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Departamento de Biologia

Inês Manuela do Vale Santana

Stress Proteotóxico na Estabilidade do Genoma Humano

Proteotoxic Stress in Human Genome Stability

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Proteotoxic Stress in Human Genome Stability

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica do Doutor Manuel António da Silva Santos, Professor Associado do Departamento de Biologia da Universidade de Aveiro.

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agradecimentos

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palavras-chave Mistranslation, stress proteotóxico, tRNA, alterações no genoma.

resumo

A introdução de erros durante a síntese proteica é um processo denominado mistranslation, do qual resulta a incorporação errónea de aminoácidos na proteína. Este processo compromete a aquisição da estrutura final correcta das proteínas e consequentemente a sua acumulação. As células apresentam mecanismos de controlo de qualidade de proteínas (PQC) que contrariam esta acumulação mas, se sobrecarregados, as proteínas podem agregar umas com as outras provocando danos nas células. A mistranslation está associada com vários efeitos tóxicos e diversas doenças. Recentemente, a mistranslation foi associada com alterações no genoma da levedura, que aumentam a sua capacidade adaptativa.

Neste trabalho, células HEK293 foram transfectadas com tRNAs mutados para demonstrar como o processo de mistranslation afecta o genoma das células. Para tal, um $tRNA_{AGA}$ de serina foi mutado no anticodão e transfectado nas células, criando cinco linhas celulares diferentes que incorporam serina em codões de Ala, Leu, Val, e His. Foi também construída uma linha contendo um plasmídeo vazio (Mock). As linhas celulares criadas foram estudadas por citometria de fluxo e microscopia de flourescência, de modo a avaliar a viabilidade e proliferação celular, o conteúdo em DNA e a acumulação de micronúcleos.

Os resultados mostram que o nível de mistranslation provocado nas células não afecta a viabilidade celular mas pode provocar algumas alterações no DNA. A proliferação celular foi afectada na linha celular que incorpora serina em codões de leucina, se observou uma paragem do ciclo celular nas fases S ou G2/M. Em relação aos micronúcleos, verificou-se um ligeiro aumento nas linhas celulares que incorporam serina em codões de alanina e leucina.

Este modelo de estudo permitiu entender os efeitos dos níveis de mistranslation no genoma de células humanas e mostrar que estas células são bastante resistentes à acumulação de proteínas aberrantes.

keywords Mistranslation, proteotoxic stress, tRNA, genome alterations.

abstract

Incorporation of errors during protein synthesis is denominated mistranslation. This compromises the acquisition of a correct final three dimensional structure and consequently the accumulation in the cell of proteins with abnormal conformations. Cells have protein quality control (PQC) mechanisms to counteract the accumulation of aberrant proteins, but when these systems are overloaded proteins aggregate, generating proteotoxic stress. Mistranslation is associated with several diseases and with genomic alterations in yeast that increase adaptation potential.

In this work, HEK293 cells were transfected with mutated tRNAs that misincorporate serine at various non-cognate codon sites. For this, a serine $tRNA_{AGA}$ was mutated in the anticodon and transfected into the HEK293 cells, creating five different cell lines that incorporate serine at Ala, Leu, Val, and His codons; one line was transfected with an empty plasmid (Mock). These cell lines were studied by flow cytometry and flourescence microscopy in order to evaluate their viability, proliferation, DNA content and micronuclei accumulation.

The results show that mistranslation induced by these tRNAs does not affect the cellular viability but may promote minor DNA alterations. Also, cellular proliferation was compromised in some cell lines, namely in the cell line that misincorporates serine at leucine codons. In this cell line, the cell cycle was arrested in S or G2/M phases. Regarding micronuclei formation, a slight increase was seen in alanine and leucine cell lines.

This cellular model allowed us to investigate the effect of low level of mistranslation on the genome of human cells and showed that human cells are highly resistant to the accumulation of aberrant proteins.

Contents

Abbreviations

aaRS: Aminoacyl-tRNA synthetases;

aa-tRNA: Aminoacyl-tRNA;

ATP: Adenosine triphosphate;

AZC: Azetidine-2-carboxylic acid;

eEF: Eukaryotic elongation factor;

eIF: Eukaryotic initiation factor;

eRF: Eukaryotic release factors;

ER: Endoplasmic reticulum;

ERAD: ER-associated degradation;

GDP: Guanosine diphosphate

GTP: Guanosine triphosphate;

HCY: Homocysteine;

HEK293: Human embryonic kidney 293;

mRNA: messenger RNA;

PAB: Poly(A) binding protein;

PI: Propidium iodide;

PPi: Pirophosphate;

PQC: Protein quality control;

PS: Phosphatidylserine;

rRNA: Ribosomal RNA;

tRNA: Transfer RNA;

UPR: Unfolded protein response**;**

UPS: Ubiquitin-proteasome system;

WT: Wild-Type.

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Introduction

The Genetic Code

The genetic information is translated into proteins through the genetic code. This code is organized in triplets of nucleotides, the codons, which, through the mRNA, are converted in amino acids that are incorporated in proteins. There are 4 different nucleotides (adenine, A; cytosine, C; guanine, G; and thymine, T, in DNA, or uracil, U, in RNA) and since they are organized in triplets, 64 non-overlapping codons can be formed: 61 codons encode amino acids, while the other 3 represent stop codons, responsible for the termination of protein synthesis (Figure 1) (Griffiths, Gelbart, & Wessler, 2004; Lewin, 2008; Pierce, 2010).

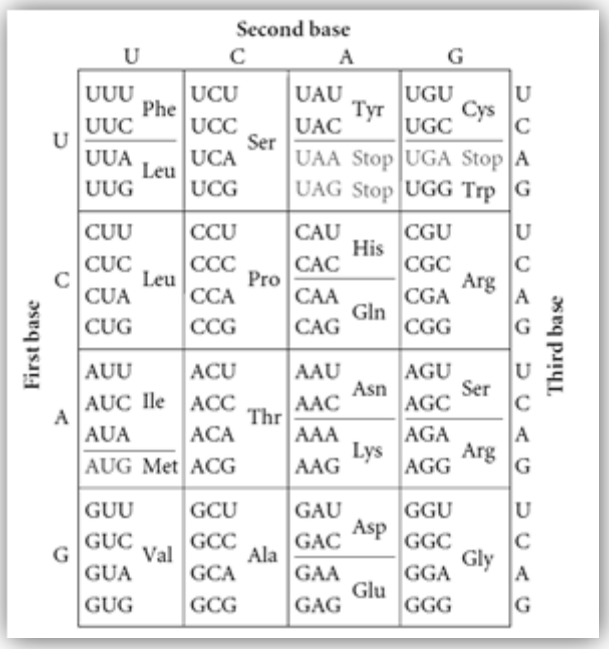


Figure 1: The genetic code comprises 64 codons: 61 encoding amino acids and 3 that terminate the proteins synthesis (Pierce, 2010).

Proteins are formed by 20 different amino acids. Since there are 64 codons, one amino acid can be encoded by more than one codon; only methionine and tryptophan are exceptions, both are encoded by one codon only. This demonstrates that the genetic code is degenerated (Griffiths, Gelbart, & Wessler, 2004; Lewin, 2008; Pierce, 2010).

Synonymous codons, i.e., that codify the same or a related amino acid, usually have similar sequences, and in most cases the third base of the codon does not have much significance. This is called third-base degeneracy. One example is alanine: it is encoded by four codons that differentiate only in the third base. The fact that similar codons codify similar amino acids reduces the effects of mutations since alterations in one random base can result or not in the substitution of one amino acid for an equivalent one (Lewin, 2008; Pierce, 2010).

Another characteristic of the genetic code is that one codon can be recognized by more than one tRNA. This occurs because the anticodon loop of the tRNA allows some flexibility at the first base of the anticodon, while the pairing of the other two bases follows the Watson-Crick rules of pairing. This is the Wobble Hypothesis which claims that the first base of the anticodon is able to pair with alternative bases in the third position of the codon (Lewin, 2008; Pierce, 2010).

The Cell Cycle

Cells can only originate from other cells. This process is called cell division or cell cycle and is constituted by two stages: the interphase and the M phase (Figure 2). Interphase is the time when the cell grows and prepares for its division and it's divided into several phases. In the G1 (Gap1) phase, the cell increases its size and it's prepared to DNA synthesis. In the S (Synthesis) phase occurs chromosome duplication. Finally, there is another gap time, the G2 phase, where the cells continues to grow and prepares to M phase. There is yet another gap phase, the G0 phase, where the cell left the cycle and is considered a resting step. In mammalian cells, this phase can extend for days or weeks, depending on the cell type. The M phase lasts about an hour in mammalian cells, and is sub-divided into two steps: mitosis, where the genome is duplicated and separated into two nuclei, and the cytokinesis, in which the cytoplasm divides, originating two new cells (Alberts, et al., 2004; Karp, 2010).

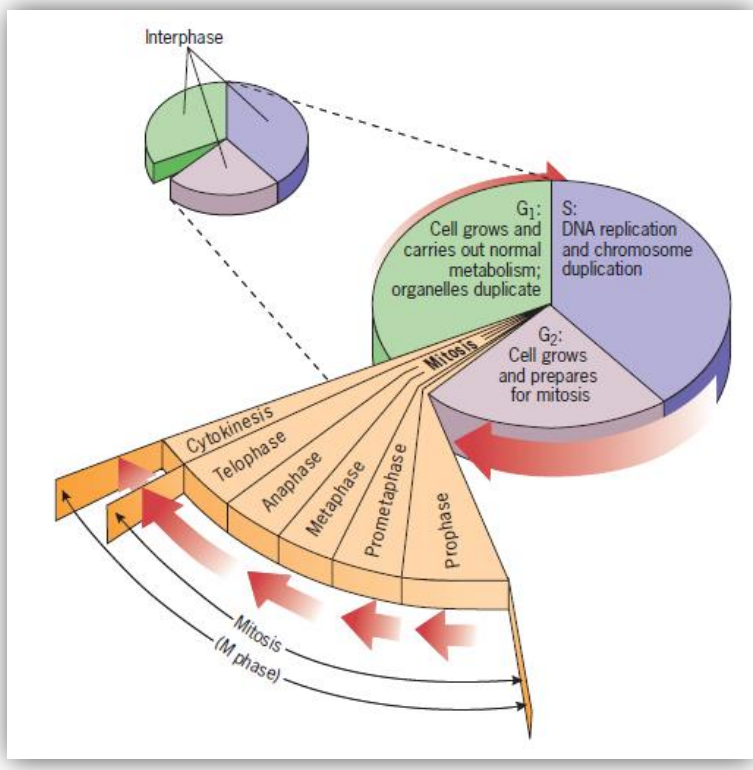


Figure 2: The cell cycle is composed by interphase, which comprises G1, S, and G2 phases (there is another phase, G0, not represented in the figure), and the M phase, divided into mitosis and cytokinesis (Karp, 2010).

- Cell Cycle checkpoints

The cell has to assure that the DNA is in perfect conditions during its division. For that, at the end of almost every phase of the cell cycle, exists a checkpoint. These are surveillance mechanisms that can stop cell division if some problem occurs in the DNA. If some damage occurs to any chromosome, if there is a problem during DNA synthesis, or if the chromosomes are not properly aligned to division, cause the cell to stop the cycle and an attempt to fix the problem. If that is not possible, then the cell cannot progress in the cell division (Alberts, et al., 2004; Karp, 2010).

