the activation of MAPK by the protein kinase Mos is essential for its activity (*13*). Bhatt and Ferrell (*3*) now show that the metaphase arrest induced by the activation of the Mos/MAPK cascade in cell-free extracts prepared from frog embryos can be abrogated by the specific immunodepletion of Rsk-2 from the extracts. Interestingly, adding back physiological concentrations of recombinant Rsk-2 protein restores the ability of the extract to undergo metaphase arrest in response to Mos. These results are complemented in the accompanying report by Gross *et al.* (*2*), who show that a constitutively activated form of Rsk-1 can induce metaphase arrest in cleaving frog embryos in the absence of any detectable endogenous MAPK activity. These findings raise the intriguing possibility that Rsks may be the only MAPK substrate required for cytostatic factor activity. However, it is also possible that the truncated Rsks that were overexpressed in these experiments may behave nonphysiologically. The work of Bhatt and Ferrell (*3*) further suggests that Rsks may be responsible for some of the changes in cell morphology that occur during cell division. This begs the question of what the Rsks are actually doing. Although the protein targets of Rsks remain unclear, a promising candidate is the ubiquitin-dependent proteolysis machinery that plays an important role in cell cycle regulation.

To understand the mechanism responsible for metaphase II arrest, the new studies suggest that future work should focus on finding the targets of Rsk rather than those of MAPK. The same may be true for other cellular processes involving the activation of MAPKs. In this regard, development of antibodies specific for known Rsk phosphorylation motifs (*4*) will be useful for identifying new Rsk substrates. It is still not clear whether different Rsk isoforms have different functions and protein targets. Both Rsk-1 and Rsk-2 appear to have similar effects on the metaphase II arrest of frog embryos (*2*, *3*). However, only Rsk-2 (but not Rsk-1 or Rsk-3) is able to phosphorylate histone H3 in fibroblasts treated with epidermal growth factor (*10*). The constitutively activated forms of Rsks described in this issue (*2*) and elsewhere (*4*) should be of great help in investigating new Rsk functions. A rewarding task for the future will be to generate reagents to specifically inhibit Rsk signaling, allowing the contribution of Rsks to various cellular responses to be deciphered. As the three new studies suggest, such an approach could reveal an even more important job for Rsks in the MAPK signaling pathway than previously thought.

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PERSPECTIVES: ULTRAFAST X-RAY DIFFRACTION

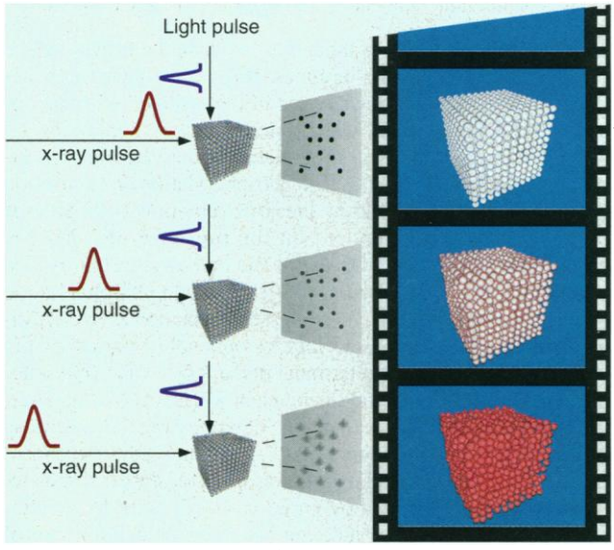
Watching Matter Rearrange

Keith A. Nelson

Can we "watch" the elementary molecular or collective motions that lead to chemical or structural change as they take place (see the figure below)? On page 1340 of this issue, Siders *et al.* (*1*) report an important step toward this goal. Using ultrafast pulses of x-ray radiation as the probe, they monitor the melting of a semiconductor sample after irradiation by an intense, ultrafast visible pulse. The experiment (see the figure on the next page) allows the direct measurement of the changes in crystal lattice structure and loss of crystalline order associated with melting. The results represent the early fruits of a worldwide effort to generate ultrashort hard x-ray pulses and use them for the characterization of ultrafast events through time-resolved x-ray diffraction (*2*–*5*).

In earlier work, the same group was able to monitor light-driven sound waves that give rise to time-dependent changes in unit cell volume (*6*). The next step, namely to watch the sample undergo collective structural change during a solid-liquid phase transition (*1*), introduced numerous experimental difficulties, not the least of which is that each laser shot destroys the irradiated region of the single-crystal thin-film sample. Still, enough data could be collected to reveal a precipitous drop in x-ray diffraction intensity within the first few picoseconds of intense optical excitation. The results indicate that the excited region of the sample, which contains a very high density of energetic electrons, melts quickly—long before the electrons have given up most of their energy to lattice vibrations—and homogeneously, in contrast to the more usual case (observed in less highly excited sample regions) of surface melting followed by propagation of the melt front into the sample at a fraction of the speed of sound.

Of course, we can already monitor ultrafast time-dependent motion in chemical reactions and structural rearrangements using femtosecond optical pulses (*7*). This year's Nobel Prize in Chemistry, awarded to Ahmed Zewail for the development of "femtochemistry," recognizes remarkable achievements in monitoring chemical bond breakage in photochemically reactive molecules (*8*). These events are monitored with light in the visible or nearby spectral regions, rather than x-rays, and the probe pulse therefore "watches" the valence electrons, rather than the positions of atomic nuclei. That is fine for



CREDIT: C.W. SIDERS

Molecular movies. Time-resolved x-ray diffraction can record transient structures of materials as they undergo rapid change initiated by an ultrashort optical pulse.

