New Londoner: Several rare short-snouted seahorses have been discovered in recent marine surveys of the Thames estuary, convincing scientists that a breeding population now exists in the river. (Picture: Daniel Sprawson/ZSL.)

century. A sewage system created by Victorian engineers was a remarkable achievement that began the big clean-up.

The now much cleaner river is once again home to many species alongside the seahorse: the young of marine species such as flounder and bass have been discovered in nursery sites up to 20 km inland. But the sewage system that has done so much to restore the river's cleanliness still faces a problem: during summer storms with their torrential downpours, the system can in some years become overwhelmed with sewage which enters the river. As the storms usually coincide with periods when the river flow is at its lowest, the sewage has a short-term but devastating effect on river species.

But the discovery of remarkable new species such as the short-snouted seahorse should help to bolster efforts to ensure the river is clean all year round.

Nigel Williams

Invadopodia

Alissa M. Weaver

What are invadopodia? Invadopodia, or 'invasive feet', are actin-rich protrusions associated with sites of proteolytic degradation of the extracellular matrix (ECM). They received this name due to their presence at the basal surface of cells plated on beds of ECM and are generally found in invasive, but not non-invasive, cancer cells.

How do you recognize them? The typical invadopodia assay involves plating cells overnight on a thin layer of fluorescent ECM, such as crosslinked FITC–gelatin or unlabeled gelatin overlaid with FITC–fibronectin. Invadopodia-associated ECM digestion leads to removal of the fluorescent ECM such that degraded areas are evident as dark spots in the fluorescent background. Active invadopodia have actin-rich protrusions associated with the sites of degradation (Figure 1A). In electron micrographs, invadopodia are long and slender, protruding vertically away from the cell body (Figure 1B). In wide-field or single confocal images, invadopodia protrusions appear as puncta.

What are the molecular components of invadopodia? Invadopodia are hotspots of signaling and actin assembly. Src kinase signaling is both necessary and sufficient to induce invadopodia formation and many Src kinase substrates are found in invadopodia, including Tks5/FISH, N-WASp, AMAP1/ASAP1, cortactin, and dynamin. Branched actin assembly is important for the formation of invadopodia protrusions, as well as for membrane trafficking, and depends on molecules such as Arp2/3 complex, N-WASp, and cortactin. Adhesion proteins (such as integrins and CD44), membrane trafficking proteins (such as dynamin), and proteinases (such as MT1-MMP and seprase) are also found in invadopodia, suggesting that invadopodia serve as hubs where many cellular processes are coordinated for the process of ECM degradation.

How are invadopodia different from lamellipodia or filopodia?

Both invadopodia and lamellipodia are dependent on branched actin assembly. However, different branched actin activators are thought to be involved in each structure, e.g. N-WASp is important for invadopodia formation whereas WAVE-2 is essential for lamellipodial protrusion. In addition, lamellipodia lack proteolytic activity and have not been strongly tied to Src kinase activity. Finally, visible cell–ECM adhesions are usually physically separated in space from lamellipodia, appearing behind leading edge protrusions, whereas in invadopodia and podosomes (see below) adhesion proteins, such as vinculin and paxillin, are concentrated at or immediately surrounding the actin-rich protrusions.

Invadopodia also have similarities to filopodia, in that both types of protrusion are dependent on Cdc42 activity and are long and thin (Figure 1B). It seems probable that, as with filopodia, unbranched bundled actin may provide the structure for the straight, finger-like portion of the invadopodium that protrudes into the matrix; however, as of yet there is no definitive proof to support this idea. Currently, more lamellipodia-associated molecules have been implicated in invadopodia function than filopodia-associated molecules; however, it seems likely that both unbranched and branched actin assembly molecules cooperate to give rise to functional invadopodia.

Do normal cells make invadopodia?

