embryonic stem cells is more than twice as fast in mice compared to humans (Rayon et al. 2020).

Regardless of the species, however, early developing embryonic stem cells need to undergo rapid changes in their gene expression profiles to ensure proper development and

differentiation. Mechanisms such as ToD, can allow for swift adjustments in protein levels to aid these rapid changes. This ensures that cells can quickly respond to developmental cues and environmental changes, maintaining the necessary pace of development. Such mechanisms are crucial for adapting to the specific temporal requirements, ensuring that embryonic stem cells can effectivly progress through the stages of development.

Translation on demand

We define ToD as an increase in protein abundance due to enhanced translation, occurring without any change in the mRNA levels of the corresponding gene. In other words, ToD is a regulatory mechanism where translation efficiency is selectively increased for specific genes. This upregulation allows for rapid adjustments in protein levels, supporting cellular responses that need immediate protein synthesis without requiring new mRNA transcription. Such translation driven increases could be particularly useful in situations where quick adaptations are necessary, as it enables cells to respond to changes in the environment or developmental cues by rapidly elevating the protein output from existing mRNA pools. In vivo, there are already described mechanisms that facilitate forms of selective, rapid translation of mRNAs. For example, neurons need local translation at active synapses for quick cellular responses (Holt and Schuman 2013). In early embryos, maternal mRNAs are stored and activated after fertilization, enabling the embryo to rapidly synthesize proteins necessary for early development without new mRNA synthesis (De Leon, Johnson, and Bachvarova 1983). In stress response, rapid and selective synthesis of stress proteins, such as heat shock proteins, is facilitated by ToD, allowing cells to quickly adapt and protect themselves under stress conditions without increasing mRNA levels (Holcik and Sonenberg 2005).

2i Condition for maintaining naive pluripotency

Since serum contains undefined factors that can induce differentiation, the 2i medium was developed to provide a defined, chemically controlled environment that preserves mESCs in the naive pluripotent state (Ying et al. 2008). The 2i medium relies on the synergy

between two inhibitors, PD0325901 and CHIR99021, to sustain mESCs in an undifferentiated state. PD0325901, a MEK inhibitor, blocks the MEK/ERK signaling pathway, suppressing differentiation signals from FGF and ERK that would otherwise prompt mESCs to exit pluripotency (see Figure 1). Meanwhile, CHIR99021, a GSK3α/β inhibitor, stabilizes β-catenin and activates Wnt/β-catenin signaling, promoting the expression of self-renewal genes and reinforcing pluripotency (Ying et al. 2008). Together, these inhibitors create a controlled environment that preserves mESCs in a ground state resembling the undifferentiated pre-implantation epiblast (Ying et al. 2008). Alongside leukemia inhibitory factor (LIF), which activates the JAK-STAT3 pathway to further inhibit differentiation, the 2i medium maintains mESCs in a naive pluripotent state, enabling them to retain full differentiation potential without lineage bias (Ying et al. 2008).

The removal of the 2i medium triggers the transition from the naive pluripotent state by reactivating the MEK/ERK and GSK3 pathways, allowing ERK signaling to promote differentiation and reducing Wnt signaling to enable lineage specification. This shift initiates a cascade of biological changes, including the downregulation of naive markers (such as Nanog and Esrrb) and upregulation of formative markers (such as Otx2), along with epigenetic remodeling, altered chromatin accessibility, and a metabolic shift from glycolysis to oxidative phosphorylation (Wray et al. 2011).

