Microtubule-directed transport of purine metabolons drives their cytosolic transit to mitochondria

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To meet their purine demand, cells activate the de novo purine biosynthetic pathway and transiently cluster the pathway enzymes into metabolons called purinosomes. Recently, we have shown that purinosomes were spatially colocalized with mitochondria and microtubules, yet it remained unclear as to what drives these associations and whether a relationship between them exist. Here, we employed superresolution imaging methods to describe purinosome transit in the context of subcellular localization. Time-resolved imaging of purinosomes revealed that these assemblies exhibit directed motion as they move along a microtubule toward mitochondria, where upon colocalization, a change in purinosome motion was observed. A majority of purinosomes colocalized with mitochondria were also deemed colocalized with microtubules. Nocodazole-dependent microtubule depolymerization resulted in a loss in the purinosome–mitochondria colocalization, suggesting that the association of purinosomes with mitochondria is facilitated by microtubule-directed transport, and thereby supporting our notion of an interdependency between these subcellular components in maximizing purine production through the de novo purine biosynthetic pathway.

Significance

This study draws on the power of superresolution microscopy to investigate how metabolons behave near different subcellular components. We revealed an interdependent relationship among purinosomes, mitochondria, and microtubules. This further suggests a role for each in maximizing purine production in times of high intracellular demand. With the increasing number of reported metabolons, this study has uncovered a potential general strategy for how metabolons use subcellular networks to facilitate metabolic trade between themselves and other cellular organelles.

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supporting the notion of an interplay between these subcellular bodies.

Results

Colocalization of Purinosomes with Mitochondria and Microtubules by Two-Color STORM. Colocalization studies of purinosomes with mitochondria in HPRT-deficient fibroblasts were performed using two-color 3D STORM imaging of cells transiently expressing the purinosome marker FGAMS-mMaple3 and immunostaining the mitochondrial outer membrane translocase (TOM20) with the photoswitchable dye Alexa Fluor 647 (Fig. 1A and B). The percentage of purinosomes colocalized with mitochondria was found to be 81.4 ± 6.7%, which was significantly higher than that obtained from the randomized distribution of purinosomes in the cytosol (42.9 ± 6.3%; P < 0.05; Fig. 1C and SI Appendix, Supplementary Materials and Methods).

We also observed a high level of colocalization between purinosomes (FGAMS-mMaple3) and microtubules immunolabeled with Alexa Fluor 647 (Fig. 1D and E). Statistical analysis showed that the purinosome–microtubules colocalization percentage was 91.6 ± 3.0%, which was significantly higher than that of a randomized purinosome distribution (60.5 ± 5.6%; P < 0.05; Fig. 1F).

Given the high percentages of purinosomes that are colocalized with mitochondria (81.4%) and microtubules (91.6%), it is likely that a significant number of the purinosomes are simultaneously colocalized with both mitochondria and microtubules.

Colocalization of Purinosomes with Mitochondria and Microtubules by Three-Color VT-iSIM. To obtain further evidence that some purinosomes are colocalized with both mitochondria and microtubules, we performed three-color imaging of live cells, using VT-iSIM (Fig. 2A). The general localization of purinosomes with mitochondria and microtubules is illustrated in the representative region of interest (ROI; Fig. 2, Inset). The image in the ROI is split into individual channels for better visualization (Fig. 2B). We found 57.1 ± 13.2% of purinosomes colocalized with mitochondria, which is statistically greater than the randomization control (22.5 ± 10.2%; P < 0.05; Fig. 2C). Substantial purinosome–mitochondria colocalization was thus observed with both imaging methods, and the quantitative difference in the extent of colocalization observed between the two methods may be attributed to differences in the acquisition and image analysis methods, or sample-to-sample variations. Furthermore, we observed that essentially all mitochondria-associated purinosomes were also colocaled with microtubules (Fig. 2D). Of the remaining purinosomes, the majority (86%) were also associated with microtubules. These observations invite the question as to whether microtubules drive purinosome–mitochondria colocalization.

