

**Figure 2** The colonization of leaves by endophytes, and pathogen deterrence. a, The natural colonization of leaves, increasing over time, by a diverse endophyte community. b, c, The results of the key experiments carried out by Arnold *et al.*<sup>1</sup> on sterile cacao seedlings. b, Pathogen attack of plants that had been inoculated with endophytes resulted in comparatively little pathogen occupation of leaf cells and limited damage. c, By contrast, pathogens became well established in endophyte-free control leaves and caused severe damage or leaf death.

transmission through seeds that is seen in grasses.

Arnold *et al.*<sup>1</sup> next looked at the distribution of endophyte diversity in cacao. The most similar communities occurred in neighbouring sites, and they became more divergent as the distance between sites increased. There was little evidence that endophyte composition reflects habitat type. The authors also sampled three tree species, including cacao, at a single site and found that most types of endophyte showed host affinity, infecting only one of the three. In fact, endophyte communities from different host species at a single site were more divergent than communities from cacao trees at separate sites. Arnold *et al.* confirmed this apparent host preference using agar plate assays in which fungal endophytes grew differentially in response to leaf extracts from the three species (although some studies<sup>7,8</sup> on other tropical systems found little evidence for host preference). The degree of host specificity is a critical parameter for estimating total endophyte biodiversity based on isolation of fungi from individual tree species.

The most dramatic result occurred when Arnold *et al.* inoculated sterile cacao seedlings with several groups of endophyte, and then exposed the seedlings to a species of *Phytophthora*. This is a common pathogen that causes black pod disease and is related to the agent of Irish potato blight (*Phytophthora infestans*). Endophyte-free control leaves died in greater numbers than those inoculated with fungus, and the control leaves that did survive suffered much more damage (Fig. 2). The relative advantage of endophyte inoculation was higher in older than in younger leaves. Overall, endophyte-protected leaves suffered less than half the damage seen in control leaves (Fig. 1, inset).

But how does the fungal endophyte kill

or inhibit the growth of *Phytophthora*? One possibility is that the endophytes simply occupy the space that might be taken by the pathogen, or competitively consume resources; alternatively, direct antagonism might be involved. Furthermore, does inhibition result primarily from the action of a particular endophyte or do a variety of them act additively or synergistically? Would the results hold if endophytes and *Phytophthora* were inoculated simultaneously, or if the pathogen was inoculated first? In native

habitats, where endophytes and pathogens occur together, do heavily diseased plants host less diverse fungal communities?

Whatever the answers to these questions, Arnold and colleagues' findings<sup>1</sup> point to biological control as a promising way of tackling disease in cacao trees. This could be achieved by spraying the trees with fungal spores, or perhaps more realistically by growing them alongside other trees that could serve as sources of fungal inoculum. The results also clearly demonstrate that plant–endophyte mutualistic interactions are not restricted to cool-season grasses, and they explain why host plants don't defend themselves against infection by the fungi concerned. Pathogens and endophytes are common in all plants, so carrying out similar experiments in a range of tropical and temperate species should prove highly rewarding. ■

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### Nanotechnology

## How does a nanofibre grow?

Pulickel M. Ajayan

Decades of research have failed to decipher the atomic-scale mechanism by which carbon nanofibres grow out of vapour. High-resolution microscopy shows that the carbon atoms have a bumpy ride.

In his historic monograph *On Growth and Form*<sup>1</sup>, D'Arcy Thompson wrote that the form of an object is a diagram of its growth forces. Often, however, this relationship between growth and form is too complex to determine: the growth of filamentous carbon in the vapour phase is a case in point. Despite a large body of literature on the subject, replete with fascinating and varied examples, no definitive model for the growth of carbon nanofibres has evolved, owing to a lack of consistent experimental data<sup>2,3</sup>. Considering the substantial impact that these materials are likely to have on technology<sup>4</sup>, it seems imperative that their growth mechanisms be understood, so that nanofibres can be manufactured with well-defined characteristics. A long-awaited solution to the mystery of nanofibre growth is presented

by Helveg *et al.*<sup>5</sup> on page 426 of this issue.

Nanoscale carbon fibres are grown through the interaction of metal-catalyst nanoparticles with hydrocarbon vapour at high temperature. The hydrocarbon molecules dissociate at the interface between catalyst and vapour, and carbon atoms precipitate into a graphite trail in the shape of a cylindrical, multi-walled nanofibre (Fig. 1). Quite how the nanofibre forms is unknown. The catalyst particle might stay at the growing end of the nanofibre (called tip growth), or it might sit at the starting end (base growth)<sup>6</sup>. The state of the particle itself (such as its structure or shape) during the growth process is also unknown, but the particle size and fibre diameter are similar.

