ultra-rapidly spinning pulsar Since then several more of these pulsars have been found. Various theoretical attempts to understand the origin and evolution of these millisecond pulsars predict a substantial number of them in the Galaxy, indeed, their population may exceed a hundred thousand Therefore, a crucial test of these theoretical scenarios would be to find many more of these pulsars, and establish their true distribution in the Galaxy. However, these are very faint objects and hence extremely difficult to detect even with the largest telescopes. Thus it would help a great deal if millisecond-pulsar 'suspects' or 'candidates' could be identified. With this in mind, a careful analysis is being made of the low-frequency maps obtained in the Gauribidanur survey to identify steep-spectrum point sources.

In recent times there has been renewed interest in the source counts, as well as in the large-scale distribution, of extragalactic radio sources. In all these discussions, the main observational input has been the results of surveys done at relatively high frequencies. It may be of some interest to do a similar analysis based on the observations of extragalactic sources at a much lower frequency. There could in principle be differences in the source counts, as well as in their angular distribution. For example, some of the sources seen at low frequencies may not form a part of the population seen at higher frequencies, this can happen if they have a very steep spectrum. The Gauribidanur survey, with its wide coverage and uniform sensitivity, is of particular interest in this context.

The low-angular-resolution colour picture (cover) is intended only to highlight the large-scale features. To make detailed studies of individual objects, or a small region of the sky, one has to make contour maps of the brightness with the full angular resolution possible with the telescope. As an illustration, the contour map of the VELA supernova remnant is shown. A supernova remnant is the expanding ejecta of explosions of massive stars (Supernovae). Initially the ejecta expand at very high velocities of $\sim 10{,}000$ kilometres per second; later on they slow down owing to their interaction with the gas between the stars. Owing to this interaction they also become very powerful radio sources. According to the standard model of supernovae (originally due to Baade and Zwicky, 1934), the explosive deaths of massive stars are the result of the formation of neutron stars at their centres. [Neutron stars are extremely compact objects (radius ~ 10 km) with average density of $\sim 10^{14}$ g cm^{-3}.] Soon after these exotic stars were discovered (by Jocelyn Bell in 1967) it was found that the Vela supernova remnant harbours a central pulsar. This seemed to confirm spectacularly the association of neutron stars with the supernova phenomenon. Although this hypothesis is now extremely well established, ironically, the very first association between a pulsar and a supernova remnant (namely the Vela pulsar in the Vela supernova remnant) is now being questioned! It has been recently suggested that this may be a chance superposition in the sky. A detailed analysis of this question by Dwarakanath seems to suggest that the Gauribidanur observations of this remnant may substantially help answer the question.

1. Dwarakanath, K. S., 'A synthesis study of the radio sky at decametre wavelengths' Ph D thesis, Indian Institute of Science, Bangalore, 1989
2. Dwarakanath, K. S. and Udaya Shankar, N., *J. Astrophys. Astron.*, 1990, **11**, 323.
3. Dwarakanath, K S., Deshpande, A A. and Udaya Shankar, N., *J. Astrophys. Astron.*, 1990, **11**, 311
4. Udaya Shankar, N and Ravi Shankar, T. S., *J Astrophys. Astron.*, 1990, **11**, 297.

