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Illuminating DNA replication during *Drosophila* development using TALE-lights

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New discoveries allow systematic engineering of DNA sequence recognition using the modular recognition units of the transcription activator-like effectors (TALEs) or the guide RNA of the CRISPRs. The engineered specificity offers the potential to guide a wide range of activities to particular sequences [1–3], and targeted nucleases cause directed mutagenesis [4–6]. Here we have tagged sequences using fluorescent fusions that we call TALE-lights, and have used these to follow replication of particular satellite sequences during a major embryonic transition in *Drosophila*. We show that replication-timing of individual sequences can be measured. As embryos develop, the cell cycle extends and the timing of replication of satellite sequences shifts to later times within the more prolonged S phase. Here we show that the compact foci of satellite sequences expand in conjunction with their replication and that a satellite sequence, 359-bp, shows a particularly marked shift to later replication in the cell cycle after the midblastula transition (MBT). This replication behavior suggests that developmental signals can separately influence the timing of different satellite sequences.

The earliest mitotic cycles in a *Drosophila* embryo are extremely fast with an S phase of only 3.4 min that occupies all of the short interphase. After 9 cycles, interphase duration progressively and incrementally increases until cycle 14 when S phase abruptly extends to one hour as numerous accompanying changes activate morphogenesis at the MBT. We are interested in the temporal control of this progression. We have shown that the increasing length of S phase is a key determinant of timing and is required for cycle 14 MBT events [7]. In the early, fast S phases, all of the DNA sequences replicate at the same time without exhibiting the typical eukaryotic subdivision in which euchromatin replicates early and heterochromatin replicates late. As the S phase incrementally increases in duration during cycles 12 and 13, there is a slight delay in the timing of replication of satellite sequences, the so-called constitutively heterochromatic sequences [8]. The abrupt prolongation of S phase in cycle 14 is triggered by down-regulation of G2 cyclin–Cdk1 activity, a kinase capable of driving early replication of otherwise late replicating satellite sequences [9]. Thereafter the replication of satellite sequences is substantially delayed and different satellites replicate with different delays [8].

To understand this program, we need to identify the developmental signal triggering these events, and we need to understand the control of replication timing. To begin to investigate the latter with temporal precision, we wanted methods to follow replication timing live. To do this we have made TALE-lights that mark specific blocks of satellite sequence. Two TALE-lights recognize sequences within a 359 base-pair sequence that is repeated about 30,000 times on the X-chromosome, and another recognizes two repeats of a 10 base-pair sequence present in pericentric arrays called 1.686 on each of two large autosomes (Figure 1A,B and see Experimental Procedures in Supplemental Information, published with this article online). We injected *in vitro* produced RNAs into the embryos to express the TALE-lights (not shown), or have directly injected bacterially expressed TALE-light proteins into the embryos. Figure 1C shows three nuclei of a blastoderm embryo as they progress through cell cycle 13. The embryo was injected with TALE-lights, 359–RFP and 1.686–GFP, during the previous cycle. A single spot for 359–RFP is seen, indicating that this is a male embryo (one X chromosome) and four spots can generally be seen for 1.686 (two homologs for each of the two chromosomes carrying this repeat). We confirmed the specificity by comparison of the TALE-light signal to the *in situ* signal obtained with satellite-specific probes (Figure S1 A–C). The dynamic features of TALE-light signals are of interest.

TALE-light signals decline during mitosis. As the nuclei reach metaphase (20 min in Figure 1C), the 359-bp signal was diminished to the point where it was not detected. Although the 1.686 signal is also much fainter, it can be seen as four pairs of spots. The pairs represent the sister chromatids, which, though still paired at the metaphase plate, typically resolve into two closely spaced spots that abruptly separate at the onset of anaphase (not shown). We do not know the basis for the decline in the signal at mitosis. The compaction of the satellite sequences changes little if at all between interphase and mitosis [8]; nonetheless, other dramatic changes that include mitosis-specific modifications of the histones, and association of specific proteins to mitotic chromosomes [10] might displace the TALE-lights. Signals reaccumulate as interphase nuclei reform.

Of more direct concern to us here, TALE-lights allow us to follow the timing of replication of specific sequences in real-time and so achieve a heretofore unattainable temporal resolution. As is particularly obvious for the 1.686, the fluorescent foci partially disperse during interphase and then re-compact (Figure 1C). The dispersal occurs at the time when the satellites replicate [8]. We have previously described a tagged version of the processivity factor for replication, GFP–PCNA, that acts as a reporter for replication [7,8]. In embryos injected with an RFP–TALE-light and GFP–PCNA, the PCNA was recruited to the foci at the time that they expanded in size (Figure 1 D,E). We conclude that the compact foci of heterochromatin partially decompact as they replicate. Note that all four spots of 1.686 disperse and recruit GFP–PCNA at the same time despite the fact that they are neither contiguous nor paired. This suggests that this sequence responds accurately and independently to a timing signal.

