Walking the wire
Post-transcriptional regulation of T cell effector functions in health and disease
Salerno, F.

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Chapter 1

General Introduction

Adapted from:

T cells require post-transcriptional regulation for accurate immune responses

Salerno and Wolkers, Biochem Soc Trans. 2015 Dec 1;43(6):1201-7
Introduction

Gene expression is one of the most fundamental processes of life. It comprises the transfer of information from DNA, via messenger RNA (mRNA), to proteins. This multi-step process is strongly influenced by cell-intrinsic signals and environmental cues, and dictates the functional and phenotypic identity of each cell of our body. The series of linked processes that allow a gene to be transcribed into mRNA, and an mRNA to be processed, translated into amino acid chains, and folded into functional proteins, has been mechanistically well characterized. However, the fundamental question how genomic information is accurately shaped into a specific cellular proteome still remains largely unanswered.

The difficulty of fully understanding which mechanisms ultimately control the flavor and the abundance of protein production is due to the highly complex regulatory network that keeps gene expression in check. Gene expression is regulated at multiple levels, i.e. the transcriptional, the post-transcriptional, and the post-translational level. The regulation at the transcriptional level includes epigenetic modifications, chromatin accessibility, docking of RNA polymerase and transcription factors, and the regulation of splicing and polyadenylation processes. The post-transcriptional level consists of regulatory proteins and nucleic acids that control the lifetime of mRNA molecules, their localization in subcellular compartments and the efficiency of their translation initiation and elongation. The post-translational level refers to protein modifications - such as phosphorylation, glycosylation, SUMOylation - that further define protein folding, stability and functionality.

The combined effect of these different regulatory events determines the final protein output, and thus cellular functionality. For example, cytotoxic CD8+ T lymphocytes (T cells) are critical to fight and clear viral infections, intracellular pathogens and malignantly transformed tumor cells. In addition, CD8+ T cells can develop immunological memory, and memory T cells persist in the body to confer protection against secondary insults. Both functions depend on the ample and rapid production of effector molecules, such as cytokines, chemokines and cytotoxic granules, which arm an effective T cell response. However, effector molecules represent a double-edged sword: whereas they are essential to kill infected cells and prevent pathogenic spread, they are also highly toxic, and the deregulation of their production can be a potential risk of pathogenesis. In fact, aberrant secretion of excessive amounts of pro-inflammatory cytokines by T cells greatly contribute to the pathogenesis of inflammation-driven diseases and autoimmune disorders like lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease and allergies. Thus, tight regulation of gene expression is a crucial hallmark of protective yet balanced immune responses.

Over the last decades, the transcriptional regulatory networks that control T cell differentiation and effector functions have been studied in great detail, but understanding the mechanisms of regulation at the mRNA level has been more challenging. Only in recent years we have started to fully appreciate the importance of post-transcriptional events, and in particular its role in controlling the rapid on/off-switch of cytokine production. The requirement of post-transcriptional regulation to fine-tune T cell effector functions is not surprising if we consider that many mRNAs encoding for effector molecules and regulatory proteins are very unstable, and that their production rates need to rapidly change according to environmental cues. Importantly, gene transcription and mRNA translation do not follow a linear correlation, and upon T cell receptor (TCR) stimulation only ~50% of the
variation in protein concentration can be explained by changes in mRNA abundance\textsuperscript{21}. This weak correlation between transcriptome and proteome further underlines the importance of post-transcriptional control for accurate immune responses.

**Post-transcriptional regulation of effector molecules**

Post-transcriptional events allow T cells to regulate the intensity and the timing of their immune response by modulating stability, subcellular localization and translation of specific mRNA transcripts. *Table 1* gives an overview of currently known cytotoxic T cell-related genes that are post-transcriptionally regulated in T cells or in other immune cell types. The fate of cytokine mRNA in T cells depends on their decoration with RNA binding proteins (RBPs) and/or antisense RNAs, such as micro-RNA (miRs) and long non-coding RNA (lncRNAs) on the 5'- and 3'-untranslated regions (UTRs), and possibly also on the coding region. The 3'UTR is a potent regulator of mRNA stability and translation\textsuperscript{22} and, as recently discovered, of membrane protein localization\textsuperscript{23}. Evolutionary studies have shown that its length is directly correlated with the expansion of post-transcriptional regulatory circuits and the morphological complexity of different metazoan species\textsuperscript{24}. In cytokine mRNAs, the length of the 3'UTR often exceeds that of the coding sequence\textsuperscript{7}, suggesting that it plays a critical role in determining the fate of mRNA. In addition, polymorphisms in the 3'UTR of immune related mRNAs, like tumor necrosis factor-α (TNF-α)\textsuperscript{25}, interleukin-6 (IL-6)\textsuperscript{26} and the TCR ζ chain\textsuperscript{27}, can induce autoimmunity by disrupting the balance between mRNA stability and decay.

