investigation. However, theory predicts that unresolvable sexual arms races may be infrequent, and that it is more usual for one sex or the other to gain an evolutionary advantage, halting antagonistic co-evolution, even though the underlying conflict may not be resolved, or that a balance in 'arms levels' results. Exceptions may be common at the molecular level — for example, involving seminal proteins and receptors — where costs of escalation are probably relatively low.

What's the big deal? Sexual conflict has been implicated as the driving force behind many important biological phenomena, including the rapid molecular evolution of reproductive genes and proteins, genome evolution and genomic imprinting. Conflict over reproductive investment may even explain the evolution of the sexes: proto-males may have parasitized the larger gametes of proto-females. Recently, sexual conflict has been implicated in speciation. In any evolutionary arms race between the sexes, there are potentially many different ways individuals of either sex could evolve to enhance their fitness. Any adaptation then counter-selects on the opposite sex, which again has many ways it could counter-adapt, and so on.

In any sexual arms race, it is genes involved in reproduction that are the focus of conflict, and molecular evidence suggests that many reproductive genes evolve extremely rapidly. As a result, populations can, in principle, evolve differences in almost any reproductive trait extremely rapidly through sexual conflict, and it is largely this feature that has prompted the promotion of sexual conflict as a speciation agent. It is important to note, however, that more traditional mechanisms of sexual selection can also generate rapid evolution of reproductive characters, and that distinguishing between the different selection pressures that can potentially lead to reproductive isolation is likely to be difficult.

Sexual conflict, sexual selection, what's the difference? The two concepts are intimately related. Sexual selection, selection in exclusive relation to securing matings (more strictly fertilizations), inherently generates sexual conflict because, for example, females will mate with some males, but not with others. Additionally, sexual conflict can be thought of as a sexual selection mechanism, as adaptation to win sexual conflicts can increase variance in reproductive success. Traditionally, sexual selection via female choice was seen to be generated by either direct or indirect benefits to females. In contrast, recent formulations of sexual selection generated by sexual conflict give primacy to direct costs, and female mating decisions are viewed as an attempt to minimise costs rather than maximise benefits. The importance of various mechanisms is currently being debated, but it seems likely that all play some role in sexual selection, although their relative importance may vary.

Where can I find out more?

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Primer

Fidelity in protein synthesis

Luisa Cochella and Rachel Green

The flow of genetic information from DNA to RNA to protein constitutes the basis for cellular life. DNA replication, transcription and translation, the processes through which information transfer occurs, are the result of millions of years of evolution during which they have achieved levels of accuracy and speed that make modern life possible. All three processes have base complementarity at the core of their mechanisms. DNA replication and transcription both depend on complementarity of the incoming nucleotide to the DNA template, whereas translation depends on the complementarity of the anticodon of the incoming transfer RNA (tRNA) to the codon in the template messenger RNA (mRNA). Fidelity of genetic information transfer thus relies heavily on discrimination between complementary, Watson-Crick (and in a few cases wobble) base pairs and non-complementary ones.

To ensure high selectivity, the macromolecular machines that carry out replication, transcription and translation — DNA polymerase, RNA polymerase and the ribosome, respectively — have evolved specific substrate recognition strategies. These strategies exploit the stability arising not only from the hydrogen-bonding and stacking capacity of Watson-Crick base pairs but, more importantly, from their distinct geometry. Both polymerases and the ribosome have chemical groups that directly monitor the geometry of the template-substrate base pair. In the case of DNA polymerases, this 'geometric selection' is estimated to contribute three orders of magnitude or more to selectivity, while hydrogen bonding only provides 7–40-fold selectivity.

