NASA/JPL

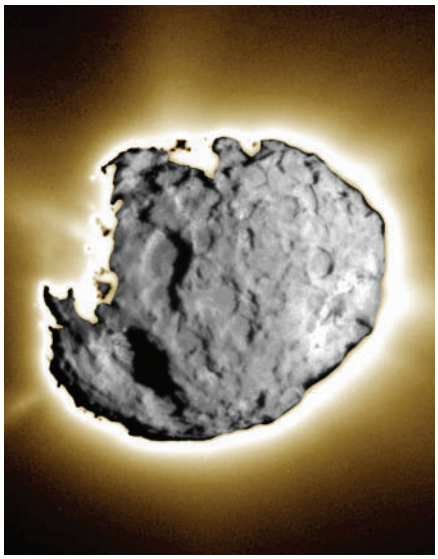

Figure 1 **Close-up on Wild 2. This composite image from the Stardust spacecraft shows the cratered surface of the comet and its halo of gas and dust, which seems collimated into several jets.**

distance from the Sun, might tend to erode the 'waist' of a nucleus, making more evolved comets more prolate.

The topographic relief on Wild 2, however, is substantially greater (at least 200 m)[1] than that on Borrelly (about 100 m), even though both comets have similar volumes and thus similar gravitational states (assuming that their densities are comparable). The slopes on Wild 2 are steep — nearly vertical in some cases. These facts, and the presence of at least one prominent overhang, suggest that the surface layers are strong and cohesive, and might be expected to get stronger with additional evolution. And yet the features on the presumably more evolved comet Borrelly are not suggestive of strength in its surface material. Borrelly has large mesas (raised plateaux), which have been interpreted as being eaten away by outgassing along the mesa walls[5]. Wild 2 has a much smaller area of mesas — which would, contrarily, suggest that Wild 2 is the more evolved of the two, with most of its mesas having been eaten away. But then the existence of preserved craters on Wild 2 and not on Borrelly implies that the opposite is true.

There are many features — and more mysteries — on the surface of Wild 2, including circular features that Brownlee *et al.*[1] attribute to impacts, and two footprint-like depressions that the Stardust team have named Left Foot and Right Foot. However, there are no small features (less than 0.5 km in diameter) that could be associated with impacts, and there seems to be no displaced material left from larger impacts (although it may be that the escape velocity on Wild 2 is sufficiently low that this material has left the surface). If the circular features are indeed impact craters, the surface must, overall, be rather old for there to have been so many impacts. Other non-circular features seem to be associated with streams of gas, or jets, in the coma (Fig. 1), produced by a combination of outgassing and other processes and modelled in the second of the papers, by Sekanina *et al.*[2]. For these sublimation vents not to have completely obliterated the comet surface, the jets must have developed fairly recently — perhaps they have been active only since the decrease in perihelion distance 30 years ago.

The dust counters on board Stardust, monitored by Tuzzolino *et al.*[3], picked up narrow bursts of dust particles during the fly-through, with very sharp transitions between a high density of particles and essentially no particles. This may be caused by the spontaneous fragmentation of dust grains in the inner coma. The fragments would have dispersed by the time they reach the outer coma, which would explain why the Vega spacecraft that flew past comet Halley at a greater distance did not detect the same effect. The sharp transitions might also have been disguised by the more limited resolution of Vega's instruments and its higher fly-by speed. Using stereoscopy in optical images of the jets, Sekanina *et al.*[2] have determined their three-dimensional orientations and successfully correlated them with some of the individual bursts in the dust.

Finally, the chemical analysis of the dust particles presented by Kissel *et al.*[4] has revealed marked differences compared with a similar analysis for comet Halley. Most notable is the almost complete absence of atomic ions in the mass spectra at Wild 2, perhaps due in part to the much lower fly-by speed (6 km $s^{-1}$ compared with 68 km $s^{-1}$, relative to each comet) and thus smaller impact speed of the dust particles onto Stardust's monitors. As at Halley, organic molecules are abundant, but the material at Wild 2 seems more nitrogen-rich. Generally, the grains at Wild 2 seem depleted in C=O-bonded material compared with the grains at Halley. Kissel *et al.*[4] also exclude the presence of water ice and free amino acids.

Although these papers[1–4] represent only a preliminary analysis of the data from Wild 2, the results are already intriguing. I look forward to the more detailed analyses to come — which will help to identify the evolutionary and intrinsic differences between the comets — and, of course, to Stardust's return to Earth in 2006. ■

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Heart disease

# An ongoing genetic battle?

Deepak Srivastava

Babies born with physical defects in their hearts may survive, but they often suffer defects in heart function as adults. The physical and functional problems might, it seems, have the same genetic cause.

