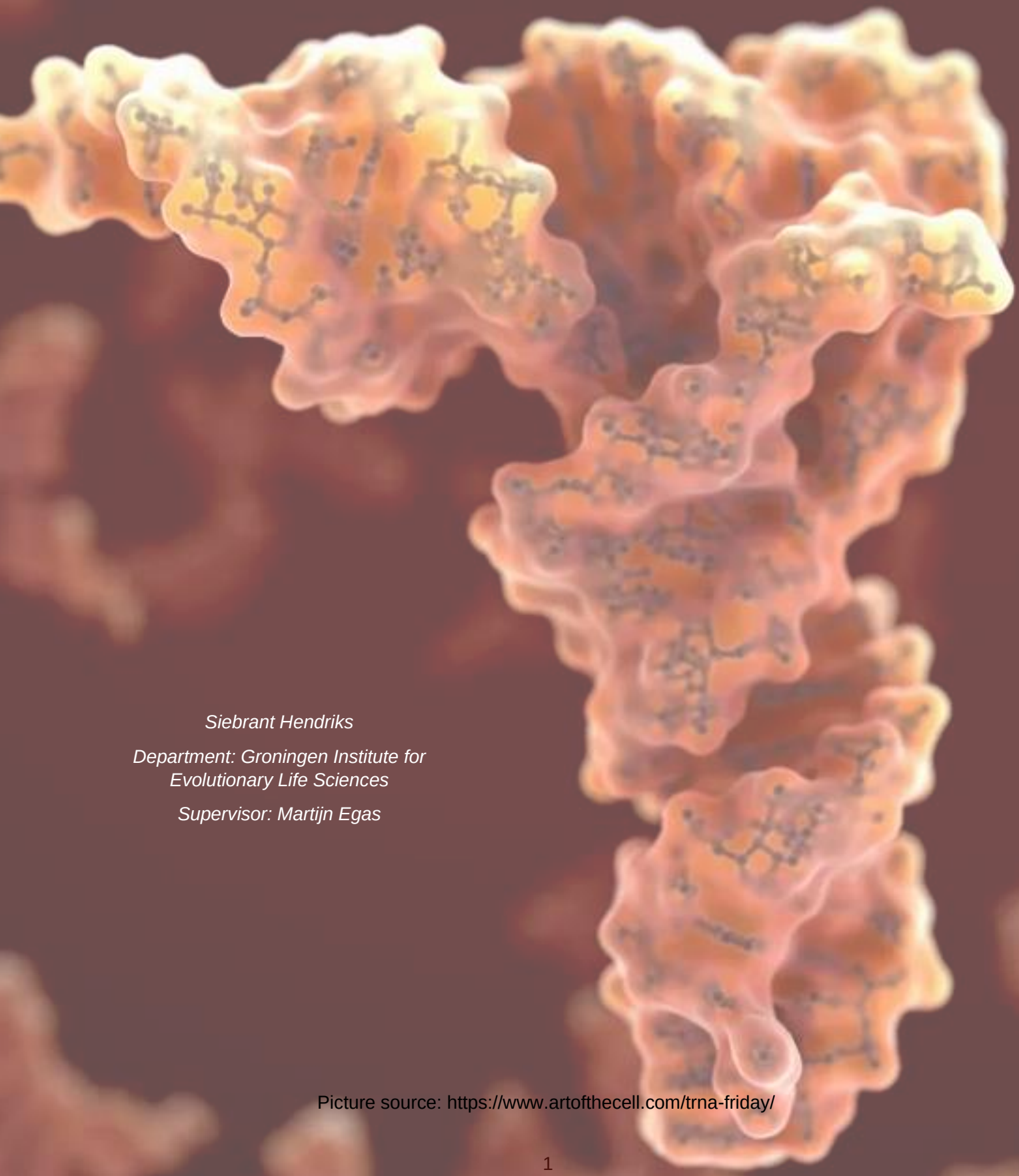
Adenine in the First Anticodon Position

Too Wobbly for Most



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Abstract

The topics of protein synthesis, transcription and translation, have become embedded in biology throughout the years. Nowadays about any academic would have some familiarity with them because of their education in high school. Despite this, certain elements in the process of translation remain undiscovered, or uncommon knowledge, even to researchers within the field of biology itself. One example of this is the fact that we require fewer unique tRNAs/anticodons than the amount of unique codons present on the mRNA. How a reduced set of anticodons would pair with all the codons is described in the wobble hypothesis. One observation within this hypothesis is that the nucleotide base adenine does not seem to occur in the first anticodon position, also known as the 34th tRNA residue, or the wobble position. In this article we investigated models and experiments that aim to give a rationale as to why this is the case. The predominant conclusion seems to be that adenine in the first anticodon position would appear to alter the structure of tRNA in such a manner that most of its interactions in the translation process would be hindered in one way or another. However, there seems to be little experimental confirmation to this rationale. Perhaps partly because of this, throughout the years we observe more and more exceptions to this initial exclusion of adenine in the wobble position. For researchers more attuned to this subject, this might have already been common knowledge in regard to eukarya and bacteria. However, in more recent discoveries we also found exceptions with archaea that have adenine present in the wobble position in certain tRNAs. Despite these findings there does appear to be an evolutionary disadvantage to having adenine in the wobble position that is worthwhile investigating.

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1 Introduction

With the discovery of the structure of DNA, Watson and Crick propelled the field of genetics into the atomic era, like many other fields of science that went before (Watson & Crick, 1953). With this new paradigm many other sub-cellular and molecular discoveries have been made. Because of this the processes of replication, transcription, and translation have become common knowledge for any biologist (Ruth, 1984). Although well understood as these processes may be, many peculiarities, oddities, exceptions, and novel additions can still be found and investigated (Berg et al., 2019) (Krahn et al., 2020) (Van Der Gulik et al., 2023). In this article we will focus on tRNA (transfer ribonucleic acid). Here we will explore some more uncommon knowledge about this macro-molecule and investigate some of its oddities. However, in order to better understand those interactions, we will first reiterate the process of translation.