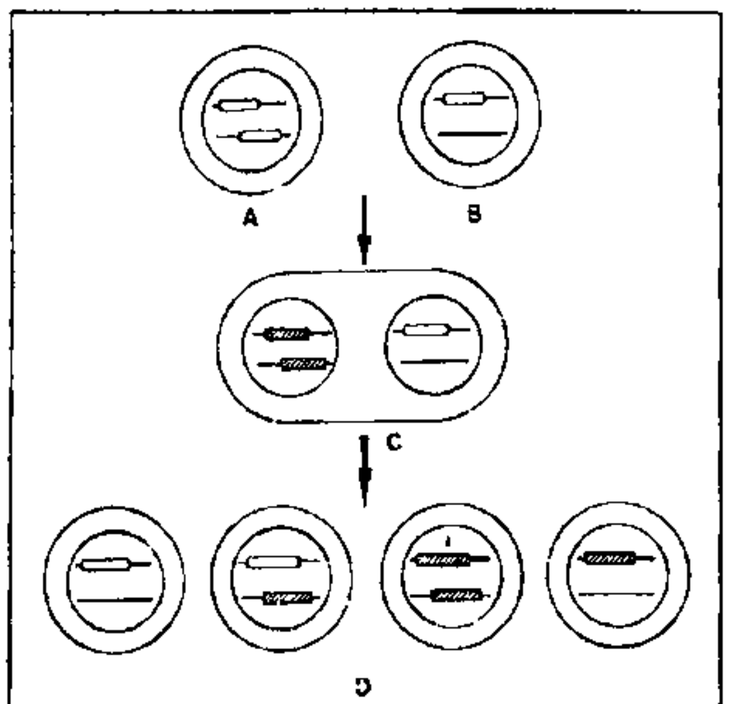
G. Srinivasan is in the Raman Research Institute, Bangalore 560 080.

RIP tidings: Accelerating divergence of duplicated DNA sequences?

D. P. Kasbekar

In 1986 Mary Case reported[1] some baffling results with the filamentous fungus *Neurospora crassa*. She had transformed a *qa-2*$^-$ strain (lacking the enzyme catabolic dehydroquinase) with the *qa-2*$^+$ allele, which went on to integrate either at the *qa-2* locus or, as in the majority of cases, at unlinked (ectopic) chromosomal locations. All the transformants maintained their transformed phenotype (qa-2$^+$) through mitosis during vegetative growth. Unexpectedly, they appeared to lose it through meiosis. This happened even in self-crosses of the transformant strains. Classically, a self-cross is expected to produce only 4 0 (in this case, 4 *qa-2*$^+$: 0 *qa-2*$^-$) tetrads, but, incredibly, often in these crosses 0:4 (*qa-2*$^+$: *qa-2*$^-$) tetrads were obtained. In other words, the *qa-2*$^+$ allele had disappeared. Some crosses even displayed extreme perversity and yielded exclusively 0:4 tetrads. These results did not make sense in 1986. Moreover, the Case study was not unprecedented, similar results demonstrating unusual behaviour of transforming DNA in *Neurospora* had been reported at least thirteen years earlier[2]. Understanding came with a novel explanation: in the sexual phase preceding meiosis, duplicated DNA sequences (such as the *qa-2*$^+$ and *qa-2*$^-$ alleles) are subjected to a previously unknown genetic process, termed RIP (repeat-induced point mutation), which results in multiple G–C to A–T transition mutations[3] and consequently dismembers both the *qa-2* alleles.

In the sexual phase in *Neurospora*, fertilization produces a dikaryon, i e a cell with two nuclei The two haploid nuclei do not fuse until after about ten mitotic divisions of the dikaryotic cell RIP occurs in the duplicated DNA sequences in the haploid nuclei of the dikaryons. Duplications are identified, possibly by homologous pairing, and



RIP in an unlinked duplication A is a haploid nucleus containing a DNA sequence (open box) duplicated on another chromosome, B is a nucleus with that sequence present only at its normal location Fertilization (top arrow) produces a dikaryon C, with two haploid nuclei RIP occurs in the nucleus bearing the duplication and produces sequence divergence by converting G–C base pairs into A–T base pairs (hatched boxes) Fusion of the two haploid nuclei then transforms the dikaryotic cell into a diploid that immediately enters meiosis (lower arrow). D shows the four haploid nuclei that issue from meiosis A round of mitosis follows meiosis and the eight daughter nuclei get encapsuled in the eight spores of the ascus

cytosine residues in both copies of the duplication are methylated. The methylcytosine residues might then be targeted for deamination into thymine residues. This is one way in which C–G base pairs can be converted into T–G mismatches that can subsequently be repaired into T–A mutations. Alternatively, it is possible that it is the unmethylated C residues that get enzymatically deaminated to U, resulting in U–G intermediates that can be converted into T–A. In other words, the details relating cytosine methylation to the transition mutations are not yet established. RIP is highly efficient; approximately 10% of the G–C base pairs in a pair of unlinked duplicated DNA sequences were converted into A–T. RIP is also highly specific; single-copy sequences remain unscathed[4].

