The origin recognition complex protein family

Bernard P Duncker*, Igor N Chesnokov† and Brendan J McConkey*

Addresses: *Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 Canada. †Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, School of Medicine, Birmingham, AL 35294, USA.

Correspondence: Bernard P Duncker. Email: bduncker@sciborg.uwaterloo.ca

Published: 17 March 2009

Genome Biology 2009, 10:214 (doi:10.1186/gb-2009-10-3-214)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2009/10/3/214

© 2009 BioMed Central Ltd

Summary

Origin recognition complex (ORC) proteins were first discovered as a six-subunit assemblage in budding yeast that promotes the initiation of DNA replication. Orc1-5 appear to be present in all eukaryotes, and include both AAA+ and winged-helix motifs. A sixth protein, Orc6, shows no structural similarity to the other ORC proteins, and is poorly conserved between budding yeast and most other eukaryotic species. The replication factor Cdc6 has extensive sequence similarity with Orc1 and phylogenetic analysis suggests the genes that encode them may be paralogs. ORC proteins have also been found in the archaea, and the bacterial DnaA replication protein has ORC-like functional domains. In budding yeast, Orc1-6 are bound to origins of DNA replication throughout the cell cycle. Following association with Cdc6 in G1 phase, the sequential hydrolysis of Cdc6- then ORC-bound ATP loads the Mcm2-7 helicase complex onto DNA. Localization of ORC subunits to the kinetochore and centrosome during mitosis and to the cleavage furrow during cytokinesis has been observed in metazoan cells and, along with phenotypes observed following knockdown with short interfering RNAs, point to additional roles at these cell-cycle stages. In addition, ORC proteins function in epigenetic gene silencing through interactions with heterochromatin factors such as Sir1 in budding yeast and HP1 in higher eukaryotes. Current avenues of research have identified roles for ORC proteins in the development of neuronal and muscle tissue, and are probing their relationship to genome integrity.

Gene organization and evolutionary history

The first origin recognition complex (ORC) proteins to be identified were purified from cell extracts of budding yeast (Saccharomyces cerevisiae) as a heterohexameric complex that specifically binds to origins of DNA replication [1], and the subunits were named Orc1 through Orc6 in descending order of apparent molecular mass, as judged by SDS-PAGE (Figure 1). Shortly thereafter, the corresponding genes were cloned [2-7]. Dispersed among six chromosomes (ORC1 chromosome 13, ORC2 chromosome 2, ORC3 chromosome 12, ORC4 chromosome 16, ORC5 chromosome 14, ORC6 chromosome 8) the sizes of the genes mirrors the sizes of the proteins they encode, ranging from 1,308 bp to 2,745 bp, and all are intronless, as is the case for the vast majority of budding yeast open reading frames [8]. Subsequently, orthologs of ORC1-ORC5 were identified in organisms as diverse as Drosophila melanogaster [9], Arabidopsis thaliana [10] and Homo sapiens [11], strongly suggesting that these genes are likely to exist in all eukaryotes. ORC6 genes have also been assigned in numerous metazoan species (Figure 2), and although the encoded proteins are relatively well conserved between metazoans and fission yeast (Schizosaccharomyces pombe), there is insufficient identity to definitively conclude that they are homologous to budding yeast Orc6, which is also considerably larger than Orc6 in these other species [11]. As with S. cerevisiae, the genes in other species are spread among multiple chromosomes. Apart from Orc6, the size of the individual protein subunits encoded does not vary much between species, although the length of the genes themselves is considerably longer in...
higher eukaryotes (for example, they range from 8,746 bp for ORC6 to 87,405 bp for ORC4 in H. sapiens) as would be expected as a result of the presence of intronic sequence.

Along with ORC subunit orthologs, additional Orc1-like proteins are widespread in eukaryotic species. The most notable of these is Cdc6, a replication factor that aids in loading the Mcm2-7 DNA helicase onto replication origins (Figure 3). In budding yeast, Cdc6 has strong similarity with a 270-amino-acid stretch of Orc1 [6], and phylogenetic analysis of a wide array of species suggests that the ORC1 and CDC6 genes may be paralogs [12]. As shown by a neighbor-joining tree based on AAA+ protein domains (discussed below), Orc1 contains an additional BAH (bromo-adjacent homology) domain (pink), which interacts with the Sir1 protein and is involved in epigenetic silencing. Orc1 and Orc2 have regions of disorder (yellow); a DNA-binding AT-hook motif (here PRKRGRPRK) is identified in S. cerevisiae Orc2, and several of these have also been identified in disordered regions in S. pombe Orc4. The number of amino acids for each protein is indicated at the right.

