fleshed out by Ewald Hering (3) and reverberated throughout the latter half of the 19th century. Fueled by growing recognition of the importance of relational processing (5) and by the birth of modern neuroscience, Mach's ideas of reciprocal action have since inspired an enormous number of behavioral and biological studies of visual perception. Celebrated among them was the discovery in the 1940s (6) of the existence of neuronal circuitry—known as "lateral inhibition"—that conforms to Mach's predicted spatial interactions.

Although lateral inhibition is now believed to underlie the perceptual sharpening of local edges of contrast (3, 7), the neuronal signals that underlie perceived light intensity of extended surfaces (areal brightness) have remained a mystery. The new report by Rossi *et al.* (2) offers intriguing new evidence for the locus and the nature of these signals. By varying luminance relations within a simple visual pattern, these investigators were able to manipulate the brightness of a target area in two ways: (i) directly, such that brightness paralleled changes in area luminance; or (ii) inductively, such that area luminance remained unchanged but its brightness inversely paralleled changes in the surrounding luminance. The target area was placed within the receptive fields of individual neurons in primary visual cortex, and the activity of the neurons was recorded in the presence of both direct and induced brightness changes. A substantial fraction of neurons exhibited responses that covaried with areal brightness, regardless of whether brightness changes were caused directly or induced by changes occurring well beyond the margins of the receptive field. In other words, these neurons appear to encode perceived intensity of light in a manner that is independent of cause.

Areal brightness induction thus satisfies the painter's need and can be accounted for in neuronal terms. But what function does it serve when viewing natural scenes? The simple answer is that it is borne of an exquisite sensitivity to contrast—for it is contrast, not local light intensity, that offers the most important information about the viewer's environment. The reason for this is clear: The intensity of light arising from a surface is a product of both the reflectance of the surface and the intensity of the light by which it is illuminated. The reflectance, which is critical for object recognition, is commonly dissociable from the intensity of the illuminating light—a phenomenon von Helmholtz characterized as "eliminating the differences of illumination" (8, p. 287). Diffuse illumination changes (for example, sunlight versus shadow) alter luminance everywhere in the image, but luminance ratios (that is, contrast) remain unchanged, mirroring the physical constancy of surface reflectance. Not surprisingly, surface reflectance generally appears constant under these conditions, a phenomenon known as "lightness constancy." By contrast, changes in luminance ratios—such as those used by Rossi *et al.*—are indicative of reflectance changes. These are, of course, the conditions that lead to brightness induction.

Considered in this light, it is tempting to speculate that the neurons discovered by Rossi *et al.* do not merely represent brightness but may underlie the more behaviorally significant quantity of perceived surface reflectance (lightness). As we have seen, recovery of surface reflectance is marked by the complementary perceptual phenomena of lightness constancy and brightness induction, and these are the gold standards by which we should judge potential neural substrates. Rossi *et al.* have documented induction. If these neurons mediate lightness perception, however, we would expect the same cells to exhibit an invariant response under conditions that mimic variations in diffuse illumination—that is, when luminance changes uniformly, such that contrast remains unchanged. Although this test for lightness constancy has yet to be performed, Rossi *et al.* have opened the door to an exciting exploration of the ways in which the multiple physical causes of light in the retinal image are isolated and identified by the brain.

More generally, these experiments exemplify a growing and welcome trend in studies of sensory processing. Traditionally, this discipline has focused on neural events that encode local characteristics of the retinal stimulus. However illuminating this approach has been for understanding sensory coding, few would argue that the way we actually see the world is tied directly to these characteristics. On the contrary, it is the remarkable constancy of perception in the face of ever-changing retinal conditions that is a hallmark of visual experience. By adopting stimulus configurations that bring about a dissociation between local retinal image properties and perceptual state—such as those that elicit brightness induction—it becomes possible to tease apart the neural structures and events that give rise to perception. And therein lies one of our greatest hopes for understanding the substrates of vision.

References

1. Leonardo da Vinci, *Treatise on Painting (Codex Urbinus Latinus 1270)*, A. P. McMahon, Transl. (Princeton Univ. Press, Princeton, 1956).
2. A. F. Rossi *et al.*, *Science* **273**, 1104 (1996).
3. E. Hering, *Outlines of a Theory of the Light Sense*, L. M. Hurvich and D. Jameson, Transl. (Harvard Univ. Press, Cambridge, MA, 1920/1964).
4. E. Mach, translated in F. Ratliff, *Mach Bands: Quantitative Studies on Neural Networks in the Retina* (Holden-Day, San Francisco, 1965).
5. K. Koffka, *Principles of Gestalt Psychology* (Harcourt, Brace, New York, 1935).
6. H. K. Hartline, *Fed. Proc.* **8**, 69 (1949).
7. A. Fiorentini *et al.*, in *Visual Perception: The Neurophysiological Foundations*, L. Spillman and J. S. Werner, Eds. (Academic Press, San Diego, CA, 1990).
8. H. von Helmholtz, *Physiological Optics*, J. P. C. Southall, Transl. (Optical Society of America, 1860/1924), vol. 2.

