teins with the cytoskeleton. This similarity is all the more intriguing given the observation that moesin, ezrin and radixin are tyrosine phosphorylated and may mediate growth-factor-specific cellular changes[13–15].

Over the past five years, many (if not most) of the genetic loci responsible for familial cancers have been identified. The genetics have far out-paced understanding of the biochemistry and cellular biology of these genes' products. Some clues, such as the predicted relationship of merlin/schwannomin to cytoskeletal elements, have emerged. But the clear challenge is now to unravel the mysterious process through which a defect in such a gene leads to a mass of tumour cells. □

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QUANTUM MECHANICS

Making waves with electrons

A. Zettl

A CENTRAL concept of modern physics is the wave–particle duality of matter. The wave-like nature of freely propagating particles can be inferred from various diffraction and scattering experiments. These methods typically extract the quantum-mechanical properties of matter from changes in the momentum or energy of interacting particles. On page 524 of this issue[1], Crommie *et al.* describe an experiment in which the wave nature of electrons is directly observed from standing-wave patterns spatially resolved on the surface of a clean copper crystal. They used a scanning tunnelling microscope to image the local density of states of electrons trapped in a two-dimensional layer at the crystal surface. Such visualization of purely quantum-mechanical interference phenomena brings gratifying reality to these typically ethereal concepts. One can imagine a variety of experiments where this ability could be used to extract new and useful information.

Scanning tunnelling microscopes (STMs) have been used in the past to study distinctive electronic behaviour ranging from charge density waves[2] to superconducting vortices[3]. Now we find that the STM can also be used to image the quantum interference patterns of electrons moving in a two-dimensional plane. Although so-called two-dimensional electron gases are more usually associated with layered inorganic crystals, organic charge-transfer salts and artificial layered semiconductor structures, it turns out that electrons can be trapped at the close-packed surfaces of the noble metals, copper, silver and gold. Thus trapped, the electrons constitute a nearly ideal two-dimensional electron gas. Confinement to the surface has its origin in naturally occurring energetic barriers in directions perpendicular to the surface. On the vacuum side, electrons are trapped by the work-function (ionization) barrier; and on the bulk side, electrons see a forbidden band gap in the metal.

The resulting two-dimensional noble-metal surface states, which have been studied extensively in photoemission experiments[4,5], are to be distinguished from more localized 'Tamm' states, or dangling bonds, seen on semiconductor surfaces. Surface states have a long history of being studied with STMs. In fact, one of the first spectroscopic studies of metals with the STM was performed on gold, and clearly showed a spectroscopic signal due to a gold surface state[6].

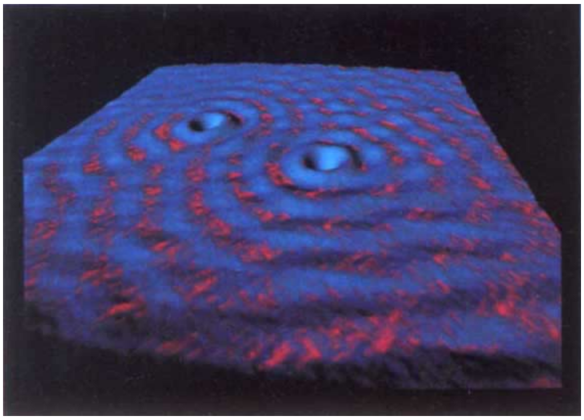

FIG. 1 Visibly ruffled. Standing waves (wavelength, 15 Å) of electrons scattered by a pair of point defects on the surface of a copper crystal. It is only through the interference effects created by the fixed defects that the electron waves become apparent. (Courtesy of D. M. Eigler.)

The ability to resolve the quantum-mechanical interference patterns of surface-state electrons provides a new wrinkle to an old problem, and yields a new tool for the study of defects on metallic surfaces. Without defects, the density of the surface-state electrons would be flat and featureless; this is in contrast to charge density waves which exist independently of defects (see Figs 1 and 2). Structure in the electronic surface-state density arises from the scattering of the surface-state electrons from imperfections on the surface. This scattering causes the incident and reflected electron waves to interfere quantum mechanically and set up standing waves in the defects' vicinity. The physics behind this is similar to the Friedel oscillation[7], a variation in total charge density around a defect (contributed to by electrons of all energies up to a maximum, the Fermi energy, which thus fixes the wavelength of Friedel oscillations).

