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Polyteny: still a giant player in chromosome research

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Abstract

In this era of high-resolution mapping of chromosome territories, topological interactions, and chromatin states, it is increasingly appreciated that the positioning of chromosomes and their interactions within the nucleus is critical for cellular function. Due to their large size and distinctive structure, polytene chromosomes have contributed a wealth of knowledge regarding chromosome regulation. In this review, we discuss the diversity of polytene chromosomes in nature and in disease, examine the recurring structural features of polytene chromosomes in terms of what they reveal about chromosome biology, and discuss recent advances regarding how polytene chromosomes are assembled and disassembled. After over 130 years of study, these giant chromosomes are still powerful tools to understand chromosome biology.

Keywords

Polytene; Polyploid; Endocycle

What is a polytene chromosome and where are they found?

Polytene chromosomes are formed when the products of multiple rounds of S-phase remain tightly associated to form one supersized chromosome. In particular polytene chromosomes are found in a specific subset of endopolyploid cells, which have undergone a variant cell cycle known as the endocycle. During endocycles, cells undergo successive genome replications without any intervening nuclear envelope breakdown. For more on the regulation of endocycles, we refer the reader to several recent reviews on the topic (Fox and Duronio 2013; Calvi 2013; Orr-Weaver 2015). Because the number of endocycles can vary between tissues and organisms, polytene chromosomes cover an impressive range of ploidy from those that are only reduplicated a single time to chromosomes made of many thousands or even millions of genome copies (Nagl 1978). We also note that polytene chromosomes can form in cells that undergo transient nuclear envelope breakdown without subsequent chromosome separation (Cantero et al. 2006; Wirth et al. 2006).

Polytene chromosome is found in a diverse set of insects, plants, animals, and unicellular organisms (Table 1). Polytenes can also result pathologically from perturbations in cell cycle regulation.

Insects—frequent and often enormous polytenes

Polytene chromosomes were first discovered in the 1880s in insects (Balbiani 1881). One of the earliest published examples of polytene chromosomes is of *Chironimus* salivary gland chromosomes found in Walther Flemming's 1882 book, *Zellsubstanz, Kern, und Zelltheilung* (Flemming 1882). In the 1930s, researchers began using polytene chromosomes as a new genetic tool, helping to make *Drosophila* one of the premier model organisms of the twentieth century (Heitz 1933; Painter 1933; Letsou and Bohmann 2005). Most of the descriptions of polytene chromosomes in insects are from the Diptera order.

Dipteran polytene chromosomes have been a frequent model for study of chromosome biology because of their large size and prevalence. Firstly, Dipteran polytene chromosomes exhibit striking, easy to visualize structural features. These include a very close alignment of chromatids along the length of the chromosome, often with little or no visible separation between chromatids. This close alignment is due in part to somatic chromosome pairing, in which homologous chromosomes align next to each other in a sequence-dependent manner so that the number of polytene chromosomes is equal to the haploid chromosome number. Somatic pairing plays a role in diploid cells in the phenomenon of transvection, in which enhancers on one chromosome can affect transcription on the homologous chromosome in trans (Gelbart and Wu 1982; Mellert and Truman 2012). In polytene cells, somatic pairing was a key tool for early geneticists because it allowed the identification of deletions, inversions, and translocations, as these aberrations locally disrupt homolog pairing or result in ectopic pairing (Painter 1933). Insect polytenes are also characterized by prominent bands and interbands, the names given to the alternating pattern of dense and less dense regions, respectively, along the length of the chromosomes, as visualized by light microscopy (Zhimulev et al. 2004) (Fig. 1a).

Secondly, Dipteran polytene chromosomes are generally present in a variety of tissues within a single organism and are present throughout the lifecycle. This contrasts with other organisms where polyteny is only confirmed to occur in a small number of cell types (mammals) or where polyteny is visible only for a short developmental window (ciliates). The prevalence of polytenes in insects allowed for several critical studies exploring how polytene chromosomes change during development and in cells with different gene expression patterns (Breuer and Pavan 1955; Pavan and Breuer 1952). This led to important discoveries including early evidence of gene activation following hormone pulses or heat-shock during which polytene chromosomes physically and visibly changes in response to transcriptional changes (Breuer and Pavan 1955; Ritossa 1962; Ashburner and Bonner 1979) (Fig. 1e). Thirdly, Dipteran polytene chromosomes are characterized by their high degree of reduplication, which can reach over 1000-fold in some *Drosophila* tissues and as high as 1,000,000-fold resulting in chromosomes 10 mm in length in *Rhynchosciara* following infection with certain viruses (Pavan et al. 1971). The large size and prominent banding present in polytene chromosomes helped *Drosophila* become one of the workhorses of early molecular biology. For example, genes in *Drosophila* could be cloned without a priori knowledge of the sequence, solely due to their position using either mutants that disrupt stereotypical polytene banding patterns, or by hybridization with labeled nucleic acid probes (Scalenghe et al. 1981; Bender et al. 1983).