We defined the time of replication of 359-bp and 1.686 sequences in cycles 12 through 15 based on PCNA recruitment (Figure 1F, movies S1 and S2). The satellites exhibited delayed initiation of replication and the amount of this

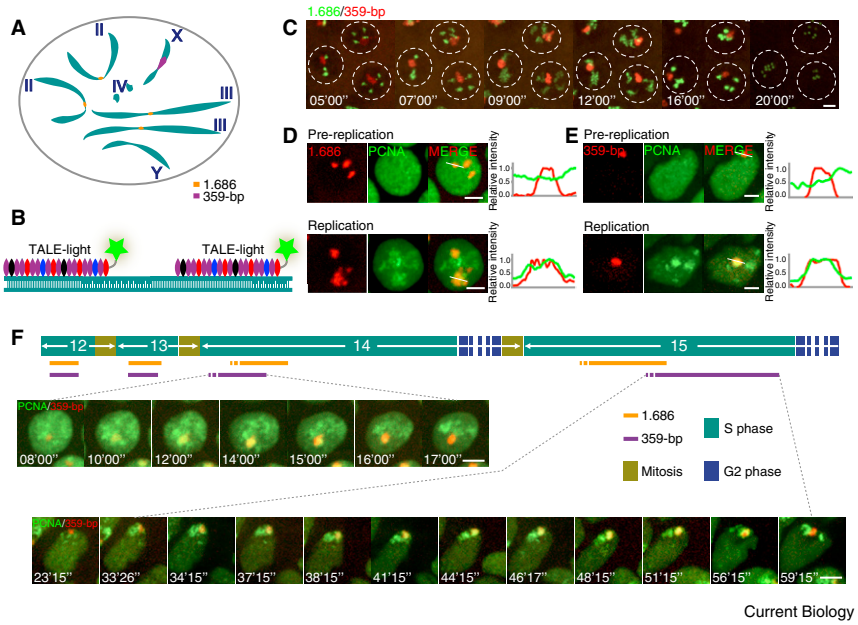

Figure 1. Timing events of the late replication program in embryos using TALE-lights.
(A) A schematic of the chromosome complement of a male *Drosophila* with the positions of 359-bp repeats and 1.686 repeats illustrated. (B) Cartoon of two TALE-lights, with colored domains having different nucleotide specificities, each recognizing two repeats of the 1.686 sequence (details in Supplemental Experimental Procedures). (C) Two-color imaging of 359–RFP (red) and 1.686–GFP (green) TALE-lights throughout cycle 13. Early and slight decompaction of the single 359-bp focus is followed by the more dramatic decompaction of all four of the 1.686 foci. The sequences recompact, initially without obvious duplication into sisters, but on entry into mitosis the duplication of 1.686 foci is evident as pairs of sister foci at the metaphase plate. Staining by TALE-lights declines during mitosis, and this is particularly dramatic for the 359-bp probes. (D) An embryo injected with GFP–PCNA and 1.686–RFP TALE-light was imaged in cycle 14 before and after initiation of replication of the 1.686 and a single nucleus is shown. The recruitment of PCNA to the satellite foci at the time of their decompaction is illustrated and levels of fluorescence are quantified along the indicated line. Note that homolog pairing, a well-known phenomenon in *Drosophila*, begins in cycle 14 and the number of 1.686 foci decline from four to three to two with substantial nucleus-to-nucleus variation during cycle 14. (E) A cycle 15 nucleus in an embryo injected with GFP–PCNA and 359–RFP TALE-light showing the recruitment of GFP–PCNA to the RFP focus. (F) A time line illustrating the changing duration of the embryonic cell cycles and their S phases along with the timing of the replication of the satellite sequences. Illustrative frames from the movies are shown for 359-bp in cycles 14 and 15. Note that a variable length G2 is introduced in cycle 14 and this is indicated by interrupted blue line segment. Bars: 3 μm.

delay increased from one cycle to the next (Figure S1D). Additionally, the duration of replication increased (Figure S1E). While it is possible that the binding of TALE-lights might alter replication behavior, we suggest that any such effect is slight, because the measured replication of 359-bp is consistent with our previous direct assessment of replication time of this repeat [8]. Beyond the new temporal detail and accurate replication timing during the asynchronous post-MBT cycles, one new feature of the data surprised us. Among the sequences we studied, we had identified 359-bp as the earliest replicating satellite sequence in S phase 14, and we found that it shifted to a considerably later replication time in cycle 15. Nonetheless, we thought that it remained an unusually early replicating satellite sequence in cycle 15 and we thought that each satellite had a particular lateness attribute so that, even though sequences shifted toward later replication, they would not change order. However, it is clear from these data that between cycle 14 and cycle 15 the relative timing of 1.686 and 359-bp replication switches (Figure 1F). This finding suggests that developmental changes can independently program the timing of the replication of these different sequences; however, this result should be interpreted with caution, as it is possible that the binding of TALE-lights might alter the behavior of these satellites differently.

Our findings illustrate that TALE-lights introduce new experimental avenues by providing a simple way to visualize particular sequences live. This capability empowers the use of other tagged reporters, such as PCNA–GFP, allowing determination of their recruitment to specific sequences over time.

Supplemental Information

Supplemental Information contains one figure, experimental procedures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.01.023.

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