equilibrium), the natural assumption is that the barrier to nucleation must steadily decrease. But Auer and Frenkel have demonstrated[1], using advanced computer simulation techniques[3], that the free-energy barrier preventing the formation of crystals can increase, even though the thermodynamic driving force favouring the crystalline phase continues to increase. So, at high enough densities, ΔG^* passes through a minimum and begins to increase, a result not predicted by classical theory. This effect is strongest for polydisperse systems.

An increase in the barrier to nucleation implies that there is a decrease in the rate of nucleation. This in itself is not surprising, because in experiments the crystal nucleation rate for many materials goes through a maximum and then decreases at high densities or low temperatures (Fig. 1). This effect is usually attributed to a dramatic slowdown in the kinetics, because fluid motion becomes sluggish as the density increases or the temperature decreases[4]. Auer and Frenkel's work shows that this interpretation may not always be correct — the maximum in the crystal nucleation rate may come from a minimum in the free-energy barrier preventing formation of the critical nucleus. This prediction represents yet another challenge[5] to classical nucleation theory.

There are two further intriguing and puzzling aspects of the work by Auer and Frenkel[1]. The first is suggested by an application of the nucleation theorem to their data. The nucleation theorem is a general result from the thermodynamics of small systems that states that the rate at which the nucleation free-energy barrier changes with chemical potential can be used to predict the excess number of particles in the critical nucleus relative to the background fluid[6] — in other words, how dense the critical nucleus is. According to Auer and Frenkel, for ΔG^* to pass through a minimum the excess number of particles must pass through zero and become negative, so the density of the critical crystalline nucleus must become lower than that of the fluid around it. This prediction is surprising, because hardly any fluids have crystals that are less dense than their liquid states (water is a rare exception — ice floats). This new idea can be tested by experiment and by further simulation.

A second surprise is the suggestion that the minimum nucleation energy barrier may apply for systems of monodisperse hard spheres, as well as polydisperse ones. As a result, the density of the critical nucleus of the hard-sphere crystal would be lower than that of the surrounding liquid. Systems of monodisperse hard spheres have been studied so thoroughly that theorists thought they understood them completely, but this idea has never been proposed before. Calculations based on density functional theory[7] — a method better suited to the low nucleation barriers in the monodisperse case — may shed light on this question.

Auer and Frenkel also predict that a minimum nucleation rate will affect the typical size of the microcrystals that eventually result once the phase transition is complete. Although the nucleation step is difficult to observe in the laboratory, the final distribution of crystal sizes is relatively easy to measure. Moreover, it has a significant effect on the properties of the resulting solid materials. If real colloidal systems (beyond the hard-sphere model considered here) can be made fully amorphous by having sufficiently polydisperse particles, new types of materials may be created. For example, an increase in the polydispersity of synthetic semiconductor colloids might lead to amorphous materials with new electronic properties. Studies of polydisperse colloids may also give us a better understanding of how metal alloys crystallize. There's more to colloids than meets the eye. ■

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Molecular biology

RNA enzymes for RNA splicing

Andy Newman

Many messenger RNAs are not functional until they are processed by a complex called the spliceosome. It seems increasingly likely that processing is catalysed by the RNA — and not the protein — parts of this complex.

The discovery of catalytic RNAs in 1982 forced proteins to relinquish their status as the only molecules that can catalyse chemical reactions in living cells. For instance, RNA forms the active site in the ribosome, the complex molecular machine that catalyses protein synthesis. And there are hints that an RNA-processing reaction — the splicing of precursor mRNA molecules — might be catalysed by the RNA components of the 'spliceosome' (reviewed in ref. 1). But without precise structural information about the spliceosome's active site this has been hard to confirm. On page 701 of this issue, Valadkhan and Manley[2] provide further support for the idea, with their discovery that a protein-free complex of just two of the spliceosome's RNA components catalyses a reaction that is closely related to the first catalytic step of splicing.

