In This Issue

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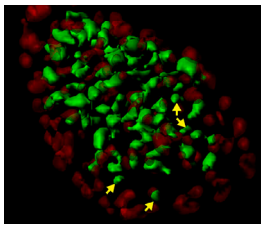
Determining the replication factory settings



Super-resolution microscopy shows the replication factor PCNA (green) in replication factories of different sizes including small ones (arrows) that may contain a single replicon.

Saner et al. describe how neighboring DNA regions stochastically assemble into replication factories in budding yeast.

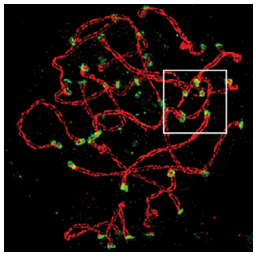
A replicon is a stretch of DNA duplicated from a single replication origin. In eukaryotes, multiple replicons assemble into sub-nuclear structures called replication factories, where the replicons are duplicated by DNA polymerases and other replication proteins. Replication factories help to coordinate efficient DNA synthesis, but how replicons are organized into these structures is unclear.

Saner et al. used live-cell imaging to follow the replication of different replicons along a budding yeast chromosome. Surprisingly, whether the replicons duplicated in the same or different replication factories varied from cell to cell. Neighboring replicons were more likely to duplicate in the same factory than replicons spaced further apart. Once assembled into the same factory, however, replicons remained associated for several minutes, long enough, perhaps, for replication to be completed.

Using super-resolution microscopy, Saner et al. found that many replication factories contain only a single replicon and that few factories contain more than four. Mathematical models of factory assembly supported the idea that neighboring replicons randomly associate to form factories of variable size and composition. Senior author Tomoyuki Tanaka is now interested in determining whether similar principles apply to the formation of replication factories in mammalian nuclei and to the assembly of other subnuclear structures involved in transcription and DNA repair.

Saner, N., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201306143.

KASH5 helps meiotic chromosomes LINC up



Super-resolution microscopy shows rings of KASH5 (green) at the tips of homologous chromosome pairs stained for the axial element protein SCP3 (red).

Horn et al. identify a protein that helps homologous chromosomes pair up in meiosis by connecting them to the microtubule cytoskeleton.

Early in meiosis, chromosomes cluster together so that homologous chromosomes can find each other and pair up to undergo recombination. Clustering is controlled by LINC complexes, which span the nuclear envelope to couple chromosomes to the microtubule-based motor protein cytoplasmic dynein. Dynein can therefore pull chromosomes toward the centrosome on one side of the nucleus.

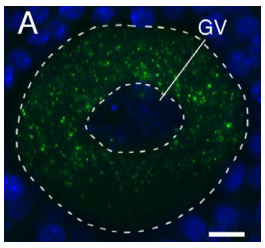
LINC complexes are formed by members of the SUN and KASH protein families. SUN1 is an inner nuclear membrane protein that, in most organisms, attaches to the telomeres of meiotic chromosomes. A member of the KASH family of outer nuclear membrane proteins links SUN1 to cytoplasmic dynein, but which KASH protein performs this function in mammals is unknown.

Horn et al. focused on KASH5, a recently identified KASH protein expressed in testes and ovaries. KASH5 colocalized with SUN1 at sites where telomeres attached to the nuclear envelope in mouse spermatocytes. Mice lacking KASH5 were infertile. Males, for example, couldn't produce mature sperm because their spermatocytes arrested early in meiosis after failing to form homologous chromosome pairs. Telomeres were still attached, via SUN1, to the nuclear envelope, but dynein was no longer recruited to these attachment sites, thus abolishing chromosome clustering and homologue pairing.

Having established KASH5 as a member of the meiotic LINC complex in mammals, senior author Brian Burke now wants to identify proteins that connect telomeres to SUN1 at the nuclear periphery.

Horn, H.F., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201304004.

RNA granules act as egg timers



Granules of *cyclin B* mRNA (green) are distributed asymmetrically in the cytoplasm of an immature mouse oocyte. GV indicates the germinal vesicle/nucleus.

Kotani et al. reveal how RNA granules control the timing of *cyclin B* translation during oocyte maturation.

Fully grown oocytes initially arrest in prophase I, and mRNAs required for meiotic progression are translationally repressed. In response to maturation hormones, mRNA translation is activated so that the oocytes can progress to metaphase II, ready for fertilization. One key mRNA is the *cyclin B* transcript, whose translation induces germinal vesicle (nuclear) breakdown and assembly of the first meiotic spindle. Exactly how *cyclin B* translation is controlled is unclear, however.

Kotani et al. found that *cyclin B* mRNA is assembled into granules in the cytoplasm of immature zebrafish and mouse oocytes. Maturation hormones induced disassembly of these granules at the same time that *cyclin B* began to be translated. The researchers discovered that granule assembly was promoted by actin filaments and by the protein Pum1, which bound to *cyclin B* transcripts. Disrupting granule assembly—by depolymerizing actin or expressing a mutant *cyclin B* mRNA unable to bind Pum1—caused *cyclin B* to be translated sooner after stimulating oocyte maturation. Inhibiting granule disassembly, on the other hand, delayed *cyclin B* translation and germinal vesicle breakdown.

Lead author Tomoya Kotani says that granule assembly isn't required to repress *cyclin B* translation; even in the absence of granule formation, Cyclin B isn't produced until oocyte maturation is initiated. Instead, granules control the timing of *cyclin B* translation in maturing oocytes. Kotani now wants to investigate what triggers granule disassembly and translation activation and to follow the process in real time using live imaging.

Kotani, T., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201302139.