3'UTR-dependent post-transcriptional events are determined by *cis*-regulatory elements that consist of nucleotide sequences, secondary structures, or a combination of the two. One of these sequences, Adenylate Uridylate (AU)-rich elements (AREs), are found in around 16% of human protein-coding genes\textsuperscript{28} including many genes coding for cytokines and other inflammatory proteins (Table 1). AREs are thus potent *cis*-acting determinants of mRNA turnover and translation, and are crucial regulator of T cell effector functions.

**Activation of naive CD8\(^+\) T cells**

Naive T cells are small quiescent cells that circulate through secondary lymphoid organs in search of cognate antigens presented in the context of appropriate major histocompatibility complex (MHC, also referred to as human leukocyte antigen (HLA)) molecules. It has been calculated that only a fraction of naive T cells recognize one specific epitope (about 1 to 10 cells per million CD4\(^+\) T cells and 1 to 100 cells per million CD8\(^+\) T cells)\textsuperscript{29,30,31}. However, when a naive CD8\(^+\) T cell encounters its cognate antigen, the engagement of the TCR triggers a signaling cascade that initiates a strong program of cell growth and clonal expansion, and that ultimately leads to differentiation into effector T cells. The antigen dose, as well as the affinity and duration of the interaction between naive T cells and matured antigen-presenting cells (APCs) determine the robustness of T cell activation\textsuperscript{32,33,34,35}. Co-stimulatory and adhesion molecules expressed on the surface of both T cells and APCs strengthen and stabilize this interaction, thereby further promoting T cell survival, proliferation and effector functions. Co-stimulation through cluster of differentiation 28 (CD28), CD27, or CD40\textsuperscript{36,37,38,39} is essential to fully activate naive T cells, whereas OX40 and 4-1BB sustain T cell responses at later stages\textsuperscript{40,41}. Conversely, lack of co-stimulatory signals can cause T
cell anergy or hyporesponsiveness to infections\(^2\). In addition to TCR triggering and costimulatory receptors, the inflammatory milieu that is generated upon infection contributes to clonal expansion and accurate differentiation into effective cytotoxic T cells\(^3\). When antigen-experienced CD8\(^+\) T cells leave the secondary lymphoid organs and reach the site of infection, they exert their antimicrobial functions by releasing high amounts of pro-inflammatory cytokines such as interferon gamma (IFN-\(\gamma\)) and TNF-\(\alpha\), and cytolytic effector molecules like perforin, granzymes A, B and K\(^4, 5\).

Naive CD8\(^+\) T cells usually reach their peak of clonal expansion within 5-8 days upon the initial antigen encounter\(^6\). Yet, they require several rounds of proliferation before they can produce large amounts of effector molecules\(^7, 8\). This discrepancy in time between optimal clonal expansion and optimal effector functions is at least in part due to differences in the