Plants—environmental control of polyteny

The presence of developmental polyploidy varies between different plant species but appears to be much more common in angiosperms (flowering plants) (Nagl 1978). Interestingly, many of the structural characteristics of polytene chromosomes seem to be under a high degree of environmental control in plants. For example, growing *Phaseolus vulgaris* (the common bean) at lower temperatures increases the visibility of polytene chromosome bands and results in more compact polytene chromosomes while growth at higher temperatures reduces banding (Nagl 1969). The amount of light exposure can also affect polytene chromosome condensation (Nagl 1981). Understanding how these environmental factors influence polytene structure may provide a useful model for how environmental cues translate into changes in nuclear architecture and gene expression.

Mammals—the trophoblast

Of all the polyploid cell types in mammals, the trophoblast giant cells (TGC) of the developing placenta have the highest ploidy with copy numbers up to 1000 C in mice and 4000 C in rats (Nagl 1972; Barlow and Sherman 1974; Pandit et al. 2013). TGCs are also the clearest example of a tissue in mammals with polytene chromosomes (Zybina 1970; Zybina and Zybina 1996). Unlike Dipteran polytene chromosomes, TGCs are less condensed and do not show prominent banding, which accounts for a debate in some early studies of whether they were in fact polytene (Barlow and Sherman 1974). However, blocking transcription using actinomycin D, which causes chromosomes to condense slightly, revealed that the number of mouse TGC chromosomes remained constant despite ploidy increases (Snow and Ansell 1974). The degree of polyteny can also be tracked by following the Barr body, the tightly compacted heterochromatic X-chromosome, which is transcriptionally silenced during dosage compensation in female mammalian cells. In rat hepatocytes, which are polyploid but not polytene, the number of Barr bodies increases with each round of S-phase (Ohno et al. 1959). In contrast, in polytene rat TGCs, all of the multiply replicated X-chromosomes remain together so that only a single Barr body is observed, even in highly polyploid cells. Follow-up work in mice using in situ hybridization confirmed that TGCs are polytene. Although, unlike in insect polytene chromosomes, there is no somatic pairing in TGCs so the number of polytene chromosomes is equal to the diploid, instead of the haploid, chromosome number (Varmuza et al. 1988; Bower 1987). Interestingly, TGC polytene chromosomes in rodents can be more or less discrete depending on the cell cycle and appear to dissociate slightly during S-phase and then realign in interphase (Zybina and Zybina 1996).

Ciliates—the macronucleus

The presence of giant chromosomes in ciliates was first described by Balbiani in the nineteenth century (Balbiani 1890), and their rediscovery in the 1960s revived interest in their study (reviewed in (Ammermann 1987)). Ciliates have a life history that is distinct from that of many other organisms, and polytene chromosomes form a crucial portion of this life cycle in many ciliate species. Normally, ciliates exist with at least two nuclei: a macronucleus (responsible for RNA synthesis and is polyploid) and a micronucleus (forms the germline, but is transcriptionally inactive). Under mating conditions, the ciliate

micronucleus undergoes meiosis and fuses with the micronucleus of another cell from a compatible mating type. The fused micronuclei then divide, and one of the daughters becomes the future macronucleus (Yerlici and Landweber 2014). In some species (like *Oxytricha*), development of the macronucleus passes through a polytene stage (Ammermann 1987, 1971). In these cells, the developing macronucleus undergoes several consecutive rounds of DNA replication. As these rounds of DNA replication take place, prominent polytene chromosomes develop. These chromosomes have characteristic bands of darker staining heterochromatin interspersed with lighter staining chromatin (Bostock and Prescott 1972). In *Oxytricha*, there is evidence for the pairing of homologous chromosomes (Spear and Lauth 1976), while in *Stylonychia*, homologous chromosomes appear unpaired (Ammermann 1971).

In ciliates, polytene chromosomes are transient. Shortly after forming, they break up along their length and are encased in vesicle compartments within the nucleus (Kloetzel 1970). After being compartmentalized, the polytene chromosomes disperse, and the genome reduction characteristic of many ciliates takes place, resulting in hundreds or thousands of short chromosomes (Yerlici and Landweber 2014; Spear and Lauth 1976). After this genome reduction, polytene chromosomes are not observed during the vegetative cycle of the ciliate (Ammermann 1987).

Pathological polytene chromosomes—chemotherapy, cancer, and other maladies

Apart from the developmental polytene chromosomes already described, polytene chromosomes are known to occur in pathological contexts in a variety of organisms. Unlike most developmentally programmed polytene chromosomes, those that arise pathogenically retain mitotic capacity, and it is this ability to divide that can lead to negative outcomes. Pathological polytene chromosomes are found in muscular dystrophy patients (Schmidt et al. 2011), spontaneous abortions (Therman et al. 1978), and many tumor types (Therman et al. 1983; Erenpreisa et al. 2009; Bieseke and Poyner 1943). In mice, it was found that induction of polytene chromosomes led to increased tumorigenicity (Davoli and de Lange 2012), which may suggest a role for these polytenes in a subset of the ~ 1/3 of solid tumors that are derived from whole genome duplications (Zack et al. 2013).

