configurations such as nanolenses[10] and bow-tie nanoantennas[11] to take advantage of all three mechanisms: resonance, gap plasmons and the lightning-rod effect.

The context for Kim and colleagues' experiments[1] is an array of gold bow-tie nanoantennas on a sapphire substrate. In the nanoscale gaps of the bow-tie, the local optical intensity can be enhanced in an idealized case by up to 10,000 times (Fig. 1). In reality, the enhancement is limited by intrinsic optical losses in gold, imperfections in the structure's geometry, and the defects and polycrystalline nature of the gold's crystal lattice. But if the local field intensity is increased by just 100 times, it reaches 10^{13} W cm^{-2}, sufficient for relatively efficient generation of EUV radiation. Because this field is extremely locally concentrated in the gaps, it does not damage the sapphire substrate. Equally, the high dielectric permittivity of the gold nanoparticles means that the field cannot penetrate far into their surface.

The authors immerse their bow-tie assembly in a jet of argon gas. From this point, everything proceeds broadly analogously to the traditional production of EUV light. First, the high local field created by the plasmonic enhancement detaches an electron from the argon atoms as they pass through the bow-tie gaps. This electron is accelerated by the optical fields along its trajectory, and collides and recombines with the atom. The oscillating optical field causes the collisions to repeat periodically, releasing energy at high-harmonic frequencies of the original light.

There are two principal distinctions between this process and the generation of high harmonics by intense optical pulses. First, the intensity threshold is considerably lower, as touched on earlier. Second, the incident optical pulses are relatively tightly focused, and so the EUV light is generated in just one or a few nanogaps of the array and is not highly directed. (Directedness would stem from the interference of radiation from many bow-ties.)

This new method of short-wavelength light generation will open doors in imaging, lithography and spectroscopy on the nanoscale. It could expand the range of techniques that exploit local-field enhancement, such as near-field scanning optical microscopy, from the infrared and optical wavelengths to the EUV and, ultimately, as the plasmonic quality of the nanoantennas improves, X-ray range. This much shorter wavelength will benefit techniques such as core spectroscopy and nanoscale X-ray crystallography.

With less-tight focusing, it will also become possible to generate EUV radiation in many nanoantennas simultaneously. The resulting spatially coherent, laser-like light could have applications in many areas: in macroscopic spectroscopies, in screening for structural defects in materials and, extended to X-ray and even γ-ray wavelengths, in the detection of minute amounts of fissile materials in the arenas of public security and defence. ■

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DEVELOPMENTAL BIOLOGY

Order in the lung

David Warburton

Given the lung's thousands of branching airways, its development might be expected to be a highly complex process. Yet a surprisingly simple picture now emerges of when, where and in what order these branches form.

Elaborate branching is everywhere in nature. From riverbeds to oilfields, from trees to blood vessels, branching connects the large to the small. The lung is also a prime example of a reproducible branching system, allowing gas to be transported from the air to tissues deep within an animal. Without it — or without the simpler branched ducts found in less complex organisms — oxygen transport by diffusion probably would have limited the evolution of terrestrial animals to less than one millimetre in size. But how does such a sophisticated network develop? Metzger *et al.*[1] (page 745 of this issue) provide a remarkable, yet simple picture that explains the orderly development of the more than a million branches in the mammalian lung.

In mammals, air enters through the nasal and oral cavities and passes through the larynx and trachea before reaching the lung. The trachea branches into two primary bronchi, which, within the lung, further branch into secondary and tertiary bronchi and finally into bronchioles. To investigate the sequence of events leading to this complex, yet highly reproducible network of branches, Metzger *et al.* studied the early bronchial tree in three dimensions by examining chemically fixed lung tissue from mouse embryos using microscopy.