ORC-like proteins are not just confined to the eukaryotes. Genes with homology to ORC1 and CDC6 have been found in most species of archaea, which typically have 1 to 9 copies, although as many as 17 have been found in the case of Haloarcula marismortui (reviewed in [13]). Studies of archaeal ORC proteins have yielded important results, because they not only bind to defined origin sequences but are amenable to crystallization, which has provided important structural information about ORC-DNA interactions [14,15]. Curiously, genome analysis of several Methanococcus species has uncovered no evidence of ORC-like sequences. Given the apparent functional conservation of ORC proteins between eukaryotes and archaea, it will be interesting to determine whether ORC orthologs have simply been overlooked as a result of lower sequence conservation, or whether these species have developed another means of initiating DNA replication at origin sequences.

Evidence that proteins with ORC-like functions are actually common to all domains of life is provided by investigations of the bacterial DnaA protein. DnaA, like ORC, acts as an initiator of DNA replication and, whereas DnaA and the archaeal Orc1/Cdc6 proteins share little sequence identity,
Homology between Orc6 in representative species D. melanogaster (Dm), H. sapiens (Hs), A. thaliana (At), S. pombe (Sp), and S. cerevisiae (Sc). Orc6 contains a unique conserved domain, identified by homology with the Orc6 protein fold superfamily (pfam 05460) [76]. This domain is interrupted by a large disordered region [77] in S. cerevisiae. Orc6 has no recognizable homology to Orc1-5 or AAA+ domains. The carboxy-terminal region of Orc6 in D. melanogaster has been shown to interact with a coiled-coil region of the septin protein Pnut, possibly mediated by coiled-coil motifs predicted in Orc6 [78]. The number of amino acids for each protein is indicated at the right.

Figure 2
Homology between Orc6 in representative species D. melanogaster (Dm), H. sapiens (Hs), A. thaliana (At), S. pombe (Sp), and S. cerevisiae (Sc). Orc6 contains a unique conserved domain, identified by homology with the Orc6 protein fold superfamily (pfam 05460) [76]. This domain is interrupted by a large disordered region [77] in S. cerevisiae. Orc6 has no recognizable homology to Orc1-5 or AAA+ domains. The carboxy-terminal region of Orc6 in D. melanogaster has been shown to interact with a coiled-coil region of the septin protein Pnut, possibly mediated by coiled-coil motifs predicted in Orc6 [78]. The number of amino acids for each protein is indicated at the right.

Figure 3
ORC and its interactions with other pre-RC proteins at origins of DNA replication. Orc1-Orc5 are required for origin recognition and binding in S. cerevisiae, whereas Orc6 is dispensable in this regard [44]. In contrast, Orc6 is essential for ORC DNA binding in D. melanogaster [28]. Studies with both S. cerevisiae and human cells have indicated that Cdc6 interacts with ORC through the Orc1 subunit (indicated by a double arrow) [31,79,80]. This association increases the specificity of the ORC-origin interaction [20]. Further studies with S. cerevisiae suggest that hydrolysis of Cdc6-bound ATP promotes the association of Cdt1 with origins through an interaction with Orc6 (indicated by a double arrow) [25,31], and this in turn promotes the loading of Mcm2-7 helicase onto chromatin.

Characteristic structural features
Orc1-5 as well as Cdc6 have conserved AAA+ folds, including Walker A and Walker B ATP-binding domains, characteristic of ATP-dependent clamp-loading proteins, which allow ring-shaped protein complexes to encircle duplex DNA (see Figure 1). Sensor-1 and Sensor-2 motifs are also found within the AAA+ fold and are believed to detect whether ADP or ATP is bound and to contribute to ATPase activity [18]. These domains are located centrally, in the case of Orc1 and Orc2, and towards the amino termini in Cdc6, Orc3, Orc4, and Orc5. Near the carboxyl termini of these proteins a winged-helix domain is present that mediates DNA binding [14,15,17]. Somewhat surprisingly, structural studies of archaeal Orc1 suggest that the AAA+ domain also contributes to its association with origin sequences [14,15]. Interestingly, Cdc6 has been shown to act like an additional ORC subunit, associating with the complex in the G1 phase of the cell cycle and inducing a conformational change that increases its sequence specificity for DNA binding [19,20]. When Cdc6 is bound to ORC, a ring-like structure is predicted with structural similarities to the Mcm2-7 helicase complex that ORC-Cdc6 loads onto DNA in an ATP-dependent manner [19,21].