These aberrant polytene chromosomes are often the product of a single extra S-phase in which the products of replication remain attached. This specific type of polytene chromosome made up of four sister chromatids is called, among other names, a polytene diplochromosome. Diplochromosomes are known to form following treatment with mitosis blocking drugs such as chemotherapeutic topoisomerase inhibitors (Sumner 1998), spindle poisons (Levan 1939, 1938; Blakeslee and Avery 1937), or when chromosomes are unable to separate due to mutations in various cell cycle regulators or inactivation of genome integrity checkpoints (Wirth et al. 2006; Vidwans et al. 2002; Stormo and Fox 2016). Because of the serious consequences that can arise from pathological diplochromosomes developing a better understanding of how they form and what happens to them during mitosis is of critical importance.

The presence of polytene cells in diverse species and contexts suggests that polytenes represent an important mode of DNA organization. As discussed in the next section, many structural features of these chromosomes are also common between organisms.

Common structural features of polytene chromosomes

In addition to their large size, polytene chromosomes have distinct features, including chromosome banding, underreplication, and gene amplification. Here, we review recent experiments that have suggested new models for these polytene structural phenomena and discuss their implication for identifying new functions of polyteny.

Chromosome banding—chromosome territories made visible

The prominent and distinctive bands along the whole length of the chromosome are the most famous characteristic of the classic polytene chromosomes found in *Drosophila* (Fig. 1a). Understanding what the bands represent, how they form, and how they change has been a major subject of study in polytene chromosomes. Early on, the linear order of bands in polytene chromosomes was recognized to be distinct for each chromosome and corresponds directly with the linear gene order. Further, changes in the genetic map such as the loss or translocation of a specific gene could be seen under the light microscope as a corresponding change in the banding pattern of polytene chromosomes (Painter 1933). It was suggested that each band (~ 5000 in *Drosophila*) would correspond to a single gene or perhaps several co-regulated genes (Ashburner 1980), but subsequent work showed this model to be too simplistic. For a more comprehensive description of chromosome banding studies in polytene chromosomes, we refer the reader to a review of the history (Zhimulev et al. 2004).

Recent work using newly available techniques has revealed, in detail, what chromosome bands represent. Chromosome bands of polytene chromosomes do not correspond to genes on a one to one level, but do correspond well with other features of chromosomes, including open and closed chromatin, areas of the genome which are accessible to proteins, or tightly compacted, respectively. This has been elegantly shown by studies that used data from diploid *Drosophila* cells to accurately predict the location of bands and interbands in polytene cells. Using chromatin profiles from diploid cells, the authors found that it was possible to predict the location of band-interband borders in polytene chromosomes (Vatolina et al. 2011).

More recently, a separate study (Eagen et al. 2015) compared *Drosophila* diploid cells and polytene salivary gland cells using next generation sequencing and chromosome conformation capture. With this technique, the authors looked for areas of the genome that cluster together in the nucleus, which are called topologically associated domains (TADs). The authors found that TADs in polytene chromosomes are not only excellent predictors of chromosome bands, but that TADs in diploid and polytene cells were almost always the same, suggesting that the bands in polytene chromosomes really do represent the unseen configuration of inter-phase chromosomes in diploid cells. Interestingly, the authors also found that homologous chromosomes are not perfectly aligned, but rather their sequences were offset by at least 100 bp, raising interesting questions about what generates somatic

pairing if not precise sequence. This study powerfully underscores the continued value of polytene chromosomes in modern chromosome research.

Differential replication—somatic genome diversity

In addition to an increased genome content, polytene cells frequently exhibit another major alteration to the diploid genome—frequent and localized DNA underreplication (Fig. 1b, d) and/or gene amplification (Fig. 1c). Many previous reviews cover these topics and their occurrence in diverse species (Nordman and Orr-Weaver 2012; Cross 2014; Schoenfelder and Fox 2015). Here, we briefly note important recent findings with regards to differential genome replication in polytene cells, which have expanded our appreciation of this polytene chromosome property.

As noted previously, endoreplication refers to repeated S-phases without cell division resulting in a cell with whole genome duplications. However, the duplication of the genome in many polyploid cells, in contrast to diploid cells, is often not even across the whole chromosome. Underreplication occurs when sections of the genome are not replicated during S-phase. Gene amplification occurs when specific loci are replicated additional times compared to the rest of the genome. Interestingly, gene amplification and underreplication co-occur in some polyploid cells so that a single polyploid cell can contain underreplicated and amplified sequences.

