Control not at initiation? Bah, humbug!

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Initiation is often assumed to be rate-limiting for protein synthesis, but the presence of rare codons nonetheless can influence protein levels. In this issue of The EMBO Journal, Chu *et al* report that when rare codons are positioned near the start of the coding region, 'liberation' of the initiation codon for loading of the next 40S subunit may be rate-limiting for initiation and therefore overall protein synthesis. The sequential nature of translation results in an interdependence in ribosome association either by *de novo* initiation or recycling. Thus, a more general view emerges where both elongation and initiation can contribute to protein expression.

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The field of protein synthesis has largely been indoctrinated in the concept that the first committed step in the pathway, 'initiation', is rate-limiting. Indeed, there are more than 30 polypeptides associated with initiation, and some 40+ phosphates deposited on different initiation factors. The dominant regulation by the eIF2α kinases and the phosphorylation status of 4E-BP has been well characterized. The former limits the amount of available ternary complex (eIF2•GTP•Met-tRNAi) and the latter restricts the level of active eIF4F. Now, the paper by Chu *et al* provides support for a more general perspective on the mechanistic coupling between initiation and elongation.

A simplified kinetic model helps to understand the relationship between ribosome association and elongation rates on protein synthesis. Translation can be considered to be a series of sequential reactions in which initiation proceeds with rate constant k_{init} and is followed by 'liberation' of the initiation site at a rate constant k_{lib} (Cleland, 1975). The rate constant k_{init} encompasses ribosome binding and can depend on the concentrations of mRNA and free 40S subunits, and therefore will be the point of regulation under conditions where their concentrations are rate-limiting. The rate constant k_{lib} represents movement of the ribosome a sufficient distance from the start codon to allow binding of an additional 40S subunit with rate constant k_{init}. The rate constant k_{lib} is dictated by the individual rate constants for elongation of each codon within the distance that must be cleared to allow another round of initiation.

It follows that since initiation, clearance and elongation are sequential that their relative magnitudes will dictate which will be rate-limiting and therefore the point of regulation for protein expression (Ray, 1983). This can be seen in Fig 1 which shows the magnitude of the overall net rate constant for initiation (k_{obs}) as a function of the intrinsic rate constants for ribosome binding (k_{init}) and for liberation of the initiation site (k_{lib}). When liberation is fast relative to the range of k_{init} values, the overall observed rate constant for initiation is highly sensitive to k_{init}. In contrast, when liberation is slow there is little effect of the change in magnitude of k_{init}. The converse is true and under conditions where k_{init} is fast relative to k_{lib},
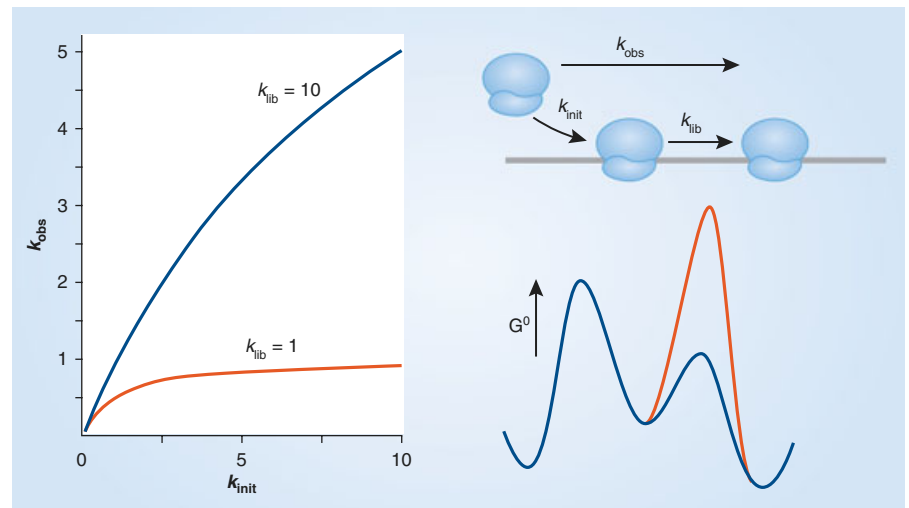
Figure 1. Contributions of the intrinsic rate constants for initiation (k_{init}) and for liberation (k_{lib}), on the net rate constant for initiation (k_{obs}).