While the accuracy of DNA replication and transcription depend only on cognate base pair selection, translation depends on an additional, base-pairing-independent reaction that must be carried out with high specificity. Each tRNA must be covalently attached to a specific amino acid — aminoacylated — preserving an unambiguous codon-amino acid correspondence known as the genetic code. This reaction is carried out by aminoacyl-tRNA synthetases specific for each amino acid and a corresponding group of tRNAs (isoacceptors). These enzymes must therefore recognize two substrates: first, a group of tRNAs which share a collection of 'identity elements' and second, an amino acid that may be distinguished by small differences in side-chain properties.

Polymerases, synthetases and the ribosome have been the paradigm cases for studying enzyme specificity, though we still do not fully understand the strategies used for high fidelity polymerization. In general terms, the specificity of an enzyme is limited by the difference in free energy of binding between correct and incorrect substrates. This difference derives from molecular distinctions that allow the correct substrate to make more favorable interactions with the enzyme or enzyme-template complex. This limitation to specificity becomes a problem during genetic information flow, where differences in free energy between cognate and some non-cognate substrates are generally smaller than would be necessary to account, in a single step, for the observed high fidelity of the process.

To this limitation is often added a requirement for high speed, which generally precludes full exploitation of available free energy differences. These constraints have guided the evolution of enzymes involved in information transfer to reach optimum ratios of accuracy and speed. Below we discuss, primarily in the context of translation, three strategies that macromolecular machines have evolved to achieve this balance, focusing on mechanisms known as editing, kinetic proofreading and induced fit.

Protein synthesis or translation has an observed fidelity of 1 error in 10^3–10^4 polymerized amino acids. These infrequent errors are generally substitution or missense errors resulting from mistakes in one of two different steps during the translation process (Figure 1): first, misacylation of a tRNA by its aminoacyl-tRNA synthetase; and second, 'selection' of an incorrect tRNA during the elongation cycle. Different constraints for speed and in available discriminatory binding energy have shaped the evolution of these two steps in translation to achieve the necessary level of fidelity. It should be noted that other types of error, such as incorrect start site selection, frameshifting and inappropriate termination can also occur during translation but are not discussed here as they have been less well studied.

Aminoacyl-tRNA synthetases use editing

Correct aminoacylation depends on the selection of two appropriate substrates, the tRNA and the amino acid, by the corresponding aminoacyl-tRNA synthetase. tRNA selection itself appears not to present a major challenge, as tRNAs are big enough to contain a large number of 'identity elements', or determinants, for specific interactions. Amino acids, however, are smaller and must be distinguished solely by the nature of their side-chains. Although there are substantial chemical differences among most amino acids, the very similar chemical and/or structural properties of some make them difficult to distinguish. As a specific example, threonyl-tRNA synthetase must discriminate threonine from the isosteric valine and from serine, which is smaller but has a γ-hydroxyl group like threonine.

How do synthetases deal with this? The aminoacylation reaction, which takes place at a site of the enzyme called the synthetic site, occurs in two steps. First the amino acid is activated by adenylation (consuming ATP) and then it is transferred to the tRNA (releasing AMP). Steric exclusion of amino acids with larger side-chains and recognition of specific properties of each amino acid generally make this synthetic site

