MATERIALS SCIENCE

Now You See Them

Can new results on calcium carbonate nucleation be reconciled with classical nucleation theory?

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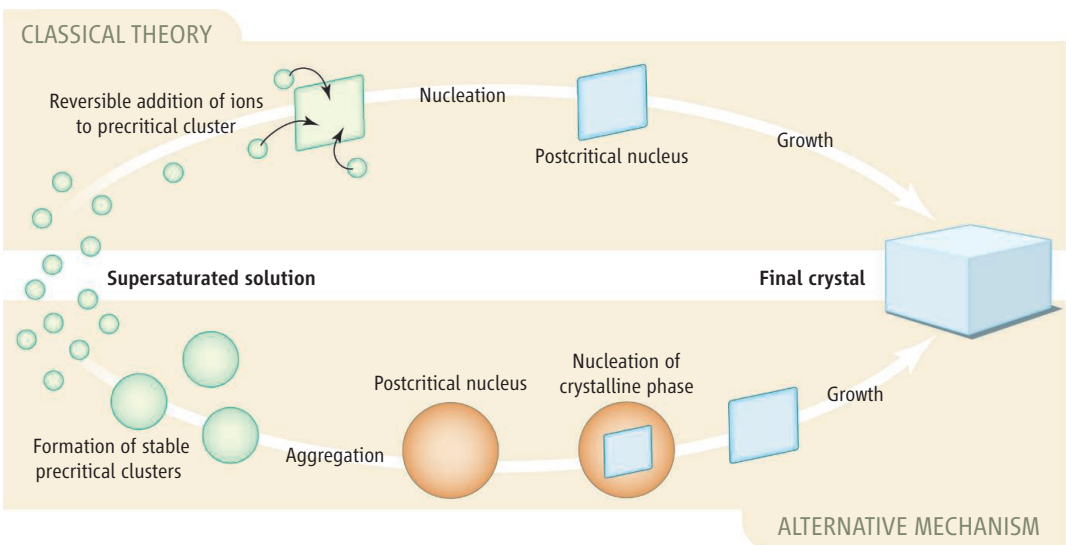
Crystallization lies at the heart of many natural and technological processes, from the production of pharmaceuticals and nanomaterials to the formation of bones and teeth, frost heave, and scale deposition. Crucial features of these crystals, such as lattice orientation, particle size, and size distribution, are defined by conditions during the earliest stages of precipitation—at nucleation. Yet, nucleation from solution is poorly understood, because experimental studies of nucleation are highly challenging (*1*). Recent studies have highlighted the possible role of clusters in nucleus formation (*2*, *3*). On page 1819 of this issue, Gebauer *et al.* provide support for this thesis (*4*) by demonstrating the presence of large, well-defined clusters before nucleation of one of the phases of calcium carbonate. Crystallization appears to proceed through aggregation of these clusters. The results challenge the conventional picture of crystal nucleation.



How do crystals nucleate? According to classical nucleation theory, calcium carbonate nucleation proceeds by addition of ions to a single cluster (**top**). Gebauer *et al.* now suggest a different mechanism, in which nucleation of ACC occurs by aggregation of stable, amorphous, precritical clusters (**bottom**). The nucleated ACC phase subsequently crystallizes to generate the final stable crystal product.

Classical nucleation theory provides a simple understanding of how crystals nucleate. Nucleation is often slow because of a free-energy barrier originating from the interface between the nucleus and its surroundings. The theory assumes that nuclei grow one molecule at a time (see the figure, top). As the nuclei grow, their Gibbs free energy increases, until a free-energy maximum is reached at the critical size. At least in simple systems such as argon, critical nuclei are expected to persist for microseconds or less, making them virtually impossible to observe. Beyond the critical size, the nuclei are stable and release energy during growth.

In their investigation of calcium carbonate nucleation, Gebauer *et al*. observe long-lived precritical clusters, about 2 nm in diameter, and suggest that they grow by colliding and coalescing. These results are clearly in contrast to the picture of nucleation presented by classical nucleation theory. The theory assumes that the structure of the nucleus is like a piece of the bulk phase and that its surface has the same interfacial tension as a bulk phase. However, if stable, precritical clusters are to exist, they must lie in a free-energy minimum. Such a minimum would only occur if the classical theory's assumptions are wrong, perhaps because the structure of the clusters is different from that of the bulk.

The possible structure of the precritical calcium carbonate clusters is open to speculation. If the ions are present in a bulk crystal lattice, then it is surprising that the clusters of about 70 ions neither shrink nor grow. Alternatively, the structures may be ordered but differ from that of the bulk phase, thus retarding growth. Modeling has shown that small ordered clusters of argon atoms are not just chunks cut from the bulk lattice but form different structures, such as icosahedra, which are incompatible with growth to fill space (*5*). In the case of argon, the true bulk phase nucleates on these ordered clusters, rendering them transient. However, calcium carbonate solutions are considerably more complex, so that nucleation of the true bulk phase from an ordered nanoparticle may be more difficult. Calcium carbonate is highly polymorphic in that it can exist in six different crystal structures. The first polymorph formed after nucleation is often amorphous calcium carbonate (ACC), which subsequently crystallizes (*6*, *7*). ACC has no long-range order, but it often has short-range structural order that appears to determine the lattice structure after crystallization (*8*). The most likely scenario is therefore that the precritical clusters are themselves amorphous or of low structural order. However, if they are amorphous, it is again unclear why they neither dissolve nor grow.

