billion years, despite all the planetary turmoil, and that evolution, as much as it means change and innovation, it also — perhaps more often — means hanging on to what works best.

References

1. Zhang, X.G., Maas, A., Haug, J.T., Siveter, D.J., and Waloszek, D. (2010). A eucrustacean metanauplius from the Lower Cambrian. Curr. Biol. *20*, 1075–1079.
2. Allison, P.A., and Briggs, D.E.G. (1993). Exceptional fossil record: distribution of soft-tissue preservation through the Phanerozoic. Geology *21*, 527–530.
3. Orr, P.J., Benton, M.J., and Briggs, D.E.G. (2003). Post-Cambrian closure of the deep-water slope-basin taphonomic window. Geology *31*, 769–772.
4. Gould, S.J. (1989). Wonderful Life: The Burgess Shale and the Nature of History (New York: W.W. Norton).
5. Simon, Conway Morris (1998). The Crucible of Creation: The Burgess Shale and the Rise of Animals (Oxford: Oxford University Press).
6. Conway Morris, S. (2009). Walcott, the Burgess Shale and rumours of a post-Darwinian world. Curr. Biol. *19*, R927–R931.
7. Shu, D.G., Luo, H.L., Conway Morris, S., Zhang, X.L., Hu, S.X., Chen, L., Han, J., Zhu, M., Li, Y., and Chen, M. (1999). Lower Cambrian vertebrates from South China. Nature *402*, 42–46.
8. Van Roy, P., Orr, P.J., Botting, J.P., Muir, L.A., Vinther, J., Lefebvre, B., el Hariri, K., and Briggs, D.E.G. (2010). Ordovician faunas of Burgess Shale type. Nature *465*, 215–218.
9. Waloszek, D. (2003). The 'Orsten' window — a three-dimensionally preserved Upper Cambrian Meiofauna and its contribution to our understanding of the evolution of arthropoda. Paleont. Res. *7*, 71–88.
10. Chen, J.Y., Bottjer, D.J., Li, G., Hadfield, M.G., Gao, F., Cameron, A.R., Zhang, C.Y., Xian, D.C., Tafforeau, P., Liao, X., *et al.* (2009). Complex embryos displaying bilaterian characters from Precambrian Doushantuo phosphate deposits, Weng'an, Guizhou, China. Proc. Natl. Acad. Sci. USA *106*, 19056–19060.
11. Zhang, X.G., Siveter, D.J., Waloszek, D., and Maas, A. (2007). An epipodite-bearing crown-group crustacean from the Lower Cambrian. Nature *449*, 595–598.
12. Siveter, D.J., Williams, M., and Waloszek, D. (2001). A phosphatocopid crustacean with appendages from the Lower Cambrian. Science *293*, 479–481.
13. Harvey, T.H.P., and Butterfield, N.J. (2008). Sophisticated particle-feeding in a large Early Cambrian crustacean. Nature *452*, 868–871.
14. Hughes, N.C. (2007). The evolution of trilobite body patterning. Annu. Rev. Earth Plan. Sci. *35*, 401–434.
15. Kortschak, R.D., Samuel, G., Saint, R., and Miller, D.J. (2003). EST analysis of the cnidarian Acropora millepora reveals extensive gene loss and rapid sequence divergence in the model invertebrates. Curr. Biol. *13*, 2190–2195.
16. Erwin, D.H., and Davidson, E.H. (2002). The last common bilaterian ancestor. Development *129*, 3021–3032.

Florian Maderspacher is *Current Biology*'s Senior Reviews Editor.
E-mail: florian.maderspacher@current-biology.com

DOI: 10.1016/j.cub.2010.05.025

DNA Replication: Metazoan Sld3 Steps Forward

In yeast, phosphorylation of the Sld3 protein by cyclin-dependent kinases is essential for replication initiation. In metazoans, three potential Sld3 counterparts have emerged. A new study suggests that one of these, Treslin/Ticrr, is the Sld3 ortholog.