A key question is why cells would undergo underreplication or amplification? The simplest model for the function of localized polytene underreplication, which often occurs within gene poor regions and repetitive regions (Endow and Gall 1975; Hammond and Laird 1985a; Hammond and Laird 1985b), is that shortening S-phase by not replicating the entire chromosome reduces the need for replication machinery without limiting the number of coding sequences. This compromise allows endocycling cells to focus cellular resources elsewhere. In support of this model, many polyploid *Drosophila* cell types down-regulate factors required for replication and components of the mitotic cell cycle, such as centrosomes (Schoenfelder et al. 2014; Nordman et al. 2011; Maqbool et al. 2010; Mahowald et al. 1979; Mahowald and Strassheim 1970).

However, there are consequences to underreplication. Sequencing in *Drosophila* indicates that underreplication leads to DNA breaks and the accumulation of deletions, translocations, and inversions when these breaks are repaired (Fig. 1d) (Andreyeva et al. 2008; Yarosh and Spradling 2014). These aberrations are thought to occur because during underreplication forks stall. These stalled forks are unstable, and when they collapse, the outcome is DNA breaks. This suggests a trade-off in which cells generate copy number variation and DNA breaks in exchange for a reduction in replication. Since *Drosophila* polyploid cells down-regulate the apoptotic machinery, the errors caused during replication can be survived by the cell (Mehrotra et al. 2008; Zhang et al. 2014; Bretscher and Fox 2016). Ciliates undergo an even more extreme genome rearrangement after the polytene stage; so, small amounts of underreplication may not be detrimental. In fact, underreplication of sequences to be eliminated during macronucleus formation may serve a purpose in specifying them as break points during macronucleus assembly (Chalker and Yao 2011).

Until very recently, it was not known if underreplication was a feature of mammalian polyploid cells. Early attempts to find underreplication using CsCl fractionations and renaturation found no evidence of underreplication (Zybina and Zybina 1996; Sherman et al. 1972). Using more modern tools to look for evidence of underreplication, two different groups used comparative hybridization to arrays and genome sequencing to search for copy number variation in trophoblast giant cells (TGC) and megakaryocytes (MK). Both groups found little evidence for underreplication in the MK. However, one group found 47 regions of reproducible underreplication in TGCs (Sher et al. 2013; Hannibal et al. 2014). Evidence for underreplication was most pronounced in parietal TGCs, which have the highest ploidy level.

This recent data from mammals complements another study in flies indicating that in addition to underreplicating gene poor regions, underreplication may target specific genes. One suggestive piece of data is that while underreplicated regions are in heterochromatin, not all heterochromatin is underreplicated, indicating a preference for some regions over others (Nordman et al. 2011; Hannibal et al. 2014; Sher et al. 2012). Excitingly, there are overlapping classes of genes that are frequently underreplicated in both species. Sequencing in mammalian TGCs and in *Drosophila* salivary glands found that genes involved in cell adhesion and in neurogenesis were significantly enriched in regions of reproducible underreplication (Yarosh and Spradling 2014; Hannibal et al. 2014). Why should those genes in particular be targeted? Hannibal et al. suggest that the known requirement for down-regulation of cell adhesion and neurogenesis genes during placental development means that underreplication could act as an additional layer of control in TGCs (Kokkinos et al. 2010; Liao et al. 2012). Yarosh and Spradling instead argue that the stochastic DNA breaks, which can lead to deletions, inversions, and translocations, in cell surface genes and immunoglobulin genes are themselves advantageous for the organism by generating genetic diversity (Fig. 1d). Further testing of both of these models is an exciting area of future study, as it will help to reveal how polytene chromosome structural alterations may contribute to specialized cellular functions.

In addition to underreplication, polytene chromosomes in both mammals and insects amplify some loci above the level of the rest of the genome (Fig. 1c). Gene amplification is a specific type of rereplication in which subsets of the DNA are replicated by repeated origin firing in a developmentally programmed manner (Hook et al. 2007; Fragkos et al. 2015). In *Rhynchosciara* salivary glands, the 16-fold increase at certain loci is visible as an enlargement of a subset of bands on the polytene chromosome. These enlarged bands also continue to incorporate thymidine when S-phase has ceased on the rest of the chromosome (Glover et al. 1982; Ficq and Pavan 1957). Using quantitative southern blotting, gene amplification was also found in follicle cells in the *Drosophila* ovary where it was shown to amplify chorion genes, which code for proteins required for egg shells (Spradling and Mahowald 1980). Unlike in underreplicated regions, gene amplification, at least in *Drosophila*, appears to be accompanied by stable nested replication forks (Fig. 1c) (Osheim and Miller 1983). However, gene amplification in follicle cells is dependent on DNA damage repair, in particular, non-homologous end-joining (NHEJ). The authors suggested that not all forks stably nest and some collapse, likely in a similar fashion to the process in underreplication (Alexander et al. 2015).

