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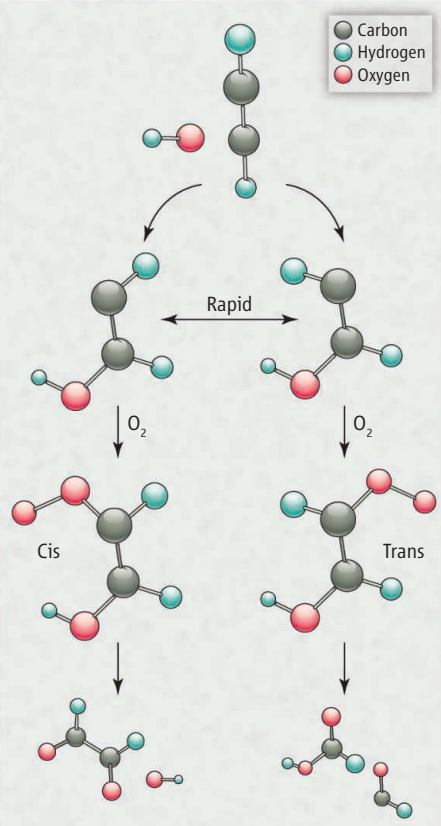

Depends on how long the excitement lasts. The two paths of the oxidation of acetylene (C_2H_2) with hydroxyl radical (OH•) and oxygen (O_2), an important atmospheric reaction studied by Glowacki *et al*., are depicted. Initially the C_2H_2-OH• radical forms with a high degree of internal vibrational energy, and the cis and trans forms interconvert. If the reaction with O_2 occurs when this internal energy is present, roughly equal amounts of the two sets of products are formed. However, if collisions cool the intermediate sufficiently, the more stable cis form and the glyoxal pathway (that reforms OH•) is favored.

partner. In their experiments, Glowacki *et al*. made high-quality measurements of OH radical decays in the presence of C_2H_2 over a wide range of temperatures (212 to 473 K) and pressures (13 to 100 mbar or 10 to 760 torr). They also varied the ratio of O_2/N_2, confirming the observation of Bohn *et al*. (*2*) that the extent of OH regeneration depended on the relative amounts of these two gases, not the absolute amounts.

These observations strongly support the hypothesis that the nascent R-OH radicals were being intercepted by O_2 before they could be fully relaxed. The explanation was further supported by high-level quantum chemical calculations on the structures and energetics of the system, coupled with simulations of the transient energy flow in the radicals. These calculations followed the evolution of the energy distribution among the C_2H_2-OH radicals and their O_2 adducts, showing that at atmospheric pressure, the radicals typically took tens of nanoseconds to undergo vibrational relaxation. In accord with the experimental findings, at high O_2 content (90%), the radicals were intercepted before relaxation, leading to a roughly equal amount of each product, while at low O_2 (1%), the radicals were fully relaxed by collisions with N_2, which led to a preponderance of glyoxal as product.

The work of Glowacki *et al*. shows that even at the relatively high pressures of the atmosphere, vibrational excitation may play a role in chemical reactions. Chemical activation is known to exert subtle effects on unimolecular processes, in the decomposition of organic alkoxy radicals (*4*), for example. However, it is unusual for a bimolecular reaction to be affected by the internal quantum state of a molecule. For example, the reactions of O_2 with these alkoxy radicals are normally assumed to proceed at the same rate, independent of the internal energy (but this assumption has never been verified).

The reaction of OH radicals with isoprene, which has the highest total emission rate into the atmosphere of any hydrocarbon except methane, also proceeds by an addition mechanism analogous to the OH-C_2H_2 reaction. Because the OH-isoprene adduct can also exist in cis and trans forms, the products of isoprene oxidation might also be dependent on the interactions of the vibrationally excited R-OH radicals with O_2 (*5*). In fact, Dibble and co-workers recently completed a study of the isomerization rates of 1-methylallyl radicals, which can be considered prototypes for the isoprene OH system (*6*), paving the way for studies of more complex systems. Overall, it is likely that most of the reactions occurring in the atmosphere are dominated by thermal energy distributions. However, vibrational excitation like that seen by Glowacki *et al*. plays a role in some of the most important systems, and these systems will continue to provide exciting challenges to both experiment and theory in the coming years.