Yu V. Fu and Johannes C. Walter*

In vertebrate cells, DNA replication initiates from thousands of sites called origins of replication, a process that requires a cyclin-dependent kinase (CDK) [1]. One of the holy grails in the replication field has been the identification of functionally relevant S phase CDK substrates. The recent demonstration that Sld2 and Sld3 are the only essential CDK targets for replication initiation in yeast was an important breakthrough [2,3]. However, metazoan Sld2 and Sld3, as well as metazoan S phase CDK targets, remained elusive. In the last year, three different proteins (DUE-B, Treslin/Ticrr, and GEMC1) were identified that appear to have similar functions to Sld3, and at least one of these (GEMC1) was found to be a bona fide CDK target [4–7]. However, none of the three proteins was shown to contain significant sequence similarity to Sld3. In a new report in this issue of *Current Biology*, Sanchez-Pulido and colleagues [8] now show that significant sequence homology does in fact exist between Treslin/Ticrr and Sld3, suggesting an orthologous evolutionary relationship between these two proteins. As discussed below, this observation raises interesting questions about the evolutionary and functional relationship of Treslin/Ticrr to DUE-B and GEMC1.

In all eukaryotic cells, replication initiation can be separated into two distinct steps that occur in different phases of the cell cycle (Figure 1). In G1, the origin recognition complex (ORC) binds to DNA and cooperates with Cdt1 and Cdc6 to recruit the MCM2–7 helicase. In S phase, CDK and DDK (Dbf4-dependent kinase) cooperate with Sld2, Sld3, Dpb11 and several other factors to activate the MCM2–7 helicase. The mechanism of this activation process is currently mysterious, but it involves the recruitment of two helicase co-factors, Cdc45 and GINS, to the MCM2–7 complex to form the CMG holo-helicase complex. Once activated, CMG unwinds the origin in preparation for DNA synthesis.

In budding yeast, CDK promotes the interaction between Dpb11, Sld2, and Sld3 [2,3]. Specifically, Sld2 and Sld3 are phosphorylated on multiple sites by CDK, and this modification promotes the binding of these proteins to caboxy- and amino-terminal pairs of BRCT repeats within Dpb11, respectively. Via an unknown mechanism, the complex of Dpb11, Sld2, and Sld3 helps facilitate the formation of the CMG helicase.

How conserved are these events in higher eukaryotic cells? In metazoans, TopBP1 (also called CUT5 or MUS101) is an excellent candidate for the Dpb11 ortholog: like Dpb11, it contains multiple BRCT motifs, the first three of which are necessary and sufficient to support chromosomal DNA replication [9,10]. Moreover, TopBP1 stimulates the same initiation step as Dpb11, the origin association of Cdc45 and GINS [11]. Based on sequence similarity, RecQL4 is a possible vertebrate homolog of Sld2. However, unlike Sld2, RecQL4 appears to function *after* Cdc45 and GINS have been loaded onto replication origins [12,13]. Therefore, the CMG assembly function of Sld2 may have been replaced by another protein in higher eukaryotes.

Three proteins, DUE-B, Treslin/Ticrr, and GEMC1, have all been proposed as possible candidates for the vertebrate Sld3 ortholog. DUE-B (DNA Unwinding Element Binding), was isolated as a c-myc origin binding protein [5]. Treslin (TopBP1-interacting,