### Table 1:

<table>
<thead>
<tr>
<th>mRNA</th>
<th>cis-element within 5'UTR</th>
<th>cis-element within 3'UTR</th>
<th>trans-element</th>
<th>mechanism of control</th>
<th>references</th>
</tr>
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<tr>
<td>CCL3</td>
<td>n.d.</td>
<td>ARE</td>
<td>TTP</td>
<td>mRNA stability</td>
<td>(170)</td>
</tr>
<tr>
<td>CCL5 (Rantes)</td>
<td>n.d.</td>
<td>stem-loop structure</td>
<td>n.d.</td>
<td>mRNA stability, translational block</td>
<td>(113, 171)</td>
</tr>
<tr>
<td>CCL11 (Eotaxin)</td>
<td>n.d.</td>
<td>ARE</td>
<td>HuR</td>
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<td>CCL22</td>
<td>n.d.</td>
<td>GAIT</td>
<td>EPRS</td>
<td>translational block</td>
<td>(125)</td>
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<tr>
<td>COX-2</td>
<td>n.d.</td>
<td>ARE</td>
<td>TTP, HuR, TIAR-1, TIAR, CUGB2, miR-16</td>
<td>mRNA stability</td>
<td>(85, 120, 173-175)</td>
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<tr>
<td>CXCL10 (IP-10)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>mRNA stability</td>
<td>(176, 177)</td>
</tr>
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<td>Fas Ligand</td>
<td>n.d.</td>
<td>ARE, MRE</td>
<td>HuR, miR-21</td>
<td>mRNA stability</td>
<td>(178, 179)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>n.d.</td>
<td>ARE</td>
<td>TTP, AUF-1</td>
<td>mRNA stability, translational block</td>
<td>(68, 180-182)</td>
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<tr>
<td>Granzyme B</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>translational block</td>
<td>(183)</td>
</tr>
<tr>
<td>IFN-(\gamma)</td>
<td>pseudoknot structure</td>
<td>ARE, MRE</td>
<td>TTP, HuR, miR-29</td>
<td>mRNA stability, translational block</td>
<td>(56, 67, 81, 184)</td>
</tr>
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<td>IL-2</td>
<td>JNK response elements</td>
<td>ARE, MRE</td>
<td>Nucleolin, YB-1, TTP, NF-90, miR-181c</td>
<td>mRNA stability, translational block</td>
<td>(55, 66, 185-187)</td>
</tr>
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<td>IL-3</td>
<td>n.d.</td>
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<td>TTP</td>
<td>mRNA stability</td>
<td>(188, 189)</td>
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<td>IL-6</td>
<td>n.d.</td>
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<td>TTP, AUF-1</td>
<td>mRNA stability</td>
<td>(190, 191)</td>
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<td>IL-11</td>
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<td>ARE, GRE</td>
<td>CUBG1</td>
<td>mRNA stability</td>
<td>(192)</td>
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<td>IL-15</td>
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<td>n.d.</td>
<td>translational block</td>
<td>(193, 194)</td>
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<td>IL-18</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>mRNA stability</td>
<td>(195)</td>
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<td>Perforin</td>
<td>n.d.</td>
<td>MRE</td>
<td>miR-139</td>
<td>translational block</td>
<td>(83)</td>
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<td>TNF-(\alpha)</td>
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<td>ARE, CDE, MRE</td>
<td>TTP, HuR, TIAR-1, TIAR, Roquin, miR-16, miR-221</td>
<td>mRNA stability, translational block</td>
<td>(56, 68, 76, 84, 85, 120)</td>
</tr>
</tbody>
</table>

ARE: AU-rich elements; GAIT: IFN-\(\gamma\) activated inhibitor of translation; MRE: miR recognition elements; GRE: GU-rich elements; CDE: constitutive decay elements
regulation of specific gene expression. Whereas genes involved in cell division are rapidly produced due to their accessible epigenetic signature, genes encoding cytokines are highly methylated at lysine 27 (H3K27m3) and their heterochromatin state acts as a brake on gene transcription. To promote high levels of mRNA transcription upon TCR triggering, loss of the repressive H3K27m3 modification at the proximal promoter of cytokine encoding genes is not sufficient, in addition a distinct set of histone modifications (such as the acquisition of the permissive H3K4m3 modification) needs to occur. The only exception is represented by the production of TNF-α that rapidly occurs upon TCR triggering of naive T cells, in part due to an overall permissive epigenetic signature of its promoter.

**Post-transcriptional events promote T cell effector functions**

Many cytokine and chemokine encoding transcripts are intrinsically unstable. However, T cell triggering through the TCR together with co-stimulatory signals leads to rapid mRNA stabilization (Figure 1-left). For instance, mRNA of the key cytokines IL-2, IFN-γ and TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) are stabilized when the co-stimulatory molecules CD28 and lymphocyte function-associated antigen 1 (LFA-1) are engaged. Interestingly, stabilization of these transcripts is primarily driven by the recognition of AREs within the 3'UTR by RBPs such as nuclear factor (NF)-90 (for IL-2) and human antigen R (HuR) (for TNF-α and IFN-γ). Both RBPs employ a similar mode of action: post-translational modification upon T cell activation of NF-90 and HuR leads to translocation from the nucleus to the cytoplasm where they bind to ARE-containing transcripts. How ARE-binding proteins (ARE-BPs) stabilize these intrinsically unstable mRNAs in T cells is still not fully understood. Studies on lipopolysaccharide (LPS)-stimulated macrophages describe a phosphorylation-regulated exchange of mRNA decay-inducing tristetraprolin (TTP) with the stabilizing ARE-BP HuR, thereby promoting translation. Alternatively, studies in HEK293T cells propose that HuR redirects the mRNA to polysomes for active translation by oligomerizing along the RNA and cause the dissociation of miRs from the target mRNA (Figure 1-left).