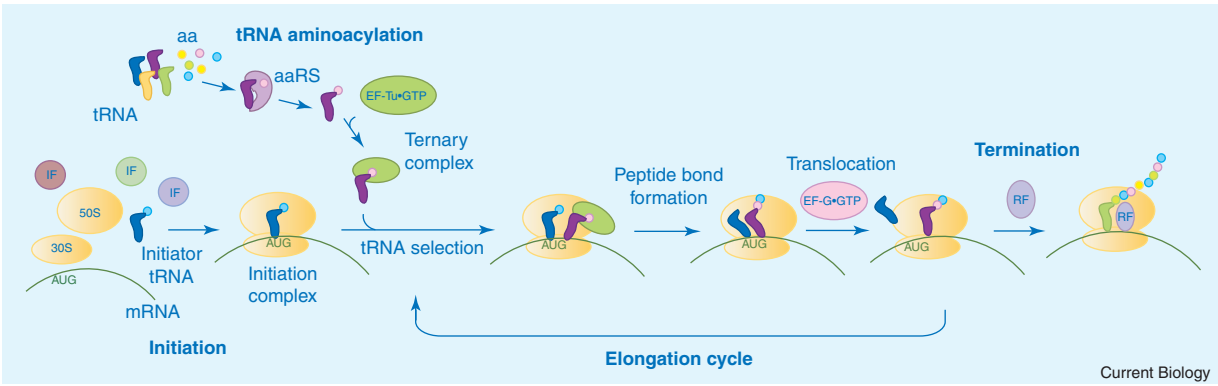

Figure 1. Overview of translation and its individual steps.

This review focuses on mechanisms of fidelity maintenance during tRNA aminoacylation and tRNA selection. 50S, large ribosomal subunit; 30S, small ribosomal subunit; IF, initiation factor; mRNA, messenger RNA; tRNA, transfer RNA; aa, amino acid; aaRS, aminoacyl-tRNA synthetase; EF-Tu, elongation factor Tu; EF-G, elongation factor G; RF, release factor.

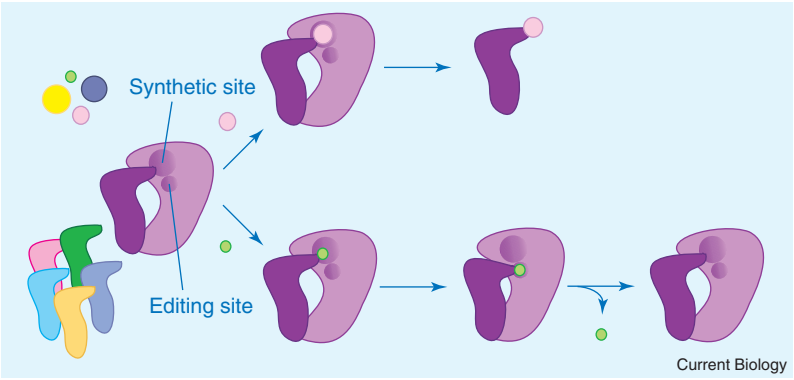

Figure 2. Editing mechanism involved in determining fidelity during tRNA aminoacylation.

Each aminoacyl-tRNA synthetase selects its cognate tRNA through a number of specific 'identity elements' (represented by the different tRNA colors). Selection of the cognate amino acid occurs in two stages. First, the synthetic site excludes amino acids that are larger than the cognate one, or that cannot establish sufficient specific interactions (top pathway). Smaller amino acids with some similarity to the cognate one can be misincorporated by the synthetic site and are hydrolyzed in a distinct site of the enzyme, the editing site (bottom pathway).

specific enough so that only the correct amino acid can be activated and transferred. But amino acids having similar properties to and a smaller size than the cognate amino acid can be misactivated at frequencies that are too high to maintain an unambiguous code. As a consequence, enzymes facing this problem have evolved a second active site, distinct from the synthetic site, called the editing site, where misactivated amino acids or misacylated tRNAs are hydrolyzed.

The presence of two catalytic sites with different activities led to the proposal of a 'double-sieve' model of fidelity (Figure 2). In this model, the synthetic site of the enzyme acts as the first sieve, excluding amino acids that are too large or that cannot establish specific interactions. For example, threonyl-tRNA synthetase can discard amino acids larger than threonine, based on size. It also discards valine, which is similar in size but lacks the γ-hydroxyl group. Threonyl-tRNA synthetase binds valine significantly more weakly than threonine because a specific interaction between a zinc ion in the active site and the γ-hydroxyl group of threonine does not form when valine is bound. Smaller amino acids that can establish sufficient interactions, however, may slip through this first, coarse sieve and be activated and transferred to the tRNA.

