

Visualizing a Concept

Methods of Looking at Active Genes

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“Ever since the gene hypothesis was generally accepted, geneticists and cytologists have dreamed of the day when it would be possible to see the actual genes, instead of having to be satisfied with studying their “shadows”, which were “reflected” in the morphological development of generations of organisms” (T S Painter, 1934).

Chromosomes on which genes are located can be viewed under the microscope during cell division. Unfortunately, even during the mitotic metaphase stage when the chromosomes are most distinctly identifiable, the chromatin fibre that forms the chromosome is so highly condensed that individual genes cannot be seen even with the highest possible resolution and magnification of the electron microscope (see Vani Brahmachari in Suggested Reading). Moreover at this stage of the cell division cycle, the genes are completely inactive and therefore, the process of gene activity cannot be visualized. On the other hand, in the interphase period when the genes are active, the chromatin fibre is so highly dispersed that individual chromatin fibres remain unresolvable. It is in this context that the ability to see genes in reality, rather than dream of them only as a conceptual entity (see S C Lakhotia in Suggested Reading), becomes very exciting. This article discusses some of the experimental paradigms that can be used to *see* genes.

Certain special cell types carry the so-called ‘giant chromosomes’ which allow direct viewing of genes under the microscope. The lines quoted in the beginning of this article from a paper published in 1934 by T S Painter reflect the excitement of cytologists of the 1930s when they realized the significance of polytene chromosome (see *Box 1*). The polytene (poly = multiple; tene =



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Box 1 Polytene Chromosomes – A Highway to the Lair of Genes

Polytene chromosomes were first viewed under microscope in cells of *Chironomus* larvae by E G Balbiani in 1881 although their chromosome nature was understood only 50 years later in the early 1930s. Displaying a remarkable foresight, T S Painter in a paper very aptly entitled 'Salivary chromosomes and the attack on gene' published in 1934 in *The Journal of Heredity*, stated "With these four discoveries before us (i. e., constant and distinctive patterns, somatic synapsis, the behavior of active and inactive regions and the separation of arms of the large autosomes) it was clear that we had within our grasp the material of which every one had been dreaming. We found ourselves out of woods and upon a plainly marked highway with by-paths stretching in every direction. It was clear that the highway led to the lair of the gene".

These prophetic and optimistic statements have indeed been borne out by subsequent studies which not only physically localized specific genes on specific sub-regions of the chromosomes but also allowed us to really see genes when they were active. Studies with polytene chromosomes provided direct demonstration that active genes make RNA and that correlated with the new RNA, new proteins are synthesized in the cell. The first insights into the mode of action of steroid hormones on cellular physiology through specific gene activation were also obtained through studies on polytene chromosomes.

threads) chromosomes are commonly found in certain cell types of dipteran insects like *Drosophila*, *Chironomus*, *Anopheles* etc. These giant chromosomes result from repeated duplication of chromatid fibres within the same nuclear envelope (endoreplication) and a very tight association of the daughter chromatid fibrils so that each chromosome consists of hundreds or thousands of sister chromatids. Since these bundles of sister chromatids are not as highly condensed as the metaphase chromosomes, it is possible to study the sub-chromosomal organization of genetic material in these cells even with a light microscope. In the illustration of a polytene chromosome spread of *Drosophila melanogaster* in *Figure 1*, the nucleus has undergone 8 or 9 cycles of endoreplication so that the chromosomes are very large and are characterized by a series of dark and light stained regions, the band and interband regions, respectively. Every band has a characteristic and reproducible shape and size due to which each of these have been individually numbered and can be identified easily. A polytene nucleus in *D. melanogaster* shows five long arms corresponding to the acrocentric¹X-chromosome,

¹ Acrocentric chromosomes have the centromere at one end while metacentric chromosomes have the centromere near the mid-point along the length of the chromosome.