Currently, there is evidence that *Drosophila* follicle cell polytene chromosomes contain six regions that are amplified, although at least one of these appears to depend on genetic background. In addition to genes for chorion proteins, these regions also encompass sequences for other proteins needed for egg production, such as vitelline (Kim et al. 2011). Gene amplification is required in follicle cells because mutants that fail to amplify these loci produce inviable eggs (Landis et al. 1997). Recently, it was found that the situation is very similar in the TGCs of the mouse. In TGCs, five loci are detectably amplified by whole genome sequencing. As in *Drosophila*, these loci are enriched in highly expressed genes critical for embryo viability (Hannibal and Baker 2016). In both systems, the extent of amplification extends in a gradient away from the center of the amplified region (Fig. 1c). In *Drosophila*, amplified regions contain some *cis* sequences that are necessary and sufficient for amplification when moved around the genome (Delidakis and Kafatos 1989; Orr-Weaver and Spradling 1986), but amplification is also regulated by chromatin in particular histone acetylation. Tethering the histone deacetylase Rpd3 to amplified sequences using a modified Gal4 system blocks amplification (Aggarwal and Calvi 2004). Interestingly, no common *cis*-regulatory element has been found between the different amplified regions in either *Drosophila* or mouse.

Gene amplification of *Drosophila* polytene chromosomes has also been a valuable tool for the study of replication in general. This is due to the fact that the canonical replication machinery is still required during gene amplification, but only a small portion of the genome is in S-phase light microscopy. Thus, replication events are visible at a single origin of replication. This technique was used to show that chromosome position effects the efficiency of origin initiation (Kim et al. 2011; Aggarwal and Calvi 2004) and that replication is not confined to particular locations within the nucleus (Calvi and Spradling 2001).

What is it about cells with polytene chromosomes that allows for tolerated underreplication and gene amplification? Why for example do TGCs in mice undergo both phenomena while megakaryocytes, which are also polyploid but are not thought to be polytene, do not? It may be that polyteny is not the key feature but rather polytene chromosomes and differential replication are the consequences of some other change in non-mitotic cells. Future research can lead to a better understanding of factors that control these genome alterations.

How to assemble a polytene chromosome

What forms of chromatid interaction hold polytene chromosomes together? While we incompletely understand the answer to this question, several recent studies have enhanced our understanding of the multiple forms of contact between the many strands of a polytene chromosome.

Cohesins

The cohesin complex has a critical role in regulating the structure of chromosomes in dividing cells especially during mitosis where it is responsible for holding together sister chromatids (Skibbens 2016; Nasmyth and Haering 2009). Cohesins are also dynamic on chromatin during interphase, including on polytene chromosomes. Recent work on non-

mitotic functions of cohesins in gene regulation may partially explain non-structural reasons that polytene chromosomes contain cohesins (Pauli et al. 2010, 2008).

Recent experiments have investigated the role cohesins play in regulating the structure of non-mitotic chromosomes, including polytenes. Sister chromatids of dividing cells are prevented from separating during interphase by the presence of cohesins, and polytene chromosomes are large bundles of chromosomes that do not separate. It thus seemed reasonable that cohesins might be necessary for holding polytene chromosomes together. However, recent work in *Drosophila* contradicts this simple model. Although cohesins bind to many loci on polytene chromosomes (Dorsett et al. 2005; Markov et al. 2003) (Fig. 1b), the cohesin complex seems to be dispensable for polytene structure at least once that structure is established. This was shown by work using a *Drosophila* mutant in which a null mutation in the cohesin subunit Rad-21 was rescued by a transgenic construct containing *rad-21* with a tobacco etch virus (TEV)-protease cleavage site integrated into it. In this system, expression of TEV-protease from a heat-shock promoter led to a total loss of Rad-21 and a corresponding dissociation of the rest of the cohesin complex from chromosomes. Despite the apparent total loss of cohesins, the structure of the polytene chromosomes in the salivary gland appeared unchanged (Pauli et al. 2008). Conversely, constitutive low-level expression of TEV-protease after salivary gland differentiation but during endocycling led to smaller salivary gland chromosomes, although whether this was due to structural problems or a result of gene expression changes is not clear.

The maintenance of polytene chromosomes in the absence of cohesins in *Drosophila* may be due in part to the presence of cohesin-independent chromosome pairing in *Drosophila*. Cohesin-independent pairing means that, unlike in other organisms where sister chromatids will separate in G2 without cohesins, in *Drosophila* sister chromatids remain paired in G2 even in cells depleted of cohesin components (Senaratne et al. 2016). Additionally, somatic pairing between homologous chromosomes, which are not held together by cohesion, has been observed in Diptera for over a hundred years (Metz 1916). Therefore, it is perhaps not surprising that in *Drosophila*, polytenes are maintained in the absence of cohesins. What role cohesins play in organizing the structure of polytene chromosomes in other systems remains to be seen.