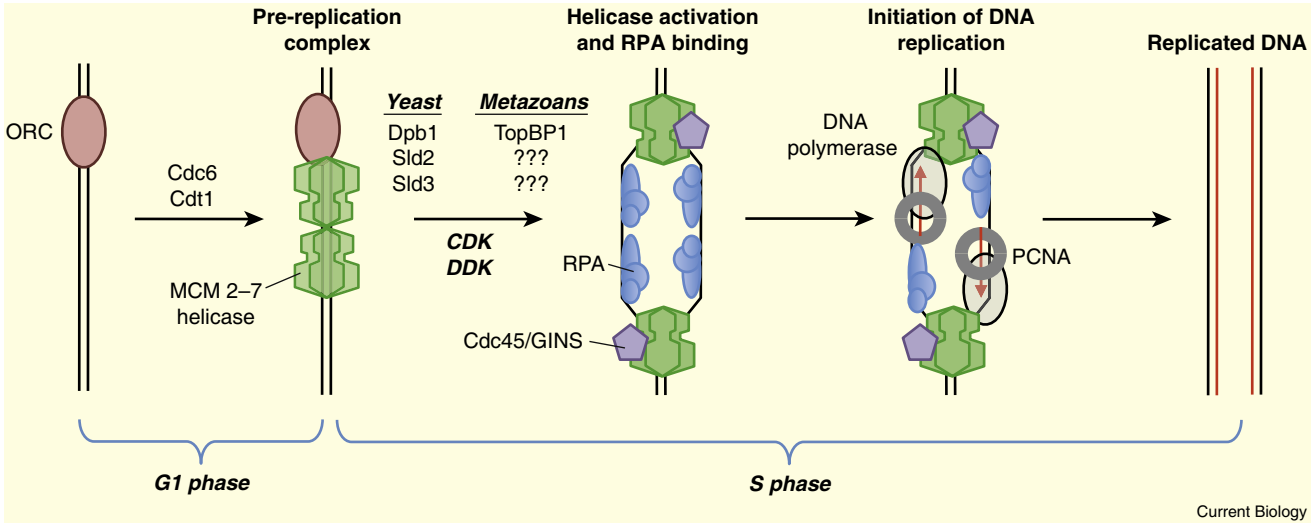

Figure 1. Mechanism of eukaryotic DNA replication.

In the G1 phase of the cell cycle, ORC, Cdc6, and Cdt1 cooperate to recruit the MCM2–7 helicase to origins, forming pre-replication complexes. In S phase, CDK and DDK cooperate with many other factors to load GINS and Cdc45 onto the MCM2–7 helicase to form the CMG complex, which is the active helicase. In yeast, Dpb11, Sld2, and Sld3 are critical CDK targets. In metazoans, Dpb11 most likely corresponds to TopBP1, but the proteins that function like Sld2 and Sld3 in CMG assembly are not known. Once assembled, CMG unwinds the origin, leading to binding of the single-stranded DNA binding protein RPA, followed by DNA polymerases and their processivity factor PCNA. The two sister replisomes assembled at the origin then replicate DNA in opposite directions.

replication-stimulating protein) was isolated as a TopBP1-binding protein from *Xenopus* egg extracts [6]. The same protein was also recovered in a screen for checkpoint deficiency in zebrafish and called Ticrr (TopBP1-interacting, check-point, and replication regulator) [7]. Finally, GEMC1 was identified due to its partial homology with the coiled-coil domain of the Cdt1-inhibitor Geminin. Notably, GEMC1 does not bind to Cdt1, nor does it inhibit DNA replication. DUE-B, Treslin/Ticrr, and GEMC1 have all been characterized in some detail in *Xenopus* egg extracts. Like Sld3, all three proteins can be found in a complex with TopBP1, and they all function specifically at the Cdc45 loading step.

Like Sld3, the DUE-B, Treslin/Ticrr, and GEMC1 proteins have also been connected to CDK activity. Thus, Treslin/Ticrr contains 35 potential CDK phosphorylation sites, and its binding to TopBP1 is stimulated by Cdk2, analogous to the interaction of Sld3 with Dpb11. Interestingly, like Sld3 [14], the binding of Treslin/Ticrr to pre-replication complexes itself is not CDK-dependent. So far, however, specific Cdk2 phosphorylation sites on Treslin/Ticrr have not been implicated in the protein's function. In the case of GEMC1, its interaction with TopBP1 is compromised when eight CDK sites on GEMC1 are mutated. Importantly, these sites are also essential for the function of GEMC1 in DNA replication. These results establish GEMC1 as the first bona fide CDK target for replication initiation in metazoans. Unlike Treslin/Ticrr, GEMC1 chromatin loading is MCM2–7-independent, but ORC- and TopBP1-dependent. Finally, the CDK-dependence of DUE-B binding to TopBP1 is not yet clear. However, DUE-B binds to chromatin dependent on the pre-replication complex and Cdk2 activity. In summary, the regulation of DUE-B, Treslin/Ticrr, and GEMC1 by CDK is still being elucidated and therefore does not point unambiguously to one of these proteins as the metazoan Sld3 ortholog.