Analysis of Purinosome Motions with Respect to Mitochondria and Microtubules. To evaluate purinosome motions in live cells with respect to both mitochondria and microtubules, three-color time-lapse images were captured, revealing the interplay between these three subcellular components during a 495-s (5 s/frame, 100 frames) time period. The percentage of purinosomes colocalized with mitochondria was 57.1 ± 13.2% (Fig. 2D). Statistical analysis showed that the purinosome–mitochondria colocalization percentage was 91.6 ± 3.0%, which was significantly higher than that of a randomized purinosome distribution (60.6 ± 5.6%; P < 0.05; Fig. 2F). Data are presented as mean ± SD, n = 14 cells, P < 0.05, as calculated by paired t test.
course (Movie S1). During this time, the number of purinosomes in a
given cell did not drastically change ($n = 30$ cells, 3,784 purinosomes)
(SI Appendix, Fig. S2). The motions of purinosomes depended
strongly on their colocalization with other subcellular struc-
tures. Mean squared displacement (MSD) analyses of purino-
some trajectories in the $x$-$y$ plane revealed three types of motion:
normal diffusion, constrained motion, and directed motion.
The majority of purinosomes that were colocalized with
both mitochondria and microtubules (84%) showed constrained
(nondirected) motion (see Fig. 4A). As illustrated in Fig. 3A, a
purinosome (yellow arrow) displayed minimal displacement
while colocalized with both mitochondria and microtubules
(Fig. 3B and Movie S2). The MSD of this trajectory (solid
black line) demonstrated constrained motion, as characterized by
an asymptotic behavior of MSD over $\Delta t$ (red dashed line, Fig. 3C).
The remaining dual-colocalized purinosomes (16%) showed a
limited directed motion that is attributed to its travel along a
microtubule as it approaches a mitochondrion. The motions of such
a purinosome (yellow arrow) moving along a microtubule from point
A to B are shown in Fig. 3D (Movie S3). Here, the purinosome
showed directed motion as it moved toward a mitochondrion along a
microtubule from 0 to 140 s (Fig. 3E and F, Upper). Once colo-
calized with the mitochondrion, the purinosome showed constrained
motion ($\Delta t = 145$–245 s; Fig. 3F, Lower). The purinosomes that were
not colocalized with either mitochondria or microtubules showed
relatively small displacement (Fig. 3G and H and Movie S4). These
purinosomes showed a linear dependence of MSD on $\Delta t$ (Fig. 3F,
red dashed line), as shown by the representative purinosome in
Fig. 3G. The median value of the diffusion coefficient of purino-
somes was calculated to be $4.5 \times 10^{-4}$ $\mu m^2/s$ ($n = 25$).

We next asked how general such purinosome behaviors are
across a number of cells (Fig. 4A). Within the 30 cells, 135
purinosomes were analyzed over the course of 4,135 total time
frames. The distance a purinosome traveled between two con-
secutive frames for both directed and nondirected motions was
calculated (Fig. 4B). For directed motions, the median value
from the distribution of distances was 344 nm and is distinct from
the population of purinosomes displaying nondirectional mo-
tions (Fig. 4B). The calculated mean velocity for these purino-
somes during directed motion was determined by MSD fitting
to be 55.2 nm/s (SI Appendix, Fig. S3), with a median time for
the duration of directed motion of 20 s (Fig. 4C; $n = 100$
representative trajectories).

Validation of Microtubule-Assisted Directed Motion of Purinosomes Was
Observed on Disruption of Microtubule Polymerization with Nocodazole.
Microtubule depolymerization was first detected after 30 min of
treatment with nocodazole, and within 2 h, complete depolymerization
was noted (SI Appendix, Fig. S4). We next imaged purinosome-positive cells that were costained for mitochondria to ask whether purinosome–mitochondria colocalization in cells changed as a function of time after nocodazole treatment. A representative cell showing the lack of colocalization between purinosomes (green) and mitochondria (red) after 2 h of nocodazole treatment

![Image]

**Fig. 3.** Characterization of purinosomes based on both their localization and MSD analyses in HPRT-deficient fibroblasts. (A) Representative time-lapse three-color images showing a purinosome (FGAMS-EGFP, green) colocalized with both mitochondria (MitoTracker Red, red) and microtubules (silicon-rhodamine tubulin, gray; yellow arrow). Portion of a purinosome colocalized with mitochondria is shown as yellow. (B) Trajectory of the specified purinosome from A in x-y coordinates. The purinosome was colocalized with mitochondria and demonstrated very minimal displacement over the course of 225 s. (C) Time-average MSD plot of the trajectory in B (solid black line) fitted with the equation for constrained motion (dashed red line). (D) Representative time-lapse three-color images showing a purinosome (yellow arrow) colocalized with only microtubules initially. From 0 to 140 s, the purinosome moved along a microtubule and then became colocalized with a mitochondrion from 140 to 155 s. (E) Trajectory of the specified purinosome from D in x-y coordinates that demonstrated a much larger displacement than the purinosome in A. (F) Time-average MSD plot of the trajectory in E (solid black line) revealed a biphasic behavior. The MSD of this trajectory was first fitted with the quadratic equation for directed motion (Upper, dashed blue line, 0–140 s) and then constrained motion as in C (Lower, dashed red line, 140–155 s). (G) Representative time-lapse three-color images showing a purinosome (yellow arrow) not colocalized with either mitochondria or microtubules. (H) Trajectory of the specified purinosome from G in x-y coordinates. This purinosome displayed random motion with minimal displacement. (I) Time-average MSD plot of the trajectory in H (solid black line) fitted with the equation for normal diffusion (dashed red line).
Unlike HeLa cells, no significant change in the number of purinosomes per HPRT-deficient fibroblast was observed postnocodazole treatment (17). The degree of purinosome–mitochondria colocalization postnocodazole treatment decreased substantially (from $57.1 \pm 13.2\%$ to $23.8 \pm 2.1\%$) after 3 h of treatment, with a randomized control of $12.9 \pm 3.7\%$. Purinosomes not colocalized with either mitochondria or microtubule displayed normal diffusion, with a median diffusion coefficient of $1.8 \times 10^{-4}$ μm²/s ($n = 34$) during the 495-s time course (Fig. 5B and D, SI Appendix, Fig. S6, and Movie S5). These results suggest that microtubule-directed movement of purinosomes is important for purinosome–mitochondria colocalization.

