# RNA AND TRANSLATIONAL CONTROL IN CANCER

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The dysregulation of protein synthesis is an emerging hallmark of cancer, where altered translation is essential for the induction of oncogenic gene programmes. Distinct programmes of gene expression drive tumour growth and create the supportive microenvironment in which it flourishes. Our research aims to understand how components of the translation machinery are required to increase the rate of translation of key oncogenic mRNAs and how best we can target these pharmacologically.

### Understanding the essential role of eIF4A1 for the translation of oncogenic mRNAs and how this can be targeted

Translation initiation is a major determinant of protein production and requires precise regulation to drive translation of selected mRNAs. Eukaryotic translation initiation factor (eIF) 4A1 is a DEAD-box RNA helicase and catalyses at least two major reactions during translation initiation: mRNA loading onto the 40S ribosomal subunit and translocation of the initiating ribosome along the 5′ untranslated region (UTR) to the start site. While not all eIF4A1-dependent mRNAs require these activities to the same degrees, dysregulated eIF4A1 activity is at the root of oncogenic translational programmes, reflected by a strong therapeutic interest in targeting eIF4A1 in cancer. Currently, a range of chemically diverse eIF4A1-inhibitors have been described, including hippuristanol and eFT226, which is the first-in-class eIF4A1-inhibitor to have entered clinical trials. Despite this, it is still only incompletely understood how eIF4A1-dependent mRNAs, such as oncogene mRNAs, recruit and activate specific eIF4A1 functions, and how eIF4A1 inhibitors perturb these mechanisms to inactivate eIF4A1-dependent oncogene translation. Also, of critical importance, all current eIF4A inhibitors target both eIF4A1 and its paralogue eIF4A2, which share roughly 90% identity at the amino acid level. Yet eIF4A2 has a distinct role from eIF4A1, in that it can act as a translational repressor in conjunction with the CCR4-NOT complex. Hence, to uncover eIF4A1's full therapeutic potential, we need to both better understand its role and basic mechanism of function in cancer and also understand the consequences of eIF4A inhibition on the distinct functions of eIF4A1 and eIF4A2.

Data from the Sansom lab show that loss of either eIF4A1 or eIF4A2, but not both, in the intestine of wild-type mice is partially tolerated. However, in colorectal cancer (CRC) models, loss of eIF4A1 leads to reduced proliferation and increased survival, but the loss of eIF4A2 accelerates tumourigenesis and leads to decreased survival. This loss of eIF4A1 in the CRC models phenocopies the loss of Myc. We therefore hypothesised that eIF4A1 is required to support the translational landscape following loss of *Apc* and that that oncogenic Wnt signalling requires both the upregulation of Myc's transcriptional targets and the eIF4A1-dependent translation of these mRNAs. To test this, we first carried out RNA-Seq on the small intestines from either wild-type (WT), *Apc*$^{-/-}$ (APC) or *Apc*$^{-/-}$ *Kras*$^{G12D}$ (AK) mice, following loss of either eIF4A1 or eIF4A2. Interestingly, this showed a collapse of the Myc driven transcriptional signature, specifically in the oncogenic setting following loss of eIF4A1, but not eIF4A2 (Fig 1A). This suggests that eIF4A1 is required to support the translation of these down-stream targets of Myc. To test this, we performed ribosome-profiling on AK mice, following loss of eIF4A1. Indeed, this showed that these same mRNAs were translationally repressed following loss of eIF4A1, in that their ribosome occupancy decreased significantly more than their total mRNA abundance (Fig 1B). This suggests that oncogenic Wnt signalling and hyper-proliferation is dependent, not only on the transcriptional activity of Myc but the activity of 4A1 to support the translation of its targets. To test this, we tried to rescue the hyper-proliferation phenotype by over-expressing a Myc transgene that is not dependent on eIF4A1 for its translation. As this did not rescue the phenotype (Fig 1C), this supports our model that eIF4A1 is required to support the translational landscape following loss of *Apc* in a manner analogous to the role played by Myc at the transcriptional level (Fig 1D). This interdependency of Myc and eIF4A1 to drive hyperproliferation could represent a new axis to target Myc-driven cancers, where targeting Myc directly has proved difficult.

To understand how pharmacological inhibition of eIF4A1 compares to its genetic loss we performed ribosome-profiling in MCF7 cells following treatment with the eIF4A inhibitors hippuristanol and eFT226 **(Fig. 2A)**. These two compounds show distinct modes of action in vitro. Namely, eFT226 follows a gain of function mechanism by inducing and stabilising RNA-binding, which leads to increased RNA unwinding activity **(Fig. 2B)**, while in contrast hippuristanol results in a loss of function of eIF4A1 by inducing dissociation of RNAs off eIF4A1, which leads to inhibition of eIF4A1's RNA unwinding activity **(Fig. 2B)**. Thus, we hypothesised that the compounds also show distinct modes of action in cells which should reveal a wide spectrum of mRNA targets that depend on eIF4A1 activity for their translation. Indeed, by ribosome profiling we identified 241 mRNAs that were sensitive to both compounds and, most interestingly, also two groups of 2,092 and 100 mRNAs that were only sensitive to eFT226 or hippuristanol **(Fig. 2C)**. As eFT226 and hippuristanol affect eIF4A1 activity distinctly, we hypothesised that the compounds inhibit distinct functions of eIF4A1, that are specifically required by these compound-specific mRNAs for their translation.

