## Contents

**Abbreviations**

| Abbreviation | Term |
|---|---|
| RPF | Ribosome Protected Fragment |

# 1. Introduction

## 1.1. Cancer

According to data from the World Cancer Research Fund, in 2018 there were an estimated 18 million cancer cases worldwide of which 9.5 in men and 8.5 in women. Lung and breast were the most common cancers overall. However, the most common cancers among women were breast, colorectal and lung whereas for men these were lung, prostate and colorectal cancer.

Cancer is a disease in which cells start growing abnormally and evade mechanisms monitoring cellular integrity. Hanahan et al. (Hanahan & Weinberg, 2011) summarised such mechanisms and how cancer evades them as the hallmarks of cancer. These hallmarks will be discussed in section xxx.

In this thesis I will discuss two studies in which breast cancer and pancreatic cancer play a central role. Therefore in the next two sections will focus on these two very different cancers.

### 1.1.1. Breastcancer

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### 1.1.2. Pancreatic cancer

stats and explanation on pancreatic cancer

### 1.1.3. Hallmarks of cancer

Hallmarks of cancer and lead into gene expression

## 1.2. Central dogma of gene expression

## 1.3. mRNA translation

For the vast majority of protein coding mRNAs,eukaryotic mRNA translation occurs in the cytoplasm, however a small subset of mRNAs is translated in the mitochondria. mRNA translation is a process that includes initiation, elongation, termination and ribosome recycling and is an essential process. mRNA translation inititiation is commonly regarded as the rate limiting step. Nevertheless, regulation

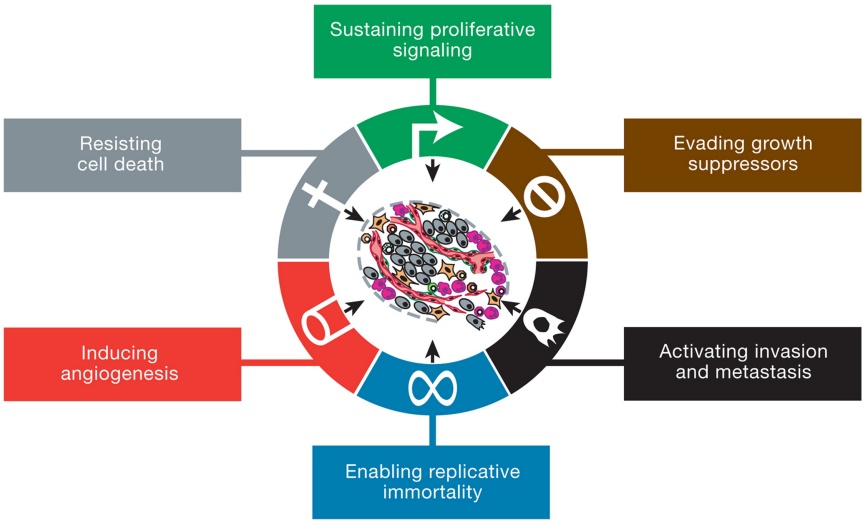


Figure 1.1: The Hallmarks of Cancer - This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark. Reprinted from Hallmarks of cancer: The next generation, 144, Hanahan, Douglas and Weinberg, Robert A., Hallmarks of cancer: The next generation, 646-674, Copyright (2011), with permission from Elsevier

of mRNA translation is also regulated at the elongation and termination phases to a lesser extent.

**1.3.1. mRNA translation initiation** In eukaryotes most mRNAs are translated by scanning the mRNA for a start codon (AUG). This mechanism begins with the formation of the 43s pre-initiation complex (PIC) consisting of methionyl-initiator tRNA (met-tRNAi) in a ternary complex (TC) with guanosine triphosphate (GTP) bound eukaryotic initiation factor 2 (eIF2). The PIC is recruited to the 5'-cap of mRNAs which is facilitatedby the eIF4F 5'-cap binding complex, a complex consisting of eIF4E (cap binding protein), eIF4A (RNA helicase) and eIF4G (scaffolding protein). The PIC then scans along the mRNA from the 5' end until it encounters an AUG codon. After AUG recognition eIF2-GTP is hydrolyzed forming a stable 48S PIC. Afterrelease of eIF2-GTP the 60S ribosomal subunit joins to form the 80S ribosome and protein

synthesis can commence(Hinnebusch, 2014,Dever & Green (2012)). Next to this scanning mechanism, mRNA translation can also be initiated via alternative cap independent mechanisms(Wurth & Gebauer, 2015).

