Lecture 7.
Polytene chromosomes.
Lampbrush chromosomes

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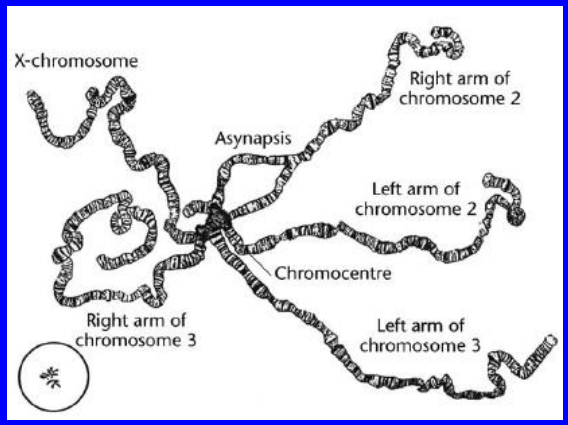
POLYTENE CHROMOSOMES

Polytene chromosomes were discovered by Balbiani (1881) in larval salivary glands, Malpighian tubules, intestine, hypoderm and muscles of Chironomus plumosus as a cylindrical cord that repeatedly unravelled and filled the nucleus. He called this structure a 'permanent spireme'.

In 1933–1934, three groups of researchers (Painter, 1933; Heitz and Bauer, 1933; King and Beams, 1934), using squashed preparations, showed that the 'spireme' is not continuous but consists of separate elements whose number is close to the haploid number of the mitotic chromosomes. Each element is formed as a result of the tight synapsis (joining together) of homologous chromosomes. Each chromosome has a definite and constant morphology and is composed of segments, displaying a distinctive transverse-banding pattern .

The giant size of the salivary gland chromosomes was explained by Koltzoff (1934) as being the consequence of multistrandedness.

The term 'polytene' was proposed by Koller (1935) and adopted by Darlington (1937).



PECULIARITIES OF POLYTENY:

1. The formation of polytene chromosomes is associated with the elimination of the entire mechanism of mitosis after each DNA doubling, as a result of which the cell cycle consists of just two periods, synthetic (S) and intersynthetic (G).

2. At the end of each replication period, DNA strands do not segregate; rather, they remain paired to each other to different degrees.

3. The polytene chromosomes formed are incapable of being involved in mitosis.

4. The nuclear membrane and nucleolus remain intact during consecutive DNA replication cycles.

Polyteny arises and attains high levels in tissues, organs and at developmental stages when there is need for the rapid development of an organ at an unaltered high level of function. Organs containing cells with polytene chromosomes are, as a rule, involved in intense secretory functions accomplished during a short time against a background of rapid growth.

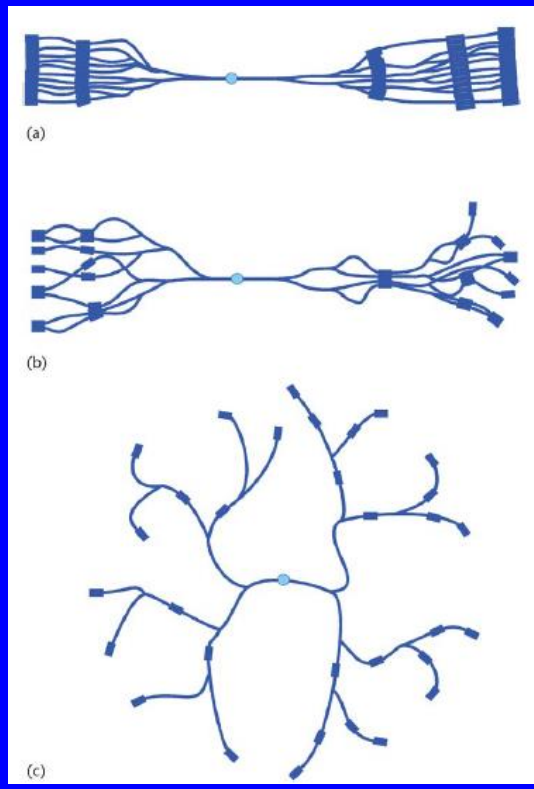
MORPHOLOGY OF POLYTENE CHROMOSOMES

The morphology of polytene chromosomes can vary widely due to the differing degrees of synapsis of the DNA strands. Polytene chromosomes develop from the chromosomes of diploid nuclei by successive duplication of each chromatid.

If homologous chromatid conjugation is maximal, classic polytene chromosomes, i.e. cylindrical cables with a distinct banding pattern (Figure a).

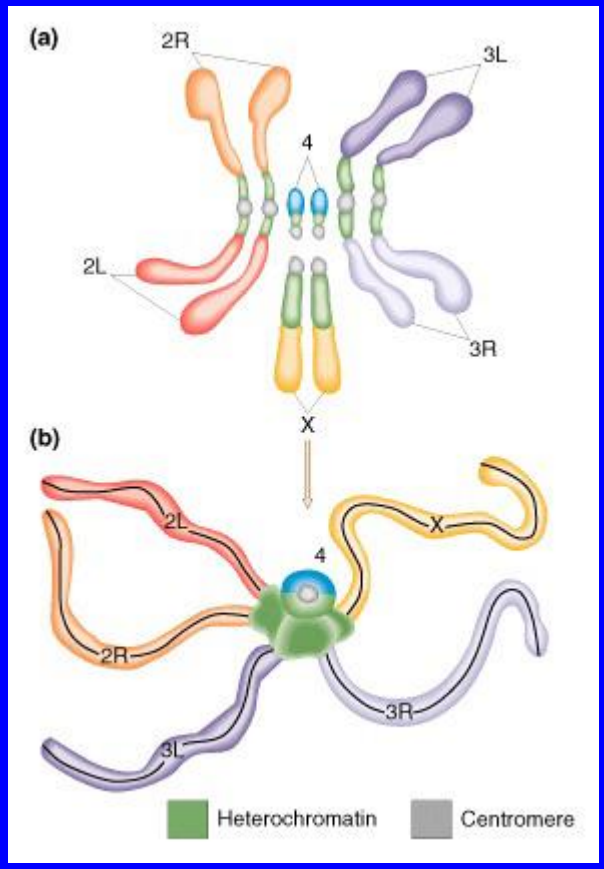
If the degree of chromatid conjugation is minimal, a polyploid nucleus with a reticular structure is formed (cryptic polyteny) (Figure b).

