Despite their lower threshold for moving brood, low-temperature-incubated ants selected warmer parts of their artificial nest for brood placement. Weidenmüller *et al.* [3] speculate that this behaviour relates to the best strategies for incubating brood in spring and summer. They suggest that in spring, heat is a limiting resource. Thus, colonies place brood in the warmest part of the nest, but stand ready to move it away rapidly should the temperature become super-optimal. In summer, heat is the enemy, and it is probably best if brood is kept in parts of the nest where high temperatures are never experienced, thus saving the labour of frequently moving brood around, and reducing the risk that it will ever experience lethal temperatures. Hence, a rapid response to rising temperature is not required, for the brood is located in a cool part of the nest. This argument is a bit counter-intuitive, and I am not entirely convinced by it; however, the speculation is supported by the fact that high-temperature-reared workers moved brood to cooler parts of the artificial nest than low-temperature-reared workers.

Finally, Weidenmüller *et al.* [3] found that adult ants that were exposed to increasing temperatures repeatedly developed lower task thresholds and thus started to move pupae at lower temperatures than they had done previously. This supports the idea that task threshold can be reinforced by experience [7], increasing the variance in task thresholds among workers in a colony. Interestingly, although experience decreased the threshold for carrying out brood, it did not change the threshold for the first response; that is, picking the brood up. This suggests that experienced workers do not become more sensitive to increasing temperature, but become more efficient in their response to it.

The Weidenmüller *et al.* [3] study is important because it demonstrates another way by which non-genetic mechanisms can result in significant inter-individual behaviour within colonies of insects. The work focussed on brood incubation behaviour, but it is not unreasonable to suspect that thresholds of other behaviours could also be affected by a worker's experience as a pupa or larva. For example, in the honey bee, larval diet has a profound effect on morphology, and likely the behaviour of the subsequent adults [8]. Thus, larval feeding, pupal incubation, age and genetics may all interact to produce workers with different task thresholds, and thus colonies that are more homeostatic and efficient.

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Metastasis: Wherefore Arf Thou?

The small GTP-binding protein Arf6 is known to be an important regulator of the actin cytoskeleton and of cell motility associated with metastasis. A recent study identifies yet another role for Arf6 in metastasis — as a regulator of plasma-membrane-derived microvesicle release.

Richard T. Premont* and Robert Schmalzigaug

Perhaps the most perilous moment in carcinogenesis is when transformed cells break free from their original microenvironment, seeking to flourish elsewhere. As tumor cells migrate away from their site of origin, they can elude the remaining normal spatial controls that might have kept the tumor from expanding explosively and can go on to find new environments where they can thrive, forming new and often more aggressive tumors.

Established anti-cancer therapies target cell proliferation, as transformed cells divide relentlessly in the absence of feedback controls that normally limit the growth of non-transformed cells. An alternative therapeutic approach would be to prevent tumor cells from migrating away from their site of origin; much effort has therefore been expended in developing a molecular understanding of cell migration in normal and tumor cells. The Arf6 protein is one important mediator of tumor-cell migration and invasiveness during metastasis, suggesting that Arf6-regulated pathways may provide valuable targets for therapeutic intervention to reduce metastatic potential.

Arf6 is a member of the ADP-ribosylation factor (Arf) family of small GTP-binding proteins [1]. Like all GTP-binding regulatory proteins, Arf6 cycles between an inactive GDP-bound form and an active GTP-bound form. Activity is controlled by guanine nucleotide exchange factors (GEFs) that promote GDP release and GTP binding, and GTPase-activating proteins (GAPs) that promote hydrolysis of bound GTP to GDP [1]. Unlike other Arf family members, which function primarily to coordinate intracellular vesicle trafficking events in the Golgi and endoplasmic reticulum, Arf6 functions primarily at the plasma membrane [1]. The traditional view of Arf6 is that it

does have a role in coordinating vesicle trafficking, but that it specifically regulates traffic to and from the plasma membrane [1]. As such, Arf6 is an important regulator of cellular signaling by affecting the ability of receptor proteins to make their way to and from the cell surface. Arf6 also has an important role in coordinating cell shape and motility by regulating the actin cytoskeleton, especially in tandem with the Rho-family member Rac1 [2–4]. Arf6 functions as a signaling intermediate, linking cell-surface receptors to the activation of several enzymes involved in plasma membrane lipid metabolism and lipid-messenger generation [1]. Given the diversity and complexity of Arf6 functions, and the apparently limited capacity of other Arf isoforms to perform these specific tasks, it is not surprising then that mice lacking Arf6 die during embryogenesis [5].