RNA Molecules

The cell read the information encoded in the genome and convert it into proteins. This flow of information from DNA to protein is initiated with the conversion of the DNA into mRNA. Not all the DNA is converted in mRNA at once; because not all the genes products are needed at the same time, or in the same cell, only one gene, sometimes a few genes, are transcribed to RNA (Karp, 2010; Pierce, 2010).

RNA is similar to DNA, but is constituted by a single strand, formed by ribonucleotides. There are many types of RNA: messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA (miRNA), small interfering RNA (siRNA), small nucleolar RNAs (snoRNA), to name only a few.

The rRNA is the principal component of the ribosome and is responsible for the major activities of the ribosome, such as selection of tRNAs or peptide bond formation (Pierce, 2010).

In eukatyotics, the mRNA produced in the nucleus is transported to the cytoplasm for translation. mRNA interacts directly with the ribosome and the tRNAs. The latter make the bridge between the mRNA and proteins. They are the link that converts the information encoded in the mRNA into amino acids in the polypeptide chains. There are several different tRNAs, and each one attaches to a specific amino acid: all tRNAs have anticodons, complementary to the codons in the mRNA, that specify which amino acid must be incorporated in the polypeptide chain (Karp, 2010; Pierce, 2010).

Due to the existence of complementarity between some nucleotides, hydrogen bonds are formed, giving the tRNAs a cloverleaf structure (Figure 3), that folds into itself, giving the tRNAs a L-shaped tertiary structure. The tRNA structure is made by: the acceptor arm, which has a specific unpaired sequence at the 3’ end, CCA, responsible for the attachment of the amino acid; the T arm; the D arm; the variable arm; and the anticodon arm, that contains the anticodon triplet (Lewin, 2008; Pierce, 2010).

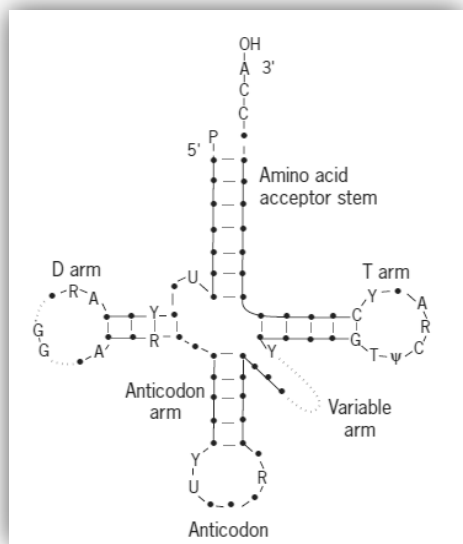


Figure 3: Cloverleaf structure of transfer RNA. It is composed by the acceptor arm, which interacts with the amino acids, the D arm, the T arm, the variable arm, and the anticodon arm. The anticodon is complementary to the codons on the mRNA that specify which amino acid should be incorporated in the polypeptide chain (adapted from Karp, 2010).

- tRNA aminoacylation reaction

Aminoacylation of the tRNAs consist in the attachment of the cognate amino acid to the 3' end of the tRNA. There are different tRNAs that can bind to one amino acid, but there is only one correct amino acid to each tRNA. This aminoacylation process is mediated by the aminoacyl-tRNA synthetases (aaRS). These enzymes are divided in two classes, class I and class II, each one comprising 10 enzymes. Class I enzymes are characterized by the presence of HIGH and KMSKS motifs, responsible for ATP-binding, and the Rossmann fold, responsible for the nucleotide binding. Class II enzymes have motifs 1, 2 and 3 that act as the enzymes active site. There are 20 different kinds of aaRS (one for each amino acid), and each one is responsible for the attachment of the correct amino acid to the correct tRNA, as they recognize the amino acid and the respective tRNA before the charging (Arnez & Moras, 1997; Karp, 2010).

The tRNA aminoacylation happens in two steps. Firstly, there is the amino acid activation with adenosine triphosphate (ATP), and then the transfer of the activated amino acid to the tRNA. This process can be demonstrated by the following reactions:

1) amino acid + ATP → aminoacyl-AMP + PP_i

2) aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP

This process requires ATP as an energy source for the binding of the amino acid to the tRNA, and produces aminoacyl-tRNA, adenosine monophosphate (AMP) molecule and pyrophosphate (PP_i) (Karp, 2010; Pierce, 2010).

Translation

Translation is the mechanism by which amino acids are assembled into proteins. The main machinery needed for translation, among other molecules, is the ribosome. In eukaryotic cells the ribosomes are composed by the small (40S) and large (60S) subunits, which contain the rRNA, and their central task is the reading of the mRNA and the linking of the amino acids in the correct order. The sequence of a coding strand of mRNA, read in the direction from 5' to 3', consists of codons corresponding to the amino acid sequence of a protein read from N-terminus to C-terminus (Lewin, 2008).

This mechanism can be divided into 3 different main steps: initiation, elongation, and termination. At the end of translation there is recycling of the components of the translation machinery, so they can be used in another round of protein synthesis (Pierce, 2010).

- Initiation

During translation initiation there is assembly of the small unit of the ribosome with the initiator tRNA and with the mRNA, identification of the initiation codon (the AUG codon), and joining of the large ribosomal subunit [Figure 4(a)], forming the initiation complex (Karp, 2010; Pierce, 2010).

A critical component of the initiation complex is the formation of the ternary complex, eIF2–GTP–Met-tRNA$_i$, composed by the initiator tRNA, the Met-tRNA$_i$, the eukaryotic initiation factor 2 (eIF2), and one molecule of GTP (**g**uanosine triphosphate). Then, the ternary complex binds to the small subunit of the ribosome, through the action of the initiation factors eIF1, eIF1A and eIF3, forming the 43S complex. When the ribosome is not translating, the small (40S) and large (60S) subunits are linked together (80S ribosome), and the ternary complex cannot bind to the small subunit. For this, the two subunits of the 80S ribosome must be separated. This is possible through the action of the eIF3. This factor binds to the small subunit and prevents binding with the large subunit [Figure 4(a)] (Kapp & Lorsch, 2004; Karp, 2010; Pierce, 2010).

The next step is the binding of the 5’ end of the mRNA to the initiation complex. Several different eIFs, in conjunction with the poly(A) binding protein (PAB), charge the mRNA into the small subunit. After this, the 43S complex starts scanning the mRNA in the

5'-3' direction, searching for the AUG codon. When this complex finds the initiation codon, interaction between codon in the mRNA and the anticodon in the tRNA stalls the scanning ribosome, triggering the hydrolysis of GTP by eIF2. Thereafter, the initiator tRNA is released into the P site of the ribosome by the eIF2–GDP that, then, leaves the complex, as do the others initiation factors. The complex eIF2–GTP is one of the most important components in this step, and it is recycled by the eIF2B after each round of initiation, in order to be used again (Kapp & Lorsch, 2004; Pierce, 2010).

The stalled 40S subunit at the AUG initiation codon permits the binding of the 60S subunit with the help of the factor eIF5-GTP, with hydrolysis of GTP, promoting the dissociation of eIF5-GDP from the complex [Figure 4(a)] (Karp, 2010).

- Elongation

This is the step where the proteins are actually made. The ribosome has three different sites that are occupied with tRNAs during elongation: the aminoacyl (A) site, the peptidyl (P) site, and the exit (E) site. During initiation, the initiator tRNA binds with the ribosome in the P site, where the AUG codon is positioned while the adjacent A site is free. Then, peptide chain elongation begins [Figure 4(b)] (Kapp & Lorsch, 2004; Karp, 2010).

One aminoacylated tRNA, matching the codon in the mRNA, is transported to the A site, in a ternary complex made by aa-tRNA–eEF1A–GTP. Correct codon-anticodon interactions are necessary for the hydrolysis of GTP by eEF1A, and only occur if the tRNA is the correct one. This promotes a conformational change in the ribosome. With GTP hydrolysis, the eEF1A-GDP complex is released from the ribosome, leaving the aminoacyl-tRNA in the A site, with the right conformation to continue in the peptide chain formation. In this step, the eEF1A-GDP must be recycled to its GTP form, in order to participate in other rounds of elongation [Figure 4(b)] (Kapp & Lorsch, 2004; Karp, 2010; Pierce, 2010).

In the next step, a peptide bond between the new amino acid and the nascent peptide chain is formed by the peptidyl transferase center in the ribosome 60S subunit. The new amino acid leaves the A site to the P site, but continues attached to the tRNA. So, the ribosome contains the deacylated tRNA in the E site, peptidyl-tRNA in the P site, and a free A-site to accept a new tRNA. The ribosome is translocated by eEF2, in the direction 5' to 3', promoting the transfer of the deacylated tRNA to the E site, and the translocation of

the A-site tRNA to the P site. The mRNA moves along with the complex, so the codons are always matching the right anticodon. This step is repeated until a stop codon (UAA, UAG, or UGA) is found, and termination of elongation starts [Figure 4(b)] (Kapp & Lorsch, 2004; Karp, 2010; Pierce, 2010).

- Termination

After the ribosome encounters a stop codon on the A site, the process of termination begins. The result of this process is the discharge of the peptide chain, through the break of the bond connecting the peptide to the tRNA on the P site. Since there are no tRNAs with anticodons matching the stop codons, proteins called eukaryotic release factors (eRF) bind to the A site, and promote the release of the newly formed protein [Figure 4(c)] (Kapp & Lorsch, 2004; Karp, 2010).

Two classes of eRFs are known. Class I eRFs (eRF1) recognize one of the stop codons (UAA, UAG, or UGA) in the ribosomal A site, and hydrolyze the polypeptide chain, that is released into the cytoplasm. The class II eRFs (eRF3) promote the release of the eRF1 from the A site. To finish the translation process, the deacylated tRNA is released from the P site, the mRNA is dissociated from the ribosome, and the latter is disassembled so that the subunits can be used again [Figure 4(c)] (Karp, 2010).

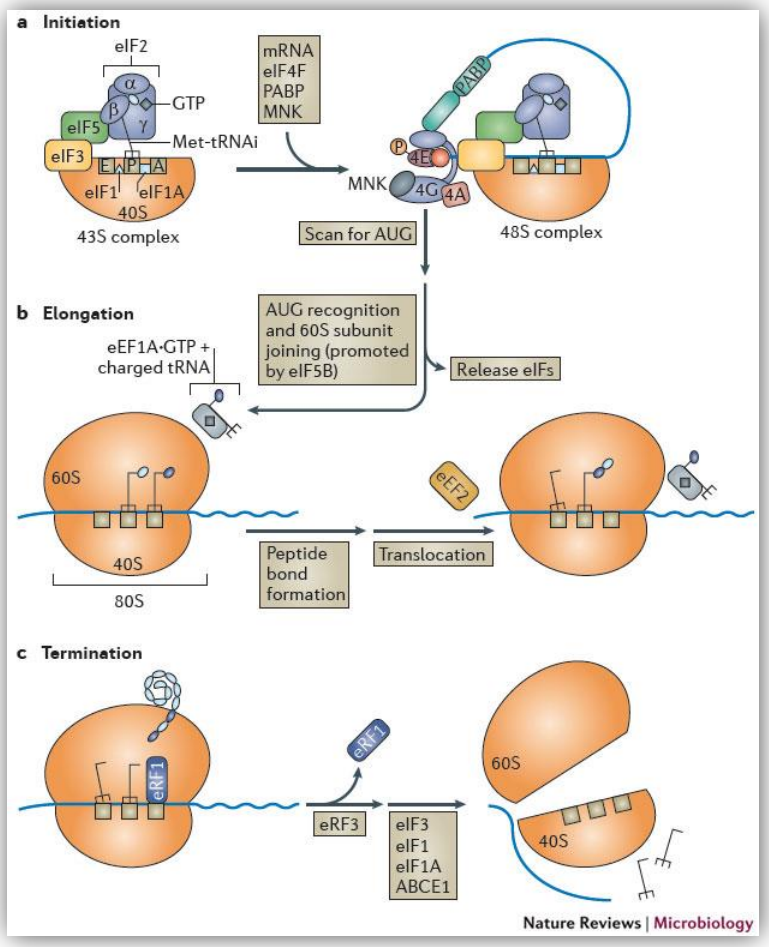


Figure 4: Process of translation showing the (a) initiation, (b) elongation, and (c) termination steps and the principal components involved in each one (Walsh & Mohr, 2011).