Interestingly, some normal cells make structures called podosomes that are very similar to invadopodia. Podosomes are formed in cells that need to cross tissue barriers, such as monocytes and macrophages, and in cells that remodel tissue, such as osteoclasts. In addition, they are formed in activated endothelial and vascular smooth muscle cells and thus could be involved in the pathological remodeling that takes place in atherosclerosis or aortic aneurysms. Podosomes contain the same molecular components as invadopodia, with the only differences appearing to be the substitution of cell-type-specific isoforms, e.g. hematopoietic-specific molecules such as WASp and $\beta2$ integrins are present in macrophage podosomes, whereas their ubiquitous counterparts N-WASp and $\beta1$ integrins are found in cancer cell invadopodia.
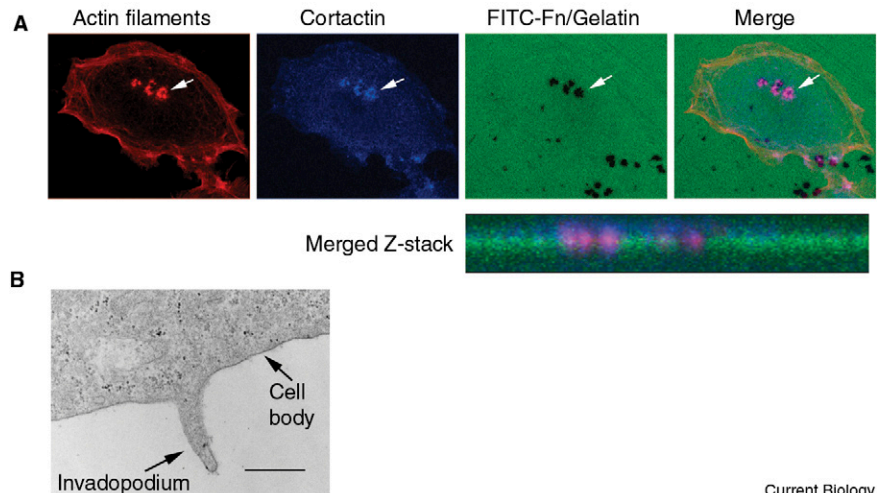
Figure 1. Invadopodia identification.

(A) A typical invadopodia assay in which breast cancer cells were cultured overnight on cover-slips coated with crosslinked gelatin overlaid with FITC–fibronectin (FITC–Fn/Gelatin), followed by fixation and staining with molecular markers of invadopodia (actin filaments and cortactin) and imaging by confocal microscopy. The arrow shows colocalisation of invadopodia markers with a dark patch of degraded ECM. (B) Electron micrograph of a head and neck squamous cancer cell plated on crosslinked gelatin, showing a typical long slender invadopodia protrusion. Scale bar = 500 nm. (Unpublished images from Kevin Branch (A) and Emily Clark (B), Weaver lab.)

What is the difference between podosomes and invadopodia?

This is a hotly debated topic in the field. In general, podosomes are thought to promote directed cell motility by monocyte-derived cells, such as dendritic cells, possibly by substituting for focal adhesions in those cells. Conversely, invadopodia seem to be more important for ECM degradation than for migration, perhaps because cancer cells frequently express both focal adhesions and invadopodia. However, podosomes were recently shown to degrade ECM, similar to invadopodia; thus the differences with respect to separation of adhesion and degradation functions between the two structures may not be extensive.

Morphologically, podosomes are visible as ring structures, with adhesion proteins defining the ring that surrounds an actin-rich core. By contrast, invadopodia generally appear in wide-field micrographs as puncta with no separation between molecular components. In addition, podosomes have faster dynamics, with turnover times of a few minutes, compared with hours for invadopodia. Because podosomes have been traditionally studied under different conditions from invadopodia, e.g. in cells cultured on glass coverslips rather than on the semi-three-dimensional layer of ECM that is used in invadopodia assays, it is not clear whether the differences in morphology and dynamics between podosomes and invadopodia are as distinct as have been reported. Future studies will likely address these points.

Are invadopodia found in vivo?

In vitro, invadopodia are defined by the association of degradation activity with actin-rich protrusions using the in vitro fluorescence ECM assay. In vivo, less progress has been made, although this is an area of great interest in the field. Investigators such as John Condeelis and Elisabeth Genot are actively working on identifying in vivo invadopodia and podosomes, respectively, using combinations of prototypical actin core and ECM proteolysis markers.

Are they important in vivo?

Remodeling of ECM is important for many physiological processes, including normal development, bone homeostasis, and wound repair. In cancer, proteolytic degradation of the ECM is required for invasion across basement membranes and possibly outgrowth at secondary sites. Whether proteinase activity is required for migration through loose connective tissue is still an open question and is likely to depend on the size of the matrix pores and whether cells are migrating collectively or as single

cells. Regardless, increasing evidence supports the idea that invadopodia are the subcellular structures required for ECM remodeling activity. Molecules such as the transmembrane metalloproteinase MT1-MMP are essential for invadopodia activity *in vitro* and have been shown by Steve Weiss' laboratory and others to be important for tumor growth and invasion *in vivo*, suggesting that invadopodia are likely to enhance tumor growth at secondary sites through removal of space constraints.