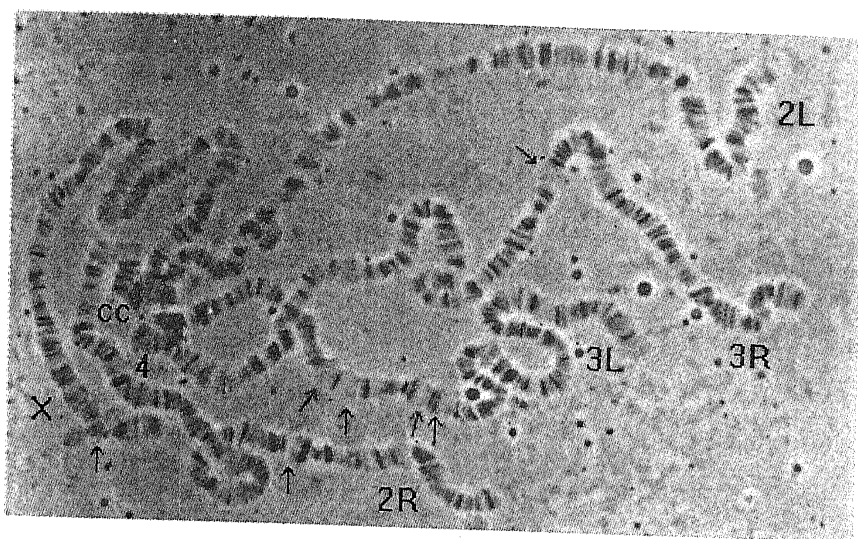


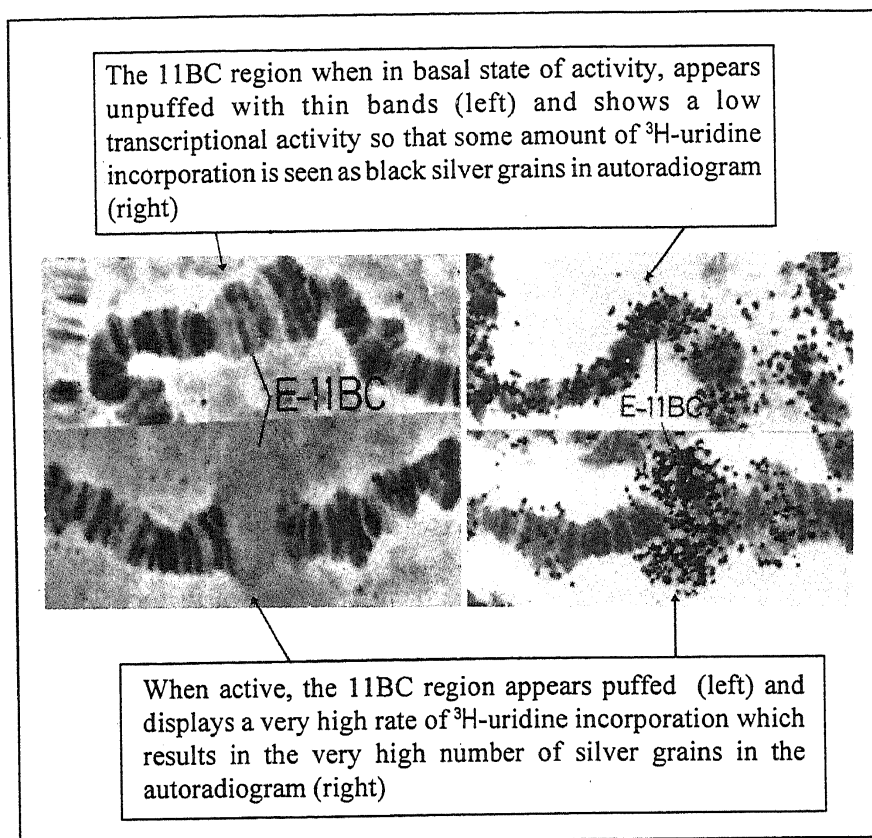
Figure 1 A polytene chromosome spread of *Drosophila melanogaster*.

the left and right arms of the metacentric chromosomes 2 and 3 (2L, 2R, 3L and 3R, respectively) and the small 4th chromosome; all the heterochromatic regions near the centromere of the X-chromosome and the autosome and whole of the Y-chromosome (in the case of males) remain coalesced into a common chromocentre (cc). Since the homologous chromosomes show tight somatic pairing (as in most other dipterans), each of the chromosome arms actually corresponds to the two homologs (the two homologs may appear unpaired over short regions in rare cases).