Mistranslation

To maintain its normal cellular activity, mRNAs must be accurately translated into the corresponding proteins. Translation is a very accurate process but it is not an error free process: on average there is 1 mistake in every 10^4 codons translated (Drummond & Wilke, 2009; Reynolds et al., 2010).

Translational errors occur at the tRNA aminoacylation and mRNA decoding steps. The aminoacylation is carried out by the aaRS (aminoacyl tRNA synthetase) enzymes and if they mischarge tRNAs, then amino acids are misincorporated in the polypeptide chain. These alterations could result from faulty differentiation of chemically similar amino acids by aaRS (near-cognate amino acids), or because the wrong tRNA is recruited by the aaRS. During decoding of the mRNA, four main types of errors can occur: 1) missense errors result in the replacement of an amino acid by another in the polypeptide chain; 2) nonsense mutations caused by read-through of stop codons, forming proteins with extended C-termini; 3) frameshifting errors, where the reading frame of the mRNA is altered, producing out-of-frame truncated proteins; 4) processivity errors, that consist in the premature termination of translation, producing truncated proteins (Moura et al., 2009; Reynolds et al., 2010).

Although these errors occur at low rates, they can be detected and corrected in all steps of translation, and it is assumed that the cell maintains the highest level of quality control and the lowest level of translational errors (Schimmel, 2011). In order to detect aminoacylation errors, aaRSs developed editing control mechanisms, which decreases the error frequency, and are very important in the translational quality control. With these mechanisms aaRS are able to regulate aminoacylation, preventing the attachment of near- or non-cognate amino acids. The editing mechanisms select cognate aa:tRNA pairs by discrimination between the amino acids available. This is the first checkpoint in aminoacylation and starts in the selection of the amino acid in the active site of aaRS. Since several amino acids are very similar to each other, the aaRS may not be able to discriminate all of them and some errors can occur (Reynolds et al., 2010; Schimmel, 2011).

The ribosome also has proofreading mechanisms. The ribosome must assure that the codon-anticodon pairing in the A-site is correct, even if it cannot discriminate misacylated

tRNAs. If there is a mismatch in the codon-anticodon pairing, the aa:tRNA is rejected by the ribosome. If there is a mismatched codon-anticodon in the P site, robosomal specificity is lost causing a premature termination of elongation and release of the peptide chain. The ribosome also continuously checks if the reading frame is the correct one avoiding frameshifting errors and also premature termination of elongation (Schimmel, 2011).

Some studies show that mutations on the editing site of the mouse alanyl-tRNA synthetase lead to misincorporation of serine at alanine codons, causing the accumulation of misfolded proteins, and therefore loss of Purkinje cells and ataxia (Lee, et al., 2006). Another example in mice, and also in humans, is the incorrect mischarging of tRNAMet with homocysteine (Hcy), which leads to the formation of aberrant proteins, elevating the proteotoxic stress and promoting the risk of vascular diseases (Jakubowski, 2008).

In spite of causing toxicity and leading to heritable mutations, mistranslation can be tolerated and can potentiate adaptation. In vitro studies with *C. albicans* showed that mistranslation resulting from misincorporation of serine (Ser) at leucine (Leu) codons can be advantageous. This ambiguity enables growth of *C. albicans* in environments not favorable for the wild type strain, proving that the disruption of the proteome caused by the mistranslation is not detrimental to the organisms. Instead, it leads to the survival of the cells in hostile environments (Bezerra, et al., 2013).

Protein Misfolding

Protein folding is the process by which a newly formed protein adopts its final three-dimensional conformation. When leaving the ribosome, the polypeptide chain is an amorphous linear chain that has neither the stability nor the capacity to perform its function. So the amino acids must interact between them in order to obtain the final 3-D conformation. To control the folding and to prevent errors, the cell has mechanisms that check for abnormal proteins and try to refold or eliminate them in order to prevent alterations on the normal cellular functions. When proteins are not in the right conformation they expose hydrophobic residues that interact with other normal or aberrant proteins originating protein aggregates. These proteins can even interact with other cellular components diminishing cellular viability and enhancing cellular death (Stefani & Dobson, 2003; Chen et al., 2011).

Maintaining proteome integrity is fundamental for cell viability, but there are several physiological conditions that influence the folding of proteins, such as inefficient protein biogenesis, errors during transcription or translation, mutations in DNA or RNA, or even errors in consequence of defective post-synthetic events (Goldberg, 2003). Higher levels of misfolded proteins can also be produced by neurodegenerative diseases, metabolic and environmental stresses, such as production of reactive oxygen species and exposure to chemicals, respectively, aging and cancer (Chen et al., 2011).

If misfolded proteins are being formed at a high level, the quality control machinery can be overloaded, proteins can aggregate and damage the cell. These aggregated proteins can be more stable than proteins in their native state, leading to diseases like Alzheimer's and Parkinson's (Goldberg, 2003; Chiti & Dobson, 2006; Gregersen et al., 2006).

Cells respond to increased levels of misfolded proteins by activating the protein quality control (PQC) systems. If these systems cannot remove all the misfolded proteins, apoptosis is activated and the cells are eliminated (Gregersen et al., 2006). Cells can up-regulate the quality control components, or can sequester misfolded and/or aggregated proteins (Figure 5). In all these strategies chaperones are present and have a major role in the recognition of the abnormal proteins. They participate in the refolding of the aberrant proteins, or, if this is not possible, the chaperones target these proteins to degradation by the ubiquitin-proteasome pathway (Gregersen et al., 2006; Chen et al., 2011).

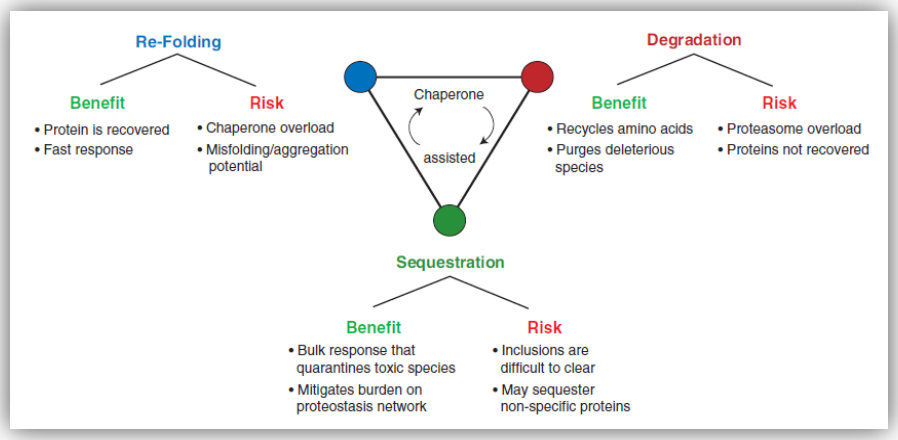


Figure 5: Chaperone assisted strategies to maintain protein homeostasis, showing the benefits and the risks of re-folding, degradation and sequestration (Chen et al., 2011).

Chaperones can be categorized in folding, holding and unfolding chaperones (Figure 6). The folding chaperones assist in the folding of new polypeptide chains. The holding chaperones, e.g. small heat shock proteins (smHSP), bind in a reversibly way to misfolded proteins so they do not aggregate and can be transported to folding chaperones in order to be correctly folded. The unfolding chaperones are responsible for the unfolding of aberrant proteins so they can be either degraded or folded from the beginning. This is an important step because, before degradation, the mutant proteins must be completely unfolded so they can enter the proteolytic machinery (Gregersen et al., 2006).

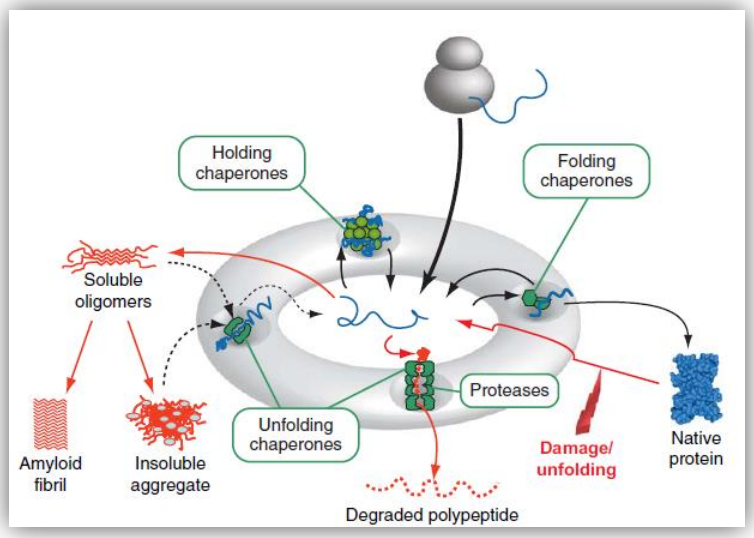


Figure 6: Protein quality control systems involving chaperones. Chaperones can be categorized in folding, holding and unfolding, accordingly to their functions (Gregersen et al., 2006)

Some of the chaperones have also the ability to remove some proteins that are already in aggregates, and transfer them to other chaperones to refolding or to destruction (Goldberg, 2003; Chen et al., 2011).

The chaperones together with the ubiquitin-proteasome system (UPS) are the principal cytosolic PQC system (Gregersen et al., 2006). The UPS is a proteolytic pathway triggered by the accumulation of misfolded or partially folded proteins, and leads to an increase of cellular degradative capacity. The UPS tags misfolded proteins through a cascade of enzymatic ubiquitinations which addresses them to destruction by the 26S proteasome (Chen et al., 2011). The 26S proteasome is a very large protease complex, with one cap bonded to one or both ends. This cap is responsible for the recognition and guiding of only the ubiquitinated proteins into the proteolytic core of the proteasome, avoiding non-specific degradation (Nandi et al., 2006).

The ubiquitination process consists in the transfer of ubiquitin molecules to the abnormal protein, assisted by specific enzymes. This process is repeated several times and a polyubiquitin chain is formed in the targeted protein. Then, the proteasome recognize the ubiquitin in these proteins and directs them to its proteolytic center (Figure 7). Before degradation, ubiquitin needs to be removed from the proteins, in a process called deubiquitination (Gregersen et al., 2006; Nandi et al., 2006).

