nature immunology

Translational control of immune responses: from transcripts to translatomes

Ciriaco A Piccirillo[1,2], Eva Bjur[3], Ivan Topisirovic[4,5], Nahum Sonenberg[6] & Ola Larsson[3]

Selective translational control of gene expression is emerging as a principal mechanism for the regulation of protein abundance that determines a variety of functions in both the adaptive immune system and the innate immune system. The translation-initiation factor eIF4E acts as a node for such regulation, but non-eIF4E mechanisms are also prevalent. Studies of 'translatomes' (genome-wide pools of translated mRNA) have facilitated mechanistic discoveries by identifying key regulatory components, including transcription factors, that are under translational control. Here we review the current knowledge on mechanisms that regulate translation and thereby modulate immunological function. We further describe approaches for measuring and analyzing translatomes and how such powerful tools can facilitate future insights on the role of translational control in the immune system.

© 2014 Nature America, Inc. All rights reserved.

Despite sharing a common genome, cells in multicellular organisms have distinct phenotypes and respond differently to the same stimulus. Thus, cells of the immune system have developed tightly regulated mechanisms to control their own activity. Such functions depend largely on selective gene expression. Delineating gene-expression programs in specific cell populations and their responses to intra- and extracellular cues and understanding the underlying mechanisms are therefore pivotal to understanding cell-specific functions. The gene-expression pathway consists of multiple regulatory steps. Each of these steps can contribute to the repertoire of expressed proteins in a cell type– and/or stimulus-dependent fashion. Accordingly, gene expression can be modulated post-transcriptionally through the regulation of splicing[1], the export of the mRNA from the nucleus to the cytosol[2], the stability of the mRNA in the cytosol[3] and the translation of the mRNA to produce a protein[2] (**Fig. 1a**). Such mechanisms can be general (by affecting most transcripts in the cell), selective (by regulating a subset of transcripts) or specific (by targeting only one transcript for regulation). A hallmark of post-translational mechanisms (for example, protein degradation) and post-transcriptional mechanisms (including the translation of mRNA) is the induction of acute changes in the proteome, which must adapt to rapid changes in the cell environment, all without the need for *de novo* transcription. In this Review we describe the current knowledge about the relative contributions of post-transcriptional and post-translational mechanisms for the genome-wide regulation of gene expression, the importance of translational control for immune responses and how genome-wide approaches can be (and have been) used to gain insights into the translational control of immunological function.

Regulation of gene expression via translational control

The introduction of DNA microarray technology has facilitated studies of differences in the steady-state abundance of mRNA genome wide in different conditions, tissues or cell lines[4–6]; these are often referred to as 'transcriptome studies', although this method measures the 'sum' of transcriptional and mRNA-decay effects on the steady-state abundance of mRNA (**Fig. 1a**). Despite advances in the understanding of mechanisms that regulate gene expression, transcriptome studies are commonly based on the assumption that the abundance of mRNA and that of protein correspond. However, studies of various species directly comparing changes in the abundance of mRNA and that of protein have shown that there is often a poor correlation between these[7,8]. Such differences can be introduced by translational and post-translational mechanisms that act together with transcriptional and other post-transcriptional mechanisms to establish protein abundance. However, the relative contribution of each of these regulatory steps remains largely elusive. It is important to note that the relative contribution of these regulatory steps in the gene-expression pathway will depend on the cell type(s) and context(s) and probably varies over time after a response to a stimulus[7]. Nevertheless, some such comparisons have been made. A pioneering study that estimated the extent of transcription, mRNA degradation, mRNA translation and protein degradation in mouse fibroblasts under optimal growth conditions concluded that translation is the predominant mechanism among post-transcriptional and post-translational regulatory mechanisms and affects the abundance of protein to an extent similar to that of transcription[9]. One limitation of that study is that relative contributions of various steps of the gene-expression pathway were monitored in a single steady-state condition and therefore the

[1]Department of Microbiology and Immunology, and Microbiome and Disease Tolerance Centre, McGill University, Montreal, Canada. [2]FOCIS Centre of Excellence and Centre for Translational Biology, Research Institute of the McGill University Health Centre, Montreal, Canada. [3]Department of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institutet, Stockholm, Sweden. [4]Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montréal, Canada. [5]Department of Oncology, McGill University, Montréal, Canada. [6]Department of Biochemistry, and Goodman Cancer Research Centre, McGill University, Montreal, Canada. Correspondence should be addresses to C.A.P. (ciro.piccirillo@mcgill.ca) or O.L. (ola.larsson@ki.se).

Received 23 February; accepted 8 April; published online 19 May 2014; doi:10.1038/ni.2891

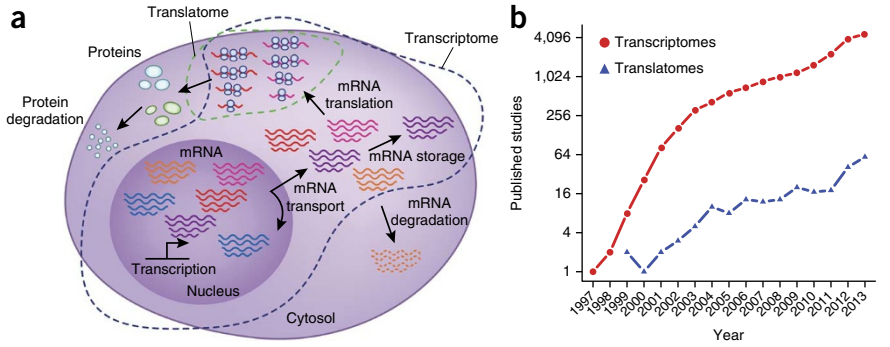

Figure 1 The PTO model. (**a**) The gene-expression pathway consists of multiple levels that can be regulated individually and in concert. Following transcription, mRNA is transported to the cytosol, where it can be degraded, stored or used for protein synthesis. Each of these steps enables the selective regulation of subsets of mRNAs. According to the PTO model, these subsets share similar functions and are identified by 'zip codes' (distinguished here by mRNA color) that enable binding and regulation by RBPs. Because each of these steps can be regulated for each mRNA subset separately, gene-expression mechanisms can act together to orchestrate expression patterns in response to different stimuli. Labels along top (perimeter) indicate mRNA populations studied by 'translatomics' and 'transcriptomics'. (**b**) Despite the fact that translation is a principal mechanism for the regulation of gene expression, studies of the translatome are more rare than are those of the transcriptome; here, the number of studies of transcriptomes per year is based on literature searches for the key word 'transcriptome', whereas studies of translatomes were collected manually.

dynamics following perturbations could not be assessed. Another study addressed this particular aspect in a model in which differences in steady-state mRNA abundance, mRNA translation and protein degradation were determined by comparison of control THP-1 human monocytic cells and THP-1 cells induced to differentiate into a macrophage-like phenotype by the phorbol ester PMA[10]. In this dynamic model, translation was still the principal mechanism among post-transcriptional and post-translational regulatory mechanisms and accounted for ~40% of the dynamic regulation of protein abundance (compared with ~45% for steady-state RNA). Despite such limitations, these studies support the tenet that translation of mRNA has a pivotal role in controlling gene expression. Studies of the 'translatome' (the genome-wide pool of translated mRNA), however, are still scarce relative to those of the transcriptome (**Fig. 1b**; details of such studies are discussed below), and only a small fraction of translatome studies are focused on the immune system. Thus, there are substantial gaps in knowledge in terms of how translation of mRNA contributes to gene-expression programs in cells of the immune system.

Genome-wide organization of gene-expression programs

The profound effects that selective translational control has on protein abundance indicate that translation must be tightly regulated and coordinated with other components of the gene-expression pathway. In 1969, Spirin presented a model for how such gene-expression networks may be organized by highlighting RNA-protein complexes ('informosomes') as positive and/or negative regulators of the association of ribosomes with mRNA[11]. That concept was further developed by the 'post-transcriptional operon' (PTO) model of Keene and colleagues, which integrates translation and other steps in the gene-expression pathway by postulating that the expression of subsets of mRNAs that encode functionally related proteins is orchestrated via spatiotemporal coordination of multiple gene-expression layers (e.g., transcription, mRNA stability and translation)[12]. The PTO model is centered on RNA-binding proteins (RBPs), which act as *trans*-acting regulatory factors that recognize specific *cis* elements (for example, nucleotide sequences, secondary structures or combinations thereof) in mRNA. In this manner, RBPs use *cis* elements as 'zip codes' to sort mRNAs that encode molecules with similar cellular functions (**Fig. 1a**). Such 'zip codes' are thought to be located mainly in the untranslated regions (UTRs) of the mRNAs, although there are some examples of 'zip codes' in coding regions[13].

