Unlike prokaryotic mRNAs which contain cistrons, each with its own individual initiation codon and ribosome-binding site, eukaryotes have the task of finding a start codon near the 5′-end of the mRNA transcript. This is believed to occur by the 40S subunit, along with initiation factors, which recognize the m7G cap, subsequently ‘scan’ the mRNA in a 5′-3′ direction until they encounter a start codon (Kozak 1980).

To begin translation, Kozak depicted a ribosome as “scanning” the mRNA from the 5′-end until it encounters the first AUG codon. In many cases though, 5-10%, a ribosome will bypass one or more AUG codons before encountering the correct codon which will initiate translation. To explain this occurrence, Marilyn Kozak viewed the sequences around initiating AUGs and found the sequence CC(A.G)CCAUGG to be common. To see if this sequence is the most efficient, she mutated the sequence surrounding the AUG in a cloned rat pre-proinsulin gene, in order to decrease its efficiency at initiating translation. The gene was placed under the control of a SV40 virus promoter, introduced into monkey (COS) cells, and then labeled the newly synthesized proteins with 35 S-methionine, with subsequent immunoprecipitation, electrophoresis of the proteins and fluorography to detect the proteins. The best initiation occurred with CCACCAUGG, producing a large access of proteins compared to all other sequences (Kozak, 1983; Weaver 1999).

The surrounding context of the sequence was known to play a role in initiation and therefore Kozak introduced the mutated sequences out-of-frame and upstream of the normal initiation codon, in order to confuse ribosomes and force them to begin translation out of frame, thereby producing less protein (proinsulin). Upstream mutants that did not resemble the optimum sequence very well, did not interfere with initiation at the downstream AUG, and therefore a lot of protein was made. However, the closer the upstream mutated sequence resembled the optimal sequence, the more it interfered with initiation at the downstram AUG, and less protein was made. This finding is exactly what Koazk's scanning model predicts, in which an initiation complex, including the 40S ribosome, scans the mRNA in search of an optimum sequence to begin. Moving the sequence, will therefore change the reading frames and the distance from the 5′-end that the initiation complex will begin and therefore prevent correct protein formation (Kozak 1983).
However, many mRNAs found in nature contain an upstream AUG surrounded by an optimum sequence, that could still initiate at a downstream AUG. Kozak noticed that many of these sequences have a stop codon between the two AUG codons, which would terminate the ribosomes first initiation attempt at the first AUG (Kozak 1986). Without successful initiation, the ribosomes would continue scanning for another start codon and reinitiate at the next AUG. Kozak validated this idea through a set of constructs containing a stop codon in this context, with the synthesis of proinsulin as an indicator of initiation at the correct downstream codon. It was displayed that an upstream AUG followed by a stop codon was ineffective at halting translation at the downstream site, as abundant protein (proinsulin) was made. The ribosome must therefore be scanning down the mRNA transcript, until it encounters the suitable AUG codon (Kozak 1986).

The sequences present between the first AUG and the stop codon in the above experiment define an open reading frame (ORF), but were fairly short. What if an open reading frame (ORF) large enough and capable of encoding for a protein was added to the mRNA upstream of the gene of interest (making it a bicistronic mRNA)? Kozak added an ORF for a protein of different size compared to proinsulin, to the transcript in order to differentiate the end products of translation. Through fluorography, Kozak detected that the first AUG codon was used, thereby producing the small newly inserted protein instead of the proinsulin (Kozak 1986). This again confirms the scanning model of initiation, as the ribosome scans the mRNA, initiates at the first AUG codon and translates the first ORF into a protein and subsequently dissociates from the transcript, probably loosing initiation factors needed for the initiation at the second AUG codon, thereby ignoring it. Upon subsequent attachments to the 5′-cap, the ribosomes will always encounter the first AUG, as the scanning model predicts (Kozak 1986; Weaver 1999).

Along with the above mentioned experiments, the scanning model is further supported by additional evidence:

1. ATP depletion and stable hairpin structures before a AUG codon, blocked the migration of a 40S ribosomal complex (Kozak 1986; Kozak 1989), stalling the ribosomes upstream from the initiation codon, thereby providing evidence of their entry upstream of the start site with subsequent migration downstream, rather than exactly on the start site.
2. Circulization of a RNA template does not allow for ribosomal attachment (Konarska 1981; Kozak 1979), leaving ribosomal entry only capable on a free 5’ end. Therefore, the 5’ end of mRNA is the ribosome entry site.

3. A repressor protein blocking the 5’ end of an mRNA, disables the ribosomes ability to bind to mRNA, thereby no ribosomes being present on the mRNA (Paraskeva et al., 1999).

4. 5’ end beta-globin mRNA can act as an assembly site for initiation complexes (Pestova et al., 1998)

5. Migration of ribosomes is displayed through the observation of multiple complexes bound throughout the length of each mRNA (Kozak and Shatkin 1978; Munroe and Jacobson 1990). These polysome-like complexes, validate the migrating/scanning capabilities of ribosomes and depict translation initiation as beginning with a search for a start site, as ribosomes are also found upstream of start sites, rather than by exact identification of AUG.

6. Addition of a stable base paired structure trapped ribosomes upstream of the migration barrier (Kozak 1998).

With such evidence available to support the scanning model of translation initiation, it would seem that there should be no need to question it’s accuracy. Nevertheless, many exceptions to the scanning model have been observed, displaying the scanning model as not a concrete mechanism for translation initiation I believe, but a model that can act as a prototype to the many exceptions seen in eukaryotic initiation. This can be seen in the observation of leaky scanning and reinitiation which require modifications of the traditional scanning model to suit their existence.

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