Small Nucleolar RNAs Guide Ribosomal RNA Methylation

David Tollervey

In all organisms, proteins are synthesized by ribosomes that share extensive similarities in structure and function. These ubiquitous particles contain the mature ribosomal RNAs (rRNAs), which are excised from a large common transcript [the rRNA precursor (pre-rRNA)] and undergo extensive covalent nucleotide modification, together with about 80 ribosomal proteins. In eukaryotic cells, ribosomes are assembled in a specialized compartment within the nucleus of the cell, the nucleolus.

Over the past few years, an extraordinarily large number of small RNA species (snoRNAs) have been found to reside in the nucleolus. Each human pre-RNA molecule transiently associates with more than 100 different snoRNA species, but the role of each species in ribosome synthesis remained largely unknown. Two recent papers (*1*, *2*) now report that members of a large family of snoRNAs act as guides for rRNA methylation. For each site of ribose methylation, base pairing between the pre-rRNA and a specific guide snoRNA targets the site for methylation and identifies the nucleotide to be modified.

The snoRNAs are associated with proteins in small nucleolar ribonucleoprotein particles (snoRNPs) (*3*). Two evolutionarily conserved motifs (box C and box D) are present in many snoRNAs and are implicated as protein-binding sites, although the proteins that interact with these sequences have not been identified. Many snoRNAs that contain box C and box D have two

The author is at the European Molecular Biology Laboratory, Postfach 10 22 09, Meyerhofstrasse 1, 69012 Heidelberg, Germany. E-mail: tollervey@embl-heidelberg.de

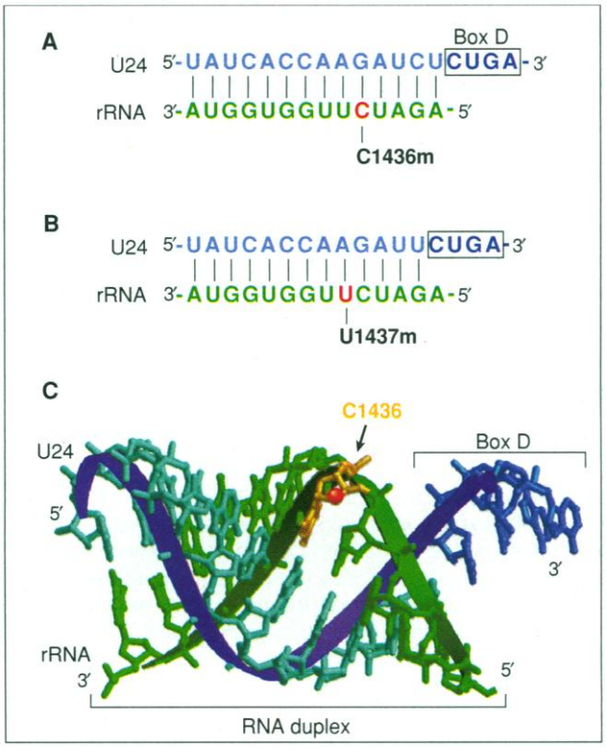
unexpected features. First, they are not transcribed from their own promoter and terminator regions but are excised by posttranscriptional processing from the introns of mRNAs, most of which encode proteins in their exonic sequences (*3*, *4*); these proteins generally participate in ribosome synthesis or function and include nucleolar proteins, ribosomal proteins, and translation factors Second, these snoRNAs exhibit striking primary sequence complementarity to sequences in the mature rRNAs. The regions of complementarity are remarkably long, and typically include 14 to 17 consecutive base pairs. Base-pairing interactions form between the snoRNAs and the large pre-rRNA molecules. They usually involve highly conserved regions of the rRNAs that lie near the catalytic center of the ribosome, and frequently include sites of modification (*5*, *6*).

All rRNAs undergo extensive covalent modification, mainly pseudouridine formation, base methylation, and methylation of the 2′-hydroxyl position of the ribose group (2′-O-methylation). The human rRNAs contain about 106 2′-O-methyl groups, but their locations lack any clearly conserved structural motif or nucleotide sequence context (*7*). The new work now shows that almost all snoRNA-rRNA base-paired interactions can be depicted such that a box D–like sequence is at a constant distance of five base pairs from a site of 2′-O-methylation (see figure) (*1*, *2*). The snoRNA-pre-rRNA interaction would therefore serve to position the protein or proteins associated with box D at a precise distance from the site of methylation on the rRNA. This positioning is proposed to define the nucleotide that will undergo ribose methylation. Subsequently, this observation was made independently for the yeast snoRNAs (*8*).