1.1 On Translation

Translation is commonly known as the cellular process that results in protein synthesis. This process starts out with (messenger) mRNA. This macromolecule is a chain containing various combinations of its four different core molecules (bases): Adenine (A) Uracil (U) Guanine (G) and Cytocine (C). The function of an mRNA strand is to contain the instruction set for the assembly of a protein, or more accurately, a polypeptide. Along a strand of mRNA the cellular mechanism can read a signal to start polypeptide synthesis, then a varying amount of signals that would sequentially incorporate one of the 20 different amino acids, and eventually a signal that would stop polypeptide synthesis. This would mean a mRNA molecule has to be able to contain 22 different instructions. However, we have learned that the start signal is paired to the incorporation of one of the amino acids, usually methionine. So overall a mRNA molecule has to be able to contain 21 different instructions. How is this accomplished if a mRNA chain can only be made from four different bases? Nature seems to have resolved this issue by grouping the bases together. When putting the bases in groups of three, 64 different combinations can be reached. That is more than plenty to facilitate the coding for these 21 different instructions. A group of three of these bases is known as a codon. With only a total of 21 functions to be performed by a sizeably larger amount of 64 codons we have found a redundancy of codon functioning, i.e. multiple different codons can perform the same task of coding for a certain amino acid (Lagerkvist, 1978). The pairs in which codons code for their matching function/amino acid is referred to as the standard genetic code (Woese, 1964). These pairings can be seen in figure 1 (Rye et al., 2016).

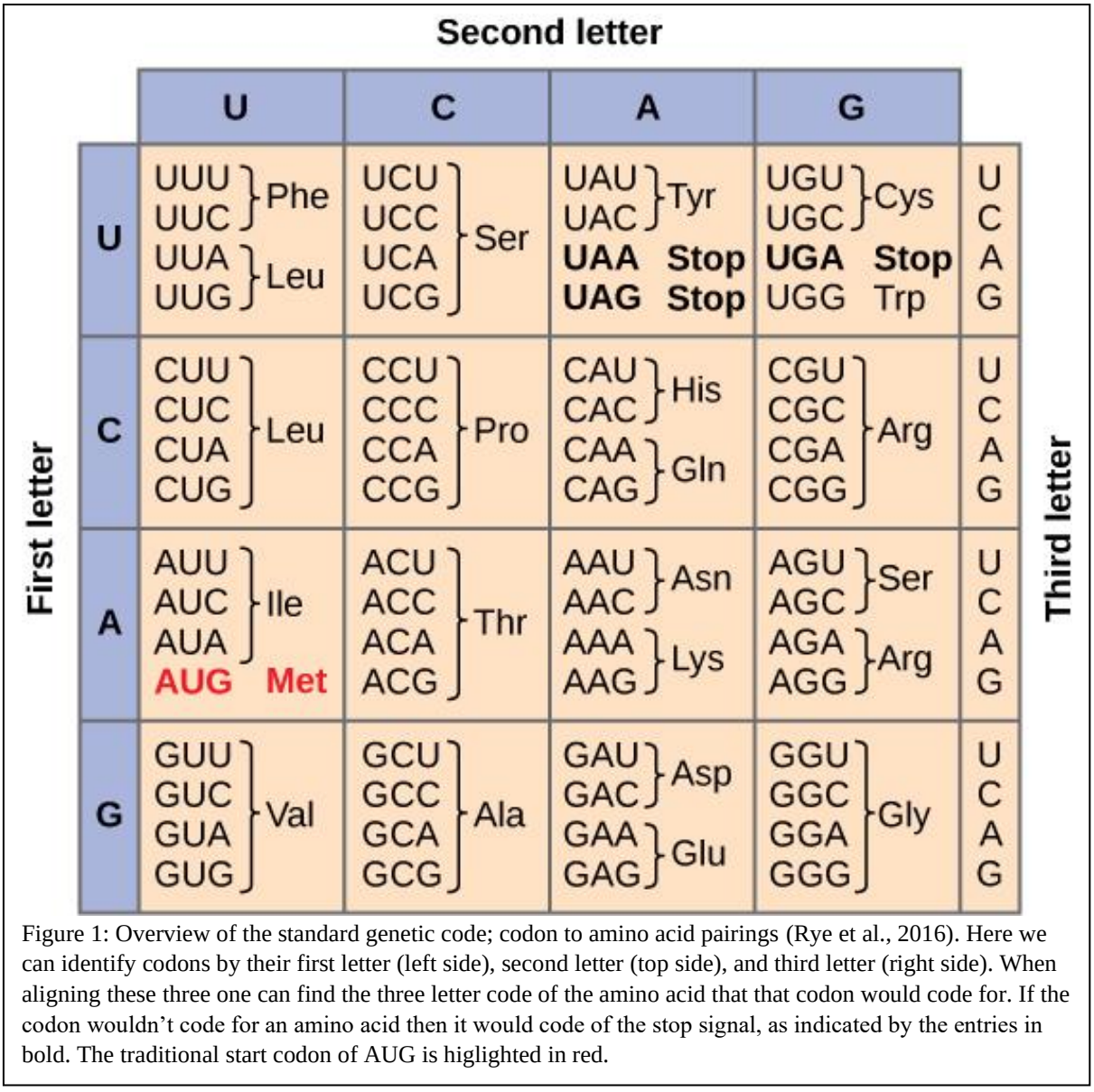

Figure 1: Overview of the standard genetic code; codon to amino acid pairings (Rye et al., 2016). Here we can identify codons by their first letter (left side), second letter (top side), and third letter (right side). When aligning these three one can find the three letter code of the amino acid that that codon would code for. If the codon wouldn't code for an amino acid then it would code of the stop signal, as indicated by the entries in bold. The traditional start codon of AUG is higlighted in red.

Of course, with only a code, amino acids won't just spontaneously be linked together. First two other catalytic molecules are needed: The ribosome and (transfer) tRNAs. The ribosome acts as the enzymatic machinery that links the amino acids together to form the polypeptide chain. tRNAs are the carriers that one by one gather amino acids and bring them towards the ribosome for incorporation in the polypeptide chain. The ribosome is made up of both amino acids and (ribosomal) rRNA. It is rather unique for utilizing RNA in facilitating catalytic functions (Cooper, 2000). It is an enzymatic complex that, in its simplest overview, contains two subunits. A smaller one that first binds to the mRNA and searches for the start codon, which is most commonly AUG, though this may vary between species (Asano, 2014). Once a start codon is found, the larger subunit will join the complex and continue with synthesis from the first amino acid coded by the start codon. The specific size of these subunits varies between species (Cooper, 2000).

A complete ribosome contains three catalytic sites in which the steps of polypeptide synthesis are performed: an A, P and E-site. In the A site the codon coding for the next amino acid is exposed, here it binds the tRNA carrying that specific amino acid. Then it transfers the current polypeptide chain on top of that amino acid. After this the ribosome moves up one codon along the mRNA, and in doing so shifts bound tRNA between the catalytic sites. From the A site the bound tRNA moves to the P site, and from the P site it moves to the E site. Then if a tRNA happens to occupy the E site, the mechanism facilitates the disassociation for that tRNA and removes it from the complex, allowing the

cycle to continue. This process continues until it reaches a stop codon on the mRNA. At this codon the A site of the ribosome won't be able to bind an amino acid carrying tRNA. Instead, it will bind certain molecules specified as release factors (Youngman et al., 2008), which will aid in the termination of translation, and thus in the finishing of a polypeptide chain. For a visual overview of this process, have a look at figure 2 (Reece et al., 2013). It is possible for multiple ribosomes to work along the same stretch of mRNA at the same time. More on the kinetics of polypeptide synthesis can be found in (Gorban et al., 2019). Do note that this overview of protein synthesis is highly simplified, and thus there are many more molecular/mechanical processes that are involved. Quite some of these have already been investigated, but A substantial amount still remain to be discovered as stated by Woese, (2001).

