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mRNA translation from an antigen presentation perspective: A tribute to the works of Nilabh Shastri

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ABSTRACT

The field of mRNA translation has witnessed an impressive expansion in the last decade. The once standard model of translation initiation has undergone, and is still undergoing, a major overhaul, partly due to more recent technical advancements detailing, for example, initiation at non-AUG codons. However, some of the pioneering works in this area have come from immunology and more precisely from the field of antigen presentation to the major histocompatibility class I (MHC-I) pathway. Despite early innovative studies from the lab of Nilabh Shastri demonstrating alternative mRNA translation initiation as a source for MHC-I peptide substrates, the mRNA translation field did not include these into their models. It was not until the introduction of the ribosequence technique that the extent of non-canonical translation initiation became widely acknowledged. The detection of peptides on MHC-I molecules by CD8 + T cells is extremely sensitive, making this a superior model system for studying alternative mRNA translation initiation from specific mRNAs. In view of this, we give a brief history on alternative initiation from an immunology perspective and its fundamental role in allowing the immune system to distinguish self from non-self and at the same time pay tribute to the works of Nilabh Shastri.

A good place to start is the observation from the lab of Thierry Boon in the late 1980s that cells transfected with tumor DNA fragments lacking a promoter sequence, or containing a frameshift or a stop codon upstream of the antigen coding sequence, could still be recognized by tumor antigen-specific CD8 + T cells (Boon and Van Pel, 1989) (Fig. 1). This lead to the proposition that peptides for the MHC-I (pMHC-I) are derived from "short regions of the genome located around the antigen sequence that can be translated autonomously", i.e. independently of the production of the corresponding full length mRNA or the corresponding protein. In support of the latter idea, the same group demonstrated that some human tumor-infiltrating lymphocytes (TILs) could also recognize intronic sequence-derived antigens (Coulie et al., 1995; Guilloux et al., 1996), and other similar studies described similar mechanisms for alternative open reading frames (Wang et al., 1996; Rosenberg et al., 2002; Wang et al., 1998), for non-AUG initiation codons (Ronsin et al., 1999; Weinzierl et al., 2008) and for IRES-dependent translation (Carbonnelle et al., 2013). Until then, it had been assumed that the pMHC-1 substrates were derived from the degradation of full length proteins (Rock et al., 2014). It worth keeping in mind that this dogma influenced the interpretation of observations related to the MHC-I and CD8 + T cell-based immune surveillance such as how peptide substrates enter the class I pathway and what the self and non-self recognition is based upon. For example, if full length proteins are the source, the focus on what the immune system detects in terms of self vs non-self, would be on protein turnover but if alternative peptide products is the source, the focus will instead turn towards translation initiation and the presence of RNAs in the cells (Starck and Shastri, 2011; Goth et al., 1996). The importance of this has been highlighted by the recent hype in mRNA vaccines. The source of peptide material for the class I pathway has been controversial. In the mid-90 s, Bennick and Yewdell's lab took the concept of non-full

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length proteins as a source for self vs. non-self recognition a step further by postulating that antigenic peptides for the MHC-I pathway are derived from an unknown peptide source termed defective ribosomal products, or DRiPs (Yewdell et al., 1996). The arguments for DRiPs were logical but what this pool of non-full length peptides actually originates from was not known. Nevertheless, apart from T. Boon's works, there were supports for alternative peptide products coming from observations by J.V. Fetten (Fetten et al., 1991) and N. Shastri (Shastri and Gonzalez, 1993) in the early 1990s, demonstrating that introduction of a frameshift mutation does not disrupt the production of MHC-I epitopes derived from the influenza virus nucleoprotein or the presentation of the SIINFEKL epitope, respectively. The debate on full length *vs*. alternative translation products as the source for antigenic peptides was a heated topic for some time but the problem was then, and still is, that as soon as the peptide is from within an open reading frame it is difficult, if not impossible, to determine if it is derived from the full length protein, from a DRiP or from any other potential source of peptides. Thus, the relative contribution to the class I pathway can be difficult to estimate if it the peptide originates from an open reading frame. However, if a peptide is derived from an intron, or from an untranslated RNA sequence, we can with certainty say it is not derived from degradation of a full length protein. An attempt to address the contribution of full length proteins to class I-restricted immune surveillance was, nevertheless, made by fusing an antigenic peptide to the IκBα. IκBα is phosphorylated and ubiquitinated and instantly degraded by the 26S proteasome following extracellular stimuli and, consequently, this degradation pathway affects the full length proteins only. However, targeting IκBα carrying an antigenic peptide sequence for degradation has no impact on antigen presentation, despite the cell being swamped with proteasome degradation products (Apcher et al., 2011). It is an interesting question how the degradation of certain substrates, and not others, generates antigen peptide material (Yewdell et al., 2019). A next important step came in the late 1990s when Shastri's laboratory discovered that the synthesis of antigenic peptides might not be carried out by the canonical translation initiation machinery which depends on an AUG codon in the correct Kozak context. Instead, they described a translation event starting at a CUG initiation codon suggesting that translation initiating at "near cognate" triplets, differing from AUG initiation codon by a single amino-acid, provides peptides for the MHC-I pathway (Malarkannan et al., 1999). Later, in the early 2000, the same lab showed that a peptide sequence inserted in the 3′ UTR initiated from a CUG codon generate tolerance and, thus, that non-canonical translation products was not just something observed in *in vitro* experiments (Schwab et al., 2003). The shift in focus from degradation of full length proteins to non-canonical mRNA translation as the molecular event generating pMHC-I was boosted

