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Replication timing in *Drosophila* and its peculiarities in polytene chromosomes

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Drosophila melanogaster is one of the popular model organisms in DNA replication studies. Since the 1960s, DNA replication of polytene chromosomes has been extensively studied by cytological methods. In the recent two decades, the progress in our understanding of DNA replication was associated with new techniques. Use of fluorescent dyes increased the resolution of cytological methods significantly. High-throughput methods allowed analysis of DNA replication on a genome scale, as well as its correlation with chromatin structure and gene activity. Precise mapping of the cytological structures of polytene chromosomes to the genome assembly allowed comparison of replication between polytene chromosomes and chromosomes of diploid cells. New features of replication characteristic for *D. melanogaster* were described for both diploid and polytene chromosomes. Comparison of genomic replication profiles revealed a significant similarity between *Drosophila* and other well-studied eukaryotic species, such as human. Early replication is often confined to intensely transcribed gene-dense regions characterized by multiple replication initiation sites. Features of DNA replication in *Drosophila* might be explained by a compact genome. The organization of replication in polytene chromosomes has much in common with the organization of replication in chromosomes in diploid cells. The most important feature of replication in polytene chromosomes is its low rate and the dependence of S-phase duration on many factors: external and internal, local and global. The speed of replication forks in *D. melanogaster* polytene chromosomes is affected by SUUR and Rif1 proteins. It is not known yet how universal the mechanisms associated with these factors are, but their study is very promising.
Key words: *Drosophila melanogaster*; replication timing; replication origins; replication initiation zone; polytene chromosome; endocycle; Supressor of UnderReplication; SUUR; Rif1.

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Пространственно-временная организация репликации у дрозофилы и ее особенности в политенных хромосомах

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Drosophila melanogaster – один из популярных модельных организмов для изучения репликации ДНК. Начиная с 1960-х годов репликацию политенных хромосом активно изучали с помощью цитологических методов. В последние двадцать лет прогресс в изучении репликации определялся применением новых методов. Использование флуоресцентных красителей значительно улучшило разрешение цитологических подходов. Наличие геномной последовательности позволило изучить и соотнести репликацию ДНК со структурой хроматина и активностью генов для эухроматиновых районов в масштабе генома. Картирование границ цитологических структур политенных хромосом на последовательности генома дало возможность сравнить временные характеристики репликации районов хромосом в клеточных культурах и клетках слюнной железы. Были описаны новые особенности репликации как для хромосом диплоидных клеток, так и для политенных хромосом дрозофилы. Анализ временных профилей репликации показал, что организация репликации имеет в своей основе те же закономерности, что и у других хорошо изученных с точки зрения репликации видов, в частности человека. Ранняя репликация, как правило, приурочена к районам, характеризующимся высоким уровнем транскрипции, высокой плотностью генов и присутствием множественных сайтов потенциальной инициации репликации. Компактность генома *D. melanogaster* вносит некоторые особенности в организацию ее репликации. Последовательность репликации генома в политенных хромосомах и хромосомах диплоидных клеток имеет много общего: инициация репликации приурочена к одним и тем же районам, между которыми лежат протяженные участки генома, где репликация происходит преимущественно от краев к середине. Важнейшими особенностями репликации в политенных хромосомах являются низкая

скорость репликационных вилок и зависимость протяженности S-фазы от множества как внутренних, так и внешних факторов. В политенных хромосомах *D. melanogaster* скорость движения репликационных вилок зависит от присутствия в хроматине белков SUUR и Rif1.
Ключевые слова: *Drosophila melanogaster*; репликационный тайминг; сайт начала репликации; политенные хромосомы; эндоцикл; Supressor of UnderReplication; SUUR; Rif1.

