



# 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 showed 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.**

purine metabolism | metabolon | superresolution microscopy | mitochondria | cytoskeleton

An emerging trend in metabolism is that metabolic enzymes form supramolecular complexes, called metabolons, to enhance metabolic flux (1–4). Unlike previously reported metabolons in the tricarboxylic acid cycle (5) and glycolysis (6), enzymes within the de novo purine biosynthetic pathway assemble into transient, nonmembrane-bound clusters called purinosomes (2, 7, 8). Proximity assays demonstrated that purinosomes are composed of core and peripheral proteins and likely assemble in a step-wise manner (9, 10). Further characterization of purinosome regulation revealed that formation is likely mediated through the involvement of molecular chaperones (11, 12) and kinases (13, 14). The degree of purinosome assembly is reflected in the cell's overall intracellular purine demand and serves as a biomarker for pathway activation (15). Under cellular conditions that promote purinosome formation, the metabolic flux through the pathway was shown to be enhanced (16).

Recently, imaging studies had revealed colocalization between purinosomes and subcellular structures (i.e., mitochondria and microtubules) in HeLa cells, suggesting that the purinosome might be highly dependent on one or the other for spatial organization within the cell (14, 17). The de novo process in which the purines are made is energy-intensive and requires five molecules of ATP, numerous substrates, and cofactors for every molecule of inosine monophosphate generated. Studies have shown that the formate exported from mitochondria is an essential precursor for the 10-formyltetrahydrofolate cofactor, and elevated production of mitochondrial formate results in enhanced metabolic flux through the pathway (18). These observations help support the hypothesis that close proximity of purinosomes to mitochondria would be advantageous in meeting the catalytic demands of the enzymes.

In this study, we employed a combination of stochastic optical reconstruction microscopy (STORM) (19) and instantaneous structured illumination microscopy (VT-iSIM) to visualize the localization and movement of purinosomes within the cytosol of hypoxanthine-guanine phosphoribosyltransferase (HPRT)-deficient fibroblasts derived from patients diagnosed with Lesch-Nyhan disease (20). These cells rely on the de novo purine biosynthetic pathway to generate purines and show a twofold enrichment of purinosome-positive cells compared with a normal, asynchronous fibroblast cell population (20). By using STORM, the average diameter and density distributions of purinosomes in Lesch-Nyhan disease are shown to be comparable with those in purine-depleted HeLa cells (*SI Appendix, Fig. S1*). Colocalization analysis of FGAMS, our marker for the purinosome, with either mitochondria or microtubules was performed by 3D STORM (21) and resulted in a high degree of colocalization, similar to that of our previous studies in purine-depleted HeLa cells (14). Depending on their colocalization mitochondria or microtubules, different types of motions were revealed for purinosomes through time-lapse imaging by VT-iSIM. Characterization of directed motions revealed a high tendency for purinosomes to be localized to microtubules and their motion directed toward mitochondria, suggesting a mechanism by which purinosomes are trafficked to mitochondria via the microtubule network. Disruption of this network resulted in a decrease in purinosome-mitochondria colocalization,