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CELL BIOLOGY

Beyond Oil and Water—Phase Transitions in Cells

Anthony A. Hyman and Kai Simons

The organization of cellular compartments may be driven by liquid phase separations and the collective low-affinity interactions of macromolecules.

Contemporary biology has identified many proteins involved in different cellular processes, but we are far from understanding how they perform the tasks that cell functions require. How do collections of proteins and other molecules come together to form compartments (*1*) containing large numbers of macromolecular machines that execute specific and complex reactions? The search for underlying principles has been reinvigorated recently in part by insights into the role of phase transitions in organizing cellular compartments.

The question of how biological macromolecules form organized assemblies was posed at the dawn of biochemistry in the early 20th century. A physico-chemical description of the cell was based on ideas from colloid chemistry to describe large-scale organization of macromolecules. Biologists considered the cytoplasm to be densely packed with liquid colloid particles that constituted a separate phase, distinct from the surrounding aqueous environment (*2*, *3*). Such phase transitions were potentially powerful ways to segregate biological

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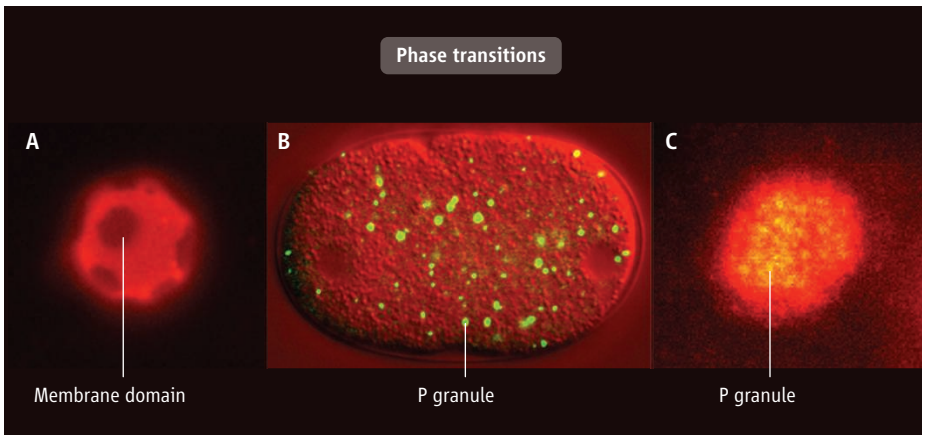

Phase transitions in the membrane and cytoplasm. (**A**) Membrane domains (dark regions) are shown in the plasma membrane of a rat basophilic leukemia cell. (**B**) P granules (labeled in green) in a *C. elegans* one-cell-stage embryo are imaged by fluorescence microscopy, as described in (*6*). Liquid P granules consist of proteins and RNAs. (**C**) A single P granule in a *C. elegans* embryo is imaged by stimulated emission depletion microscopy, as described in (*6*). In both examples, the plasma membrane and P-granule compartments have been formed by a phase transition involving liquid-liquid demixing. Such demixing can be described by the Gibbs phase rule, which states that the number of demixed entities (*P*) for a system at equilibrium is strictly correlated with the number of chemically independent components (*C*) by the expression $P = C - F + 2$, where *F* is the number of independent variable properties (such as temperature or the fraction of phase components). Presumably, selective pressure has selected for certain *P* values that give small numbers of phases by ensuring that the different components are not chemically independent, and together form a collective in the condensed phase (*13*).

macromolecules. However, scientists of that era did not understand enough about macromolecules to connect them to the physical chemistry of the cell.

The molecular biology revolution radically changed the focus from a physicochemical description of the cell to the structure of individual macromolecules. Biologists concentrated on determining "lock-and-key" intermolecular interactions and the formation of protein complexes with defined molecular architecture. But detailed insights into the function of single macromolecules and macromolecular complexes have not been sufficient to understand cytoplasmic and membrane organization on larger scales. Currently, the ideas of colloidal chemistry and phase separations are reemerging to describe the organization of cellular biochemistry.