In contrast to eFT226, the exact molecular and structural mode of action of hippuristanol is unclear. To understand the mechanism of translational repression inferred by hippuristanol better, we turned to structural approaches, which suggested that hippuristanol binding to eIF4A1 interferes with RNA-binding and indicated a conformation of the eIF4A1-hippuristanol complex that favours eIF4G binding. Supporting this, we find that mRNAs that preferentially associate with eIF4G in cells, are those mRNAs that are most translationally repressed by hippuristanol. This suggests that eIF4A inhibition with hippuristanol specifically inactivates the eIF4F complex and its associated functions. Utilising bioinformatics-approaches we have investigated the mRNA features that may drive these distinct mRNA sensitivities towards the compounds. These data reveal that the mRNA features, that render their translation sensitive to hippuristanol, are also the features that characterise mRNA strongly associated with eIF4G (Fig. 2E). Further, the data highlights that AG-rich sequences are linked to eFT226-sensitivity, while C/GC-rich motifs are associated with hippuristanol-sensitivity. We are currently validating the relationship between these features and associated eIF4A1 functions.

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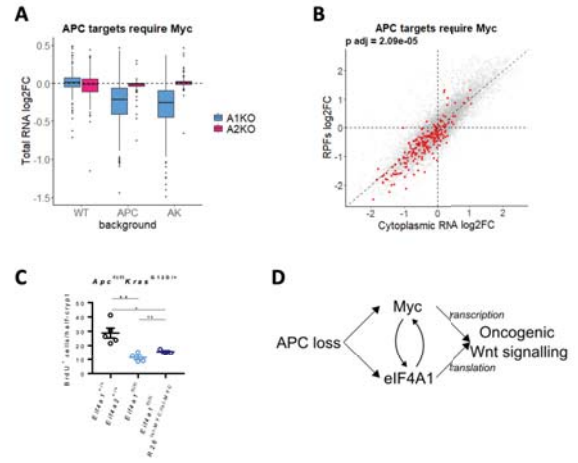


**Figure 1. (A)** Boxplot depicting changes in mRNA abundance of all Myc-targets (downstream of APC loss), within the small intestines of *Eif4a1*$^{fl/fl}$ (A1KO) or *Eif4a2*$^{fl/fl}$ (A2KO) mice in the stated genetic background. Loss of eIF4A1 but not eIF4A2 leads to the downregulation of the abundance of Myc-dependent mRNAs, but only in the presence of oncogenic signalling. **(B)** Scatter plot comparing the log2FC in ribosomal occupancy (RPFs) and cytoplasmic RNA from ribosome profiling analysis, following loss of eIF4A1 in AK small intestines. Myc-targets (downstream of APC loss) are coloured in red and are statistically enriched below the line of x=y (adjusted p-value = $0.2^{-5}$), therefore showing translational downregulation of c-Myc target mRNAs. **(C)** BrdU incorporation in small intestine of AK mice with the additional stated genetic alterations. **(D)** Model depicting the interdependency of Myc and eIF4A1 to support the transcriptional and translation landscapes of oncogenic signalling following loss of *Apc* in colorectal cancer.

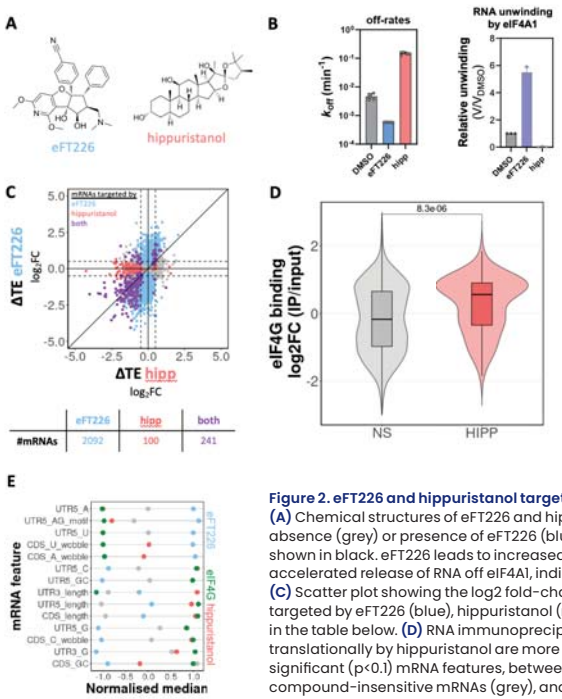


**Figure 2. eFT226 and hippuristanol target distinct eIF4A1-dependent mRNAs.**
**(A)** Chemical structures of eFT226 and hippuristanol. **(B)** RNA release of eIF4A1 bound to RNA in the absence (grey) or presence of eFT226 (blue) and hippuristanol (red). RNA in the absence of protein is shown in black. eFT226 leads to increased binding of eIF4A1 to RNA, while hippuristanol induces accelerated release of RNA off eIF4A1, indicated by the increased and decreased signals, respectively. **(C)** Scatter plot showing the log2 fold-changes (log2FC) in translational efficiency (TE) of mRNAs targeted by eFT226 (blue), hippuristanol (red) or both (purple). Numbers of identified mRNAs is given in the table below. **(D)** RNA immunoprecipitation (RIP) of eIF4G shows that mRNAs inhibited translationally by hippuristanol are more associated with eIF4G. **(E)** Normalised means of statistically significant (p<0.1) mRNA features, between eFT226- (blue), hippuristanol-sensitive (red) mRNAs, compound-insensitive mRNAs (grey), and mRNAs enriched for eIF4G-binding (green).