In general, each darkly stained band region appears to correspond to one gene, although there are many bands that contain more than one gene and there are some genes that span more than one band! It was shown by W Beermann in the 50s that genes that are active in polytene nuclei can be viewed under the microscope as swollen and less stained regions, called *puffs* (very large puffs in polytene cells of *Chironomus* larvae are termed *Balbani Rings* after the original discoverer of these chromosomes). Puffing results from a localized decondensation of the constituent chromatin fibrils of a dark band in preparation for transcriptional activity. Incubating live polytene cells in a medium containing ^3H -uridine for a brief period (5-10 minutes) allows the newly synthesized RNAs to be radio-labeled. Cellular autoradiography² of chromosome preparations of labeled cells permits identi-

² Autoradiography is a technique that records the decay of radioactive elements on a photographic film. In cellular autoradiography, live cells, which have been allowed to incorporate a specific radioactively labeled precursor (e.g. ^3H -uridine for labeling the newly synthesized RNA), are fixed, placed on slides, and then permanently covered with a special photographic film (nuclear emulsion) and stored in dark for sometime. After sufficient exposure to the radioactive decay of the label present in the cells, the film, still adhering to the slide, is developed and fixed while the cells are stained through the film with appropriate dyes. A microscopic examination now enables one to see the cells and the overlying film with minute silver grains, each of which represents a radioactive decay. This allows precise localization of the site in the cell where the radioactivity was localized, and thereby, of the site/s where a given metabolic activity (e.g., RNA synthesis in the case of ^3H -uridine labeling) was in progress.

Figure 2 A segment of the polytene chromosome E of *Drosophila kikkawai*.



fication of the sub-chromosomal sites where RNA synthesis is in progress. The illustration in *Figure 2* shows a segment of the polytene chromosome E of *Drosophila kikkawai* in which the 11BC region is either in a relatively inactive state or in a transcriptionally very active state: the left panel shows phase contrast images of this chromosome region while the autoradiographic images of ^3H -uridine labeled chromosomes are shown in the right panel. Note that when the 11BC region is active, it appears puffed and also shows a considerably increased uptake of ^3H -uridine as revealed by the presence of a larger number of silver grains in the autoradiogram.

The other cell types which allow visualization of active genes under the light microscope due to their 'giant chromosomes' are the primary oocytes of several salamanders where *lampbrush* chromosomes are seen during the diplotene stage (see *Box 2*). These giant chromosomes display large lateral loops (the presence of these numerous lateral loops arising from each

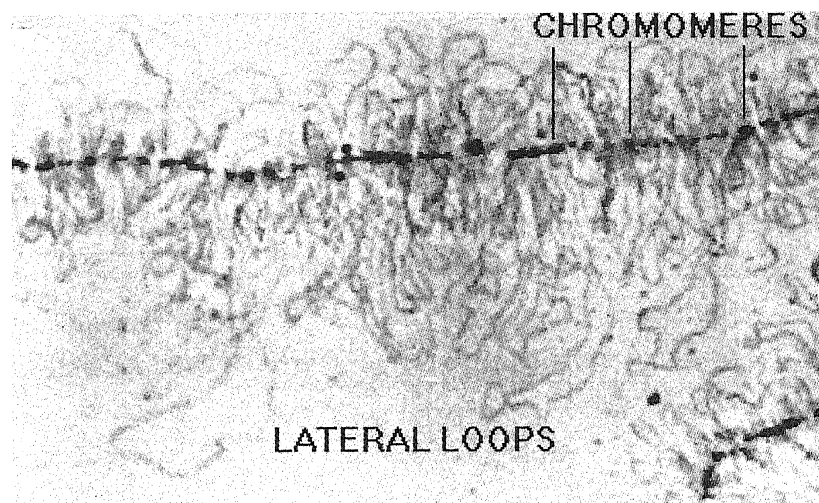
Box 2 Lampbrush Chromosomes – Oocyte Specific and Transcriptionally Active Chromosomes

