reported device characteristics for a single-electron tunneling transistor (SET) junction formed in this way (*15*). Although the SET performance of these devices appears entirely convincing, the correlation of device size and quantum confinement has not yet been confirmed on devices based on titanium wires, which presumably reflects a contribution of grain size on performance. Problems such as these, where reliable control of electrical and dimensional properties at less than 10 nm becomes critical, demand that fabrication be coupled with in situ diagnostics. By monitoring a resistance increase in the wire during fabrication, Snow and Campbell have shown that routine and reliable control can be attained in this size regime (*5*). A distinguishing feature of SPM-based nanoscale technology, that which sets it apart from the incremental refinements of existing methods such as electron-beam lithography, is the promise of exceptional materials control. Such control, rather than linewidth control, is needed for investigations at the sub–10-nm level. Further progress will hinge primarily on the integration of fabrication with local diagnostic probes in order to fully optimize the process (*16*).

Direct oxidation by SPM, only recently at the frontiers of nanoscience, has arrived at the point where it has now become a powerful technique for the prototyping of novel device structures. This research is one aspect of a generalization of the scanned probe concept leading to its eventual role in advanced manufacturing. Multiple-tip arrays, in which large numbers of individual SPM instruments perform parallel fabrication and inspection over a large sample area, are now a reality. Quate's group at Stanford has demonstrated parallel lithography by coupling the direct oxidation process with a five-tip SPM array (*17*). As these developments continue to be explored in the research laboratory, industrial acceptance of scanned probe techniques is growing rapidly. Various types of critical measurements needed in production and process development for the semiconductor, magnetic recording, and polymers and coatings sectors have been identified (*18*). These applications have the potential to drive the creation of an integrated SPM-based technology employing both fabrication and measurement functions. The prospects for this will be determined largely by the extent to which nanoscience research and technological innovation become successful at anticipating each other's development paths.

References and Notes

1. G. Binnig and H. Rohrer, *Helvetica Physica Acta* **55**, 726 (1982). The term scanned probe microscopy includes STM as well as other types of SPM, such as AFM.
2. K. E. Drexler, *Engines of Creation* (Anchor/Doubleday, New York, 1986).
3. J. A. Stroscio and D. M. Eigler, *Science* **254**, 1319 (1991).
4. C. R. K. Marrian, Ed., *Technology of Proximal Probe Lithography* (SPIE, Bellingham, WA, 1993).
5. E. S. Snow and P. M. Campbell, *Science* **270**, 1639 (1995).
6. J. A. Dagata *et al.*, *Appl. Phys. Lett.* **56**, 2001 (1990).
7. J. A. Dagata *et al.*, *J. Vac. Sci. Technol. A* **10**, 2105 (1992).
8. H. Sugimura *et al.*, *Appl. Phys. Lett.* **63**, 1288 (1993).
9. H. J. Song *et al.*, *J. Vac. Sci. Technol. B* **12**, 3720 (1994).
10. N. Kramer *et al.*, *Appl. Phys. Lett.* **66**, 1325 (1995).
11. M. Enachscu, E. Hartmann, F. Koch, *ibid.* **64**, 2253 (1994); H. C. Day and D. R. Allee, *ibid.* **62**, 2691 (1993); D. Wang *et al.*, *ibid.* **65**, 1415 (1994); S. W. Park *et al.*, *ibid.* **67**, 2415 (1995).
12. E. S. Snow and P. M. Campbell, *ibid.* **64**, 1933 (1994).
13. ———, B. V. Shannabrook, *ibid.* **63**, 3488 (1993).
14. S. C. Minne *et al.*, *ibid.* **66**, 703 (1995); P. M. Campbell, E. S. Snow, P. J. McMarr, *ibid.*, p. 1308.
15. K. Matsumoto *et al.*, *Jpn. J. Appl. Phys.* **34**, L1387 (1995); M. Ishii and K. Matsumoto, *ibid.*, p. L1329.
16. T. Teuschler *et al.*, *Appl. Phys. Lett.* **66**, 2499 (1995); *ibid.*, in press.
17. S. C. Minne *et al.*, *J. Vac. Sci. Technol. B* **13**, 1380 (1995).
18. J. A. Dagata *et al.*, *Industrial Applications of Scanned Probe Microscopy Workshop Reports, NISTIR* 5550 (December 1994) and 5752 (November 1995); available from the National Technical Information Service, Springfield, VA.

