

Featured Publication Note

Dr. Casas-Delucchi and colleagues

Technische Universität Darmstadt, Max Delbrück Center for Molecular Medicine & Ludwig Maximilians University Munich



Imaging the dynamics and epigenetic control of inactive X chromosome replication

Background

The inactivation of one X chromosome in female somatic cells is a well known example of epigenetically silenced chromatin and is necessary for gene dosage compensation. Late replication of the inactive X chromosome (Xi) has been proposed to be an important factor in the maintenance of the silenced state. In this study, researchers analyzed the dynamics of Xi replication in living cells and investigated the epigenetic factors that determine this replication timing.

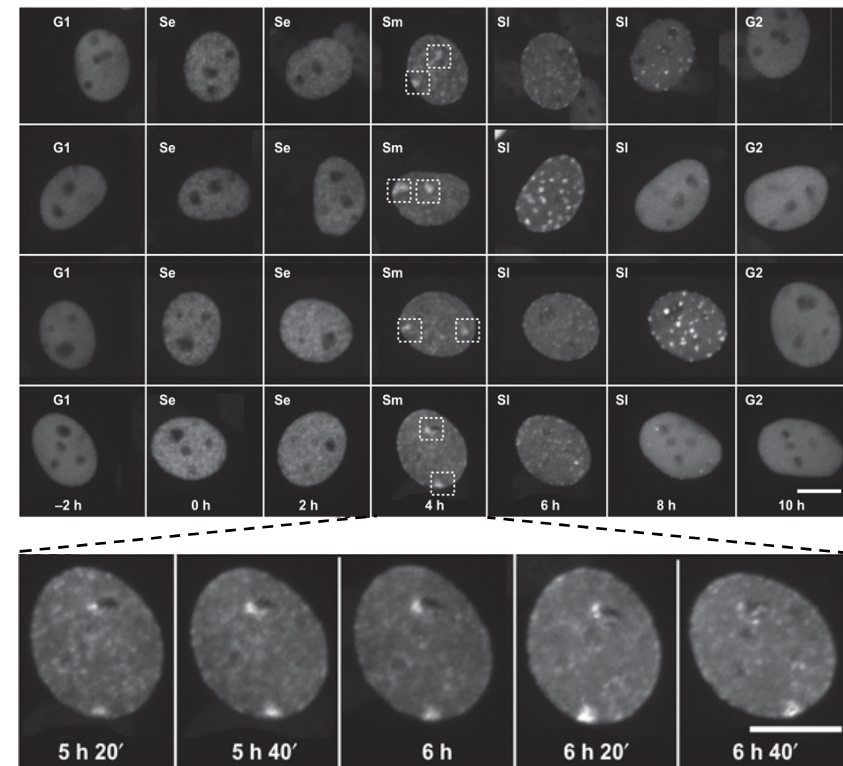
How did the UltraVIEW VoX system help researchers to achieve their research goals?

To follow the precise progression of DNA replication in living mammalian cells, researchers used GFP-tagged replication proteins and performed 3D time-lapse experiments using the UltraVIEW® VoX 3D Live Cell Imaging System (figure). This system was chosen because of its low phototoxicity levels and fast acquisition speed. Time-lapse analysis demonstrated that in somatic mammalian cells, Xi replicates within a narrow time frame during early-mid S-phase.

The extent to which different epigenetic modifications define these replication dynamics was then examined. Three epigenetic hallmarks of Xi were individually disrupted using mutational analysis and chemical inhibition, and the effect on the timing of Xi replication observed using the UltraVIEW VoX system, as well as other microscope systems. These experiments demonstrated that histone hypoacetylation has a key role in controlling the maintenance of the replication timing of Xi.

How does this study contribute to scientific knowledge?

The researchers speculate that the epigenetically controlled replication dynamics of Xi might be a common feature of different forms of transcriptionally silent chromatin, and that histone hypoacetylation is the common epigenetic denominator which regulates their synchronous replication.



The inactive X chromosome replicates synchronously during early-mid S-phase

Selected frames of time-lapse imaging movies (available at <http://www.nature.com/ncomms/journal/v2/n3/full/ncomms1218.html#supplementary-information>) of C2C12 mouse myoblasts stably expressing GFP-PCNA (proliferating cell nuclear antigen) to visualize DNA replication sites. Z stacks were collected every 20 min over a time period of up to 15 hr. Two large, synchronously replicating chromatin structures appear during early-mid S-phase and persist for 60-120 min. Cell cycle phases are indicated: G1, SE (S early), SM (S mid), SL (S late) and G2.