J Rückert while working with shark oocytes first published an account of these special chromosomes in 1892 and made three important observations: i) these special structures are chromosomes, ii) the chromosomes are associated in pairs and iii) most of the lateral projections are loops with both their ends stuck into a chromosome axis. He named them *lampbrush chromosomes* in view of their apparent similarity with the brushes used in those times to clean the oil lamps. W R Duryee between 1937 and 1941 made a significant contribution to their studies by developing a manual isolation procedure for the lampbrush chromosomes from living oocytes of frogs and newts and thereby demonstrating their interesting biological properties. Subsequent studies between the 50s and 80s of this century on lampbrush chromosomes, particularly from salamanders and frogs, have contributed enormously to our understanding of eukaryotic chromosome organization and function. These studies helped establish the concept of only one continuous DNA double helix throughout the length of a chromatid (UNINEMY of chromosomes) and thereby exposed the C-value paradox (presence of much more DNA than what seems to be transcribed and what may be considered as essential). Studies on lampbrush chromosomes also established the polarity of transcription in a given transcription unit. H G Callan, one of the pioneers in modern studies on these chromosomes had proposed two famous hypotheses, namely, the *master and slave* hypothesis (to explain the C-value paradox) and the *spinning and retraction* hypothesis (to explain the dynamics of lampbrush loop function). These two hypotheses stimulated a lot of research that contributed to our understanding of the general organization and function of genomes in eukaryotes. Ironically, however, both the hypotheses were subsequently proven to be wrong! This demonstrates an important aspect of science. Even an hypothesis that may ultimately turn out to be wrong, may contribute immensely to the progress of the subject if it stimulated newer studies. There are any number of such examples in the history of science where quantum progress occurred due to ideas that finally were found to be wrong!

It is also interesting to note that for quite some time the lampbrush chromosomes were cited as important evidence for DNA not being the genetic material because they did not stain with Feulgen stain (the Feulgen staining has, since its discovery in 1926, remained a very specific staining procedure for cellular DNA). The apparent absence of Feulgen staining in the lampbrush chromosomes found in developing oocytes was used by opponents of DNA's role in heredity as a strong argument against any genetic significance of DNA. It was established only much later that DNA is indeed present all along the length of lampbrush chromosomes: the apparent lack of Feulgen staining of these chromosomes was due to the chromatin fibre being highly extended, a feature that makes these chromosomes suitable for gene activity studies.



Figure 3 Parts of a lampbrush chromosome.



chromomere on the chromosome axis gives them the lampbrush appearance and hence the name). The lateral loops are active in RNA synthesis which can be visualized by ^3H -uridine labeling. Each of these loops has a distinctive morphology and generally consists of one transcription unit; the newly synthesized RNA remains associated with the loops. Parts of a lampbrush chromosome at the diplotene stage of meiosis are shown in *Figure 3*. It also depicts lateral loops originating from the dark chromomeres from one of the two sister chromatids.

A classical picture of 'genes in action' was provided by O J Miller and his associates in the late 60s when they examined the chromatin fibres carrying the ribosomal RNA genes of amphibians by electron microscopy of surface spread preparations.