**1.3.2. mRNA translation elongation** The 80S ribosome contains three sites; the acceptor (A), peptidyl (P) and Exit (E) sites. After initiation, the 80S ribosome is positioned with the met-tRNAi in the P site at the AUG codon with the following codon of the transcript in the A site awaiting its cognate tRNA. The tRNA arrives in a TC together with eukaryotic elongation factor 1A(eEF1A)at the A-site of the ribosome. After arrival in the A-site,the codon is then recognized. The binding of eEF1A is GTP dependent, recognition of the cognate codon by the tRNA triggers hydrolysis whereby eEF1A releases from the tRNA thatis then recycled by eEF1B.Peptide bonds are then formed accompanied by a tRNA hybrid state whereby acceptor sites of tRNAs in the A-and P-site now move to the P-and E-site. Binding of eEF2-GTP then promotes translocation of the tRNAs into the P-and E-sites after which eEF2B-GDP releases. After release of the deacylated tRNA from the E-site the ribosome is ready for the next cycle. This process is repeated until a stop codon (UAA,UGA or UAG)is detected by the ribosome(Dever & Green, 2012). mRNA translation termination Termination of mRNA translation is facilitated by two release factors, eRF2 and eRF3-GTP. The TC with eRF2 and eRF3-GTP binds to the A-site of the ribosome. Recognition of the stop codon by the ribosome then causes hydrolysis resulting in a conformational change and release of the polypeptide. eRF1 and the ATP binding cassette protein (ABCE1) together promote the splitting of the 60S and 40S subunits, of which the 40S subunits has still bound tRNA. After release of the tRNA from the 40S subunits the parts of the translational machinery can be recycled(Dever & Green, 2012).

## 1.4. Regulation of mRNA translation

**1.4.1. mTOR singalling pathway** mTOR is a conserved Ser/Thr kinase and is found in two structurally and functionally distinct complexes, mTORC1

and mTORC2. mTORC1 contains mTOR, regulatory associated protein of TOR (raptor), the GTPase beta-subunit like protein (GbetaL) and disheveled, EGL-10, pleckstrin [DEP] domain containing mTOR-interacting protein (deptor). mLST8 and deptor are found in both mTORC1 and mTORC2.However, rapamycin-insensitive companion of TOR (rictor), mammalian stress-activated protein kinase [SAPK]-interacting protein (mSIN1), and proline-rich protein 5 (protor) are specific to mTORC2(**???**,Pearce et al. (2007)). In regards to regulation of mRNA translation, mTORC1 is a key player in regulation of translation initiation through facilitating the release of eIF4E from 4E-BPsvia phosphorylation of 4E-BPs by mTOR (Hsieh et al., 2010). Furthermore, substrates of mTORC1 include ribosomal S6 kinases (S6Ks) 1 and 2(Schepetilnikov et al., 2013), and La ribonucleoprotein domain family member 1 (LARP1)(Tcherkezian et al., 2014). mTORC2 is found to associate with ribosomes to promote co-translational phosphorylation and foldingofnascent Aktpolypeptide(Oh et al., 2010).As mentioned mTORC1 is activated via growth hormonesincludinginsulin and insulin like growth factor (IGF).For example,wheninsulin binds to the insulin receptor,tyrosine kinases (RTKs) and phosphoinositide 3-kinase (PI3K) are activated. Phosphatidylinositol 3,4,5-triphosphate (PIP3) is then generated by PI3K from Phosphatidylinositol 4,5-biphoasphate (PIP2). This step is reversed by PTEN which hydrolyzes PIP3 to PIP2, thereby working antagonistically to PI3K. PIP3 recruits AKT and phosphoinositide-dependent kinase1 (PDK1) towards the plasma membrane where AKT is phosphorylated by PDK1. Ras homologue enriched in brain (Rheb) is a GTPase that stimulates mTOR in its GTP bound form. The tuberous sclerosis complex (TSC) consists of TSC1 (scaffold protein) and TSC2 is a GTPase-activating protein (GAP) which inhibits Rheb through hydrolysis of Rheb-GTP to Rheb-GDP, thereby inhibiting mTOR activity. AKT mediates phosphorylation of TSC2, leading to a decreased GAP activity and reduced mTOR inhibition. Signaling through the Ras GTPase by growth factors may also activate mTORC1 through the RAF/MEK/ERK axis whereby

