mouse lacking the essential RNA subunit of telomerase (*11*). Cells from these mice still could undergo malignant transformation and form tumors after transformation with viral oncoproteins, contrary to the predictions of the telomere-clock model. However, alternative mechanisms of telomere maintenance could have been activated in these cells.

To resolve the controversy, Bodnar *et al.* tested the effect of inappropriate activation of telomerase in normal human cells (*3*). Their approach was made possible by the discovery of the reverse transcriptase subunit of telomerase (TRT) from two unicellular eukaryotes (*12*) and by the subsequent identification of a human ortholog (hTRT) (*13*). Most somatic human cells do not express this reverse transcriptase but contain all the other components of the enzyme so that expression of the missing hTRT component leads to reconstitution of enzyme activity (*14*). The task of Bodnar *et al.* was therefore straightforward: they simply examined the proliferative potential of primary human cells that were forced to express telomerase from a transfected hTRT gene.

Their results were strikingly unequivocal. Activation of telomerase resulted in the unscheduled addition of the drones of TTAGGG repeats that normally cap human chromosome ends. Cells with such artificially elongated telomeres showed a spectacular alteration in their growth potential. Whereas the primary cells used in the experiment normally senesced after a well-defined number of cell divisions, the telomerase-positive cells missed their senescence cue and continued to divide. At the time of submission of the work, many of the cell lines had proceeded for 20 population doublings beyond their normal senescence point and not only continued to grow vigorously but also showed a normal karyotype and maintained a youthful morphology. Similar results were obtained with three different primary cell types (retinal epithelial cells, foreskin fibroblasts, and vascular endothelial cells) attesting to the universal role of telomere shortening in the senescence of human cells.

These data support Cooke's original speculation (*1*) and the subsequent proposal of the telomere-clock model (*15*). Human telomeres are programmed to undergo gradual shortening by about 100 bp per cell division and when several kilobases of the telomeric DNA are lost, cells stop dividing and senesce. How telomere shortening is detected, and what the downstream signaling pathway is, will need to be addressed. However, there is now little doubt that this process has the makings of a powerful tumor suppressor system, creating a barrier to any cell that has escaped normal growth control by its environment.

But what about mice with their long telomeres and the fact that knockout mice lacking the telomerase RNA gene still spawn transformed cells? The answer to these questions is not clear at this stage. However, it seems very unlikely that mice use telomeres as a tumor suppressor system and perhaps with good reason. Since the telomere barrier to proliferation does not manifest itself until many cell divisions have passed, this mechanism may not be useful for a small animal in which a 2-cm mass of misplaced cells could be life-threatening. Such a small tumor would usually not affect people adversely. Other tumor suppressor pathways show subtle differences between mice and men as well (for example, BRCA-1 and Rb).

As Bodnar *et al.* point out, the ability to rejuvenate human cells with telomerase will create significant new opportunities for basic and applied interests. Will the manipulation of telomerase allow alteration of cellular life-span in vivo? Time will tell, but resetting the telomere clock may come at a price, since the induction of cellular immortalization might increase the rate of tumorigenesis in the altered cell population.

The implications for cancer research are also profound. The new data indicate that activation of telomerase in human tumors bypasses cellular senescence and is thus a requirement for tumor progression. As such, the molecular basis for telomerase de-regulation in tumors should be of immediate interest; the prediction is that one or more tumor suppressor genes prevents activation of telomerase in normal human cells.

Finally, the results should strengthen the determination of those who are searching for telomerase inhibitors as potential anti-cancer agents. However, the role of telomerase in human tumors deserves further testing and some other concerns remain, including worries about the protection afforded by long tumor telomeres and the possibility that other telomere maintenance systems might take over once telomerase is out of commission. As human telomeres have only just advanced as prime pieces in the field of oncogenetics, there is no telling what the end-game will be like.

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SURFACE CHEMISTRY

A New Opportunity in Silicon-Based Microelectronics

John T. Yates Jr.

The silicon surface designated by crystallographers as the (100) face provides the foundation for the silicon-based microelectronics industry and has been the subject of a wide range of investigations that make use of almost the entire arsenal of experimental methods in surface science (*1*). One small portion of the research on this important silicon surface concerns its chemical reactivity with organic molecules, which recently led to a major advance that is destined to open new technological opportunities in microelectronics (*2*). With the knowledge gained, it may be possible to bond a wide range of useful organic molecules directly to the dangling bonds on the surface.