As mentioned above, sequence similarity has been identified for Orc1 and Sir3, with a particularly high degree of conservation between their amino-terminal 214 amino acids (50% identical, 63% similar), which includes a BAH (bromo-adjacent homology) protein-protein interaction domain [6,22]. Sir3 is required for transcriptional silencing of telomeres and mating-type loci, functions that are also ORC-dependent [3,5,23], as discussed below. Although formally a member of ORC, Orc6 contains none of the aforementioned structural features, and shows no evidence of a common evolutionary origin with Orc1-5. It is nevertheless considered an ORC protein as its association with the other five subunits is required to promote the initiation of DNA replication. Relative to other ORC subunits, Orc6 is poorly conserved between budding yeast and metazoan eukaryotes [11] (see Figure 2). Nevertheless, a number of important domains specific to Orc6 have been identified in S. cerevisiae, including an amino-terminal ‘RXL’ docking sequence (amino acids 177-183) which mediates an interaction with the S-phase cyclin Clb5 [24], and a carboxy-terminal region (the last 62 amino acids) which associates with the other ORC subunits. Both ends of Orc6 (amino-terminal 185 amino acids, carboxy-terminal 165 amino acids) interact with Cdt1, another replication factor required to load Mcm2-7 onto DNA [25]. In both human and Drosophila cells, Orc6 plays a role in cytokinesis, and studies with the latter organism have identified a carboxy-terminal domain that interacts with the septin Pnut, a component of the septin ring that forms in cell division, as well as an amino-terminal domain that is important for DNA binding [26-29]. Interestingly, structural modeling of Drosophila Orc6 revealed that the amino terminus, but not the carboxyl terminus, is homologous to the human transcription factor TFIIB, raising the possibility that proteins involved in replication and transcription may have coevolved [27].

Localization and function
Detection of ORC by immunofluorescence and live-cell imaging of fluorescently tagged subunits in budding yeast have demonstrated that it localizes to punctate subnuclear foci throughout the cell cycle [30,31]. Moreover, chromatin
immunoprecipitation (ChIP) of ORC-bound genomic DNA that was subsequently labeled and hybridized to high-density, tiled, whole-genome \textit{S. cerevisiae} oligonucleotide arrays revealed 400 ORC-enriched regions, which included 70 of the 96 replication origins that had been experimentally verified previously [32]. These findings are consistent with a
role for ORC as a scaffold for the sequential association of a number of additional replication factors in G1 phase of the cell cycle, including Cdc6, Cdt1, and Mcm2-7, which collectively form the pre-replicative complex (pre-RC), required for the initiation of DNA replication (reviewed in [23]).

Binding sites for budding yeast ORC have been identified at HML (hidden MAT left), and HMR (hidden MAT right) silent cassettes, used for mating-type switching through gene conversion of the MAT allele, and at telomeric loci, whereas the majority of Drosophila ORC appears to be associated with heterochromatin, consistent with the role of this complex in mediating gene silencing [23,33]. The amino terminus of S. cerevisiae Orc1 interacts with the heterochromatin factor Sir1, and truncation mutants lacking this region are defective in silencing but not DNA replication [6,34], indicating that these two functions of the protein are separable. The role of the Orc1 amino terminus in mediating transcriptional repression seems to be conserved among eukaryotes, as it has also been found to interact with heterochromatin protein 1 (HP1) in Xenopus and Drosophila [33] which, in a fashion similar to Sir1, helps to propagate silenced chromatin.

It appears that all six ORC subunits remain chromatin-associated throughout the cell cycle in S. cerevisiae [35], but this differs from observations in metazoan cells where, in a number of cases, Orc1 appears to be absent from ORC at certain points in the cell cycle. For example, in human HeLa cells, Orc1 dissociates from chromatin during S phase, and then reassociates at the end of mitosis (reviewed in [36]). Immunofluorescent detection of Orc2 in one study indicated that it is found on chromatin throughout the cell cycle in Drosophila embryos [33]; however, a similar analysis with Drosophila neuroblasts and recently reported live-cell imaging of Orc2-green fluorescent protein (GFP) in embryos argue that this protein is actually excluded from chromosomes from prophase until anaphase [37,38]. Fluorescence loss in photobleaching analysis in hamster imaging of Orc2-GFP in embryos revealed that the BAH domain of this subunit promotes ORC association with DNA is promoted have been discovered. Some of these are related to the relatively high AT content that is a common feature of replication origins among diverse species. For example, in the fission yeast S. pombe, a domain of Orc4 binds to AT-rich DNA [45], and another ‘AT-hook’ protein, HMGA1a, has recently been shown to target ORC to replication origins in human cells [46]. HMGA1a, which is known to interact in a highly specific manner with the minor groove of stretches of AT, was shown to interact with Orc1, Orc2, Orc4 and Orc6. Interestingly, an AT-hook motif is also present in S. cerevisiae Orc2, although its functional significance has not been determined (see Figure 1). It is clear, however, that AT content is not the only origin determinant, as numerous studies with both S. pombe and Drosophila have shown differences in ORC binding between stretches of DNA that have the same proportion of AT [23]. A study of human Orc1 revealed that the BAH domain of this subunit promotes association of ORC with chromatin [47]. Human and Drosophila investigations have pointed to transcription factors, including c-Myc, E2F, and the Myb complex, as likely ORC-targeting factors [48-51], whereas a ribosomal RNA fragment that associates with Tetrahymena ORC has been found to direct the complex to complementary rDNA sequence in the genome of this organism [52]. Furthermore, whereas Orc6 is dispensable for origin binding in S. cerevisiae [44], it is absolutely required for this function in Drosophila [28,53].