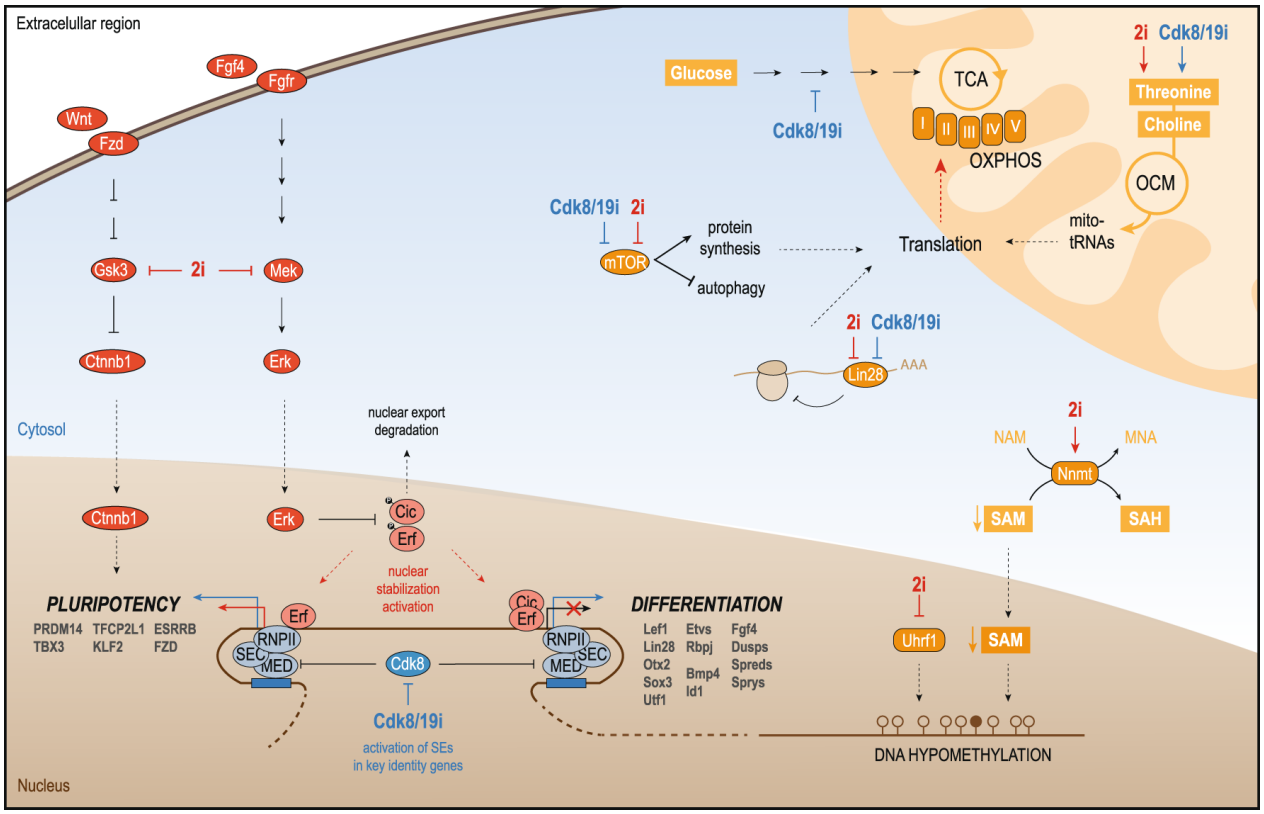

Figure 1: **2i mechanism of stabilizing naive pluripotency** The inhibition of Mek/Erk through 2i-dependent mechanisms prevents the phosphorylation of Erf and Cic, resulting in the stabilization of these factors within the nucleus. These transcriptional effectors may play a role in activating genes associated with naïve pluripotency while repressing those involved in primed/formative states. Cdk8/19i influences the phosphorylation of proteins that are part of the elongation and transcription machinery, including the super elongation complex (SEC), RNA polymerase II (RNPII), and Mediator (MED). This could lead to the rapid activation of genes regulated by super-enhancers, which include factors that stabilize the naïve state as well as certain factors from the primed/formative states that are also upregulated in response to Cdk8/19i. The activation of the naïve circuitry through 2i and Cdk8/19i may support the enhanced mitochondrial oxidative phosphorylation (OXPHOS) capacity of these cells. The degradation of Lin28a might alleviate its repressive effects on target mRNAs, leading to the upregulation of mitochondrial proteins among others. The balance between autophagy and protein synthesis may sustain the translation of these mitochondrial components. Additionally, the re-wiring of mitochondrial sources for one-carbon units may further support the translation of mitochondrial-encoded proteins. Furthermore, the increased availability of S-adenosylmethionine (SAM) levels, due to NNMT upregulation, combined with the impairment of DNA methylation caused by Uhrf1 degradation, contributes to the characteristic hypomethylated DNA state typical of 2i conditions. (Martinez-Val et al. 2021)