Another dramatic visualization of active genes can be made by viewing surface-spread ('Miller-spread') preparations of interphase nuclei under the electron microscope. A classical picture of 'genes in action' was provided by O J Miller and his associates in the late 60s when they examined the chromatin fibres carrying the ribosomal RNA genes of amphibians by electron microscopy of surface spread preparations (see *Figure 4*). The ribosomal RNA genes occur in multiple copies and are present as clusters with the transcription units being separated by non-transcribing spacers. Each of these multiple rRNA transcription units is very actively transcribed in growing oocyte of amphibians by a large number of RNA polymerase molecules. As a result, each ribosomal RNA transcription unit gives a typical *Christmas tree image* with the successively longer branches being the nascent and elongating rRNA transcripts. As seen



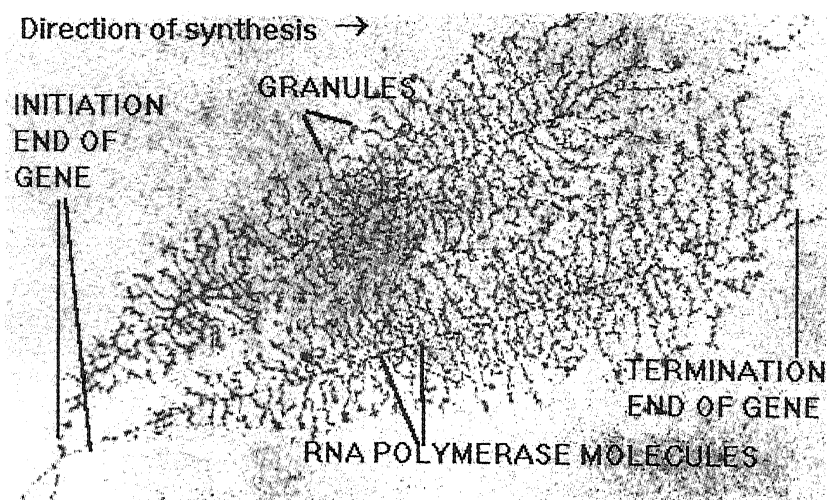


Figure 4 A picture of genes in action – the Christmas tree image.

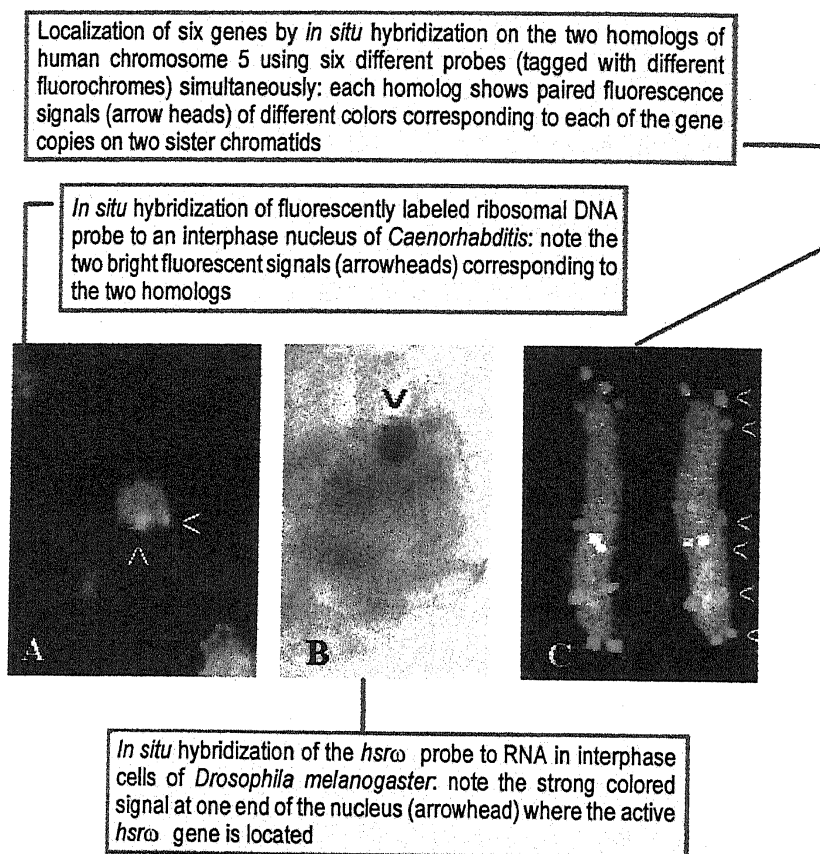
in *Figure 4*, the transcriptionally active regions of the DNA fibre are studded with RNA polymerase molecules, each of which carries an elongating nascent 45S rRNA molecule. As each RNA polymerase molecule moves along the DNA strand and transcribes subsequent bases, it carries with it the nascent 45S rRNA so that the size of the nascent transcript increases as one proceeds from the initiation to the termination end of the transcription unit which results in the Christmas tree image. Such images provided a direct evidence for existence of transcription units with well defined transcription start and termination sites. One can view active transcription of other genes also by this method but since the number of RNA polymerases associated with most of the non-ribosomal transcription units is not so high as with the ribosomal genes, only a few elongating nascent RNA chains are seen.