Translation is commonly separated into four phases: initiation, elongation, termination and recycling[14]. With a few notable exceptions (in which most of the control seems to occur at the elongation step[15–18]), translation is regulated mainly at the initiation step[14]. Accordingly, RBPs and other known regulators of translation modulate translation by altering the activity of translation-initiation factors[19,20]. Of note, the full spectrum of RBPs that affect the initiation of translation is still not fully understood. Many mRNA-associated RBPs have been identified by approaches based on mass spectrometry[21,22], but their role in regulating translation remains to be determined. Here we will highlight results from numerous studies focused on the role of RBPs, regulators of translation and related cellular pathways in the selective regulation of mRNA translation in the immune system.

eIF4E is a node for translational control

The expression of many proteins engaged in various components of innate or adaptive immunity is regulated at the level of translation. It is generally thought that the mTOR kinase pathway has a central role in this process[23,24]. This pathway integrates a variety of extracellular signals and intracellular cues such as cellular energy status[25–28], the availability of nutrients[29,30] and oxygen[31,32], hormones and growth factors to stimulate cell growth, proliferation and influence differentiation[33]. mTOR exists in two complexes, mTORC1 (refs. 34,35) and mTORC2 (refs. 36,37), that differ in their composition, function, downstream substrates and sensitivity to rapamycin[33]. mTORC1 is typically defined by a specific component, raptor, and stimulates anabolic processes, including protein synthesis, whereas mTORC2 contains rictor and regulates cytoskeletal organization and survival via modulation of AGC kinases. In addition, under most conditions, mTORC1 is sensitive to rapamycin but mTORC2 is not[36–38]. Rapamycin is a naturally occurring triene macrolide with strong immunosuppressive properties that acts as an allosteric inhibitor of mTORC1 (refs. 23,24,39). The two best-described downstream targets of mTORC1 linked to translational control are the '4E-binding proteins' (4E-BPs) that inhibit the translation-initiation factor eIF4E[40–43] and the ribosomal protein S6 kinases[42,43]. The S6 kinases regulate translation via the phosphorylation of various downstream targets, such as the ribosomal protein S6 (ref. 44), the tumor suppressor PDCD4 (ref. 45) and the translation-initiation factor eIF4B[46]. Members of the 4E-BP family are small translational suppressors (4E-BP1, 4E-BP2 and 4E-BP3 in mammals) that associate with eIF4E[47], which is the cap-binding subunit of the eIF4F complex[48]. In addition to containing eIF4E, the eIF4F complex also includes the large scaffolding protein eIF4G and the DEAD-box helicase eIF4A. When dephosphorylated, 4E-BPs bind to eIF4E and prevent the association of eIF4E with eIF4G[47]. mTORC1 phosphorylates 4E-BPs[40,49],

© 2014 Nature America, Inc. All rights reserved.

which leads to their dissociation from eIF4E, thereby enabling the eIF4E-eIF4G association and assembly of the eIF4F complex.

Although eIF4E is a general initiation factor, an increase in its activity only marginally affects global protein synthesis but strongly stimulates the translation of a subset of mRNAs referred to as 'eIF4E sensitive'[50]. This can occur via various mechanisms, including the selective induced translation of otherwise inefficiently translated mRNAs with long and structured 5′ UTRs (described elsewhere[2,51,52]) and probably the recruitment of mRNAs associated with inhibitory RBPs for translation[19] (**Fig. 2a**). The subset of eIF4E-sensitive mRNAs therefore probably depends on the cellular context, which influences the repertoire of expressed RBPs and mRNAs. Moreover, mTOR stimulates the translation of mRNAs with 5′ terminal oligopyrimidine tracts ('TOP mRNAs') that encode components of the translational machinery, including ribosomal proteins[53–57]. Under physiological conditions, however, the effects of mTOR on the translation of TOP mRNAs seem to be largely independent of the 4E-BPs[58] and eIF4E[59]. Published findings indicate that depending on the stimulus, the main effectors of mTOR signaling on the translation of TOP mRNA are the RBPs TIA-1 and TIAR[60], and LARP1 (ref. 61).

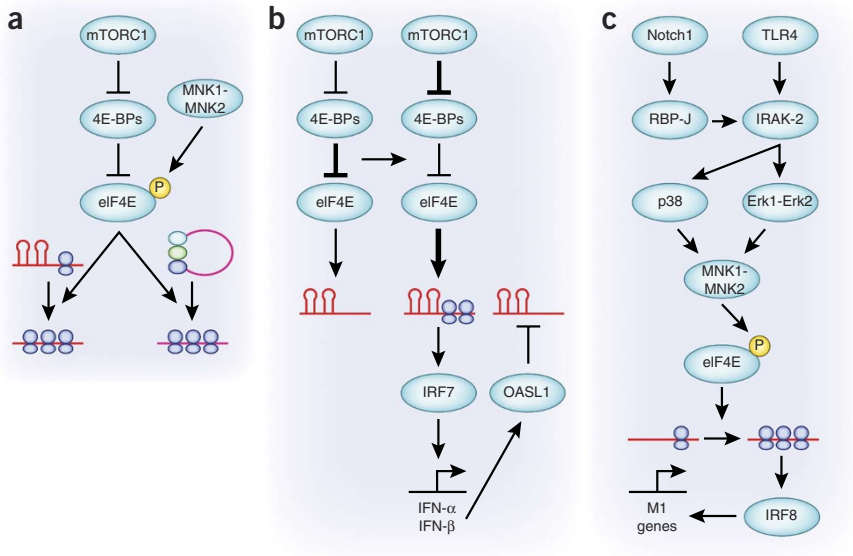

Figure 2 eIF4E is a node for regulation of immune functions via translational control. (**a**) The activity of eIF4E is regulated by both the mTORC1–4E-BP–eIF4E pathway and the MNK1-MNK2-eIF4E pathway. Increased eIF4E activity leads to the selective translation of otherwise inefficiently translated mRNAs with highly structured 5′ UTRs and also probably those in inhibitory RBP complexes. (**b**) A model for the regulation of IRF7 translation via the mTORC1–4E-BP–eIF4E pathway and OASL1. Increased mTORC1 activity (or lack of 4E-BPs) leads to the selective translation of IRF7 and downstream transcription of genes encoding type I interferons. This is paralleled by an increase in the abundance of OASL1 protein, which inhibits IRF7 translation by binding directly to the 5′ UTR of IRF7 mRNA, thereby creating a negative feedback loop. (**c**) The expression of IRF8 protein and macrophage polarization is controlled via the MNK1-MNK2-eIF4E axis. Activation of Notch1 and TLR4 leads to signaling via MAPK-MNK1-MNK2 and downstream phosphorylation of eIF4E. This activates translation of IRF8, which induces transcription of M1 macrophage–associated genes (M1 genes).

In addition to being modulated by the mTORC1–4E-BP pathway, eIF4E activity is also modulated via phosphorylation by the kinases MNK1 and MNK2 on a single serine residue (Ser209 in humans)[62–64] (**Fig. 2a**). Although the precise mechanism by which phosphorylation of eIF4E affects its function remains unclear, a genome-wide study of mouse embryonic fibroblasts (MEFs) has indicated that it selectively promotes translation of many mRNAs, including those encoding chemokines[65]. Moreover, the MNK1-MNK2-eIF4E pathway has been linked to many mechanisms that control immunological function. Translational control of immunological function via eIF4E can therefore be modulated by mTORC1-dependent inactivation of 4E-BPs and direct phosphorylation by MNK1 or MNK2 (**Fig. 2a**). In addition, increases in eIF4E activity can be achieved by stimulation of its expression at the level of transcription (for example, by the transcription factor c-Myc[66]) or mRNA stability (for example, by the RBP HuR[67]).