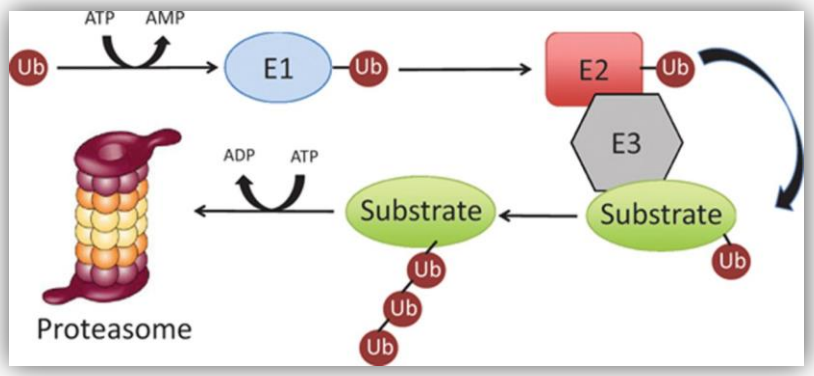


Figure 7: The ubiquitin-proteasome pathway. This is an important mechanism that degrades misfolded proteins tagged with several molecules of ubiquitin (Ub). E1, E2 and E3 are enzymes involved in the ubiquitination process (Pagan, et al, 2013).

Another system of protein quality control involves the secretory pathway, and the endoplasmic reticulum (ER). The ER is associated with the ribosomes and is responsible for co- and post-translational modifications, like folding of new polypeptides chains. If that is not possible, the proteins are degraded by the ER-associated degradation (ERAD). In this process, misfolded proteins are retrotranslocated to the cytosol and destroyed by proteasomes. So, the ER can be considered an important component of the PQC (Römisch, 2005; Gregersen et al., 2006; Karp, 2010).

The ERAD recognizes the hydrophobic sequences and other signs of misfolded proteins, as aggregation tendency or unpaired cysteine residues. The calnexin and calreticulin are some of the elements that interact with the misfolded proteins and assist on their refolding (Gregersen et al., 2006).

Accumulation of misfolded proteins in the ER activates the unfolded proteins response (UPR). This process works through the stimulation of several signals, normally deactivated when the concentration of abnormal proteins is low in the ER. If these proteins reach high levels, the UPR activates the expression of several genes in order to ease the stress on the ER. Among these, there are genes responsible for the production of molecular chaperones that assist in the correct folding of misfolded proteins, genes encoding proteins that ensure the transportation of aberrant proteins into the cytosol for their degradation. If these pathways fail to eliminate the ER stress, apoptosis is activated and the cell is eliminated (Karp, 2010; Cao & Kaufman, 2012).

Another important process responsible for the elimination of aberrant proteins is autophagy. This is a self-destructing pathway used by the cell to eliminate long-lived cytoplasmic proteins and damaged organelles, and also protein aggregates that can provoke cellular damage. It allows the survival of the cell in response to various stressors, protecting the organisms from several diseases, including degenerative diseases like Parkinson's or Alzheimer's. This pathway mediates stress-induced metabolic adaptation and damage control through the formation of autophagosomes that capture and deliver the harmful components to the lysosome. In the case of cellular overload by protein aggregates, autophagosomes can fuse with endocytic compartments, in order to facilitate the elimination of these proteins. In this way, the cell eliminates the damaged organelles or proteins via catabolism, without harming itself, and can recycle nutrients (He & Klionsky, 2009; Chen et al., 2011). Autophagy can also be induced by the endoplasmic reticulum

stress. When the ER is overcharged with unfolded proteins, and refold exceeds the ER capacity, it prompts the cell autophagic pathways (He & Klionsky, 2009).

Mistranslation and Genomic Alterations

Proteotoxic stress can also induce DNA alterations in yeast. Several studies show that proteotoxic stress caused by the action of radicicol, an HSP90 inhibitor, is one of the most efficient inducer of aneuploidies, causing loss of DNA in specific loci (Chen, et al., 2012; Shor, et al., 2013). Moreover, mistranslation in *Candida albicans* allows the cells to survive in toxic environments where wild type cells were not able to survive. This is explained by alterations in the genome induced by mistranslation. Therefore, mistranslation can lead to evolutive genomic alterations (Bezerra, et al., 2013).

Aims of the Study

The main objective of this work was to study the cellular responses to mistranslation at the genome level, using HEK293 cells as an experimental model system. To achieve this we used a genetic system developed in the host laboratory, to create different HEK293 cell lines that mistranslate a single codon in a controlled manner. With this model we wanted to elucidate:

- how mistranslation affect the cell cycle;
- the influence of mistranslation on cellular viability;
- the genomic consequences of the accumulation of misfolded proteins;
- if these cells lines are a good biological model to study genome alterations induced by mistranslation.

Experimental Model System

Human embryonic kidney (HEK) 293 cells were transfected with pIRES-DsRed plasmid containing a serine tRNA gene mutated in the anticodon ($tRNA^{Ser}_{AGA}$) (Figure 8), so that the seryl-tRNA synthetase (SerRS) charges this tRNA with Ser. The mutation in the anticodon leads to amino acid misincorporation at the proteome level. Four cell lines were created with the following anticodon mutations: alanine (Ala; $tRNA^{Ser}_{AGC}$), leucine (Leu; $tRNA^{Ser}_{AAG}$), valine (Val; $tRNA^{Ser}_{CAC}$), and histidine (His; $tRNA^{Ser}_{GUG}$). One cell line (Mock) was transfected with the empty plasmid and used as control, and another line was transfected with the serine tRNA (WT).

The pIRES-DsRed plasmid has a geneticin resistance gene that allows selection by including geneticin (G428; Gibco) in the culture medium. In this way, only the cells transfected with the plasmid are capable of growing, while non-transfected cells do not survive.

This mistranslation system is being used used in NIH3T3 and HEK293 cells in the host laboratory. Characterization of these cells shows that the ubiquitin-proteasome pathway is upregulated suggesting that the mutant tRNAs are functional and the model is validated to study proteotoxic stress.

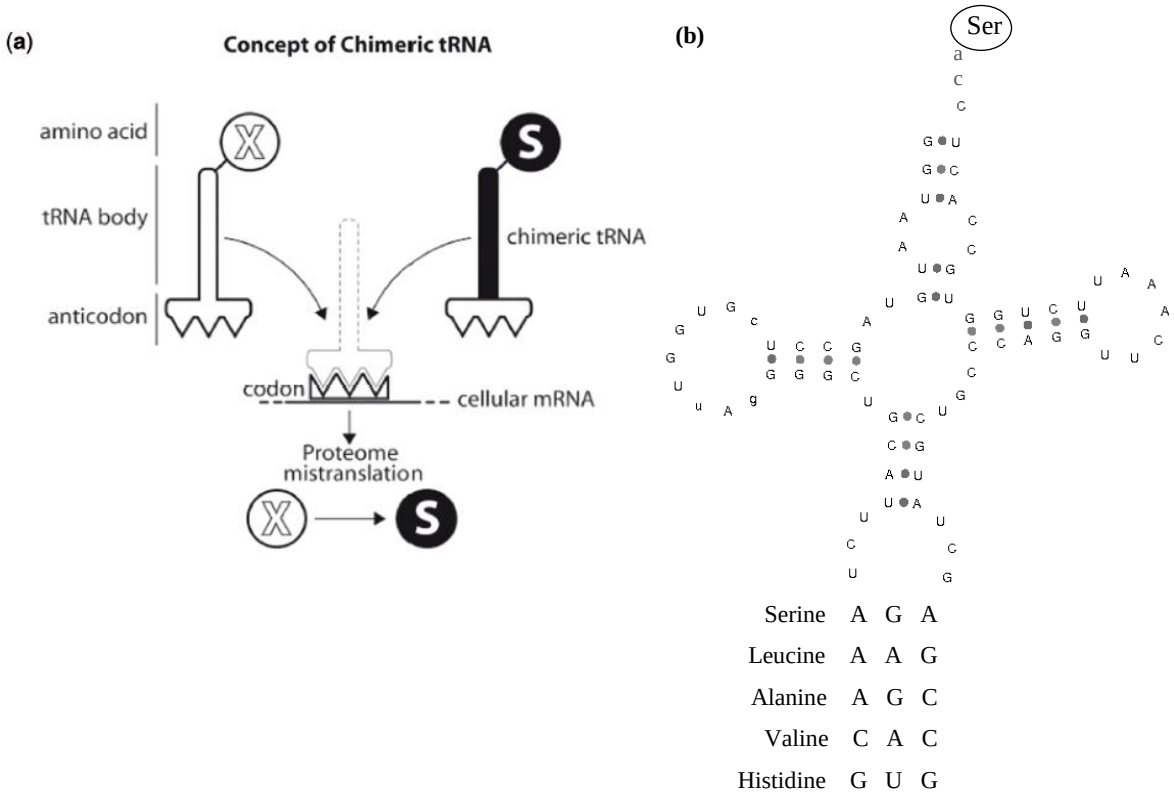


Figure 8: Representation of (a) the function of chimeric tRNA and (b) the tRNA used in this study (Geslain, et al., 2010). The chimeric tRNA are mutated on the anticodon (AGA), which then are able to recognize codon of other amino acids, but incorporating always the wild-type amino acid (Ser).

Materials and Methods

Cell Culture

HEK293 cells (purchased from ATCC, Manassas USA) were cultured in Minimum Essential Medium (MEM; Gibco) with 10% of fetal bovine serum (FBS; Sigma), 1% geneticin (Gibco), and supplemented with MEM non-essential amino acids (100x; Gibco). Cells were kept in 25cm^2 falcon flasks at 37ºC, in a humidified incubator with 5% CO_2/95% air.

Flow Cytometry

Flow cytometry is a laser based technology that allows simultaneous multiparametric analysis of the physical and chemical features of a cell population, in which the results are obtained as statistical data (Shapiro, 2003).

- Cell Cycle Assay

The DNA content varies along the cell cycle and its measurements can be done rapidly and precisely by flow cytometry using a fluorescent stain (Shapiro, 2003).

The cells were seeded in 6-wells plates, at a final concentration of 1x10^{5}cells/mL, for 24 hours. As the HEK293 cells are adherent, they were detached from the flasks with 200µl of trypsin (0,25% Trypsin-EDTA; Gibco), 2 minutes at 37ºC, and trypsin was inactivated with 2ml of culture medium. The cell suspension was centrifuged at 1000rpm for 5 minutes. The supernatant was discharged and cells were resuspended in cold 70% ethanol and kept at -20ºC until analysis. For analysis, the cells were centrifuged at 1000rpm for 5 minutes. The supernatant was carefully discharged and cells were resuspended in 800 µL of 1% PBS in cytometer tubes. Cells were gently aspirated up and down with a Pasteur pipette in order to dissociate then. 50µL of RNAse (100mg/ml; Qiagen) and 50µL of propidium iodide (1mg/ml; Sigma) was added and the cell suspension was incubated for 10 min, in the dark and horizontally. The cells were kept on ice prior to analysis in the flow cytometer (Beckman Coulter Epics XL).

