Biochimica et Biophysica Acta 1863 (2016) 2859–2867

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Erythroid cell mitochondria receive endosomal iron by a "kiss-and-run" mechanism

Amel Hamdi [a,b,1], Tariq M. Roshan [a,1], Tanya M. Kahawita [a,b], Anne B. Mason [c], Alex D. Sheftel [d,e], Prem Ponka [a,b,*]

[a] Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec, Canada
[b] Department of Physiology, McGill University, Montreal, Quebec, Canada
[c] Department of Biochemistry, University of Vermont, Burlington, VT, USA
[d] Spartan Bioscience Inc., Ottawa, Ontario, Canada
[e] High Impact Editing, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 24 August 2016
Accepted 8 September 2016
Available online 11 September 2016

Keywords:
Iron
Transferrin
Endosomes
Mitochondria
Erythroid cells

ABSTRACT

In erythroid cells, more than 90% of transferrin-derived iron enters mitochondria where ferrochelatase inserts Fe^{2+} into protoporphyrin IX. However, the path of iron from endosomes to mitochondrial ferrochelatase remains elusive. The prevailing opinion is that, after its export from endosomes, the redox-active metal spreads into the cytosol and mysteriously finds its way into mitochondria through passive diffusion. In contrast, this study supports the hypothesis that the highly efficient transport of iron toward ferrochelatase in erythroid cells requires a direct interaction between transferrin-endosomes and mitochondria (the "kiss-and-run" hypothesis). Using a novel method (flow sub-cytometry), we analyze lysates of reticulocytes after labeling these organelles with different fluorophores. We have identified a double-labeled population definitively representing endosomes interacting with mitochondria, as demonstrated by confocal microscopy. Moreover, we conclude that this endosome-mitochondrion association is reversible, since a "chase" with unlabeled holotransferrin causes a time-dependent decrease in the size of the double-labeled population. Importantly, the dissociation of endosomes from mitochondria does not occur in the absence of holotransferrin. Additionally, mutated recombinant holotransferrin, that cannot release iron, significantly decreases the uptake of ^{59}Fe by reticulocytes and diminishes ^{59}Fe incorporation into heme. This suggests that endosomes, which are unable to provide iron to mitochondria, cause a "traffic jam" leading to decreased endocytosis of holotransferrin. Altogether, our results suggest that a molecular mechanism exists to coordinate the iron status of endosomal transferrin with its trafficking. Besides its contribution to the field of iron metabolism, this study provides evidence for a new intracellular trafficking pathway of organelles.

1. Introduction

Iron is a transition metal whose properties make it useful to vital biologic processes in virtually all living organisms. Essential cellular functions include use of iron for oxygen transport, electron transfer, DNA synthesis and innumerable other purposes [1–4]. However, under physiological conditions iron is virtually insoluble and can be extremely toxic when not properly shielded [5–7]. Hence, iron plays a role in the formation of toxic oxygen radicals that damage various cell structures. Therefore, it is critical that we gain a better understanding of the mechanisms involved in normal and abnormal intracellular iron trafficking.

Developing erythroid cells, which have a capacity to obtain and process iron with astonishing efficacy [8], are capable of concentrating iron (in the form of heme in the red cell) to approximately 7000-fold what is present in the plasma (as diferric transferrin [Fe_2-Tf]). Additionally, the delivery of iron into hemoglobin occurs extremely efficiently, since mature erythrocytes contain about 45,000-fold more heme iron than non-heme iron [9]. These facts suggest that in erythroid cells the iron transport and heme biosynthetic machineries are fully integrated and are part of the same metabolic pathway that leads to a remarkably efficient production of heme. Delivery of iron to these cells occurs following the binding of Fe_2-Tf to its cognate receptors (TfR) on the cell membrane. The Fe_2-Tf-TfR complexes are then internalized via endocytosis, and iron is released by a process which includes both acidification and the participation of the TfR [1,9–12]. Iron, following its reduction to Fe^{2+} by Steap3 (six-transmembrane epithelial antigen of the prostate [13]), likely together with ascorbate [14] is then transported across the endosomal membrane by the divalent metal transporter 1, DMT1/Nramp2 [15].

* Corresponding author at: Jewish General Hospital, Lady Davis Institute for Medical Research, and the Department of Physiology, McGill University, 3755 Chemin Côte-Ste-Catherine, Montréal, QC H3T 1E2, Canada.
E-mail address: prem.ponka@mcgill.ca (P. Ponka).
[1] Co-first author.

http://dx.doi.org/10.1016/j.bbamcr.2016.09.008
0167-4889/

Following its egress from endosomes, iron is transported to intracellular sites of use and/or storage in ferritin, but this aspect of iron metabolism remains elusive or is at best controversial. It has been commonly believed that a low molecular weight intermediate chaperones iron from endosomes to mitochondria and other sites of utilization [16]. However, this long sought iron binding intermediate, that would constitute the labile iron pool, has *never* been identified. In fact, earlier work from this laboratory demonstrated [17] that there was virtually no low molecular weight iron pool in hemoglobin-synthesizing cells and that iron present in this pool does not behave as an intermediate, thus corresponding to an end product. Although it has recently been proposed that the ferrous iron in this pool is associated with glutathione [18], this conclusion was only based on chemical interactions. Hence, the relevance of this study to the physiological mechanisms involved in the intracellular iron transport remains in doubt. Additionally, it has been proposed that human poly(rC)-binding protein 1 (PCBP1) "chaperones" iron into ferritin and non-heme iron proteins [19,20]. On the other hand, more recent reports [21,22] have proposed PCBP2, rather than PCBP1, to play an "iron-chaperone" role. This diversity is probably due to different cell types and/or experimental strategies used. However, no direct evidence has been provided that PCBPs acquire iron from transferrin-endosomes, carry it in cytosol and, more crucially, deliver it to mitochondria.