Translational control via the mTORC1–4E-BP pathway

The mTORC1–4E-BP–eIF4E axis influences the translation of many mRNAs that encode proteins with central roles in immunology, such as the transcription factors IRF7 (ref. 51) and GATA-3 (ref. 68) and the cytokine interleukin 4 (IL-4)[69]. MEFs isolated from mice that lack both 4E-BP1 and 4E-BP2, in which eIF4E is not suppressed under conditions of suppressed mTORC1 signaling, produce more type I interferon than do MEFs isolated from wild-type mice. Accordingly, replication of several interferon-responsive viruses, including influenza and vesicular stomatitis virus, is attenuated in MEFs from mice deficient in both 4E-BP1 and 4E-BP2 relative to such replication in wild-type MEFs[51]. Those *in vitro* findings have been confirmed *in vivo*, as mice deficient in 4E-BP1 and 4E-BP2 are less susceptible to infection with vesicular stomatitis virus than are wild-type mice. That is paralleled by higher expression of type I interferon–regulated genes and more production of type I interferon in plasmacytoid dendritic cells. Mechanistically, the relationship between the 4E-BP status of the cell and the production of type I interferon is explained by the inhibitory effects of 4E-BPs on the translation of IRF7 mRNA. IRF7 mRNA has a long and highly structured 5′ UTR and therefore has eIF4E-sensitive translation. Cells that lack 4E-BPs are unable to suppress the translation of IRF7 mRNA upon virus-induced inhibition of mTOR signaling, which allows the induction of IRF7-mediated transcription of downstream genes encoding type I interferons. In addition to 4E-BPs, the RBP OASL1, whose expression is concomitantly increased along with that of IRF7, has been shown to bind directly to the 5′ UTR of IRF7 mRNA and suppress translation[70]. Therefore, OASL1 may induce a negative feedback mechanism that leads to termination of the interferon response by suppressing expression of IRF7 (**Fig. 2b**).

The mode of action whereby the expression of a central regulator of transcription of the immune response is controlled at the level of translation has also been described during differentiation of $CD4^+$ T cells into distinct effector lineages. GATA-3 is both necessary and sufficient for T helper type 2 (T_H2) cell differentiation. While IL-4 induces the expression of GATA-3 mRNA, the resulting protein amounts are insufficient to induce T_H2 cell differentiation. An increase in the amount of GATA-3 protein and commitment to the T_H2 lineage require simultaneous activation of signaling via the T cell antigen receptor and are mediated through an mTORC1-dependent increase in the synthesis of GATA-3 protein[68]. While the precise mechanism of this phenomenon

© 2014 Nature America, Inc. All rights reserved.

remains unclear, these findings are consistent with the notion that the effects observed are mediated by a mechanism dependent on 4E-BP–eIF4E. Also consistent with the proposed role for eIF4E in T cell differentiation is the finding that inhibition of eIF4E in Foxp3$^-$ CD4$^+$ T cells activated with the invariant signaling protein CD3, the coreceptor CD28 and IL-2 results in a greater abundance of Foxp3 protein. As Foxp3 is a marker and essential lineage-commitment factor for the development of regulatory T cells in the thymus and peripheral tissues, this indicates that suppression of eIF4E under an activating condition bolsters the differentiation of Foxp3$^-$ CD4$^+$ T cells into induced regulatory T cells[71]. The precise mechanism and whether the effect of eIF4E is direct or indirect (for example, via an effect on proliferation), however, remains unclear and requires further investigation.

Analogous to the finding that signaling via CD28 leads to the stabilization of IL-2 mRNA[3], mTORC1 has been linked to regulation of the translation of IL-4 mRNA. The inducible costimulator ICOS is a key protein for the induction of humoral immunity and has high expression in the follicular helper subset of CD4$^+$ T cells. These cells assist in the formation of germinal center reactions in the B cell zones of lymphoid tissues[72]. A study has shown that while ICOS has a critical role in the development of follicular helper T cells, it also provides a major contribution to the effector functions of such cells by modulating translation[69]. ICOS assists signal transduction mediated by the T cell antigen receptor in mouse splenic CD4$^+$ T cells by activating the mTORC1–4E-BP–eIF4E axis; this leads to more translation of IL-4 mRNA through an as-yet-unknown mechanism. Thus, ICOS-dependent translational control is positioned to be a major determinant of the targeted delivery of IL-4 to cognate B cells during collaborations between T cells and B cells in the germinal center.

In addition to the aforementioned findings, many studies have linked mTOR signaling to the differentiation of various cell types in both the innate immune system and the adaptive immune system[73]. Collectively, these results indicate that mTOR- and eIF4E-dependent translational mechanisms have a major role in interferon responses and T cell biology.

Translational control via the MNK-eIF4E axis

A second set of genes affecting diverse immune functions are regulated at the level of translation via the mitogen-activated protein kinase (MAPK)-MNK1-MNK2-eIF4E pathway, including those encoding the transcription factor IRF8 (ref. 74), the transcription factor NF-κB inhibitor IκBα[75] and the chemokine CCL5 (RANTES)[76].

The polarization of macrophages into cells with specific functional characteristics is controlled by transcription factors[77,78]. The transcriptional regulator RBP-J is the principal nuclear mediator of signaling via receptors of the Notch family. RBP-J affects the innate immune response to *Listeria monocytogenes* by increasing Toll-like receptor 4 (TLR4)-induced expression of key mediators of the M1 macrophage phenotype[74], which is dependent on IRF8. RBP-J promotes kinase IRAK2–dependent signaling via TLR4 to MNK1, which induces the phosphorylation of eIF4E and leads to increased translation of IRF8 (**Fig. 2c**).

In vitro studies mainly of MEFs, cancer cell lines and primary human fibroblasts have shown that depending on the virus, phosphorylation of eIF4E can be upregulated[79–81] or downregulated[82–84]. A study has provided mechanistic insights into the role of the phosphorylation of eIF4E in the native immune response to viral infection. MEFs isolated from mice engineered to express a mutant form of eIF4E that cannot be phosphorylated have lower translation of IκBα mRNA than do wild-type MEFs[75]. IκBα binds and sequesters NF-κB in the cytoplasm, which leads to inhibition of the transcriptional activity of NF-κB[85]. Lower expression of IκBα in MEFs expressing that mutant eIF4E correlates with augmented transcriptional activity of NF-κB, which promotes the production of interferon-β (IFN-β). Accordingly, mice expressing that mutant eIF4E exhibit more resistance to viral infection (for example, infection with vesicular stomatitis virus) than do their wild-type littermates[75].

Upstream activators of MNK1 and MNK2 include the MAPKs Erk1, Erk2 and p38 (ref. 48), which are involved in the translational control of chemokine expression. In T cells, translation of mRNA encoding the transcription factor RFLAT-1 is stimulated by Erk1, Erk2 and p38 through a mechanism that depends on MNK1 and the 5′ UTR of RFLAT-1 mRNA[76]. Upregulation of RFLAT-1 expression induces transcription of the gene encoding CCL5 (RANTES) in activated T cells. This mechanism is thought to enable memory T cells to rapidly alter their abundance of CCL5 (RANTES) in response to various proinflammatory factors in their local microenvironment. Collectively, these examples illustrate the important role the MNK1-MNK2-eIF4E axis serves in immunological functions by controlling the activity of key transcription factors.

Translational control via mechanisms not directly linked to eIF4E

In addition to modulating translation by directly or indirectly affecting eIF4E activity, the translational regulation of immunological function also occurs via a variety of other mechanisms that often involve RBPs (**Table 1**). One such example is the GAIT translational repressor complex, which in humans includes the aminoacyl-tRNA synthetase EPRS, the RBP NSAP1, the ribosomal protein L13a and glycolysis enzyme GAPDH. Ceruloplasmin, an acute-phase plasma protein produced and secreted by hepatocytes and activated monocytes or macrophages, serves a critical role in iron metabolism[86] and has antioxidant and bactericidal activity[87]. Ceruloplasmin is induced by IFN-γ, but the synthesis of ceruloplasmin protein is disrupted 16 hours after activation despite the presence of abundant ceruloplasmin mRNA[88]. This is caused by translational suppression mediated by binding of the GAIT complex to a GAIT element in the 3′ UTR of ceruloplasmin mRNA[88,89]. In addition to ceruloplasmin, various chemokines and their receptors are targets of the GAIT complex[90].