Phase transitions are common in the nonbiological world. For instance, the transformation of water vapor to liquid drops after cooling, as seen on a car windshield on a cold morning, is a classic example of a gas-liquid phase transition. In biology, phase transitions can take the form of liquid-liquid demixing, where two liquids with different properties separate from each other. One example is the two-dimensional separation of lipids and proteins in membranes into dynamic liquid membrane rafts, distinct from the surrounding bilayer (*4*). The capability to form membrane domains is a subcompartmentalization device that allows for regulated protein segregation within the membrane plane. This mechanism is used to control endocytic or exocytic membrane transport, to transduce specific signals across the plasma membrane, or to carry out different biochemical reactions, depending on the proteins involved. Key to understanding the principles underlying liquid-liquid demixing in cell membranes is the mutual interactions between sterols, sphingolipids, and raft proteins (*5*). These form dynamic nanoscale assemblies that coalesce through multivalent interactions between raft lipids or between proteins into more stable platforms, which form a condensed, tightly packed, and ordered phase within the membrane.

However, liquid phase transitions are not confined to membranes. Cells have numerous examples of nonmembrane-bound compartments containing many proteins that perform complex biochemistry. These compartments form rapidly and are disassembled when not required. Examples are protein-RNA bodies such as Cajal bodies in the nucleus (implicated in RNA metabolism) or nuclear promyelocytic leukemia (PML) bodies that form under stress conditions in certain cells. Recent studies on P granules and nucleoli suggest that protein-RNA complexes are liquids that form by liquid phase transitions from cytoplasm. P granules are protein-RNA complexes that are involved in germline formation in the nematode *Caenorhabditis elegans*. These granules exhibit liquid-like behavior; that is, they form fluid droplets (*6*), suggesting that they arise through liquid-liquid demixing from the cytoplasm (see the figure). Nucleoli, which are sites of ribosome synthesis, were also shown to behave like liquid droplets of protein-RNA complexes, exhibiting viscous-like fluid dynamics (*7*). A further discovery in the structure and function of ribonucleoproteins (RNPs) (*8*–*10*) demonstrated that in mouse brain and human cell extracts, proteins with low-complexity sequence domains (regions with low amino acid diversity) separate into a different phase together with RNA during liquid-liquid demixing. These studies suggest a model in which RNAs bind to RNA binding proteins, which in turn phase separate using their low-complexity sequences. The function of low-complexity sequence domains, which are abundant in the protein universe, have long puzzled biologists, but these experiments support the idea that they may have evolved to mediate such liquid-liquid demixing.

One problem in thinking about liquid phase separations in biological systems is the large number of components in a compartment. For instance, nucleoli are thought to have over 100 components. A possible framework for thinking about how different proteins contribute to the fluid state would be to consider three classes of molecules. One class drives the phase separations—for example, the multivalent, sometimes disordered proteins, the interaction of which forms the liquid phase in cells, or the ordered phase in membranes (*10*). Even in complex compartments, this type of molecule may be relatively few. In the case of P granules, although they are thought to contain dozens of components, two alone, when expressed in cultured mammalian cells, can form P granule–like structures (*11*). A second class of proteins make specific interactions with the first class. The third are molecules that partition selectively into the condensed phase formed by the first two classes of proteins and/or RNAs. In complex compartments, this third class is likely to be the most abundant and responsible for the localized biochemistry and its regulation.

In cells, the specific characteristics of liquid phase transitions will depend on the interactions between the molecules involved. Only recently has it been possible to reconstitute liquid-liquid demixing using in vitro systems (*12*) and multivalent signaling molecules. The proteins Nck and N-WASP, two molecules that interact with each other, when generated in vitro could form liquid droplets in which the concentration of the proteins in the drops was about

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100 times that in the surrounding aqueous medium. Similar droplets were also observed when these proteins were overexpressed in cells. Notably, the concentration needed for the phase transition into fluid droplets correlated with the valency of these interacting proteins. The importance of this (*12*) and other in vitro work (*8*) is that it allows the study of the molecular basis of liquid phase separations in cells. It also shows that phase transitions in cytoplasm are not confined to assemblies that contain RNA, suggesting that phase separations could have a general role in organizing cellular biochemistry. There are many other candidate molecules with low-affinity polyvalency that could lead to liquid-liquid demixing, such as polyADP ribose, glycogen, and ubiquitin chains.