## 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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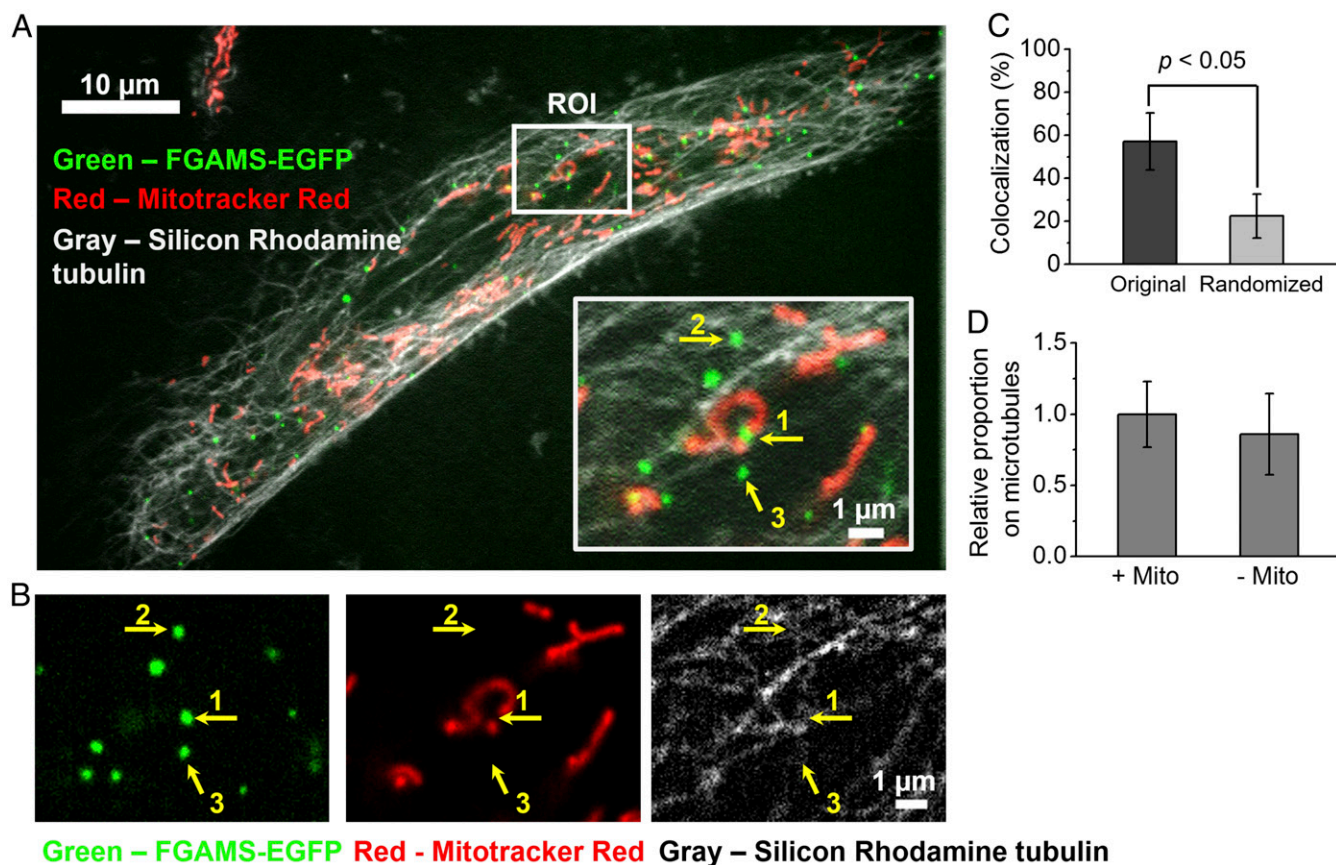
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**Fig. 2.** Colocalization of purinosomes with mitochondria and microtubules, using high-resolution confocal microscopy. (A) Representative three-color image by VT-iSIM showing purinosomes (FGAMS-EGFP, green) that were localized in the network of mitochondria (MitoTracker Red, red) and microtubules (silicon-rhodamine tubulin, gray) in HPRT-deficient fibroblasts at a given time. (Inset) Magnified ROIs from A illustrating different type of purinosome colocalizations with respect to a subcellular structure of interest (1, mitochondrion; 2, microtubule; and 3, neither mitochondrion nor microtubule). (B) The individual channels for purinosomes, mitochondria, and microtubules of the ROI in A. (C) Average colocalization percentage between purinosomes and mitochondria in HPRT-deficient fibroblasts. (D) Further classification of the mitochondria-colocalized purinosomes (+Mito) and nonmitochondria-colocalized purinosomes (–Mito). The relative proportion of the +Mito group that were also colocalized with microtubules was  $1.00 \pm 0.23$ , indicating that large proportion of purinosomes ( $57.1 \pm 13.2\%$  of total purinosomes) were dual-colocalized with both mitochondria and microtubules. For the –Mito group,  $86.0 \pm 29\%$  of them ( $36.9 \pm 12.3\%$  of total) were colocalized with microtubule.

