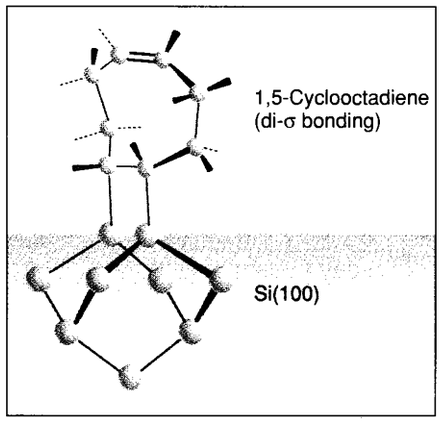
the C=C bond in olefins was very reactive at the silicon dangling bond sites in studies of propylene and ethylene chemisorption on the Si(100) surface (*4–10*). For ethylene, saturation surface coverages of about one ethylene per Si_2 dimer site were found, and a simple di-s bonding model was postulated (*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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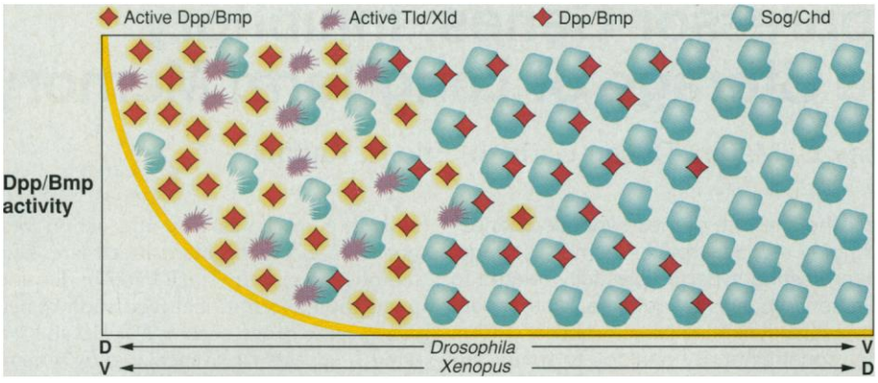

Proteases make the gradient. To generate the Dpp/Bmp activity gradient that directs dorsoventral patterning (yellow), the Sog/Chd proteins (blue) diffuse from their source and bind to free Dpp/Bmp (red) to form inactive complexes that can freely diffuse. Activated Tld/Xld metalloproteases (purple) cleave Sog/Chd from these complexes to release free active Dpp/Bmp to form a steeper concentration gradient than that resulting from formation of inactive complexes alone. Sog/Chd is highly expressed in the ventral (V) region in *Drosophila* and the dorsal (D) region of *Xenopus* embryos.

Bone morphogenetic protein (Bmp) is a morphogen that directs dorsoventral patterning in vertebrate embryos, and decapentaplegic (Dpp) is its equivalent in invertebrates (*5*). Because different concentrations of these ligands produce different cell types, gradients in the activity of these morphogens have been proposed to generate both dorsal and ventral embryonic structures. How are these gradients set up? Genetic and biochemical studies in *Drosophila* and *Xenopus* have identified a set of gene products that regulate the activities of Dpp and Bmp during embryonic development.

Two homologous proteins, short gastrulation (Sog) in the fly and Chordin (Chd) in the frog, complex with Dpp or Bmp and prevent these ligands from activating their receptors (*2*, *3*). If this regulation is responsible for creating the gradient, a simple model predicts that regions of the embryo that experience high concentrations of Dpp/Bmp activity would have decreased levels of such inactive complexes, whereas regions that respond to lower levels of Dpp/Bmp will have higher levels of inactive complexes (see the figure). The amount of active Dpp/Bmp would depend on the overall efficiency of complex formation, which could be regulated by concentration and diffusion rates of the Sog/Chd and Dpp/Bmp proteins.