Introduction

The development of high-throughput methods facilitated the genome-scale analysis of many biological phenomena. By now, detailed replication timing profiles have been described for genomes of many model organisms. As a rule, these profiles were created by pulse labeling of fluorescent DNA precursors in nonsynchronized cell cultures followed by automatic cell sorting by amount of DNA, which reflects cell cycle stages. Generally, two or three fractions corresponding to the early, middle, and late S-phases were isolated (Gilbert, Cohen, 1987; Schübeler et al., 2002; Schwaiger et al., 2009). Replication profiles are calculated as log2-transformed ratios of probabilities of replication in the early and late S-phase (Schübeler et al., 2002). Negative values correspond to replication in early S-phase, and positive values point to late replication.

Replication timing profiles reflect nonuniform chromatin organization, which is in turn associated with the nonuniform organization of the genome. Generally, regions with transcriptionally active chromatin replicate in the first half of the S-phase, while regions with silenced chromatin replicate later (Gilbert, 2002; Hiratani, Gilbert, 2009; Schwaiger et al., 2009; Gilbert et al., 2010; Hansen et al., 2010). Both in mammals and *Drosophila* early replication correlates with high gene density and high transcription level. Borders of early replicating domains match borders of topological domains. Late replicating regions coincide with domains of silenced chromatin and lamina-associated domains (Pickersgill et al., 2006; Belyakin et al., 2010; Peric-Hupkes et al., 2010; Rhind, Gilbert, 2013; Pope et al., 2014; Boulos et al., 2015; Prioleau, MacAlpine, 2016). Replication profiles may vary among different cell types, reflecting different chromatin states in particular genomic regions (Hiratani et al., 2008, 2010; Schwaiger et al., 2009; Pope et al., 2010). Aberration of replication timing may cause genome instability, defects of chromatin condensation, and, as a consequence, oncogenic transformation of cells (Hiratani, Gilbert, 2009).

Replication timing is regulated at the level of extended chromosomal domains (Berezney et al., 2000; Hiratani, Gilbert, 2009; Gillespie, Blow, 2010). In human and mouse genomic regions of a megabase scale demonstrate nearly synchronous initiation of DNA replication and borders between early and late replicating regions coincide with borders of open and closed chromatin (Jackson, Pombo, 1998; Ma et al., 1998; Julienne et al., 2013). Activation of origins and initiation of replication play a key role in the determination of replication timing (Jackson et al., 2006; Gillespie, Blow, 2010). Recent studies demonstrated that topological domains played an important role in the establishment of replication domains, or regions with coordinated regulation of DNA replication (Pope et al., 2014). In addition, it was shown that some genomic regions have no active replication initiation sites. These regions replicate late by very long replicons initiated in adjacent early replicating regions (Norio et al., 2005; Durkin, Glover, 2007; Cadoret et al., 2008; Letessier et al., 2011; Debatisse et al., 2012).

Replication timing in *Drosophila* cell cultures

D. melanogaster was one of the first organisms for which whole-genome replication timing profiles were obtained and extended (>100 kb) domains of early and late replication were identified (Schübeler et al., 2002; MacAlpine D.M. et al., 2004; Schwaiger et al., 2009; MacAlpine H.K. et al., 2010; Lubelsky et al., 2014). According to D.M. MacAlpine et al. (2004) and M. Schwaiger et al. (2009), the average size of replication domains in *Drosophila* (i.e., extended zones of similar replication timings where replication initiation is coordinated) is estimated to be 180 kb, much smaller than megabase-sized domains in mammals (White et al., 2004; Woodfine et al., 2005; Hiratani et al., 2008). The distribution of the Origin Recognition Complex (ORC) is the most important determinant of the replication-timing program in *D. melanogaster* chromosomes (MacAlpine D.M. et al., 2004; MacAlpine H.K. et al., 2010; Lubelsky et al., 2014). Early replication domains are characterized by high density of ORC-binding sites, presence of active chromatin marks, high gene density, and elevated transcriptional activity. On the contrary, late replicating domains demonstrate the absence of active chromatin marks, low gene density, weak ORC binding, and presence of H3K27me2/3 or H3K9me2/3 (MacAlpine et al., 2010; Lubelsky et al., 2014).