The collective analysis of purinosomes ($n = 50$/time point) showed a time-dependent reduction in directed motion when the duration of nocodazole treatment increased (Fig. 5E and SI Appendix, Fig. S7). After 3 h of treatment, the proportion of directed motion in purinosomes not colocalized with mitochondria, is shown in Fig. 5A and B. Unlike HeLa cells, no significant change in the number of purinosomes per HPRT-deficient fibroblast was observed postnocodazole treatment (17). The degree of purinosome–mitochondria colocalization postnocodazole treatment decreased substantially (from $57.1 \pm 13.2\%$ to $23.8 \pm 2.1\%$) after 3 h of treatment, with a randomized control of $12.9 \pm 3.7\%$. Purinosomes not colocalized with either mitochondria or microtubule displayed normal diffusion, with a median diffusion coefficient of $1.8 \times 10^{-4}$ μm²/s ($n = 34$) during the 495-s time course (Fig. 5B and D, SI Appendix, Fig. S6, and Movie S5). These results suggest that microtubule-directed movement of purinosome is important for purinosome–mitochondria colocalization.

The collective analysis of purinosomes ($n = 50$/time point) showed a time-dependent reduction in directed motion when the duration of nocodazole treatment increased (Fig. 5E and SI Appendix, Fig. S7). After 3 h of treatment, the proportion of directed motion in purinosomes, not colocalized with mitochondria,
decreased from greater than 85% to less than 20%. Finally, we compared the displacement-to-distance ratio and the average step size of the purinosome trajectories in untreated condition and at 2 h postnocodazole treatment (Fig. 5F). Displacement-to-distance ratio is defined as the ratio a purinosome is displaced between the initial and final positions over the sum of the distances between consecutive positions during the time course. The nocodazole-treated trajectories distinctly clustered with those purinosomes having nondirected motions in the untreated control, further supporting the notion that the directed motion of purinosomes is attributed to its colocalization with microtubules.

Discussion

This study examined the spatiotemporal relationship among purinosomes, mitochondria, and microtubules. Of the purinosomes colocalized with microtubules, but not simultaneously with mitochondria, the vast majority showed directed motion along microtubules with a mean velocity of 55 nm/s. A wide range of transport velocities on microtubules have been observed, from several tens of nanometers per second >1 μm/s (22–24), and the velocity that we observed here for purinosome is similar to the directed movement of RNA granules along dendrites in neurons (ca. 50 nm/s) (25). Disruption of the microtubule polymerization by nocodazole led to a decrease in purinosome–mitochondria colocalization and a loss of directed motion. Results with the nocodazole-treated cells support the importance of the microtubules both for purinosome movement and for the association of purinosome with mitochondria.

The de novo purine synthesis pathway enzymes require cofactors such as ATP and folate, both of which are products of mitochondrial metabolism. In contrast, the ultimate products of a purinosome are AMP and GMP, and GMP is essential for mitochondrial DNA synthesis. Therefore, the formation of the purinosome metabolon cannot only facilitate flux from phosphoribosyl pyrophosphate to AMP/GMP by the encapsulation of constituent enzymes but can also, through its association with mitochondrion, act as an import/export agent for metabolites responsible for the function of both. We speculate that in general, metabolic pathways could be likewise organized into metabolons that are actively transported to distinct complementary cellular organelles to maximize both their functions.

Materials and Methods

Materials and experimental procedures for plasmids and antibodies, cell culture and transient transfection of mammalian cells, STORM, high-resolution confocal microscopy, immunostaining for STORM, STORM colocalization analysis, high-resolution confocal colocalization analysis, randomized colocalization analysis, image visualization and statistical trajectory analysis of the representative three-color images, fitting of the time-averaged MSD, and nocodazole treatment experiment are described in the SI Appendix, Supplementary Materials and Methods.

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