Thirty years ago, children born with a heart defect had little chance of survival. Thanks to surgical and medical advances, the outlook now is usually not so grim, such that although the incidence of congenital heart disease (CHD) has not changed substantially (still nearly 1 out of every 100 live births), its prevalence is rising rapidly[1]. In fact, there are now more than 1 million survivors of CHD in the United States alone. But as these people enter their teens and twenties, new disease processes are becoming apparent, including abnormal conduction of electricity within the heart and a diminished ability of the heart muscle to contract. These 'secondary' defects have generally been ascribed to abnormal blood flow resulting from the primary defect, but Pashmforoush *et al.*[2], writing in *Cell*, suggest that the same mutated gene that causes an early defect in heart development in mice might later be directly involved in cardiac dysfunction, even in adulthood. If true in humans, the finding might allow postnatal approaches to alter the natural history of CHD in genetically at-risk individuals.

Pashmforoush and colleagues[2] studied a master regulatory protein called Nkx2-5, which controls many other genes and is essential for normal heart formation in fruit-flies, mice and humans[3–5]. People with mutations in one of their two copies of this gene typically show abnormal communication between the two atrial chambers of the heart (an atrial septal defect), and progressive disruption of electrical conduction through the cardiac chambers, which can result in sudden death[5]. Although the mechanisms remain unclear, this protein is known to interact with two others, Tbx5 and Gata4, mutations in which cause a similar CHD in humans[6–8].

Previous work on the Nkx2-5 protein elegantly illustrated the usefulness of cross-species studies to inform biologists

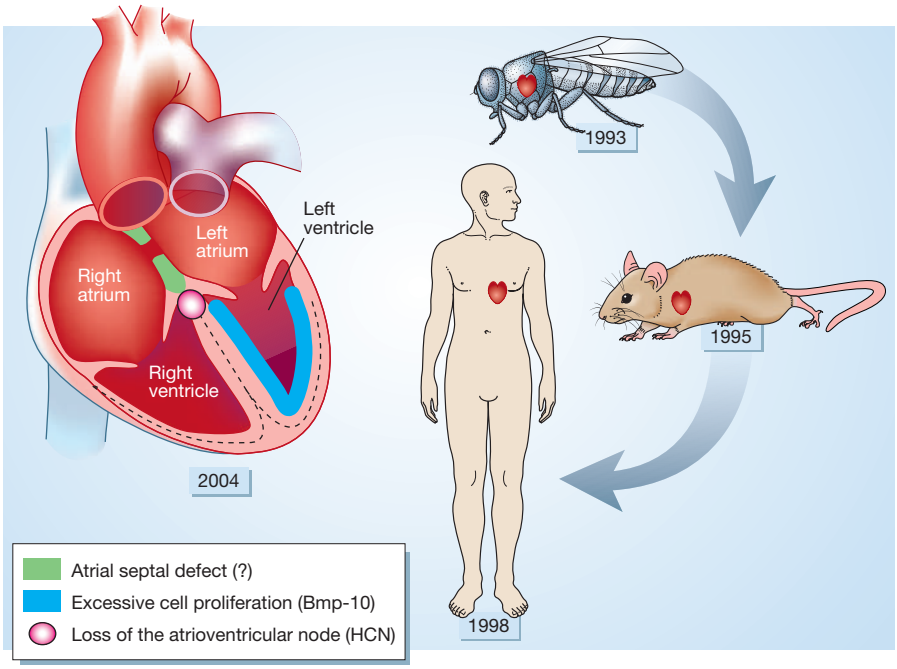


Figure 1 **Problems of the heart in young and old. Clockwise from top, the Nkx2-5 protein was discovered to be required for normal heart development first in fruitflies[3], then mice[4] and finally humans[5]. For instance, mutations in Nkx2-5 lead, by an unknown mechanism, to a defect in the wall between the two atrial chambers of the heart (an atrial septal defect; left). Pashmforoush *et al.*[2] have now found that Nkx2-5 mutations also produce defects in the hearts of adult mice. For instance, such mutations (perhaps by increasing the concentrations of the Bmp-10 protein) lead to increased cell proliferation in the wall of the ventricles. Possibly by increasing the concentrations of the channel protein HCN, they also lead to progressive loss of the atrioventricular node. Finally, deregulation of sarcolipin might lead to decreased cardiac function (not shown).**

and clinicians about disease characteristics and mechanisms, and the new paper continues that theme. Using sophisticated genetic technologies, Pashmforoush *et al.* engineered mice to lack Nkx2-5 specifically in muscle cells in the ventricular chambers of the heart, after the initial muscle specification event had occurred.