Comparison of replication timing in two cell types revealed changes in 21 % of autosomal regions (Schwaiger et al., 2009). Changes in replication timing on autosomes correlate with difference in gene expression. An elevated level of H4K16ac was detected in early replicating regions, including nontranscribed regions on the male X chromosome (Schwaiger et al., 2009). Y. Lubelsky et al. (2014) analyzed replication profiles in three *D. melanogaster* cell cultures by high-throughput sequencing and concluded that the majority of early and late replicating domains, covering 60 % of the genome, demonstrated similar replication timing patterns in the cell lines. The highest density of ORC-binding sites was observed in domains replicating early in the cell lines. In dynamic domains with variable replication timing, the density of ORC binding sites was low regardless of the replication time (Lubelsky et al., 2014).

Work on cell cultures revealed general patterns of replication in *Drosophila*, but no criteria were suggested for borders of replication domains. The understanding of the subdivision of *Drosophila* genome into replication domains came from analysis of replication in polytene chromosomes.

Cytological studies of replication timing in polytene chromosomes

Polytene chromosomes are interphase chromosomes comprising multiple DNA copies stacked together. Their giant size makes it possible to visualize molecular processes, such as gene expression or replication, at the cytological level at a very high resolution. Characteristic polytene chromosome patterns of alternating dark bands and light interbands have been used for many years for fine mapping of various cyto-

genetic markers. There are two types of bands in *Drosophila* polytene chromosomes. The most pronounced black bands contain DNA in a very compact state, whereas gray bands are less condensed. The DNA of interbands is significantly decompacted (Spierer A., Spierer P., 1984; Kozlova et al., 1994; Vatolina et al., 2011; Zhimulev et al., 2014).

Since the 1960s, replication in polytene chromosomes of *Drosophila* and other Diptera has been extensively studied by cytological methods. A detailed cytological analysis of replication in polytene chromosomes of *D. melanogaster*, *Rhynchosciara angelae* (=*Rhynchosciara americana*), *Chironomus thummi*, *Anopheles stephensi* and some other species showed similar patterns of ^{3}H thymidine incorporation in different polyploid tissues (for references see (Zhimulev, 1999)). A stage of continuous labeling, when entire chromosomes were covered with ^{3}H thymidine was followed by a stage of discrete labeling, when only the compact bands were labeled. In addition, a stage of inverse discrete labeling was detected, when some interbands, puffs, and decondensed bands were labeled (for review see (Zhimulev, 1999)). This stage corresponds to the very early S-phase. To determine the order of replication for different regions, various elegant methods were employed, such as double radioactive labeling, determination of the DNA amount in replicating bands, and analysis of patterns after natural or artificial synchronization of endocycles (Keyl, Pelling, 1963; Plaut et al., 1966; Danieli, Rodinò, 1967; Mulder et al., 1968; Amabis, 1974; Stocker, Pavan, 1974; Achary et al., 1981; Redfern, 1981). In different species, the replication time of a band correlates with the amount of DNA in it (Keyl, 1965; Mulder et al., 1968; Hägele, 1976; Bedo, 1982). Big bands replicate late and complete replication in a similar temporal order in different polytene tissues of *Drosophila* (Sinha et al., 1987; Koryakov, Zhimulev, 2015) and mosquito *Anopheles stephensi* (Redfern, 1981).

The use of fluorescence detection increased the resolution of the method. Back in 1985, the replication of larval salivary gland polytene chromosomes of *Chironomus tummi* was investigated by BrdU incorporation followed by fluorescence detection (Allison et al., 1985). D. Koryakov and I. Zhimulev (2015) used BrdU to study replication in polytene chromosomes from nurse cells of *D. melanogaster otu* mutants. In recent years, replication detection with PCNA antibodies has been extensively used (Gibert, Karch, 2011; Kolesnikova et al., 2013, 2018; Andreyeva et al., 2017). The high resolution of the method revealed that at the continuous labeling stage, when replication was seen almost everywhere along the chromosome arms, large black bands had not even started replication, whereas some regions of loosely packed chromatin had already completed it (Kolesnikova et al., 2013; Koryakov, Zhimulev, 2015).