In some cases, conjugation of chromatids is disturbed in only one chromosome of the set. This polytene chromosome then completely loses its banding pattern and looks diffuse, becoming a so-called *pompon-like chromosome* (Figure c).



MORPHOLOGY OF POLYTENE CHROMOSOMES

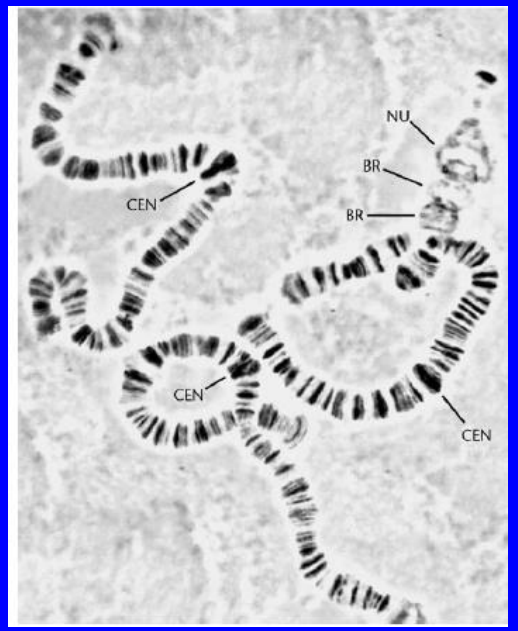
Viewed broadly, the morphology of polytene chromosomes in females and males is the same. The X-chromosome of *Drosophila* males, however, has a different appearance. Although the polytene X-chromosome of the male contains half as much DNA as that of the female, it occupies almost the same area as the two female X-chromosomes. It is more loosely packed, twice as much RNA is synthesize in it as in a single female chromosome and it contains more nonhistone proteins than the female X-chromosome. In the male, the Y chromosome remains part of the chromocentre, along with the centromeres and pericentromeric heterochromatin.



MORPHOLOGY OF POLYTENE CHROMOSOMES

In many polytene nuclei, nonhomologous chromosomes are joined by their centromeric regions to form a common ***chromocentre***. Chromocentres in polytene chromosomes correspond to heterochromatin in mitotic chromosomes. There are two types of heterochromatin in Drosophila species: a and b heterochromatin. The ***a heterochromatin*** consist mainly of satellite DNA (i.e. short nucleotide sequences repeated hundred thousand or even million times). The ***b heterochromatin*** comprises mainly middle repeated mobile elements. In many other species a common chromocentre does not form.

There are about 240 regions of the so-called intercalary heterochromatin scattered throughout the genome. These manifest some of the characteristics of the centromeric heterochromatin: late DNA replication, underreplication during polytenization cycles and ectopic pairing. However, intercalary heterochromatin in contrast to centromeric contains genes, necessary only during a certain stage of development. After that those genes must be surely repressed.



OCCURRENCE OF POLYTENY

Organism	Organ	Type of polyteny
Insects, Diptera		
Larvae	Salivary glands, gut, midgut, hindgut, gastric caeca, Malpighian tubules, fat bodies, epidermal cells, hypoderm, ring gland	Classic type
Pupae	Malpighian tubules, cardiac wall, fat body, rectum, foot pad cells, bristle-forming cells	Classic type
Adults	Malpighian tubules, hindgut, midgut, fat body	Classic type
	Ovarian nurse cells	Classic or cryptic types. Cryptic types can be transformed to the classic type at the expense of mutations or inbreeding
Insects, Collembola	Salivary glands	Cryptic, cryptic–classic, classic types
Protozoa, Infusoria	Macronuclear anlage	Classic type
Mammals	Trophoblast cells	Cryptic, semicryptic
	Tumour cells	Cryptic type
Plants	Antipods, suspensors, endosperm, synergids, endosperm haustorium, tissue culture, callus culture	Cryptic, can be transformed to classic, e.g. at low temperature

MULTISTRANDEDNESS OF POLYTENE CHROMOSOMES

Growth resulting from an increase in the size of relatively few cells, rather than an increase in cell number through cell division, is a phenomenon well known in the Insecta. Such growth is accompanied by a parallel increase in nuclear size and DNA content. At present, there is substantial evidence for chromosomes of this type being a bundle of individual chromatids. Polyteny levels (C) differ considerably in different cells within an organ, between organs and between organisms and species.

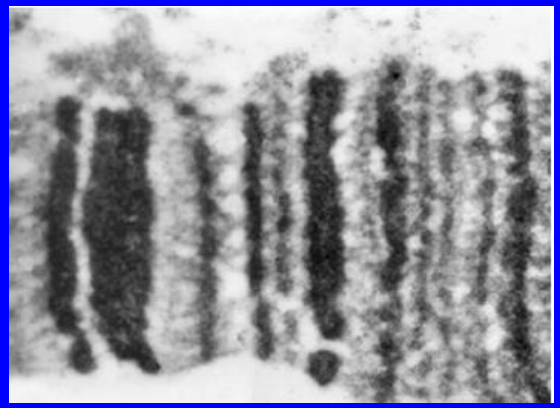
Not all DNA fragments in an individual chromatid polytenize to the same extent. Local underreplication of DNA during polytenization is most evident in the pericentric heterochromatin, and in intercalary and telomeric heterochromatin.