Non-coding RNAs also play a critical role in the regulation of effector function. For instance, a global repression of miRs is observed in effector T cells compared to naive T cells. In addition, several transcripts employ alternative polyadenylation sites upon T cell activation to generate shorter 3'UTRs, which results in the loss of specific miR binding sites and hence protects target mRNAs from degradation. Furthermore, genome-wide analysis showed that CD8+ T cells express hundreds of IncRNAs upon antigen recognition. While their specific function has not been identified yet, IncRNAs could possibly support T cell function by acting as a “sponge” for miRs, or by promoting degradation of miRs with complementary sequences (Figure 1-left).

In summary, a powerful inflammatory response requires the stabilization of cytokine mRNA, and necessitates the support of co-stimulatory molecules. However, how the signal strength determines the fate of mRNA in T cells is not yet known. This is of particular interest if we consider that upon T cell differentiation, effector/memory T cells respond more rapidly to insults, and to much lower antigen levels when compared to naive T cells. Intriguingly, memory T cells can also respond to infections in an antigen-independent fashion, as they can be triggered by cytokines, and possibly by other inflammatory signals. Whether cytokine
production requires mRNA stabilization per se, or whether suboptimal T cell activation also allows direct translation of unstable mRNA remains an open question.

**RBPs and non-coding RNAs turn off T cell responses**

Once the infection is resolved, the effector T cell population contracts and about 90-95% of T cells die through apoptosis\(^6\). Furthermore, cytokine production must be rapidly terminated to prevent damage to the non-infected tissue. Stopping DNA transcription alone however is not sufficient to immediately halt the immune response, because the mRNA levels may not be immediately affected, and protein translation can therefore continue. To overcome this problem, post-transcriptional events controlled by RBPs and/or miRs are again indispensable (Figure 1-middle). For instance, TTP binds to ARE-bearing transcripts and induces the degradation of Ifng and Il2 mRNA in T cells\(^66,67\), and of Tnfa, Il10 and GM-CSF mRNA in primary bone marrow stromal cells and macrophages\(^68,69\). TTP promotes mRNA decay by recruiting the DCP2 decapping complex and the CCR4-Not deadenylase complex\(^70\) on target mRNAs.
Mice lacking TTP develop a complex inflammatory syndrome that is associated with a prolonged Tnfa mRNA half-life and elevated levels of circulating TNF-α. Likewise, deletion of AREs within the 3'UTR of Tnfa and Ifng results in chronic protein production that causes spontaneous development of chronic inflammatory arthritis, Crohn's like inflammatory bowel disease, Lupus-like disease and aplastic anemia.

More cis-regulatory elements targeted by RBPs for mRNA degradation have been identified in 3'UTRs. GU-rich sequences (GREs) are recognized by the CUG-binding protein 1 (CUGBP1) and are highly expressed in short-lived transcripts mediating intracellular signaling cascades in T cells, such as MyD88, Akt and JUNB. Constitutive decay elements (CDE) and alternative decay elements (ADE) form conserved secondary structures and are hubs for RBPs like Roquin, promoting e.g. degradation of mRNA encoding inducible T cell co-stimulator (ICOS), OX-40, and TNF-α in T cells and macrophages, respectively.