Similarity of replication timing in polytene chromosomes and chromosomes of diploid cells

Despite a great amount of papers devoted to cytological studies of replication in polytene chromosomes, until recently, it was difficult to compare these results with data obtained on diploid cells because morphological structures of polytene chromosome were mapped to the *Drosophila* genome assembly at insufficient resolution. Invoking cytological and molecular data, T.D. Kolesnikova et al. (2018) mapped borders of all 159 prominent black bands on chromosome 2R to the *Drosophila* genome assembly with a high accuracy. These 159 bands are characterized by the presence of silent chromatin *ruby* (Zhimulev et al., 2014) and hence were named rb-bands (Kolesnikova et al., 2018). The rb-bands demonstrate low gene density, low gene expression level in individual tissues, and enrichment in transcriptionally inactive types of chromatin. The INTervals between rb-bands, or INTs, comprised from gray bands and interbands, are characterized by high gene density, high proportion of actively transcribed genes, and open chromatin in different cell types. The rb-bands contain predominantly tissue-specific genes, the INTs are enriched in genes expressed at high levels in many tissues. The rb-bands correspond to 60 % of the euchromatic part of chromosome 2R. Their sizes vary within 15–500 kb, the median size being ~50 kb. INTs are generally smaller, the median size ~30 kb. Border regions between rb-bands and INTs often coincide with borders of topological domains. The borders are characterized by a dramatic difference in the presence of multiple epigenetics markers such as H3K27me3, Lamin, SUUR, and histone H1 (Kolesnikova et al., 2018). INTs and rb-bands split chromosome 2R into two types of domains with contrasting properties, which are conservative in different tissues (Kolesnikova et al., 2018). Analysis of rb-bands from another chromosome arm shows similar features (Kolesnikova, unpublished results).

Analysis of replication in polytene chromosomes using anti-PCNA immunostaining demonstrated that INTs replicate before rb-bands (Kolesnikova et al., 2018). Completion of replication in rb-bands correlates with the size of the regions. Comparison of publicly available replication data for D*rosophila* cell cultures (Schwaiger et al., 2009) revealed that regions corresponding to the rb-bands showed a similar replication timing in diploid Kc and Cl8 cells; that is, replication starts and complete later in these regions, and replication time correlates with the size of the region. INTs replicate early in the salivary glands and cell cultures (Kolesnikova et al., 2018).

Analysis of ORC distribution data for salivary glands and cell cultures (Sher et al., 2013) revealed that INTs are enriched with ORC binding sites in polytene chromosomes and a cell culture and serve as initiation zones of early replication in different cell types. The rb-bands are practically devoid of ORC binding sites and presumably replicated by forks entering from early replication zones. A negative correlation –0.6 between the size of a region and time of replication completion in the cell culture (a minimal replication score for a region) supports this assumption. M. Schwaiger et al. (2009) found that in *Drosophila* cell cultures average length of replicons originated from early and late origins are 80 and 30 kb, respectively and suggested that replication forks originated from early replication origins enter late replicating regions. Indeed, the size of early replicons (80 kb) exceeds that of early replicating INT regions (30 kb).

Finding early and late replication origins: challenges and obstacles

While early replication origins are well defined in the *Drosophila* and human genomes, the locations and the very nature of the late replication origins remain somewhat obscure.