Species	Organ	Degree of polyteny (C)
Chironomus plumosus	Larval salivary glands	1024–4096
Chironomus tentans	Larval salivary glands	8192–32 768
Drosophila melanogaster	Larval salivary glands	1024–2048
	Larval midgut	512–1024
	Imaginal Malpighian tubules	2–256
	Larval fat bodies	16–512
	Larval prothoracic gland	64–512
	Ovarian nurse cells	512–8192
Rhynchosciara angelae	Larval salivary glands	4000–16 000
	Larval salivary glands after intracellular microsporidial infection	512 000–1 024 000
Mammals, different species	Trophoblast	64–4096
Plants, different species	Suspensor, haustorium, antipods and synergids	2–8192

BANDING PATTERN

Along the linear axis of each chromatid, variation in the extent of coiling of the DNA and its associated proteins leads to variation in the concentration of the chromatin. Regions of high concentrations are known as chromomeres. For each chromatid the pattern of chromomeres is highly specific so that in the polytene chromosome homologous chromomeres align alongside each other exactly and usually fuse as a band across the polytene element. The banding of polytene chromosome is a stable and specific feature of their organization that the individual bands can be recognized, mapped and assigned reference numbers. In the interchromomeric or interband regions, the DNA and protein concentration is lower than in the bands.

The structure of the chromomeres is flexible and the general banding pattern is highly variable when intra- and/or extracellular conditions change.



SOMATIC SYNAPSIS OF HOMOLOGOUS CHROMOSOMES

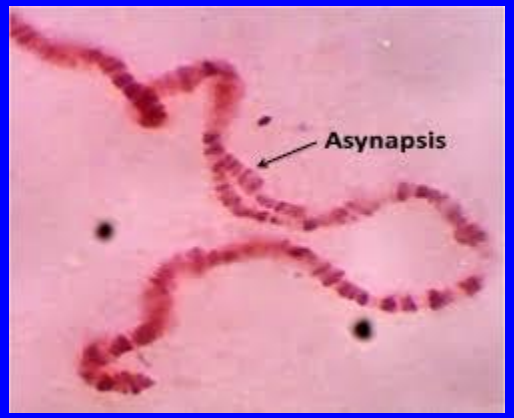
Somatic synapsis occurs when two homologous polytene chromosomes fuse. The chromosomes synapse band to band with high precision, giving the impression that a single chromosome is produced.

Somatic synapsis is not an obligatory feature of polytene chromosomes: homologous chromosomes consistently conjugate to various degrees in dipteran insects, but in plants or Collembolan insects synapsis is normally absent.

The frequency of salivary gland nuclei showing disturbed synapsis in any of the chromosomes during normal development of *D. melanogaster* varies between 6.5% and 45% according to different authors.

Enhancement of asynapsis occurs in hybrids from crosses between representatives of various forms or races.

Modifiers of position-effect variegation (temperature, quantity of Y-chromosome heterochromatin) influence the degree of asynapsis.



MOLECULAR ORGANIZATION OF BANDS AND INTERBANDS

The band number in the *D. melanogaster* genome can be estimated at 3500–5000. Euchromatin contains approximately 120 megabase pairs of DNA and about 14 thousand genes. A medium-sized band contains 30 kilobase (kb) pairs of DNA. In general, one 30 kb band may contain between 3 and 5 genes.

The regions of polytene chromosome lying between two bands are called interbands or interchromomeres. The precise identification of the interbands in polytene chromosomes is difficult because of their small size. Interbands vary between 0.05 and 0.38 mm in size, most frequently being 0.1–0.2 mm, and the molecular size of the interband is 0.3–3.8 kb. Many nonhistone proteins can be seen located in the interbands: RNA polymerase II, proteins-binding RNA in ribonucleoprotein particles, protein included in heterogeneous RNA complexes.

The genetic organization of interbands could be divided into two classes. Interbands of the first type (I) correspond to the regulatory regions of genes inactive in salivary glands. Interbands of second type (II) correspond to the genes constantly active in this tissue, i.e. contain housekeeping genes.

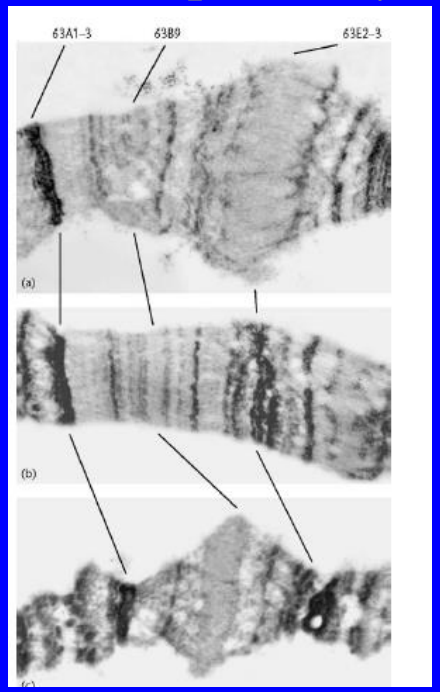
PUFFS

Painter (1935) described a series of relatively achromatic swollen segments as the salient features of the third chromosome of D. melanogaster. Bridges (1935) called some of the swellings, e.g. those in the X-chromosomal 2B region, '***puffs***'. There were no thickenings in the given regions in the other larvae; they contained the usual bands.