The authors parse bronchial branching beyond the primary branch into three geometrical modes, which they call domain branching, planar bifurcation and orthogonal bifurcation. In domain branching, daughter branches form in rows along the parent branch, like bristles on a bottle brush. This branching mode forms the main secondary branches. Next, planar bifurcation is used for the formation of tertiary and later-generation branches; this mode is characterized by the splitting of a branch tip into two. Finally, orthogonal bifurcation involves two rounds of branching. Both rounds involve

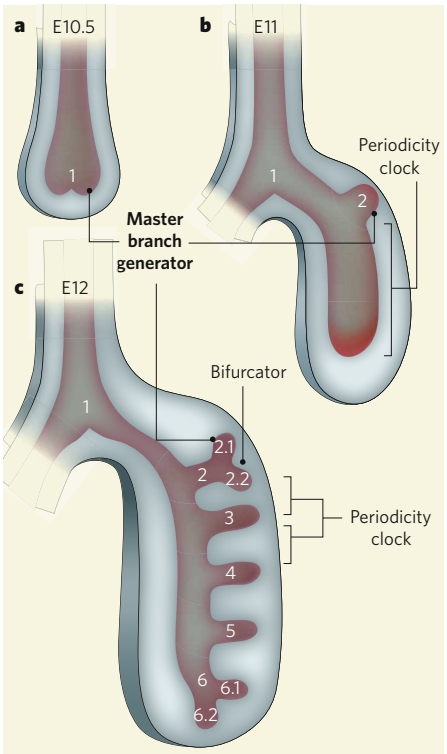

Figure 1 | A master and three slaves. Studying early lung morphogenesis in the mouse embryo, Metzger *et al.*[1] show that this organ's airways form in a sequential manner in three series of events, or subroutines, which are all driven by one master branch generator. **a**, At embryonic day (E) 10.5, the primary bronchial branch (1) forms, followed by (**b**) the development of the left upper-lobe branch (2) by E11. **c**, The first two segmental branches of the left upper-lobe branch (2.2 and 2.3) and the subsequent formation of branches 3–6 occur at E12. The master branch generator is active throughout these events, and the inferred sites of action of the periodicity clock and bifurcator subroutines are shown. At E12, the rotator subroutine has not yet begun to function.

50 & 100 YEARS AGO

50 YEARS AGO

To the young scientist making contact with industry or commerce fresh from school or university it comes as rather a shock to find that his general 'scientific' usage of the C.G.S. system has such small acceptance in the outside 'practical' world. True, he has used 'Winchester quarts' to hold his standard solutions made up in grams per litre, he has stirred with fractional h.p. motors and dabbled in foot-poundals and lb./sq. in. But he has learnt to think in metric units and centigrade degrees. He knows that engineers seem to be able to exist with other units, and he respects their attempts to decimalize the rather antique units that he finds them using. He will probably be surprised to learn that Great Britain narrowly missed adopting the metric system of weights and measures almost one hundred years ago by a handful of votes in Parliament.

From *Nature* 7 June 1958.

100 YEARS AGO

A poll has just been taken by the Geological Society to ascertain the opinion of the fellows resident in the United Kingdom as to the admission of women to the society. The number of voting papers sent out was 870, and 477 replies were received. An analysis of the votes shows that 248 fellows were in favour of the admission of women as fellows and 217 against their admission, but of this number only 133 voted against the admission of women at all, the remaining eighty-four being in favour of their admission as associates. The fact that there was a majority of thirty-one in favour of the admission of women as fellows should be an encouraging sign to the increasing number of women who are taking up scientific work and in other ways contributing to the extension of natural knowledge.

From *Nature* 4 June 1908.

planar bifurcation, but, between the two, a 90° rotation of the bifurcation plane leads to the arrangement of the resulting branches into a rosette (Fig. 2f on page 746). These simple branching modes are used iteratively to give rise to the labyrinthine network that constitutes the bronchial tree.

The repetitive nature of the branching modules, together with their hierarchical control and the fact that they are coupled, suggests that the genetic 'hard-wiring' for bronchial branching could actually be quite simple. Thus, determining how the genome encodes the early development of the lung might be more tractable than previously thought. It also gives hope that, some day, regeneration or engineering of damaged lung tissue might be possible.

How are the three branching modes regulated? Metzger *et al.* infer that, once left–right laterality of the lung is established, airway branching is driven by a 'master' branch generator, with three 'slaves' in the form of subroutines (series of discrete patterning events). Of these, one subroutine seems to instruct a periodicity clock, which times the appearance of subsequent branches; another determines the rotational orientation of the branches around the axis of the parent airway; and the third mediates bifurcation (Fig. 1).