So what is this splicing reaction? Most nuclear genes have a discontinuous structure, consisting of protein-coding sequences (exons) that are interrupted by non-coding

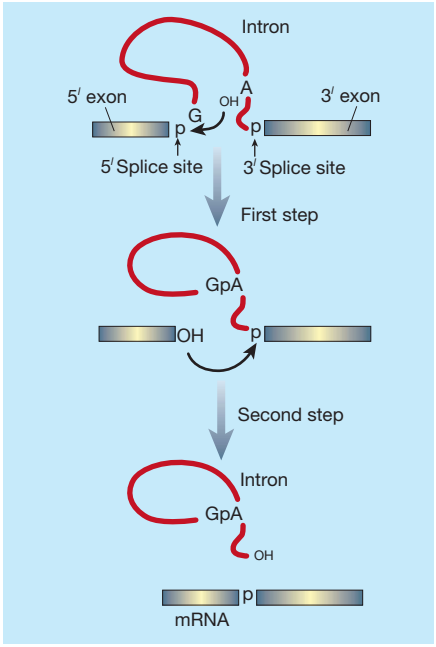

Figure 1 **Splicing of precursor messenger RNA molecules. In the first step, the 2′ hydroxyl group (OH) of an adenosine nucleotide in the intron (the branch-point adenosine, A) attacks the phosphodiester bond (p) at the 5′ splice site. This bond breaks, and a new phosphodiester bond is formed (GpA). In the second step, the 3′ OH group of the free 5′ exon attacks the 3′ splice site. The result is joined exons (the spliced messenger RNA) and a free intron.**

sequences (introns). Transcription of these genes produces precursor mRNAs (pre-mRNAs), which must be processed by accurately removing the introns and splicing together the exons. These reactions are carried out by spliceosomes, which are built from five RNA molecules (known as small nuclear RNAs, snRNAs) and more than 50 proteins.

The two catalytic steps of splicing are both 'phosphotransfer' reactions — in each step, one phosphodiester bond in the pre-mRNA substrate is broken and another is formed. Picture a pre-mRNA molecule that consists simply of an exon, followed by an intron, and then another exon (Fig. 1). The two ends of an RNA are referred to as 5′ and 3′; so, in our simple pre-mRNA molecule, the 5′ splice site is the end of the intron that is connected to the 5′ exon. In the first splicing reaction, the 5′ splice site is attacked by a 2′ hydroxyl group from an adenosine nucleotide that lies in a specific sequence — the 'branch-point' — in the intron. This results in the formation of a phosphodiester linkage within the intron, and a hydroxyl group at the newly exposed end of the 5′ exon. In the second step, this 'new' hydroxyl group targets the intron's 3′ splice site to yield the final products: spliced mRNA and the excised intron.

Of the five snRNA molecules in the spliceosome, U2 and U6 have emerged as the prime candidates for components of the spliceosome's active site. In U6, several nucleotides are crucial for the catalytic steps of pre-mRNA splicing; these nucleotides are identical in U6 molecules from a wide range of organisms[1]. They include an 'AGC' triad, which is also an essential element of the catalytic domain of many self-splicing RNAs. In catalytically active spliceosomes, U2 and U6 base pair together and form a network of interactions with the branch-point and 5′-splice-site sequences[1,3,4].

Last year, Valadkhan and Manley[5] showed that human U2 and U6 snRNAs anneal together, in the absence of proteins and the presence of magnesium ions, to form a stable, base-paired structure. Crucially, ultraviolet irradiation of this complex produced a specific covalent crosslink between U2 and U6, pointing to a three-dimensional structure similar to that thought to exist in catalytically active spliceosomes. These authors[2] have now found that this U2–U6 RNA structure forms a specific complex with a short artificial RNA molecule that contains a branch-point sequence, mimicking the helix formed between U2 and the branch-point sequence in an intact spliceosome. Moreover, this model complex activates the 2′ hydroxyl of the branch-point adenosine to attack a specific phosphodiester bond in the essential AGC triad in U6 snRNA.