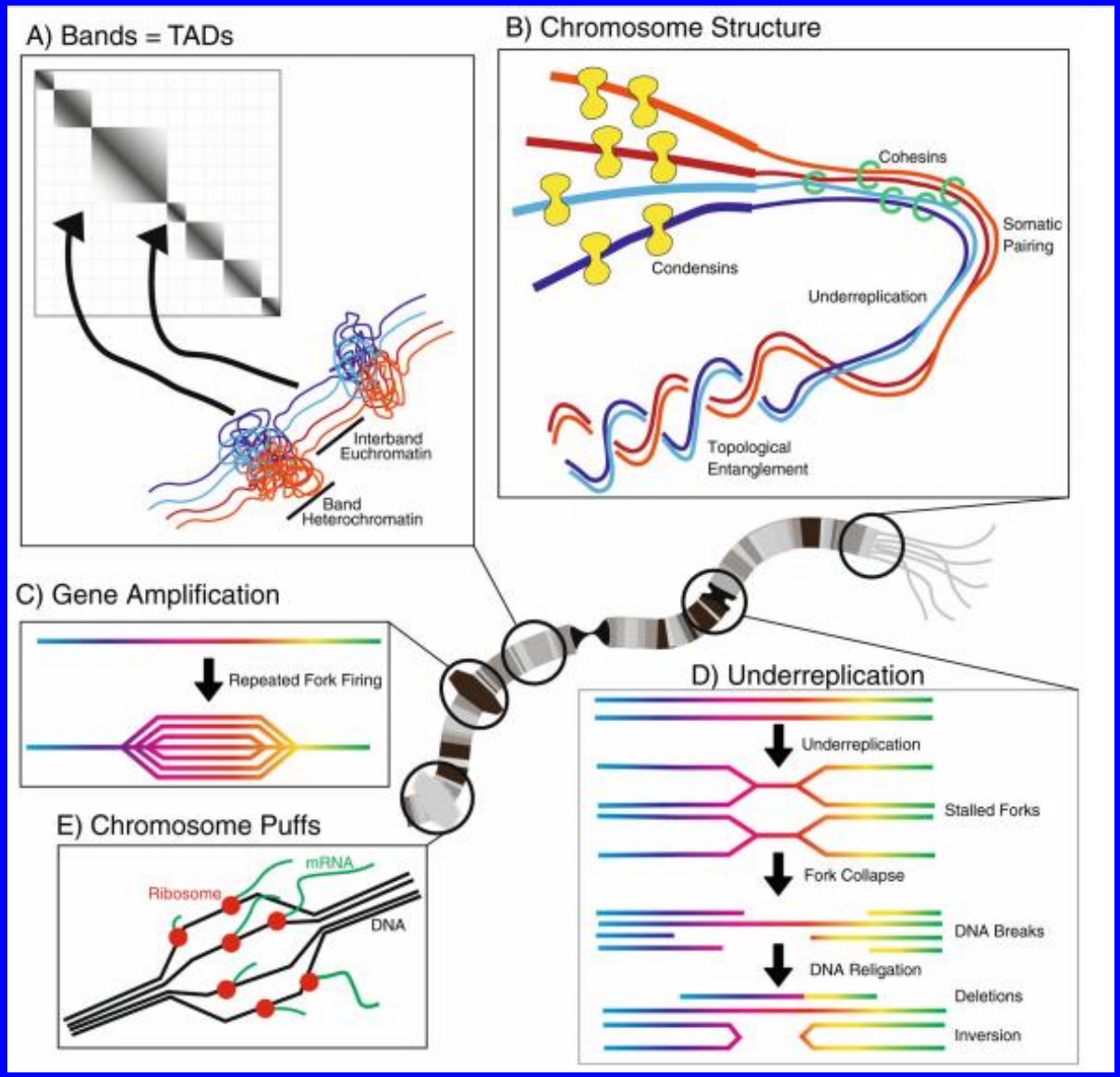
The spectrum of puffs and Balbiani rings is strictly specific to each tissue at a given stage of development. A detailed timetable of changes in the activity of the various puffs has been tabulated for *Drosophila* by Ashburner (1970).

The puffs are sites of very active transcription.

In Sciarids species, certain puffs are involved in DNA amplification. They differ in this respect from the majority of puffs. The 'DNA puffs' remain darkly stained because of the accumulation of extra DNA. Such 'DNA puffs' arise only in salivary glands at the late stages of larval development and are involved in coding for proteins of salivary gland secretion.