In this simple example the net rate constant for initiation is $k_{obs} = k_{init} \, k_{lib} \, / \, (k_{init} + k_{lib})$. On the left is an example of the cases in which k_{lib} is fast relative to k_{init} (blue line, $k_{lib} = 10$) and in which k_{lib} is slow relative to k_{init} (red line, $k_{lib} = 1$). These rate constants are pseudo-first order and have units of reciprocal time. On the right is a Gibbs free energy profile of the extreme cases in which k_{init} is rate limiting (blue line) or k_{lib} (red line) is rate limiting.

changes in k_{lib} will affect the net rate constant for initiation. Differences in k_{lib} can arise from intrinsic differences in rare codons between mRNAs or from engineering in rare codons as done in the paper by Chu *et al*. Also, it follows that if the net rate constant for subsequent steps in the translation of the mRNA are fast relative to the rate constant for clearance, then the clearance step(s) will overall be rate limiting.

It follows that all pathways for initiation will be sensitive to clearance, particularly recycling. The documented interaction of the 5′ end of the mRNA (via eIF4F) with the 3′ end of the mRNA (via the poly(A) binding protein) (Wells *et al*, 1998) provided the explanation for the enhanced translational efficiency of mRNAs that contained an m^7G cap and a poly(A) tail. Upon termination, 40S subunits would be recruited more efficiently to the 5′ end of the mRNA than would occur if a free 40S subunit in solution was to be recruited. Consistent with this interpretation was the previous report that formation of full-sized polysomes took considerably longer than just the ribosome transit time for the coding region of the mRNA (Nelson & Winkler, 1987). Here, the explanation was that the more efficient utilization of 40S subunits at termination for initiation tended to outcompete free 40S subunits from binding to the mRNA and thereby, delaying the time required for maximal polysome formation.

A few additional facts germane to understanding rate-limiting steps in initiation are: in most reports of polysome profiles from log-phase growth, the amount of 40S, 60S and 80S ribosome (the latter which may or may not represent new initiations) is roughly 20 to 30% of the total ribosome pool. As a consequence, approximately 80% (or more) of the 40S subunits are in polysomes. Secondly, from estimates of the levels of initiation factor proteins, the most common numbers come out to be roughly 0.3–1.0 mole of factor per mole of ribosome (John Hershey and Christopher Fraser, personal communication). Thus, if 70–80% of the ribosomes are associated with elongation, then there is more than an equal molar amount of initiation factor for newly initiating 40S subunits (note, the initiation factors could still be limiting if the affinity for the 40S subunit was poor). Thus, understanding the individual steps that contribute to k_{init} is the key to understanding translational control.

The article by Chu *et al* clearly shows several variations where changes in the elongation rate influence protein expression as manipulated through the use of rare codons. In a similar study by Shah and coworkers (Shah *et al*, 2013), they developed a whole cell model for the study of protein synthesis in yeast. In this, the authors concluded that 'protein production in healthy yeast cells is typically limited by the availability of free ribosomes'. Where these two come together is that the availability of free 40S subunits is limited by 40S subunits being released at termination, not by free 40S subunits in solution. It should be noted that the Chu *et al* article and previous publications implying control at the level of elongation (i.e. Nielsen & McConkey, 1980) were based upon cells in log-phase growth. It still remains that initiation factors can be the dominant control point under conditions of reduced protein synthesis and that the global control through restricted levels of ternary complex or restricted levels of eIF4F activity actually have different consequences (Merrick, 2003, 2010).

Thus, the rate-limiting step in the sequential process of translation is the forward step for which a change in its rate constant produces the largest effect on the overall rate. This fundamental feature is well illustrated by the translational control mechanism revealed by Chu *et al* that responds to the speed of ribosome movement immediately after the start codon. So, yes Virginia, there is control of initiation by elongation.[1] The trick perhaps is to find the experiment to understand the underlying values for k_{lib} and k_{init}.

Acknowledgements

Note

[1]In response to 8-year-old Virginia O'Hanlon's question 'Please tell me the truth, is there a Santa Claus?', newspaper columnist Francis Church replied 'Yes Virginia, there is a Santa Claus.' Church's letter in the New York Sun was originally published in 1897 and was published annually at Christmas time in the Sun for almost 50 years.

Conflict of interest

The authors declare that they have no conflict of interest.

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