Small RNA Chaperones for Ribosome Biogenesis

Joan A. Steitz and Kazimierz T. Tycowski

Vertebrate ribosomal RNA (rRNA) genes undergoing active transcription are pictured in virtually every textbook of biology. These electron micrographs show spreads of chromatin derived from the nucleolus, the cellular compartment responsible for ribosome biogenesis. The nascent rRNA molecules that emanate from the DNA pinpoint the sites where synthesis begins and ends within the tandem repeats of rDNA (*1*). But these elegant visualizations of genes in action also convey an important message concerning the fate of the attached precursor rRNA (pre-rRNA) transcripts (Fig. 1). Because their lengths uniformly increase along each transcription unit [~13,000 nucleotides (nt) in mammals], significant RNA processing must not occur until synthesis is complete. Approximately half of pre-rRNA sequences are then discarded during the maturation of 18S, 5.8S, and 28S rRNAs (*2*). Although ciliates splice their pre-rRNAs, the processing pathway in vertebrates consists simply of an ordered series of endo- and exonucleolytic cleavages that carve away the spacer regions and trim the ends of the rRNAs. What nucleolar components orchestrate these cuts, and why do cells complete synthesis of an enormous RNA before embarking on the process?

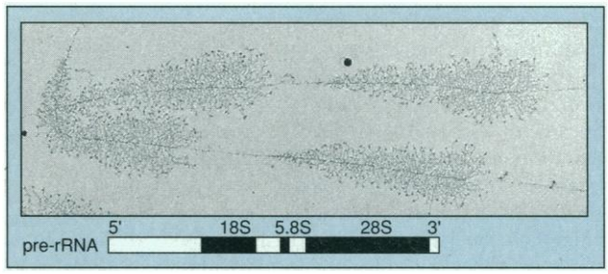

Fig. 1. Electron micrograph of actively transcribed rRNA genes from the *Triturus viridescens* oocyte. Each transcription unit shows growing pre-rRNA chains decorated at their 5′ ends with the so-called terminal knobs. Lower panel shows a schematic representation of vertebrate pre-rRNA. [Micrograph courtesy of O. L. Miller.]

Small nuclear ribonucleoproteins (snRNPs) have recently been identified as major players in eukaryotic rRNA processing (*3*). In addition to the well-studied snRNPs that assemble into spliceosomes to excise introns from pre–messenger RNAs (pre-mRNAs), cells contain distinct snRNPs localized in the nucleolus. The small RNAs in vertebrate nucleolar snRNPs range in length from 70 to more than 200 nucleotides. Many of these snRNPs contain a protein called fibrillarin, an autoantigen that is an abundant component of the fibrillar regions of the nucleolus. Current estimates of the number of nucleolar snRNPs in both vertebrates and yeast range from 50 to 100; approximately 20 vertebrate RNA sequences are now known.

So far, only three of the many vertebrate nucleolar snRNPs have been assigned functions in rRNA processing (*3*). The most