Concatenation and chromosome pairing

If removing cohesins is not sufficient to eliminate polyteny, then what establishes and maintains the tight association between chromatids that is the hallmark of polytene chromosomes? One answer may be the topological entanglement of chromatids generated during successive genome replications (Fig. 1b). Relief of this entanglement by topoisomerase is generally not complete until mitosis and is required for sister chromatid separation at anaphase (Holm et al. 1985). Additionally, loss of topoisomerase activity during mitosis via pharmacological inhibition has been shown to generate polytene chromosomes from diploid precursors by preventing chromatid separation at anaphase (Cantero et al. 2006; Sumner 1998). In striking contrast, loss of topoisomerases increases the number of unpaired homologous chromosomes in *Drosophila* cell culture through an unknown mechanism (Williams et al. 2007). It is perhaps most likely that precise levels of

topological interaction and of topoisomerases are critical and are carefully controlled in polytene cells.

Underreplication

What effect does underreplication have on polytene chromosome structure? In *suppressor of underreplication* (*Su(UR)*) mutants, which more fully replicate the genome of polytene cells, polytene chromosomes remain polytene, suggesting that underreplication is not required to hold polytene chromosomes together (Belyaeva et al. 1998). Nevertheless, underreplication does have profound effects on polytene chromosome morphology (Fig. 1d). By comparing the structure of polytene chromosomes in the same tissue in wild-type and *Su(UR)* mutants and between cell types with and without underreplication, it is possible to deduce some of the effects of underreplication on chromosome structure. The most striking feature, which led to the discovery of *Su(UR)*, is the increase in copy number of heterochromatic sequences, especially near the centromeres. Further, regions on the polytene chromosomes that form small constrictions due to underreplication, named intercalary heterochromatin, are lost in *Su(UR)* mutants and additional bands are evident (Belyaeva et al. 1998). There is also a loss of structural disruptions in which thin fibers can be seen to extend from one polytene chromosome to another (Belyaeva et al. 1998). These fibers are thought to be formed by the repair of DNA breaks generated in underreplicated regions that can join with other distance sequences to generate these DNA strands (Yarosh and Spradling 2014). Fully replicated chromosomes also lose the chromocenter that is characteristic of wild-type salivary gland chromosomes as can be seen by the chromosomes in early ovarian nurse cells and the polytene chromosomes in mitotic hindgut papillar cells and in *Su(UR)* mutant salivary glands in *Drosophila* (Stormo and Fox 2016; Belyaeva et al. 1998; Dej and Spradling 1999).

Thus, it is clear that several mechanisms influence chromosome interaction in polytene DNA yet how these different pathways interact and influence each other and whether other mechanisms exist remains an open question. The intricate structure of polytene chromosomes might suggest that disassembly of a polytene chromosome would be impossible. However, as we discuss in the next section, polytenes can be completely disassembled.

How to disassemble a polytene chromosome

In many cell types including in insects, ciliates, and some mammals, polytene chromosomes are a transient structure that is broken down. Generating a mechanistic understanding of this process has become an important subject of research in recent years and has led to the discovery of one major pathway responsible for separating polytene chromatids.

In many polytene cells, separation on a small scale is associated with the need for greater access to the chromatin. In *Drosophila* salivary gland chromosomes, there is a stereotyped series of puffs that appear during development that are regulated by the steroid hormone ecdysone (Breuer and Pavan 1955) (Fig. 1e). Further puffs can be induced at defined locations by heat-shock (Ritossa 1962). Elegant work over 50 years ago showed that these puffs were related to an increase in transcription at those specific loci (reviewed by

(Ashburner and Bonner 1979)) (Fig. 1e). This work demonstrates that the proximity of chromatids to each other is not fixed but can be altered by proteins binding to the DNA. Similar results are seen in plants, where it has been noted that the thickness and the definition of the polytene bands relate to the temperature, likely via transcription (Nagl 1969). TGCs also show more condensation when transcription is pharmacologically inhibited (Snow and Ansell 1974). Thus, local transcription can locally disrupt polyteny.

Complete or nearly complete polytene separation has been observed in mitosis-capable polytene cells in *Drosophila*. During the development of the ovary, the oocyte-supporting nurse cells undergo five rounds of S-phase during which polytene chromosomes are produced. Following that 5th S-phase, the chromosomes condense slightly and separate from one another (Dej and Spradling 1999). Mutations in the anaphase promoting complex subunit encoded by the *morula* gene enhance the phenotype, likely due to the elevation of mitotic cyclins in this mutant that lead to not only chromosome separation but the formation of a partial mitotic spindle (Reed and Orr-Weaver 1997). However, the presence of underreplication may act as a constraint on separating these polytene chromosomes. For example, more fluorescent in situ hybridization (*FISH*) foci are evident in *SU(UR)* mutant ovarian nurse cells following developmentally programmed polytene separation, suggesting that more chromatids are able to undergo separation (Dej and Spradling 1999). The purpose of this separation remains to be discovered, but several genes that control it are known (Goodrich et al. 2004; Keyes and Spradling 1997; Klusza and Deng 2010; King et al. 1981). However, how these genes function in controlling polytene dispersal is not well understood. For example, two genes, *squid* and *hrb27c*, are known to have RNA binding properties, but whether these directly relate to their function in chromosome dispersal or if the effect is indirect is not known (Goodrich et al. 2004).