Importantly, no compelling sequence homology was initially reported between Sld3 and DUE-B, Treslin/Ticrr, or GEMC1 [4–7], raising the question whether any of these proteins is a true Sld3 ortholog. However, Sanchez-Pulido *et al.* [8] now show that Treslin/Ticrr does in fact contain a domain that shares significant sequence homology with Sld3. The authors first interrogated the protein databases with Treslin/Ticrr using the sequence comparison method HMMer2 and found distant plant homologs [15]. They then generated a profile from the animal and plant Treslin/Ticrr proteins, and used this to search the database. This yielded significant similarity ($E = 8.6 \times 10^{-15}$) to the fungal Sld3 family profile. These observations indicate that Treslin/Ticrr and Sld3 descended from a common ancestor that existed before the divergence of the principal eukaryotic lineages. Importantly, several of the temperature-sensitive mutations in fungal Sld3 proteins map to residues that are common to all Treslin/Ticrr and Sld3 proteins, and one of these disrupts the interaction of Sld3 with Cdc45. Together with the CDK-independent Treslin/Ticrr chromatin loading, this sequence analysis points to Treslin/Ticrr as the strongest candidate for the metazoan Sld3 protein.

If Treslin/Ticrr is the true Sld3 ortholog, what are the evolutionary origins and functions of DUE-B and GEMC1? One possibility is that the function of Sld3 in replication initiation was divided among several proteins, some of which are not related by sequence to Sld3. This would afford metazoan organisms more CDK targets in replication initiation, allowing for additional regulatory inputs into this crucial event. Another possibility is that DUE-B or GEMC1 performs Sld2's role in CMG assembly. In this regard it is interesting that the recruitment of DUE-B to pre-replication complexes is Cdk2-dependent [5], which also

appears to be the case for Sld2 [16]. Most likely, the assembly of the CMG complex requires a similar set of molecular events in all eukaryotes. Only once these steps have been elucidated will it be possible to understand how these tasks are divided among different proteins in any specific organism.

References

1. Bell, S.P., and Dutta, A. (2002). DNA replication in eukaryotic cells. Annu. Rev. Biochem. *71*, 333–374.
2. Tanaka, S., Umemori, T., Hirai, K., Muramatsu, S., Kamimura, Y., and Araki, H. (2007). CDK-dependent phosphorylation of Sld2 and Sld3 initiates DNA replication in budding yeast. Nature *445*, 328–332.
3. Zegerman, P., and Diffley, J.F. (2007). Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. Nature *445*, 281–285.
4. Balestrini, A., Cosentino, C., Errico, A., Garner, E., and Costanzo, V. (2010). GEMC1 is a TopBP1-interacting protein required for chromosomal DNA replication. Nat. Cell Biol. *12*, 484–491.
5. Chowdhury, A., Liu, G., Kemp, M., Chen, X., Katrangi, N., Myers, S., Ghosh, M., Yao, J., Gao, Y., Bubulya, P., *et al.* (2010). The DNA unwinding element binding protein DUE-B interacts with Cdc45 in preinitiation complex formation. Mol. Cell Biol. *30*, 1495–1507.
6. Kumagai, A., Shevchenko, A., and Dunphy, W.G. (2010). Treslin collaborates with TopBP1 in triggering the initiation of DNA replication. Cell *140*, 349–359.
7. Sansam, C.L., Cruz, N.M., Danielian, P.S., Amsterdam, A., Lau, M.L., Hopkins, N., and Lees, J.A. (2010). A vertebrate gene, ticrr, is an essential checkpoint and replication regulator. Genes Dev. *24*, 183–194.
8. Sanchez-Pulido, L., Diffley, J.F.X., and Ponting, C.P. (2010). Homology explains the functional similarities of Treslin/Ticrr and Sld3. Curr. Biol. *20*, R509–R510.
9. Hashimoto, Y., Tsujimura, T., Sugino, A., and Takisawa, H. (2006). The phosphorylated C-terminal domain of Xenopus Cut5 directly mediates ATR-dependent activation of Chk1. Genes Cells *11*, 993–1007.
10. Yan, S., Lindsay, H.D., and Michael, W.M. (2006). Direct requirement for Xmus101 in ATR-mediated phosphorylation of Claspin bound Chk1 during checkpoint signaling. J. Cell Biol. *173*, 181–186.
11. Van Hatten, R.A., Tutter, A.V., Holway, A.H., Khederian, A.M., Walter, J.C., and Michael, W.M. (2002). The Xenopus Xmus101 protein is required for the recruitment of Cdc45 to origins of DNA replication. J. Cell Biol. *159*, 541–547.
12. Matsuno, K., Kumano, M., Kubota, Y., Hashimoto, Y., and Takisawa, H. (2006). The N-terminal noncatalytic region of Xenopus RecQ4 is required for chromatin binding of DNA polymerase alpha in the initiation of DNA replication. Mol. Cell Biol. *26*, 4843–4852.
13. Sangrithi, M.N., Bernal, J.A., Madine, M., Philpott, A., Lee, J., Dunphy, W.G., and Venkitaraman, A.R. (2005). Initiation of DNA replication requires the RECQL4 protein mutated in Rothmund-Thomson syndrome. Cell *121*, 887–898.
14. Kamimura, Y., Tak, Y.S., Sugino, A., and Araki, H. (2001). Sld3, which interacts with Cdc45 (Sld4), functions for chromosomal DNA replication in Saccharomyces cerevisiae. EMBO J. *20*, 2097–2107.
15. Eddy, S.R. (1996). Hidden Markov models. Curr. Opin. Struct. Biol. *6*, 361–365.
16. Muramatsu, S., Hirai, K., Tak, Y.S., Kamimura, Y., and Araki, H. (2010). CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol ε, and GINS in budding yeast. Genes Dev. *24*, 602–612.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave, Boston, MA 02115, USA.
*E-mail: johannes_walter@hms.harvard.edu