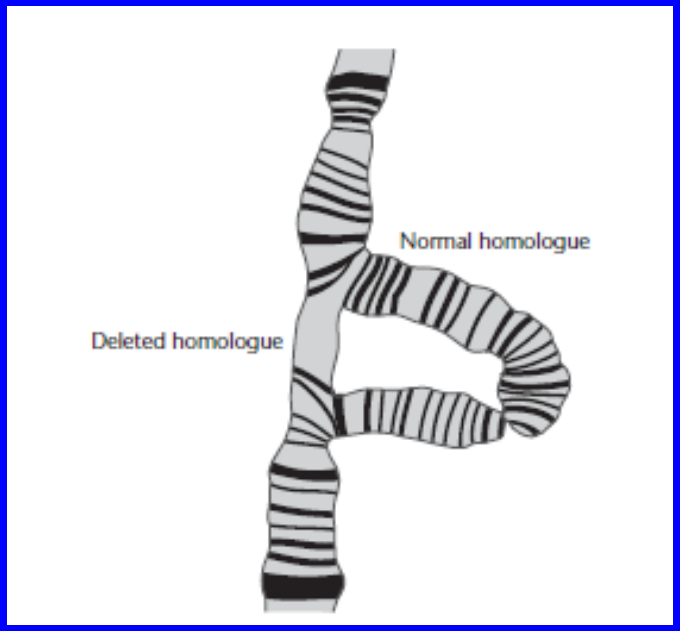
COMMON FEATURES OF POLYTENE CHROMOSOMES

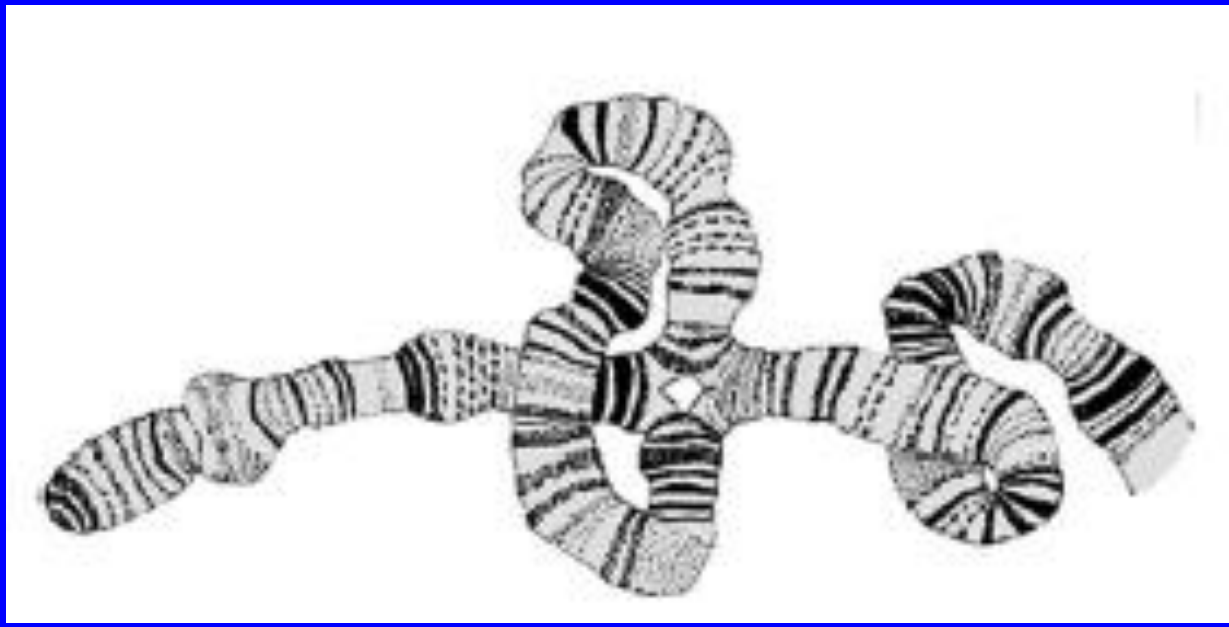


UTILITY IN GENETIC ANALYSIS.

Small deletions can be used for accurate gene mapping. With the use of this method in the 1930s the first genes were mapped in *D. melanogaster* polytene chromosomes with an accuracy to several bands or even part of a band. Today many hundreds of genes are precisely located in the polytene chromosomes maps.

Heterozygous inversions can be seen clearly in polytene chromosomes. This is important in the genetic analysis of populations.

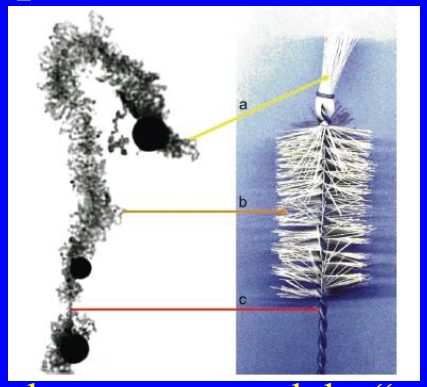




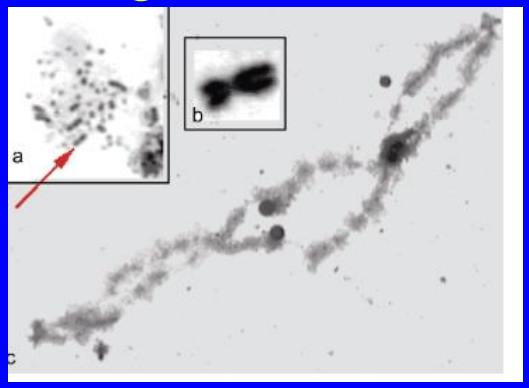
LAMPBRUSH CHROMOSOMES

Lampbrush chromosomes (LBC) were discovered in salamander egg cells (*Ambystoma mexicanum*) by Flemming in 1882. Ten years later, LBCs were identified in shark egg cells and described by Rückert in 1982. It was Rückert who introduced the term "lampbrush chromosome" into biological nomenclature.

Lampbrush chromosomes are intermediate structures present during the first meiotic division. In a prolonged diplotene stage, they undergo decondensation that results in the production of very large chromosomal structures. LBCs' length ranges (depending on the species) from 400 to 800 mm, which makes them up to 30 times larger than their mitotic counterparts.



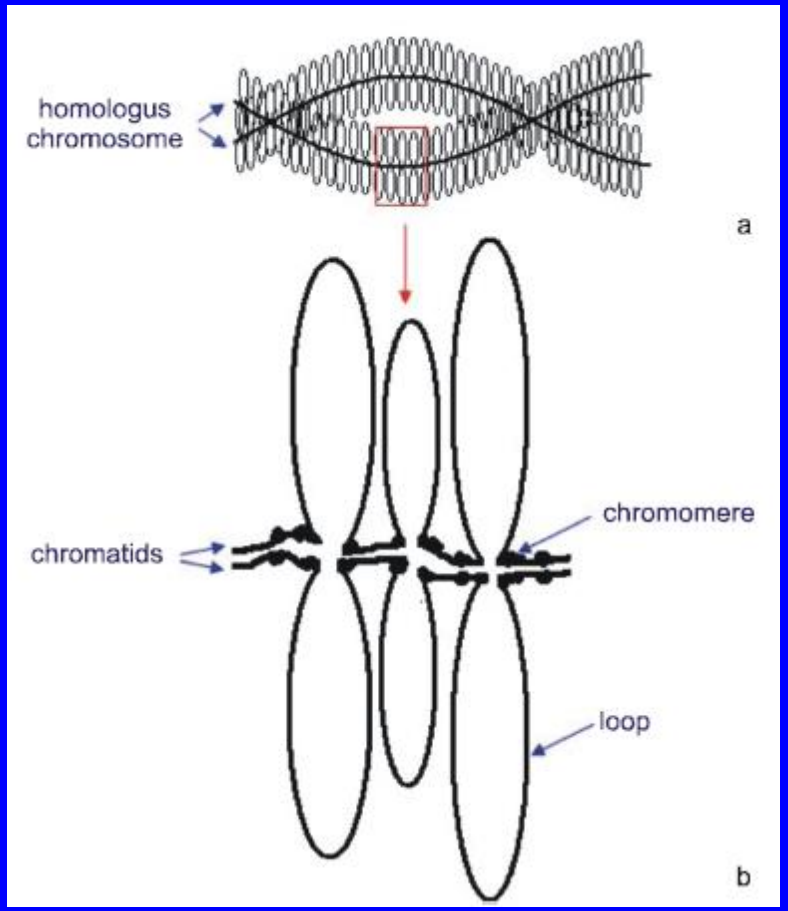
Lampbrush chromosome and the "original item". The arrows indicate analogous structures; a – telomeric loop, b – side loops, c – a chromatid without loops