ERK signalling pathway and its effects on translation

ERK signaling influences translation primarily by modulating the activity of eukaryotic translation initiation factor 4E (eIF4E) (Lavoie, Gagnon, and Therrien 2020). ERK activates MNK1 and MNK2 kinases, which phosphorylate eIF4E, enhancing its binding to the mRNA cap structure and promoting the formation of the eIF4F complex (see Figure 2). This complex, consisting of eIF4E, eIF4G, and eIF4A, is essential for recruiting the ribosome to mRNA, scanning for the start codon, and initiating protein synthesis. ERK signaling can selectively enhance the translation of specific mRNAs, particularly those with complex 5′ UTRs, leading to the production of proteins involved in cell growth and survival. Additionally, ERK interacts with other pathways, such as mTOR, to coordinate translation regulation in response to various signals, thus optimizing cellular responses to environmental changes.

mTOR signalling pathwy and its effect on translation

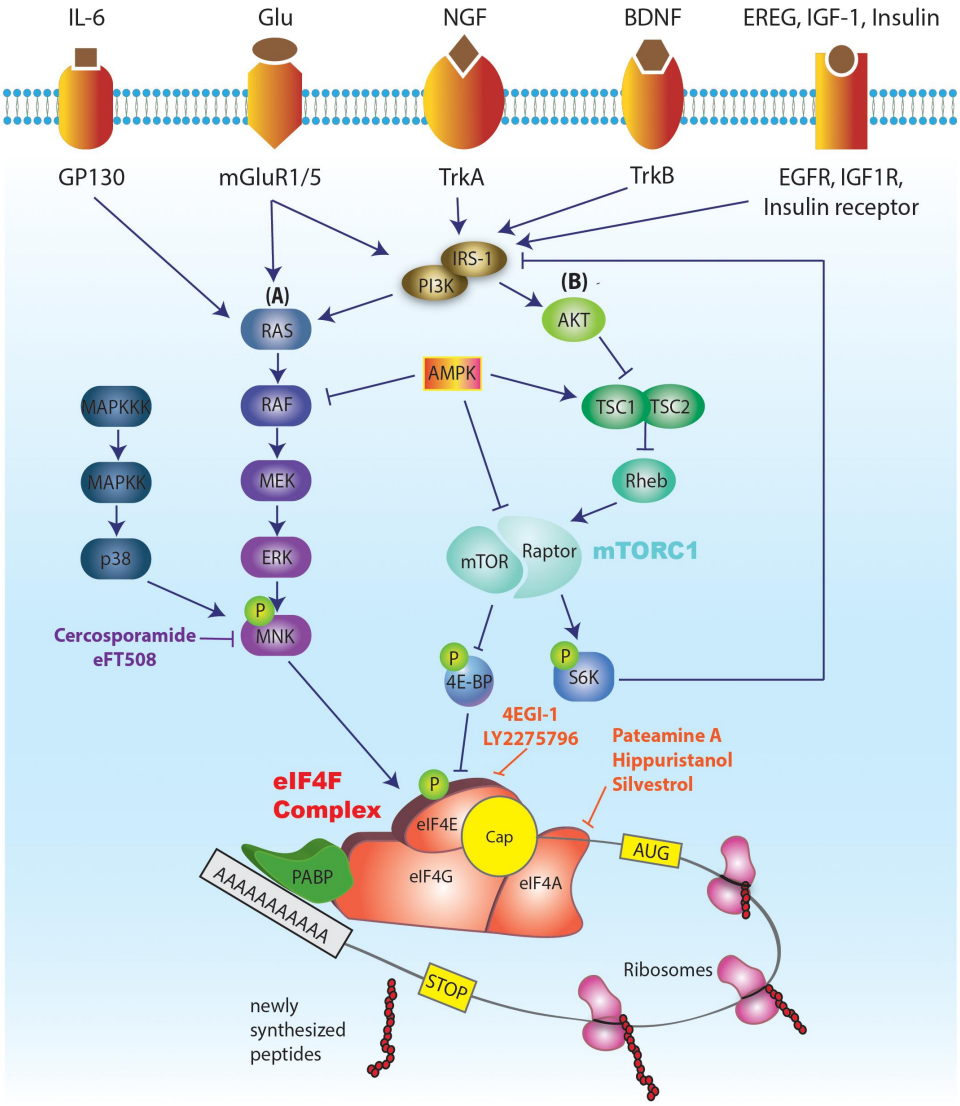

Figure 2: **ERK and mTORs effects on eIF4E regulation** The ability of eIF4E to bind to the mRNA cap makes it a key regulator of translation. A crucial step in translation initiation involves eIF4E binding to the mRNA cap, facilitating the formation of the eIF4F complex on the cap structure (comprising a 7mGp linked to the first nucleotide). The eIF4F complex includes eIF4E, eIF4G (a scaffolding protein), and eIF4A (a helicase). The successful assembly of the eIF4F complex on the mRNA cap promotes the recruitment of the pre-initiation complex (PIC), followed by scanning of the 5′ UTR to locate the start codon AUG and the joining of the 60S ribosomal subunit, marking the completion of translation initiation. eIF4E acts as a downstream effector of both the mTORC1 pathway (via 4E-BP-dependent repression) and the ERK pathway (via eIF4E phosphorylation by MNK 1/2). The activities of mTORC1 and ERK signaling pathways are influenced by various external signals and internal cues (Uttam et al. 2018).

Additional mechanisms influencing translation

Aim of this study