The role of the second, fine sieve is played by the editing site, which is too small to fit the cognate amino acid, but can hydrolyze other small amino acids that slipped through the first selection. In the case of threonyl-tRNA synthetase, serine binds to the zinc ion in the activation site and is activated and transferred with an error frequency of 1 per 10^3. As the overall error frequency of charging by threonyl-tRNA synthetase is 1 in 10^4, editing must increase the accuracy by a factor of 10. Numerous experiments suggest that editing contributes a factor of 5–100-fold to overall selectivity, reducing error frequencies to a range of 1 in 10^4–10^5. Most editing aminoacyl-tRNA synthetases appear to be able to hydrolyze both the activated amino acid (pre-transfer to the tRNA) and the aminoacyl-tRNA (post-transfer) *in vitro*, though the actual contribution of each of these pathways to overall editing *in vivo* has been a subject of debate; in general, albeit with known exceptions, the post-transfer editing pathway seems to predominate *in vivo*.

It has been argued that hydrolysis or editing of an incorrect intermediate by an independent activity/site can provide the largest theoretical increase in fidelity over the maximum imposed by the difference in binding free energy. Interestingly, many DNA polymerases use a similar strategy in which editing of a mismatched terminal nucleotide is carried out by a completely independent exonuclease activity.

tRNA selection uses kinetic proofreading and induced fit

Given that tRNAs are aminoacylated with such great accuracy by aminoacyl-tRNA synthetases (10^{-4}–10^{-5}), high fidelity translation then depends on selection of the cognate aminoacyl-tRNA corresponding to the codon presented by the mRNA in the ribosome. Here, the substrates, aminoacyl-tRNAs, are discriminated primarily on the basis of their anticodon sequences. The difference in free energy of binding between the cognate and a non-cognate aminoacyl-tRNA (with two or three mismatches to the codon in the mRNA) is easily large enough to exclude the latter from the ribosome. Discrimination of near-cognate aminoacyl-tRNAs, with only one mismatch between codon and anticodon, with high accuracy, however, is not a trivial problem. There are generally sufficient binding energy differences to allow discrimination between cognate and near-cognate pairings (especially if these differences are sampled several times). But because tRNA selection has the additional constraint of needing to be fast, as translation is rapid and processive, these differences cannot be adequately exploited. Indeed, the rapid rate of translation apparently precludes the establishment of equilibrium between the various tRNAs and the ribosome–mRNA complex, thus calling into action kinetic discrimination mechanisms.

The first strategy shown to operate during aminoacyl-tRNA selection has been termed kinetic proofreading. It was long ago realized that, if substrate selection were separated into two distinct phases by an irreversible step (in this case GTP hydrolysis), there would be two opportunities to