They found that the animals did not die, as they would if they lacked Nkx2-5 in all tissues. Surprisingly, however, they suffered as adults from abnormally thickened ventricles and disruption of the conduction system. Unlike in the humans described above, this mutation did not cause gross morphological defects in the heart at birth, except that a conduction centre called the atrioventricular node — the key site of electrical communication between the atria and ventricles — was smaller than normal. But although this node allowed conduction at first, the specialized muscle-derived cells in the node were lost over time and replaced by scar tissue, resulting in progressive defects in electrical conduction.

Because Nkx2-5 was disrupted solely in the ventricular muscle lineage, this work clearly demonstrates that the formation and maintenance of the atrioventricular node depend on Nkx2-5 functioning in heart muscle cells, rather than in other heart cell types. In addition, the investigators show that this protein is involved in postnatal regulation of the function and proliferation of cardiac muscle cells — all of which, they find, were similarly affected in a patient with a mutation in this gene.

The insights of this work come not from the ability to copy a human disease in mice, but rather through the use of a mouse model of that disease to understand the underlying mechanisms. Using state-of-the-art bioinformatics and microarray technologies, Pashmforoush *et al.* found, and confirmed the identity of, numerous genes that are deregulated in hearts lacking Nkx2-5. Their data suggest that this protein normally inhibits the production of another, bone morphogenetic protein-10 (Bmp-10), and that, in the absence of Nkx2-5, concentrations of Bmp-10 increase — perhaps causing the thick ventricles seen in mice and humans with Nkx2-5 mutations (Fig. 1). Similarly, the authors propose that a deregulation of contractile genes in the muscle and ion channels in conduction tissue contributes to the progressive dysfunction of muscle and the atrioventricular node, respectively. This work therefore provides a framework for beginning to understand how cardiac developmental genes might be co-opted for additional tasks, extending into normal heart maintenance and function throughout life.

So this work represents a milestone in our understanding of the long-term consequences of CHD, but of course questions remain. For example, the authors assume that the progressive loss of conduction cells in the absence of Nkx2-5 means that Nkx2-5 has a role in maintaining those cells. But it is also possible that there is ongoing turnover of muscle-derived conduction cells, and that, in the absence of Nkx2-5, a putative pool of progenitor cells is unable to replenish the ageing conduction cells — resulting in progressive abnormalities. In addition, although the authors propose that inhibiting Bmp-10 might be useful in preventing the thick ventricles observed when Nkx2-5 is disrupted, the current evidence remains circumstantial and awaits genetic or chemical proof of a direct role for Bmp-10 in mediating the effects of Nkx2-5 mutations.

Finally, the absence of atrial septal defects in Pashmforoush and colleagues' mutant mice could be because Nkx2-5 has a role in an alternative lineage, such as non-muscle cells derived from the endocardial lining of the heart, or it could simply reflect the fact that the 80–90% decrease in Nkx2-5 concentrations was above the threshold to produce such a morphogenetic defect. Nonetheless, the findings show that the functional anomalies seen in patients with Nkx2-5 mutations are not secondary to the morphological defects.

The discovery that one gene has distinct roles in developing and adult hearts has implications for our understanding of the natural history of CHD. Patients who survive CHD as a result of surgical intervention often have good long-term outcomes, but others suffer complications ranging from ventricular dysfunction to sudden death. Studies so far have failed to identify clinical or other predictors of outcome in such patients, and the events are thought to be stochastic.

Nevertheless, with the increasing recognition that CHD has a significant genetic component[9], it becomes plausible to imagine that genetic variations underlie both the initial morphogenetic defects and the predisposition to long-term consequences. For instance, many adult-onset cardiac rhythm disturbances, previously considered to be 'acquired' throughout life, might in fact be the result of a genetic mutation that renders portions of the conduction system 'programmed' to fail over time, and so might be congenital in nature. If so, efforts to identify the genetic variations will be essential, as therapeutic or preventive measures, throughout childhood and in adulthood, might then be possible. It is probably time to reconsider the idea of the heart as a static organ and to recognize that previous distinctions between childhood and adult-onset heart disease might in fact be hindering us from getting to the heart of the problem. ■

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Metrology

# Electrifying effects in colloids

Patrick Warren

The electric field generated in the sedimentation or centrifugation of charged colloidal particles could be exploited to determine the charge and the mass of macromolecules in a single experiment.

It might be thought that the last word on sedimentation or centrifugation had been said long ago. But over the past decade there have been persistent indications from experiment[1], theory[2] and simulations[3] of an unusual phenomenon in the sedimentation equilibrium of suspensions of charged colloidal particles when the ionic strength is low. Colloids are small particles, typically less than one micrometre in size, that have many technological applications as well as relevance to fundamental science. On page 857 of this issue, Raşa and Philipse[4] present convincing evidence that a macroscopic electric field, generated when the charged colloid is centrifuged to equilibrium, is behind the strange effect.