In this project, we aim to identify ToD candidates from a multi-omic dataset, which includes a time series of bulk RNA sequencing and proteomic data (Yang et al. 2019). These multiple omic layers enable us to explore how protein abundance responds to changes in mRNA levels and to identify genes that may be regulated through ToD mechanisms. Next to finding genes that are subject to ToD regulation, we want to illustrate why these specific genes are prioritized and to uncover the underlying mechanisms of this regulation. This involves exploring the biological roles and functional significance of these genes in cellular processes. Additionally, we are interested in understanding the gene regulatory network and proteome changes that occur as mESCs exit pluripotency, as these regulatory networks are most likely to be regulated by ToD.

By analyzing the temporal dynamics within these data layers, we can distinguish cases where protein levels increase independently of mRNA changes, a hallmark of ToD regulation. This approach provides a comprehensive view of the post-transcriptional mechanisms that contribute to protein abundance, offering insights into gene-specific regulatory processes. Understanding the more nuanced relationship between RNA and protein expression, as well as the timing of regulation, will provide valuable insights into the fundamental principles of molecular biology and enhance our comprehension of cellular regulatory networks as well as early development.

Furthermore, this study should serve as a framework to detect ToD candidates in general. We also aim to validate our findings and approaches with a human temporal dataset to check the validity of the methods and see if we obtain similar results. This cross-species validation will help ensure that the mechanisms identified are robust and applicable across different biological contexts.

Methods and tools

Data overview and processing

Translation on demand metrics

RNA-Seq

Proteomics

Ribo-Seq

Gene Overrepresentation Analysis

Gene set enrichment analysis

Motif enrichment

Software

Results

Early developement is shaped by ToD

Gene overrepresentation analysis reveals time dependent pathway regulation of developmental terms

ToD candidates regulate gene expression as transcription factors

ToD is influenced by RNA binding proteins

GSEA reveals

Riboseq reveals

Discussion

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Appendices

Supplementary figures

Supplementary tables

Code and Data availability

Abbreviations

Table 1: List of abbreviations

Abbreviation	Definition
ToD	Translation on demand
mESCs	Mouse embryonic stem cells
PSCs	Pluripotent stem cells
EpiSCs	Epiblast stem cells
hESCs	Human embryonic stem cells
LIF	Leukemia inhibitory factor
eIF4E	Eukaryotic translation initiation factor 4E