One molecular mechanism that is required for polytene separation is the activity of the condensin II complex. Mutations in the condensin *CapH2* are viable but lead to ovarian nurse cell chromosomes that remain polytene (Hartl et al. 2008). Conversely, overexpression of *CapH2* is able to create separation between chromatids in both salivary glands measured by cytology and in diploid cells measured by transvection (Smith et al. 2013; Bauer et al. 2012). What causes the chromosomes to separate? The authors suggest competition between chromosome alignment and axial compaction. Condensins drive axial compaction and therefore force apart chromatids (Bauer et al. 2012).

Total polytene separation occurs in *Drosophila* hind-gut rectal papillar cells. In these cells, polytene chromosomes completely separate into pairs of sister chromatids during a process named SIRS for “Separation Into Recent Sisters” before separating sister chromatids at anaphase (Stormo and Fox 2016). This process begins synchronously with nuclear envelope breakdown suggesting that some cytoplasmic factor may be involved. It also involves a role for the spindle checkpoint gene *mad2*, which is required to time the length of mitosis. An important advantage of the hindgut rectal papillar model is that it is amenable to live imaging, which enables the visualization of polytene disassembly in real-time. If mitosis takes place too quickly, as in *mad2* mutants, then SIRS fails to complete and mitotic errors result. As in the separation of *Drosophila* ovarian nurse cell chromosomes, papillar cells do not rely on the spindle for SIRS, but further molecular mechanisms of SIRS remain to be

elucidated. There is evidence that in some mammalian species, a similar process may occur in TGC where polytene chromosomes have been observed to separate into numerous smaller bundles (Zybina and Zybina 1996; Zybina et al. 2011).

Pathological endocycles are often incapable of fully separating polytenes prior to anaphase when they return to mitosis in contrast to developmentally programmed mitotic polytenes. This failure to depolytenize can result in highly error-prone divisions in a variety of systems including flies and mammals, and these errors can lead to aneuploidy in the daughter cells (Therman et al. 1978; Vidwans et al. 2002). In particular, pathological endocycles are able to separate homologous chromosomes but are not able to fully separate the products of S-phase, resulting in the diplochromosome shape—four (or more) chromatids held together by pericentric cohesins. In *Drosophila*, these aberrant chromosomes trigger the spindle checkpoint, which is able to reduce but not eliminate errors during mitosis (Stormo and Fox 2016). As with other polytene chromosomes, once these diplochromosomes are separated, they do not reform in subsequent divisions, which remain polyploid but not polytene. Future studies in the above-discussed models will continue to reveal both the key requirements for polytene assembly and disassembly, as well as the cues required to regulate the surprisingly dynamic nature of polytene chromosome structure.

Summary

In this review, we have highlighted several ways in which polytene chromosomes remain both informative tools for the chromosome biologist and unique models with their own fascinating biology. While polytene chromosomes are most frequently found in insects, they are critical in diverse organisms from ciliates to plants to humans, and they play important roles in disease progression. Given their giant size and the recent revelation that TADs can be easily visualized in chromosome bands, it is clear that study of polytenes will continue to illuminate new properties of chromosome organization and its relation to gene expression. Related to this important finding, the emergence of new information about how to assemble and disassemble a polytene can provide convenient models to manipulate polytene structure and study its effect on the genome and transcriptome. Further, the recent discovery that underreplication and gene amplification is shared between mammalian and insect polytenes opens up fascinating new lines of research. Over 130 years after the initial report of polytenes, it is clear that much remains to be learned from these giants of the chromosome world.

Abbreviations

TGC	Trophoblast giant cell
TAD	Topologically associated domain
TEV	Tobacco etch virus
Su(UR)	Suppressor of under-replication

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Fig. 1.