Replication profiles are produced by analysis of a cell population. Comparison of replication initiation sites identified by different techniques in mammals shows that origins are often present in clusters, named replication initiation zones (Borowiec, Schildkraut, 2011; Cayrou et al., 2011; Mesner et al., 2011). Every replication initiation zone contains multiple replication origins. It is postulated that origins have low efficiency (probability of activation in a cell cycle). Replication initiates stochastically at any origin within the zone. Initiation of replication inactivates neighboring origins by 'interference' (Lebofsky et al., 2006; Petryk et al., 2016; Prioleau, MacAlpine, 2016). In humans, the median size of early replication zones is 150 kb (Petryk et al., 2016). Multiple ORC binding sites identified within these zones serve as potential replication origins. These origins are confined to particular regions and easily detected by different experimental techniques (Petryk et al., 2016). In *Drosophila*, INTs presumably act as early replication initiation zones and contain multiple ORC binding sites. Both in *Drosophila* and humans, early replication initiation zones are interwoven with late replicating regions characterized by U-shaped replication profiles. Replication origins in late replicating regions are less confined to specific sites, and they do not necessarily correspond to ORC binding sites; hence, they can escape detection by traditional approaches (Petryk et al., 2016). A 'cascade' model for sequential activation of internal origins by moving replication forks was proposed for late replicating regions (Chagin et al., 2010; Guilbaud et al., 2011; Petryk et al., 2016).

M. Schwaiger et al. (2009) identified two peaks of replication initiation events in *Drosophila* cell cultures. Analysis of data from different sources on replicon size, size of candidate replication initiation zones, and domains depleted of ORC binding sites suggests that a major proportion of the euchromatic part of the *Drosophila* genome is replicated by forks originating in early S-phase in INTs (Kolesnikova et al., 2018). Size of the rb-bands located between INTs rarely exceeds hundreds of kbs, and these regions often complete replication by forks entering from adjacent INTs before activation of the late origins. Only the longest rb-bands represented by regions of intercalary heterochromatin and pericentric heterochromatin regions initiate replication in the late S-phase in diploid cells. It is hypothesized that late origins never fire in polytene chromosomes (Lilly, Duronio, 2005; Lee et al., 2009).

Activation of origins occurs throughout all S-phase. However, origins can be divided in two classes according to their response to the system of DNA damage control, intra-S-phase checkpoint. Treatment of cells with hydroxyurea (HU) activates the intra-S-phase checkpoint. Origins capable of activation in the presence of HU are considered as early origins (Shirahige et al., 1998; Willis, Rhind, 2009). MacAlpine et al. (2010) mapped origins active in Kc167 cells after HU treatment. All identified HU-insensitive origins coincide with ORC binding sites. However, only 30 % of the ORC binding sites overlap HU-insensitive origins. It is unclear whether the remaining ORC binding sites correspond to late replication initiation sites or represent weak origins, which may be activated in a stochastic manner in a limited number of cells. In addition, any manipulations with the cell cycle and the inter-S-phase checkpoint can have a profound effect on the pattern of active origins (Kolesnikova et al., 2013).

Distinct properties of replication in *Drosophila* polytene chromosomes

Polytene chromosomes emerge in a modified cell cycle called endocycle. An endocycle lacks all steps of mitosis. Different tissues attain different ploidies. In *D. melanogaster* salivary gland cells typically achieve ~1,300C, whereas fat body cells ~256C and midgut enterocytes 32C (Shu et al., 2018). In salivary glands, the exit from the S-phase happens before the completion of the entire genome replication and approximately 30 % of the genome are underreplicated in polytene chromosomes (Lilly, Spradling, 1996; Royzman, Orr-Weaver, 1998; Doronkin et al., 2003; Lee et al., 2009; Zielke et al., 2013; Edgar et al., 2014; Shu et al., 2018). The underreplication points to a change in the system of DNA damage control. It is plausible that the intra S-phase checkpoint and activation of late origins are absent in polytene nuclei (Lilly, Duronio, 2005; Lee et al., 2009). The apoptosis mechanism is turned off in these cells (Hassel et al., 2014). Genes associated with DNA replication are expressed at relatively low levels in endocycling cells (Maqbool et al., 2010).

