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SPOTLIGHT

Directed migration: Cells navigate by extracellular vesicles

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Directional cell motility toward a chemical gradient, chemotaxis, is critical during inflammation, embryogenesis, and cancer metastasis. In this issue, Kriebel et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201710170) demonstrate that the key cAMP chemoattractant for *Dictyostelium discoideum* amoebas is synthesized within and released from extracellular vesicles to promote chemotaxis.

Extracellular vesicles (EVs) are cell-derived vesicles that carry a variety of bioactive cargoes and mediate autocrine and paracrine communication. EVs are released by virtually all cell types and are major mediators of cell–cell communication (Maas et al., 2017; van Niel et al., 2018). They have been implicated in numerous pathological processes including tumor progression, inflammation, and neurodegenerative diseases. Since EVs are present in body fluids and have access to most tissues, the diagnostic and therapeutic potential of EVs is the subject of intensive investigation. EVs are broadly classified according to their biogenesis mechanism, e.g., budding from the surface of cells (microvesicles and large oncosomes) or forming within endosomal multivesicular bodies (MVBs; exosomes).

Recent studies reported that directional cell motility and chemotaxis are promoted by exosomes secreted from cancer cells, lymphatic endothelial cells, and neutrophils (Sung et al., 2015; Majumdar et al., 2016; Sung and Weaver, 2017; Brown et al., 2018). Chemotaxis is a type of migration in which cells move directionally up gradients of chemicals called chemoattractants. Although the intracellular signaling mechanisms have been investigated by many groups, it is not well known how stable gradients are generated in vivo to produce an effective chemotactic response, especially given the rapid diffusion of chemicals after secretion. In this issue, Kriebel et al. examined the role of exosomes in promoting chemotaxis of the model organism *Dictyostelium discoideum*.

Dictyostelia are soil-living amoebas that undergo chemotaxis toward cAMP under starvation conditions. As cAMP is released from the rear of migrating cells, chemotaxis toward cAMP leads to aggregation of the amoebas into multicellular structures. Kriebel et al. (2008) previously showed that the enzyme that synthesizes cAMP, adenylyl cyclase (ACA), is present in MVBs located at the rear of migrating amoebas. In their current study, through 3D reconstruction of focused ion beam–scanning EM (FIB-SEM) on ACA-YFP–expressing *Dictyostelium* cells, Kriebel et al. (2018) show that leader cells release ACA-containing vesicular trails and that follower cells stream onto them in a head-to-tail fashion. Kriebel et al. (2018) also observed through FIB-SEM that ACA-containing vesicles originated from MVBs and were secreted through fusion of MVBs with the plasma membrane, suggesting that the EVs are exosomes. Correlative analysis of migratory cell tracks using the distance of each cell to its nearest ACA-containing trail as well as the angle between its direction of migration and the direction to the nearest ACA-containing trail at each time point revealed that the vesicular trails are highly chemotactic and direct the migration of the follower cells.

To confirm that ACA is enriched in exosomes, Kriebel et al. (2018) purified EVs from ACA-YFP–expressing cells using sucrose density gradients and analyzed them by nanoparticle tracking, EM, Western blot analysis, and proteomics. Interestingly, ACA-YFP was found in fractions containing small vesicles with an average diameter of ∼150 nm as well as in fractions containing large vesicles, cell fragments, and markers for the ER, mitochondria, and Golgi. Since Kriebel et al. (2018) could not rule out the possibility that other EV types might also contain ACA-YFP, they referred to the ACA-containing vesicles as EVs rather than exosomes. To test whether EVs also contain cAMP, Kriebel et al. (2018) used a FRET assay to measure cAMP levels associated with intact or lysed EVs. Interestingly, while lysed EVs had higher and constant levels of cAMP from time 0, intact EVs released cAMP over time, suggesting active synthesis and/or release. Measurement of ATP, the precursor used by ACA to generate cAMP, revealed that it is also present inside EVs.

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The final piece in the puzzle was determining how cAMP is released from the inside of EVs to the outside in order to promote chemotaxis. Of the 68 ABC transporters in *Dictyostelium*, proteomics analysis identified 13 in EVs. Using pharmacologic and genetic inhibition approaches, Kriebel et al. (2018) narrowed it down to the ATP-binding cassette transporter subfamily C (ABCC) class of transporters and ABCC8 in particular. Indeed, a newly generated knockout of ABCC8 greatly diminished chemotaxis and streaming; RFP-ABCC8 expressed in the *abcC8*$^{-}$ cells was enriched in vesicular trails and excluded from the plasma membrane. Altogether, these data indicate that the entire machinery for generating and releasing chemoattractants is contained within EVs: the catalytically active enzyme (ACA), its substrate (ATP), the product (cAMP), and the transporter (ABCC8; Fig. 1).

Overall, Kriebel et al. (2018) describe an elegant system by which chemotactic signals are generated and sustained to promote streaming in a complex multicellular system, and they elucidate a major mechanism by which cells leave a memory of themselves. EVs are left in a breadcrumb-like trail behind cells and continue sending chemotactic signals to surrounding cells. The finding that EVs act as cell-independent entities to not only carry but also generate bioactive products is important because it shows that they can amplify signals. The same group previously showed that neutrophil exosomes contain 5-lipoxygenase and autogenerate the chemotactic lipid leukotriene B4 (LTB4) in response to the potent chemotactic bacterial peptide f-Met-Leu-Phe (fMLF; Majumdar et al., 2016). In both cases, the presence of enzymes that can generate a product continuously amplifies and sustains a signal beyond that which would occur by carrying just the product. Those data also suggest that amplification of chemotactic signals is an important function of EVs that is conserved across organisms and necessary to promote effective directional sensing. In a different context, it was reported that precursor miRNAs can be processed into mature miRNAs in exosomes in a cell-independent manner due to the presence of the RNA interference–silencing complex (RISC) machinery in breast cancer–associated exosomes (Melo et al., 2014) and that this activity is important to promote tumor aggressiveness. Along with Melo et al. (2014), Kriebel et al. (2018) provide direct evidence that EVs can act as an independent machinery to regulate biological processes such as chemotaxis or tumorigenesis via enzymatic activities.

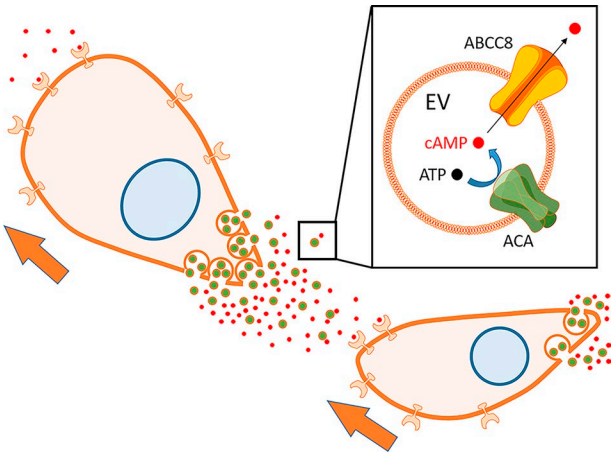

Figure 1. **EVs secreted from *Dictyostelia* synthesize and release cAMP to promote chemotaxis.** cAMP is converted from ATP by ACA in EVs and secreted through ABCC8. Secreted cAMP makes a gradient and promotes chemotaxis of follower cells.

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