An STM, though, does not measure the total density of electrons, but rather the local density of states at a given energy, selected by choice of the voltage applied to the STM tip[8]. The STM, therefore, has the ability to resolve a Friedel oscillation by spatially mapping out the scattered electron density at different energies. The details of these oscillations are not solely dictated by the lattice periodicity but depend sensitively on the energy being probed, the electron's effective mass, and the scattering potential of the defect. By measuring an energy-resolved set of spatial oscillations one can work backwards and extract information about the defect scattering potential, as well as surface-state electronic parameters. It is of course well appreciated that defects, such as step edges, are important in regulating such processes as film growth and catalysis on metal surfaces.

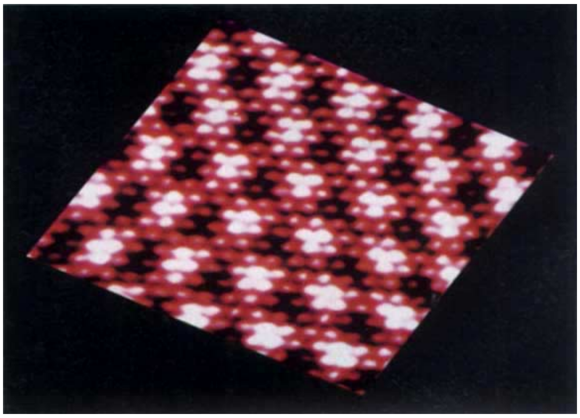

FIG. 2 Permanent wave. Charge density waves in TaS$_2$, revealed by atomic force microscopy. These are permanent corrugations of charge density, arising through a periodic lattice distortion driven by the electron–phonon interaction. (Courtesy of R. V. Coleman.)

The experiment reported by Crommie *et al.* used a low temperature (4 K) ultrahigh vacuum STM. Recently Hasegawa and Avouris of IBM Yorktown

Heights have seen similar oscillations near step edges on a gold surface using a room-temperature STM (unpublished results). Although there are some differences, the basic mechanism of quantum interference of surface-state electrons appears to operate on both the copper and gold close-packed surfaces. A careful study of differences between the room-temperature and 4 K results could possibly lead to a better understanding of inelastic scattering of surface-state electrons.

Perhaps the most interesting possibility raised by these results is the potential for studying the interaction of surface-state electrons with adsorbates, such as gas molecules attached to catalyst surfaces. The interest here is twofold. First, the interference patterns of the surface-state electrons might be used as a probe of the electronic structure of an individual adsorbate. Second, if the adsorbates do scatter the surface-state electrons, then one might be able to arrange the adsorbates with the STM tip[9] to create microscopic electron containers of arbitrary shape. Confined electron structures in buried semiconductor interfaces have been studied for years using transport and optical probes. The possibility now exists directly to access and spatially resolve the state density of artificially confined two-dimensional electrons, ideally including two-dimensional electrons in the presence of strong magnetic fields. □

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1. Crommie, M. F., Lutz, C. P. & Eigler, D. M. *Nature* **363**, 524–527 (1993).
2. Coleman, R. V., Drake, B., Hansma, P. K. & Slough, G. *Phys. Rev. Lett.* **55**, 394–397 (1985).
3. Hess, H. F. *et al. Phys. Rev. Lett.* **62**, 214–217 (1989).
4. Gartland, P. O. & Slagsvold, B. J. *Phys. Rev.* B**12**, 4047–4058 (1975).
5. Kevan, S. D. *Phys. Rev. Lett.* **50**, 526–529 (1983).
6. Kaiser, W. J. & Jaklevic, R. C. *IBM J. Res. Develop.* **30**, 411–416 (1986).
7. Friedel, J. *Nuovo Cim. Suppl.* **7**, 287–311 (1958).
8. Tersoff, J. & Hamann, D. R. *Phys. Rev.* B**31**, 805 (1985).
9. Eigler, D. M. & Schweizer, E. K. *Nature* **344**, 524–526 (1990).

