to its inhibitory subunit (known as ICAD or DFF45). In response to death-inducing stimuli, cells activate a chain of enzymes called caspases. One of these caspases cleaves ICAD, releasing the active nuclease, which is then free to wreak havoc in the dying cell's nucleus[2]. By contrast, endoG is activated by a change in its subcellular localization. Normally, endoG is found only in mitochondria, but in response to apoptotic stimuli it is released into the body of the cell[4]. Once released, endoG — like apoptosis-inducing factor[7], another mitochondrial protein required for cell death — will work even when caspase activation is limited or compromised, as might be the case, for example, during viral infection.

It is also possible that different apoptotic nucleases have distinct functions. Indeed, Parrish *et al.*[5] suggest that endoG not only participates in the 'deconstruction' of apoptotic cells, but can also contribute to the actual killing process. Using a genetic screen in *C. elegans*, these authors identified genes that, when mutated, protect cells from an activated form of the caspase CED-3. One of the genes identified, *cps-6*, probably encodes the nematode counterpart of endoG — the CPS-6 protein is also found in mitochondria, and shows nuclease activity towards both isolated nuclei and purified plasmids (small, circular DNA molecules). Indirect evidence indicates that CPS-6 probably also participates in DNA degradation in apoptotic cells in living worms[5].

Surprisingly, unlike the two previously known apoptotic nucleases[2,3], CPS-6 not only promotes DNA degradation, but also appears to contribute to cell killing. Loss of *cps-6* function slightly increased cell survival in many of the genetic backgrounds tested by the authors, including worms in which CED-3 was either weakly active, or even — rather surprisingly — completely inactive[5]. The implication is that endoG is not only sufficient to cause cell death, but might also be necessary, at least under certain conditions.

Like any good story, these two papers[4,5] leave us thirsting for more. First, how do we reconcile the previously described function for endoG — in the replication of mitochondrial DNA — with this new role in apoptosis? To the experienced apoptologist, this is hardly an issue. Ever since the discovery of the double life of the cytochrome *c* protein, as both an electron carrier and a pro-death agent, we have grown accustomed to the idea that apoptotic proteins might have quite mundane functions in normal cells.

A more serious problem arises, however, when one considers the expected localization of endoG within mitochondria. Unlike cytochrome *c*, which is found in the space between the two membranes of the mitochondrion, endoG should be within the inner matrix, deep inside the mitochondrion, if it is to participate in mitochondrial DNA replication. Yet we know that apoptotic mitochondria retain most matrix proteins. Indeed, Li *et al.*[4] show that the matrix protein Hsp70 is not released upon treatment with truncated Bid. The authors suggest that endoG might actually be found mostly in the intermembrane space, with only a small fraction participating in mitochondrial DNA replication in the matrix. Detailed organelle-fractionation analyses should settle this question.

The finding that endoG can promote apoptosis in *C. elegans* is important: it is the strongest evidence yet that mitochondria help to regulate cell death in nematodes. (Although the worm counterpart of the mammalian anti-apoptotic protein Bcl-2 binds to mitochondria[8], there is as yet no evidence that cytochrome *c* is involved in nematode apoptosis.) Unfortunately, Parrish *et al.*[5] do not provide any direct evidence that endoG leaves the mitochondria in *C. elegans*, as Li *et al.* do for mammalian endoG. However, because the loss of the worm endoG alters the kinetics of DNA degradation in apoptotic nuclei, we have to assume that it does leave the mitochondria. How it does this is a mystery, as *C. elegans* apparently lacks counterparts of Bax and Bak, mammalian proteins that are thought to be essential for allowing mitochondrial molecules to escape[9].

It is surprising that the loss of endoG activity in *C. elegans* results in increased cell survival. Studies of the previously known apoptotic nucleases[2,3] had suggested that DNA degradation has a late, non-essential role in apoptosis. Perhaps the nematode endoG acts earlier in the apoptotic pathway, at a point when a cell's fate has not yet been irreversibly sealed. In cells wavering between life and death, removal of even a single apoptotic subprogramme might be sufficient to tip the balance towards survival. Indeed, experiments in my laboratory show that eliminating the subprogramme by which dying cells are engulfed by other cells significantly increases cell survival when caspases are activated only weakly. Whether the mammalian endoG also shows cell-killing activity remains to be determined.

Finally, it is worth pointing out that endoG is not restricted to multicellular organisms — a fact that is perhaps not surprising, given its proposed role in mitochondrial DNA replication. It will be interesting to see where on the evolutionary tree endoG acquired its death-promoting character. Perhaps this nuclease is responsible for some of the caspase-independent cell deaths and ladders of fragmented DNA that have been observed in plants, fungi and protozoa. ■

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High-energy physics

Neutrinos reveal split personalities

John N. Bahcall

For more than 30 years scientists have puzzled over the mystery of the missing neutrinos emitted from the Sun. Data from underground detectors in Canada and Japan combine to provide the answer.