The authors identify a protein called Sprouty2 as a candidate component of the periodicity-clock subroutine in mice. Sprouty2 is named after the excessive tracheal branching seen in fly mutants that lack this gene. In flies, branching of the tracheal airway is initiated and controlled by the *branchless* gene, the closest mammalian equivalent of which is the gene that encodes the signalling protein FGF10. Also, the receptor for the protein product of *branchless* is Breathless, whose mammalian counterparts are FGF receptors. Sprouty2 is an evolutionarily conserved, inducible downstream inhibitor of FGF-receptor signalling from flies to mice.

The *Fgf10* gene is expressed in the mesenchymal tissue, which overlies the epithelial-cell layer lining the emerging branch tip. The FGF receptor FGFR2 is expressed throughout the epithelium, and Sprouty2 is expressed locally at the branch tips[2]. Also, mutations in the *Fgf10* or *Fgfr2b* genes that prevent their expression completely abrogate lung branching, and either decreased FGF10 expression or enhanced expression of Sprouty2 produces a small, poorly branched lung. Thus, in both flies and mice — and probably in humans — Sprouty2 mediates fine regulation of FGF signalling at the correct time, place and dose to induce and control orderly airway branching.

Metzger and colleagues' observations further suggest that the balance between FGF expression, FGF-receptor activation and Sprouty2-mediated inhibition of FGF signalling is possibly a central component not only of the master branch generator but also of the periodicity-clock subroutine. The periodicity clock can be speeded up by increasing the internal pressure in cultured embryonic mouse lung tissue[3]. This gain-of-function effect, which involves a significant increase in the rate of branch extension, a reduction in inter-branch length and a shift from bifurcation to trifurcation of branch tips, is mediated by a pathway requiring FGF10, FGFR2b and Sprouty2[3–6]. Thus, the often-overlooked connection between physics and biology in developmental processes is clearly important.

Other crucial players involved in lung branching include a long list of gene transcription factors, such as Nkx2.1; major signalling pathways, including those mediated by retinoids, bone morphogenetic protein, Hedgehog and Wnt; and essential components of the extracellular matrix, especially fibronectin and laminin[6,7]. All these protein factors are expressed at the right time and place, and function in and around the tips of the airway branches. Equally important for structural reproducibility is suppression of branching at regions away from the tips. The signal protein Sonic hedgehog accomplishes this by negatively regulating *Fgf10* expression proximal to the tips, thus suppressing out-of-place branching.

Genes related to those encoding most, if not all, of these morphogenetic factors are thought to have been present even in *Urbilateria*, the common ancestor of the planarian flatworms[8]. So it is possible that conditions of relative oxygen shortage exerted strong evolutionary pressure on pre-existing groups of such genes, to select for tube formation and hence gas transport. In support of this idea, FGF signalling in flies is regulated by oxygen levels so as to match terminal tracheal branching to the local oxygen needs of a tissue[9]. In mice, and presumably in humans, the signalling pathway mediated by molecules such as hypoxia-inducible factor and vascular endothelial growth factor also plays a crucial developmental part with the FGF–FGFR–Sprouty2 pathway. Together, these pathways match the capillary vasculature to the epithelial layer in branches of the early lung, a process that is crucial for determining the eventual gas-diffusing capacity of the organ[10].

Whether a master branch generator controlling a select few slave subroutines represents a general developmental strategy that has been reused over evolutionary time, in different branched organs, remains an intriguing possibility. Also, solving the specific problem of gas diffusion as a limit on size, and discovering how simplified, genetically controlled branching routines interact with physical and biological factors to direct complex yet reproducible patterns of development, will be matters of great interest. To quote Charles Darwin as interpreted by biologist Sean Carroll, they will aid our understanding of how "endless forms most beautiful" have evolved from a relatively simple tool-box of genetic modules. ■

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PHYSICAL CHEMISTRY

Charge states in transition

Raffaele Resta

Transition metals come in different oxidation states with different electric charges. So at least we are told at school. Detailed calculations lead to a heretical conclusion — those variable charge states are a myth.

The idea of electric charge 'belonging' to a given atom or ion has been a central one ever since Michael Faraday, studying the use of electric current to decompose ionic compounds, published his laws of electrolysis in 1832. The charge that passes between two electrodes can be measured; it is always an integer multiple of the basic electronic charge; and it is proportional to the number of atoms exchanged. At the root of this effect is a basic quantization phenomenon[1] of the kind that still exercises physicists investigating, for example, the quantum Hall effect[2]. Both electrolysis and the quantum Hall effect deal with charges in transit. But when instead we address the static charges belonging to atoms in compounds, no such quantization theorem can be invoked. Such charges seem arbitrarily defined: the charges one gets are non-integer, and cannot be reliably measured.