extracellular signal-regulated kinase (ERK) leads to direct phosphorylation of TSC2 and raptor or via the RSKs(Roux & Topisirovic, 2018,Roux & Topisirovic (2012),Laplante & Sabatini (2012)).Protein synthesis is the most energy expensive process within cells(Buttgereit & Brand, 1995).Therefore,regulation of mRNA translation is tied to cellular energy levels. AMP-activated protein kinase (AMPK) is a kinase activated by increased AMP/ATPratioas well as ADP/ATP ratios. AMPK inhibits protein synthesis by activation of TSC2, thereby reducing mTOR activity. Furthermore, cellular oxygen levels are linked to ATP production, where low oxygen levels reduce ATP production leading to AMPK activation(Leibovitch & Topisirovic, 2018).mTOR modulates global mRNA translation mainly throughmodulation of 4E-BPs and S6Ks(Bruno D. Fonseca et al., 2014,Hay & Sonenberg (2004)). However,mTOR also mediates selectivemRNA translation (Gandin et al., 2016). Upon activation, mTORphosphorylates 4E-BPsleading to release of eIF4E(Roux & Topisirovic, 2018,Saxton & Sabatini (2017)). As described above eIF4E then facilitates assembly of eIF4F, whichis essential for cap dependent mRNA translation initiation. S6Ks (S6K1 and S6K2) phosphorylates multiple componentof the translational machinery such as RPS6which is implicated in ribosome biogenesis(B. Magnuson, Ekim, & Fingar, 2012). Furthermore, S6Ks also phosphorylateeEF2 kinase which is a negative regulator of protein synthesis(X. Wang et al., 2001).Lastly, S6Ks phosphorylate programmed cell death 4 (PDCD4)triggering its SCFbetaTrCP-dependent degradation (Carayol et al., 2008).PDCD4is a factor blocking the eIF4A-eIF4G interactionby binding to eIF4A.Binding of PDCD4 to eIF4A leads to inhibitionof eIF4A activity and thus translation of mRNAsthat require RNA helicase activity(Yang et al., 2003). More recent work indicates an effect of mTORC1on LA motif (LAM)-containing factor family La-related protein 1 (LARP1). In that study theconserved RNA-binding protein of LARP1interacts with raptor and is phosphorylated by mTORC1.However, the scope of LARP1 mediated effectsremaincontroversial.It has been suggested LARP1 stabilizes or regulates translation of mRNAs with the

terminal oligo pyrimidine (TOP) motif in a context dependent manner[Tcherkezian et al. (2014),Bruno D Fonseca et al. (2015),Deragon & Bousquet-Antonelli (2015)).

**1.4.2. The integrated stress response** eIF2 delivers Met-tRNAi to the 40s ribosomal subunit (33). During the integrated stress response (ISR) the alpha subunit of eIF2 is phosphorylated on Ser51 which leads to a global suppression of 5' cap dependent mRNA translation. Upon eIF2alpha phosphorylation, eIF2alpha directly engages the guanine nucleotide exchange factor eIF2beta. eIF2beta converts the inactive eIF2-GDP to eIF2-GTP, therefore eIF2alpha phosphorylation limits eIF2 recycling of Met-tRNAi to the ribosome (33). Simultaneously, eIF2alpha phosphorylation stimulates selective translation of mRNAs with upstream open reading frames (uORFs) such as ATF4 which is a transcription factor that plays a crucial role in the adaptation to stress (34). There are multiple kinases, activated depending on the cellular stress, which phosphorylate eIF2alpha. These kinases include Protein kinase R-like endoplasmic reticulum kinase (PERK) which is activated by misfolded peptides in the endoplasmatic reticulum (ER), Heme regulated eIF2alpha kinase (HRI) which is activated during oxidative stress, protein kinase R (PKR) which is activated in response to certain viral infections and GCN2 which is activated when cells are deprived of amino acids (4, 35–37). Therefore, several distinct stress origins converge on the same pathway regulating mRNA translation.