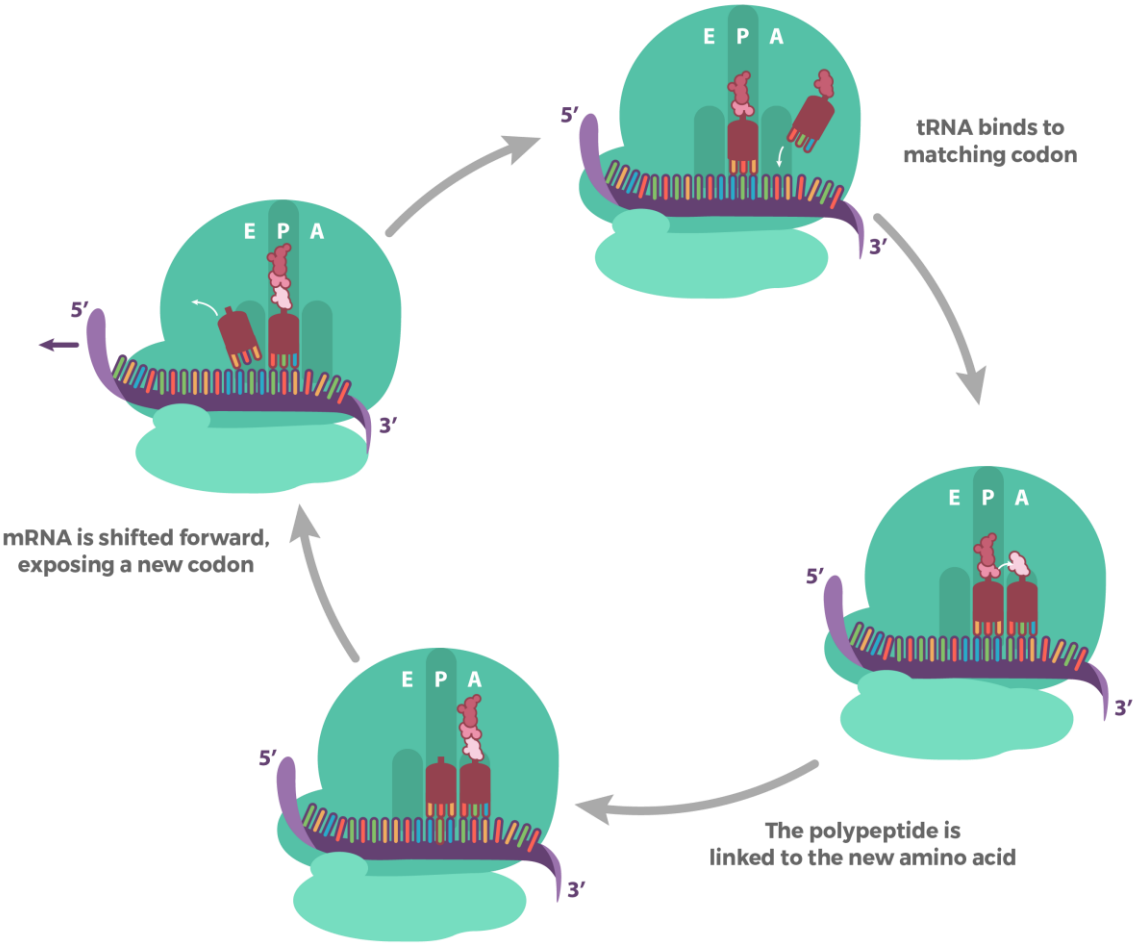

Figure 2: Steps of translation (Reece et al., 2013). Here depicted is the process in which translation occurs. The ribosome is depicted in teal, the larger and smaller subunits can be distinguished by a slight difference in shade. The A, P and E sites are marked thusly. The mRNA strand passing through the ribosome from its 3' end to 5' end is also visible as the purple strand with all the different coloured bases present on top. Likewise, tRNA is depicted as the maroon-coloured object with the bases of the anticodon present in various colours. To the tRNA the amino acids are attached and visible as the irregular blobs in various shades of pink. Each picture depicts one of the four steps in the translation cycle.

1.2 Overview of tRNA structure

Now that we have established a general overview of translation, we can go deeper into how tRNA functions in this process. The structure of tRNA can be seen in figure 3 (Berg et al., 2019). tRNA is a single-stranded RNA molecule that has a short length of about 60 to 90 bases. This molecule forms base pairs within itself, folding into a functional secondary structure. This structure has five noteworthy motifs that facilitate parts of its functioning (Thapa, 2023):