The *Drosophila* endocycle is driven by oscillation of Cyclin E/Cdk2 activity. Different levels of Cyclin E/Cdk2 activity control initiation and termination of endoreplication, as well as the origins reset (Lilly, Spradling, 1996; Edgar, Orr-Weaver, 2001; Lilly, Duronio, 2005; Edgar et al., 2014; Shu et al., 2018). Ectopic expression of *Cyclin E* under control of the *hsp70* promoter induces S-phase in salivary gland cells (Duronio, O'Farrell, 1995; Su, O'Farrell, 1998). The oscillation of Cyclin E-Cdk2 activity is controlled at many levels, including the transcriptional induction of the *Cyclin E* gene by E2F1 (Duronio, O'Farrell, 1995), the destruction of Cyclin E protein by SCFAgo E3 ubiquitin ligase (Moberg et al., 2001; Shcherbata et al., 2004; Zielke et al., 2011), and the oscillation of cyclin-dependent kinase inhibitor Dacapo (Hong et al., 2007; Swanson et al., 2015). The oscillation of Cyclin E-Cdk2 activity and, hence, endocycle regulation are sensitive to external factors, such as nutrient levels (Britton, Edgar, 1998). External factors act via a network of signaling and transcription factors. Well-studied upstream regulators of the endocycle include growth factors, in particular, insulin-like peptides, and epidermal growth factor (EGF) signaling. The downstream effectors in these pathways are PI3K, AKT, target of rapamycin (TOR), forkhead box O (FOXO), RAS, and MAPK (Britton et al., 2002; Saucedo et al., 2003; Pierce et al., 2004; Grewal et al., 2005; Demontis, Perrimon, 2009; Edgar et al., 2014). The nonuniform distribution of external factors can asynchronously induce endoreplication in cells within an organ. Nuclei in salivary gland are not synchronized, but the distribution of endocycle stages is not random (Rudkin, 1973; Smith, Orr-Weaver, 1991; Kolesnikova et al., 2013). For example, nuclei in proximal and distal ends of the *D. melanogaster* salivary gland differ by one or two rounds of endoreplication. Some of these differences are mediated by transcription factor Sunspot (Ssp). It promotes the expression of E2F1 and PCNA in *D. melanogaster* salivary glands. In the proximal region of the gland, the *sunspot* gene activity is inhibited by wingless (Wnt) signaling, resulting in reduced E2F1 expression, a longer endocycle, and decreased ploidy (Taniue et al., 2010). Ssp is negatively regulated by Arm (Taniue et al., 2010). The *arm*-GAL4 driver induces mosaic expression

of the reporter gene *LacZ* in salivary glands of *Drosophila* third instar larvae (Kolesnikova et al., 2005). Perhaps the Arm mosaic expression contributes to the asynchrony of endocycles in salivary gland. In *D. melanogaster* ovarian follicle cells, differentiation-associated endocycle exit is regulated through a combination of Notch signaling, Ecdysone receptor, and transcriptional repressor Tramtrack (Sun et al., 2008).

It has been shown in *Drosophila* that the duration of endocycles and, presumably, the S-phase increases with increase of ploidy (Rudkin, 1972, 1973). The speed of replication forks in salivary gland polytene chromosomes of *Drosophila* and *Rhynchosciara* is significantly lower than in diploid cells, especially during the late S-phase (Meneghini, Cordeiro, 1972; Cordeiro, Meneghini, 1973; Steinemann, 1981a, b; Lakhotia, Sinha, 1983). Studies of underreplication profiles in different *Drosophila* tissues have shown that underreplication zones can be tissue-specific, but they correspond to repressive chromatin areas lacking origins in other polytene tissues where they are completely replicated. The authors suggest that the difference in underreplication is caused by tissue-specific variation of replication rates in these regions (Hua et al., 2018).