Arf6-regulated pathways have been studied extensively in the context of cancer, especially in tumor-cell metastasis. In previous work, Sabe and co-workers [6,7] explored Arf6-dependent migration and invasion in breast cancer-derived cell lines. Breast tumor cells with a highly invasive phenotype had increased expression of Arf6 compared with less invasive cells [8], and siRNA-mediated knockdown of Arf6 expression reduced cell migration and invasiveness [6]. Active Arf6 promoted the formation of tumor cell invadopodia — membrane protrusions that project into and digest the extracellular matrix [6]. Arf6 localized to invadopodia, and an inactive Arf6 mutant prevented invadopodia formation and matrix digestion [9]. In a series of recent studies (reviewed in [7]), Sabe and co-workers delineated a pathway promoting metastatic potential in breast cancer cells that began with stimulation of the EGF receptor, to activation of the Arf GEF GEP100/BRAG2, of Arf6, and of the ArfGAP protein ASAP1/AMAP1, which acts as an Arf6 effector to recruit paxillin and cortactin to invadopodia. D'Souza-Schorey and colleagues [9,10] performed similar studies with the LOX invasive melanoma cell line. LOX melanoma cells stably expressing mutant forms of Arf6 that were either locked in the active, GTP-bound state or the inactive, GDP-bound state were compared with wild-type LOX cells (expressing only endogenous Arf6) in a xenograft model using athymic nude mice [10]. Mice injected subcutaneously with $LOX^{ARF6\text{-}GTP}$ cells developed primary tumors that were much smaller and grew more slowly than those in mice injected with LOX cells or $LOX^{ARF6\text{-}GDP}$ cells [10]. However, despite this slower growth, $LOX^{ARF6\text{-}GTP}$ cells were much more invasive of immediate surrounding tissue. Nevertheless, in a direct metastasis establishment assay, in which tumor cells are injected into the tail vein, $LOX^{ARF6\text{-}GDP}$ cells showed a small reduction in the number and size of lung metastases compared with LOX cells, whereas $LOX^{ARF6\text{-}GTP}$ cells failed to induce lung metastases. These findings indicate that Arf6 functions in controlling both cell proliferation and cell migration ability during metastasis. In glioma cells, Arf6 has been shown to promote invasion through activation of Rac1 [11] and also to mediate EGF-stimulated proliferation [12]. In various other cell types, the Arf GEFs ARNO/cytohesin-2 and EFA6 [13] have been implicated in promoting cell migration, as have Arf GAPs, such as ASAP3 [14] and GIT1/GIT2 [15].

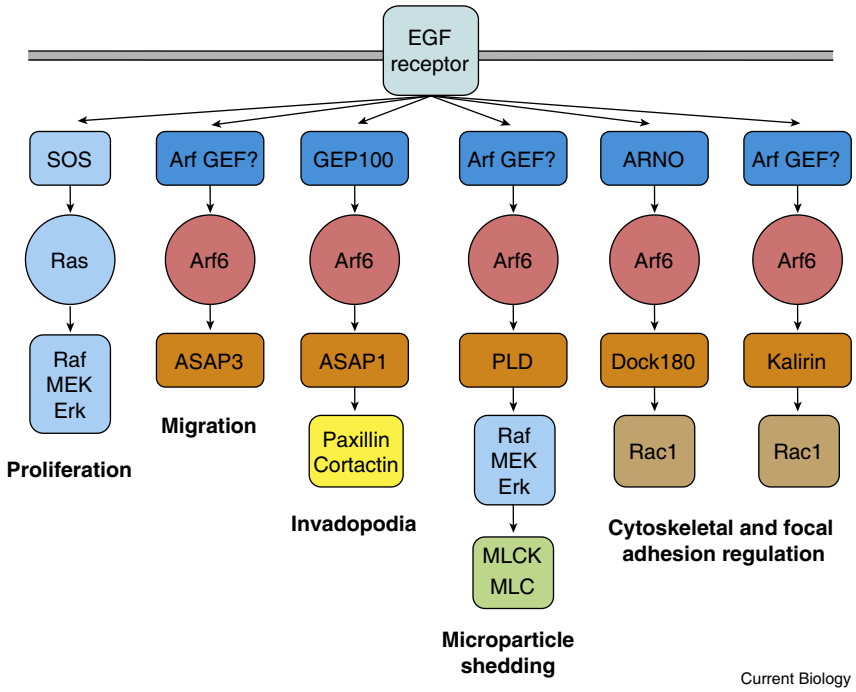

Figure 1. Arf6 signaling pathways in the regulation of tumor-cell migration and metastasis.
In the well-known mitogen-activated protein kinase pathway, the EGF-bound EGF receptor recruits the Ras GEF Sos to activate the small GTP-binding protein Ras, which initiates the Raf–MEK–Erk kinase effector cascade to increase cell proliferation. Arf6 is similarly activated downstream of extracellular receptors, such as the EGF receptor as shown here, as well as by intracellular signals that activate Arf GEF proteins such as ARNO or GEP100. For many Arf6 pathways, the specific GEF remains to be identified (shown as 'ArfGEF?'). Activated Arf6 binds to effector proteins (such as phospholipase D (PLD), and the Rac1 GEFs Dock180 and kalirin), some of which also function as regulatory ArfGAPs (ASAP1, ASAP3). These effectors initiate further signaling pathways, through Rac1 or Erk, that promote further cellular events, or may directly recruit proteins that alter cellular function (such as paxillin and cortactin in invadopodia).

