of the high atmosphere would tend to reducing rather than oxidizing. Results could depend also on the phase of solar activity.

The driving perturbations and the proposed effects do deserve more study. CO_2 and CH_4 should be simulated separately to isolate mechanisms of change, the sizes of the changes in temperature, pressure and mixing ratios of chemicals should be compared to corresponding ranges of natural variability so that trends can be extracted from observations, and inconsistencies in the calculations should be removed (mesospheric concentrations of CO_2 were closer to 300 than to 330 p.p.m. in the 1950s). Also, further attention should be paid to low CO_2 and CH_4 amounts; concentrations of these species have been as low as 190 – 280 p.p.m. (ref. 10) and 0.35 p.p.m. (ref. 11) respectively in the past 160,000 years and well outside this range for CO_2 at earlier times.

Roble and Dickinson's report opens our eyes to further disturbances to the global atmosphere that are primarily due to human activities. These may turn out to be manageable or even insignificant. But like the ozone hole over Antarctica, they may be large enough to be seen from Mars or some other vantage point in space. □

Ralph J. Cicerone is in the Geosciences Department, University of California, Irvine, California 92717, USA.

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GENE TARGETING

Tapping the cellular telephone

Mario Capecchi

GENE targeting means that we now have the potential to generate mice of virtually any desired genotype. In the first instance, standard recombinant DNA technology is used to alter a cloned DNA sequence of a chosen locus; the modified DNA is then introduced into a pluripotent stem cell derived from a mouse embryo, and homologous recombination between the exogenous and endogenous chromosomal sequence transfers the mutation to the genome. Microinjection of the stem cells containing the modified locus into mouse blastocysts is used to generate germ-line chimaeras. Finally, interbreeding of heterozygous siblings yields animals homozygous for the desired mutation. This technology has already been used to create germ-line chimaeras containing targeted disruptions in the *HPRT*, *abl*, *en-2*, n-*myc*, β-2 microglobulin, *igf-2* and *int-1* genes. Despite these achievements, we know very little about the recombination mechanisms underlying gene targeting in mammalian cells. On page 170 of this issue[1], Zheng and Wilson provide an elegant demonstration of an unexpected feature not predicted by any simple recombination model, namely that the gene targeting frequency in mammalian cells is independent of the number of target sequences present in the genome. The authors report that the efficiency of targeting into the dihydrofolate reductase gene (*DHFR*) was identical in a normal and in an amplified Chinese hamster-ovary cell line containing 400 copies of the *DHFR* gene. Each *DHFR* gene in the amplified cell line is presumed to be equivalent as the DHFR enzyme and messenger RNA levels are proportional to the number of modified genes.

This observation is unexpected because the exogenous DNA sequence must search an enormous number of DNA sequences to find the cognate chromosomal sequence and participate in homologous recombination. In addition, the gene targeting frequency is independent of the number of copies per cell of exogenous DNA molecules introduced into the recipient cell[2]. This means that the search for the cognate chromosomal DNA sequence is probably not rate-limiting. Furthermore, because this gene targeting occurs at readily detectable frequencies, there is probably a cellular machinery for the efficient sampling of chromosomal sequences.

Interestingly, as the authors point out, in yeast the gene targeting frequency seems to be proportional to the number of target sequences in the genome[3,4]. For example, targeting into the ribosomal RNA genes, which are present at 140 copies per genome, is 100–200 times more frequent than into the *leu-2* gene. Gene targeting in yeast and mammalian cells also differs in a number of other respects. The frequency depends more on the extent of homology between the exogenous and chromosomal sequences in mammalian cells than it does in yeast[5–7]: in yeast this dependence is linear, but in mammalian cells it is exponential. On the other hand, the presence of homologous ends in the targeting vectors is apparently more critical for gene targeting in yeast than in mammalian cells[8,9]. But in spite of these differences, the absolute frequencies of gene targeting in yeast and mammalian cells are surprisingly comparable. The main difference between the two cell types is that when they are exposed to exogenous DNA, yeast mediates recombination events that are almost exclusively homologous, whereas recombination events in mammalian cells are predominantly non-homologous. So the problem in mammalian cells is to identify homologous recombination in a vast arena of scattered, non-homologous recombination activity.

In any organism, gene targeting exploits the cellular machinery to mediate homologous recombination between exogenous and endogenous DNA. What is the normal function of this machinery? One function must be DNA repair, but evidence is accumulating that all the DNA sequences in a cell may be continually in communication with one another. In yeast, gene conversion occurs between cognate DNA sequences on homologous or non-homologous chromosomes with comparable frequency[10]. In humans, examples are turning up in which the normal gene seems to have been mutated by gene conversion as a result of a pseudogene residing on non-homologous chromosomes. If all DNA sequences within a cell are continually talking to each other, then the function of the communication machinery could extend well beyond DNA repair. For example, it might participate in the generation, maintenance and divergence of gene families and in the shuffling of exons between genes sharing stretches of homologous DNA sequences.

At present there is a flurry of activity to employ gene targeting in pluripotent stem cells as a means to define the function of a wide range of genes in the living mouse. Our increasing understanding of the gene targeting reaction itself will continue to improve the design of such experiments. □

Mario Capecchi is at the Howard Hughes Medical Institute, University of Utah, 337 South Biology, Salt Lake City, Utah 84112, USA.

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