Comment on “The [4Fe4S] cluster of human DNA primase functions as a redox switch using DNA charge transport”

Luca Pellegrini

O’Brien et al. (Research Article, 24 February 2017, eaag1789) report that the iron-sulfur cluster of primase has a redox role in enzyme activity. Their analysis is based on a partially misfolded structure of the iron-sulfur cluster domain of primase. In the correctly folded structure, two of the three tyrosines putatively involved in electron transfer, Y345 and Y347, contact the RNA/DNA helix, providing an alternative explanation for the data of O’Brien et al.

O’Brien et al. (1) published evidence that the Fe-S cluster of the DNA-dependent RNA polymerase known as primase is redox active and that its oxidation enhances DNA binding. They proposed that reduction of the oxidized form of the Fe-S cluster, via electron transfer through the double-stranded helix formed by the RNA primer annealed to the DNA template, would facilitate dissociation of the enzyme from its substrate and form the basis for a possible mechanism of primer termination. In the model, the electron needed for reduction would be provided by a protein partner such as DNA polymerase α, which acts in close cooperation with primase to extend the RNA primer with deoxyribonucleotides and is known to have its own Fe-S cluster (2).

In support of their model, the authors identify three residues in the regulatory subunit (p58) of primase—tyrosines 309, 345, and 347—that would form an electron-transfer pathway between the RNA/DNA helix and the Fe-S cluster. However, the identification of such a tyrosine pathway is based on a structure of the Fe-S domain of primase (p58C) (3), which is incorrectly folded. In this structure, amino acids 318 to 360 adopt a β-hairpin conformation, unlike the α-helical conformation observed for the same region in the structures of yeast and human p58C (Fig. 1A) (4, 5). The reason for the local misfolding is probably a single-point I271S mutation present in the p58C construct used by Vaithiyalingam et al. in (3).

In the correct structure, two of the three tyrosines, 345 and 347, have radically altered relative positions and considerably longer inter-residue distances (Fig. 1B). Indeed, at 14.7 Å, it is unclear that the distance between the side chains of Y345 and Y309 would be suitable for electron transfer on a physiologically relevant time scale. Furthermore, Y309, the third tyrosine component of the proposed electron wire, is not invariant in eukaryotic primases and is occasionally replaced by amino acids such as leucine that cannot participate in electron transfer [see figure 2B of (4)].

Surprisingly, the authors omit discussion of a recent structural analysis of the human p58C bound to an RNA primer/DNA template (6). This structure shows that Y345 and Y347 are at the interface with the RNA/DNA helix and make contacts with the triphosphate group at the 5′ end of the RNA primer and with the DNA template, respectively (Fig. 1B). Thus, the lower initiation activity of the Y345 primase mutant might be explained by weaker RNA/DNA binding rather than impaired charge transfer.

Considering the alternative explanation provided here for the mutagenesis data of O’Brien et al., based on the correct structure of the p58C, it is appropriate to consider their original conclusion about a redox role of the Fe-S cluster of primase as tentative and requiring further experimentation.

REFERENCES

23 March 2017; accepted 13 June 2017 10.1126/science.aan2954
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*Science* 357 (6348), eaan2954.
DOI: 10.1126/science.aan2954

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