- Annexin V-FITC Assay

The cellular membrane of apoptotic cells loses its asymmetry, transferring the phosphatidylserine (PS), normally present in the inner leaflet of the membrane, to the cell surface. Annexin V is a protein that binds specifically to PS, acting as a label in this assay. If annexin V is conjugated with a fluorescence marker, such as FITC, apoptosis can be analyzed by flow cytometry (van Engeland, et al., 1998).

For this assay, cells were seeded in 6-wells plates, at a final concentration of $1x10^5$cells/mL, for 24 hours. The procedure up to the centrifugation step of the cell suspension was the same as in the cell cycle analysis. After the centrifugation, the supernatant was discharged and cells were resuspended in 500µl of 1x binding buffer. After, 5µl of Annexin V-FITC and 5µl of propidium iodide were added, and the cells were incubated for 5 minutes at room temperature in the dark. Then, the cells were analyzed by flow cytometry (Attune® Life Technologies).

To perform this assay the annexin kit from BioVision (K101-25), comprising Annexin V-FITC, 1x binding buffer, and propidium iodide, was used.

Micronuclei Assay

Micronuclei form in cells where there is fragmentation of the genome. Cytochalasin B was used to block cellular division, forming binucleated cells, to facilitate the observation of micronuclei (Fenech, 2007).

HEK293 cells were seeded in 24-wells plates with 12mm round coverslips coated with poly-D-lysine (BD Biocoat), at a concentration of $4,8x10^4$cells/mL for 24 hours. After this, cytochalasin B (600µg/ml DMSO; Sigma) was added to each well and incubated for 24 hours. Next, the culture medium was discharged and 350µL Hoechst 33342 (0,5µL in 1% PBS; Sigma)were added per well, and incubated in the dark for 15 minutes (Figure 9). Coverslips were placed in glass slides with mounting media (Fluoroshield; Sigma). Then, the cells were analyzed using a fluorescence microscope (63x magnification; Zeiss Axio Imager Z1) using 350nm/470nm excitation/emission filters.

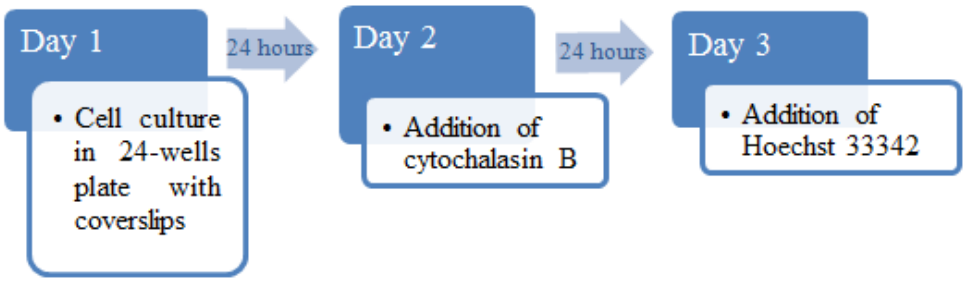


Figure 9: Diagram of the micronuclei assay. Cells were cultured for during 24 hours. After this, cytochalasin B was added and incubated for 24-hours incubation. Hoechst 33342 was then added for 15 minutes.

Images of the cells were taken using AxioVision software and nuclei were counted to calculate the percentage of viable and non-viable cells. In the viable cells, mono, bi and multinucleated cells were counted, and the number of binucleated cells with micronuclei were registed.

Statistical Analysis

Cell cycle assay was carried out in duplicates (10.000 events were evaluated on each replica). Data was analyzed with FlowJo software, and statistical significance was evaluated by two-way ANOVA with GraphPad Prism 5. The annexin assay was carried out in triplicates (15.000 events evaluated), while two replicates were carried out to the micronuclei assay. In these assays, data was analyzed using Microsoft® Excel.

Results

Cell Cycle Analysis

In this work, flow cytometry was performed to evaluate if mistranslation affects the progression of cells through the cell cycle. For this, we used the cell lines misincorporating Ser at Leu, Ala, Val, and His codons, and compared the number of cells in each phase of the cell cycle with the control. PI was used to stain the cells, since it binds to the DNA and allow us to calculate the amount of DNA in each cell. In figure 10 is showed a comparison between the histograms of each cell line with the histogram obtained in the control line (Mock). It is seen a slight difference of the amount of DNA between the lines and the control, especially in the S and G2/M phases.

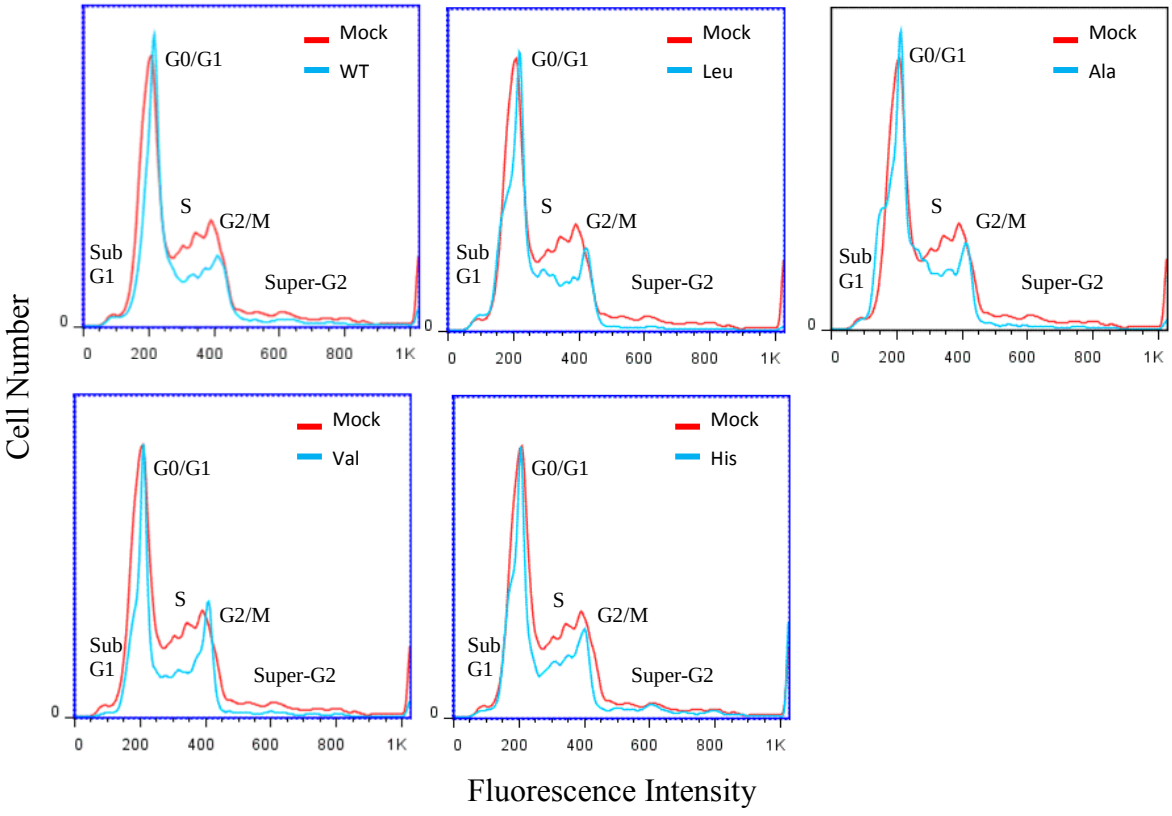


Figure 10: Comparison between fluorescence intensity of the different cell lines with the control, in order to assess variations in the amount of the DNA.

Sub-G1 phase represents the phase where the cells have the lowest levels of DNA. It represents less than 10% of cells in all lines (Figure 11) and can be caused by apoptosis or fragmentation of the nucleus during the assay. In the G0/G1 phase all lines had similar levels of DNA. The cell line misincorporating Ser at Ala codons showed the lowest number of cells (~25% of cells) in the G0/G1 phase, while the cell line misincorporating Ser at Leu sites showed the higher levels of cells (~31% of cells) in this phase. In the S and G2/M phases there was a statistical significant difference ($p<0.05$) between WT, one of the controls, and the Leu cell line. In S phase, excluding WT and Leu, all lines had similar values. The Leu cell line had higher percentage of cells in the S phase, while the Ala, Val, and His cell line showed almost no differences between them. The Mock and WT cell lines showed a slight difference between them. In the G2/M phase, the WT cells showed the highest percentage of cells with the correct DNA content, whereas the Leu cell line showed the lowest percentage of cells with the correct DNA content.

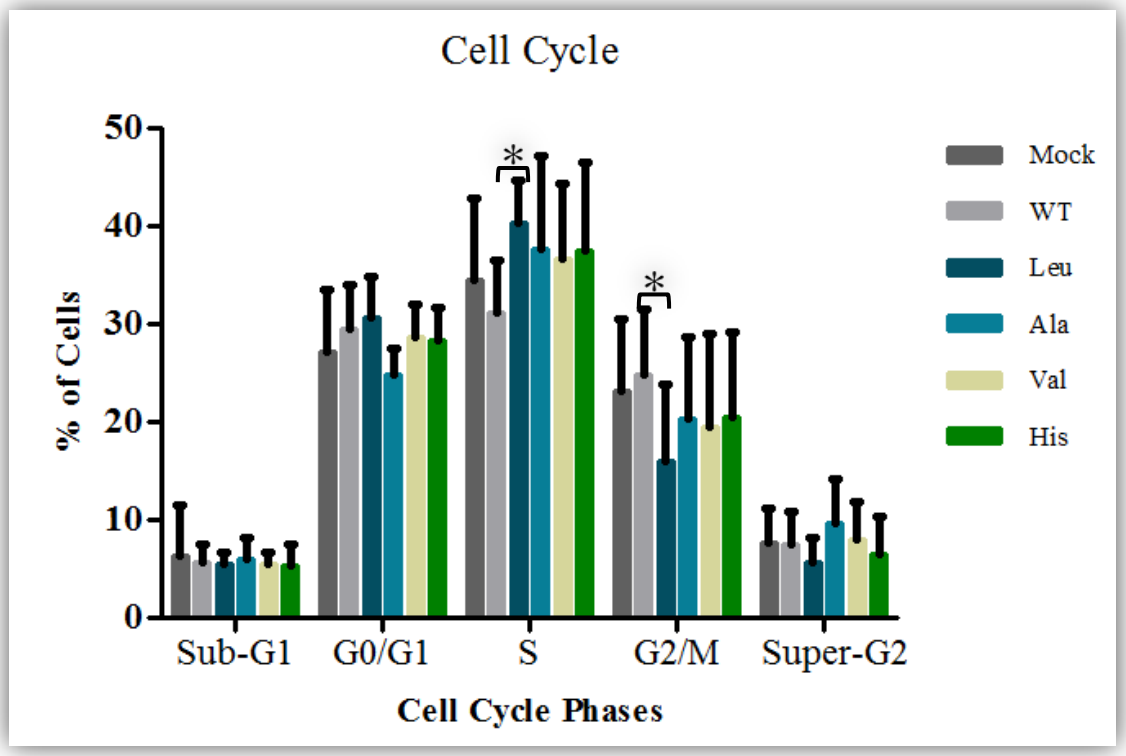


Figure 11: DNA content through the cell cycle, analyzed by flow cytometry. Statistical differences are seen between WT and Leu lines in the S and G2/M phase ($p<0.05$). This assay was carried out in duplicates and statistical analysis assessed by two-way ANOVA.

