No Genome Barriers to Promiscuous DNA

The movement of DNA sequences between mitochondrial, chloroplast and nuclear genomes is even more prolific than had been expected

The term "promiscuous DNA" was introduced into the literature a year and a half ago independently by John Ellis of the University of Warwick, England, and by Frances Farrelly and Ronald Butow of the University of Texas Health Sciences Center, Dallas. Farrelly and Butow used the term in their report of the apparent natural transfer of yeast mitochondrial DNA sequences into the nuclear genome (*1*). Ellis applied it in an editorial comment (*2*) on a paper describing the apparent translocation of DNA sequences between mitochondrial and chloroplast genomes in corn by David Stern and David Lonsdale of the Plant Breeding Institute, Cambridge, England.

Since that time the subject of DNA's promiscuity has exploded, with an impressive series of reports springing into the literature. The most recent, by Stern, who is now at the Carnegie Institution of Washington, Stanford, and Jeffrey Palmer, Duke University, reveals that movement of DNA sequences between chloroplasts and mitochondria is not just a rare event but is rampant process. In a series of analyses on mung beans, spinach, corn, and peas, they found that "every chloroplast (ct) DNA sequence tested reacted with one or more mitochondrial (mt) DNA restriction fragment." Interorganellar DNA transfer is, as they note, "a general phenomenon" (*3*).

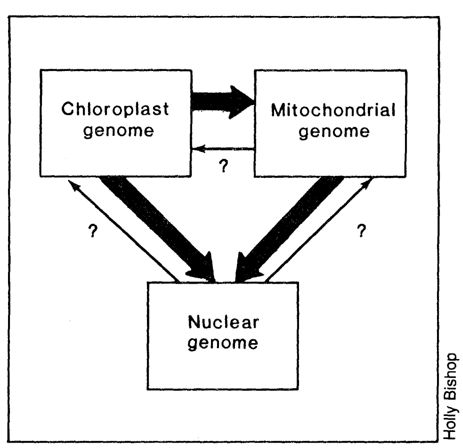
The interest in DNA translocation between the nuclear genome and those of mitochondria and chloroplasts derives from the still unsolved mystery of origin of these semi-independent structures. Although both mitochondria and chloroplasts now depend to a significant extent on the import of nuclear-encoded proteins for their myriad metabolic functions, many biologists believe that these organelles once were free-living, primitive prokaryotes that became entrapped in a eukaryotic precursor. This endosymbiont hypothesis, as it is called, requires that through evolutionary time these entrapped organisms lost their independence by losing genetic information to the nuclear genome.

The endosymbiont hypothesis received a boost with the discovery of sequences common to the nuclear and organellar genomes, because this seemed to confirm that translocation of genes could indeed occur. And in some cases the presence of certain telltale sequences associated with the genes in question confirmed that the direction of movement had been from organelle to nucleus. The discovery that mitochondria and chloroplasts might exchange genes between each other was, however, something of a surprise, because these organelles had always been regarded as being rather independent of each other.

After noting the apparent transfer of part of a chloroplast repeated sequence to the mitochondrial genome in corn, Stern decided to pursue the issue in more detail, analyzing more sequences and more species.

Mitochondrial genomes of higher plants are large compared with those in fungi and mammals, and compared with chloroplast genomes too. Moreover, there is often great size variation among mitochondrial genomes of related species: there is, for instance an eightfold difference between watermelon and muskmelon. At least some of this "extra" DNA appears to be transcribed in the larger genomes, even though there is often little difference in the number of polypeptides eventually produced.

The great size and sequence flexibility of the mitochondrial genome is in stark contrast with that in the chloroplast, which appears to be tightly constrained in size and sequence between even distantly related species. Even a minor structural modification can apparently perturb chloroplast function. The most likely direction of permanent sequence translocation between the genomes, therefore, is from chloroplast to mitochondrion, as the mitochondrial genome can more readily accommodate foreign DNA and survive.



Promiscuous DNA

Large arrows show directions in which sequences have been shown to have moved; thin arrows indicate probable, but not clearly demonstrated, movement.

Confirmation of this assumption comes from a comparison of certain sequences that are common to mung bean and spinach chloroplast genomes and are also found in their respective mitochondria. "Because the chloroplast sequences are very close to each other while those in the mitochondria have diverged to some extent you can conclude that they originated in the chloroplasts," says Stern.

In the work reported in their most recent paper Stern and Palmer used about a dozen relatively large chloroplast fragments from any one species of their test as probes of the mitochondrial genomes. More recently they have gone to a finer resolution by using many more smaller fragments, which reveals that chloroplast sequences are scattered throughout the mitochondrial genomes. The distribution of the chloroplast DNA in the mitochondria bears no relation to its organization in the chloroplast genome. Moreover, certain chloroplast sequences are to be found in multiple copies in some of the mitochondrial genomes, which seems to indicate multiple transfers.

Although some profound uncertainties about the rate of evolution of chloroplast and mitochondrial DNA sequences make it a hazardous business to suggest with any degree of certainty the timing of transfer events, Stern and Palmer feel they can conclude that at least some were relatively recent. Transfer is probably a continuous process, but the ability to match the original and transferred sequence will diminish as the sequences diverge through time.