Experimentally, it has proved difficult to track the dynamics of this high-temperature catalytic reaction with spatial and temporal

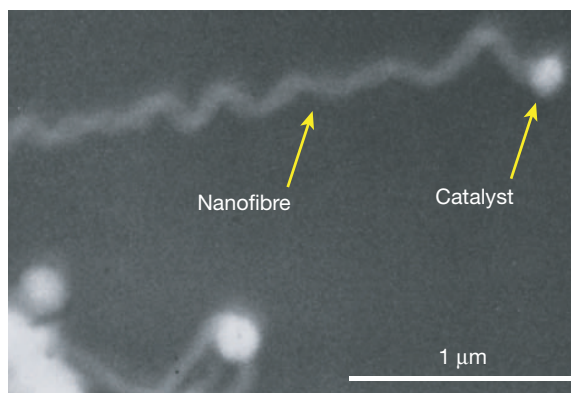
resolution sufficient to observe the growth of the nanofibre directly at the atomic scale. The images presented by Helveg *et al.*<sup>5</sup>, capturing the early stages of nanofibre growth, are clearly the result of some impressive developments in high-resolution transmission electron microscopy *in situ*<sup>7</sup>. These authors have managed to record, in real time, the catalytic reaction at about 500 °C between methane and supported nickel nanoparticles (5–20 nm in diameter) inside a high-resolution transmission electron microscope. Analysed frame by frame, the images reveal the events that lead to nanofibre growth.

It seems that the catalyst particle moves with the growing nanofibre, following the tip-growth prescription. What is surprising is that the nanofibre growth is promoted by abrupt shape changes in the catalyst particle itself, which are in turn driven by the reaction between the catalyst and the vapour. These shape changes, between spherical and elongated, are sudden and repeated. In its elongated form the particle serves as a template, facilitating the formation of aligned graphite layers as carbon atoms diffuse across its surface. A closer look at the images reveals that the nucleation and growth of these graphite layers occur at 'bumps' on the nickel nanoparticle — single-atom step-edges that develop and then disappear continuously. This step-edge mechanism is also backed up by theoretical calculations.

Throughout the growth process, the nickel catalyst remains crystalline, bounded by the low-energy facets of the nanoparticle surface. The shape changes are driven by shifts in the balance of surface energy as graphite layers repeatedly cover then expose facets of the nickel surface. It is crucial that part of the nickel surface remains in direct contact with the surrounding vapour: when the particle is fully encapsulated by graphite layers, nanofibre growth stops because no further reaction between metal and vapour occurs.

These experiments by Helveg *et al.*<sup>5</sup> show the importance of shape transformations in small particles when they are used as nanoscale growth catalysts. Indeed, the reaction-induced reshaping of metal particles seen here has certain parallels with the inherent structural instability of metal nanoparticles<sup>8</sup>. The diagram of forces that determines the final form of a nanofibre is closely linked to the drastic deformations of particle shape. As the particle gets larger, shape fluctuations become energetically unfavourable, and this could explain why large particles are inefficient catalysts for nanofibre growth<sup>9</sup>.

A word of caution is needed, however: the mechanism proposed by Helveg *et al.*<sup>5</sup> may only be relevant in specific cases; it does not easily extend to smaller carbon nanotubes, in which the carbon lattice is far more ordered than in nanofibres and for which the catalyst particle is not always seen attached



**Figure 1 Growth of a nanofibre.** At high temperatures, and in the presence of a catalyst, vapour-phase hydrocarbon molecules dissociate. The molecule's carbon atoms form into nanofibres, as shown in this scanning-electron-microscope image<sup>10</sup>. Helveg and colleagues' high-resolution study<sup>5</sup> of the process now reveals the atomic-scale mechanism.

to the nanotube structure<sup>6</sup>. So, even with this new insight, the mystery of the growth of nanoscale filamentous carbon is not completely solved. But this study has overcome some major experimental stumbling blocks to provide the first direct glimpses of nanofibres as they grow. It heralds a new beginning in nanoscale real-time growth observations that should lead to better control over fibre synthesis for nanotechnology. ■

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#### Developmental biology

## Tail of decay

Alexander F. Schier

Humans and other vertebrates develop in a head-to-tail sequence. A mechanism that is based on a gradual decay of RNA appears to contribute to this process.

**T**he thousands of different cell types in the vertebrate body are arranged in a complex but reproducible pattern. The underlying blueprint develops in the embryo, when initially naive cells mature and specialize according to their position. In recent years, it has become clear that positional information is often provided by the graded distribution of signals<sup>1,2</sup>. Depending on their location in the gradient, cells are exposed to different concentrations of the signal and differentiate in a dose-dependent way. But how do these gradients form? On page 419 of this issue, Dubrulle and Pourquie<sup>3</sup> describe one mechanism. In the growing embryo, cells at the tip of the tail produce a messenger RNA (mRNA) molecule that codes for a protein signal; as cells move away from the tip, the levels of mRNA gradually decay. The resulting gradient is thought to regulate the maturation of cells.

The use of gradients and dosage-dependent responses is widespread in development, from fly embryos to the mammalian nervous system. A gradient has also been

implicated in the head-to-tail development of vertebrates. During early development, embryonic structures are progressively laid down from head (anterior) to tail (posterior). This process can be illustrated by the formation of the skeleton. The vertebrae are arranged in a repetitive anterior-to-posterior pattern. They each emerge from small blocks of tissue called somites (Fig. 1a, overleaf). The first somite forms most anteriorly, and then one somite after the other is added in a head-to-tail sequence<sup>4</sup>. A somite forms through maturation of the presomitic mesoderm — a region of immature cells that lie posteriorly to the last-formed somite. The presomitic mesoderm in turn derives from cells that are released from a more posterior growth zone called the tail bud. So, during their maturation, cells move in an assembly line from the tail bud to the presomitic mesoderm and then to the somites.

Previous studies have shown that this maturation is controlled by a signalling molecule that belongs to the fibroblast growth factor (FGF) family of proteins<sup>5</sup>. It appears