Common features of polytene chromosomes. While polytene chromosomes are found in a diverse group of cell types and perform diverse functions they often share a common set of core features including underreplication of some genomic loci, amplification of other loci, they have visible band and interband regions along their length, and chromatids are paired to form thick bundles and will change their morphology during times of elevated transcription. **a** Polytene chromosomes are characterized by transverse bands along their length. These bands are formed by tightly compacted heterochromatin interspaced with loosely compacted euchromatin. Recent work has shown that polytene bands in *Drosophila* are found to be topologically associated domains (TADs) in chromosome conformation capture experiments. Interestingly, the same TADs are present in diploid cells, indicating that structure is preserved between polytene and diploid cells. **b** A polytene chromosome is found in any cell where the products of more than one successive S-phase without an intervening cell division are held in parallel. Recent work has helped to explain what holds sisters in alignment and

what can separate them. Several factors hold sister chromatids together, including topological entanglement caused by DNA coiling, underreplication in some species and cell types, somatic pairing in Diptera and possibly in some ciliates, and cohesin complexes which hold chromatid strands together and are present on polytene chromosomes. In order to separate polytene chromosomes into individual strands, condensins are required. They drive compaction of chromatid fibers that can overcome the forces holding chromatids together. **c** Gene amplification occurs when a single region on a polytene chromosome is rereplicated multiple times by repeated replication fork firing. Gene amplification allows extra transcriptional output when a small number of transcripts are needed in very large quantity. **d** Underreplication is present in polytene chromosomes in ciliates, mammals, plants, and insects. Failure to replicate a portion of sequence is hypothesized to stall replication forks. These forks are unstable and eventually collapse, resulting in DNA breaks. Broken DNA can ligate to other sequences either nearby or more distant, generating inversions, translocations, and deletions. **e** Chromosome puffs occur when high levels of transcription at a particular location lead to localized expansion of the polytene structure to accommodate the transcriptional machinery

Table 1

A survey of polytene chromosomes in nature

Type	Subtype	Species	Cell type	Ploidy (C value)	Notes	Reference
Ciliate	Hypotrichous	<i>Oxytricha</i> sp.	Macronuclear Anlage	16–32	UR, somatic pairing	(Ammermann 1987; Yerlici and Landweber 2014; Spear and Lauth 1976; Murti and Prescott 1999)
		<i>Stylonychia</i> sp.	Macronuclear Anlage	32–64	UR, gene Amp., no somatic pairing	(Ammermann 1971; Bostock and Prescott 1972; Kloetzel 1970; Meyer and Lipps 1981; Meyer and Lipps 1980; Ammermann et al. 1974)
Plants	Angiosperm	<i>Euplotes</i> sp.	Macronuclear Anlage	64–128	No RNA synthesis	(Ammermann 1971; Rao and Ammermann 1970)
		<i>Phascolus</i> sp.	Embryo suspensor	1024–8192	Banding is temperature Sensitive	(Nagl 1969; Nagl 1974)
		<i>Pisum sativum</i>	Cotyledon	64	Induced during regeneration	(Marks and Davies 1979)
		<i>Zea mays</i>	Endosperm	384	Cells are initially triploid (3 N)	(Nagl 1981; Tschermak-Woess and Enzenberg-Kunz 1965)
		<i>Petunia</i> sp.	Pathological	8–16	Following treatment with spindle Poison	(Levan 1939)
Animals	Insects	<i>Drosophila melanogaster</i>	Salivary gland	1024	UR	(Yarosh and Spradling 2014; Endow and Gall 1975; Hammond and Laird (1985); Nordman et al (2011))
		“	Nurse cell	1024	UR, partial separation	(Dej and Spradling 1999)
		“	Follicle cell	16	Gene amp.	(Spradling and Mahowald 1980; Calvi et al. 1998)
		“	Rectal papillar cell	16	Separation, mitotic	(Stormo and Fox 2016; Fox et al. 2010)
		“	Pathological	8	Mitotic	(Vidwans et al. 2002; Stormo and Fox 2016)
		<i>Chironomus tentans</i>	Salivary gland	32,768	Band pattern changes seasonally	(Zhimulev et al. 2004; Ilyinskaya 1994)
		<i>Rhynchosciara angelae</i>	Salivary gland	16,000–1,024,000	Largest occur after infection, gene amp	(Breuer and Pavan 1955; Diaz and Pavan 1965)
Mammals	Mouse	Mouse	Trophoblast giant cell	512–1024	UR, gene amp.	(Snow and Ansell 1974; Sherman et al. 1972; Hannibal et al. 2014)
		“	Pathological	8	Mitotic	(Levan and Hauschka 1953; Davoli et al. 2010)
		Rat	Trophoblast giant cell	2048	Partial separation	(Zybina et al. 2011)
		Human	Trophoblast giant cell			(Sarto et al. 1982)
		“	Pathological	8	Mitotic	(Therman et al. 1978; Therman et al. 1983)

This table is not a complete list of species or tissues within species but instead is intended to give an overview and highlight some interesting examples. For additional examples, see (Zhimulev et al. 2004; Nagl 1981; Zybina and Zybina 1996; Ammermann 1987; Carvalho 2000)

UR underreplication, Separation polytene chromosomes separate at least partially after forming, Mitotic polytene chromosomes undergo mitosis after forming, Gene amp. specific gene sequences are amplified by additional replication