The speed of replication forks in *Drosophila* polytene chromosomes depends on SUUR protein (Sher et al., 2012; Nordman et al., 2014). In $SuUR^{ES}$ polytene chromosomes the late replicating regions complete replication earlier (Zhimulev et al., 2003). Both in salivary glands and in diploid cells, all chromosome regions corresponding to rb-bands are enriched in SUUR protein (Kolesnikova et al., 2018). Targeting of SUUR protein to an early replication region on a polytene chromosome leads to the late completion of replication in this site (Pokholkova et al., 2015). It can be assumed that SUUR plays an important role in the replication delay associated with rb-bands at least in salivary gland chromosomes (Kolesnikova et al., 2018). Continuous *SuUR* overexpression in salivary glands starting from early embryogenesis leads to a miniature gland, indicative of a strong suppression of DNA replication in the cells (Volkova et al., 2003; Zhimulev et al., 2003). Overexpression of *SuUR* in the middle of the third larval instar under the salivary gland specific *Sgs3*-GAL4 driver leads to disappearance of nuclei at G-phase and accumulation of nuclei at early S-phase, when labelling is observed only in early replicating regions (Kolesnikova et al., 2011). The *SuUR* overexpression does not abolish incorporation of 5-ethynyl-2′-deoxyuridine (EdU) into the polytene chromosomes, hence DNA replication continues, albeit at a very low rate.

SuUR overexpression exerts a significantly less pronounced effect in diploid cells. There is no obvious effect on eye formation, but apoptosis was observed in the wing disc, and the wing shape was distorted. The effect is enhanced by mutations in the genes encoding E2F and PCNA proteins, suggesting that ectopic SUUR affects replication in diploid cells (Volkova et al., 2003). The cause of the more pronounced effect of SUUR on DNA replication in polytene chromosomes compared with diploid cells remains to be found out.

O.V. Posukh et al. (2015) suggests that the SUUR-dependent replication delay is important for a proper post-replication chromatin assembly. The *SuUR* mutation changes levels of H3K27me3 and H3K9me3 in late replicating regions of polytene chromosomes of salivary glands and pseudonurse cells of otu^{11} mutants (Koryakov et al., 2011; Sher et al., 2012; Posukh et al., 2017). According to O.V. Posukh et al. (2017), SUUR in *Drosophila* chromosomes is involved in the epigenetic inheritance of H3K27me3 in those regions where Polycomb complexes only establish, but do not maintain H3K27me3 silencing (Posukh et al., 2015, 2017).

The Rif1 protein, a candidate repressor of replication, is another important factor involved in the regulation of replication timing in *D. melanogaster*. Rif1 recruits phosphatase PP1 to multiple chromosomal sites, where the latter can dephosphorylate replicative helicase and block premature replication of heterochromatin sequences (Sreesankar et al., 2015; Seller, O'Farrell, 2018). During amplification of chorion genes in ovarian follicle cells, Rif1 is localized in active replication forks in a partially SUUR-dependent manner, where it directly regulates replication fork progression (Munden et al., 2018). It appears that in polytene chromosomes SUUR protein binds to chromatin domains where histone H1 is accumulated during the S-phase (Andreyeva et al., 2017). SUUR binds to the components of replication forks (Kolesnikova et al., 2013; Nordman et al., 2014) and attracts Rif1 protein (Munden et al., 2018). In turn, Rif1 protein attracts PP1 phosphatase, which dephosphorylates replicative helicase, thereby controlling the activation time of the origins and the replication fork rate (Sreesankar et al., 2015; Seller, O'Farrell, 2018).

Conclusion

In the last twenty years, the progress in understanding of replication was associated with advances in high-throughput techniques. Comparison of genomic replication profiles revealed significant similarity between *Drosophila* and other well-studied eukaryotic organisms, such as humans. Early replication is often confined to intensely transcribed gene-dense regions characterized by multiple replication initiation sites. Features of DNA replication in *Drosophila* may be explained by the compact genome (Petrov, 2002). The most important feature of replication in polytene chromosomes is their low replication rate and the dependence of S-phase duration on many factors: external and internal, local and global. In *D. melanogaster* polytene chromosomes, the speed of replication forks is affected by SUUR and Rif1 proteins. It is not known yet how universal the mechanisms associated with these factors are, but their study is very promising.

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