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abundant of these (~10^6 molecules per cell), U3, is essential for an early cut close to the 5′ end of the transcript, curiously distant from any of the mature rRNA sequences. It then remains attached to the downstream product, generating the distinctive 5′-terminal knobs that characterize rRNA (but not mRNA) transcripts in chromatin spreads (*4*). Binding of the U3 snRNP might therefore be considered to commit the rRNA transcript to the processing pathway; in yeast, this interaction is essential for the appearance of 18S rRNA (*5*, *6*). The second most abundant nucleolar snRNP, U8, is required for cleavage upstream of 5.8S rRNA as well as at the 3′ end of 28S rRNA, ~5000 nt away (*7*). The less abundant U22 snRNP is essential for processing at both the 5′ and 3′ ends of the 18S rRNA, separated by ~2000 nt (*8*). Functions for two additional vertebrate snRNPs can be extrapolated from studies of their counterparts in yeast (*3*). U14 is needed for maturation of 18S rRNA (*9*), whereas RNaseMRP (a relative of the RNaseP ribozyme) executes cleavage upstream of 5.8S sequences (*10*). Unfortunately, establishing which vertebrate small nucleolar RNAs are homologous to the many other known yeast small RNAs has not been possible.

The mechanistic details of how the nucleolar snRNPs act on the pre-rRNA remain elusive. But the surprising realization that a single snRNP can coordinate cuts at far distant sites allows pleasing parallels to be drawn with bacterial rRNA processing. There, complementary sequences flanking the 5′ and 3′ ends of the 16S and 23S rRNAs come together, generating extensive stems in the pre-rRNA that are cleaved by a protein enzyme, ribonuclease III (*11*). In eukaryotes, comparable stems are not apparent, but the snRNP-dependent coupling of processing at remote sites flanking the large or small subunit rRNA (*7*, *8*, *10*) must reflect a similar pre-rRNA architecture.

The task of folding the huge eukaryotic pre-rRNA transcript may well be accomplished by a myriad of small nucleolar RNAs discovered in the last several years (*3*, *12*). The vast majority of these RNAs in vertebrates are generated by an unprecedented biosynthetic route. Their genes are embedded within the introns of protein-coding genes, most of which are involved in ribosome or nucleolar functions (*3*). Apparently, the host intron is excised from the pre-mRNA transcript and debranched; then, the small RNA is released by exonuclease action (*13*). Most bind the common nucleolar snRNP protein fibrillarin, directed by conserved sequences (called boxes C and D) adjacent to a stem formed by the termini of the intron-encoded small RNA.

The truly striking feature of the intron-encoded small nucleolar RNAs, however, is

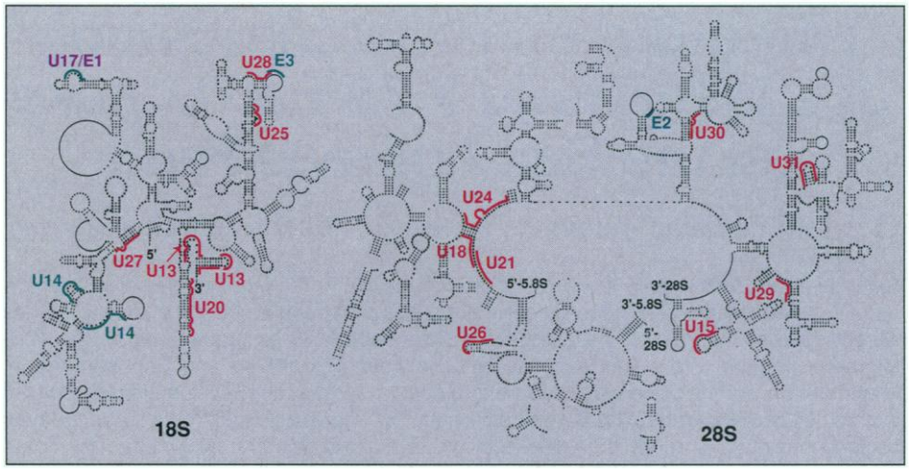

Fig. 2. Base-pairing interactions of small nucleolar RNAs with 18*S* and 28*S* rRNAs. Red, predicted base pairings; purple, experimentally supported base pairings; darker balls, rRNA nucleotides that are more than 95% identical among eukaryotic species; lighter balls, nucleotide positions that exist 95% of the time; continuous lines, regions highly variable in size and content.