The situation is actually more complex. It turns out that biologically active Bmp can be released from inactive Chd/Bmp complexes by proteolytic digestion of Chd by the Xolloid metalloprotease (the *Xenopus* Tld counterpart, Xld) (*3*). The steepness of a Dpp/Bmp gradient could then be established through the action of two opposing activities—Sog/Chd binding and inhibiting Dpp/Bmp working against the digestion of Sog/Chd by Tld/Xld, which would release Dpp/Bmp for action. This Xld cleavage of Chd/Bmp complexes provides an important new mechanism for local reactivation of growth factor from a latent complex—and for gradient formation. Consistent with this model, overexpression of zebrafish tolloid in embryos results in morphological and molecular phenotypes similar to those produced by either increases in BMP-4 or loss of chordino, the zebrafish homolog of *Xenopus* chordin (*3*, *6*).

Since Tld/Xld are present at the same concentration throughout the Sog/Chd expression domains, the proteolytic activities of Tld/Xld must be tightly controlled to generate the appropriate Dpp/Bmp gradients. Moreover, the finding that astacin metalloproteases are secreted in an inactive form and require removal of an inhibitory pro-region (*7*) introduces yet another opportunity to regulate Dpp/Bmp activity gradients in developing embryos. On the basis of sequence homology with other astacins, it seems likely that a furin-like serine protease activates Tld/Xld (*2*), thereby ultimately controlling Dpp/Bmp gradient formation during embryogenesis.

Like Tld/Xld, the Kuz metalloprotease effects embryonic patterning, but the Kuz effects are largely through the Notch signaling pathway that regulates cell-fate determinations (*4*). Notch is a cell-surface receptor that is activated through direct interactions with ligand-expressing cells. Whereas Xld-catalyzed proteolysis of Chd releases active Bmp, Kuz generates a functional receptor through proteolytic cleavage of *Drosophila* Notch. Genetic analyses of Kuz and components of the Notch signaling pathway suggest that Kuz functions upstream of the Notch receptor and that it is required in the cell where Notch is activated (*4*, *8*). Furthermore, Notch proteolysis occurs in the extracellular domain, resulting in amino- and carboxyl-terminal cleavage fragments that associate to form a cell surface heterodimeric receptor (*9*). Complete loss of Kuz (or loss of intrinsic metalloprotease activity) abolishes Notch processing and yields phenotypes in flies and frogs reminiscent of loss of Notch signaling (*4*, *8*). However, it is not known whether Kuz cleaves Notch directly or activates a proteolytic cascade leading to Notch processing.

Genetic studies in *Caenorhabditis elegans* have identified *sup-17*, a gene related to Kuz, that facilitates the Notch-related LIN-12 signaling pathway (*10*). Mutations in SUP-17 suppress the hyperactivity displayed by ligand-independent LIN-12 mutants, suggesting that proteolytic processing of these mutant proteins is required for their gain-of-function phenotypes. In addition, such mutant Notch proteins still require Kuz for their increased activity (*8*). Although these studies indicate that both ligand-dependent and ligand-independent hyperactive Notch/LIN-12 proteins require Kuz/SUP-17 for activity, they do not reveal whether the receptors must be proteolytically processed to facilitate ligand binding or receptor activation. Because Notch is proteolytically cleaved in *Drosophila* S2 cells that lack ligand (*4*), it is clear that Kuz processing of Notch is not ligand dependent; however, the possibility remains that ligand enhances cell-surface processing of Notch.

As described for the astacin metalloproteases, the intrinsic proteolytic activity of reprolysins such as Kuz is activated through removal of a pro-domain (*11*). However, deletion of the Kuz pro-domain does not produce any dominant effects when expressed in flies (*4*), indicating that proteolytically processed Notch still requires ligand for its activation. Nonetheless, if only processed Notch functions in ligand binding and receptor activation, then regulation of Kuz enzymatic activity would ultimately determine the extent of Notch signaling in cells.

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