**1.4.3. REGULATION OF TRANSLATION THROUGH 5' AND 3' UTR STRUCTURES AND ELEMENTS** Recruitment of the PIC to the 5' UTR is followed by scanning until recognition of a start codon. During scanning a process called leaky scanning can occur where the first encountered AUG is not recognized due to sub-optimal sequences flanking the start codon. Leaky scanning is influenced by eukaryotic elongation factors (eEF) 1 and eEF5 where high levels of

eEF1 promote leaky scanning and blocking of non-cognate initiation whereas eEF5 works antagonistically to eEF1. Nonetheless, translation is most favorably initiated when an AUG is encountered with the "Kozak" context. Near cognate triplets e.g. NUG can also initiate translation at a much lower frequency as compared to cognate triplets (10). Structures in the 5' UTRs can influence translation initiation e.g. stem loops (SL) like the iron responsive element (IRE), which regulates translation of mRNAs involved in iron homeostasis depending on iron availability (38) and RNA G-quadruplexes which block scanning (39). Therefore, the degree of structure of a 5' UTR can be an indicator whether an mRNA's translation efficiency is regulated or not. In eukaryotes the vast majority of mRNAs have 5' UTRs with a median length ranging from 53 to 218 nucleotides, where humans have 5' UTRs with the longest median length. Furthermore, mRNAs with long and structured 5' UTRs often encode for proteins related to proliferation, survival, and metastasis (39, 40). Next to 5' UTR structures affecting cap dependent mRNA translation there are also cap independent regulators of mRNA translation such as the viral or cellular internal ribosome entry sites (IRES). The scope of the cellular IRES is still controversial, however cellular IRES are thought recruit the 43s ribosomal subunit towards the 5' UTR (41). Additionally, eIF3 was suggested to directly bind to stem loop structures of a subset of mRNAs and repress or activate their translation (42, 43). RNA modifications in the 5' UTR could also potentially regulate mRNA translation such as the m6A modification, which can serve as an alternative cap and binds eIF3 to initiate translation or to assist ribosome scanning (44–46). Sequence elements within the 5' UTR are also a found to regulate mRNA translation e.g. mRNAs encoding for mitochondrial proteins with an extremely short (~12 nucleotides) 5' UTR which harbors the translation initiator of short 5' UTR (TISU element). These mRNAs undergo scanning free translation initiation (47). Another well-studied 5' UTR element is the 5' terminal oligo pyrimidine (TOP) element, which consists of a C at the 5' terminus followed by a stretch of 4-15 pyrimidines (48). These TOP mRNAs are fully dependent on the C at

the 5' cap. Translation of TOP mRNAs is tightly linked to mTOR activity and is often considered as mTOR dependent translation, where mTOR almost fully controls their translational efficiency (49). Nevertheless, TOP mRNAs can contain other regulatory elements in their 5' UTR alongside the TOP motif, which can override the TOP element translational control in a context dependent manner (50). The importance of the poly-A tail has been observed in several studies where the poly-A tail promotes efficient translation. mRNAs with short poly-A tails generally have a lower translational efficiency, however loss of a poly-A tail does not lead to complete inhibition of protein synthesis (51). Furthermore, there are many RNA- binding proteins binding to RNA elements in the 3' UTR such as PABP, CEBP and LARP1 which confer translational control (18, 31, 52, 53). Cytoplasmic polyadenylation elements (CPEs) are U-rich sites in the 3' UTR (UUUUUAU) on which RNA binding proteins can bind (53). Cytoplasmic polyadenylation element binding proteins (CPEBs) are able to recruit either poly(A) polymerases e.g. terminal nucleotidyltransferase 2 (TENT2) or deadenylation enzymes like the CCR4/NOT complex (54). Therefore, the interaction of CPEBs with the recruited enzymes dictates whether a poly(A) tail is shortened or extended. Next to their predominant role in polyadenylation some members of the CPEB family are known to bind to general translation regulation factors, where CPEB4 binds eIF3 (55) and CPEB1 regulates mRNA stability by binding to PABPC1 and PABPC1L (56, 57). Poly-A-binding protein (PABP) is a multifunctional protein contributing to mRNA processing, stability and translation and is thought to bind to the 3' UTR. Regulation of translation by PABP is achieved through binding to various components of the translational machinery. These components include eIF4B, an initiation factor that aids RNA helicase unwinding function, and eIF4G , eukaryotic Release factor 3 (eRF3) which supports a role of PABP in ribosome recycling, and eIF3 (58–60). Lastly, the PABP-eIF4G interaction forms the closed loop complex that connects the ends of the mRNA. In conclusion, dynamic modulation of mRNA translation can be achieved through several distinct structural features

and sequence elements in both UTRs of an mRNA. mRNA translation can be regulated at a global level i.e. reduction of protein synthesis for a large portion of the transcriptome. Furthermore, a more selective regulation of mRNA translation can be achieved through various mechanisms, which increases the complexity of the regulation of mRNA translation greatly.