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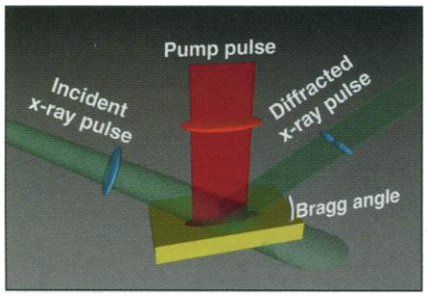

Now you see it... An intense, ultrashort optical pulse leads to melting, which is monitored through time-resolved x-ray diffraction.

photochemistry, where the valence electrons are involved in chemical change and their features in the visible spectrum can often be associated reliably with the molecular geometry, especially for small, well-studied molecules. But the connection between valence electronic spectral properties and collective structure is not easily made, and it is therefore often difficult to infer the evolution of condensed matter structure from transient optical spectroscopic features. Also, light can often not penetrate the samples sufficiently. In fact, the melting transition, now monitored with x-rays, was observed earlier with visible light (*9*, *10*). Fast melting was indicated, but it was uncertain whether it was homogeneous because the light did not penetrate the sample.

The development of ultrafast x-ray probes promises new capabilities for monitoring time-dependent structural changes in complex systems including crystalline solids and biological molecules, and is particularly promising for probing collective changes in crystal structure. But its development also issues a challenge. Now that we can watch collective structural evolution, can we devise ways to initiate it in a synchronized, or phase-coherent, manner?

In femtochemistry, absorption of an ultrashort optical pulse launches all the photochemically active molecules along the reaction path at the same time. Only then can probing light pulses be used to record snapshots of all the excited molecules as they pass through a sequence of well-defined transient molecular structures. Ultrashort optical pulses can also launch coherent collective motion of ions or molecules in crystal lattices, in some cases along the paths that lead them part of the way toward—but generally not all the way into—the positions they occupy in new crystalline structures, that is, part of the way along "collective reaction coordinates" (*11*). There is some evidence that collective structural change can occur as a result (*12*). Methods permitting more extensive optical control over collective behavior have been demonstrated (*13*, *14*). Only if they can be extended substantially will we truly gain the ability to watch the transformation of condensed matter as it passes through a sequence of well-defined transient collective structures (*15*–*17*).

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NOTA BENE: DEVELOPMENT

Whirling Dervishes

Although paired organs such as the kidney and the lung are neatly arranged on each side of the body's midline, other tissues such as the heart and the liver prefer to be on either the left or the right. But how does a developing embryo ensure that, for example, the tube of mesodermal tissue that will eventually form the heart, curves to the left of the body's axis? Recent findings by Okada *et al.* (*1*) add to growing evidence that the clockwise rotation of cilia on embryonic nodal cells is the initial event that determines the asymmetric placement of certain organs.

Nodal cells, each bearing a single cilium, are clustered in a triangular pit (node) that first appears as the neural plate is laid down (at day 7.5 of mouse embryonic development). In work published last year (*2*), Okada and colleagues reported that in mice lacking either the KIF3A or KIF3B motor protein kinesins, the asymmetry of organ placement was random (*2*). These two proteins (together with KAP3) form a complex that transports structural components of the cilium along its hollow interior. Animals deficient in either KIF3A or KIF3B are devoid of cilia.

In their new work (*1*), the Japanese group decided to investigate why the organs of the *iv* and *inv* mutant mouse strains are in abnormal locations. In the *iv* strain, which carries a mutation in a gene encoding dynein (a component of ciliary architecture), the location of organs is randomly asymmetric, whereas in *inv* mice (which carry a mutation in the *inv* gene, whose function is unknown), organ placement is completely reversed. The investigators suspected that a defect in nodal cilia could be the culprit common to both abnormalities.

To test their hypothesis, they analyzed movement of nodal cilia with a video fluorescence microscope, following the trajectory of fluorescent beads chemically attached to the ends of the cilia (large green circle in figure). The movement of unattached fluorescent beads (green spots in figure) revealed how ciliary motion altered the flow of extraembryonic fluid in the node. The authors discovered that, unlike nodal cilia in normal mice that rotated clockwise (apparently in synchrony), those in *iv* mice were motionless. The normal clockwise rotation of nodal cilia moved the fluid to the left of the node but, in the absence of ciliary movement, there was no fluid flow. In *inv* mice, the cilia appeared to rotate normally, but a closer look revealed that they were moving asynchronously. This, together with an abnormality in the shape of the node, generated turbulence in the fluid flow.

Intriguingly, nodal fluid flow begins about the time that left-right determination of tissue location first becomes manifest; asymmetric expression of left- or right-determining genes such as *lefty-2* soon follows. The Japanese group speculated that the fluid might contain morphogens (molecules that dictate development of embryonic tissues) that would accumulate on the lefthand side of the node, driven by the clockwise rotation of the cilia. Accumulation of sufficient morphogen would induce expression of genes in ventral node cells, which would then direct expression of left- or right-determining genes in adjacent mesodermal cells (these are the cells that will eventually develop into organs).

Next, Okada and collaborators plan to identify the fluid morphogens and to alter left-right determination in mouse embryos by mechanically perturbing fluid flow.

—ORLA SMITH

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