by the observation that viruses evade the class I pathway by suppressing the translation of the mRNA and not by preventing the degradation of the encoded protein (Yin et al., 2003).

At this time, the mRNA translation community held on to the canonical mRNA translation initiation model in which translation is initiated by the eIF4F complex being recruited to the 5′ cap structure and the formation of the 43S pre-initiation complex (PIC) that scans the message until it finds the first AUG codon. In this model the 43S contains the methionyl-initiator tRNA (Met-tRNAi) in complex with the eukaryotic initiation factor 2 (eIF2), making it difficult to see how a CUG could be used (des Georges et al., 2015; Hashem et al., 2013). Even cap-independent translation mediated by internal ribosomal initiation would not be exempted from this rule. It was therefore assumed that even though a CUG codon was being used, it was still a methionine that was the initiating amino acid. Nilabh was not of this opinion and he set out to show that it was a indeed a leucine that was inserted at the CUG codon and he mentioned how difficult it had been to convince reviewers that this was indeed the case (Schwab et al., 2004; Starck et al., 2012) These papers from Nilabh's lab were published in the highest ranking journals and reviewed by prominent experts on mRNA translation and, even though they convinced the immunology community, they made little impact on the mRNA translation field. Nilabh, Jon Yewdell, us and others have since attended many mRNA conferences and presented various parts and aspects of how antigenic peptides are derived from non-canonical translation events and it is interesting to note that despite the data from immunologists are not contradicted, the message just does not hit home. The works of immunology and antigen presentation was just too far from the models used by the mainstream mRNA translation field and the detection of peptides on MHC-I molecules by sensitive T cell assays (Shastri and Gonzalez, 1993; Sanderson and Shastri, 1994) was seen as something exotic and far from the usual assays to study mRNA translation products. These short products will, for example, not turn up on the metabolic labelling assays using S35-methionine, particularly if the peptide substrate is short and the initiation is not at an AUG. The presentation of non-canonical translation products on MHC-I molecules were observations without any underlying molecular mechanism to explain the phenomenon and this might help explain why the works from Shastri's lab had a difficult time to make an impact on the mainstream mRNA translation field. But, on the other hand, most works on alternative initiation are based on observed phenomena rather than molecular mechanisms. It was later shown that cells use a Leu-tRNA initiator that requires expression of the eIF2A initiation factor (Starck et al., 2012). Since these initial works, several peptides derived from CUG or other non-AUG codons have been identified on human cancers or infected cells and have been described as relevant in disease progression. For example, in human renal cell carcinoma, a peptide translated from a CUG initiation codon from the VEGF protein was found over-expressed and CD8 + T cells were enriched in clones directed

