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DIVERGENT VIEWS

The case for the involvement of the Upf3p in programmed –1 ribosomal frameshifting

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Translational fidelity, programmed frameshifting, and nonsense-mediated decay (NMD) may require common proteins, reflecting related events in translation and ribosome function (reviewed in Czaplinski et al., 1999). This letter focuses on the possible link between programmed frameshifting and NMD, and seeks to reconcile apparently conflicting conclusions concerning a proposed overlap in the factors involved.

Deletion of *UPF3*, which is required for NMD, has been reported to increase the frequency of programmed −1 ribosomal frameshifting (Cui et al., 1996; Ruiz-Echevarria et al., 1998a); in contrast, a recent report in this journal suggests that a *upf3* deletion strain does not affect −1 frameshifting (Bidou et al., 2000). Both reports agree that NMD and frameshifting are not obligatorily linked: deletion of *upf1* or *upf2* disrupts NMD, but do not affect frameshifting. The question is whether strains lacking *UPF3* are defective in −1 frameshifting. Bidou et al. answer no, while we answer yes. We suggest several explanations of this discrepancy.

THE ASSAYS: DICISTRONIC VERSUS MONOCISTRONIC mRNAs

One potential source of the different observations may lie in the systems used to monitor programmed ribosomal frameshifting. Whereas we have used a series of *lacZ*-based monocistronic vectors, Bidou et al. use bicistronic *lacZ*-luciferase reporters. In the monocistronic system, the beta-galactosidase activities generated from cells containing either −1 or +1 reporter plasmids divided by those generated from a 0-frame control are used to determine frameshifting efficiency. The bicistronic system also compares frameshift reporter and 0-frame controls. Here, the efficiency of frameshifting is calculated by determining beta-galactosidase/out-of-frame luciferase ratios and dividing these by the beta-galactosidase/0-frame luciferase ratios (Stahl et al., 1995).

In both systems, the frameshift reporter mRNAs are nonsense-containing messages. Stabilization of these in NMD mutants would result in the production of more enzymatic activity. In the monocistronic system, stabilization of the frameshift reporters could result in apparent, though not real, increases in frameshifting efficiencies. Indeed, others and we have observed such an effect. For example, apparent increases in −1 ribosomal frameshifting efficiencies are observed in cells harboring the *ifs1-1* and *ifs2-1* alleles of *UPF2* and *UPF1* respectively (Lee et al., 1995), and we also observe this twofold effect on both −1 and +1 ribosomal frameshifting in *upf1* and *upf2* deletion mutants (Dinman and Peltz laboratories, unpubl.). A similar twofold increase in the efficiency of Ty*1* directed +1 frameshifting is also seen in cells harboring the *mof4-1* allele of *UPF1* (Dinman & Wickner, 1994).

Three independent observations, however, suggest that deletion of *upf3* (and the *mof4-1* allele of *UPF1*) do promote real changes in programmed −1 ribosomal frameshifting. First, the effects are −1 frameshift specific: these mutants yield four- to fivefold increases in −1 frameshifting, as opposed to twofold increases in +1 frameshift efficiencies. A second bit of supporting evidence comes from the observation that there are no differences in the steady state abundance of the −1 frame reporter LacZ mRNA between wild-type, *upf1*Δ*A*, *upf2*Δ*A*, *upf3*Δ*A*, and *mof4-1* strains (Cui et al., 1996; Ruiz-Echevarria et al., 1998a). This suggests that stabilization of the reporter mRNA cannot account for the observed increases in −1 frameshifting in cells harboring the *upf3*Δ*A* or *mof4-1* alleles. Third, the demonstra-

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tion that cells harboring these mutations cannot maintain M_1, which is exquisitely sensitive to changes in programmed −1 frameshift efficiencies, provides independent evidence that −1 frameshifting is altered in these cells (Dinman & Wickner, 1992, 1994). It is possible that virus loss by these mutants could simply be coincidental; there are multiple routes to this phenotype. However, the most common biochemical defect associated with loss of killer, decreased levels of 60S ribosomal subunits (Ohtake & Wickner, 1995) are not observed in *mof4-1* mutants (J.D. Dinman, unpubl.). These three sets of observations support the hypothesis that *upf3*Δ*A* and *mof4-1* strains have specific defects in programmed −1 frameshifting.

THE ACTIVITY OF FRAMESHIFTING AND/OR NMD FACTORS CAN BE MODULATED BY *CIS*-ACTING SEQUENCES AND *TRANS*-ACTING FACTORS

How then to explain the observations of Bidou and colleagues? On the face of it, the bicistronic assay controls for NMD defects by normalizing the activity of the −1 luciferase reporter to the 0-frame beta-galactosidase activity on the same mRNA. However, we suggest that the use of LacZ in this system has created a unforeseen complication. As noted above, in the course of monitoring the status of NMD, we have also monitored the abundance of the LacZ reporter. Interestingly, we have found that this is very stable, even when it is either out of frame or downstream of a premature termination codon (Ruiz-Echevarria et al., 1998a; Meskauskas & Dinman, submitted; W. Wang and S. Peltz, unpubl. results). It has been shown that unique *cis*-acting elements and *trans*-acting factors prevent NMD (Ruiz-Echevarria et al., 1998b; Ruiz-Echevarria & Peltz, 2000). We think that interference by the LacZ message with the function of the Upfp complex may explain why the bicistronic reporter does not detect the *upf3*Δ*A* effect on −1 frameshifting. We propose that in wild-type cells, the function of the Upfp complex bound to the ribosome is inactivated during the course of translating through the >3 kb of LacZ mRNA. Therefore, by the time the translational machinery encounters the frameshift signal in the bicistronic reporter it is effectively Upf3-minus. This would account for why Bidou and colleagues did not detect a difference in frameshifting between wild-type and *upf3*Δ*A* strains. Indeed, two aspects pertaining to the nonsense-suppression data reported by Bidou and colleagues also support this: (1) the observation of >15% nonsense suppression in wild-type cells is unusually high, and (2) the average 1.7-fold difference between wild-type and mutant cells is extremely small compared to the 6–10-fold differences that we observe using nonsense-containing monocistronic reporters (S. Peltz and W. Wang, unpubl.).

CONCLUSIONS

Although neither approach is definitive, we think that we have addressed the problem of NMD and programmed −1 ribosomal frameshifting correctly. However, it is always possible that we could be wrong, and it is obvious that more experiments are needed. Perhaps direct measurements of protein levels, a side-by-side comparison of the two assays, and/or a bicistronic reporter with an ORF other than LacZ that is sensitive to NMD would help to resolve this discrepancy.

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