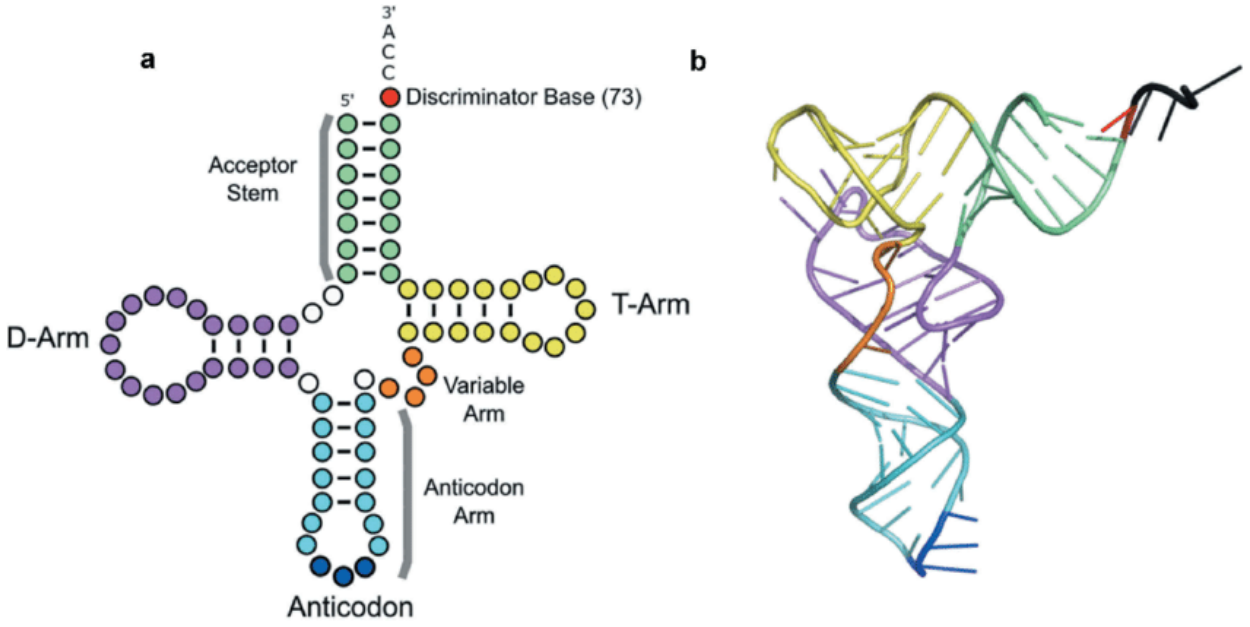

Figure 3: Structure of tRNA schematic and 3D depiction(Berg et al., 2019). In both pictures the main features of tRNA are colour coded as follows: Discriminator base (red), acceptor stem (green), T-arm (yellow), variable arm (orange), anticodon arm, (teal), anticodon (navy blue), D-arm (purple).
a: tRNA represented as two-dimensional structure with its main features annotated. This representation is often revered to as a cloverleaf. **b:** The three-dimensional structure of tRNA as represented by $tRNA^{Phe}$. The tRNA is folded into an L-shape through its intramolecular base pairings.

- The anticodon arm: This part contains the anticodon which complements the codon of the mRNA being translated. The bases of the anticodon pair up with those of the codon by forming hydrogen bonds, thus linking the tRNA and mRNA strongly together during translation.
- The T-arm: This part supports the T-loop that is usually occupied by the (modified) bases of Thymine, Pseudouridine and Cytosine. These bases facilitate the tRNA in binding to the ribosome during translation.
- The D-arm: This part supports the D-loop with within a modified base Dihydrouridine. Along with this base, this loop serves as a binding site for the enzyme aminoacyl tRNA synthetase. This enzyme facilitates the binding of the relevant amino acid to its specific tRNA before it is sent off for translation.
- The acceptor stem: This stem contains both the 3’ and 5’ end of the tRNA. Because of this no loop is present, unlike the three prior arms. At the 3’ there is a sequence of CCA, which is able to bind the carboxylic group of amino acids by means of forming an ester bond, as is performed by the aminoacyl tRNA synthetase enzymes.
- The variable arm: As its name might suggest, this part will be responsible for most of the variation in the length of tRNA. The arm varies in length, varying between 3 and 21 bases. In terms of canonical functioning the variable arm simply provides stability to the tRNA molecule.

Of course, the most interesting bases in a tRNA strand are generally those of the anticodon. These bases occur on the 36, 35, and 34 positions on the tRNA strand, and complement the first, second, and third bases of a codon respectively. So, just as the standard genetic code is commonly expressed as codon to amino acid parings, it can also, less commonly, be expressed as anticodon to amino acid parings, as seen in figure 4 (Ehrlich et al., 2021).

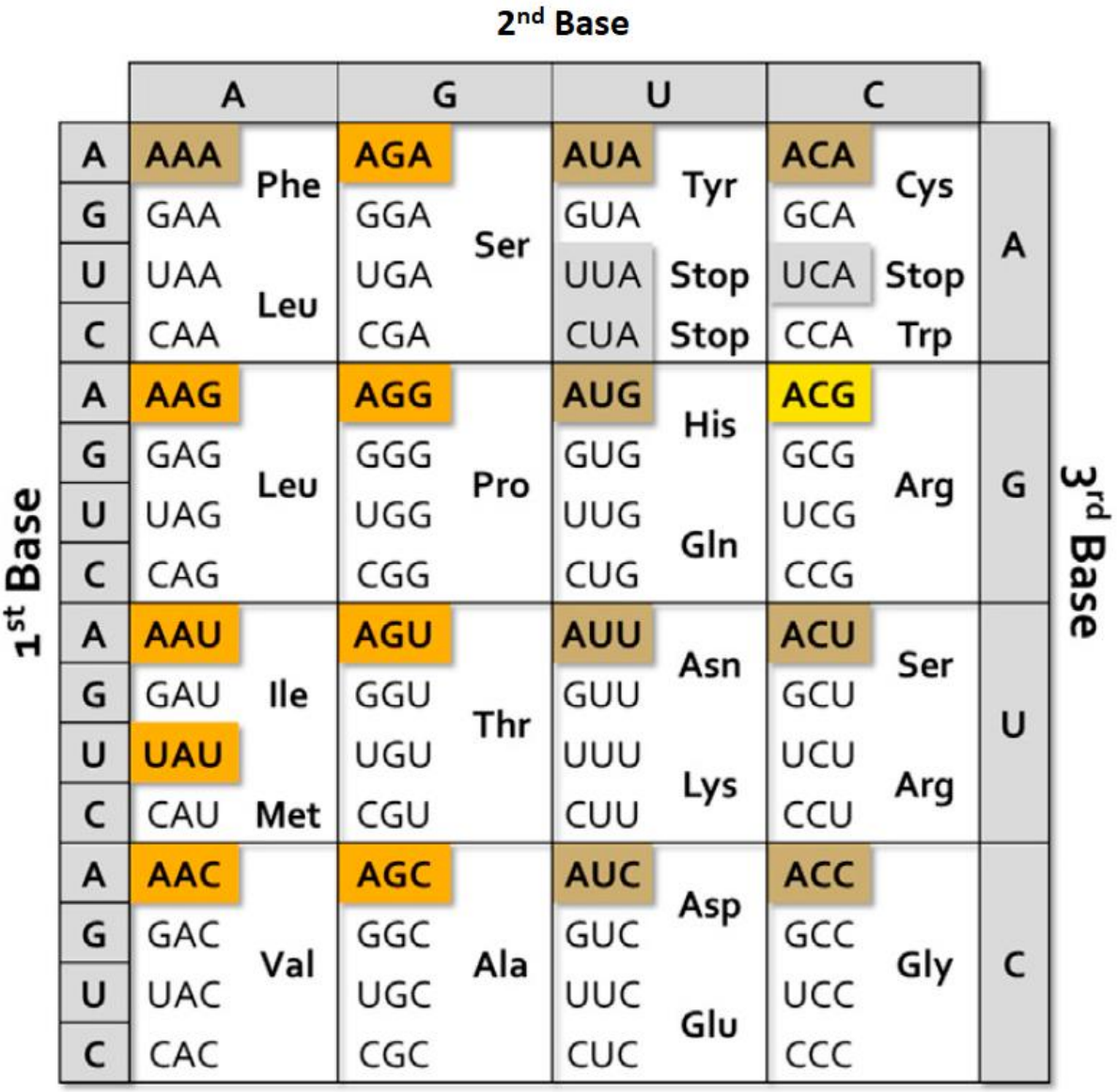

Figure 4: The standard genetic code as anticodon to amino acid pairings (Ehrlich et al., 2021). Here we can identify anticodons by their first base (left side), second base (top side), and third base (right side). Within each box we can identify the anticodons on the left and the amino acids they pair up to on the right. From the source article there are also some highlights that would be worth consideration. In grey are marked anticodons that are not present in tRNA. This is because their complementary codons code for the stop signal instead of an amino acid. All the other highlights represent exclusions amongst different domains in the tree of life. For our research it is simply relevant to note that each highlight represents exclusion of that anticodon in archaea in over 50 species that were investigated (Ehrlich et al., 2021).