Table 1 RBPs involved in the translational control of immunological function

RBP	Biological function	Targets
GAIT complex	Resolution of the response to IFN-γ in monocytes and macrophages. Expression of genes encoding inflammatory molecules is suppressed at the level of translation by the GAIT complex 12–16 h following stimulation with IFN-γ. Targets of GAIT include those encoding molecules that activate GAIT (DAPKZ and ZIPK), which creates a negative feedback loop that possibly allows cells to reset their response to IFN-γ.	CP, VEGFA, CCL22, CCR3, CCR4, CCR6, DAPKZ, ZIPK
TIA-1	Antigen-specific restimulation of primed naive T cells leads to activation of the translation of mRNAs that are transcribed but translationally suppressed by TIA-1 following priming.	IL-4
TIA-1 and HuR	Suppressed translation of key genes in macrophages is mediated by TIA-1 and HuR.	TNF, COX-2
GAPDH	GAPDH may link T cell 'energetics' to function by controlling the translation of IFN-γ.	IFN-γ

CP, ceruloplasmin; VEGF, proangiogenic cytokine; CCL22, chemokine; CCR3, CCR4, CCR6, chemokine receptors; DAPKZ and ZIPK, kinases.

© 2014 Nature America, Inc. All rights reserved.

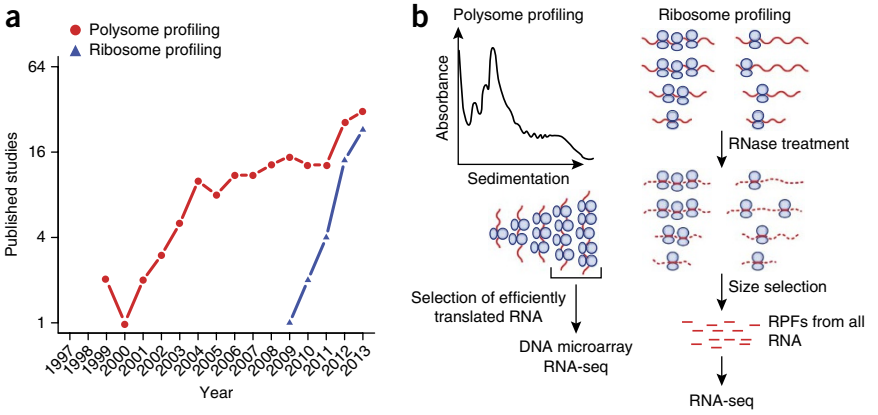

Figure 3 Polysome- and ribosome-profiling techniques are used to measure the translatome. (**a**) Polysome- and ribosome-profiling studies are equally common at present; here, the number of studies per year is based on manual literature searches. (**b**) During polysome profiling (left), mRNAs are stratified on the basis of the number of bound ribosomes, which corresponds to their translational efficiency. A pool of efficiently translated mRNA is isolated and analyzed by DNA microarray or RNA sequencing (RNA-seq). Ribosome profiling (right) involves the isolation of all RPFs independently of whether the mRNA is efficiently translated or not. Such RPFs are selected by size and quantified by RNA sequencing.

Furthermore, the kinases DAPK and ZIPK, which mediate IFN-γ-induced activation of GAIT, also have GAIT elements in the 3′ UTRs of their mRNAs, and a negative feedback mechanism is thus induced that leads to suppression of the translation of DAPK mRNA and ZIPK mRNA[91]. Hence, translational control has an important role in the resolution of inflammation.

In contrast, after naive T cells are primed, the transcription of genes encoding effector T cell cytokines is rapidly induced, but an increase in the abundance of the corresponding proteins is detected only after antigen-specific restimulation. Priming induces a global shutdown of translation via phosphorylation of eIF-2α[20]; this leads to the accumulation of mRNA in stress granules, which can be stained with an antibody to the translational repressor TIA-1 (ref. 92). TIA-1 is an RBP that modulates translation by binding to AU-rich elements (AREs) in 3′ UTRs of its target mRNAs and represents a central component of stress granules. eIF2α is a subunit of eIF2 that forms the ternary eIF2–methionyl initiator tRNA–GTP complex that delivers methionyl initiator tRNA to the ribosome[20]. After being delivered, eIF2-bound GTP is hydrolyzed and eIF2B stimulates the exchange of GDP bound to eIF2 for GTP. This allows formation of the ternary complex and another round of initiation. In response to a variety of stimuli, including viral infection, deprivation of amino acids and endoplasmic reticulum stress, eIF2 kinases (PErk, GCN2, PKR and HRI) phosphorylate eIF2α, which stabilizes eIF2-GDP-eIF2B complex and abrogates the guanine nucleotide–exchange activity of eIF2B[93]. During antigen-specific restimulation, the phosphorylation of eIF2α is diminished, which leads to the disassembly of stress granules and the translational activation of a subset of mRNAs, including IL-4 mRNA[92]. Similarly, blockade of cytokine production in self-reactive anergic T cells is regulated via translation-dependent mechanisms. In this case, the suppression of translation and the destabilization of mRNA are mediated by AREs in the 3′ UTRs of cytokine-encoding mRNAs[94]. Translational control also has a central role in signaling via tumor-necrosis factor (TNF) in innate immunity. The production of TNF in macrophages is suppressed at the level of translation by a multitude of RBPs. Macrophages from TIA-1-deficient mice produce more TNF than do wild-type macrophages, and this correlates with the translational activation of TNF mRNA[95]. Subsequent studies have shown that the RBPs HuR[96] and SRC3 (ref. 97) act together with TIA-1 to suppress the synthesis of TNF protein. Likewise, the inflammatory mediator COX-2 is translationally regulated by TIA-1 (ref. 98) and, as with the translational control of TNF, HuR can act together with TIA-1 in the suppression of COX-2 translation[96]. Thus, the expression of a plethora of cytokines is regulated at the translational level.

Cytokines not only are targets of translational control but also can act as upstream regulators of translation. It has been shown that IL-1 and IL-17 modulate inflammatory responses by engaging the translation of mRNAs encoding key inflammatory regulators. In HeLa human cervical cancer cells, IL-1 and IL-17 induce the translation of overlapping subsets of mRNAs, including MCPIP1 mRNA and IκBζ mRNA[99]. MCPIP1 is a negative feedback regulator of inflammation, and IκBζ is an atypical member of the IκB family that modulates the NF-κB response and is required for the expression of a subset of downstream genes[100]. Such translational regulation is associated with RNA elements in the 3′ and 5′ UTRs. MCPIP1 and IκBζ also seem to be regulated by TLR4 signaling in RAW264.7 mouse macrophages and human peripheral blood mononuclear cells, as stimulation of these cells with lipopolysaccharide is associated with redistribution of MCPIP1 mRNA and IκBζ mRNA to polysome fractions[99]. Together these findings indicate the existence of regulatory loops whereby translational mechanisms modulate the expression of cytokines, which in turn can induce changes in the translatome.

Translational control has also been suggested as a mechanism linking the 'energetics' of T cells and their function. Aerobic glycolysis seems to be required for the engagement of effector functions in T cells, as the ability of costimulated and growth factor–activated T cells to secrete IFN-γ is abrogated when glycolysis is impaired[101]. Under the conditions used, IFN-γ protein synthesis seemed to be limited by binding of GAPDH to AREs in the 3′ UTR of IFN-γ mRNA.