There is one system in which clusters and their contribution to the nucleation of the bulk phase have been extensively studied, and where we have at least a basic understanding of their behavior: water droplets in Earth's atmosphere (*9*). These droplets range in size from a few nanometers to tens of nanometers. They are stabilized by other species (such as sulfuric acid) that are both highly water-soluble and nonvolatile, so that they partition strongly into the nanometer-scale droplets. These ions provide an osmotic pressure inside the droplets that prevents their evaporating, even when the air is undersaturated with water vapor. Dusek *et al.* have shown that the super-

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saturation at which nucleation occurs is determined largely by the size of the nanodroplets present (*10*).

Could the precritical clusters observed by Gebauer *et al.* also be the result of stabilization of calcium carbonate clusters by another species present as an impurity? This mechanism would provide a basis for stabilizing precritical clusters in a free-energy minimum and does not contradict classical nucleation theory. Such impurities are ubiquitous and virtually impossible to eliminate from any solution. The results of Gebauer *et al.* may thus reflect the mechanism of nucleation of calcium carbonate in "real" systems. Nucleation could then occur by coalescence of the precritical clusters to give ACC, which will subsequently crystallize to a more stable crystalline polymorph. The latter mechanism is consistent with the observations of Gebauer *et al*., who show that ACC is the first phase precipitated after nucleation.

The idea that nucleation of calcium carbonate may proceed via an aggregation mechanism is highly topical. The past decade has seen great progress in understanding crystallization processes, and it is now well recognized that single-crystal growth (as distinct from nucleation) often occurs via the aggregation of small precursor units rather than by addition of ions or molecules to a nucleus (*11*). Cluster species have also been observed before nucleation in saturated solutions of compounds such as sodium chloride (*2*), urea (*12*), and glycine (*3*), and there have been suggestions that clustering can determine which polymorph is formed (*13*). However, none of these even remotely approach the size or stability of the clusters observed by Gebauer *et al.* Further investigation of precritical clusters and their role in the crystallization of calcium carbonate, and indeed other compounds, is eagerly anticipated.

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DEVELOPMENTAL BIOLOGY

From Genetic Association to Genetic Switch

Human genetic studies have led to the identification of a transcriptional regulator that could serve as a therapeutic target for adult hemoglobin disorders.

Alan M. Michelson

Deciphering the sequence of the human genome and the subsequent cataloging of common human DNA sequence variation marked a paradigm shift in human genetics. These resources, together with advances in cost-effective genotyping technologies, enabled the design of genome-wide association studies for the unbiased discovery of commonly occurring DNA sequence variations called single-nucleotide polymorphisms (SNPs) that are preferentially associated with a disease or other clinical trait (*1*). Although genome-wide association studies have uncovered disease-associated SNPs, identifying actual disease-causing variants—and gaining deep insights into how those variants generate the underlying molecular pathophysiology—have so far yielded only modest results. This has led to criticisms of the genome-wide association approach for investigating the etiologies of common diseases (*2*). However, this assessment may be premature. On page 1839 of this issue, Sankaran *et al*. (*3*) show how genome-wide association findings can lead to a detailed understanding of disease mechanisms and be used to ascertain novel therapeutic targets.

Previous genome-wide association studies conducted in independent populations have identified SNPs in three chromosomal loci that are associated with varying expression levels of human fetal hemoglobin (HbF) (*4*–*6*). HbF is a clinically important quantitative trait because elevated concentrations reduce the severity of sickle cell disease and β-thalassemia, disorders caused by different mutations in the human β-globin gene (*7*). Normally, HbF predominates in the fetus but declines to very low amounts postnatally due to repression and activation of the γ-globin and β-globin genes, respectively. The "switch" that controls these reciprocal changes in globin gene expression has been intensively investigated, but the molecular basis of this developmental process remains largely unknown. As reported by Sankaran *et al.*, functional studies motivated by recent HbF genome-wide association findings have provided a major breakthrough in understanding the hemoglobin switching problem.

One of the SNPs associated with elevated HbF expression is found in an intron (noncoding region) of the *BCL11A* gene on human chromosome 2 (see the figure). *BCL11A* encodes a protein that represses transcription in the B lymphoid lineage (*8*).

Sankaran *et al.* hypothesized that BCL11A might repress expression of the γ-globin gene, with expression or activity of this repressor correlating inversely with HbF production both during normal development and in individuals of different genotypes at the *BCL11A* locus. They first determined that BCL11A is expressed as two long isoforms, encoded by alternatively spliced messenger RNAs (mRNAs), in primary adult erythroblasts. By contrast, only shorter variants of BCL11A are found in human embryonic erythroleukemia cells and in primary human fetal liver cells, both of which express high amounts of HbF. Moreover, the genotype at the *BCL11A* SNP that affects HbF production influences expression of mRNAs encoding the long isoforms in lymphoblastoid cell lines: High expression of *BCL11A* mRNA corresponds to homozygosity for the allele associated with low HbF production; low mRNA expression corresponds to homozygosity for the allele associated with high HbF production; and SNP heterozygotes express intermediate amounts of mRNA (see the figure). If the association between the *BCL11A* SNP and the expression level of this gene in lymphoblas-

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