Also miRs are critical for mRNA degradation during the shutdown of T cell responses. In fact, during the resolution of an infection their expression levels rise back to original levels (Fig. 1-middle). The role of miRs in regulating T cell responses is further emphasized by the knockout of the miR-processing enzyme Dicer in CD8+ T cells that enhanced T cell activation. Other landmark studies have identified specific miRs for cytokine mRNA degradation. miR-29 reduces Ifng mRNA levels by directly binding to Ifng mRNA, or by targeting two critical transcription factors for IFN-γ, T-box transcription factor TBX21 (T-bet) and eomesodermin (Eomes). miR-139 represses the expression of perforin and Eomes, and miR-150 reduces IL-2 receptor α-chain (CD25) expression in CD8+ T cells. Interestingly, Tnfa mRNA degradation in macrophages and HeLa cells depends on the cooperation between TTP and miR-221 or miR-16. TTP does not directly bind to miRs, but associates with the RNA-induced silencing complex (RISC), which in turn stabilizes the interaction of miRs loaded in the RISC complex with the target mRNA to accelerate degradation. Also the degradation of mRNA encoding ICOS can be accelerated by the cooperation between the RBP Roquin and miR-146a. In this case, however, Roquin can directly bind to miR-146a and Argonaute-2 (Ago2, catalytic component of RISC), thus facilitating both miR-mediated target decay and miR homeostasis. While the cooperation between RBPs and miRs is possibly more rule than exception, only few studies thus far demonstrate the interplay between these regulatory mechanisms.

Interestingly, most recent studies revealed that the regulation of mRNA turnover does not only depend on the presence of cis-regulatory elements on the mRNA and their cognate trans-acting factors. It is also dictated by dynamic and reversible modifications of the mRNA molecules. N6-methyladenosine (m6A) is one of the most abundant RNA modifications, and is found in thousands of human transcripts. When methylation occurs at the 5'end of mRNA molecules, it can enhance its stability by interfering with the recruitment of the mRNA-decapping enzyme DCP2. Whether similar mechanisms may also occur in T cells is still unknown.
**Memory CD8⁺ T cells**

After the resolution of an acute infection, memory CD8⁺ T cells remain in the system and provide a long-term protection against secondary infection with their cognate intracellular pathogens. Memory T cells can patrol our body by circulating through the blood and be capable of entering both non-lymphoid (effector memory T cells) and secondary lymphoid organs (central memory T cells). In addition, tissue-resident memory T cells do not recirculate in the blood or lymphatics, but permanently reside in barrier tissues and ensure a first line of defense against invading pathogens. Unlike naive T cells, memory T cells can rapidly produce high amounts of effector cytokines and kill target cells upon antigen recognition, even before they proliferate into large numbers of secondary effectors. Additionally, they can also respond to distinct inflammatory cytokines, as for example IL-12, IL-18 or IL-21, in an antigen-independent manner. This imminent responsiveness of memory T cells potentiates the rapid local expression of inflammatory cytokines and chemokines and helps to recruit and activate other immune cell types, such as monocytes/macrophages and neutrophils. Thus, memory T cells are essential to prevent and contain systemic dissemination of the infection.

The transition of naive to effector and memory T cells is associated with large-scale changes in chromatin folding, gene expression, membrane structure and homing capability. Memory T cells retain most of these acquired features for life, through many rounds of antigen-independent homeostatic proliferation. The permissive epigenetic signature that T cells acquire upon primary infection, and partially maintain in their memory phenotype, defines their ‘ready-to-go’ state and promotes quick response upon secondary infections. This open chromatin conformation however also allows the docking of the RNA polymerase II on many genes of which the expression should be silent. Remarkably, memory T cells express a 100-fold higher mRNA encoding IFN-γ when compared to naive T cells. Also many other mRNA transcripts that encode for effector molecules, such as RANTES/CCL5, remain elevated in memory T cells, whereas their corresponding protein is undetectable. Constitutive expression of cytokine encoding mRNA poises memory T cells for immediate responses. However, aberrant translation of these pre-formed mRNAs must be prevented to avoid production of cytotoxic molecules at the wrong time or place.