1.3 Introduction of the wobble hypothesis

Contrary to popular assumption, organisms do not employ a separate, specific tRNA for each of the 61 amino acid coding codons on the mRNA. Disregarding the standard genetic code for a moment; functionally an organism would only need 20 different tRNAs, one for each specific amino acid. In practice most organisms end up utilizing somewhere between 28 and 46 unique tRNAs (Grosjean et al., 2009). This would mean that for those organisms there would need to be some way in which their reduced tRNA repertoire can accommodate their entire repertoire of codons in mRNA. Of course this can occur through evolutionary drift: the removal of redundant codons from the genome, and by extension the removal of the tRNAs complementary to those codons, which does happen occasionally (Diwan & Agashe, 2018). However, far more common are certain interactions that facilitate an anticodon, in not needing to completely, canonically, match a codon. This was first observed by Crick in 1966 where he stated that while the pairing of the first two bases of a codon appears to be strict, there appears to be some 'wobble' in the pairing of the last base of the codon with the first base of the anticodon. This means that a base in the 34th, first anticodon position, would be able to match more than one different base in the third/last codon position. Because of Crick's description this 34th position in the tRNA sequence also became knows as the wobble position. Crick managed to observe certain patterns with which bases could be paired in the wobble position and documented those findings as seen in figure 5 (Crick, 1966).

Pairing at the third position of the codon

Base on the anticodon	Bases recognized on the codon
U	A, G
C	G
A†	U
G	U, C
I	U, C, A

Figure 5: The original wobble hypothesis (Crick, 1966). Here we can see the original wobble hypothesis as postulated by Crick back in 1966. On the left we have the bases that would occupy the wobble position, and on the right, we have the bases that would occupy the third codon position they are presumed to be able to pair with. With the † symbol Crick also made the observation that adenine on the wobble position is rare or absent and is like enzymatically modified to inosine (Crick, 1966).

1.4 Back to the current day

As seen in the note Crick made in picture 5, already at the inception of the wobble hypothesis it is presumed that adenine in the wobble position is quite rare, and when it occurs, it is most often modified to inosine. Later findings do seem to confirm this presumption globally (Torres et al., 2014), though exceptions have been found (Tuite & Von Der Haar, 2016). Thus, it is currently known that eukarya do contain a set of tRNAs with an adenine in the wobble position, which in the majority of

instances is modified to inosine. This is also the case for one tRNA present in bacteria (ACG), though besides that one exception, all other tRNAs with adenine in the wobble position are excluded in bacteria. Likewise, all tRNAs in archaea are presumed not to contain adenine in the wobble position (Ehrlich et al., 2021). This is certainly an interesting observation, and generally not much is known as to why this is the case. That is why, in the results, this paper will focus on the question: Why is adenine absent in the 34' wobble position in archaea?

2 Results

2.1 Structural complications

The absence of adenine in the wobble position starts out as a tale of structural hinderance. As early as 1981 Balasubramanian and Seetharamulu argued that the presence of adenine in the wobble position would interfere with the structure of the U-turn that is essential to the anticodon loop (Balasubramanian & Seetharamulu, 1981). The U-turn is a conserved motif of tRNA where the 33-35th residue make a sharp turn so that the anticodon is on the outside of the structure i.e. presentable to bind with the codon. Strongly conserved in this motif is that uridine is always present on the 33rd tRNA