Taken together, these studies reveal a number of interesting features of liquid phase transitions in cells. They can occur in two or three dimensions, they involve the assembly of small macromolecular complexes through multivalent interactions, and they can form mesoscale to micrometer-scale fluid phases (*13*). Furthermore, high concentrations of solutes may also contribute to mesoscale organization in certain biological systems (*14*). The concentration of complexes that form the more condensed phase is apparently regulated close to the threshold of phase transition. This may reflect a general tendency of biological systems to be poised near a phase transition and thus promote large responses to small changes in the environment (*15*). More generally, multivalent weak interactions between proteins, or proteins and RNAs, provide the properties for liquid-like states, perhaps explaining their prevalence.

The idea of liquid-like states that either separate from the cytosol or occur in cell membranes is a powerful way to think about cellular compartments (*16*). Changes in valency of interaction by regulatory events such as phosphorylation would allow a phase transition in which the components become rapidly concentrated in one place in the cell. Entry of proteins or other regulators into condensed phases could lead to fast disassembly of liquid compartments. A small increase in the concentration of components could allow reactions to start without any other regulatory event, as the concentrations rise above the Michaelis constant (K_m) for the reaction. Depletion of components from the cytoplasm as they segregate into the condensed phase could stop reactions in the cytoplasm. One could envisage developing drugs that partition directly into fluid phases, thus changing their separation behavior.

Many cellular compartments form rapidly and are disassembled when not required. Also, a surprising number of proteins involved in metabolism and stress responses form cytoplasmic puncta in yeast (*17*). It will be fascinating to examine each one of these compartments to ask whether their formation also represents examples of liquid phase separation, and then to determine the criteria for liquid-liquid demixing. More generally, phase transitions may have important implications in disease. Because they can undergo such large-scale changes in arrangement of molecules, defects in their organization are likely to have major effects on cell viability. For instance, the large number of protein aggregates seen in neurodegenerative disease could be a product of unwanted or misregulated phase transitions.

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1. Compartments can refer to organelles such as mitochondria, or nonmembrane-bound organelles such as nucleoli. More generally, compartments can also correspond to local concentration of molecules in specific biochemical processes such as P granules or stress granules and other subcompartments such as domains in cellular membranes.
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ECOLOGY

Bad News for Soil Carbon Sequestration?

Arbuscular mycorrhizal fungi may stimulate additional decomposition of organic carbon in the soil, resulting in a net source of carbon dioxide.

George A. Kowalchuk

Rising atmospheric carbon dioxide (CO_2) concentrations are expected to increase plant photosynthetic activity and the transfer of fixed carbon belowground, providing a potential buffering mechanism against elevated CO_2 (*1*). Arbuscular mycorrhizal fungi (AMF) are central to this potential extra carbon sequestration. AMF form symbioses with most land plants, in which the fungi supply the plant with nutrients in exchange for carbohydrates (*2*, *3*). But to what extent will this extra fixed carbon stay sequestered in the soil (*1*)? On page 1084 of this issue, Cheng *et al*. (*4*) show not only that the extra soil carbon is respired back to the atmosphere, but also that AMF activity stimulates additional decomposition of soil organic carbon. Increased carbon fixation by plants and transport of this carbon to the soil via AMF may thus result in a net source of CO_2, rather than the sink we might have hoped for.

Plants may drive this AMF-dependent decomposition to gain access to nitrogen from soil organic matter (*4*). Available nitrogen often limits gross primary production and growth responses to elevated CO_2 (*5*). Thus, increased translocation of nitrogen to the plant by AMF, specifically sequestered in the form of ammonium and not nitrate (*4*), may enhance plant growth. However, if enhanced plant growth leads to further increases in carbon transfer belowground, the net effect will be increased turnover of total carbon and nitrogen, rather than increased storage. Clearly, to understand the responses of terrestrial carbon cycling to climate change, interactions between the car-

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