**What keeps memory T cells quiescent?**

Whereas there is a solid set of data on mRNA decay in T cells, far less is known about how the translation of mRNA into protein is regulated. This process of translational regulation has been proposed to keep self-reactive T cells anergic, while it also may be important to prevent excessive production of cytokines during the effector phase, or to block translation of pre-formed mRNA in memory T cells. mRNA translation is a highly dynamic process, and its efficiency corresponds to the number of completed proteins generated by one RNA molecule in time. Using ribosome profiling, it is now possible to better quantify translation efficiency by calculating the occupancy or density of ribosomes on a transcriptional unit. Furthermore, imaging translation of individual mRNA molecules at single cell level revealed that both translation initiation and elongation rates may differ on different transcripts and might depend on RBPs and antisense RNAs that decorate the mRNA.
RBPs modulating translational processes have been primarily studied in activated macrophages. For instance, HuR overexpression does not alter the mRNA levels of TNF-α and cyclooxygenase-2 (COX-2), but recruits the translational silencer TIA-1 onto the mRNA. TIA-1 binding to AREs diverts the target RNAs from polysomes to untranslated messenger ribonucleoproteins (mRNPs) to block mRNA translation and dampen the inflammatory response. Also TTP has been shown to inhibit mRNA translation through its interaction with the cap-binding translation repression 4EHP-GYF2 complex, thus linking mRNA decay to the block of translation. TTP acts as a suppressor of feedback inhibitors of inflammation and, upon LPS-stimulation of macrophages, it is rapidly inactivated by p38 mitogen-activated protein kinase (MAPK)-activated kinase MK2-dependent phosphorylation. Conversely, exposing macrophages to IFN-γ promotes the engagement of the IFN-γ activated inhibitor of translation (GAIT) complex, a heterotetramer that binds to conserved stem-loop secondary structures in the 3’UTR of e.g. C-C motif cytokine 22 (CCL22) and ceruloplasmin. This interaction hampers the entry of the translational pre-initiation complex at the 5’UTR of target mRNAs.

Interestingly, non-coding RNAs can also contribute to translational inhibition. Whereas perfect base-pairing of miRs with their target mRNA supports mRNA degradation, imperfect complementarity primarily results in translational inhibition. In zebrafish, Drosophila S2 cells, and HeLa cells, miRs were shown to repress the translation of newly synthesized mRNAs without affecting the mRNA levels. Those findings may be explained by affecting translation itself or, alternatively, by mRNA deadenylation, which may lead to decreased translation efficiency. Additionally, translation inhibition can also occur through IncRNAs. In HeLa cells the IncRNA-p21 interacts with polysomes and inhibits mRNA translation by interfering with the binding of the translational initiation factor eIF4E on the mRNA. IncRNA-p21 levels rise when HuR levels are low, thereby this mechanism does not occur when HuR levels are high. Furthermore, translation efficiency can depend on the epigenetic imprinting of the mRNA, yet its specific role is still under debate. mRNA methylation has in fact been linked to both enhanced and reduced translation capability. The position of the modified nucleotide within the transcript can possibly define its effect on mRNA translation: whereas the presence of m^6A modification at the 5’UTR can promote cap-independent translation, m^6A modification within the coding sequence may interfere with translation efficiency and elongation. While some of the processes blocking translation will also apply to T cells, cell-type specific regulation may occur that should be further investigated.

**Dysfunctional CD8^+ T cells**

When pathogens cannot be eliminated and persist in the body resulting in chronic infections, CD8^+ T cells enter in a state of functional hypo-responsiveness that is commonly defined as ‘exhaustion’. T cell exhaustion was first described in chronic Lymphocytic choriomeningitis virus (LCMV) infection in mice, and was later observed in humans during infections with for example HIV and Hepatitis C virus (HCV), and in cancer.