## 1.5. Expertimental methods to measure mRNA translation

**1.5.1. Polysome profiling** is a technique to measure changes in translational efficiencies of mRNAs between two or more conditions. Polysome profiling allows for separation of polysomes from monosomes, ribosomal subunits and messenger ribonucleoprotein particles (mRNPs). During the assay, ribosomes are immobilized on the mRNAs using translation elongation inhibitors (e.g. cycloheximide). Cytoplasmic RNA extracts are then sedimented on a linear sucrose gradient (5-50%) using ultra centrifugation. The resulting gradient is fractionated and mRNAs with different number of bound ribosomes can be extracted and analyzed for changes in translational efficiency (74). Changes in translational efficiency of an mRNA can be observed by shifts of polysome association for mRNAs from the light (inefficiently translated) towards the heavy (efficiently translated) polysome fractions or vice versa. Quantification of mRNA levels within each fraction can be assessed using Northern blotting or reverse transcription quantitative polymerase chain reaction (RT-qPCR). For transcriptome wide studies, pooling of efficiently translated mRNAs (mRNAs with >3 bound ribosomes) followed by quantification using either DNA-microarrays or RNA sequencing is common. Pooling of mRNAs as well as collection of multiple fractions makes polysome profiling inconvenient when dealing with large samples sizes or experiments with low amounts of input RNA. Therefore, an optimized sucrose gradient was developed where most efficiently translated mRNAs are collected on a sucrose cushion and thereby can be isolated from one single fraction (75). This optimized gradient allows for application of polysome profiling in small tissue samples where RNA quantity is limiting and reduces labor intensity of the assay. Polysome-associated mRNA

levels are subject to changes in translation efficiency as well as factors contributing to cytosolic mRNA levels. Mechanisms such as transcription or mRNA stability can affect cytosolic mRNA levels which impacts the pool of mRNAs that can be associated to polysomes. Therefore, to identify bona fide changes in translation efficiency it is important to collect cytoplasmic mRNA levels in parallel to polysome-associated mRNA to correct for such mechanisms (e.g. transcription or mRNA stability) during downstream analysis (74, 76).

**1.5.2. Ribosome profiling** is a technique that enables sequencing of ribosome protected mRNA fragments (RPFs). In the assay ribosomes are immobilized on the mRNAs using, similar to polysome profiling, translation elongations inhibitors (e.g. cyclohexamide) (77, 78). One limitation with the use of translation elongation inhibitors is the distortion of ribosome distributions especially at translation initiation sites. These introduced artefacts need to be accounted for in the downstream analysis when assessing ribosome position along the mRNA. Following the translation elongation inhibitor treatment, cells ought to be immediately flash frozen using liquid nitrogen. Alternatively, using only flash freezing has been seen as a robust approach in a wide range of diverse organisms (79). Generation of RPFs is achieved by RNAse treatment breaking the links of RNA between ribosomes leaving single ribosomes with a ~28 nucleotide long RNA fragment within each ribosome. RPFs are then isolated using ultra centrifugation through a sucrose cushion. Co-migration of RNA fragments such as structured non-coding RNAs or large ribonucleoprotein complexes within the sucrose gradient can be a cause of contamination and thereby can provide false readouts of translation. A polyacrylamide gel loaded with RPFs and a reference ladder is used to select RPFs of the right size. Typically, RPFs with lengths of 25 to 30 nucleotides are selected. The RPFs can then be pooled if sample specific barcodes are used. After size selection a pre-adenylated DNA is ligated to the RPFs. This RNA-DNA construct is then used as template for reverse transcription. Through gel-based purification,

full-length products of the reverse transcription are selected and circularized. Following circularization, a double stranded DNA library is constructed and PCR amplified. This library is suitable for quantification using RNAseq. In parallel to RPF selection, randomly fragmented total mRNA of the same size is also retrieved. This is achieved by extraction of total mRNA from cell lysate followed by purification via recovery of polyadenylated messages or removal of ribosomal RNA. Fragmentation of total RNA is done using an alkaline fragmentation buffer (78)(79).