Besides these classical ways of looking at active genes, recent advances in molecular cell biology techniques permit viewing of active genes in ordinary cell types also. One such method is the technique of *in situ* hybridization (*Figure 5*). In this method, a specific labeled RNA (anti-sense) or DNA probe is used to hybridize *in situ* with the RNA in the cell on a microscope slide: the site/s of hybridization correspond to the location of that particular RNA. If the gene in question is active and the transcripts produced by it have not moved away when the cells

The technique of *in situ* hybridization allows one to see genes and/or their transcripts in any ordinary cell type at any stage of the cell division cycle.



Figure 5 The *in situ* hybridization technique.



were fixed, the hybridization signal corresponds to the location of the active gene in nucleus (see panel B in *Figure 5*). If the gene was active for a longer period, its transcripts would also have moved to other cellular locations (e.g., the cytoplasm) and one would detect a signal in the parts of cytoplasm where the transcript is localized. *In situ* hybridization of specific labeled RNA or DNA probes to cellular DNA also locates the gene in the nucleus irrespective of its activity status (see the panels A and C in *Figure 5*).

In situ hybridization studies have provided considerable insight into the organization of genes and chromosomes in interphase nuclei. A particularly remarkable variation of *in situ* hybridization is the technique of *chromosome painting*. In this technique, one uses fluorescently labeled DNA probes that span the entire length of a specific chromosome (collection of probes derived

Confocal
Microscopy in
conjunction with *in situ* hybridization
techniques allows
viewing of genes
not only in the
three dimensional
space of the cell
but also in the
fourth dimension of
time.

from a 'chromosome-specific library') to hybridize interphase or metaphase cells. In interphase nuclei, the entire chromatin fibril corresponding to that particular chromosome can thus be viewed as a distinctly fluorescing thread meandering through the volume of the nucleus. In conjunction with Confocal Microscopy³ chromosome painting or other forms of *in situ* hybridization allow unprecedented detailed information on the topographical distribution of individual chromosomes or genes in the 3-dimensional space of a nucleus. Since confocal microscopy can be applied to living cells, behaviour of specific genes or the progression of transcription on a gene in time can also be studied.

³ Confocal Microscopy allows imaging of an intact cell in three dimensions without the need of either flattening it or cutting it into thin slices as required for conventional microscopy systems.

The very rapid progress in biological sciences during the past several decades has been possible only because of a judicious synthesis of technical approaches drawn from different disciplines. As the examples considered above show, it is possible now to apply very sophisticated molecular techniques at cellular level and thus *see* things as they happen *in situ* in the cell. The molecular cell biological approach thus bridges the gap between those who as biochemists or molecular biologists work in 'test tube' and those who, as general biologists, work at 'supra-cellular' levels.

Suggested Reading

- ◆ Vani Brahmachari. Know your chromosomes. *Resonance*. Vol. 1, No. 1. January 1996.
- ◆ S C Lakhotia. What is a gene? *Resonance*. Vol. 2, No. 4 and Vol. 2, No. 5. 1997.

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Science consists in grouping facts so that general laws or conclusions may be drawn from them.

Charles Darwin