LETTER

# Reply to Bagni: On BC1 RNA and the fragile X mental retardation protein

In Iacoangeli *et al*. (1), five independent groups report that results published by Zalfa *et al*. (2) are not reproducible. Bagni now suggests (3) that different reagents, antibodies, or procedures might explain this lack of reproducibility. Iacoangeli *et al*. replicated the experimental conditions reported by Zalfa *et al*. whenever possible, as indicated. In several cases, however, reagents used by Zalfa *et al*. were not available. For instance, Zalfa *et al*. generated antibody rAM1 and used it to probe BC1–FMRP interactions in supershift and other assays. The antibody did not produce a supershift in brain extracts—as one would have expected if BC1 RNA did in fact bind to FMRP *in vivo*—but abolished the regular mobility shift (2). Despite repeated requests by several of the undersigned, antibody rAM1 was not provided. We urge the Bagni group (2) to make antibody rAM1 available for independent examination. In contrast, Iacoangeli *et al*. used two established anti-FMRP antibodies that have been independently validated and are publicly available. Bagni suggests that two other groups (4, 5) have published data in support of her claims. Both articles were quoted and discussed by Iacoangeli *et al*. (1). One of the undersigned (E.W.K.) coauthored one of these articles (4) and has confirmed that, although BC1 RNA does bind to FMRP *in vitro*, this binding is entirely nonspecific because it is completely abrogated by competitor tRNA (1). Bagni does not mention that Gabus *et al*. (4) reported a $K_d$ of FMRP for tRNA of 25 nM, the same as for BC1 RNA. The second article (5) reported a weak BC1 RT-PCR signal in FMRP cross-linked immunoprecipitates and a stronger signal in MAP2 cross-linked immunoprecipitates. However, considering that MAP2 is not a known RNA binding protein, mere copurification cannot be taken as evidence for physical association (1). Thus, there is no confirmation, independent of the Bagni group, of a specific physical link between FMRP and BC1 RNA, as posited by Zalfa *et al*. (2). Similarly, the Zalfa *et al*. claim that FMRP is not associated with polyribosomes in neurons could not be confirmed in subsequent work (6, 7).

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1. Iacoangeli A, *et al*. (2008) On BC1 RNA and the fragile X mental retardation protein. *Proc Natl Acad Sci USA* 105:734–739.
2. Zalfa F, *et al*. (2003) The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell* 112:317–327.
3. Bagni C (2008) On BC1 RNA and the fragile X mental retardation protein. *Proc Natl Acad Sci USA* 105:E19.
4. Gabus C, Mazroui R, Tremblay S, Khandjian EW, Darlix JL (2004) The fragile X mental retardation protein has nucleic acid chaperone properties. *Nucleic Acids Res* 32:2129–2137.
5. Johnson EM, *et al*. (2006) Role of Pur$\alpha$ in targeting mRNA to sites of translation in hippocampal neuronal dendrites. *J Neurosci Res* 83:929–943.
6. Stefani G, Fraser CE, Darnell JC, Darnell RB (2004) Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. *J Neurosci* 24:7272–7276.
7. Khandjian EW, *et al*. (2004) Biochemical evidence for the association of fragile X mental retardation protein with brain polyribosomal ribonucleoparticles. *Proc Natl Acad Sci USA* 101:13357–13362.

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The authors declare no conflict of interest.

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