In metazoan cells, ORC localization clearly extends beyond origin sequences (reviewed in [40]). Studies with Drosophila and human cells have revealed that Orc6 also localizes to the cleavage furrow in dividing cells, and a role for this protein in cytokinesis has been confirmed in both organisms through depletion by RNA interference [26,27]. In addition, human Orc6 was shown to localize to kinetochores and reticular-like structures around the cell periphery during mitosis, and it is required for the proper progression of this cell-cycle stage [26], whereas human Orc2 also localizes to the centrosome throughout the cell cycle and its depletion results in mitotic defects and multiple centrosomes [41]. Recently, a similar role in controlling centrosome copy number was reported for human Orc1 [42].

Mechanism of action
The mechanism by which ORC promotes DNA replication, through loading and maintenance of the Mcm2-7 helicase at origin sequences, has been most closely examined in S. cerevisiae. ATP binding by the Orc1 subunit promotes association with DNA [43]. Cdc6 is then thought to bind ATP and associate with ORC, causing a conformational change that increases the specificity for the conserved origin sequences found in budding yeast. These origin regions are often referred to as autonomously replicating sequences (ARSs), and include an 11-bp ARS consensus sequence (ACS), as well as one or more B elements [20,21,23]. Cross-linking analysis has shown interactions between Orc1, Orc2, Orc4, and Orc5 proteins and origin DNA [44].

Given the lack of such conserved origin sequences in other eukaryotes, it is not surprising that other means by which ORC association with DNA is promoted have been discovered. Some of these are related to the relatively high AT content that is a common feature of replication origins among diverse species. For example, in the fission yeast S. pombe, a domain of Orc4 binds to AT-rich DNA [45], and another ‘AT-hook’ protein, HMGA1a, has recently been shown to target ORC to replication origins in human cells [46]. HMGA1a, which is known to interact in a highly specific manner with the minor groove of stretches of AT, was shown to interact with Orc1, Orc2, Orc4 and Orc6. Interestingly, an AT-hook motif is also present in S. cerevisiae Orc2, although its functional significance has not been determined (see Figure 1). It is clear, however, that AT content is not the only origin determinant, as numerous studies with both S. pombe and Drosophila have shown differences in ORC binding between stretches of DNA that have the same proportion of AT [23]. A study of human Orc1 revealed that the BAH domain of this subunit promotes association of ORC with chromatin [47]. Human and Drosophila investigations have pointed to transcription factors, including c-Myc, E2F, and the Myb complex, as likely ORC-targeting factors [48-51], whereas a ribosomal RNA fragment that associates with Tetrahymena ORC has been found to direct the complex to complementary rDNA sequence in the genome of this organism [52]. Furthermore, whereas Orc6 is dispensable for origin binding in S. cerevisiae [44], it is absolutely required for this function in Drosophila [28,53].

Rather than merely acting as a landing pad for pre-replicative complex (pre-RC) assembly, S. cerevisiae ORC appears to play an active role in loading additional pre-RC components. Following ORC-Cdc6 binding, Orc6 interacts with Cdt1 to promote Mcm2-7 association with origin DNA [25,31]. The hydrolysis of Cdc6-bound ATP is then thought
to load the initial Mcm2-7 complexes more tightly onto the DNA, and additional Mcm2-7 binding occurs following the hydrolysis of ORC-bound ATP [21]. Interestingly, even though it does not bind ATP itself, a predicted arginine finger in Orc4 is required for Orc1 ATP hydrolysis [54,55]. Once loaded, the continued presence of Orc6, Cdc6, and most probably other pre-RC components, is required to maintain the Mcm2-7 helicase complex at origins until the initiation of DNA replication [25,31,56].

Although it is not known whether the mechanism determined for the promotion of DNA replication by the ORC in budding yeast operates in precisely the same fashion in other organisms, the sequential association of the ORC, Cdc6, Cdt1, and Mcm2-7 at origins appears to be conserved in other eukaryotes, including S. pombe and Xenopus (reviewed in [23]). Furthermore, several reports have demonstrated interactions between archaeal ORC-Cdc6 and MCM proteins [57-59].