We were also able to analyze the presence of cells with higher levels of DNA, e.g. cells with duplicated DNA (polyploid) which appear in the Super-G2 phase (Figure 12). The percentage of cells in Super-G2 phase did not differ between Mock, WT and Val. The Ala cell line has the highest percentage of cells (~10% of cells) in this phase, comparatively to the controls, indicating that the Ala line had more cells with above 2n, while the Leu and His cell lines had have the lowest levels of cells in super-G2. However, the results showed no statistical significance, suggesting that additional characterization of these cells is needed ($p>0.05$).

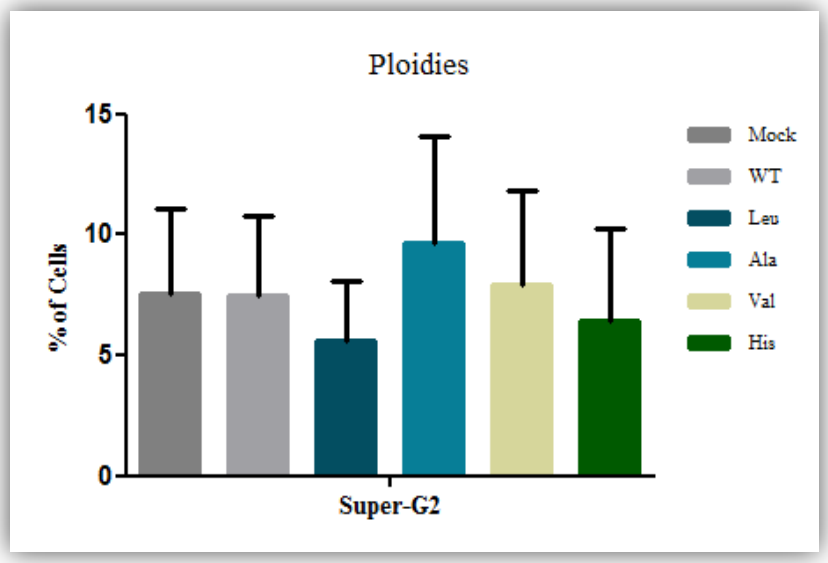


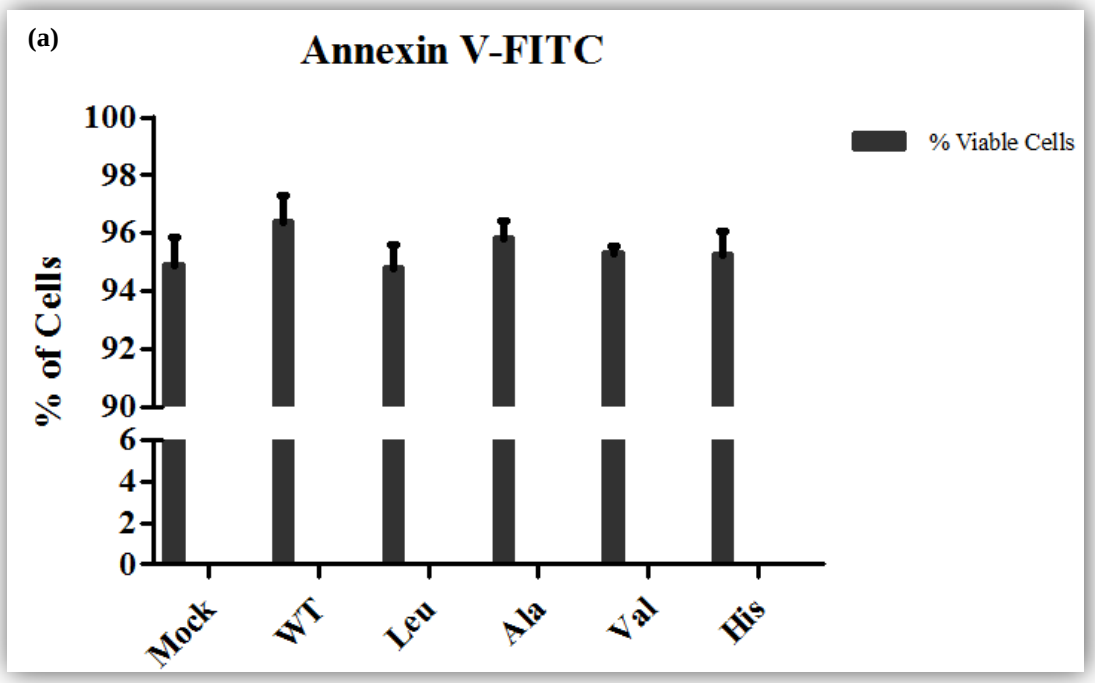
Figure 12: Percentage of cells with DNA content above 2n. No statistical significance was observed ($p>0.05$). This assay carried out made in duplicates and statistical analysis assessed by two-way ANOVA.

Cellular Viability

To assess viability of the cell lines, we have used the Annexin V-FITC assay and flow cytometry. This is a non-destructive analysis of apoptosis that uses fluorescence methodologies, like FITC (Fluorescein Isothiocyanate). Annexin V has high affinity for phosphatidylserine (PS) present in cell surface of apoptotic cells. When conjugated with the propidium iodide (PI) stain, a DNA dye, it is also possible to discriminate between apoptotic and necrotic cells since the latter only incorporate PI (Figures 13 a) and b)).

The annexin V-FITC assay showed that the majority of the cell population was viable cells (~95%). Similar viability was observed for all the cell lines studied. The WT cell line had lower levels of necrotic cells. The Leu cell line, that misincorporates Ser at Leu CUU codons, had higher levels of necrotic cells (~3.5% of cells). However, in all cell lines the total percentage of apoptotic and necrotic cells is lower than 10%. No statistical differences were observed ($p>0.05$).

The differences indicated above should be regarded only as tendencies, since we could not detect statistical significant differences between the cell lines. Additional tests are required to confirm these tendencies.



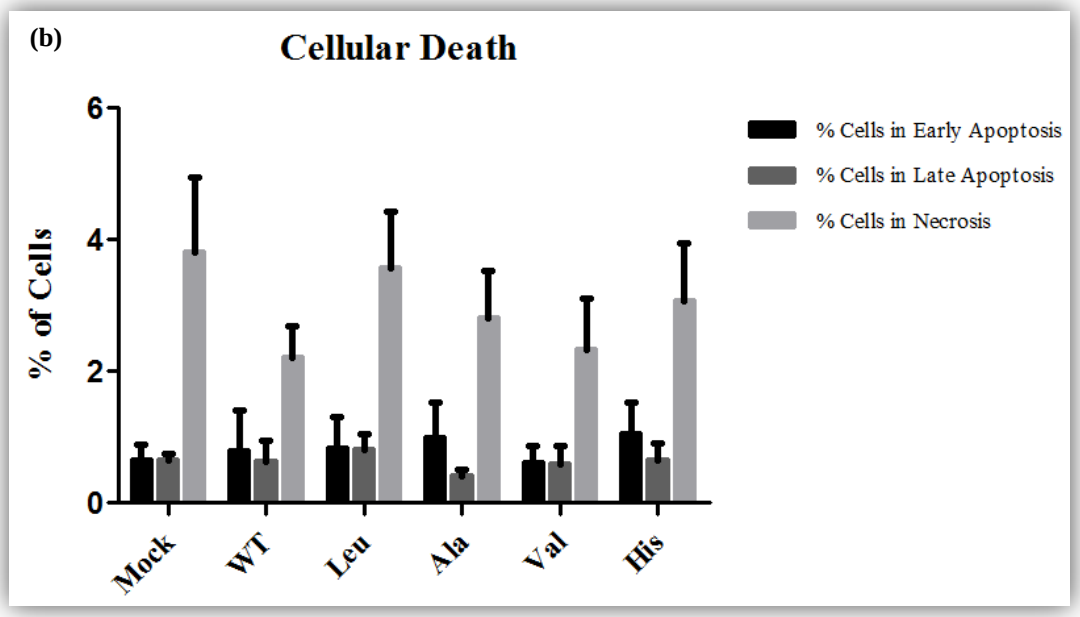


Figure 13: Cellular viability determined by the annexin V-FITC assay, using flow cytometry. The data showed that (a) approximately 95% of the cell population is viable cells, and that (b) cellular death is below 4% of cells either by apoptosis or necrosis. This assay was performed using triplicates.

Cellular viability can also be analyzed by fluorescence microscopy using the cell permeable dye Hoechst 33342. This assay does not discriminate between apoptosis and necrosis; it only indicates the percentage of live and dead cells (Figure 14).

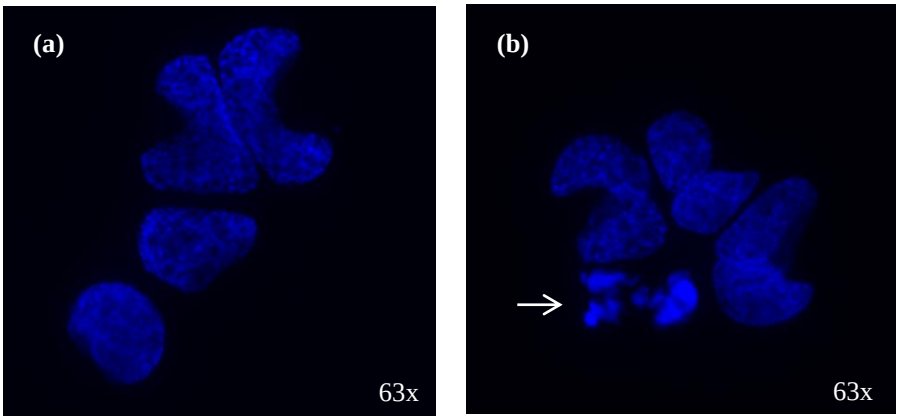


Figure 14: Example of cells obtained in the cellular viability assay with Hoechst33342 staining. In (a) is showed viable cells, while in (b) the arrow indicates a dead cell.

The values of cellular viability determined using the annexin V-FITC assay were corroborated by the data obtained using the fluorescence microscopy assay (Figure 15). Indeed, the latter assay showed that approximately 95% of the cell population was viable, with minor differences between the lines. As before, these data should be regarded as tendencies, since no statistical significance was observed between the cell lines ($p>0.05$).