Clean Si(100) is a reconstructed surface in which pairs of atoms dimerize to produce ordered rows of silicon dimers in (2×1) unit cells, as first postulated by Schlier and Farnsworth almost 40 years ago on the basis of low-energy electron diffraction (LEED) patterns (*3*). These dimers, now routinely observed by scanning tunneling microscopy (STM), present a pair of dangling bonds (exhibiting partial p character) at the surface, and the high reactivity of these "free radical" sites has been demonstrated repeatedly by means of surface electronic spectroscopies as well as by observations of chemisorption on the surface (*1*). More than 10 years ago, we and others found that

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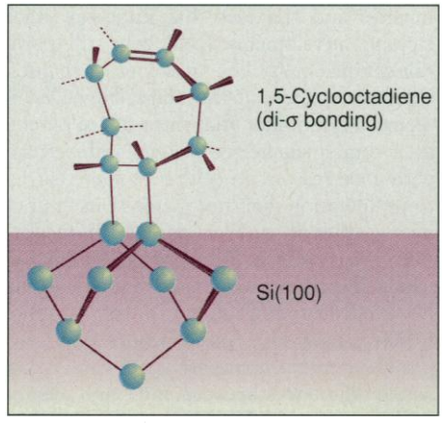
the C=C bond in olefins was very reactive at the silicon dangling bond sites in studies of propylene and ethylene chemisorption on the Si(100) surface (*4–10*). For ethylene, saturation surface coverages of about one ethylene per Si_2 dimer site were found, and a simple di-s bonding model was postulated (*7*, *8*). In addition, the study of *cis*- and *trans*-butene-2 showed that the di-s–bound olefin isomers retained their cis and trans structures when chemisorbed, leading to small differences in the thermal stability of the chemisorbed species (*11*). The chemisorption of ethylene is quite reversible, without significant molecular dissociation, and the undecomposed ethylene molecule will desorb near 550 K (*9*, *10*). In addition to the experimental findings, the di-s attachment of olefin molecules to the Si(100) surface has been proposed from theoretical studies (*12*, *13*). At present, it is controversial whether the mode of olefin chemisorption involves only the breaking of the Si-Si p-bond or whether the Si-Si-s bond also breaks upon addition of the C=C functionality to the dimer sites.

These ideas have been creatively extended by Hamers's group at the University of Wisconsin in their recent studies of the chemisorption of cyclic olefin and diolefin hydrocarbons on Si(100). Their first studies of cyclopentene chemisorption, combining STM and infrared reflection spectroscopy, demonstrated that monolayer functionalization of the Si(100) surface occurs with alignment of the hydrocarbon rings in directions parallel to the normal planes to the surface, which contain the original Si-Si dimers (*14*). They then proceeded to ask what might happen if a cyclic diolefin, 1,5-cyclooctadiene, was added to the surface. That is, would the molecule bond through both C=C bonds, or would only one of these double bonds participate in the addition reaction to the Si_2 dimer sites? The happy result is that only one C=C bond is involved in surface attachment, leaving the second C=C bond to serve as a reactive functional group for further types of surface attachment chemistry (*2*). The proposed surface structure is shown in the figure, and it is noted that the mode of chemisorption involves bonding of the cyclic species with its hydrocarbon ring located to one side of the original Si_2 dimer site as a result of the sp^3 hybridization, which occurs at the carbon atoms that attach to the silicon dimer site. These ideas have been confirmed in four ways, through the use of reflection infrared spectroscopy, STM, x-ray photoelectron spectroscopy, and theoretical calculations (Gaussian 94 level) by the Hamers group, giving the energy-minimized structure shown in the figure (*2*).

This first work with 1,5-cyclooctadiene suggests that the attachment of a variety of bifunctional organic molecules to Si(100) can be achieved by using C=C groups to achieve bonding to the surface while preserving the second functionality for further surface reactions. This capability naturally leads to a favorable linking of organic chemistry to existing and future silicon-based microelectronic technologies, for example, in active areas focused on the development of chemical and biological sensors and on molecular electronics. Here, layered and ordered organic molecules might be designed to serve in place of gate oxides in metal-oxide semiconductor transistor (MOSFET) devices for example, and for high-dielectric constant materials in capacitors. Indeed, this is an extremely favorable opportunity given the broad ability of organic chemists to stereochemically make almost any desired surface film starting from a well-defined and ordered surface functionality. The work on olefin attachment to Si(100) may therefore constitute a new beginning point for tailor-making surfaces useful for new and improved semiconductor devices.



A bonding surprise. Structure of a cyclic diolefin bonded to the Si(100) surface.

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DEVELOPMENTAL BIOLOGY

Reprolysins and Astacins ... Alive, Alive-O

Gerry Weinmaster

No one likes to be chewed-up or cut in two, but proteolysis is a fact of life! The abundance and variety of proteases—enzymes designed for cleaving proteins—underscore their importance. Proteases are classified according to their mechanism of action and their structure. Those that require zinc for their activity belong to a large family of metalloproteases that are grouped into subfamilies on the basis of their zinc binding site and overall protein structures (*1*). Reprolysins and astacins are two of these subfamilies. Recent, exciting reports have identified members of these subfamilies from *Drosophila* and demonstrated that their proteolytic activity is crucial for early patterning of the embryo (*2–4*).

During embryogenesis, the dorsoventral axis of the animal is established by a concentration gradient of a morphogen. The protease tolloid (Tld), with an active site homologous with the crayfish digestive enzyme astacin, helps to establish this gradient (*2*, *3*). In a different kind of developmental role, Kuzbanian (Kuz), a protease similar to the snake venom and reproductive metalloproteases (collectively known as reprolysins), affects pattern formation and cell-type determinations by proteolytic processing of the protein Notch into a functional cell-surface receptor (*4*).

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