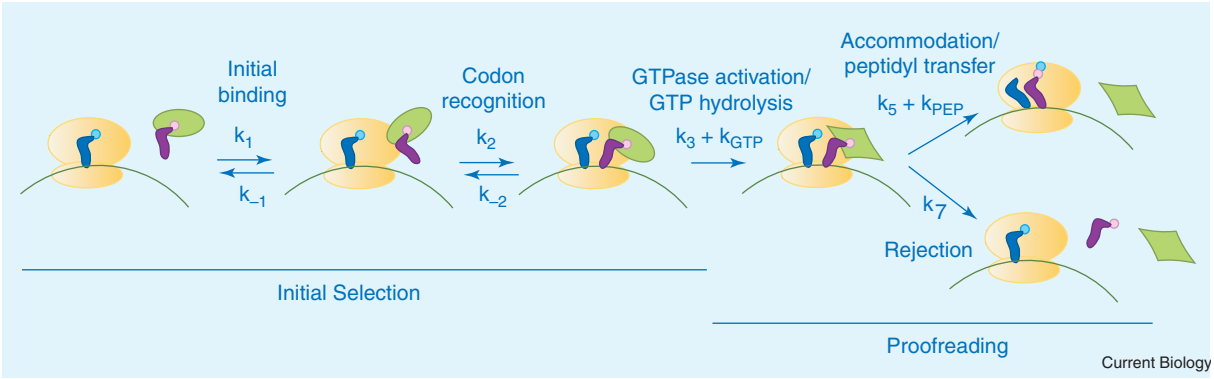

Figure 3. Detailed kinetic scheme for tRNA selection highlighting the two stages of the process, initial selection and proofreading.
The selectivity of the initial selection stage is determined by the difference in rate of GTPase activation (k_3) between the cognate and a near-cognate tRNA. The selectivity of the proofreading stage is determined primarily by the difference in rate of accommodation (k_5) between the cognate and a near-cognate tRNA. EF-Tu (green) is shown in two different conformations before and after GTP hydrolysis.

examine and discard an incorrect aminoacyl-tRNA (Figure 3). This means that the binding energy between the ribosome and the ternary complex can be sampled twice and the specificity thus increased. While the idea of having consecutive selective steps is similar to the 'double-sieve editing' mechanism discussed above, it is distinguished by the fact that kinetic proofreading applies the same basic selective step twice, whereas editing generally relies on a second distinct site or activity that monitors different properties than the first selective step.

Kinetic proofreading during tRNA selection is made possible by the fact that aminoacyl-tRNAs are delivered to the ribosome in a ternary complex with the GTPase elongation factor Tu (EF-Tu in bacteria, EF1A in eukaryotes) and GTP. In an encounter between ternary complex and the ribosome (initial selection), a cognate ternary complex is more likely to trigger GTP hydrolysis than to dissociate, whereas a near-cognate ternary complex is more likely to dissociate. Simply put, the cognate species partitions forward in the stepwise scheme whereas the near-cognate partitions backward. This initial selection step is followed by the proofreading step where inherent binding differences between codon and anticodon are again sampled. As before, the cognate aminoacyl-tRNA species is more likely to partition forward (and 'accommodate' into the A site and participate in peptide bond formation), while the near-cognate aminoacyl-tRNAs are more likely to partition backward (and be rejected from the ribosome).

The relative contribution of each of these selective steps, initial selection and proofreading, has been measured *in vitro* in multiple ways, where overall error rates of ~1 in 450 to 1 in 1600 approach the overall fidelity measured *in vivo*. In these systems, essentially all non-cognate aminoacyl-tRNAs are rejected during initial selection. Near-cognate aminoacyl-tRNAs, however, can pass through initial selection and trigger GTP hydrolysis with a frequency of ~1 in 30. These sneaky aminoacyl-tRNAs are generally rejected during the second stage, thus increasing selectivity by ~15–45-fold.

Interestingly, the maximal theoretical selectivity of kinetic proofreading is not realized here because of the processive nature of translation and associated requirement for speed. To maximize each selective step, the forward rates should be slow enough that differences in dissociation rates can be exploited. Indeed, experimental evidence shows that, when GTP hydrolysis is made extremely slow, the selectivity observed in this initial selection step is substantially increased. It was long ago suggested that ribosome mutations which affect the fidelity of tRNA selection act similarly by increasing or decreasing the rates of individual steps in the selection process.

While separating the process into two steps — kinetic proofreading — does provide an advantage during tRNA selection, it is not because differences in dissociation rates between cognate and near-cognate aminoacyl-tRNAs are exploited twice, as previously thought. Rather, during each stage of tRNA selection a second strategy comes into play that introduces a large difference in the rates of two critical forward steps. During initial selection, the rate of GTPase activation (k_3) is significantly faster for the cognate than for near-cognate aminoacyl-tRNAs, and during proofreading, there are similar differential rates of accommodation (k_5) (Figure 3). These differences in forward rates have been attributed to a mechanism historically termed induced fit, which is used by the translation machinery, polymerases and a number of other enzymes. Induced fit refers to the ability of a correct substrate, but not an incorrect one, to cause conformational changes in the enzyme and/or the substrate which have downstream effects on catalysis. During tRNA selection on the ribosome a series of conformational changes induced by binding of the cognate aminoacyl-tRNA, but not a near-cognate one, result in a number of rearrangements in the ribosome and the tRNA itself that result in the kinetic effects discussed above.