their complementarity to mature rRNA sequences. Each little RNA has the potential to form a nearly perfect duplex of 11 to 21 base pairs with either 18S or 28S rRNA (*12*, *14*). Usually, the complementarity lies within one of the universal core secondary structures that have been deduced from phylogenetic comparisons of the large rRNA sequences (Fig. 2). Association of snRNP would therefore disrupt rRNA stems or other conserved interactions, conferring an alternative geometry on the pre-rRNA undergoing processing. In a few cases, psoralen cross-linking (*15*) or genetic suppression studies (*16*) have provided direct evidence for the predicted snRNP-rRNA base pairing. The cellular abundance of the new nucleolar snRNPs is similar to that estimated for pre-rRNA (~10^4), compared with 10^7 ribosomes in the mammalian cytoplasm (*3*). In localization studies, the small RNAs are exclusively nucleolar, suggesting that they recycle within this nuclear subcompartment. The proposed role of nucleolar snRNPs in remodeling the structure of the large pre-rRNA transcript therefore mimics the action of protein chaperones in directing the folding of other polypeptide chains (*3*, *14*).

The chaperone analogy raises questions nearly as numerous as the new nucleolar snRNPs themselves (*3*, *14*). How many are there? How many are essential, as opposed to having overlapping or facilitating functions? Is adenosine triphosphate hydrolysis required for their release (as with protein chaperones)? Or are they displaced simply by ribosomal proteins assembling on the nascent rRNAs? Do they direct the relatively rare nucleotide modifications (pseudouridine and base or sugar methylations) that are likewise clustered within the universal core regions of the rRNAs (*14*)? Do they serve not only to produce a specific geometry for the pre-rRNA transcript, but also to preclude deleterious intermolecular interactions between the GC-rich, closely spaced neighboring transcripts? If nucleolar snRNPs explain repeated failures to reconstitute eukaryotic ribosomal subunits from their constituent rRNAs and proteins, how can bacterial ribosomes assemble without the assistance of small RNA chaperones? Whether or not nucleolar snRNPs are eventually assigned catalytic activities, their existence adds the nucleolus to the growing roster of cellular RNA machines.

References and Notes

1. O. L. Miller Jr. and B. R. Beatty, *Science* **164**, 955 (1969).
2. B. Sollner-Webb, K. T. Tycowski, J. A. Steitz, in *Ribosomal RNA Processing in Eukaryotes*, A. E. Dahlberg and R. A. Zimmermann, Eds. (CRC Press, Boca Raton, FL, in press).
3. E. S. Maxwell and M. J. Fournier, *Annu. Rev. Biochem.* **35**, 897 (1995).
4. E. B. Mougey *et al.*, *Genes Dev.* **7**, 1609 (1993).
5. J. M. X. Hughes and M. Ares Jr., *EMBO J.* **10**, 4231 (1991).
6. M. Beltrame and D. Tollervey, *ibid.* **14**, 4350 (1995).
7. B. A. Peculis and J. A. Steitz, *Cell* **73**, 1233 (1993).
8. K. T. Tycowski, M.-D. Shu, J. A. Steitz, *Science* **266**, 1558 (1994).
9. H. V. Li, J. Zagorski, M. J. Fournier, *Mol. Cell. Biol.* **10**, 1145 (1990).
10. J. P. Morrissey and D. Tollervey, *Trends Biochem. Sci.* **20**, 78 (1995).
11. R. M. Bram, R. A. Young, J. A. Steitz, *Cell* **19**, 393 (1980).
12. K. T. Tycowski, M.-D. Shu, J. A. Steitz, unpublished data.
13. T. Kiss and W. Filipowicz, *Genes Dev.* **9**, 1411 (1995).
14. J.-P. Bachellerie *et al.*, *Trends Biochem. Sci.* **20**, 261 (1995).
15. O. J. Rimoldi, B. Raghu, M. K. Nag, G. L. Eliceiri, *Mol. Cell. Biol.* **13**, 4382 (1993).
16. W.-Q. Liang and M. J. Fournier, *Genes Dev.* **9**, 2433 (1995).

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