**1.5.3. Comparing ribosome and polysome profiling** to measure changes in mRNA translation. Albeit both methods generate count data after quantification with RNAsequencing, there are some key aspects that differ between the techniques. Polysome profiling separates efficiently translated mRNAs from non- efficiently translated mRNAs thereby creating an mRNA based perspective for analyzing changes in translational efficiencies. In contrast, ribosome profiling determines translational efficiencies by counting the number of RPFs of both efficiently and non-efficiently translated mRNAs. Changes in translational efficiencies, e.g. shifts between the polysomal fractions, can be dramatic (I.e. near complete dissociation of ribosomes from an mRNA) or subtle (shifts from 2 to 4 ribosomes) (80). Ribosome profiling has been shown to be biased towards identification of dramatic shifts of associated ribosomes to mRNAs, whereas subtle shifts are masked which can lead to false biological conclusions. Polysome profiling is affected by this to a much lesser extent (81). RPFs in ribosome profiling can identify exact nucleotide positions occupied by ribosomes thereby offering single nucleotide resolution. Polysome profiling cannot reveal ribosome locations along the mRNA. However, polysome profiling allows access to full-length mRNAs that includes the UTRs. The single nucleotide resolution of ribosome profiling is necessary in contexts studying local translation events such as ribosomal frame shifts (82) or uORF translation (37). Higher sensitivity in detecting changes in translational efficiencies on a global scale

makes polysome profiling more suitable for transcriptome-wide studies (83). Both methods have their strengths and weaknesses and therefore each method should be considered depending on the underlying biological question of each experiment.

**1.5.4. Regulatory modes of gene expression** In transcriptome-wide studies of translation efficiencies the interplay between total mRNA and translated mRNA levels are interrogated. Traditionally it was thought that changes in translation efficiencies lead to altered protein levels. A change in translation efficiency is observed for mRNAs whose polysome-association is altered whereas their total mRNA does not change to a similar magnitude as the polysome-association (I.e. change in translation). An example thereof is TOP mRNA translation under conditions where mTOR is stimulated (81). In recent years, evidence emerged where translation efficiencies of mRNAs can be altered to compensate for changes in total mRNA levels. Within this newly identified mode of regulation of mRNA translation "translational buffering", mRNA translation is altered such that changes in total mRNA levels do not influence their corresponding protein levels (76, 84, 85). Translational buffering is observed to compensate for inter-tissue, inter-species and inter-individual difference (86–88). Furthermore, in bacteria translational buffering maintains protein complex stoichiometry as well as protein levels for conserved pathway across species (89, 90). Recently translational buffering has been observed under conditions where estrogen receptor alpha (ERalpha) is depleted. ERalpha modulates activity of specific tRNA modification enzymes. These enzymes are needed for the U34 tRNA modification. Loss of ERalpha led to reduced U34 tRNA modification thereby hindering translation of mRNAs requiring such modified tRNAs. For these mRNAs, even though their total mRNA levels were induced across conditions, their protein levels remained constant (85). Given these multiple roles of mRNA translation to regulate the proteome it is critical to distinguish them as their underlying mechanisms can have different biological implications.