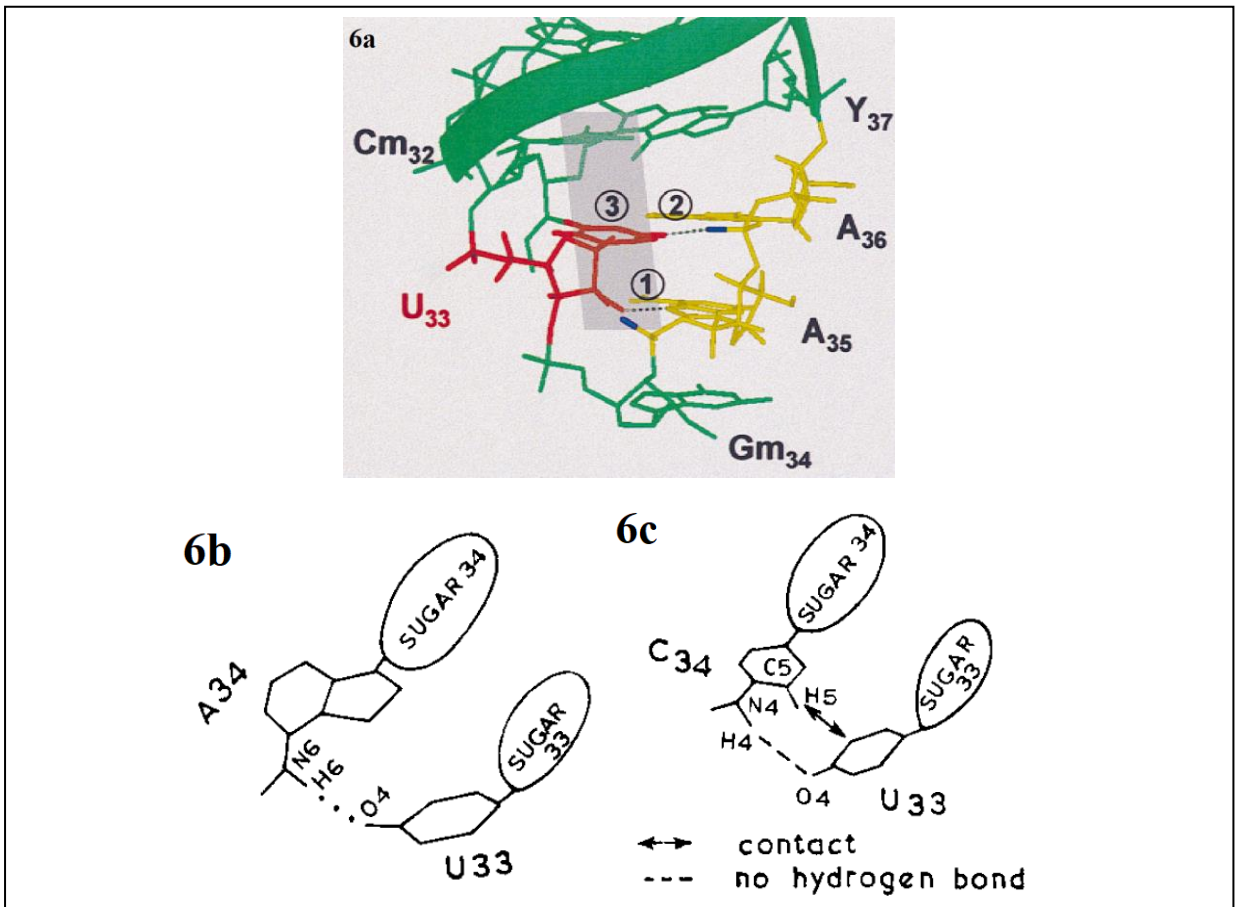

Figure 6: Structural complications at the U-turn (Ashraf et al., 1999) (Balasubramanian & Seetharamulu, 1981). **a:** Here we can see the traditional three-dimensional structure of tRNA^{Phe} present in yeast. Coloured red is U33 which forms hydrogen bonds with the 35th and 36th bases of tRNA, which are coloured yellow, both are adenine in this example. On the 34th wobble position is Gm, which is a modification of the base guanine (Ashraf et al., 1999) **b:** Here we can see how adenine on the 34th wobble position is presumed to make a hydrogen bond with uridine on the 33rd position (Balasubramanian & Seetharamulu, 1981). **c:** Here we can see how a base different from adenine e.g. cytosine provides steric hinderance which prevents it from binding uridine on the 33rd position (Balasubramanian & Seetharamulu, 1981).

position (Ashraf et al., 1999). This is because the molecular interactions that are the cause of making this turn can only happen with uridine in this position. Balasubramanian and Seetharamulu predicted that A34 would interfere with U33. U33 usually forms hydrogen bonds with elements of the N35 and N36 (N denoting any one nucleotide) positioned nucleotides in tRNA, (the second and third anticodon position), in order to form the U-turn, as seen in figure 6a. However, with Adenine present in the 34th position, U33 might instead bind A34, thus preventing it from binding N35 and N36, and thus making it unable to form the U-turn as can be seen in figure 6b. Other base in the N34 position instead provide enough steric hinderance to the U33, that this undesirable hydrogen bond would not occur, as can be seen in figure 6c.

This theory has been expanded upon by Lim in 1995. She made a computational model of tRNA and its interaction with the ribosome in its three catalytic sites (Lim, 1995). Here it is calculated that with A at the 34th (wobble) position a tRNA would bind a codon with U at the 3rd and last codon position, its

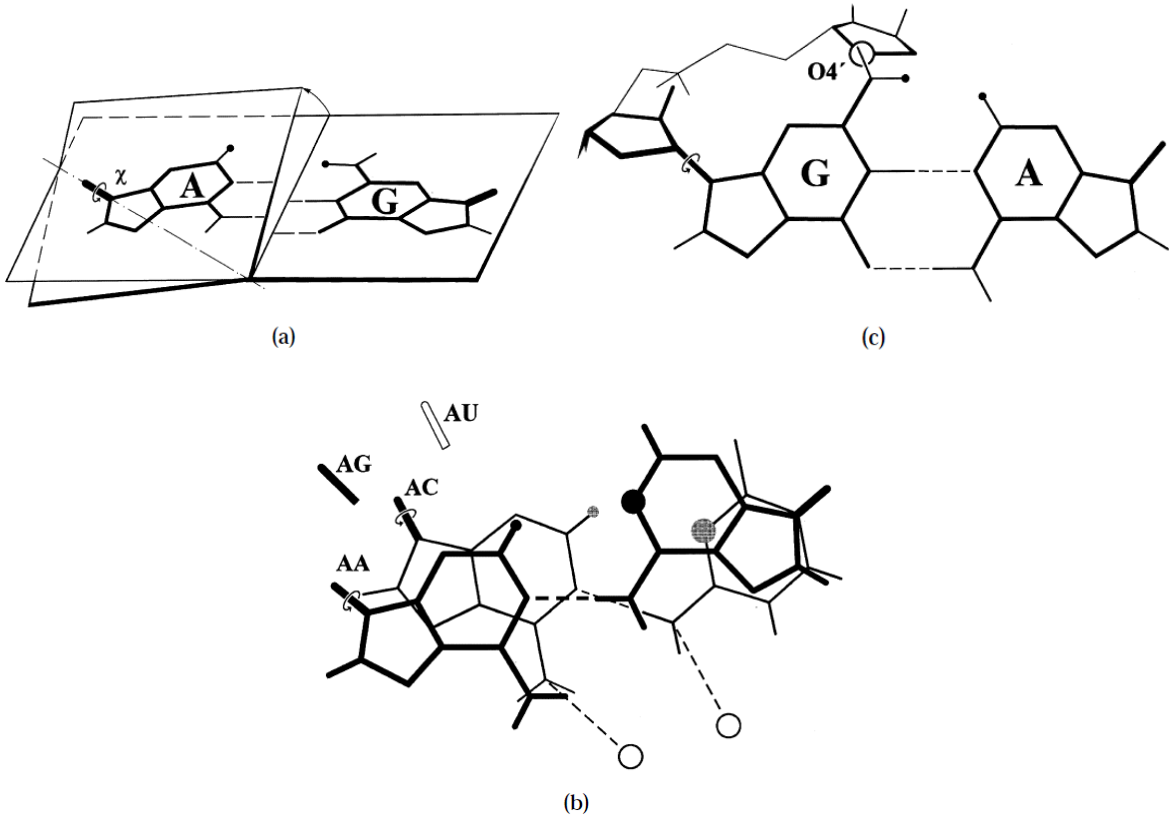

Figure 7: Structural complications of the propeller twist formation (Lim, 1995) **a:** Here we see the complications of the propeller twist represented on the planar field. An adenine on the 34th wobble position in its regular orientation would not line up with the base on the 3rd codon position, in this example a guanine. Thus, in order for adenine on the 34th wobble position to bind the base on the 3rd codon position it would need to rotate about 20 degrees on the axis of χ. **b:** Here is represented the binding of Adenine on the 34th wobble position with the four RNA bases on the 3rd codon position. This is done in the form of two letter wobble pairs (AA, AC, AG and AU). The Pair of AA is depicted with the bold lines. The pair of AC is depicted with the thin lines. The full representation of AG and AU are left out. The stick of AU is not filled in as to indicate that that pair does not experience rotational strain. The Black dots are nitrogen atoms that are shielded and thus unable to make hydrogen bonds. The open circles are water molecules froming deformed hydrogen bonds with nitrogen atoms on the AC pair, on the AA pair the nitrogen atoms analouge to those positions are also shielded. The axis of rotational strain is indicated by the twisting arrows. **c:** The wobble pair AG depicted with the backbone structure between the first and second anticodon residues (in thin lines). The oxygen atom O4' indicates the start of the next base attatched to guanine. With the overlap of the thin lines it can be seen that the rotational strain also leads to sterical hinderance in the backbone structure.