In this issue of *Current Biology*, D'Souza-Schorey and colleagues [16] describe a mechanism whereby activated Arf6 contributes to matrix degradation during tumor cell invasion. Surprisingly, they provide evidence for Arf6-mediated regulation of an entirely unexpected class of membrane trafficking events: the release of plasma membrane-derived microparticles (Figure 1). These tumor cell-derived microparticles appear to derive from regions of the plasma membrane that are distinct from traditional invadosomes, but, like invadosomes, these microparticles are associated with matrix-digesting proteases.

Microparticles are one of a class of membranous structures released from cells. Another such structure, the

exosome, is a lipid-delimited vesicle released by the fusion of an intracellular multivesicular body with the plasma membrane, spewing the contained vesicles into the extracellular milieu [17]. Microparticles, in contrast, are derived from outward budding and fission from the plasma membrane directly, the inverse of the well-known inward vesiculation of clathrin-coated pits [17]. These microparticles contain integral membrane proteins derived from their cells of origin and may also carry cargo of select intracellular proteins. Once thought to be the product only of dying cells, microparticles are now appreciated also to be products of living cells — both normal and, most often, transformed [17]. Released microparticles can find their way even to the bloodstream, where they can be used as markers of the cells that released them. However, the mechanisms regulating microvesicle production by cells have remained obscure.

In this new study, the role of Arf6-regulated pathways in microvesicle shedding from the LOX melanoma tumor cell model is established [16]. Comparing highly invasive $LOX^{ARF6\text{-}GTP}$ cells with less invasive $LOX^{ARF6\text{-}GDP}$ cells revealed that $LOX^{ARF6\text{-}GDP}$ cells accumulated numerous vesicular structures on the cell surface, whereas $LOX^{ARF6\text{-}GTP}$ cells and wild-type LOX cells had far fewer of these structures. Instead, LOX and $LOX^{ARF6\text{-}GTP}$ cells accumulated microparticles in the culture media, and these microparticles were much reduced in the culture media from $LOX^{ARF6\text{-}GDP}$ cells. Analysis of the microparticles purified from the culture media showed them to have a membrane bilayer with phosphatidylserine on the extracellular face, and to be heterogeneous in size, consistent with a plasma-membrane origin rather than a multivesicular-body (exosome) origin. Tellingly, these microparticles contained Arf6 protein and cell-surface markers (β1 integrin), as well as matrix-digesting protease activity. This suggests that tumor cells release matrix-digesting microparticles to create localized clearing of the nearby matrix in advance of invadopodia protrusion.

How does Arf6 regulate microparticle release? Unexpectedly, Arf6 does not function as part of a proto-vesicle coat in microparticle formation, as might be expected if Arf6 had an analogous function to that of Arf1 in intracellular vesicle formation. Instead, Arf6 acts as a signaling intermediate to activate a localized signaling cascade culminating in myosin light chain phosphorylation (Figure 1). Determination of precisely how myosin light chain activity leads to outward membrane scission will require future work. Other important outstanding questions include whether Arf6 is absolutely required for microparticle formation and release (does Arf6 depletion affect this?), and what signals upstream of Arf6 lead to its activation on the microparticle pathway. Clearly, the identification of additional signaling components and pathways that regulate this process may identify workable approaches to therapeutically target early metastatic cells.

Arf6 sits in the center of a web of interactions and signals that affect a tumor cell's ability to migrate during metastasis (Figure 1), having functions such as: directing matrix degradation through microparticle release [16] and through the formation of invadosomes [7]; controlling dynamics of cytoskeletal and focal adhesion connections to extracellular matrix through activation of Rac1 [2,3]; promoting general cell migration, such as through the ArfGAPs ASAP3 [14] or GIT1/GIT2 [15]; and altering membrane lipid composition and producing lipid-derived messengers [1]. One apparently critical aspect of Arf6 function in these various pathways is that it acts locally, so that Arf6 activated in one location by a particular GEF is competent to perform certain tasks, but likely not others [18]. This specificity suggests that it may be possible for particular Arf6-mediated pathways to be targeted downstream of Arf6. With the current work, one additional Arf6-mediated pathway now needs to be considered as a potential target: the regulated release from tumor cells of plasma-membrane microparticles containing matrix-digesting proteases.

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