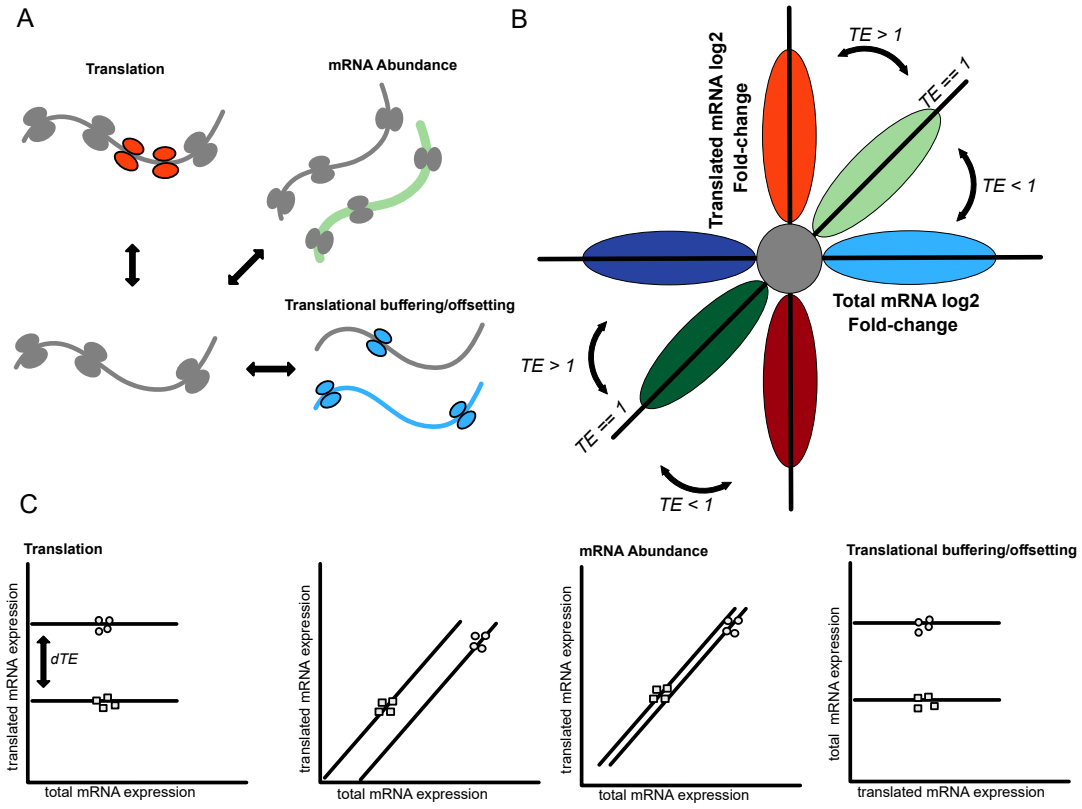


Figure 1.2: Regulatory modes of gene expression - A) simplifed overview. B) Schematic representation of regulatory modes of translation efficiency in a fold-change scatter plot. C) Schematic representation of the regulatory modes of translation efficiency data in anota2seq models.

### 1.5.5. Algorithms for analysis of changes in Translational Efficiencies

Initial approaches for analysis of transcriptome-wide translation studies used an approach called the translation efficiency (TE-score). This score calculates the ratio of the ratios between polysome-associated mRNA levels divided by total mRNA levels within each condition. The TE- score approach has been shown to be prone to spurious correlations. The ratio of polysome-associated mRNA and total mRNA can systematically correlate with total mRNA levels and thereby lead to elevated false positive findings. Over the recent years several algorithms to analyze polysome-profiling and ribosome-profiling data have been developed

these include babel (91), DESeq2 (92), RiboDiff (93), Xtail (94). Although these methods have their distinct approach to identify changes in translation efficiencies, their principle of analysis is similar to comparing a ratio of ratios. Therefore these methods suffer from similar issues as the TE-score. Another downside for ratio of ratios-based methods is their inability to separate changes in translational efficiencies altering protein levels from translational buffering as any change in the nominator or denominator of any ratio will implicate a difference in the ratio regardless of its origin. The ANalysis Of Translational Activity (anota) (95) algorithm follows a regression-based approach which couples analysis of partial variance (APV or ANCOVA) (96) with the random variance model (RVM)(97). This approach does not suffer from spurious correlations. The anota algorithm was initially developed for analysis of DNA-microarray data, more recently the anota algorithm has been adapted to accommodate data from RNA-sequencing and released as anota2seq (76). During the development of anota2seq the anota algorithm was e.g. adapted to enable the user to statistically separate multiple regulatory modes of gene expression including changes in translation as well as translational buffering. Thus, anota2seq allows for efficient interrogation of the translatome without being affected by spurious correlations (76).

## 2. Aims of this thesis

The aims of this thesis are to explore the regulation of gene expression in cancer, more specifically we investigate perturbations of gene expression in different cancer models as a response to drug treatment.

In **Study I** we adapted an algorithm for ANalysis Of Translation Activity data (anota) so that it could be applied to next generation sequencing data. The resulting algorithm was named anota2seq.

We then applied the anota2seq algortihm to invesitigate changes in translation efficiencies in two cancer models:

In **Study II** we unravelled the effects of eIF4A, an RNA helicase, inhibition using a synthetic rocaglate CR-1-31-B (CR-31) in pancreatic ductal adenocarcinoma.

In **Study III** we explored the effects of insulin on gene expression in a breast cancer cell line.

# 3. Results and discussion

## 4. Conclusions

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Christina is awesome.

I am sorry for all the other people of this page but no one else helped me more than my 8 paws of awesomeness Felix and Dexter. These little litter shitters have been an extreme joy to be around and kept me sane during the insanity that is writing a thesis. **Meow**

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