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COMMENT

Chromosome research—look forward to 2001

Herbert C. Macgregor

The two decades leading up to 1993 have been a period of spectacular progress in cytogenetics and they will be a very hard act to follow through into the 21st century.

Consider the scene four decades ago. Phase contrast microscopy was in the development stage. The first transmission electron microscopes were beginning to appear in a few scattered research laboratories: they were primitive and unreliable. The genetic role of DNA had just been discovered. The human chromosome number was 48 and no-one had ever seen a whole human karyotype. Computers did not exist, except for the ingenious mechanical ones that were used for bomb sighting in the 1939–45 war. Cytology was all in black and white and although the quality of the picture was often superb, our understanding of the image was severely limited. Nevertheless, the goals were clear.

First, we needed better resolution. Second, we were sure that chromosomes were linear and that each represented a single enormously long DNA double helix, so the search was on for visible linear differentiation. We had a right to expect it, since it was so obviously present in the giant polytene chromosomes that had been known and studied for more than 30 years. Third, we knew that genes were distributed along chromosomes and we wanted a method for finding them.

It all happened quite quickly. Resolution improved at the speed it took to develop light and electron microscopes, the latter being given an enormous boost with the introduction of microelectronic circuitry. Chromosome banding was developed, C-banding being very largely a spin-off from early successful attempts at *in situ* nucleic acid hybridization. It all happened in a heady period of intensely competitive science in the decade following 1965 that included the discovery of restriction endonucleases in 1969, quickly followed by the invention of recombinant DNA technology for cloning of specific polynucleotides. By 1975 all the shapes were there, recorded meticulously by early observers and classical cytogeneticists. Now they had to be explained and, at the time, there seemed to be a surfeit of tools with which to do the job.

Now we have reached 1993. We have unlocked linear differentiation. Our ability to locate genes on chromosomes is limited only by the need to isolate and label the sequences. We have improved resolving power very nearly to the theoretical limits in light microscopy, and electron microscopists have long since been operating at the molecular level. We have unravelled the chromosome and, through a crafty blend of microscopy and molecular biology, we now understand the three levels of organization that are represented in the condensed metaphase condition. We are rapidly learning about centromeres and kinetochores. We know a lot about telomeres, how they seal chromosome ends and render them non-interactive and how they facilitate the completion of DNA replication at the end of the duplex. We can measure almost anything on chromosomes, we can separate chromosomes, sort them, microdissect them, even manufacture them and of course we can sequence them. In many cases we have actually mapped the gene sequences from end to end on specific chromosomes and in two cases we have generated entire chromsome nucleotide sequence maps (Chumakov *et al.* 1992, Oliver *et al.* 1992). We have entered the era of the 'domain', whole chromosome domains within nuclei, surface domains, centromere domains, pairing domains, coding domains, specific notional aspects of a chromosome that are functionally important or intimately involved in events that we are able to define—but about which we know practically nothing! The truth in 1993 is that after nearly 100 years of chromosome research we have almost completed the basic foundations of an understanding in this field. The artistic and colourful illustrations that decorate the pages of modern undergraduate textbooks in cell biology are drawn with confidence and memorized with ease. So long as the chromosome remains still and conforms, we are comfortable. As soon as it begins to move, however, or changes its shape, our confidence evaporates, for it has to be admitted that at this point in time we simply do not know what is going on.

What can we do today that we were unable to do 10 years ago? The answer is: quite a lot, and most of it we can do much faster than before. A good example is the development of the confocal microscope. Perhaps the most astonishing thing about confocal microscopy is that in 1988 it generated instant excitement and immediately sold itself as a major new tool for biologists: yet the principle of confocal microscopy was discovered and patented and its potential fully appreciated as early as 1957.