Frontiers
Now that roles for ORC proteins have been established at other points in the cell cycle than simply the G1/S boundary, it is of primary interest to determine the way in which the proper progression of cell-cycle stages might be coordinated by the complex as a whole or by its individual subunits. For example, studies of human Orc6 have shown that it associates with the kinetochore during the G2/prophase transition [60], and in both human and Drosophila cells it localizes to the cleavage furrow just before cytokinesis [26,27]. Similarly, a mitotic function has been uncovered for Orc2 in promoting sister-chromatid cohesion in budding yeast after it is no longer required for DNA replication [61]. Thus, it is possible that a redistribution of ORC subunits after their role in DNA replication is complete helps to ensure the proper order of cell-cycle events.

Another avenue of ORC research that is presently yielding intriguing results is the elucidation of roles for these proteins in development [62]. Studies with Drosophila Orc3 have shown that it localizes to larval neuromuscular junctions, and that its mutation leads to impaired neuronal cell proliferation and to learning defects, as judged by a reduction in olfactory memory [63,64]. Similarly, Orc2-5 have been detected at high levels in mouse brain, and knockdown of Orc3 and Orc5 by short interfering RNAs (siRNAs) impeded dendritic growth [65]. Furthermore, siRNA knockdown of Orc1 was recently shown to inhibit the proliferation of rat smooth muscle cells [66].

In recent years, numerous ORC-associated proteins have shown promise as biomarkers for early cancer detection (reviewed in [67]), and alterations in the expression levels of a number of them have been implicated as contributing to human lung carcinomas and mouse mammary adenocarcinomas [68-70]. The extent to which mutations in ORC subunits and/or perturbations of their normal levels may contribute to carcinogenesis is an important unresolved question. Some initial indications have been obtained through the observation that genomic instability, in the form of DNA re-replication, can occur as a result of mutations in combinations of pre-RC components, including Orc2 and Orc6, in budding yeast [71,72]. Given the finding that ORC plays an active enzymatic role in loading Mcm2-7 onto DNA in S. cerevisiae, it will be very important to determine if the complex acts in the same way in higher eukaryotes, including humans. Interestingly, Drosophila Orc2 interacts with the tumor suppressor protein retinoblastoma 1 (Rb1) and siRNA-mediated reduction in Orc6 levels sensitizes human colon cancer cells to treatment with chemotherapeutic agents, pointing to possible links between ORC subunits and cancer development [73,74].

Further investigation into both normal and dysregulated ORC function should yield important insights into the way cells coordinate the distinct stages required for their duplication, how they are organized into specific tissue types, and how carcinogenesis occurs.

Acknowledgements
The writing of this review was supported by funding from the Canadian Institutes of Health Research (BPD), National Institutes of Health Grant GM69681 (INC) and the Natural Sciences and Engineering Research Council of Canada (BJM). BPD is a Research Scientist of the Canadian Cancer Society.

References
1. Bell SP, Stillman B: ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature 1992, 357:114-115.
2. Bell SP, Kobayashi R, Stillman B: Yeast origin recognition complex functions in transcription silencing and DNA replication. Science 1993, 262:1844-1849.
3. Foss M, McNally FJ, Laurentson P, Rine J: Origin recognition complex (ORC) in transcriptional silencing and DNA replication in S. cerevisiae. Science 1993, 262:1838-1841.
4. Li JJ, Herskowitz I: Isolation of ORC4, a component of the yeast origin recognition complex by a one-hybrid system. Science 1993, 262:1870-1874.
5. Micklem G, Rowley A, Harwood J, Nasmyth K, Diffley JF: Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. Nature 1993, 366:87-89.
6. Bell SP, Mitchell J, Leber J, Kobayashi R, Stillman B: The multidomain structure of Orc1p reveals similarity to regulators of DNA replication and transcriptional silencing. Cell 1995, 83:563-568.
7. Loo S, Fox CA, Rine J, Kobayashi R, Stillman B, Bell SP: The origin recognition complex in silencing, cell cycle progression, and DNA replication. Mol Biol Cell 1995, 6:741-756.
8. Spignola M, Grate L, Haussler D, Ares M Jr: Genome-wide bioinformatic and molecular analysis of introns in Saccharomyces cerevisiae. RNA 1999, 5:221-234.
9. Gossen M, Pak DT, Hansen SK, Acharya JY, Botchan MR: A Drosophila homolog of the yeast origin recognition complex. Science 1995, 270:1674-1677.
10. Diaz-Trivino S, del Mar Castellano M, de la Paz Sanchez M, Ramirez-Parras E, Desvoyes B, Gutierrez C: The genes encoding Arabidopsis ORC subunits are EZF targets and the two ORC1 genes are differentially expressed in proliferating and endoreplicating cells. Nucleic Acids Res 2005, 33:5404-5414.
11. Dhar SK, Dutta A: Identification and characterization of the human ORCs homolog, J Biol Chem 2000, 275:34983-34988.

12. Giraldo R: Common domains in the initiators of DNA replication in Bacteria, Archaea and Eukarya; combined structural, functional and phylogenetic analysis. FEBS Microbiol Rev 2003, 26:53-554.