In 1926, Jean Baptiste Perrin was awarded the Nobel Prize in Physics "for his work on the discontinuous structure of matter, and especially for his discovery of sedimentation equilibrium"[5]. What Perrin had discovered was that the same law that governs the rarefaction of Earth's atmosphere with height — its barometric profile — also governs the distribution of suspended colloid particles undergoing brownian motion. At the time, Perrin's measurements of the barometric profile in a painstakingly prepared gamboge (gum resin) suspension gave an independent determination of Avogadro's number, thus contributing to the establishment of the atomistic hypothesis as experimental fact. In centrifugation — which is now well established as a means of determining molecular mass — the modest effects of Earth's gravity are replaced by a strong radial centripetal acceleration, as a sample is whirled around at high speed in a specialist device.

Piazza *et al.*[1] found that, for charged colloidal particles at reduced ionic strength, the barometric profile is inflated, as though the particles weigh less than they should do. Biben and Hansen[2] have since suggested, using density functional theory, that the sample column behaves like a condenser, in the sense that imbalanced charges accumulate at the top and bottom. The resulting electric field spans the whole column and acts to lift the colloid particles up against the force of gravity.

So, what is going on? A simple explanation, first discovered by van Roij[6], relates the phenomenon to a now-classic piece of physical chemistry. In 1911, Frederick Donnan considered the equilibrium across a semi-permeable membrane separating a salt solution from a suspension of charged colloidal particles or macromolecules[7]. The membrane is permeable to the small ions of the salt but impermeable to the colloids. Naively, one might expect that the small ions would distribute themselves so as to have the same concentration on both sides of the membrane. However, Donnan discovered that this is not the case. Rather, the ions become redistributed; for example, ions with the same charge as the colloids tend to be expelled from the colloid-containing compartment — the Donnan common-ion effect (Fig. 1). The effects arise from a microscopic charge imbalance that builds up in the vicinity of the membrane, creating a potential difference between the compartments, now known as the Donnan potential.

Raşa and Philipse[4] have made a connection between the Donnan membrane problem and the sedimentation profile of charged colloids. The macroscopic electric field in the sedimentation case can be attributed to a gradient in the Donnan potential, corresponding to the gradient in the concentration of colloidal particles (Fig. 1). The full problem has to be solved self-consistently, to obtain the coupled density and electric-field profiles — which is exactly what Raşa and Philipse have done.

Their calculations show that a careful analysis of the density profile for a dilute suspension of charged colloids or macromolecules could allow both the molecular mass and the charge of the sedimenting species to be determined. What remains to be demonstrated is that the phenomenon can be measured not just for colloidal particles but also for macromolecules. In this respect, globular proteins such as lysozyme might be good candidates. If it works, the approach offers an interesting alternative to electrophoretic methods for characterizing proteins. ■

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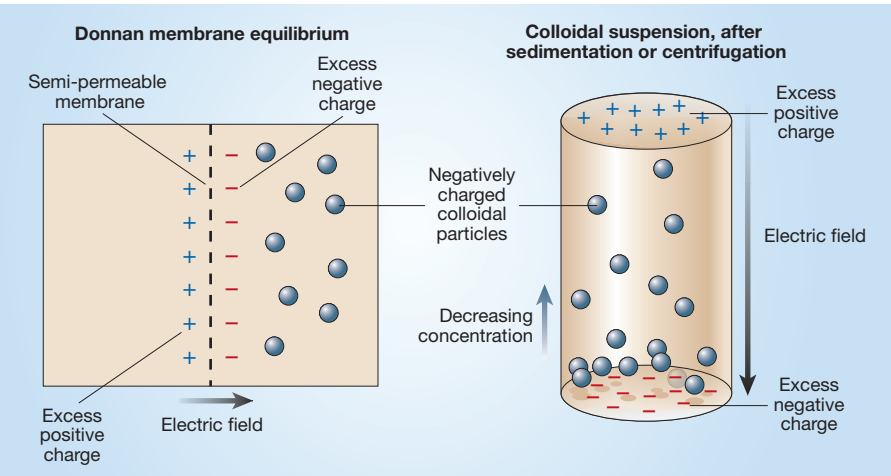


Figure 1 **The Donnan membrane equilibrium and sedimentation of a charged colloidal suspension. In a Donnan equilibrium, the charge imbalance in the vicinity of a semi-permeable membrane gives rise to an electric field, with a jump in the electrostatic potential typically occurring over a length of less than a micrometre[8]. Similarly, if a colloidal suspension has a gradient of concentration (such as is produced in sedimentation or centrifugation), then a macroscopic electric field is generated by the charge imbalance appearing at the top and bottom of the sample column. The presence of this field has observable consequences for the density profile of negatively charged colloidal particles, as has been confirmed experimentally by Raşa and Philipse[4].**

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