Neutrinos are unique subatomic particles. They have no electrical charge, travel essentially at the speed of light, and come in three types: electron, muon and tau. These particles are so elusive that you do not notice the hundred billion solar neutrinos that pass through your thumbnail every second. The 'solar neutrino mystery' began in 1968, when a pioneering experiment[1] found fewer electron-type solar neutrinos than predicted by a detailed model[2] of how the Sun shines (and how many neutrinos are detected). Two ideas were widely discussed: either the model of the Sun was wrong, or something happens to the neutrinos on their way to the Earth.

In the 1980s and 1990s, several international teams of scientists performed a range of underground experiments designed to solve this mystery. This work provided circumstantial evidence that some solar neutrinos change en route to the Earth from the electron-type produced in the Sun to another type that is harder to detect. But there was no smoking-gun evidence for such particle personality changes, usually called neutrino oscillations. This situation changed dramatically three weeks ago when scientists working in Canada announced that they had solved the mystery[3]: "The Sudbury Neutrino Observatory (SNO) finds that the solution lies not with the Sun, but

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100 YEARS AGO

Catalase is the name given to a new enzyme of general occurrence described by Dr. Oscar Loew in Report (No. 68) of the U.S. Department of Agriculture (Division of Vegetable Physiology and Pathology) with special reference to the tobacco plant. This enzyme possesses the power of producing catalytic decomposition of hydrogen peroxide, a decomposition which, according to the author's experiments, is probably not produced by any other known enzyme. The enzyme appears to exist in an insoluble and in a soluble form, which are designated α- and β- catalase respectively… Experiments on the nature of catalase indicate that it is an oxidising enzyme, the most characteristic reaction studied in this direction being its rapid oxidation of hydroquinone to quinone. Numerous tests have established the general occurrence of catalase in the vegetable kingdom. No living plant or vegetable organ tested was found free from it, some plants containing more of the soluble, others more of the insoluble, form. In the animal kingdom it also appears to be widely distributed.
From *Nature* 4 July 1901.

50 YEARS AGO

A situation provoking speculation has been found during the course of a preliminary investigation of one-shear (1½-year old) rams offered for sale at the Feilding Stud Ram Fair… Data used in these analyses were derived from the catalogues of the 1948, 1949 and 1950 sales and the 1948 Flock Book of the New Zealand Romney Marsh Breed Society. Of the 612 rams offered for sale in the three years, 52.6 per cent were sired either by lambs or by one-shear rams. The offspring of the one-shear rams brought the highest average price and had a lower rejection-rate than any other age… However, Goot has shown that one-shear sires comprise only 27.8 per cent of the sires available for use in those flocks static in numbers and consisting of 400 or more ewes. Selection of rams for the Stud Fair and for single-entry in the Flock Book is almost solely on phenotype and certainly without reference to age of the parents, nor does this latter point interest buyers. Because of this, it would be expected that the sires of rams chosen for single-entry and for sale would be represented in the same proportion as they are used in the flocks… Further analyses are planned to throw more light on these rather perplexing problems.
From *Nature* 7 July 1951.

with the neutrinos, which change as they travel from the core of the Sun to the Earth."

How did the SNO scientists — a collaboration of 113 scientists from 11 universities and laboratories in Canada, the United States and the United Kingdom — solve the solar neutrino mystery? Over 2,000 metres below the Earth's surface, within an active copper and nickel mine, the SNO collaboration built a laboratory[4] the size of a ten-storey building. Here, their underground detector is shielded from cosmic rays and radioactive contamination from dust — the laboratory is so clean it contains less than a teaspoon of dust. SNO scientists built a spherical detector, 12 metres in diameter, which contains 1,000 tonnes of heavy water (D_2O) and is itself immersed in a 30-metre cavity filled with normal water (H_2O). Neutrinos from the Sun are occasionally detected by the heavy water (about five per day), producing light that is measured by 10,000 photomultipliers.

In the initial results reported by SNO, only electron neutrinos were detected (by a specific reaction in the heavy water). A Japanese–American experiment, known as Super-Kamiokande[5], can detect all three types of neutrinos, but is mostly sensitive to electron neutrinos. But Super-Kamiokande, which uses pure H_2O in an underground detector in northern Japan, does not distinguish between electron-type and other solar neutrinos.