Techniques and analysis approaches for studying translatomes

As described above, there is ample evidence that translational control has a pivotal role in the immune system. Techniques that enable unbiased characterization of the translatome therefore are probably required for more complete understanding of the gene-expression networks in cells of both the innate immune system and the adaptive immune system. The efficiency with which translation is initiated is measured by the number of ribosomes that a given mRNA molecule binds[102]. The most commonly used techniques to estimate translational efficiency on a genome-wide scale are the polysome- and ribosome-profiling techniques. These assays are used at a roughly equal frequency, as judged by the number of published data sets based on each (**Fig. 3a**). For the preparation of polysomes, cytosolic extracts are sedimented in a sucrose gradient, which allows separation of mRNAs on the basis of the number of ribosomes they bind. This allows the isolation of a pool of efficiently translated mRNAs (**Fig. 3b**)—for example, those associated with more than three ribosomes, commonly referred to as 'heavy polysome–associated' RNA. The composition of these mRNAs is determined by DNA microarray[103] or RNA sequencing[104], and the resulting data are used for analysis of translatomes. An additional ribosome-profiling technique entails the isolation of RNA sequences protected by ribosomes from RNase-mediated

© 2014 Nature America, Inc. All rights reserved.

degradation (ribosome-protected fragments (RPFs))[105]. RPFs are then analyzed by RNA sequencing[106], although microarrays have also been used[107], for quantification (**Fig. 3b**). There is a distinction between the techniques, in that the polysome-profiling technique allows direct isolation of mRNAs associated with heavy polysomes, while ribosome profiling reports the number of RPFs, including those originating from monosomes and light polysomes that are not efficiently translated[102]. This property may render ribosome profiling less efficient in identifying differences in translation that parallel changes in protein expression, but no head-to-head comparison of these methods has been done so far.

Changes in transcription or post-transcriptional mechanisms that are upstream of translation will affect the polysome-associated RNA or RPFs by altering the steady-state abundance of mRNA[108]. Therefore, when identifying differences between two conditions in the context of translation, it is essential to correct differences in the amount of polysome-associated RNA or RPFs[108] for differences in the steady-state amount of mRNA. To allow such correction, data on cytoplasmic or whole-cell amounts of RNA are obtained in parallel[7]. The methods used for adjustment of the resulting data have a substantial effect on which genes are identified as being translated differently[108,109], and the conclusions drawn from downstream analysis also depend on the approaches used[110,111]. In the past, translational-efficiency scores calculated as the $\log_2$ ratio of the amount of polysome-associated RNA or RPFs to the amount of cytosolic RNA were used to determine translational activity of a given mRNA and then were used for comparison of translation in various conditions. However, that method is theoretically problematic and can potentially lead to the identification of large numbers of false-positive results and false-negative results[108]. The 'analysis of translational activity' (Anota) method[112] was developed to overcomes such issues[108]. The importance of these improvements is illustrated by a study in which analyses of differences in translation calculated by translational-efficiency scores or Anota were compared with changes in the proteome[113]. While changes in translation calculated according to Anota correlated well with changes in protein amounts, analysis based on translational-efficiency scores showed no such correlation[113]. With a few exceptions[71,114,115], differences in translation have been analyzed by variations in translational-efficiency scores, and reanalysis may therefore provide a different perspective on studies already published. For analysis of differences in translation calculated by Anota, at least three replicates are needed when two conditions are compared, but if more than three conditions are analyzed, two replicates per condition are sufficient[71]. Another method has been developed specifically for the analysis of data obtained by ribosome profiling (the 'RPF-based' method)[109]. It was concluded that translational-efficiency scores cannot be used for statistical analysis of differences in translation, and three replicates were identified as the sample size that results in improved statistical power[109]. The performance of this method in terms of mirroring changes in the proteome has not been evaluated, although it has shown overall good performance in a study of the cell cycle[116]. A few additional methods that allow visualization of translatome data are also useful tools in the quest for understanding to what extent differences in translation contribute to the gene-expression repertoire[117] or where RPF sequences are located[118]. Through the use of these approaches, the analysis of translatomes is a powerful tool with which to identify genes under translational control.

Mechanistic insights into translation by ribosome profiling

A unique property of ribosome profiling is that after the alignment of RPFs to the genome, information about the precise positions at which ribosomes are associated with the mRNA molecule is obtained. This property has been used for detailed mechanistic studies of the initiation, elongation and termination of translation. For example, in combination with agents such as lactimidomycin, which results in enrichment for ribosomes at the translation-initiation site[119], ribosome profiling has been used for the identification of translation start sites[120]. A similar application of ribosome profiling to embryonic stem cells has surprisingly demonstrated ribosomes bound to non-coding RNAs[121], which suggests that these RNAs are translated into short peptides. That finding was reassessed when it was concluded that RPF RNA sequencing–derived 'reads' alone are not sufficient to determine if an RNA is translated or not[122]. Moreover, data obtained with cells treated with harringtonin[123] (which, like lactimidomycin but with less specificity[120], results in enrichment for RPFs at translation-initiation sites) were insufficient for the prediction of whether an RNA was translated or not. It is possible that other properties, such as RNA structures or RBPs, also generate RPFs[122]. These issues need to be taken into account when ribosome-profiling data are used to study locations of ribosomes on mRNA.

Insights from genome-wide studies of translation

Genome-wide techniques for studying translation have been used extensively in the mTOR field. Initial studies documented that constitutive[52,124] or induced[125] overexpression of eIF4E affects gene expression mainly at the level of translation and identified genes encoding proteins that underlie phenotypes associated with hyperactivation of eIF4E. Other studies have evaluated the effect of upstream components of the mTOR pathway[55,126,127] or have used inhibitors of mTOR[53,54,114] and have found that the mTOR pathway affects mainly the translation of specific mRNAs largely dependent on the 4E-BPs. These findings are consistent with the examples noted above indicating that the mTORC1–4E-BP–eIF4E axis is a major regulator of translation in cells of the immune system. The utility of genome-wide profiling of translation in elucidating immune responses is illustrated by the fact that the studies described above used such approaches for the identification of IRF7 (ref. 51), the measurement of effects of OASL1 on the translatome[70], the identification of IκBα[65,75], the identification of targets of GAIT[90], the identification of differences in the translatomes of primed versus restimulated naive T cells[92] and the identification of mRNAs whose translation is affected by IL-17 (ref. 99). Additional early studies of model systems relevant to immunology included studies of the effects of stimulating T lymphocytes with antigen[128] and treating Jurkat T cells with rapamycin[129], whereas subsequent studies have evaluated the effect of lipopolysaccharide on dendritic cells[130] or J774.1 mouse macrophage-like cells[131]. Polysome profiling has further been used to study the translatomes of Foxp3$^-$ and Foxp3$^+$ CD4$^+$ T cell subsets and has identified translational signatures distinct from the transcriptomes of these cells[71]. This has allowed the identification of eIF4E mRNA as having increased translation upon the activation of Foxp3$^-$ CD4$^+$ T cells. In turn, eIF4E activates the translation of a subset of gene products necessary for T cell proliferation[71]. Thus, assessing the translatome has been a key tool for the discovery or characterization of several of the mechanisms that mediate the translational control of immune responses.

Conclusions

Technical advances have made it possible to study the relative contributions of transcriptional, post-transcriptional and post-translational gene-expression mechanisms to steady-state protein amounts and their dynamic regulation. From such studies, translational control of gene expression has emerged as a principal mechanism. This is

© 2014 Nature America, Inc. All rights reserved.

manifested in the regulation of immune responses. Studies indicate that translational control has a key role in a range of potentially host-detrimental immunological properties, including the expression of key transcription factors and cytokines, the termination of immune responses and differentiation into distinct cell lineages. The discovery of additional such mechanisms will be facilitated by the application of state-of-the-art approaches for measuring and analyzing the translatome. Several fields in particular may benefit from such studies. For example, it is striking that disease-focused studies of translational control of the immune system are largely lacking. Indeed, translational control may have a yet-to-be-discovered role in autoimmune disease and/or chronic inflammation. Moreover, transcriptomes induced by cytokines with distinct functions largely overlap[132], which raises the possibility that differences in responses at the level of translation may guide distinct downstream immunological functions. Finally, delineating the role of translational control in the context of the differentiation of cells of the immune system is imperative, given the importance of the mTORC1 pathway in such processes. In conclusion, understanding of the full repertoire of immunological functions that are controlled at the level of translation is still nascent, and many exciting discoveries lie ahead.