So what is the relationship between these static charges and 'oxidation states' — the formal states of integer charge that today underlie our ideas of electrolysis and much else in chemistry? On page 763 of this issue, Raebiger, Lany and Zunger[3] address this question in transition metals, which are notable for having more than one stable oxidation state. Their answer? There is no connection: a change in the oxidation state of a transition-metal atom occurs without noticeable change in its net physical charge. The authors provide a consummately elegant explanation for this finding as the consequence of a feedback mechanism that leads the charge on the transition-metal atom to regulate itself. Furthermore, they show that this mechanism is present in both semiconducting and ionic host materials.

The concept of atomic oxidation states was introduced by the American chemist Wendell Latimer in 1938. It is undoubtedly useful for bookkeeping in chemical reactions involving molecules, crystals or liquids. An agreed set of rules[4] is used to attribute an integer oxidation state to all the atoms of a given compound. This oxidation state corresponds to the charge (in units of electronic charge) that an atom would have if its bonds were entirely ionic. In reality, when two nearest-neighbour atoms are of different species, their bond is neither entirely ionic nor entirely covalent. It is thus misleading, and a little dangerous, to attribute any physical meaning to the Coulomb energy ascribed to an atomic point charge by its oxidation state.

Even in the archetypal ionic crystal, sodium chloride, a change in oxidation state of 1 exaggerates the physical charge transfer between atoms of the two species, which is between around 0.7 and 0.8 (ref. 1). Further away from the extreme ionic limit, where the character of bonds between different atomic species is strongly covalent, characterized by the sharing rather than the swapping of electrons, this disparity becomes even greater. For transition-metal atoms, covalence is important even in bonds to significantly electronegative species such as oxygen, where ionic bonding would be expected to dominate[5].

"What is the relationship between static electric charges and oxidation states? None."

Raebiger *et al.*[3] perform a series of first-principle calculations of the charges on single transition-metal atoms embedded in a large semiconducting or ionic matrix. They consider different occupation states for transition-metal energy levels that sit in the band gap of the surrounding matrix structure — that is, in an energy range where the matrix itself has no allowed energy levels. This configuration allows the oxidation state of the transition metal to be varied while changing (almost) nothing else.

Because the bonds formed by transition-metal atoms are always partly covalent, the energy levels of the transition metal and its partner in the bonding are strongly hybridized. The hybridization of transition-metal and oxygen energy levels has been thoroughly investigated. In the class of compound oxides known as perovskites, hybridization is largely responsible for their characteristic 'ferroelectricity' — their spontaneous polarization even in the absence of an electric field[5,6].

Quite generally, covalence creates what amounts to a constructive interference between the quantum wavefunctions of the partner atoms. In Raebiger and colleagues' simulations, interference between the wavefunctions of the transition metal and the surrounding atoms contributes to a net atomic charge around the transition-metal atom. The authors explain their core finding — that the net physical charge belonging to a transition-metal atom is essentially independent of its oxidation state — with the following feedback mechanism. Suppose we wish to add an electron to the transition-metal atom, and thus change its oxidation state; then, unavoidably, the hybridization of its energy levels with those of its partner atom changes. This change always has the effect of displacing some negative charge away from the transition metal; the extra charge flows away and is spread over a large region, and the net physical charge belonging to the transition-metal atom within the structure stays essentially constant.

The charge transfers associated with changes in oxidation states have often been considered as a first approximation to the physical, ionic charge of a transition-metal atom, with covalent hybridization accounting for just a small correction to it. Raebiger *et al.* show[3] otherwise: charge self-regulation, rather than charge transfer, is the appropriate model.

Different oxidation states of the same transition-metal atom have distinct 'ionic radii' and distinct signatures, such as shifts in the X-ray light emitted from their core levels. These features must now be reconsidered in terms of the degree of hybridization, not the net physical charge at the transition-metal site. Knowledge of the electron density alone is not enough to address such subtleties; the full quantum-mechanical density matrix must be analysed instead. Tools devised to this end include charge-and-bond-order analysis and the electron localization function[7,8]. By applying such tools, we can hope to achieve an understanding of transition-metal bonding in different oxidation states that is finally on a sound theoretical footing. ■

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