If only electron neutrinos travel from the Sun to the Earth, then SNO and Super-Kamiokande would measure the same number of neutrinos. If some solar neutrinos are muon or tau neutrinos, then Super-Kamiokande would measure a larger number. Indeed, the Super-Kamiokande number exceeds the SNO number with a probability of 99.96% (3.3 standard deviations), conservatively calculated. This is a smoking gun.

The Sun emits neutrinos over a wide range of energies, but SNO and Super-Kamiokande are sensitive to a specific energy range. Using data from both measurements, SNO scientists calculated the total number of these solar neutrinos that reach the Earth. The measured number agrees well (within 0.3 standard deviations) with the prediction of the standard solar model[6].

What does all this mean? In 1969 two Russian scientists first proposed[7] that neutrino oscillations cause the observed discrepancy between the predicted and measured numbers of solar neutrinos. In 1998, experiments at the Super-Kamiokande detector[8] provided the first evidence of neutrino oscillations by studying neutrinos produced when cosmic rays interact with the Earth's atmosphere. SNO has now confirmed that solar neutrinos undergo oscillation.

The Sun only produces electron neutrinos, but some muon or tau neutrinos reach us from the Sun. Therefore, solar neutrinos must oscillate from one type to another. This phenomenon, which requires that neutrinos have non-zero mass, is not predicted by the simplest version of the textbook theory of weak particle interactions (called electroweak theory). The standard theory of weak interactions must be modified slightly, which is important but not unexpected. Most importantly, the specific way neutrinos oscillate, which must be determined by future experiments, may help select the correct generalization of existing physical theories.

Neutrinos contribute to the mass density of the Universe. Combining results on neutrino masses from SNO, Super-Kamiokande and nuclear physics experiments, SNO scientists conclude that electron, muon and tau neutrinos contribute between 0.1% and 18% of the critical mass density of the Universe. The most plausible value is 0.1%. A neutrino mass density of 0.1% is probably too small to affect significantly the geometry or fate of the Universe, but it is about one-quarter of the mass density of all the stars we observe. So even though there is an enormous number of neutrinos in the Universe, the small amount of mass they contribute is not going to solve the problem of the Universe's missing 'dark matter'.

Arguably, the most spectacular result from SNO is that the total number of solar neutrinos measured by the observatory and Super-Kamiokande is bang on that predicted by the standard solar model. In appropriate units, the predicted value is 5.05 ± 0.2 and the measured value inferred by comparing the results of SNO and Super-Kamiokande is 5.44 ± 1.0. This is a triumph for the theory of stellar evolution. The predicted number of neutrinos depends on the 25th power of the central temperature of the Sun. Getting the neutrinos correct to 20% implies we can calculate the Sun's central temperature (15.7 million kelvin) to better than 1%. As stellar evolution theory is widely used to interpret observations of stars and galaxies, this agreement is a cause for rejoicing among astronomers.

Physicists are happy because they have an interesting phenomenon to study; astronomers are happy because their solar theory has been proven correct. But the work has only just begun. Scientists have so far made detailed measurements of only 0.005% of the neutrinos astronomers believe are emitted by the Sun. The remaining neutrinos are at lower energies and so are more difficult to detect. Until these lower-energy neutrinos are observed and compared with theory, we cannot be sure we really understand the intricacies of the mystery of the missing neutrinos. In the meantime, SNO and Super-Kamiokande have crucial additional measurements to make. It is a great time to be involved in neutrino research. ■

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Evolutionary biology

Searching for speciation genes

Roger Butlin and Michael G. Ritchie

The formation of new animal species often results from divergence in male sexual behaviours and female preferences. The genetic basis of this sexual isolation in fruitflies is gradually being revealed.

What are the genetic changes that underlie the origin of new species? Are there common patterns across taxonomic groups, and what do these patterns tell us about the evolutionary circumstances that promote speciation? Writing in *Proceedings of the National Academy of Sciences*, Ting *et al.*[1] and Doi *et al.*[2] arrive at contrasting conclusions. Both groups studied fruitflies, and asked how many genes need to be changed before a female switches her mating preference from males of one species to males of another. Depending on the pairs of species studied, the answer may be either many[1] or very few — perhaps only one[2].

Research on the genetics of speciation began in earnest with Dobzhansky[3] in the 1930s, and those looking for 'speciation genes' have used similar techniques ever since. To map the chromosomal locations (loci) of such genes, researchers use pairs of related species that do not normally mate but will do so in the laboratory if given no other choice. The hybrid offspring are then examined for traits that are related to 'reproductive isolation' between the two original species. Such traits might include sterility of the hybrids (a cause of postmating isolation) or differences in mate preference (which contributes to premating isolation). At the same time, researchers look for 'marker' genes that are associated with the isolating traits[4]. This provides clues to the location of the genes that underlie reproductive isolation between the original species; the assumption is that the divergence of these genes and traits contributed to the divergence of the species. But the problem with identifying such speciation genes is that reproductive isolation is usually a by-product, not their main function, and arises only when two species interact.