This model is strongly supported by experimental evidence. Deletions of the genes encoding several yeast snoRNAs—U24 (*1*), U18, snR38, snR39a/b, snR40, snR41, and snR47 (*8*)—each result in the loss of the predicted 2′-O-methyl group. More dramatically, ectopic expression of U24 restores methylation at the wild-type position, whereas a mutation that alters the spacing between the box D sequence and the region of base pairing displaces the site of the methylation (see figure) (*1*). Furthermore, expression in mouse cells of an "artificial" snoRNA containing the box C and box D sequences and a region of rRNA complementarity accurately directs modification at a novel site in the rRNA (*9*). Together, these results indicate that the snoRNP–pre-rRNA interaction is the sole determinant of the site of methylation. Box D is also found in snoRNAs that lack extended rRNA complementarity and are not encoded by introns, so the methylase itself probably does not directly bind to box D. It remains unclear whether the methylase is an integral component of the snoRNPs or is targeted to the site of methylation by transient interaction with the box D–binding protein or proteins.

Eukaryotic cells have, therefore, developed an elaborate system for site-specific rRNA methylation. The function of these methyl groups remains obscure, however. U24 and several other methylation-guide snoRNAs tested in yeast are completely dispensable for growth (*1*, *3*), showing that they are not required for the synthesis of functional ribosomes. In vivo depletion of the *Xenopus* guide snoRNA U18 also has no effect on pre-rRNA cleavage (*10*). In the current model, these modifications confer improvements in the efficiency of ribosome assembly or function by fine tuning the rRNA structure or interaction with ribosomal proteins, but in most cases the growth advantage conferred is not detected under laboratory conditions.

So, is everything about pre-rRNA modification and snoRNA function now explained? Well, not really. No correlation between snoRNA binding sites and the locations of other rRNA modifications—base methylation and pseudouridine formation—has yet been found; these sites are presumably identified by a different mechanism. Furthermore, a large number of other snoRNAs exist that lack the extended rRNA complementarity of the methylation guide snoRNAs. Several snoRNPs are required for specific pre-rRNA processing steps in *Xenopus* and yeast (*3*, *11*), but of these, only RNase MRP is known to play an enzymatic role in cleavage (*12*). The roles of other snoRNPs in the cleavage reactions is unclear; they may present the pre-rRNA to the nucleases in the correct conformation or target the nucleases to the cleavage sites (*13*). It is likely that other snoRNPs function as "chaperones," aiding the correct folding of the pre-rRNA and assembly of the large number of ribosomal proteins. We can look forward to further surprises from the complex world of snoRNAs over the coming years.



Interaction between yeast U24 snoRNA and the pre-rRNA (A) The 3′ region of yeast U24 (upper strand) can form 14 consecutive base pairs with the 25*S* rRNA (lower strand), such that a conserved sequence element, box D, is positioned five nucleotides (about one half turn of the RNA helix) from a site of methylation at position C1436. (**B**) Removal of one nucleotide from U24 5′ to box D (U24m) results in displacement of the methylation to U1437 (*1*). (**C**) Model for the U24-rRNA interaction. The duplex region is drawn as an A-form helix (*14*). The base-paired interaction places box D, and its associated protein or proteins, at a defined position with respect to the site of methylation, allowing the methylase to identify the correct 2′-hydroxyl group of the ribose for modification.

References and Notes

1. Z. Kiss-László, Y. Henry, J.-P. Bachellerie, M. Caizergues-Ferrer, T. Kiss, *Cell* **85**, 1077 (1996).
2. M. Nicoloso, L.-H. Qu, B. Michot, J.-P. Bachellerie, *J. Mol. Biol.* **260**, 178 (1996).
3. E. S. Maxwell and M. J. Fournier, *Annu. Rev. Biochem.* **35**, 897 (1995).
4. K. T. Tycowski, M.-D. Shu, J. A. Steitz, *Nature* **379**, 464 (1996).
5. J.-P. Bachellerie *et al.*, *Trends Biochem. Sci.* **20**, 261 (1995).
6. J. Steitz and K. Tycowski, *Science* **270**, 1626 (1995).
7. B. E. H. Maden, *Prog. Nucleic Acid Res. Mol. Biol.* **39**, 241 (1990).
8. J. Ni, A. G. Balakin, M. J. Fournier, personal communication.
9. J. Cavaille, M. Nicoloso, J.-P. Bachellerie, personal communication.
10. D. A. Dunbar, V. C. Ware, S. J. Baserga, *RNA* **2**, 324 (1996).
11. J. Venema and D. Tollervey, *Yeast* **11**, 1629 (1995).
12. Z. Lygerou, C. Allmang, D. Tollervey, B. Sèraphin, *Science* **272**, 268 (1996).
13. S. Abou Elela, H. Igel, M. Ares Jr., *Cell* **85**, 115 (1996).

14. I thank G. Vriend for drawing the A-form helix.