It appears then that the combination of kinetic proofreading and induced fit in tRNA selection provides a suitable balance between fidelity and rapid elongation rates. If we take the simplest case of kinetic proofreading, where there are no differences in forward rate constants introduced by induced fit — where k_3 and k_5 are equivalent for both cognate and near-cognate tRNAs — significant discrimination between cognate and near-cognate tRNAs will only be observed when k_3 and k_5 are very, very slow relative to k_{-2} and k_7. In other words, if tRNA selection were an equilibrium process where the full discrimination potential was extracted from the binding energy, there would be no need for other discriminatory mechanisms. But such a slow step in translation is apparently not compatible with the overall rapid rate of elongation.

The addition of induced fit to the process of tRNA selection boosts selectivity *when the reaction is constrained to be fast* by accelerating the rate of passage of cognate species relative to near-cognate ones. Because forward rates are fast relative to reverse ones, the selectivity of each step is lower than the theoretical maximum allowed by *intrinsic* energetic differences between cognate and near-cognate tRNAs in the complex. In this case, the energetic cost of inducing conformational changes has little impact on cognate tRNA selection but has substantial detrimental effects on near-cognate tRNA selection thus conferring increased specificity. Effects on forward rates that result from an induced fit mechanism have been shown to be a dominant determinant of fidelity in tRNA selection. A body of experimental data supports this idea by showing that miscoding increases when the differences in GTPase activation and accommodation rates are decreased either by introduction of a mutation in the tRNA body or by addition of antibiotics like paromomycin and streptomycin.

We have discussed two general mechanisms used to maintain the high fidelity of protein synthesis (as well as of DNA replication and transcription). The first mechanism is comprised of editing and kinetic proofreading. Although different in detail, both strategies amplify the available discrimination power, determined by differences in free energy of binding, by having more than one selective step. Of these two strategies, editing has an advantage arising from the use of two distinct sites that scrutinize different properties of the substrate. The second mechanism, induced fit, depends on substrate-specific conformational changes that result in selective modulation of forward rate constants, permitting high fidelity discrimination when rapid rates are essential. Such distinct solutions for different enzymes ultimately result from the evolutionary constraints imposed by the diverse requirements for fidelity, speed and efficiency on each molecular problem.

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Captivity selects for smaller eyes

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Eye size is adapted to ecological and behavioral conditions. Large eyes provide hawks and dragonflies with high spatial resolution, and owls, nocturnal bees and deep-sea fish with high sensitivity [1–4]. Conversely, eyes are reduced when the need for vision diminishes, exemplified by the subterranean naked mole rat and cave-dwelling fish, crayfish and crickets [5]. These adaptations suggest that eye size responds to selection but, as far as we know, there are no reports of eye size changing progressively over time in response to changes in selection pressure, possibly because slow changes are difficult to detect.

We have measured the size of compound eyes in populations of *Drosophila melanogaster* that have been held in captivity for different lengths of time. We found that flies from older cultures have smaller eyes. We conclude that, because there is less need for vision in captivity and eyes are costly, there has been selection for smaller eyes. Cultures appear to have slowly adapted for 60 years, and this has important implications for how wild-type *Drosophila* are defined.

When wild *Drosophila* are placed in culture their need for vision to find food and mates is reduced. We obtained flies from stocks established with wild flies 0.5, 15, 20, 34, 48 and >64 years ago to see if and how eye size changes over time following a reduction in the need for vision. To control for founder effects and historical variation in rearing conditions, we examined three further independent cultures aged 0.5, 20 and >64 years, giving nine populations in all (Table 1).

We took at least 10 flies of each sex from each culture and measured their eye size, defined as the square root of the product