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DIVERGENT VIEWS

The case against the involvement of the NMD proteins in programmed frameshifting

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The complexity of the nonsense-mediated decay (NMD) system makes it difficult to study by comparing the expression of various single reporter constructs. The known effects of the *NMD* genes include a reduction both in mRNA stability (reviewed by Czaplinski et al., 1999) and in the efficiency of translational initiation (Muhlrad & Parker, 1999) of nonsense-containing plasmids as well as an apparent increase in the efficiency of translational termination as evidenced by increased readthrough of nonsense mutations (Bidou et al., 2000; Maderazo et al., 2000). The single reporter system can not distinguish among these effects and inference is required to determine which mechanism underlies any observed phenotypic effect on gene expression. It is particularly problematic to differentiate the effects of translation initiation accuracy from putative effects on translational frameshifting. The dual reporter system used in our work isolates the effect of translational frameshifting from effects on mRNA stability, initiation or termination. Much is made by Dinman et al. of the relative effects of various mutations, yet it remains unclear whether these are fundamental differences or simply differences in phenotypic strength of the various mutations.

The suggestion that the inability to maintain the *M₁* yeast virus (*Mak⁻*) reflects a defect in frameshifting flies in the face of the fact that most *mak* mutants affect 60S ribosomal subunit biogenesis, reducing the efficiency of translational initiation (Ohtake & Wickner, 1995). This connection is further reinforced by the fact that mutations affecting translation initiation factors eIF2 and eIF2B also have a *Mak⁻* phenotype (Harashima & Hinnebusch, 1986). Though it is possible that a frameshifting effect of the NMD proteins could explain this phenotype, it is reasonable to suggest that it depends

on their initiation effect. The lack of an effect of *mof4-1* on 60S ribosomal subunit biogenesis is expected, because the protein should not affect bulk translation, but only translation of nonsense-containing mRNAs.

Dinman et al. dismiss our report by suggesting that our reporter is not sensitive to NMD. That the reporter is sensitive to NMD is shown by the effect of *NMD* mutations increasing nonsense readthrough as measured with the dual reporter system. In addition, nonsense-containing dual reporter transcripts show the expected loss of expression of the upstream gene caused by NMD-dependent mRNA degradation (G. Stahl and P. Farabaugh, unpubl. data). The suggestion that the length of the upstream gene, *lacZ*, interferes with the ability of the NMD system to regulate frameshifting in our reporter is based on the proposed existence of a ribosome-bound complex of Upf proteins. Such a complex remains hypothetical. The only evidence for it is that Upf2p interacts with both Upf1p and Upf3p (He et al., 1997) and that all three factors are found in polysome fractions (e.g., Atkin et al., 1997). Recently, Maderazo et al. (2000) showed that the Upf proteins are present at far below stoichiometric with ribosomes. The average yeast cell contains 100,000 ribosomes, 1,600 copies of Upf1p, 160 copies of Upf2p and only 80 copies of Upf3p. It seems clear that a complex of Upf proteins is unlikely to ride on each translating ribosome, making the model envisioned by Dinman et al. extremely unlikely. If they interact with the terminating ribosomes as a complex at all, then they most likely are recruited at the point of termination, making the length of the gene translated irrelevant.

CONCLUSION

We agree that these experiments cannot be considered definitive and realize the value of side-by-side comparison of the two assays. The issue of how the

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NMD system modulates both mRNA stability and translational competence remains an important one regardless of the outcome of this debate, and one that we intend to continue to address.

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