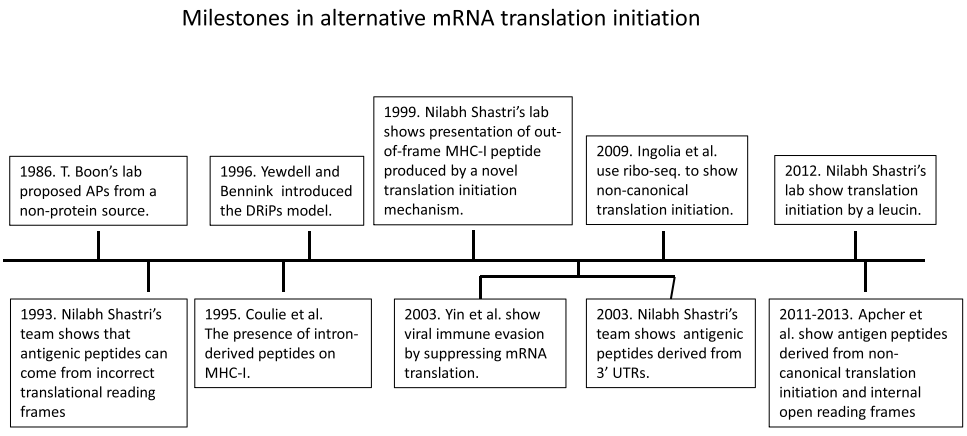

Fig. 1. Some mile stones in the understanding of non-canonical mRNA translation and alternative open reading frames as a source of antigen peptides to the major histocompatibility class I pathway. pMHC-I = MHC class I peptides.

against this peptide (Weinzierl et al., 2008). pMHC from "non-coding" mRNA sequences, i.e. introns, intron-exon or exon-UTR junctions, 3′ or 5′UTRs, short open reading frames or long non-coding RNA have since also been described (Yewdell et al., 2019). It has been said that every step in evolution is preceded by an advancement in self vs. non-self recognition and with this in mind, mRNA translation is a particular interesting topic in that the antigen presentation pathway for alternative translation products evolved around a source of peptide substrates that preceded the regulated canonical translation controlling gene expression in mammalians of today.

Works from Weissman's lab in 2009 and 2011 finally broke the dam. They used ribosomal profiling (or Ribo-seq) to study global mRNA translation initiation and they focused in particular on small peptide fragments and it was shown that a large proportion of initiation events take place on non-AUG codons (Ingolia et al., 2014, 2009; Ingolia et al., 2011). In fact, results from Ribo-seq along with large scale analysis of the human proteome using mass spectrometry analysis recently confirmed non-conventional translations as a common feature of eukaryotes. A recent large scale study combining elution of MIPs from B-LCLs and customized database generated from six-frame translation of RNA sequencing data estimated that around 10 % of the MIPs are generated from non-conventional translation (Chong et al., 2020; Erhard et al., 2018; Ruiz Cuevas et al., 2021). A large scale study combining elution of antigenic peptides from MHC-I molecules and six-frame translation of RNA sequencing data estimated that around 10 % of the MIPs are generated from non-conventional translation (Laumont et al., 2016). More recent studies suggest this number to be fare greater. This implicates that the substrates generated for the MHC-I come from throughout the genome and represent a much greater source of peptide products as the currently described exome. But keep in mind that even if a peptide is originating from an classic open reading frame, it cannot easily be determined if it is derived from the encoded protein, or not. The questions that now need to be answered are why the MHC-I pathway has evolved to use a distinct mRNA translation event to generate the peptides used to allow the immune system to distinguish self from non-self and how the immune system can deal with the enormous amounts of potential antigenic peptide substrates while retaining sensitivity to non-self peptides. A big step forward will be to know the composition of the ribosome carrying out translation of non-canonical open reading frames and how it is regulated. This will greatly help understanding cancer and viral immune evasion and open for new therapeutic approaches to further harness the immune system to generate better vaccines as well as to manipulate the immunopeptidome on infected or transformed cells. It is worth keeping in mind that non-canonical translation events can give rise to peptides with specific functions also outside the MHC-I pathway.

Later years advances in the detection of alternative ORFs has raised the question of what an open reading frame really is. Bioinformatic analysis has implicated thousands of small novel ORFs and muddled the definition of a gene (Sberro et al., 2019). In fact, observation from MHC-I antigen presentation had as early 2011 illustrated the presence of ORFs within the main ORF by introducing synonymous mutations in leucine codons upstream to the antigenic peptide that affect production of antigenic peptides but not the expression of the corresponding full length protein (Apcher et al., 2011). As the classical view of what a gene and an ORF really are is coming under scrutiny, well established models for mRNA translation control, RNA quality control etc. have become subjects of revaluations or adjustments. With the help of protein mass spectrometry on the immunopeptidome and some opened minded scientists doing the bioinformatics analysis, we are likely to learn more of alternative ORFs and their physiological roles in the near future (Orr et al., 2020).

We will finish this by paying tribute to the works of Nilabh Shastri. He has made important contributions also on other aspects of antigen presentation and processing (Guan et al., 2021; Nagarajan et al., 2016; Hammer et al., 2006) but his works on mRNA translation have (Malarkannan et al., 1999; Starck and Shastri, 2016) been a great source of inspiration and reassurance for our own works (Apcher et al., 2011, 2013) and his foreseeing and creativity will be greatly missed.

Data availability

Data will be made available on request.
No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2021.12.010.

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