13. Barry ER, Bell SD: DNA replication in the archaea. Microbiol Mol Biol Rev 2006, 70:876-887.

14. Dueber EL, Corn JE, Bell SD, Berger JM: Replication origin recognition and formation by a heterodimeric archaeal Orc1 complex, Science 2007, 317:1210-1213.

15. Gaudier M, Schwirnith BS, Westcott SL, Wigley DB: Structural basis of DNA replication origin recognition by an Orc1 protein, Science 2007, 317:1213-1216.

16. Most ML, Berger JM: DNA replication initiation mechanisms and regulation in bacteria, Nat Rev Microbiol 2007, 3:343-354.

17. Clarye MG, Botchan M, Nogales E: Single particle EM studies of the Drosophila melanogaster origin recognition complex and evidence for DNA unwrapping, J Struct Biol 2008, 164:241-249.

18. Iyer LM, Leippe DD, Koonin EV, Aravind L: Evolutionary history and higher order classification of AAA+ ATPases, J Struct Biol 2004, 146:1-31.

19. Speck C, Chen Z, Li H, Stillman B: ATPase-dependent cooperative binding of ORC and Cdc6 to origin DNA, Nat Struct Mol Biol 2005, 12:965-971.

20. Speck C, Botchan M: Cdc6 ATPase activity regulates ORC-Cdc6 stability and the selection of specific DNA sequences as origins of DNA replication, J Biol Chem 2007, 282:1705-1714.

21. Randell JCV, Bowers JL, Rodriguez HK, Bell SP: Sequential ATP hydrolysis by Cdc6 and ORC directly loads the Mcm2-7 helicase, Mol Cell 2006, 21:29-39.

22. Callebaut I, Courvalin JC, Moreno JP: The BAH (bromo-adjacent homology) domain: a link between DNA methylation, replication and transcriptional regulation, FEBS Lett 2005, 577:189-193.

23. Bell SP: The origin recognition complex: from simple origins to complex functions, Genes Dev 2002, 16:659-672.

24. Wilms GM, Archambault V, Austin RJ, Jacobson MD, Bell SP, Cross FR: Interaction of the S-phase cyclin Cdc8 with an ‘XSL’ docking sequence in the initiator protein Orc6 provides an origin-localized replication control switch, Genes Dev 2004, 18:981-991.

25. Chen S, de Vries MA, Bell SP: Orc6 is required for dynamic recruitment of Cdc1 during repeated Mcm2-7 loading, Genes Dev 2007, 21:2897-2907.

26. Prasanth SG, Prasanth KV, Stillman B: Orc6 involved in DNA replication, chromosome segregation and cytokinesis, Science 2002, 297:1026-1031.

27. Chesnokov IN, Chesnokova ON, Botchan M: A cytokinetic function of Drosophila ORC protein resides in a domain distinct from its replication activity, Proc Natl Acad Sci USA 2003, 100:9150-9155.

28. Balasov M, Huijbregts RPH, Chesnokov I: Role of the Orc6 protein in origin recognition complex-dependent DNA binding and replication in Drosophila melanogaster, Mol Cell Biol 2007, 27:1431-1435.

29. Huijbregts RPH, Svitin A, Stinnett MW, Renfrow MB, Chesnokov I: Functional integrity of GTTPase activity and filament formation of the septin complex, Mol Cell Biol 2009, 29:270-281.

30. Pasero P, Duncker BP, Schwob E, Gasser SM: A role for the Cdc7 kinase regulatory subunit Dbf4 in the formation of initiation-compeent origins of replication, Genes Dev 1999, 13:1599-1597.

31. Semple JW, Da-Silva LF, Jervis EJ, Ah-Kee J, Al-Aratt H, Kummer L, Herikhuiz J, Pasero P, Duncker BP: An essential role for Orc1 in DNA replication through maintenance of pre-replicative complexes, EMBO J 2006, 25:150-1518.

32. Xu W, Aparicio JG, Aparicio OM, Tavare S: Genome-wide mapping of ORC and Mcm2 binding sites on tiling arrays and identification of essential ARS consensus sequences in S. cerevisiae, BMC Genomics 2006, 7:276.

33. Pak DTS, Pflumm M, Chesnokov I, Huang DW, Kellum R, Marr J, Ramanowicz P, Botchan MR: Association of the origin recognition complex with hectorhomatin and HPI in higher eukaryotes, Cell 1997, 91:311-323.

34. Zhang Z, Hayashi MK, Merkel O, Stillman B, Xu RM: Structure and function of the BAH-containing domain of Orc1p in epigenetic silencing, EMBO J 2002, 21:4600-4611.

35. Liang C, Stillman B: Persistent initiation of DNA replication and chromatin-bound MCM proteins during the cell cycle in cdc6 mutants, Genes Dev 1999, 13:3375-3386.