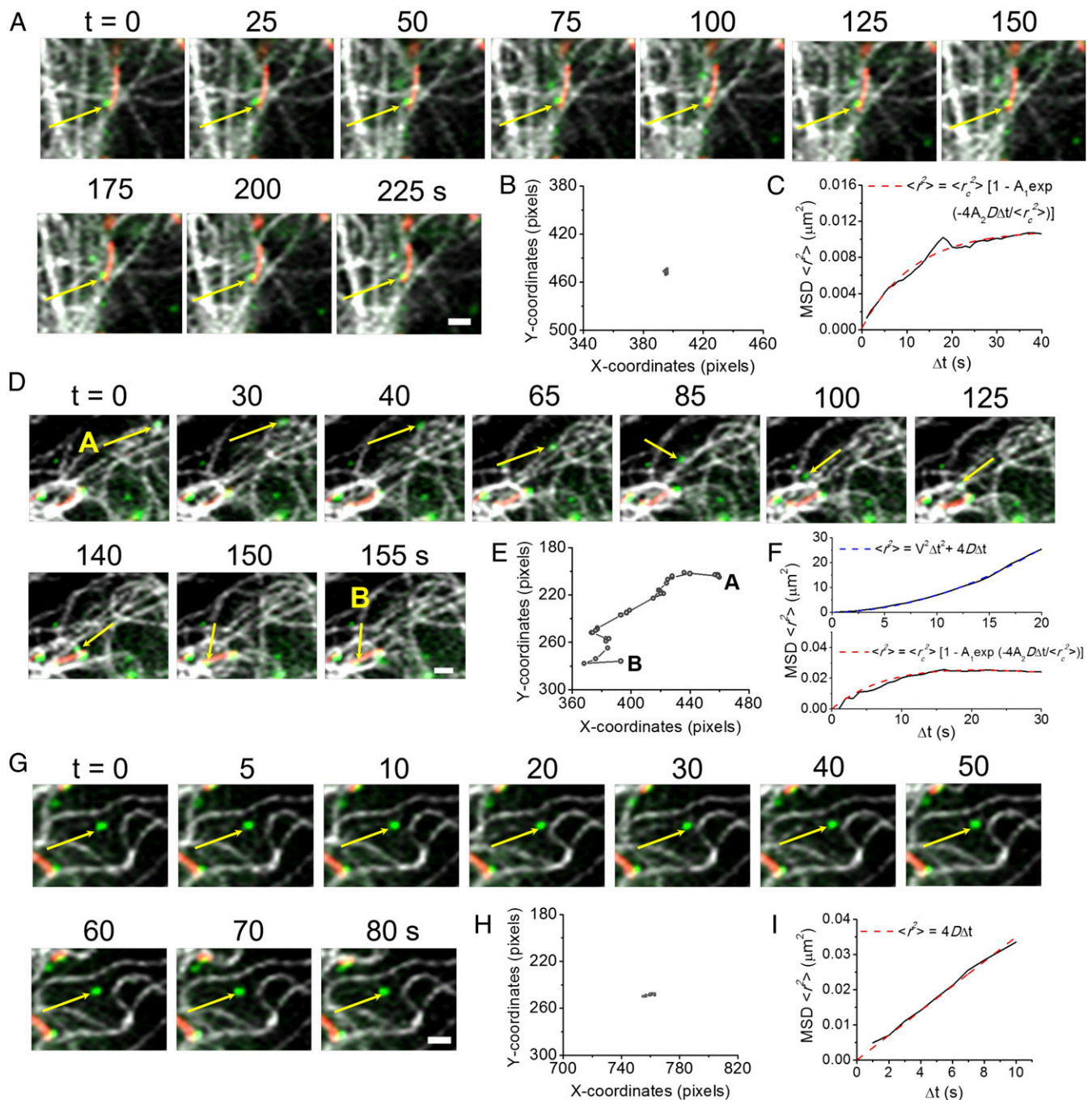
course (Movie S1). During this time, the number of purinosomes in a given cell did not drastically change ( $n = 30$  cells, 3,784 purinosomes) (SI Appendix, Fig. S2). The motions of purinosomes depended strongly on their colocalization with other subcellular structures. Mean squared displacement (MSD) analyses of purinosome 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 colocalized 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. 3I, red dashed line), as shown by the representative purinosome in Fig. 3G. The median value of the diffusion coefficient of purinosomes was calculated to be  $4.5 \times 10^{-4} \mu\text{m}^2/\text{s}$  ( $n = 25$ ).

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 consecutive 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 motions (Fig. 4B). The calculated mean velocity for these purinosomes 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



**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).

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



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  $>1 \mu\text{m/s}$  (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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