Most progress has been made on the genetics of postmating isolation, specifically the sterility of hybrid males. When new forms of genes (alleles) arise in diverging populations, they clearly do not spread because they cause sterility; they must be 'fixed' for other reasons. Mapping these loci has borne out Dobzhansky's suggestion[3] that hybrid sterility is based on harmful interactions between incompatible alleles that have been fixed independently in diverging populations[5]. A common pattern is that many loci are involved[6]; identification of the genes at these sites reveals the main functions of the loci, and allows one to assess whether divergence was promoted by natural or sexual selection, or by random 'genetic drift'[7].

Sexual (premating) isolation may be the main cause of speciation in animals. It is often brought about by divergence in male sexual signals and female preferences. Here, the key genetic issues are different. The main trait to look for is assortative mating, in which individuals prefer to mate with individuals who resemble themselves. But this is difficult to study. It involves up to four types of individual (males and females of both species); it is hard to separate from variation in readiness to mate; and it is based on flexible behavioural decisions that are often context-dependent and difficult to quantify. If, on the other hand, one studies the genetics of male signals or female preferences, it is hard to know how they relate to isolation. So the genetics of sexual isolation has not made as much progress as the analysis of sterility, and little consensus has emerged[8].

This is where the new papers[1,2] come in. Ting *et al.*[1] have studied the sexual isolation of the widespread M form of *Drosophila melanogaster* and the Z form found in southern Africa. Females of the Z form strongly prefer Z males over M males[9]. The authors started with flies that had an M genome, except that the two copies of chromosome III were replaced by the Z form of these chromosomes. From here, Ting *et al.* produced flies in which sections of either one or both of the Z chromosomes were replaced by sections from the corresponding M chromosome. Tests of assortative mating using females from these lines and males from reference stocks allowed the authors to map regions of chromosome III that influence female traits (Fig. 1). The reciprocal crosses allowed male traits to be mapped. The results indicate that at least four loci on chromosome III influence male behaviour and at least three affect female

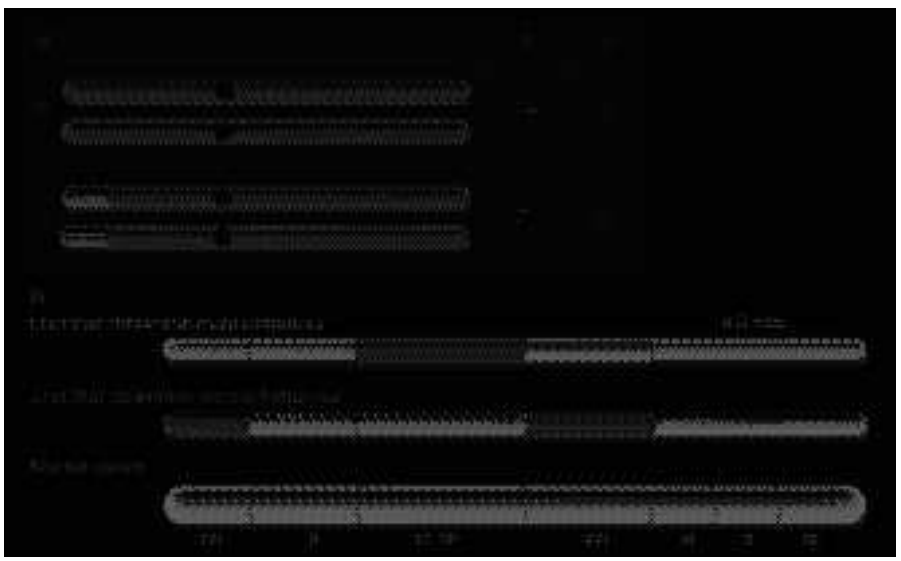

Figure 1 **How many genes cause speciation? Ting *et al.*[1] tested assortative mating — an individual's choice of mating partner — between pairs of fruitflies (*Drosophila melanogaster*). The reference stocks were the Z and M forms of this species. The 'substitution' stocks (α and β) had a genetic background that was broadly that of the M forms, but differed from the reference lines in portions of either one or both copies of chromosome III.** a, **In this case, in the β line, both copies of chromosome III have a small region of M genes (blue). These β females mate significantly more with M males than do α-line females, in which the whole of each chromosome III is of the Z form (red). The numbers represent numbers of mating pairs.** b, **Combining results across pairs of different lines reveals regions on chromosome III that have significant effects alone (red) and pairs of regions that influence mating together but not separately (green).**

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