36. DePamphilis ML: Cell cycle dependent regulation of the origin recognition complex, Cell Cycle 2002, 4:70-79.

37. Loupourt M-L, Krause SA, Heck MMS: Abrupt replication timing induces defective chromosome condensation in Drosophila ORC2 mutants, Curr Biol 2000, 10:1547-1556.

38. Baudinger T, Gossen M: Binding of Drosophila ORC proteins to metaphase chromosome requires association of mitotic cyclin-dependent kinase activity, Mol Cell Biol 2009, 29:140-149.

39. McNair AJ, Okuno Y, Mistelli T, Gilbert DM: Chinese hamster ORC subunits dynamically associate with chromatin throughout the cell cycle, Exp Cell Res 2005, 308:345-356.

40. Chesnokov I: Multiple functions of the origin recognition complex, Int Rev Cytol 2007, 258:69-109.

41. Prasanth SG, Prasanth KV, Siddiqui K, Spector DL, Stillman B: Human Orc2 localizes to centromeres, centromeres and heterochromatin during chromosome inheritance, EMBO J 2004, 23:2651-2663.

42. Hemerly AS, Prasanth SG, Siddiqui K, Stillman B: Orc1 controls centriole and centrosome copy number in human cells, Science 2009, 323:789-793.

43. Klemm RD, Austin RJ, Bell SP: Coordinate binding of ATP and origin DNA regulates the ATPase activity of the origin recognition complex, Cell 1997, 88:493-502.

44. Lee DG, Bell SP: Architecture of the yeast origin recognition complex bound to origins of DNA replication, Mol Cell Biol 1997, 17:159-7168.

45. Takayama MA, Taira T, Tama K, Iuchi-Ariga SM, Ariga H: ORC interacts with e-Myc to inhibit E-box-dependent transcription by abrogating e-Myc-SNF5/511 interaction, Genes Cells 2000, 5:481-490.

46. Bosco G, Du W, Orr-Weaver TL: DNA replication control through interaction of E2F-4B and the origin recognition complex, Nat Cell Biol 2001, 3:289-295.

47. Beal EL, Manak JR, Zhou S, Bell M, Lipshitz JS, Botchan MR: Role for a Drosophila Myb-containing protein complex in site-specific DNA replication, Nature 2002, 420:833-837.

48. Calvi BR, Byrnes BA, Kolpakas AJ: Conservation of epigenetic regulation, ORC binding and developmental timing of DNA replication origins in the genus Drosophila, Genetics 2007, 175:1291-1301.

49. Mohammad MM, Danti TR, Yalikisik JS, Smith AG, Kapler GMT: Tetrahymanora ORC contains a ribosomal RNA fragment that participates in rDNA origin regulation, EMBO J 2007, 26:5048-5060.

50. Chesnokov I, Remus D, Botchan M: Functional analysis of mutant and wild-type Drosophila origin recognition complex, Proc Natl Acad Sci USA 2001, 98:11997-12002.

51. Davey MJ, Jeruzalmi D, Kurian J, O’Donnell M: Motors and switches: AAA+ machines within the replisome, Nat Rev Mol Cell Biol 2002, 3:826-835.

52. Bowers JL, Randell JCV, Chen S, Bell SP: ATP hydrolysis by ORC catalyzes reiterative Mcm2-7 assembly as defined origin of replication, Mol Cell Biol 2004, 14:967-978.

53. Aparicio OM, Weinstein DM, Bell SP: Components and dynamics of DNA replication complexes in S. cerevisiae: redistribution of Mcm proteins and Cdc6 in S phase, Cell 2007, 21:2897-2907.

54. Shin JH, Grabowski B, Kasiviswanathan R, Bell SD, Kelman Z: Regulation of minichromosome maintenance helicase activity by Cdc6, J Biol Chem 2003, 278:38059-38067.

55. Hauagglund GT, Shin JH, Birkeland NK, Kelman Z: Stimulation of Mcm helicase activity by a Cdc6 protein in the archaeon Thermoplasma acidophilum, Nucleic Acids Res 2006, 34:6337-6344.

56. Atanassova N, Grange I: Biochemical characterization of the minichromosome maintenance complex (MCM) protein of the chromosome Aeropyrum pernix and its interactions with the origin recognition complex (ORC) proteins, Biochemistry 2008, 47:13362-13370.

57. Prasanth SG, Méndez J, Prasanth KV, Stillman B: Dynamics of pre-replication complex proteins during the cell division cycle, Phil Trans R Soc Lond B 2004, 359:7-16.
61. Shimada K, Gasser SM: The origin recognition complex functions in sister-chromatid cohesion in Saccharomyces cerevisiae. Cell 2007, 128:85-99.