During both chronic infections and cancer, CD8^+ T cells progressively lose their capability to mount protective immune responses. This gradual loss of function commences with the abrogation of their proliferative capacity and IL-2 production, and is followed by the gradual
loss of the production of TNF-α and then of IFN-γ. Loss of effector functions generally coincides with increased expression of inhibitory receptors on T cells, such as programmed cell death (PD)-1, lymphocyte-activation gene (LAG)-3, T cell immunoglobulin- and mucin-domain containing molecule (TIM)-3, cytotoxic T lymphocyte-associated antigen (CTLA)-4 and B- and T-lymphocyte attenuator (BTLA). How these two events are interconnected is still subject of ongoing investigations. It has been shown that the functional impairment of exhausted T cells is primarily due to continuous antigen exposure, prolonged duration of TCR triggering, lack of co-stimulation, and immunosuppressive environmental cues. Furthermore, transcriptional profiles of exhausted CD8+ T cells revealed a pattern of differentiation that is profoundly different from the profile of effector and memory CD8+ T cells. Exhausted T cells exhibit alterations in their metabolic and bioenergetic pathways, reduced expression of components of the translational machinery, and they become considerably smaller in size. Importantly, their dysfunctional phenotype is reversible during an early phase of chronic infection or tumor. Checkpoint blockade inhibitors, as for example blockers of the PD-1:PD-L1 pathway, can successfully restore effector functions of exhausted T cells and enhance both the control of viral infections and of tumor growth. Recent evidences have however demonstrated that tumor-specific CD8+ T cells can ultimately evolve into a terminally differentiated exhausted state that cannot be reversed. These observations were made upon analysis of the epigenetic profile of exhausted T cells. Genes encoding for inhibitory receptors, such as PD-1, CTLA-4 and TIM-3, or encoding for transcription factors associated to exhaustion, like Eomes and hypoxia-inducible factor (HIF)1-α, and negative regulators, like suppressor of cytokine signaling (Socs)1 and Socs2, rapidly establish transcriptionally active chromatin structures. Conversely, co-stimulatory molecule genes such as CD28 and ICOS, as well as effector molecule genes encoding IFN-γ and granzyme B, were downregulated together with closing of multiple chromatin regions within their loci. Interestingly, despite the reduced accessibility to the Ifng locus and the incapability to produce IFN-γ protein, we observed that exhausted CD8+ T cells continue to express Ifng mRNA in amounts that are intermediate between effector and memory CD8+ T cells. This discrepancy between transcript levels and protein levels again indicates a possible role for post-transcriptional control in modulating T cell functionality. The role of miR-dependent mRNA decay and regulation of translation initiation have been investigated in the context of tumorigenesis. In addition, transcripts encoding for many oncogenes, growth factors and their receptors, cell-cycle genes and inflammatory mediators, encompass AU-rich elements within their 3'UTR, suggesting the potential role of ARE-dependent events in tumor initiation and malignancy. However, whether specific post-transcriptional events are required in the induction and maintenance of exhausted tumor infiltrating T cells remains still unknown.
Scope of this thesis

In this thesis, we explored how different post-transcriptional events poise cytokine production in response to specific environmental cues and the state of T cell differentiation. In chapter 2 we reveal that the translation of constitutively expressed mRNA encoding for the effector molecules IFN-γ and TNF-α is actively blocked in memory T cells. The recruitment of pre-formed mRNA to ribosomes is inhibited by the binding of the RNA-binding protein ZFP36L2 to AU-rich elements present with the mRNA 3’UTR. Upon T cell activation, the translational repression conferred by ZFP36L2 is relieved (chapter 2) and memory T cells can use pre-formed Tnfa and Ifng mRNA as a template for immediate cytokine production (chapter 3).

In chapter 3 we further investigated which regulatory mechanisms are employed for the cytokine production of effector and memory T cells upon antigenic stimulation. We unravel cytokine-specific regulatory networks and TCR-dependent intracellular pathways that tailor cytokine production in magnitude and in time. Because memory T cells are also capable to produce IFN-γ upon antigen-independent stimulation, we define in chapter 4 the requirement for T cell innate responses upon activation through Toll-like receptor (TLR) ligands. We found that TLR-dependent IFN-γ production relies exclusively on translation of newly synthetized mRNA. The production of IFN-γ is strictly regulated by rapid mRNA turnover and limited energy demand. We furthermore observed that the kinetics of IFN-γ production greatly differed when T cells are stimulated in an antigen-dependent or an antigen-independent manner, and this directly correlated with the capacity to stabilize the Ifng mRNA. Since the quantity and quality of the stimuli that T cells receive might regulate the time of turnover of cytokine mRNA, we focused on resolving this issue in chapter 5.

In chapter 6 we examine the contribution of post-transcriptional events in impairing cytokine production of exhausted tumor-infiltrating lymphocytes. We found that within the immunosuppressive tumor environment, T cells lose the capacity to stabilize Ifng mRNA in an AU-rich element dependent fashion. By relieving IFN-γ from this post-transcriptional brake, we here describe a new system to further potentiate adoptive T cell therapy against melanoma tumors in mice. Finally, in chapter 7 we discuss the overall findings described in this thesis, with a particular emphasis on the signals that may govern post-transcriptional control of cytokine production during the course of a T cell response. We furthermore speculate how our findings can be exploited to restore dysfunctional immune responses.
References


Chapter 1


