LETTER

Minor splicing: Nuclear dogma still in question

Steitz *et al*. (1) question our data on cytoplasmic minor splicing (2) when comparing them with apparently contradictory results by Pessa *et al*. obtained in different systems (1, 3). However, neither their criticism of our data nor their conclusions on "strong" evidence for nuclear minor splicing appear to stand up to a more careful examination.

Our conclusions on cytoplasmic minor splicing are based on several lines of evidence, including *in situ* localization of minor snRNAs and novel functional *in vivo* approaches (2). Contrary to what Steitz *et al*. imply, our *in situ* hybridizations are specific as demonstrated by four probes with mismatch controls, Northern blotting, and nucleolar exclusion of control probes (ref. 2 and data not shown; data available upon request).

Like earlier data (4), Pessa *et al*.'s HeLa cell results are difficult to interpret because they lack *in situ* controls for probe specificity. Moreover, perinuclear material in nuclear fractions questions conclusions on truly nuclear localization from cell fractionation (5). It is noteworthy that their hybridization signals for minor snRNAs in the mouse tissues (except for U12 in brain) appear to be perinuclear/cytoplasmic in most cells. This staining is interpreted as nucleoplasmic, apparently overlapping with the DNA counterstain, which, rather than staining the nucleoplasm independent of the probe, seems preferentially associated with the hybridized probe (3).

Minor snRNAs are likely imported to the nucleus during snRNP biogenesis but may be reexported upon (nuclear) association with their substrates, resulting in steady-state cytoplasmic accumulation. Partially spliced pre-mRNAs containing minor introns are present at high proportions (2, 6) and appear to exit the nucleus (2). The proportion of substrate-associated minor snRNPs may thus cause distinct nuclear–cytoplasmic ratios in different tissues or physiological conditions.

Harald König† **and Ferenc Müller**†‡*

**Forschungszentrum Karlsruhe, Institut für Toxikologie und Genetik, Postfach 3640, 76021 Karlsruhe, Germany; and ‡Department of Medical and Molecular Genetics, Division of Reproductive and Child Health, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom*

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†To whom correspondence may be addressed. E-mail: harald.koenig@itg.fzk.de or f.mueller@bham.ac.uk.