canonical pair, without too much structural hinderance. This tRNA would also bind codons with the other bases at the third position i.e. C, G, and A. However, this would not be done without forming a propeller twist: a structural conformation that would put further strain on the codon anticodon pairing in the A-site. The structural specifics of the propeller twist can be observed in figure 7. The additional strain of the propeller twist would lead to complications in the translation process. Further findings were that the presence of adenine in the wobble position destabilizes the interduplex interaction between the A- and P-site of the ribosome. The severity of this effect is dependent on the last base of the codon this A34 is paired with. The severity can be ranked as follows:
U < C < G < A, where this effect is least severe when A34 is paired with U3, and most severe when paired with A3. Likewise, it is also predicted that A34 interferes with the interduplex interactions between the P- and E-site of the ribosome, which increases the probability of frameshifting in the translation process.

Somewhat contradictory to these findings is the research of Borén et al. (1993). They investigated $tRNA^{gly}$ found in *E.coli,* which naturally occurs with the CCC anticodon. In addition, through mutation they also tested the other anticodons in the family box of glycine. The anticodons of glycine are contained in the family box of NCC bases, N denoting that each of the four standard bases (A, U, C, G) can apply. In this research they tested the functionality of these bases *in vitro.* Their findings were that adenine in the wobble position was unable to discriminate between all bases in the third codon position. They did not investigate specific affinities adenine would have towards each individual base. Thus, their conclusion was that adenine was a “highly promiscuous wobbler” that would have been avoided in evolution because of its inability to discriminate between any bases.

It is however worth to note that since glycine is a family box of NCC bases that for this instance there would be no need to discriminate between bases. Which would suggest that there at least should be an additional reason for avoiding adenine in the wobble position.

2.2 The most common solution: A to I editing

Regardless which explanation for adenine’s sparse presence we choose, it is clear that nature avoids having the base present in the wobble position (Das & Lyngdoh, 2012) (Ehrlich et al., 2021). In the smaller number of cases where adenine is actually present in the wobble position there is usually a contingency in place to lessen its detrimental effects. By far the most common contingency is to have an enzyme that modifies this unstable adenine into a more stable inosine (Torres et al., 2014).

Lim (1995) also investigated the effects of the presence of inosine in the wobble position. Here she described that for inosine a propeller twist would need to be formed only when forming a bond with guanine. All three other canonical bases can thus bind to inosine without much issue. Likewise, when compared to adenine, inosine would ease the stress the ribosome would undergo when tRNA would transition from the A- to the P-site. This also removes the risk of frameshifting that would be present with adenine in the wobble position. These finding are also supported by Das and Lyngdoh (2012).

For Balasubramanian and Seetharamulu (1981) it can be argued that with inosine in the wobble position, the structure of the U-turn would not be jeopardized. Likewise, for Borén et al. (1993) it can be argued that with inosine having low affinity to bind guanine, it is discriminant enough to be a serviceable base in the wobble position.

2.3 Alterations on the wobble hypothesis through the years

Since 1966 numerous new discoveries have been made and as a result, the wobble hypothesis has changed a lot throughout the years (Agris et al., 2007). So it was that in 1991 the hypothesis got extended, mainly with extra modifications that could be applied to a uridine in the wobble position (Agris, 1991), allowing it to pair to different arrays of bases. Several other articles also found alterations in the base pairing rules of the wobble positions, not all of them consistent with each other (Novoa & De Pouplana, 2012) (Agris et al., 2007) (Murphy & Ramakrishnan, 2004) (Tuite & Von Der Haar, 2016) (Lim & Curran, 2001). The clearest general overview of the wobble pairing rules can be found in figure 8a. With these conflicting results between general wobble base pairing, the wobble

hypothesis became more a tale of domain-specific post-translational modifications to the tRNA. Hence it was that Grosjean et al. (2009) described four decoding, or anticodon sparing strategies, that have been observed throughout the three domains of life. The modifications that resulted from those strategies, and the domains to which they apply can be seen in figure 8b.

Regardless of these additions and alterations to the wobble hypothesis, the core fundamental of the wobble hypothesis remained true: In codon to anticodon binding, the binding remains strictly canonical between the first and second codon bases, and the 36th and 35th anticodon bases; Only between the third codon position and the 34th anticodon position there is allowance for noncanonical base pairing.

8a

Wobble Hypothesis[1] nucleoside		Modified Wobble Hypothesis[26] nucleoside		Extended Anticodon Hypothesis[11] nucleosides	
Anticodon N_{34}	Codon N3	Anticodon N_{34}	Codon N3	3′-Adjacent- 37[53]	32•38[54],39[56]
G	C,U	G	C,U	t^6A	C_{32}•A_{38}
C	G	C	G	m^6A	Ψ_{39}
I	U,C,A	I	U,C,A	m^1G	
		xm^5U^a	G	wye	
U	A,G	s^2U^b	A,G		
		xo^5U^c	A,G,U (C)		