In erythroid cells, more than 90% of iron enters mitochondria where ferrochelatase, the enzyme that inserts Fe^{2+} into protoporphyrin IX (PPIX), resides in the matrix side of the inner mitochondrial membrane. Importantly, in these cells, strong evidence exists for specific targeting of iron toward mitochondria. This mitochondria-directed transport is demonstrated in hemoglobin-synthesizing cells, in which iron acquired from Tf continues to flow into mitochondria even when the synthesis of PPIX is suppressed either experimentally [17,23–25] or in hereditary sideroblastic anemia caused by defects in heme synthesis [26–28]. Of note, a compelling candidate for the transport of iron through the mitochondria towards ferrochelatase, mitoferrin, has been identified [29].

Based on the above considerations, we have formulated a hypothesis that in erythroid cells a transient mitochondrion-endosome interaction is involved in iron translocation to its final destination [17,30]. We have collected the following experimental evidence to support this hypothesis: 1) iron, delivered to mitochondria via the Tf-TfR pathway, is unavailable to cytoplasmic chelators [31]; 2) Tf-containing endosomes move to and contact mitochondria [31] in erythroid cells; 3) endosomal movement is required for iron delivery to mitochondria [31,32]. We have also demonstrated that "free" cytoplasmic iron is not efficiently used for heme biosynthesis and that the endosome-mitochondrion interaction increases chelatable mitochondrial iron [31].

As already mentioned, the substrate for the endosomal transporter, DMT1, is Fe^{2+}, the redox form of iron which is also the substrate for ferrochelatase. These facts make our hypothesis quite attractive, since the "chaperone"-like function of endosomes may be one of the mechanisms that keeps the concentrations of reactive Fe^{2+} at extremely low levels in the oxygen-rich cytosol of erythroblasts and reticulocytes, preventing ferrous iron's participation in a dangerous Fenton reaction.

There is accumulating evidence for transfer of metabolites directly between interacting organelles [33–36]. However, the role of endosomes in distributing intracellular iron is accepted without enthusiasm [20], misinterpreted [16] or simply ignored [37]. Hence, we deemed it essential to seek further evidence for our "kiss-and-run" hypothesis.

In the current study, we used 3D live confocal imaging of reticulocytes following their incubation with MitoTracker Deep Red (MTDR) and Alexa Green Transferrin (AGTf) and demonstrated transient interactions of endosomes with mitochondria. We also demonstrate these interactions by a novel method exploiting flow sub-cytometry to analyze reticulocyte lysates labeled with MTDR and AGTf. This strategy identified a population double-labeled with both fluorescent markers representing endosomes interacting with mitochondria. FACS sorting followed by 2D confocal microscopy confirmed the association of both organelles in the double-labeled population. The results of these experiments as well as those exploiting a mutated recombinant Fe_2-Tf unable to release iron efficiently further support the hypothesis that the efficient iron transfer to PPIX requires a transient interaction between the endosome and the mitochondrion.

2. Results

2.1. Three-dimensional confocal microscopy of live cells reveals endosome-mitochondria interactions

Using two-dimensional (2D) confocal microscopy, we have previously shown that endosomes come in contact with mitochondria [31]. To avoid any pseudo-interactions of these two organelles, i.e., the endosome hovering over the mitochondria, we have used a Quorum WaveFx spinning disc confocal system to capture three-dimensional (3D) live cell imaging of reticulocytes with fluorescently labeled mitochondria and endosomes. An individual cell at different time intervals is presented in three different orientations (Fig. 1, A–C) and shows the proximity of endosomes and mitochondria at different angles (Link for Video; please see Supplementary Material).

2.2. Development of the flow sub-cytometry method to study endosome-mitochondria interactions

We developed a new method, "flow sub-cytometry", based on the standard flow cytometry method, to analyze lysates obtained from reticulocytes with fluorescently labeled mitochondria (MTDR) and endosomes (AGTf). Employing this strategy, three distinct populations: endosomes, mitochondria, and a population double labeled with both

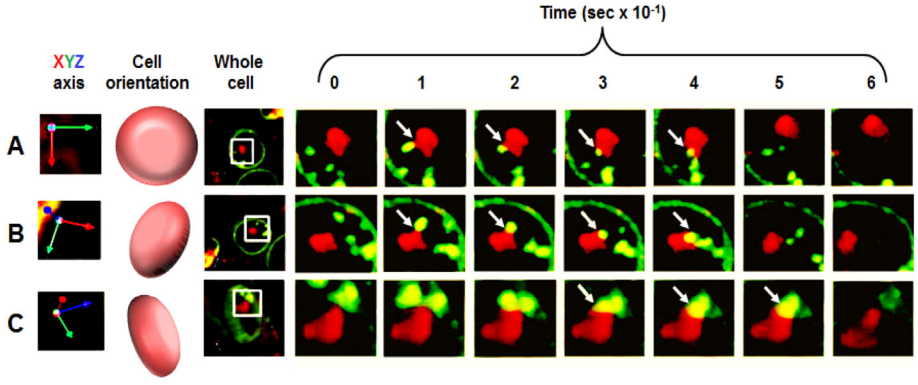

Fig. 1. 3D confocal microscopy demonstrates the association of transferrin-endosomes (green) with mitochondria (red). At one time interval ("3", marked by white arrows), endosome-mitochondria pairs are shown in three different orientations.