Of course in the spliceosome itself, the target of this attack would be the substrate's 5′ splice site, which is not present in this model complex. Nevertheless, this unusual reaction has clear similarities to the first catalytic step in authentic spliceosomes. It requires specific, invariant sequences in U6, and the attacking 2′ hydroxyl group is provided by an adenosine that has been 'bulged' out of a base-paired helix formed between U2 snRNA and the branch-point RNA. Interestingly, there is a precedent for the branch-point 2′ hydroxyl attacking U6 snRNA rather than the 5′ splice site: this is what happens in nematode spliceosomes when U6 contains specific mutations believed to cause distortion of the catalytic site[6].

The idea that a U2–U6 complex could form the active site for the first step of splicing dovetails neatly with earlier findings that the spliceosome is metal-ion-dependent, like several other RNA enzymes that use metal ions as essential cofactors[7]. In fact, it has been shown that a specific phosphodiester linkage in U6 snRNA binds a crucial magnesium ion, which may participate directly in the chemistry of the first catalytic step of splicing[8].

Are we to conclude that all of the spliceosome's protein components are relegated to peripheral roles away from the chemistry of the splicing reactions? Not necessarily. It remains to be seen whether the catalytic repertoire of spliceosomal RNAs extends to other chemical reactions that could be related to the second step of splicing. In any case, there is compelling evidence that at least one highly conserved spliceosomal protein, Prp8, is intimately associated with the snRNAs and the substrate in the spliceosome's active site. Overall, the current evidence suggests that Prp8 may act as a cofactor, perhaps stabilizing the RNA structures required for catalysis, rather than contributing directly to catalysis (reviewed in ref. 9).

Eventually, detailed structural information about the spliceosome's active site may help to settle the issue, as it has for the ribosome[10]. In the meantime, model systems such as those described here[2] could be the fast track to learning more about how the spliceosome does what it does. ■

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Daedalus

A word in your ear

Aurorae and meteorites are usually silent, but occasionally they make a noise. Daedalus reckons that this is because they perturb the magnetosphere, which sometimes forms an electromagnetic wave that converges on an observer. That observer is then exposed to a fluctuating magnetic field. The oxygen of the local air (which is paramagnetic) varies in volume in sympathy with the field, making the noise. The effect propagates at the speed of microwaves, not that of sound, so even a distant aurora or meteorite makes a noise while it is still visible.

So, says Daedalus, here is a method of creating sound at a distance. A parabolic aerial, or several of them in a phased array reinforcing at a chosen point, could launch a microwave beam whose local intensity would vary at an audio frequency. Its magnetic component would then affect the local air, and make a noise. The microwave frequency itself, far too high to hear, would not matter. Like an amplitude-modulated signal, the audio effect would depend only on its changing absolute intensity. Here is a way of generating truly local sound.

The obvious use is crowd control. Many popular events these days, such as pop festivals, take place in large fields or open spaces ringed by security staff. With a proper array of peripheral aerials, just one chosen spot could be irradiated with a sound signal, controlled by the security men on their watchtowers. Local revellers acting suspiciously could be warned off without any unsettling public declaration; only those being addressed would hear anything. Another use would be in supermarkets, museums and so on, where potential customers near a product or exhibit could be told all about it by a private local voice. If they drifted away, this would rapidly fade.

Indeed, says Daedalus, many public-address tasks could be done better by his microwave system. A single railway platform or section of platform could be addressed by an announcer. People using public telephones in airports, currently deafened by public-address announcements, could be spared by shaping the public microwave pattern to avoid the phone boxes. Even in a domestic setting, the hi-fi enthusiast might enjoy a purely local signal. His mobile phone might be made blessedly local too, although sadly his replies, as genuine sound, would still fill the room. For even Daedalus can think of no way of switching off a real local sound source. David Jones