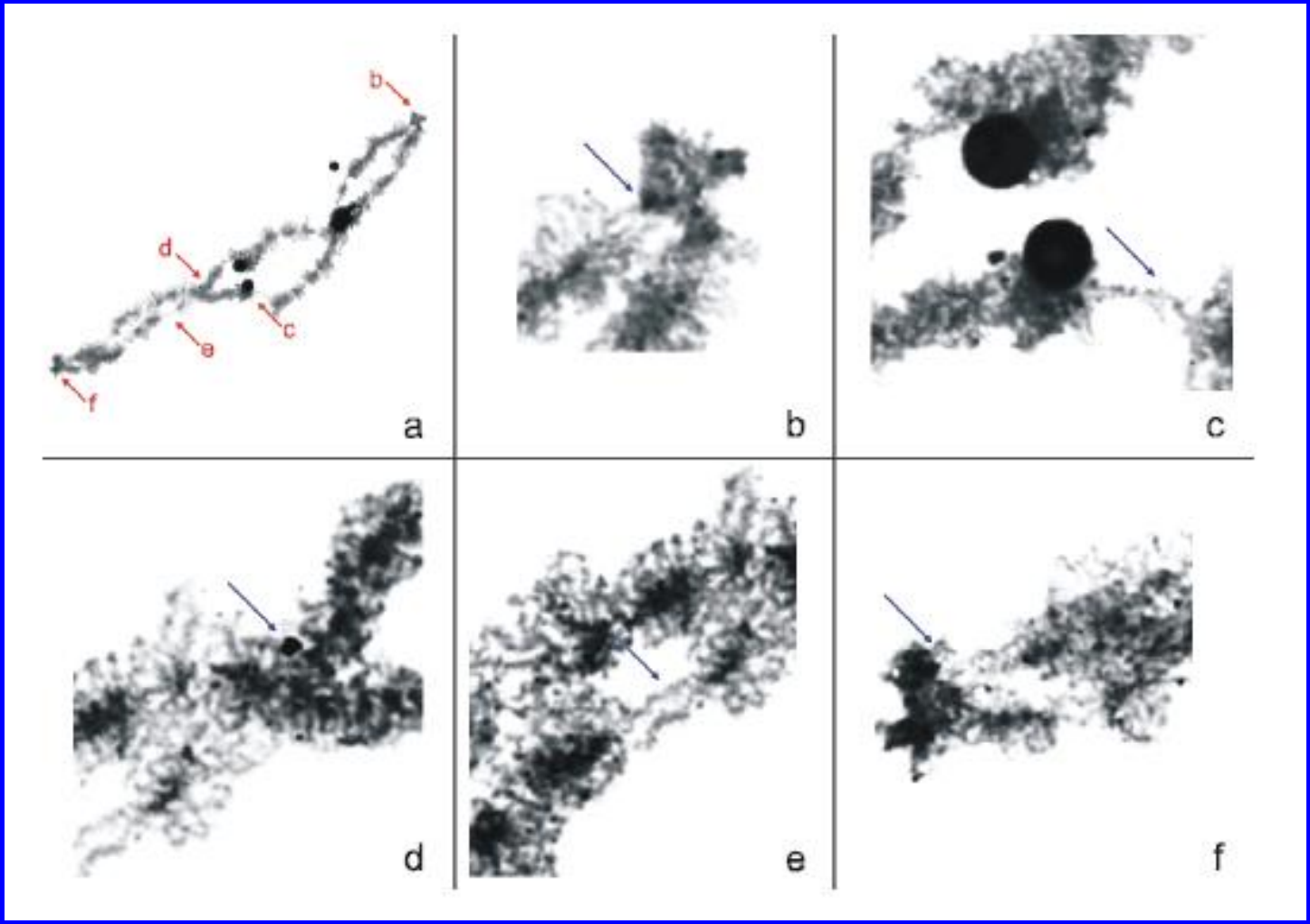


A comparison of the size of LBCs and mitotic chromosomes. A 20-fold microscopic magnification of the metaphase plate (a), 100-fold magnification of the second-pair mitotic chromosome (b), a 20-fold magnification of the second lampbrush bivalent (c)

LAMPBRUSH CHROMOSOME STRUCTURE

In the early prophase, a LBC is a bivalent that consists of two pairs of conjugating homologues, eventually forming a tetrad. Each chromatid is composed of alternately positioned regions of condensed inactive chromatin (chromomeres visible as dark irregular structures and also observed in the interphase nucleus) and side loops of decondensed chromatin. In the homologous sections of the bivalent, chromatin is condensed (spirally twisted) or decondensed in the form of side loops – two per each chromosome and four at the level of the bivalent. The loop constitutes a part of the chromosome axis. It is extensible as well as contractible. The contractibility of the loop results in the contraction and dilation of the chromomere.