Before confocal microscopy, the only way to visualize biological microstructure in three dimensions was to fix a histological specimen, embed it in wax or plastic, cut it into a continuous series of thin slices (sections), mount these on microscope slides in a numbered sequence, stain them,

Zoology Department, University of Leicester, Leicester, UK. Fax: (+44) 533 523330

photograph them and then generate a three-dimensional image by stacking the pictures on top of one another in the correct order. A relatively recent example of this kind of approach can be found in Dieter Von Wettstein's (1984) studies of the orientation of synaptonemal complexes in silkworm spermatocytes. It takes little imagination to sense how much time and work is involved in a process of this kind.

In the author's most recent experience a three-dimensional reconstruction of woodpigeon's lampbrush chromosomes arranged within an oocyte nucleus one-fifth of a millimeter in diameter (see the front cover of this issue of *Chromosome Research*) was obtained, by confocal microscopy, just a few hours after the bird had been flying over a field in the Midlands of England.

Naturally, the success of the confocal microscope is not entirely due to the principle discovered by Minsky. The full potential of his invention could never have been realized in 1957, because there were no computers. It is one thing to obtain a clear image, still another thing to generate a stack of images, quite another thing to integrate these images into a three-dimensional reconstruction. The latter simply cannot be done without a computer. But once you have your computer linked to your confocal system, then you can do all kinds of things, magnify and demagnify, scan in the z axis, measure depth, thickness, density, produce complex image enhancement effects—all of this away from the microscope, since once the images are generated and stored on disk, the microscope is no longer needed and can instantly be made available to the next eager investigator in the queue.

Confocal microscopy is just one of the refinements that have speeded up cytological investigation in recent years. Another clear example is the use of things like biotin, digoxygenin and fluorochrome/nucleotide conjugates for *in situ* nucleic acid hybridization, coupled with immunocytochemistry and fluorescence microscopy. The technique of FISH (fluorescence *in situ* hybridization) was first introduced by Langer and Ward in 1981 (Korenberg 1992, Trask 1991). Before that, all *in situ* hybridization was carried out with radiolabelled probes detected by autoradiography, involving exposure times of hours, days, weeks and sometimes even months before the slides could be developed, the autoadiograph examined and the location of the *in situ* hybrid determined. With FISH, the entire operation takes about a day.

One thing is absolutely clear: progress in modern cytogenetics is limited largely by technological development, and progress in relation to things like optics, computing and biomolecular engineering, is currently happening at a pace that more than matches our capacity to formulate questions and design new experiments around chromosomes.

In the midst of all this modern high technology, the exploitation of animal and plant diversity will remain for ever by far the most powerful scientific tool that we have in our box. It is a multipurpose tool. Diversity represents a living record of Nature's successes and failures within the framework of evolution and natural selection. Show biologists something they have never seen before in an animal and the first question they will ask is whether the same thing or a modified form of it is present in other animals or plants. If they find a modified form, then they will search further afield for other forms on the principle that by looking at Nature's variations, they will be able to sort out the important features of the new phenomenon from the trivia and get closer to an understanding of form, function and evolution.

What are likely to be the priority areas for future research in cytogenetics? The topics are easy to list. Meiosis remains full of unknowns and most of them are quite formidable. What are recombination nodules? How does the synaptonemal complex work? How are interlocks resolved? What brings homologous chromosomes into pairing association with one another? When is pairing initiated and when does recombination happen? Have all the features of meiosis evolved by a process of natural selection, and if so, what are the selective advantages of each? Chromosome disjunction must be high on our list of priorities. We are, to be sure, well advanced with our understanding of centromeres, kinetochores, microtubules and chromosome movement—an altogether fascinating area of modern cell science—but there is still a long way to go before we understand well enough to explain the countless diversities of disjunctional behaviour and spindle mechanics.

Imprinting and sex will remain high priority areas. How does a chromosome 'know' it has a paternal origin? Will we ever be able to understand the molecular basis of events such as those that accompany gametogenesis in males of *Sciara coprophila* (White 1973)? What is the molecular basis of chromosome inactivation? What is the molecular basis of sex determination, for here we have the prime example of a limited number of genes localized in one small region of a specific chromosome operating as a major developmental switch mechanism?

