Reply to Lahiri *et al.*: APPealing for a role in cellular iron efflux

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Lahiri *et al.* (1) expressed concern that by not detecting FRET between ferroportin-CFP and APP-YFP,[2] we "hinted that APP is unnecessary for ferroportin-supported Fe efflux." There is no question that APP *is* unnecessary for ferroportin-dependent Fe efflux, as illustrated by the work by MacKenzie and co-workers (2), who, using expression of Fpn in *Xenopus* oocytes, have provided details of ferroportin function. Also, we have shown that whereas knockdown of the essential ferroxidase hephaestin reduces iron efflux in primary hippocampal neurons, knockdown of APP does not (3). However, there is no question of a physiologic relationship between APP and cell iron metabolism, as Dr. Lahiri's work has made abundantly clear. The specific conclusion of Dlouhy *et al.* (4) was that the form of APP that enhances the membrane presentation of Fpn and thus Fe efflux is sAPP, not the endogenous, unprocessed form. sAPP has this property, as do helices from the protein's E2 domain or a synthetic peptide from that domain, all three of which contain the REWEE motif that Bush and co-workers (5) first identified as being linked to a role for APP in Fpn-mediated iron efflux. Our genetically encoded fluorescent proteins were used solely to determine whether native, membrane-associated APP and Fpn formed a complex; they do not, a finding in support of our conclusion that *only* soluble APP species bind to Fpn. In this context, concerns about secretase processing are moot. Note that the self-association of APP has been demonstrated using our FRET approach (6), as have APP, BACE1 trafficking, and Aβ production (7). In summary, we hope our work *will* encourage researchers to appreciate the strong likelihood that among the *physiologic* functions performed by APP family members is one related to cell iron metabolism; in the closed compartment that is the brain, this role may be paramount and thus critical to understanding the link between Alzheimer's disease and iron. Importantly, in this closed compartment, sAPP released from any of the cell types in the neurovascular unit will modulate Fpn activity. We miss this juxtacrine relationship by focusing on APP itself.

The authors declare that they have no conflicts of interest with the contents of this article.

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[2] The abbreviations used are: APP, β-amyloid precursor protein; sAPP, secreted APP; Fpn, ferroportin; YFP, yellow fluorescent protein.

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