The second goose lampbrush chromosome with a magnification of its distinctive structures. A 20-fold magnification of the second goose bivalent (a) and its distinctive structures visible with a 100× zoom (b, f – telomeres, c – centromere, d – chiasm, e – sister chromatids). The particular bivalent structures in the 100-fold blow-ups are marked with arrows

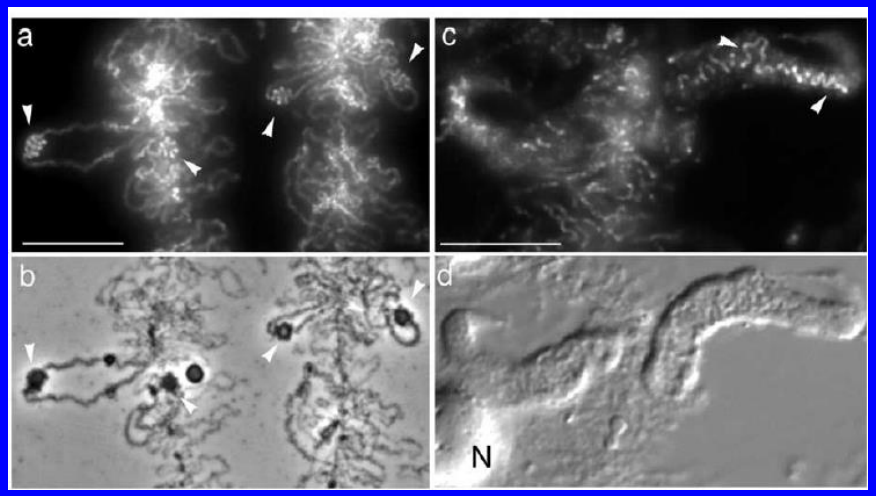
LAMPBRUSH CHROMOSOME STRUCTURE

Numerous morphological types of LBC loops have been identified. Such differentiation is determined by the type and the number of proteins that are directly bound to the emergent transcripts.

In terms of transcriptional activity, there are two basic loop types:

✓"Complex" loops have a matrix with a very complicated morphological structure (loop-formed or fibriform). "Complex" loops are classified as marker loops that enable chromosome identification or side loops.

✓The "plain" loops constitute the majority of chromosome loops and have a delicate fibrous matrix, with occasionally well-visible asymmetry. They are always loop-shaped.

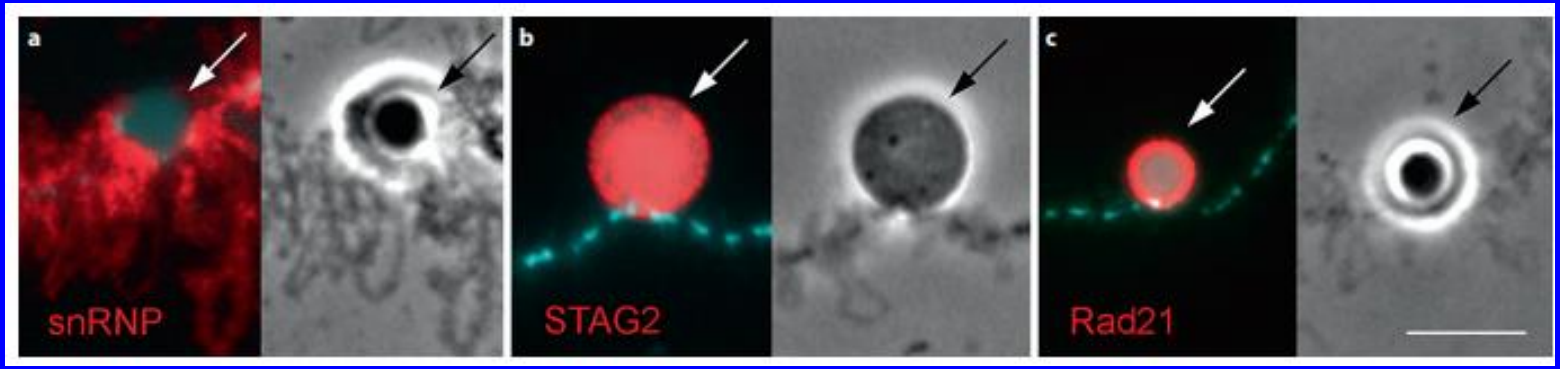


Conformation of the DNA axis in loops with complex matrix morphology. In (a) the path of the loop axis follow a spiral around the sporadic bead-like condensates (arrowheads) that are formed by the matrices of these loops. Both homologous loci are visible, although, for one of them, one of the sisters has fallen onto the chromosome axis. In (d) the much larger mass of matrix forming a 'lumpy loop' and (c) the matrix surrounds the loop axis, which follows a highly contorted path (arrowheads). N, nucleolus. Scale bars represent 10 mm.

LAMPBRUSH CHROMOSOME STRUCTURE

Lampbrush chromosomes include domains of open chromatin in which the genes can be potentially transcriptive and domains of locked chromatin without expression.

Avian lampbrush chromosomes are associated with protein bodies/structures (PBs). They have a regular connection with the chromosome axis of each LBC in the heterochromatin region. PBs may be involved in the coordination of spatial layout of chromosomes. The location of PBs is frequently associated with repetitive sequences surrounding the centromere.



Molecular composition of protein bodies (PBs) associated with centromere regions of avian lampbrush chromosomes. Immunostaining with antibodies against 'Sm-epitope' of splicing snRNP (a), cohesin components STAG2 (b) and Rad21 (c) (red). Chromosomes are counterstained with DAPI (blue). Centromere PBs (arrows) contain proteins of the chromosome structural maintenance group and have a non-RNP nature. Scale bar = 10 m.

LAMPBRUSH CHROMOSOME TRANSCRIPTION

LBCs are used as a model in studies of transcriptional regulation. Changes in transcriptional activity result in a different morphological structure of lampbrush chromosome loops. A higher transcriptional activity of microchromosomes is observed due to a greater density of genes. Transcriptional activity analyses are performed on the basis of assumption that the side loops of LBCs are the transcriptionally active sites. A decrease in transcriptional activity is observed as a shrinking of the side loops. The morphology and transcriptional activity of LBCs vary depending on the reproductive cycle, seasonal changes.