CELL PHYSIOLOGY

Secretion without full fusion

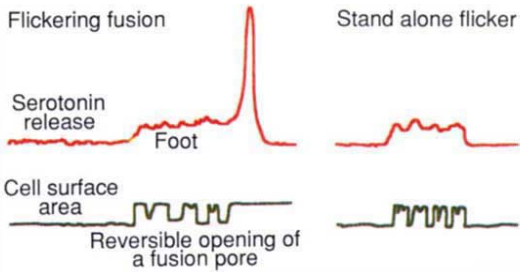
Erwin Neher

IT seems terribly wasteful that, during the release of hormones and neurotransmitters from a cell, the membrane of a vesicle should merge with the plasma membrane to be retrieved for recycling only seconds or minutes later. If parsimony has any meaning in biology, one might expect nature to have designed a way of releasing neurotransmitters and hormones which avoids intermixing components of the vesicular membrane with those of the plasma membrane. A study by Alvarez de Toledo *et al.* on page 554 of this issue[1] now points to a way that reconciles efficiency with classical concepts of vesicular exocytosis.

Several years ago[2,3], investigations into electrical membrane capacitance indicated that fusion of large secretory granules with the plasma membrane may be a transient phenomenon. Because the plasma membrane is, electrically speaking, a capacitor that is proportional to surface area, a change in capacitance can be observed when vesicular membrane is inserted into the surface membrane during exocytosis. For large granules, like those of mast cells, step-like increases can indeed be resolved when single granules fuse. Such records show that not all fusion events are permanent, but that the extra membrane often appears and disappears several times in the electrical measurement before the process is finalized. This phenomenon was termed 'flickering fusion'. Breckenridge and Almers[3], and Zimmerberg *et al.*[4], studying details of complex admittance changes in giant granules of beige mouse mast cells, concluded that a fusion pore forms as an initial electrical pathway between the interior of the granule and the extracellular space. This fusion pore gradually enlarges for complete fusion or — in the case of flickering fusion — opens and closes reversibly. Studying the fluorescence of quinacrine, loaded into the granules, it was concluded that release of the fluorophor was negligible during the flickering phase of secretion and that true release required irreversible fusion[3].

This conclusion, which previously was thought to be valid for large granules, may now have to be reconsidered, particularly for the case of smaller ones. Measurements with a more sensitive detection technique indicate that smaller granules may release much of their contents by diffusion through a reversible fusion pore. Extrapolating the available data to the size of synaptic vesicles, it seems possible that these small structures shed their contents before stable fusion has occurred, within a fraction of a millisecond.

The detection technique concerned is amperometry, the electrochemical detection of released substances by carbon-fibre microelectrodes. This technique, in its micro version, was pioneered by Wightman *et al.*[5], who showed that spike-like currents could be detected when the tip of a carbon-fibre microelectrode was located near a stimulated adrenal chromaffin cell. All the properties of these current transients were compatible with the idea that they represent the release of the contents of single catecholamine-containing granules. Chow and others[6] (including myself) corroborated this interpretation and discovered some fine structure to these secretory events. Conspicuously often, the main transient portion of the signal is preceded by a pedestal (or 'foot'). This foot is not compatible with what one would expect on the basis of diffusion theory, if release occurs instantaneously at some distance from the detecting surface. Rather, we concluded that the foot represents a trickling release through a fusion pore before bulk exocytosis occurs.



Measurement of cell surface area (lower traces) of mast cells undergoing degranulation shows that exocytosis can be reversible in the sense that the contribution of a granule to the surface membrane appears and disappears repeatedly. Simultaneous measurement of released substance (upper traces) indicates that serotonin seeps out of the granule at a low rate. The bulk of the granule contents is released after the increase in surface area becomes permanent. Occasionally 'stand alone' flickers are observed, during which exocytosis does not run to completion. Then, only a small fraction of granule contents is released from large vesicles. Comparison of kinetics and amounts of release between granules of different sizes indicates that small synaptic vesicles may be able to shed most of their contents in less than a millisecond through a reversible fusion pore.

Alvarez de Toledo *et al.* now demonstrate that this is indeed the case by combining the amperometric technique with capacitance measurements. They worked on giant granules of beige mouse mast cells, where the conductance of the flickering fusion pore can be measured. In these granules, amperometry detects the neurotransmitter serotonin[7], which is contained in the granules together with histamine. The authors observe an amperometric foot when, and only when, there is flickering fusion. They also occasionally observe 'stand alone' feet when there are capacitance flickers that do not result in a permanent increase in capacitance (see figure).

One notable finding is that plots of amperometric signal amplitude against fusion pore conductance for different granules have different slopes, showing that the concentration