62. Sasak T, Gilbert DM: The many faces of the origin recognition complex. Curr Opin Cell Biol 2007, 19:337-343.

63. Pint  O, Quintana DG, Smith A, Milahek RM, Hou Z-H, Boyton S, Jones CJ, Hendricks M, Velinson K, Wohlschlegel JA, Austin RJ, Lane WS, Tully T, Dutta A: Lateo encodes a subunit of the origin recognition complex and disrupts neuronal proliferation and adult olfactory memory when mutant. Neuron 1999, 23:45-54.

64. Rohrbough J, Pinto S, Milahek RM, Tully T, Broadie K: Lateo, a Drosophila gene involved in learning, regulates functional synaptic plasticity. Neuron 1999, 23:55-70.

65. Huang Z, Zang K, Reichardt LF: The origin recognition core complex regulates dendrite and spine development in postmitotic neurons. J Cell Biol 2005, 170:527-535.

66. Shu M, Qin Y, Jiang M: RNA interference targeting ORC1 gene suppresses the proliferation of vascular smooth muscle cells in rats. Exp Mol Pathol 2008, 84:206-212.

67. Semple JW, Duncker BP: ORC-associated replication factors as biomarkers for cancer. Biotechnol Adv 2004, 22:621-643.

68. Karakaidos P, Taraviras S, Vassiliou LV, Zarakatos P, Karinakakis NG, Kougou D, Kouloukoussa M, Nishitani H, Papavassiliou AG, Lygerou Z, Gorgoulis VG: 8291-8298.

69. Gonzalez S, Klatt P, Delgado S, Conde E, Lopez-Rios F, Sanchez-Cespedes M, Mendez J, Antequera F, Serrano M: Oncogenic activity of Cdcl6 through repression of the INK4/ARF locus. Nature 2006, 440:702-706.

70. Shima N, Alcaraz A, Lisichko I, Buske TR, Andrews CA, Munroe RJ, Hartford SA, Tye BK, Schimenti JC: A viable allele of Mmr4 causes chromosome instability and mammary adenocarcinomas in mice. Nat Genet 2007, 39:93-98.

71. Nguyen VQ, Co C, Li JJ: Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. Nature 2001, 411:1068-1073.

72. Green BM, Morrelle RJ, Ozaydin B, Derisi JL, Li JJ: Genome-wide mapping of DNA synthesis in Saccharomyces cerevisiae reveals that mechanisms preventing reinitiation of DNA are not redundant. Mol Biol Cell 2006, 17:2401-2414.

73. Amlander J, Chen X-B, Bosco G: The N-terminal domain of the Drosophila restinoblastoma protein Rbf1 interacts with ORC and associates with chromatin in an EZF independent manner. PLoS ONE 2008, 3:e2831.

74. Gavin EJ, Song B, Wang Y, Xi Y, Ju J: Reduction of Orc6 expression sensitizes human colon cancer cells to 5-fluourouracil and cisplatin. PLoS ONE 2008, 3:e4054.

75. Marchler-Bauer A, Anderson JB, Derbyshire MK, DeWeese-Scott C, Gonzales NR, Gwadz M, Hao L, He S, Hurwitz DI, Jackson JD, Ke Z, Krylov D, Lanzczycki CJ, Liebert CA, Liu C, Lu F, Lu S, Marchler GH, Mullokandov M, Song JS, Thanki N, Yamashita RA, Yin J, Zhang D, Bryant SH: CDD: a conserved domain database for interactive domain family analysis. Nucleic Acids Res 2007, 35:D237-D240.

76. Finn RD, Tate J, Mistry J, Coggill PC, Sammut JS, Hotz HR, Ceric G, Forslund K, Eddy SR, Sonnhammer EL, Bateman A: The Pfam protein families database. Nucleic Acids Res 2008, 36:D281-D288.

77. Dosztányi Z, Csizmók V, Tompa P, Simon I: IUPred: web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. Bioinformatics 2005, 21:3433-3434.

78. Lupea A, Van Dyke M, Stock J: Predicting coiled coils from protein sequences. Science 1991, 252:1162-1164.

79. Saha P, Chen J, Thome KC, Lawls SJ, Hou ZH, Hendricks M, Parvin JD, Dutta A: Human CDC6/Cdc18 associates with Orc1 and cyclins and is selectively eliminated from the nucleus at the onset of S phase. Mol Cell Biol 1998, 18:2758-2767.

80. Wang B, Feng L, Hu Y, Huang SH, Reynolds CP, Wu L, Jing AY: The essential role of Saccharomyces cerevisiae CDC6 nucleotide-binding site in cell growth, DNA synthesis, and Orc1 association. J Biol Chem 1999, 274:8291-8298.

81. Edgar RC: MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004, 32:1792-1797.

82. Gascuel O: BIONJ, an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 1997, 14:685-695.