What happens when an essential gene is duplicated? Since both copies of the duplication get riddled with point mutations, the haploid nucleus bearing the duplication is bereft of a functional copy of the gene; the heterokaryon survives nevertheless, thanks to the presence of an intact single copy of the gene in the other nucleus. Karyogamy changes each dikaryotic cell into a diploid that immediately enters meiosis. Meiotic processes such as recombination and segregation then ensure that not all the information in the affected nucleus is lost to future generations. Additionally, gene conversion of one, or both, mutated copies could also potentially undo the effects of RIP. Can RIP be a last-ditch attempt to evolve new genes before meiotic scrambling?

A round of mitosis follows meiosis, and the eight haploid daughter nuclei end up in eight spores of the ascus. Thus, a single fertilization gives rise to many dikaryotic cells, each of which produces an eight-spored ascus; the collection of asci derived from a single fertilization is called the perithecium. Studies on the fate of the duplication in related asci suggest that RIP can occur at any of the mitotic divisions preceding karyogamy, and, judging from the patterns of mutations in related asci, that the same sequence can engage in multiple rounds of RIP[5]. A measure of RIP efficiency can be gleaned from the fact that, in a tandem duplication, the repeated sequences can acquire enough differences through two sexual cycles to have diverged sufficiently for them to be immune to further RIP in subsequent cycles[3].

A strikingly similar process of premeiotic inactivation has been observed[6] in another filamentous ascomycete, *Ascobolus immersus*. In this case, however, gene inactivation is spontaneously reversible after a number of mitotic divisions, and the rate at which such reversion occurs is increased upon growth in presence of 5-azacytidine, an agent that interferes with cytosine methylation. Although these studies do not rule out the possibility of mutations, it is conceivable that premeiotic inactivation in *Ascobolus* might only involve cytosine methylation.

Could it be that methylation and deamination are two distinct facets of RIP, with different relative frequencies in the two systems? Both would inactivate the gene but only in the former case would the inactivation be reversible. In a set of elegant experiments[6], the *Ascobolus* system has been used to show that pairing is required to identify duplications. Strains carrying two or three copies of a test gene were passed through the sexual cycle and the number of inactivated copies was determined. The results showed that two or three copies could be inactivated but it was never possible to inactivate just one copy. This finding indicated that the methylation machinery recognizes paired sequences and that, when three copies are present, one of the two copies which had been methylated in one round of inactivation can nonetheless go on to pair with the third copy in a subsequent round and thereby target it for inactivation. Similar results had been obtained and the same conclusions reached in earlier studies[4,7] in *Neurospora*.

There are, obviously some duplicated genes normally resident in the *Neurospora* and *Ascobolus* genomes, for instance the rRNA genes and transposons. How do they escape inactivation and RIP? Also, is there minimum length and sequence similarity required for RIP? Now that we know how RIP may occur, it becomes important to find out how it may not.

1. Case, M., *Genetics*, 1986, **113**, 569.
2. Mishra, N. C. and Tatum, E. L. *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 3875.
3. Cambareri, E. B., Jensen, B. C., Schabtach, E. and Selker, E. U., *Science*, 1989, **244**, 1571.
4. Selker, E. U. and Garrett, P. W., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 6870.
5. Selker, E. U., Cambareri, E. B. Jensen, B C. and Haack, K. R., *Cell*, 1987, **57**, 741.
6. Faugeron, G., Rhounim, L. and Rossignol, J.-L., *Genetics*, 1990, **124**, 585.
7. Fincham, J. R. S., Connerton, I. F., Notariannı, E. and Harrington, K., *Curr. Genet*, 1989, **15**, 327.

D. P. Kasbekar is in the Centre for Cellular and Molecular Biology, Hyderabad 500 007.