ACKNOWLEDGMENTS

Supported by the Canadian Institutes for Health Research (I.T., and MOP 67211 to C.A.P.), the Canada Research Chair program (C.A.P.), the Swedish Research Council (O.L.), the Swedish Cancer Society (O.L.) and the Wallenberg Academy Fellows program (O.L.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Bava, F.A. *et al.* CPEB1 coordinates alternative 3′-UTR formation with translational regulation. *Nature* **495**, 121–125 (2013).
2. Rousseau, D., Kaspar, R., Rosenwald, I., Gehrke, L. & Sonenberg, N. Translation initiation of ornithine decarboxylase and nucleocytoplasmic transport of cyclin D1 mRNA are increased in cells overexpressing eukaryotic initiation factor 4E. *Proc. Natl. Acad. Sci. USA* **93**, 1065–1070 (1996).
3. Lindstein, T., June, C.H., Ledbetter, J.A., Stella, G. & Thompson, C.B. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science* **244**, 339–343 (1989).
4. Schena, M. *et al.* Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Natl. Acad. Sci. USA* **93**, 10614–10619 (1996).
5. Schena, M., Shalon, D., Davis, R.W. & Brown, P.O. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467–470 (1995).
6. Lockhart, D.J. *et al.* Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* **14**, 1675–1680 (1996).
7. Larsson, O., Tian, B. & Sonenberg, N. Toward a genome-wide landscape of translational control. *Cold Spring Harb. Perspect. Biol.* **5**, a012302 (2013).
8. Vogel, C. & Marcotte, E.M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **13**, 227–232 (2012).
9. Schwanhäusser, B. *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337–342 (2011).
10. Kristensen, A.R., Gsponer, J. & Foster, L.J. Protein synthesis rate is the predominant regulator of protein expression during differentiation. *Mol. Syst. Biol.* doi:10.1038/msb.2013.47 (17 September 2013).
This study identifies translational control as the principal mechanism among post-transcriptional and post-translational mechanisms for the dynamic regulation of gene expression.
11. Spirin, A.S. The second Sir Hans Krebs lecture. Informosomes. *Eur. J. Biochem.* **10**, 20–35 (1969).
12. Keene, J.D. & Tenenbaum, S.A. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol. Cell* **9**, 1161–1167 (2002).
This study introduces the present conceptual model for the regulation of gene expression at the post-transcriptional level.
13. Candeias, M.M. *et al.* P53 mRNA controls p53 activity by managing Mdm2 functions. *Nat. Cell Biol.* **10**, 1098–1105 (2008).
14. Hershey, J.W., Sonenberg, N. & Mathews, M.B. Principles of translational control: an overview. *Cold Spring Harb. Perspect. Biol.* **4**, a011528 (2012).
15. Shalgi, R. *et al.* Widespread regulation of translation by elongation pausing in heat shock. *Mol. Cell* **49**, 439–452 (2013).
16. Liu, B., Han, Y. & Qian, S.B. Cotranslational response to proteotoxic stress by elongation pausing of ribosomes. *Mol. Cell* **49**, 453–463 (2013).
17. Gerashchenko, M.V., Lobanov, A.V. & Gladyshev, V.N. Genome-wide ribosome profiling reveals complex translational regulation in response to oxidative stress. *Proc. Natl. Acad. Sci. USA* **109**, 17394–17399 (2012).
18. Leprivier, G. *et al.* The eEF2 kinase confers resistance to nutrient deprivation by blocking translation elongation. *Cell* **153**, 1064–1079 (2013).
19. Sonenberg, N. & Hinnebusch, A.G. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* **136**, 731–745 (2009).
20. Jackson, R.J., Hellen, C.U. & Pestova, T.V. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat. Rev. Mol. Cell Biol.* **11**, 113–127 (2010).
21. Baltz, A.G. *et al.* The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. *Mol. Cell* **46**, 674–690 (2012).
22. Castello, A. *et al.* Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* **149**, 1393–1406 (2012).
23. Sabatini, D.M., Erdjument-Bromage, H., Lui, M., Tempst, P. & Snyder, S.H. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* **78**, 35–43 (1994).
24. Brown, E.J. *et al.* A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* **369**, 756–758 (1994).
25. Gwinn, D.M. *et al.* AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* **30**, 214–226 (2008).
26. Inoki, K., Zhu, T. & Guan, K.L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**, 577–590 (2003).
27. Corradetti, M.N., Inoki, K., Bardeesy, N., DePinho, R.A. & Guan, K.L. Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes Dev.* **18**, 1533–1538 (2004).
28. Shaw, R.J. *et al.* The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6**, 91–99 (2004).
29. Hara, K. *et al.* Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J. Biol. Chem.* **273**, 14484–14494 (1998).
30. Wang, X., Campbell, L.E., Miller, C.M. & Proud, C.G. Amino acid availability regulates p70 S6 kinase and multiple translation factors. *Biochem. J.* **334**, 261–267 (1998).
31. DeYoung, M.P., Horak, P., Sofer, A., Sgroi, D. & Ellisen, L.W. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14–3-3 shuttling. *Genes Dev.* **22**, 239–251 (2008).
32. Brugarolas, J. *et al.* Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev.* **18**, 2893–2904 (2004).
33. Laplante, M. & Sabatini, D.M. mTOR signaling in growth control and disease. *Cell* **149**, 274–293 (2012).
34. Kim, D.H. *et al.* mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110**, 163–175 (2002).
35. Hara, K. *et al.* Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110**, 177–189 (2002).
36. Jacinto, E. *et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* **6**, 1122–1128 (2004).
37. Sarbassov, D.D. *et al.* Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol.* **14**, 1296–1302 (2004).
38. Sarbassov, D.D. *et al.* Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol. Cell* **22**, 159–168 (2006).
39. Heitman, J., Movva, N.R. & Hall, M.N. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253**, 905–909 (1991).
40. Gingras, A.C. *et al.* Regulation of 4E–BP1 phosphorylation: a novel two-step mechanism. *Genes Dev.* **13**, 1422–1437 (1999).
41. Brunn, G.J. *et al.* Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J.* **15**, 5256–5267 (1996).
42. Burnett, P.E., Barrow, R.K., Cohen, N.A., Snyder, S.H. & Sabatini, D.M. RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E–BP1. *Proc. Natl. Acad. Sci. USA* **95**, 1432–1437 (1998).
43. Hara, K. *et al.* Regulation of eIF-4E BP1 phosphorylation by mTOR. *J. Biol. Chem.* **272**, 26457–26463 (1997).
44. Wullschleger, S., Loewith, R. & Hall, M.N. TOR signaling in growth and metabolism. *Cell* **124**, 471–484 (2006).
45. Dorrello, N.V. *et al.* S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science* **314**, 467–471 (2006).
46. Raught, B. *et al.* Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. *EMBO J.* **23**, 1761–1769 (2004).
47. Pause, A. *et al.* Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. *Nature* **371**, 762–767 (1994).
48. Roux, P.P. & Topisirovic, I. Regulation of mRNA translation by signaling pathways. *Cold Spring Harb. Perspect. Biol.* **4**, a012252 (2012).
49. Gingras, A.C. *et al.* Hierarchical phosphorylation of the translation inhibitor 4E–BP1. *Genes Dev.* **15**, 2852–2864 (2001).

© 2014 Nature America, Inc. All rights reserved.