How the DNA gets from one organelle to another is still a mystery, though at least two mechanisms are being contemplated. One possibility is some kind of vector, such as a transposable element or a transducing phage. A second mechanism is much more direct, resulting from the fusion of the two organelles. Stern is impressed with the plentiful electron-micrographic evidence of such fusion and at present marginally prefers this mechanism over the first.

"At certain times in the life cycle the mitochondria and chloroplasts can come into very close contact," he notes. "There is probably plenty of opportunity for sequence transfer through fusion of the organelles." Stern acknowledges, however, that there is no reason to suggest that transfer is necessarily the result of just one mechanism. Different mechanisms might operate during different transfers. "But," he says, "I doubt there is a specialized vector system."

Stern is now interested in determining whether the chloroplast DNA might in some cases be functional in the mitochondrial genome. "We have to start by showing it is transcribed," he says, "and then go from there." Chloroplast sequences have been located very close to some functional mitochondrial genes, "but that doesn't prove anything." Stern's current guess, however, is that some of the transferred sequences will be shown to be functional.

In any case, Stern and Palmer conclude that "the widespread presence of ctDNA sequences in plant mtDNA is best regarded as a dramatic demonstration of the dynamic nature of interactions between the chloroplast and the mitochondrion, similar to the ongoing process of interorganellar DNA transfer already documented between mitochondrion and nucleus and between chloroplast and nucleus."—**ROGER LEWIN**

References

1. J. Ellis, *Nature (London)* **299**, 678 (1982).
2. F. Farrelly and R. A. Butow, *ibid.* **301**, 296 (1983).
3. D. B. Stern, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1946 (1984).

New Light on Dark Matter?

It fills the universe, it is utterly invisible, and it may not even exist; meanwhile, the hypotheses are getting more exotic

The "Inner Space/Outer Space" workshop* at the Fermi National Accelerator Laboratory was intended as a comprehensive overview of the connection between particle physics and cosmology, vintage 1984. In that it succeeded admirably. Only a few years old, this field continues to be one of the most vigorous and productive in physics.

The "inflation" theory of the early universe continued to hold center stage, as it has for several years now (*Science*, 28 January 1983, p. 375); people are currently exploring such ideas as supersymmetry and the more exotic grand unified theories in an effort to bring the detailed predictions of inflation more closely in accord with observation. Also on display were the newly revived, 60-year-old "Kaluza-Klein" models of the fundamental forces, which assume that we live in a universe having not just the 4 dimensions of ordinary space and time, but 5 dimensions, 11 dimensions, or more. (The champion theory at Fermilab called for 950 dimensions.)

But it was clear that the most baffling single problem in this field continues to be the large-scale structure of the universe, together with its relationship to the so-called dark matter, or "missing mass" (*Science*, 4 March 1983, p. 1050). This mysterious, utterly invisible ectoplasm fills the universe and has surely had a profound effect on cosmic evolution. But no one really knows what it is. The better the observations become, in fact, and the more carefully the situation is analyzed, the more the theorists are being forced into exotic hypotheses—not the least of which is that the cosmos is filled with "string."

One emerging insight, emphasized by a number of participants at Fermilab, is that *the* dark matter problem is actually *two* dark matter problems that may well involve different phenomena. The "large-scale" problem is usually phrased in terms of the quantity Ω, the ratio between the observed average mass density of the universe and a certain critical density at which the kinetic energy of cosmic expansion just balances the potential energy of gravitation. The overwhelming theoretical predilection these days is for an Ω precisely equal to 1, largely because $\Omega = 1$ is a prediction of the inflation models, which nicely explain such things as the homogeneity and isotropy of the universe.

However, in observational reality the ordinary "baryonic" matter in the universe—the stuff composed of protons, neutrons, and electrons—falls short by an order of magnitude. The best evidence for that comes from observations of the cosmic abundance of light elements such as deuterium, helium-3, and lithium-7, which were primarily produced by nucleosynthesis in the Big Bang; as Gary Steigman of the Bartol Research Foundation explained at Fermilab, the latest measurements and the latest calculations agree beautifully, but only if the baryonic matter density is less than about 15 percent of the critical density.

Thus the large-scale dark matter problem: what, if anything, makes up the remaining 85 percent?

The "small-scale" problem has to do with the dynamics of galaxies and clusters of galaxies. The situation is fraught with observational ambiguities, as Kitt Peak National Observatory's Jay Gallagher pointed out at length to the Fermilab participants, but essentially it boils down to the fact that spiral galaxies are rotating much too fast. Especially in the outer regions, there never seem to be enough visible stars to hold a given galaxy together by gravity; it is as if the galaxy were embedded in an extended halo of invisible mass that *can* hold it together. (To be precise one should actually talk about the enhancement of density over a constant background; the large-scale dark matter, if it were uniformly distributed, would have no gravitational effect on individual galaxies.)

In much the same way, galaxies in the large clusters seem to be moving too fast, as measured by the scatter in their red shifts; unless the clusters are embedded in a substantial haze of invisible mass, they would have long since flown apart.

Thus the small-scale dark matter problem: what is this stuff?

Now, from a strictly observational standpoint there is no real reason to get excited. Technical advances during the last 5 years or so have considerably speeded up the tedious business of measuring galactic red shifts—the Harvard-Smithsonian Center for Astrophysics is now compiling some 2000 red shifts per year—and this has correspondingly im-

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