Why do cells need invadopodia? why can't they just secrete proteinases at large to degrade ECM? At this point it is not fully clear why ECM degradation appears to take place only at invadopodia. This might represent a regulatory point of control, such that efficient ECM degradation only occurs where many signals and processes converge. One possibility is that proteinase activation and/or delivery occurs 'on-site' at invadopodia. The invadopodia metalloproteinase MT1-MMP is an activator of other invadopodia proteinases and could function as a critical upstream catalyst of proteinase activity for focal ECM degradation. Why Src kinase signaling and branched actin assembly are required in this process, however, is an open question.

Any outstanding controversies?
Many of the points raised above. Open questions include: what are the differences between podosomes and invadopodia? Do invadopodia exist *in vivo* (and if so what they would look like)? What are the stages in invadopodia formation and function? And are invadopodia as structures truly required for ECM degradation?

Where can I find out more?
Ayala, I., Baldassarre, M., Caldieri, G., and Buccione, R. (2006). Invadopodia: a guided tour. Eur. J. Cell Biol. *85*, 159–164.
Buccione, R., Orth, J.D., and McNiven, M.A. (2004). Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. Nat. Rev. Mol. Cell Biol. *5*, 647–657.
Linder, S. (2007). The matrix corroded: podosomes and invadopodia in extracellular matrix degradation. Trends Cell Biol. *17*, 107–117.
Weaver, A.M. (2006). Invadopodia: specialized cell structures for cancer invasion. Clin. Exp. Metastasis *23*, 97–105.
http://www.invadosomes.org

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Locusts

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What is a locust? A special type of grasshopper (Orthoptera: Acrididae) distinguished by expression of a remarkable and potentially devastating form of phenotypic plasticity, known as density-dependent phase polyphenism. Changes in local population density cause the development of strikingly different phenotypic forms, or 'phases' (Figure 1). Low population densities produce the shy, well-camouflaged 'solitarious' phase, whereas crowded conditions produce the aggregating, migratory 'gregarious' phase. Solitarious phase locusts avoid one another, but gregarious locusts can form huge groups and embark on spectacular mass migrations, travelling as marching bands of flightless juveniles and vast flying swarms of winged adults.

Of the more than 12,000 described grasshopper species, fewer than 20 are considered locusts. Swarming locusts have evolved independently a number of times in a variety of different grasshopper lineages throughout the world. It seems as though a combination of ecological factors has repeatedly favoured the evolution of locusts from their more grasshopper-like ancestors. The relationship between locusts and their environment and how this interaction leads to swarm formation is an active area of research.

What is phase polyphenism?
Although phenotypic changes in colouration as seen in Figure 1 may often be the most conspicuous feature, solitarious and gregarious phase locusts differ in a variety of other traits, including morphology, food selection and nutritional physiology, reproductive physiology, metabolism, neurophysiology, endocrinology, molecular biology, immune responses, longevity and pheromone production. In the Migratory locust of Africa, Asia and Australia (*Locusta migratoria*), the phenotypic differences are so extreme that the two phases were originally classified by Linnaeus as separate species, a mistake that was not appreciated until 1921 when Russian biologist Boris Uvarov proved that the two phases are not even different genotypes. The genetic instructions for producing the two phases are packaged within a single genome, with expression of one or other suite of genes depending on cues associated with crowding. Different locust species vary in the number of phase traits that they express. The Australian Plague locust (*Chortoicetes terminifera*), for example, shows extreme density-dependent behavioural changes, but appears to lack the colour and shape changes seen so prominently in *L. migratoria* and the Desert locust, *Schistocerca gregaria*.

Why are locusts of interest? Locusts have been feared agricultural pests since the dawn of civilisation with plagues documented in ancient texts including the Qur'an, Bible and Torah. Locust outbreaks can occur on all of the continents with the exception of Antarctica and have the potential to affect the livelihoods of one in ten people on the planet. A single locust

Figure 1. The two extreme phases of juvenile Migratory locusts, *Locusta migratoria*.

The solitarious phase insect on the left was reared alone, whereas the gregarious phase insect was reared in a crowd. (Image courtesy of Gabriel Miller.)