50. Zimmer, S.G., DeBenedetti, A. & Graff, J.R. Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor formation, growth, invasion and metastasis. *Anticancer Res.* **20**, 3A, 1343–1351 (2000).
51. Colina, R. *et al.* Translational control of the innate immune response through IRF-7. *Nature* **452**, 323–328 (2008).
This study identifies mTORC1–4E-BP–dependent translational control of IRF7, which affects the production of type 1 interferon and susceptibility to infection with vesicular stomatitis virus.
52. Larsson, O. *et al.* Apoptosis resistance downstream of eIF4E: posttranscriptional activation of an anti-apoptotic transcript carrying a consensus hairpin structure. *Nucleic Acids Res.* **34**, 4375–4386 (2006).
53. Hsieh, A.C. *et al.* The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* **485**, 55–61 (2012).
54. Thoreen, C.C. *et al.* A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* **485**, 109–113 (2012).
55. Bilanges, B. *et al.* Tuberous sclerosis complex proteins 1 and 2 control serum-dependent translation in a TOP-dependent and -independent manner. *Mol. Cell. Biol.* **27**, 5746–5764 (2007).
56. Patursky-Polischuk, I. *et al.* The TSC-mTOR pathway mediates translational activation of TOP mRNAs by insulin largely in a raptor- or rictor-independent manner. *Mol. Cell. Biol.* **29**, 640–649 (2009).
57. Avni, D., Shama, S., Loreni, F. & Meyuhas, O. Vertebrate mRNAs with a 5′-terminal pyrimidine tract are candidates for translational repression in quiescent cells: characterization of the translational cis-regulatory element. *Mol. Cell. Biol.* **14**, 3822–3833 (1994).
58. Miloslavski, R. *et al.* Oxygen sufficiency controls TOP mRNA translation via the TSC-Rheb-mTOR pathway in a 4E-BP-independent manner. *J. Mol. Cell Biol.* doi:10.1093/jmcb/mju008 (13 March 2014).
59. Shama, S., Avni, D., Frederickson, R.M., Sonenberg, N. & Meyuhas, O. Overexpression of initiation factor eIF-4E does not relieve the translational repression of ribosomal protein mRNAs in quiescent cells. *Gene Expr.* **4**, 241–252 (1995).
60. Damgaard, C.K. & Lykke-Andersen, J. Translational coregulation of 5′TOP mRNAs by TIA-1 and TIAR. *Genes Dev.* **25**, 2057–2068 (2011).
61. Tcherkezian, J. *et al.* Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5′TOP mRNA translation. *Genes Dev.* **28**, 357–371 (2014).
62. Flynn, A. & Proud, C.G. Serine 209, not serine 53, is the major site of phosphorylation in initiation factor eIF-4E in serum-treated Chinese hamster ovary cells. *J. Biol. Chem.* **270**, 21684–21688 (1995).
63. Fukunaga, R. & Hunter, T. MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. *EMBO J.* **16**, 1921–1933 (1997).
64. Waskiewicz, A.J., Flynn, A., Proud, C.G. & Cooper, J.A. Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J.* **16**, 1909–1920 (1997).
65. Furic, L. *et al.* eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proc. Natl. Acad. Sci. USA* **107**, 14134–14139 (2010).
66. Fernandez, P.C. *et al.* Genomic targets of the human c-Myc protein. *Genes Dev.* **17**, 1115–1129 (2003).
67. Topisirovic, I. *et al.* Stability of eukaryotic translation initiation factor 4E mRNA is regulated by HuR, and this activity is dysregulated in cancer. *Mol. Cell. Biol.* **29**, 1152–1162 (2009).
68. Cook, K.D. & Miller, J. TCR-dependent translational control of GATA-3 enhances Th2 differentiation. *J. Immunol.* **185**, 3209–3216 (2010).
69. Gigoux, M. *et al.* Inducible costimulator facilitates T-dependent B cell activation by augmenting IL-4 translation. *Mol. Immunol.* **59**, 46–54 (2014).
70. Lee, M.S., Kim, B., Oh, G.T. & Kim, Y.J. OASL1 inhibits translation of the type I interferon-regulating transcription factor IRF7. *Nat. Immunol.* **14**, 346–355 (2013).
This study identifies OASL1 as a key regulator of the translation of IRF7, which affects the expression of type 1 interferon. It proposes that OASL1 acts in a negative feedback loop by suppressing the translation of IRF7 mRNA.
71. Bjur, E. *et al.* Distinct translational control in CD4+ T cell subsets. *PLoS Genet.* **9**, e1003494 (2013).
This study shows that analysis of translatomes is feasible in primary cells of the immune system that are of low abundance and that such analysis provides a perspective distinct from the analysis of their transcriptomes. It also identifies eIF4E-dependent translational control as key for the proliferation of Foxp3^{-} and Foxp3^{+} CD4^{+} T cells.
72. Tangye, S.G., Ma, C.S., Brink, R. & Deenick, E.K. The good, the bad and the ugly - T_{FH} cells in human health and disease. *Nat. Rev. Immunol.* **13**, 412–426 (2013).
73. Araki, K., Ellebedy, A.H. & Ahmed, R. TOR in the immune system. *Curr. Opin. Cell Biol.* **23**, 707–715 (2011).
74. Xu, H. *et al.* Notch-RBP-J signaling regulates the transcription factor IRF8 to promote inflammatory macrophage polarization. *Nat. Immunol.* **13**, 642–650 (2012).
This study shows that phosphorylation of eIF4E is key in the translational activation of IRF8 mRNA and downstream macrophage polarization.
75. Herdy, B. *et al.* Translational control of the activation of transcription factor NF-κB and production of type I interferon by phosphorylation of the translation factor eIF4E. *Nat. Immunol.* **13**, 543–550 (2012).
76. Nikolcheva, T. *et al.* A translational rheostat for RFLAT-1 regulates RANTES expression in T lymphocytes. *J. Clin. Invest.* **110**, 119–126 (2002).
77. Krausgruber, T. *et al.* IRF5 promotes inflammatory macrophage polarization and T_H1–T_H17 responses. *Nat. Immunol.* **12**, 231–238 (2011).
78. Satoh, T. *et al.* The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat. Immunol.* **11**, 936–944 (2010).
79. Huang, J.T. & Schneider, R.J. Adenovirus inhibition of cellular protein synthesis involves inactivation of cap-binding protein. *Cell* **65**, 271–280 (1991).
80. Kleijn, M., Vrins, C.L., Voorma, H.O. & Thomas, A.A. Phosphorylation state of the cap-binding protein eIF4E during viral infection. *Virology* **217**, 486–494 (1996).
81. Connor, J.H. & Lyles, D.S. Vesicular stomatitis virus infection alters the eIF4F translation initiation complex and causes dephosphorylation of the eIF4E binding protein 4E-BP1. *J. Virol.* **76**, 10177–10187 (2002).
82. Walsh, D. *et al.* Eukaryotic translation initiation factor 4F architectural alterations accompany translation initiation factor redistribution in poxvirus-infected cells. *Mol. Cell. Biol.* **28**, 2648–2658 (2008).
83. Walsh, D., Perez, C., Notary, J. & Mohr, I. Regulation of the translation initiation factor eIF4F by multiple mechanisms in human cytomegalovirus-infected cells. *J. Virol.* **79**, 8057–8064 (2005).
84. Walsh, D. & Mohr, I. Phosphorylation of eIF4E by Mnk-1 enhances HSV-1 translation and replication in quiescent cells. *Genes Dev.* **18**, 660–672 (2004).
85. Ben-Neriah, Y. & Karin, M. Inflammation meets cancer, with NF-κB as the matchmaker. *Nat. Immunol.* **12**, 715–723 (2011).
86. Vashchenko, G. & MacGillivray, R.T. Multi-copper oxidases and human iron metabolism. *Nutrients* **5**, 2289–2313 (2013).
87. Klebanoff, S.J. Bactericidal effect of Fe^{2+}, ceruloplasmin, and phosphate. *Arch. Biochem. Biophys.* **295**, 302–308 (1992).
88. Mazumder, B. & Fox, P.L. Delayed translational silencing of ceruloplasmin transcript in γ interferon-activated U937 monocytic cells: role of the 3′ untranslated region. *Mol. Cell. Biol.* **19**, 6898–6905 (1999).
89. Sampath, P., Mazumder, B., Seshadri, V. & Fox, P.L. Transcript-selective translational silencing by gamma interferon is directed by a novel structural element in the ceruloplasmin mRNA 3′ untranslated region. *Mol. Cell. Biol.* **23**, 1509–1519 (2003).
90. Vyas, K. *et al.* Genome-wide polysome profiling reveals an inflammation-responsive posttranscriptional operon in gamma interferon-activated monocytes. *Mol. Cell. Biol.* **29**, 458–470 (2009).
91. Mukhopadhyay, R. *et al.* DAPK-ZIPK-L13a axis constitutes a negative-feedback module regulating inflammatory gene expression. *Mol. Cell* **32**, 371–382 (2008).
These authors identify a negative feedback loop for GAIT complex activity. DAPK and ZIPK, which are activators of the GAIT complex, are themselves targets for suppressed translation via a GAIT-dependent mechanism.
92. Scheu, S. *et al.* Activation of the integrated stress response during T helper cell differentiation. *Nat. Immunol.* **7**, 644–651 (2006).
93. Wek, R.C., Jiang, H.Y. & Anthony, T.G. Coping with stress: eIF2 kinases and translational control. *Biochem. Soc. Trans.* **34**, 7–11 (2006).
94. Villarino, A.V. *et al.* Posttranscriptional silencing of effector cytokine mRNA underlies the anergic phenotype of self-reactive T cells. *Immunity* **34**, 50–60 (2011).
This study shows that translational control of cytokines is important for self reactive T-cell anergy.
95. Piecyk, M. *et al.* TIA-1 is a translational silencer that selectively regulates the expression of TNF-α. *EMBO J.* **19**, 4154–4163 (2000).
96. Katsanou, V. *et al.* HuR as a negative posttranscriptional modulator in inflammation. *Mol. Cell* **19**, 777–789 (2005).
97. Yu, C. *et al.* An essential function of the SRC-3 coactivator in suppression of cytokine mRNA translation and inflammatory response. *Mol. Cell* **25**, 765–778 (2007).
98. Dixon, D.A. *et al.* Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. *J. Exp. Med.* **198**, 475–481 (2003).
99. Dhamija, S. *et al.* Interleukin-17 (IL-17) and IL-1 activate translation of overlapping sets of mRNAs, including that of the negative regulator of inflammation, MCPIP1. *J. Biol. Chem.* **288**, 19250–19259 (2013).
100. Yamamoto, M. *et al.* Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IκBζ. *Nature* **430**, 218–222 (2004).
101. Chang, C.H. *et al.* Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251 (2013).
102. Warner, J.R., Knopf, P.M. & Rich, A. A multiple ribosomal structure in protein synthesis. *Proc. Natl. Acad. Sci. USA* **49**, 122–129 (1963).
103. Johannes, G., Carter, M.S., Eisen, M.B., Brown, P.O. & Sarnow, P. Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. *Proc. Natl. Acad. Sci. USA* **96**, 13118–13123 (1999).
104. Karginov, F.V. & Hannon, G.J. Remodeling of Ago2-mRNA interactions upon cellular stress reflects miRNA complementarity and correlates with altered translation rates. *Genes Dev.* **27**, 1624–1632 (2013).
105. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R. & Weissman, J.S. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* **324**, 218–223 (2009).
This study introduces the ribosome-profiling technique as a genome-wide tool with which to map the positions of ribosomes on mRNA.