8b

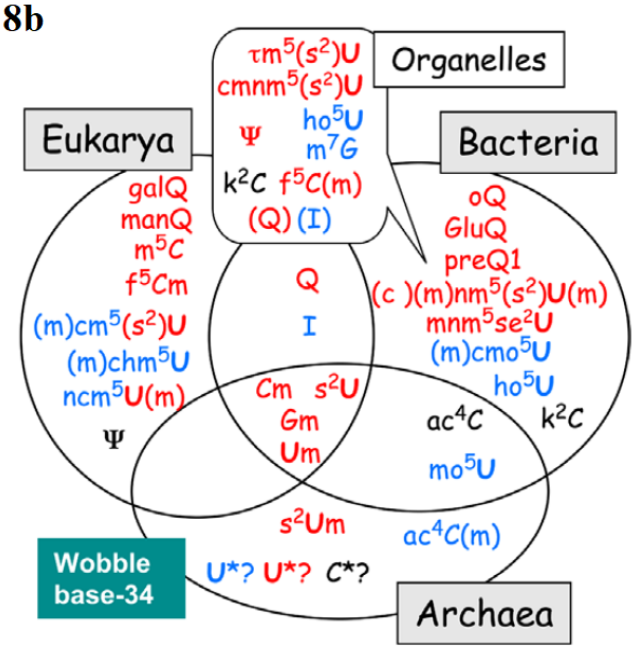

Figure 8: expansion of the wobble hypothesis (Agris et al., 2007) (Grosjean et al., 2009) **a:** three tables, the first depicts the original wobble hypothesis similar to figure 5. The second depicts the modified wobble hypothesis as formulated by (Agris et al., 2007). Here certain modifications to uridine have been added. Those modifications either expand or narrow the range of bases with which uridine is able to wobble pair. The third table depicts tRNA modifications that do not alter the 34th wobble position, but a base that is in vicinity to it. These modifications also exert an influence on codon recognition. Though the nature of those influences remains out of scope for this article. **b:** Here we have an overview of modifications to the 34th wobble position as recent as 2009 (Grosjean et al., 2009). These modifications are sorted between the three main domains of the tree of life. Those being eukarya, bacteria and archaea. Modifications to organelles are depicted in a sperate box but can be considered to be part of bacteria due to their common ancestry. The modifications coloured red are commonly involved in decoding two synonymous codons (split codon boxes). Those coloured blue are commonly involved in decoding four synonymous codons (full codon boxes). Those coloured black are involved in decoding the single AUA codon, coding for Ile. All symbols conform to conventional scientific literature. Residues are indicated by ‘m’ for methyl, ‘cm’ for carboxymethyl or ‘s^2’ for thio. When a base or residue is in brackets it is optional. The presence of certain modifications in archaea have been speculated but remain unknow, as indicated with a question mark. At least, at the time this figure was released.

2.4 Posttranslational modifications of tRNA in Archaea

To most biologists it is familiar knowledge that archaea is the least researched domain among the three main domains of life. This is also the case in regard to researching the modifications that are made to tRNAs present in archaea. Yet, in the last 30 or so years commendable efforts have been made to close this knowledge gap (Edmonds et al., 1991) (McCloskey, 2001) (Phillips & De Crécy-Lagard, 2011) (Wu et al., 2018). So, despite the fact that we are not yet on the same level as eukarya and bacteria with this topic, we are no longer completely in the dark, and now quite some interesting observations can be made. An interesting collection of modifications has been found in the 37th position of tRNA, only one base behind the anti-codon loop (McCloskey, 2001). Some, but not all of these have analogues in the two other domains of life. The presumed functions of these modifications vary, from altering interactions in wobble base pairing, to contributions to maintaining the right position of the reading frame (McCloskey, 2001). A noteworthy observation among these modifications is that inosine is present as a modification to adenine in archaea, just not in the wobble position. Here, that modification appears to happen through artifactual deamination rather than directed enzymatic activity (McCloskey, 2001). As for the case of the missing adenine in the wobble position in archaea; it does appear that for at least five species we actually can observe adenine in the wobble position in one of their tRNAs (Wu et al., 2018). These species are: *Ferroplasma acidarmanus* with the AAG anticodon for leucine, *Methanohalobium evestigatum,* with the AAC anticodon for valine, *Methanobrevibacter ruminantium* and *Halalkalicoccus jeotgali,* with the AGC anticodon for arginine, and *Natrinema pellirubrum,* with the ACC anticodon for glycine. With only five of over 160 archaea investigated these are still very much the exception to the rule. As to why adenine is rarely present in the wobble position in archaea; the most likely rationale appears to be that the archaea split off from the tree of life before the mechanism to convert adenine into inosine evolved. As a result, archaea would stick with having guanine in the wobble position rather than the troublesome adenine (Wu et al., 2018). This is a valid alternative because guanine through wobble interactions is also able to bind uracil in the third codon position.

3 Discussion

With this review article we investigated the wobble position of the anticodon. Our initial observation was that adenine is sparsely present here, and even seemed to be completely excluded within the domain of archaea (Ehrlich et al., 2021). We managed to find several rationales as to why this might be the case. The main presumption would be that adenine in the wobble position influences, in a negative manner, the structure of tRNA, and the interactions it has with the ribosome during translation. This would result in a reduced efficiency of translation, and potential frameshift (Lim, 1995). One way to circumvent this issue is to have adenine modified to inosine in this position (Torres et al., 2014). Although it appears that the enzymatic machinery needed for this modification evolved only after archaea split off from the tree of life (Wu et al., 2018). Thus, it appears that archaea remained to have adenine absent in the wobble position, and instead is more reliant on the other three bases, especially guanine, which is presumed to be able to wobble pair to uracil without much trouble.

Interestingly, recently it has been discovered that a few species of archaea do very sparingly have adenine present in the wobble position (Wu et al., 2018). This raises the curiosity as to how adenine in this instance would be dealt with. Has the mechanism to modify to inosine been acquired for these cases? Has some other method to rectify the troublesome effects of adenine evolved? Is the presence of adenine simply not that detrimental in these few cases? Might there even be a hidden benefit to adenine in these few cases? These are all questions that would need to be answered with further research, such as identifying the structural conformations of these specific tRNA, both natively and in interaction with the ribosome. This can be done through x-ray crystallography for example.

Another interesting research would be to investigate the kinetic activity of translation of these adenine leading tRNAs, and compare them to other tRNA that would pair with the same codons; which would be more efficient? Additionally, it might be worthwhile to investigate the protein products of these tRNAs; does frameshift indeed occur? Would reduced enzymatic activity, or a few small mistranslations, maybe be a benefit in a regulatory sense, as to reduce the overactivity of a certain enzyme?

Besides investigating these further questions, it also might benefit the field to take a step back and verify our prior assumptions. It would behove us to acknowledge that most of the rationale as to why adenine is troublesome in the wobble position has been produced in the 80s and 90s and predominantly as models (Balasubramanian & Seetharamulu, 1981) (Lim, 1995). In the rare instances that adenine in the wobble position was investigated in vitro the conclusion did not seem to fully align with the previous models (Borén et al., 1993). Although, the results there did not seem to dispute the models either. Thus, it is my advice that in further research we also ought to do in vivo experiments documenting the structural interactions of adenine in the wobble position. That way we can finally confirm or disprove that adenine in the wobble position interferes with the formation of the U-turn (Balasubramanian & Seetharamulu, 1981), leads to the formation of the propeller twist when pairing its non-canonical bases and hinders bases stacking in the A/P-site and E/P-site interduplexes (Lim, 1995).

Once these questions have been answered we may lay this topic to rest and could redirect our focus on other topics within the field. Such as which other codons and anticodons seem to be preferential and prevalent in organisms i.e. codon bias (Hanson & Coller, 2017). And in extension if tRNA of non-preferential anticodons have evolved to find other purposes within the cell i.e. investigation of non-canonical roles of tRNA (Krahn et al., 2020).

4 References

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