Nucleus and karyotype have become especially important in recent years, perhaps because computers, confocal systems, interest in DNA/protein interactions and the development of chromosome 'painting' and whole genome *in situ* hybridization (GISH) have enabled us to consider whether there is more to an interphase nucleus than just a membrane-bounded compartment occupied by jumble of threads. In the past we have been so preoccupied with the orderly events of cell division that the interphase nucleus has seemed unimportant. The increasing volume of evidence showing that patterns of gene expression and events at cell division are strongly influenced by the behaviour of whole chromosomes in interphase, compels us to shift our attention and ask about the relative positioning of chromosomes in relation to one another and to the things that happen to them earlier or later in the cell cycle. It is a field that was pioneered and developed by people like Michael Bennett and J.S. (Pat) Heslop-Harrison in plants (Heslop-Harrison 1993) and by Laura Manuelidis and her associates in humans (Borden and Manuelidis 1988). Their tenacity, enterprise and skill have paid off handsomely. Nuclei are now seen as dynamic structures with a design, structure and internal arrangement of chromosomes that is flexible, versatile and

functionally significant. There is absolutely no doubt that this is going to be an exceedingly active topic of research in years to come.

Whole genome *in situ* hybridization as a technique offers tremendous potential for detecting origins and homologies amongst related karyotypes, and it is likely to be regarded in years to come as one of the most successful compound methodologies to be developed in recent times.

To many cytogeneticists, karyotypes have become more interesting than individual chromosomes. An organism's karyotype is seen as a species-specific character that has evolved through natural selection. It is less likely to be of adaptive significance to the individual than to the population. In plants, fertile interspecific and sometimes even intergeneric hybrids can be made with the greatest of ease. We can do almost anything with the chromosome set of *Drosophila melanogaster* in the sense of rearranging it or chopping it up and we still get a *D. melanogaster*. On the other hand, we cannot mix two animal karyotypes that have evolved separately, even though they contain the same genes and derive from the same 'species', and expect to get viable offspring: among animals, the 'coadapted genome' is something we do not yet understand and it will doubtless occupy many cytogeneticists in the years ahead.

Of course the commercial and social impact of cytogenetics is already immense, in relation to both animals and plants. Tumour cytogenetics, as well as pre- and post-natal diagnostic cytogenetics have had a tremendous impact on clinical medicine. The early concept of Theodore Boveri (1914) that chromosome changes are at the heart of the cancer process, has been fully confirmed. More than 50 specific chromosome rearrangements have been defined in human leukaemias and solid tumours and serve as markers for chromosome regions that must be carefully explored at the molecular level in search of clues to the biology of cancer. The molecular cytogenetic analyses of solid tumours has been boosted by the use of comparative genomic *in situ* hybridization as a means of detecting relative DNA sequence copy number, an effective means of detecting localized, tumour-specific amplifications of certain DNA sequences (du Manoir *et al.* 1993). With regard to human clinical cytogenetics, nearly half the pregnancies of women over 35 in industrialized nations are now screened prenatally for foetal chromosome abnormalities and we are on the verge of prenatal diagnosis from maternal blood on the basis of 'chromosome painting' techniques. With regard to plants that are of commercial importance to man, more than half the British wheat crop, for example, and vast areas of crop agriculture in other parts of the world are the result of cytogenetic manipulation—the directed incorporation of alien chromosome segments from different species into wheat.

Chromosome engineering, currently the most important area of genetic engineering, is likely to be even more widely applied in agriculture and perhaps eventually also in medicine, if the ethical problems can be resolved. Transferring genes has to be regarded as the high impact biotechnology of the 21st century, and in this sense, an expanded understanding of chromosome recombination, genome behaviour in hybrids, gene expression and chromosome evolution is likely to be hotly in demand.

Nevertheless, cytogenetics remains a field where it is still possible for one person on their own, working with an unusual organism, to make a discovery that will put them into the history books of science. The unknowns are tough tests of human ingenuity and experimental skill, but we should not let that deter us from having a go. Modern chromosome research is colourful, exciting and usually rewarding. We hope that the pages of this journal will help to affirm that impression.

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