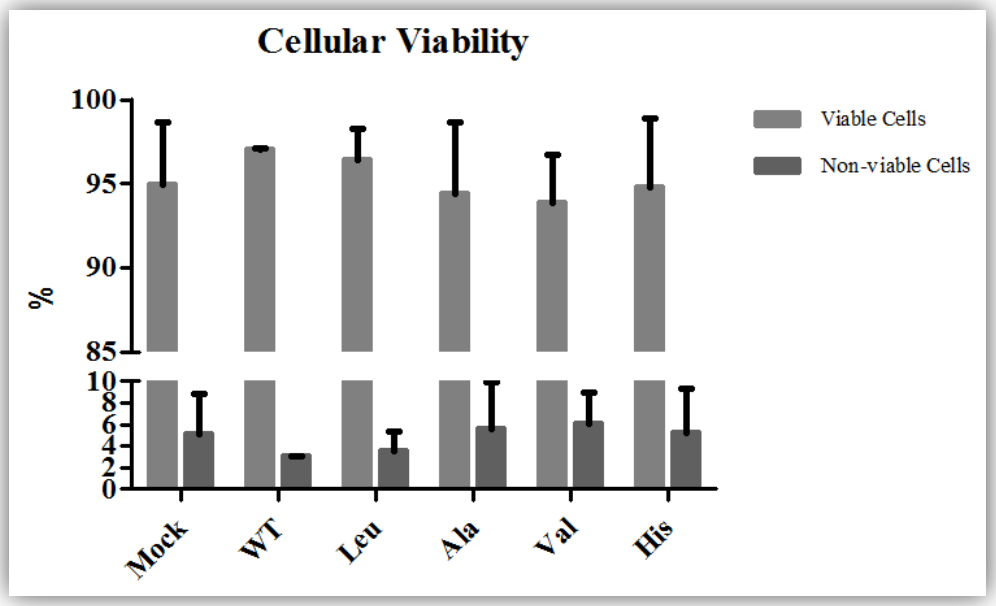


Figure 15: Cellular viability determined by fluorescence microscopy, with Hoechst 33342 staining. Nuclear morphology was analyzed and viable and non-viable cells were counted. No statistical significance was observed ($p>0.05$).

Micronuclei Assay

The micronuclei can be originated from fragments or whole chromosomes during anaphase, that later are involved in nuclear membrane. As they replicate along with the cell cycle, the best time to assess the presence of micronuclei is at the end of the first division. In this stage, once-divided cells are binucleated, which facilitates the observation of micronuclei (Figure 16). To create binucleated cells, the samples were incubated with cytochalasin B, an inhibitor of the microfilament ring essential to cytokinesis (Fenech, 2007).

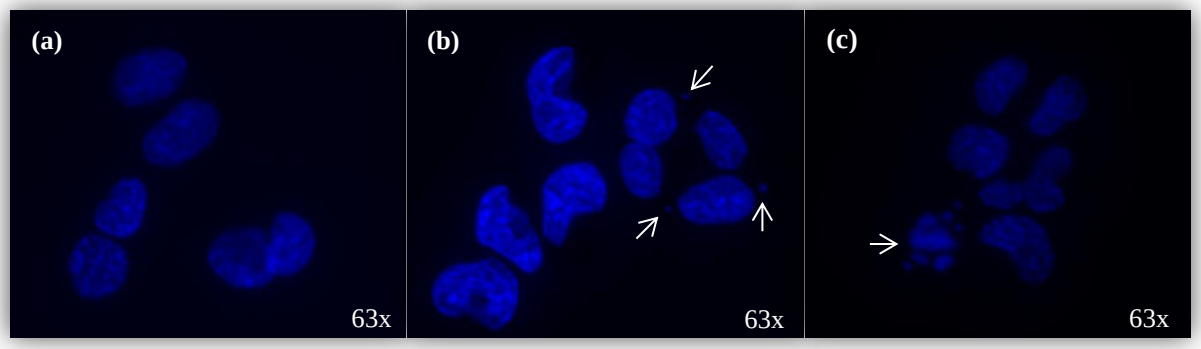


Figure 16: Microscopic fluorescence of micronuclei assay where it's evaluated (a) binucleated cells, (b) micronuclei in binucleated cells, and (c) dead cells.

In the micronuclei assay, cell division was blocked by cytochalasin B, in order to better distinguish the micronuclei. The percentage of micronuclei presented in binucleated cells was evaluated in this microscopic analysis using Hoechst 33342 staining (Figure 17).

This assay showed few differences in the number of micronuclei between the cell lines. However, Leu and Val cell lines had higher levels of binucleated cells with micronuclei. Mock, WT, and Ala had similar percentage (~10% of cells) of binucleated cells with micronuclei, while His had the lowest values (~9% of cells). The differences observed should be regarded as tendencies since statistically significant differences were not detected.

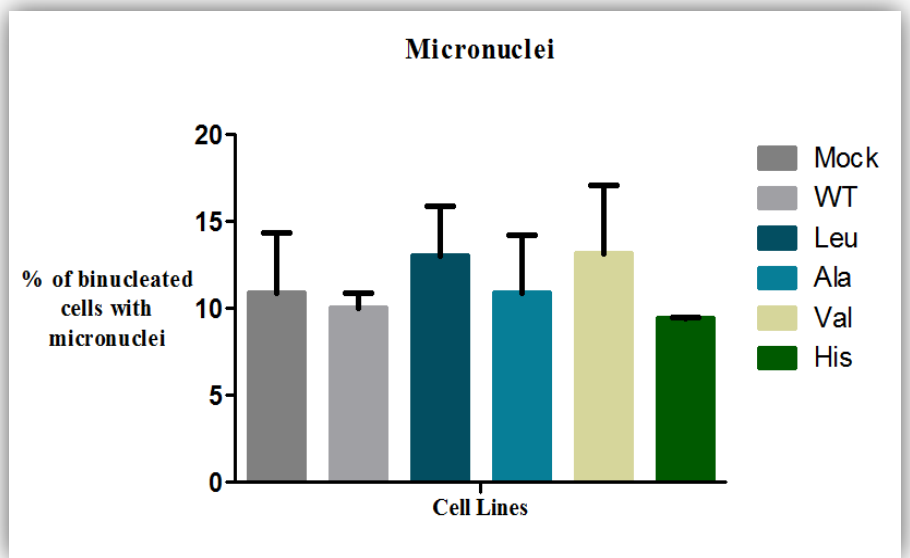


Figure 17: Hoechst33342 staining assay data indicating the percentage of binucleated cells with micronuclei. This assay was performed with two duplicates and no statistical significance was observed ($p>0.05$).

Discussion

Cell Cycle

The DNA content of the cells varies during the cell cycle, and such differences can be detected by flow cytometry, using a fluorescent DNA stain. A common DNA stain is PI that binds stoichiometrically to the DNA and its fluorescence can be quantified using regular cytometry. Since flow cytometry analyses cells in a population, i.e. each cell is analyzed individually, the data obtained provide information about the DNA content and cellular proliferation of that population (Nunez, 2001).

Flow cytometry also permits the quantification of the number of cells in each phase of the cell cycle, by measuring the amount of DNA in each cell. G0/G1 is a gap phase in the cell cycle that occurs immediately before DNA replication. In this phase the amount of PI fluorescence is lower than on the other phases of the cell cycle. During S phase DNA is synthesized and it is present in greater amounts, thus there is an increase in the intensity of the fluorescence. The G2 phase is a second gap that occurs between the S phase and mitosis (M phase), where the cells checks and repairs DNA damage. The M phase occurs when the cell divides in two daughter cells. The G2 and M phases have the same amount of DNA, and it is not possible to differentiate between them. The flow cytometry data represents the sum of cells on these two phases (Figure 18). This technique can also distinguish cells with higher or lower amounts of DNA than the amounts within the phases of the cell cycle, which can represent cells in apoptosis or cells with extra DNA, respectively (Nunez, 2001; Tabll & Ismail, 2011)

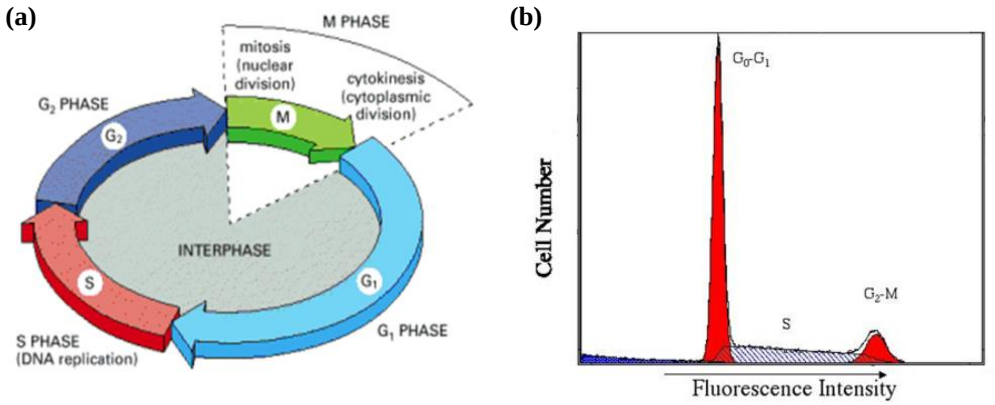


Figure 18: (a) cell cycle phases throughout time, and (b) DNA histogram obtained by flow cytometry showing the fluorescence intensity in each phase of the cell cycle (adapted from Tabll & Ismail, 2011).

We have observed statistically different percentages of cells in the S and G2/M phases between the WT and Leu cell lines. Leu and Ser are chemically distinct amino acids (Figure 19) and it is likely that misincorporation of Ser at Leu sites induces strong disruption of proteins structures, explaining the DNA content differences observed in the Leu cell line. Indeed, Ser is a polar, hydrophilic amino acid, with an uncharged R group. Histidine has a positively charged R group, being this amino acid more hydrophilic than Ser. Ala, Val, and Leu are all nonpolar amino acids, but Leu has the most different structure relative to Ser. Therefore, Ser misincorporation at Leu codons causes more damage to the final conformation of the proteins, preventing folding that can lead to higher toxicity levels. This may activate PQC systems and disrupt cellular processes comprising homeostasis.

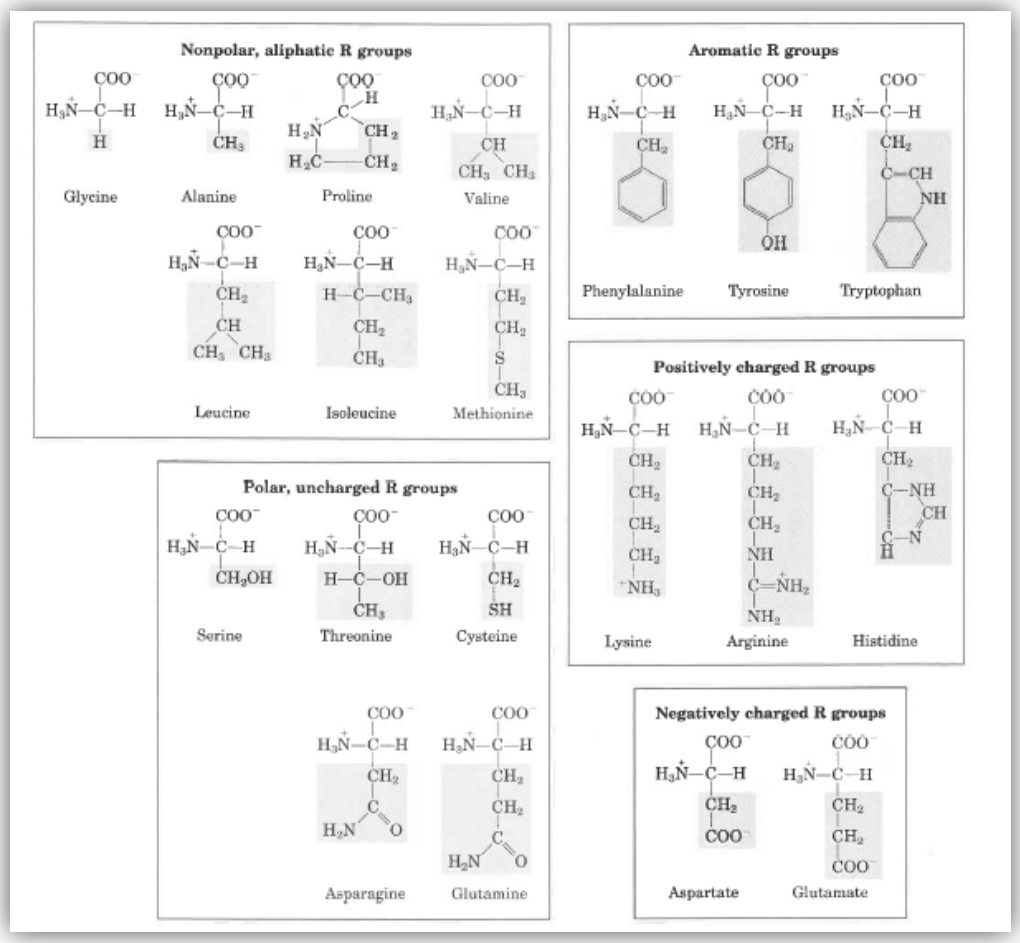


Figure 19: The structure of the twenty amino acids, organized in groups by their properties, namely size, shape of the R groups, or polarity (Nelson & Cox, 2008).