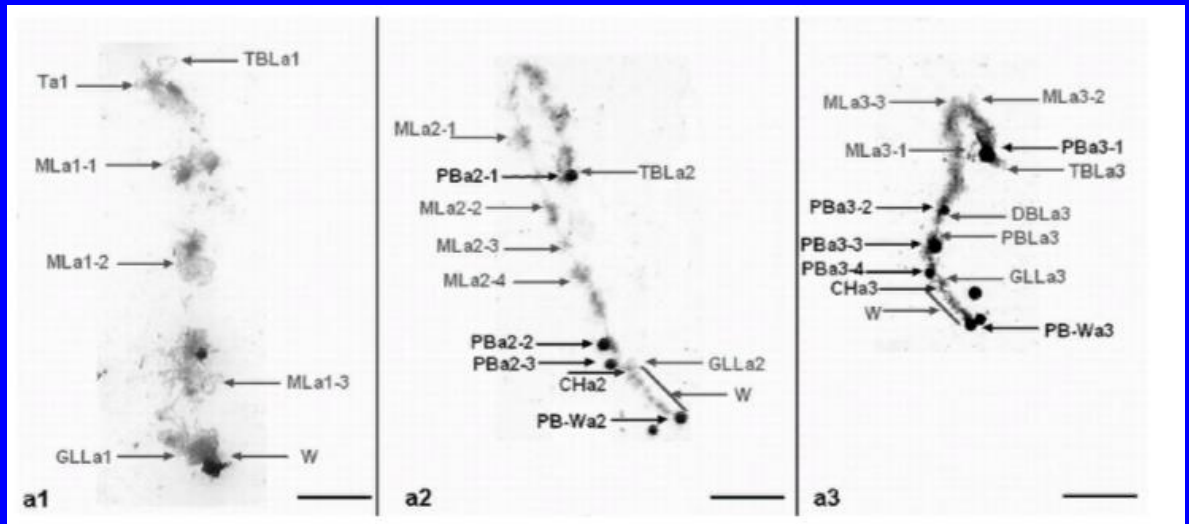
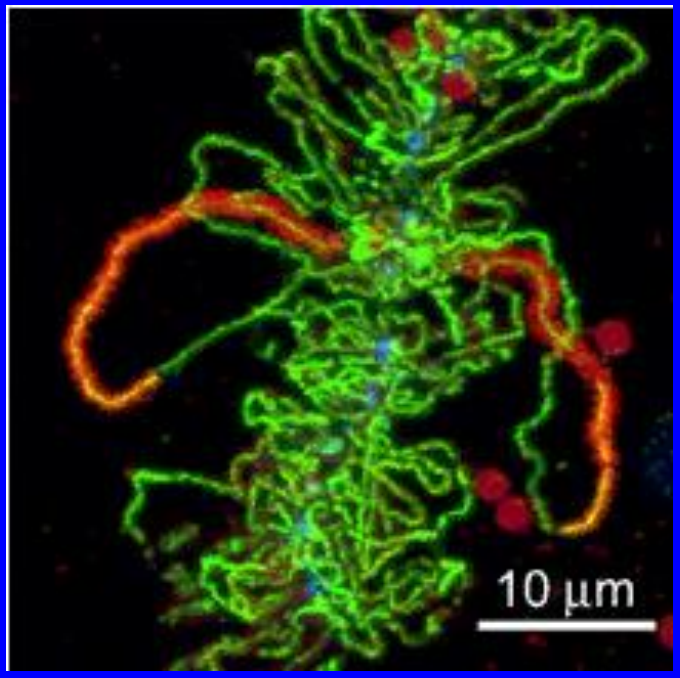


Fig. 1. Structure of lampbrush sex bivalents prior to the reproductive period. Arrows indicate marker structures of bivalents. Bar = 10μm.

LAMPBRUSH CHROMOSOME TRANSCRIPTION

The loops can be classified according to the type of the transcriptional polymerase. The largest loops include those transcribed by polymerase II. The smallest loops are transcribed by polymerase III. They contain 5S RNA coding units, tRNA or short replication sequences.

LBCs can be divided into those with one transcriptional unit and those with two or more. Over the length of 1 μm, one transcriptional unit is transcribed by a densely compacted package of around 13-20 polymerase molecules.



Paired transcription loops (sister chromatids) on a lampbrush chromosome of the newt, immunostained with antibodies against RNA polymerase II (green) and an RNA-binding protein (red). This protein is found on only a few loop pairs.

LAMPBRUSH CHROMOSOME TRANSCRIPTION

Regulation of LBC transcription is performed by means of modifications of chromosome structure and the activity of a number of post-transcription factors. The process of transcriptional activity modification consists of a set of interrelated reactions in which numerous interconnected, both structural and enzymatic, factors take part.

The first stage is the loosening of chromatin with LBC preservation. The preservation of the structure by LBCs during transcriptional activity is connected with the presence of so-called “constitutive” nucleosomes. The transcription of the oocytespecific topoisomerase I (topo-I) variant is activated during the formation of LBC structures. This topoisomerase is present in LBC loops and participates in the spatial conformation of these structures.

LAMPBRUSH CHROMOSOME TRANSCRIPTION

The transcriptionally active loops represent 5-10% of DNA. The remainder is inactive chromatin compacted in the chromomeres.

The result of transcription is visible as a ribonucleoproteinic mantle. The mantle tends to be asymmetrical, corresponding with rising electron density from the base towards the middle of the loop.

The average length of a typical lampbrush chromosome loop is 10-15 μm. The rate of transcription in lampbrush chromosome loops is 5 μm per hour. Thus, one loop is transcribed within two to a dozen or so hours. An average loop contains about 30-40 thousand base pairs, which corresponds with the average length of RNA transcribed in the oocytes.

Chromosome maps of different oocytes at various ages are identical and remain constant for a given oocyte, which suggests a species-specific nature of sequences transcribed during oogenesis.

Thank you for attention!