DOI: 10.1016/j.cub.2010.05.033

Neandertal Genome: The Ins and Outs of African Genetic Diversity

Analysis of the Neandertal genome indicates gene flow between Neandertals and modern humans of Eurasia but not Africa. This surprising result is difficult to reconcile with current models of human origins and might have to do with insufficient African sampling.

Jason A. Hodgson, Christina M. Bergey, and Todd R. Disotell*

The Neandertals were a human group that lived in Europe and Central Asia between 200 and 30 thousand years ago. They showed several morphological features, seemingly adaptations to the cold, that distinguished them from other human groups, including a strongly-built stocky postcranial skeleton, a long, low skull with large cranial capacity, double arched brow ridges, an occipital 'bun', a protruding midfacial region with a large nose and large front teeth. Contemporaneous with Neandertals were anatomically modern humans who first appeared in Africa around 200 thousand years ago, in the Middle East by 100 thousand years ago and across Eurasia by around 40 thousand years ago. Neandertals disappear from the paleontological record shortly after modern humans are seen in Europe prompting questions about their fate. Did Neandertals go fully extinct without leaving any genetic legacy, or do some Neandertal genes live on as part of the genetic diversity seen in living humans? The near complete sequencing and characterization of the Neandertal genome, detailed in three recent key papers [1–3], has now made it possible to directly compare genetic variation between Neandertals and contemporary humans. These data clarify the timing of changes along the human lineage as well as genes that were under selection since modern humans separated from Neandertals [1,2]. These data also promise to clarify the relationship between Neandertals and living humans; however, the findings of the Neandertal genome project currently raise as many questions as they answer.

There are two predominant models of modern human origins: multiregional evolution and recent African replacement. Multiregional evolution posits that the evolution of contemporary peoples occurred around the globe, with archaic populations such as the Neandertals contributing locally in their geographic regions [4]. This model predicts that Neandertals will share significant genetic variation with Europeans to the exclusion of other populations. Recent African replacement suggests that contemporary humans owe their heritage to a small African population that spread around the world replacing archaic populations with little to no interbreeding [5]. This model predicts that Neandertals will be equally distantly related to all contemporary human populations. Studies seeking to distinguish these models have been equivocal. Most surveys of contemporary genetic diversity support recent African replacement and previously published studies of Neandertal mitochondrial and nuclear DNA have failed to find any evidence of admixture [6]. However, some anomalous genetic patterns suggest that there may have been a small genetic contribution from archaic populations [7].

In their recently published analysis of the Neandertal genome, Green and colleagues [1] sequenced DNA extracted from three Neandertal