Cells have several checkpoints throughout the cell cycle that assure the fidelity of DNA replication. These checkpoints are placed at the end of each phase and delay the progression to the next phase to repair errors (Kaufmann & Paules, 1996; Khodjakov & Rieder, 2009). Therefore, mistranslation of the Leu cell line may disrupt DNA repair, or even lead to increased mutation rate, arresting the cell cycle in S phase. In practical terms, since these cells cannot complete the S phase of the cell cycle they cannot advance to the G2 phase. Therefore, fewer cells are present on the G2/M phase in the Leu misincorporating cell line (Figure 11). These results are in line with other studies showing that cell cycle is arrested by misfolded proteins produced through different processes. For example, SV40-transformed mammalian cells showed that cells which cannot eliminate misfolded proteins arrest cell proliferation and even lead to cellular death (Arslan, et al., 2012). Another similar work in yeast demonstrate that misfolded proteins formed by

elevated temperatures and by azetidine-2-carboxylic acid (AZC), an analog of proline that is incorporated into proteins, induce arrest of cellular proliferation in G1 phase (Trotter, et al., 2001). Moreover, inhibition of the UPR blocks the translation of the cyclin D1 and activates a cascade of events that arrest the cell cycle, but maintaining the cells in a viable state (Brewer, et al., 1999).

We have also observed the presence of cells with higher amounts of DNA, the polyploid cells. These cells appeared in the Super-G2 phase, and can be caused by mistranslation induced in the cells. Ploidies indicate the presence of cells with an abnormal number of chromosomes or quantity of DNA and are seen as one of the main causes of tumorigenesis (Weaver & Cleveland, 2007). Interestingly, the plasmids used in this work harboring the misreading tRNAs, transfected in NIH3T3 cells, induce the formation of tumors when injected in mice. These tumors were bigger in mice injected with the mutant tRNA that misincorporate Ser at Ala GCU codon (unpublished data). This result is in line with our results shown in Figure 12, where, in spite of lack of statistical differences, the misincorporation of Ser at Ala codons resulted in cells with high amount of DNA, greater than 2n.

In yeast, inhibition of HSP90 leads to loss of specific regions of the genome (Chen, et al., 2012; Shor, et al., 2013), and in *C. albicans*, ambiguity mistranslation of the CUG codon caused loss of large regions of the genome and even of an entire chromosome (Bezerra, et al., 2013), thus confirming that mistranslation has a major impact on genome stability.

Cellular Viability

During apoptosis, the plasmatic membrane of the cell suffers dramatic changes, namely loss of asymmetry. The phosphatidylserine (PS), normally present in the inner leaflet of the membrane is translocated and exposed in the cell surface. This allows for a non-destructive analysis of apoptosis using fluorescence methodologies, like the annexin V-FITC assay. Annexin V interacts strongly with PS and when labeled with FITC (Fluorescein Isothiocyanate) can be analyzed by flow cytometry. In necrotic cells, PS is not exposed on the cell surface, but it is possible to discriminate between apoptotic and dead cells, using propidium iodide (PI). PI is a DNA stain that cannot penetrate the plasma membrane, thus DNA is only stained if the membrane is disrupted. This allows the discrimination between early apoptosis, late apoptosis and necrosis; in the latter only PI is incorporated. So, when the annexin V-FITC assay is performed with PI staining, vital, apoptotic and dead cells can be discriminated. Vital cells are negative for both stainings, cells in early apoptosis are stained only with annexin V-FITC, the late apoptotic cells are positive for both stainings, while necrotic cells have only PI staining (Figure 20) (van Engeland, et al., 1998). Cellular viability can also be analyzed by fluorescence microscopy using Hoechst 33342, a cell-permeable DNA dye, which interacts with the DNA nucleotides. HEK293 cells stained with Hoechst 33342 observed with a fluorescence microscope allowed us to distinguish between viable and non-viable cells, through the morphology of the nucleus (Durand, 1982).

The cellular viability analysis by fluorescence microscopy is not as accurate as the analysis by flow cytometry, since it is not possible to distinguish between apoptotic and necrotic cells. Despite this, the results from nuclear staining and flow cytometry were rather similar. In both assays we have observed that the majority of the cells (~95% of cells) were viable, showing that mistranslation is tolerated and does not cause much cell death. The levels of apoptosis evaluated by the annexin V-FITC were very low (less than 2% of cells), as was the case for necrosis (less than 6% of cells). We were expecting slight increases in cell death, but the low levels observed suggest that mistranslation levels may be low or, alternatively, that human cells are highly tolerant to mistranslation.

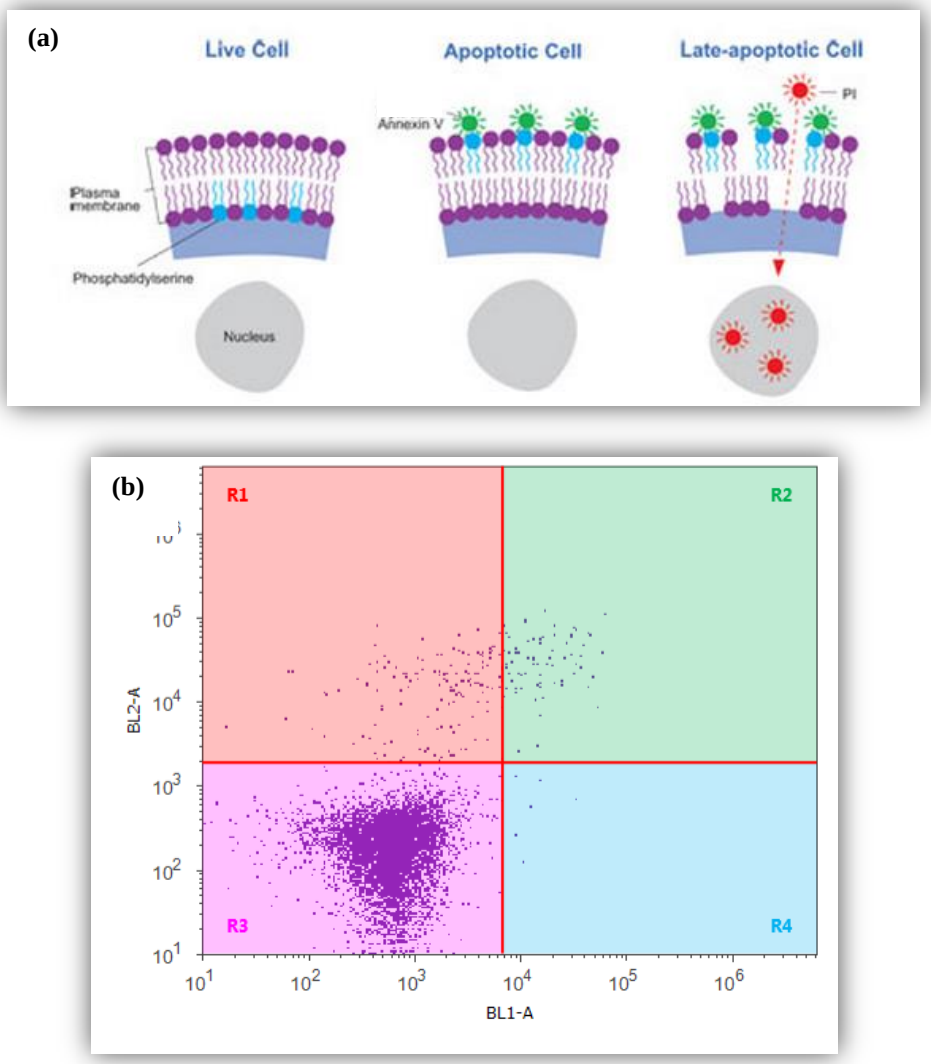


Figure 20: (a) schematic representation of the annexin V-FITC assay. In live cells phosphatidylserine (PS) is located on the inner leaflet of the cell membrane only; in early apoptotic cells, PS is translocated to the outer leaflet, being exposed to the annexin V that binds to it. In cells that are in late apoptosis, in addition to the binding of the annexin V, and because of the loss of membrane integrity, PI enters the nucleus and binds to the DNA (Apoptosis Detection Kits, 2013). (b) Flow cytometry results of the annexin V-FITC assay. R3 quadrant represents the viable cells of the population; in R4 are cells in early apoptosis; R2 cells are in late apoptosis; while R1 quadrant contains necrotic cells.

Micronuclei Assay

In this assay higher number of binucleated cells with micronuclei was observed in the cell line that misincorporates Ser at Leu CUU codon. This, together with the results of cell proliferation, where this line showed higher number of cells arrested in the S phase, demonstrate that mistranslation induced by the mutated tRNAs has a some effects on genome stability.

Interestingly, the cell line that misincorporates Ser at Ala GCU codon showed similar levels of binucleated cells with micronuclei relative to controls, but showed higher levels of cells with high amount of DNA (Figure 12). This indicates that different types of mistranslation may have differences at the genome level.

Conclusion and Future Perspectives

Mistranslation and proteotoxic stress are associated with several neurodegenerative diseases, like Parkinson's or Alzheimer's diseases (Chiti & Dobson, 2006; Gregersen, et al., 2006). In yeast, mistranslation promotes the development of advantageous phenotypes, evolutive characteristics, and introduces compensatory mutations in the genome. These adaptive mutations allow for the adaptation to new environments indicating that mistranslation may produce advantageous phenotypes through DNA mutation (Heidenreich, 2007; Bezerra, et al., 2013).

In this study were established two main objectives: i) assess the effects of mistranslation on cell viability, ii) determine the effects of mistranslation on the cell cycle and genome stability. Our data show that mistranslating HEK293 cells counteract the negative effects of this type of stress and that cell viability is barely affected. We could identify statistically significant results on the cell cycle assay. In particular, the cell line misincorporating Ser at Leu codon may activate mechanisms that prevent the completion of the S phase of the cell cycle, possibly due to alterations in the genome. We have also observed that the number of cells with micronuclei increased of the mistranslating cell lines, corroborating the results obtained in the cell proliferation assay where some cells had higher levels of DNA damage.

Therefore, our data suggests that mistranslation does not have negative effects on cell viability, cell cycle, and genome stability. However, our model system needs to be improved, perhaps by increasing tRNA copy number, to better study the effects of mistranslation on cell biology and human disease.

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