© 2014 Nature America, Inc. All rights reserved.

106. Ingolia, N.T., Brar, G.A., Rouskin, S., McGeachy, A.M. & Weissman, J.S. The ribosome profiling strategy for monitoring translation *in vivo* by deep sequencing of ribosome-protected mRNA fragments. *Nat. Protoc.* **7**, 1534–1550 (2012).
107. Zoschke, R., Watkins, K.P. & Barkan, A. A rapid ribosome profiling method elucidates chloroplast ribosome behavior *in vivo*. *Plant Cell* **25**, 2265–2275 (2013).
108. Larsson, O., Sonenberg, N. & Nadon, R. Identification of differential translation in genome wide studies. *Proc. Natl. Acad. Sci. USA* **107**, 21487–21492 (2010).
109. Olshen, A.B. *et al.* Assessing gene-level translational control from ribosome profiling. *Bioinformatics* **29**, 2995–3002 (2013).
110. Larsson, O & Nadon, R. Re-analysis of genome wide data on mammalian microRNA-mediated suppression of gene expression. *Translation* **1**, 1–9 (2013).
111. Eliseeva, I.A., Vorontsov, I.E., Babeyev, K.E., Buyanova, S.M., Sysoeva, M.A. & Kondrashov, F.A. *et al.* In silico motif analysis suggests an interplay of transcriptional and translational control in mTOR response. *Translation* **1**, 1–7 (2013).
112. Larsson, O., Sonenberg, N. & Nadon, R. Anota: Analysis of differential translation in genome-wide studies. *Bioinformatics* **27**, 1440–1441 (2011).
113. Colman, H. *et al.* Genome-wide analysis of host mRNA translation during hepatitis C virus infection. *J. Virol.* **87**, 6668–6677 (2013).
This article shows that the analysis approach (Anota or translational-efficiency score) is critical for predicting whether differences in translation identified will correlate with changes in proteomes. Only Anota analysis corresponds to changes in protein amounts.
114. Larsson, O. *et al.* Distinct perturbation of the translatome by the antidiabetic drug metformin. *Proc. Natl. Acad. Sci. USA* **109**, 8977–8982 (2012).
115. Parker, M.W. *et al.* Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J. Clin. Invest.* **124**, 1622–1635 (2014).
116. Stumpf, C.R., Moreno, M.V., Olshen, A.B., Taylor, B.S. & Ruggero, D. The translational landscape of the mammalian cell cycle. *Mol. Cell* **52**, 574–582 (2013).
117. Tebaldi, T., Dassi, E., Kostoska, G., Viero, G. & Quattrone, A. tRanslatome: an R/Bioconductor package to portray translational control. *Bioinformatics* **30**, 289–291 (2014).
118. Michel, A.M. *et al.* GWIPS-viz: development of a ribo-seq genome browser. *Nucleic Acids Res.* **42**, D859–D864 (2014).
119. Schneider-Poetsch, T. *et al.* Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nat. Chem. Biol.* **6**, 209–217 (2010).
120. Lee, S. *et al.* Global mapping of translation initiation sites in mammalian cells at single-nucleotide resolution. *Proc. Natl. Acad. Sci. USA* **109**, E2424–E2432 (2012).
121. Ingolia, N.T., Lareau, L.F. & Weissman, J.S. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of Mammalian proteomes. *Cell* **147**, 789–802 (2011).
122. Guttman, M., Russell, P., Ingolia, N.T., Weissman, J.S. & Lander, E.S. Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. *Cell* **154**, 240–251 (2013).
123. Fresno, M., Jimenez, A. & Vazquez, D. Inhibition of translation in eukaryotic systems by harringtonine. *Eur. J. Biochem.* **72**, 323–330 (1977).
124. Larsson, O. *et al.* Eukaryotic translation initiation factor 4E induced progression of primary human mammary epithelial cells along the cancer pathway is associated with targeted translational deregulation of oncogenic drivers and inhibitors. *Cancer Res.* **67**, 6814–6824 (2007).
125. Mamane, Y. *et al.* Epigenetic activation of a subset of mRNAs by eIF4E explains its effects on cell proliferation. *PLoS ONE* **2**, e242 (2007).
126. Rajasekhar, V.K. *et al.* Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes. *Mol. Cell* **12**, 889–901 (2003).
127. Tominaga, Y., Tamguney, T., Kolesnichenko, M., Bilanges, B. & Stokoe, D. Translational deregulation in PDK-1–/– embryonic stem cells. *Mol. Cell. Biol.* **25**, 8465–8475 (2005).
128. Mikulits, W. *et al.* Isolation of translationally controlled mRNAs by differential screening. *FASEB J.* **14**, 1641–1652 (2000).
129. Grolleau, A. *et al.* Global and specific translational control by rapamycin in T cells uncovered by microarrays and proteomics. *J. Biol. Chem.* **277**, 22175–22184 (2002).
130. Ceppi, M. *et al.* Ribosomal protein mRNAs are translationally-regulated during human dendritic cells activation by LPS. *Immunome Res.* **5**, 5 (2009).
131. Kitamura, H. *et al.* Genome-wide identification and characterization of transcripts translationally regulated by bacterial lipopolysaccharide in macrophage-like J774.1 cells. *Physiol. Genomics* **33**, 121–132 (2008).
132. Ring, A.M. *et al.* Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. *Nat. Immunol.* **13**, 1187–1195 (2012).

© 2014 Nature America, Inc. All rights reserved.