Cdc14: a highly conserved family of phosphatases with non-conserved functions?

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Summary

CDC14 was originally identified by L. Hartwell in his famous screen for genes that regulate the budding yeast cell cycle. Subsequent work showed that Cdc14 belongs to a family of highly conserved dual-specificity phosphatases that are present in a wide range of organisms from yeast to human. Human CDC14B is even able to fulfill the essential functions of budding yeast Cdc14. In budding yeast, Cdc14 counteracts the activity of cyclin dependent kinase (Cdk1) at the end of mitosis and thus has important roles in the regulation of anaphase, mitotic exit and cytokinesis. On the basis of the functional conservation of other cell-cycle genes it seemed obvious to assume that Cdc14 phosphatases also have roles in late mitosis in mammalian cells and regulate similar targets to those found in yeast. However, analysis of the human Cdc14 proteins (CDC14A, CDC14B and CDC14C) by overexpression or by depletion using small interfering RNA (siRNA) has suggested functions that are quite different from those of ScCdc14. Recent studies in avian and human somatic cell lines in which the gene encoding either Cdc14A or Cdc14B had been deleted, have shown – surprisingly – that neither of the two phosphatases on its own is essential for viability, cell-cycle progression and checkpoint control. In this Commentary, we critically review the available data on the functions of yeast and vertebrate Cdc14 phosphatases, and discuss whether they indeed share common functions as generally assumed.

Key words: Dual-specificity phosphatase, Cdc14, DNA damage, Mitotic exit, DNA replication, DNA repair

Introduction

It is well recognized that reversible phosphorylation of proteins has a pivotal role in their respective biological functions and in controlling virtually every cellular process in eukaryotes, including metabolism, gene transcription and translation, cell-cycle progression, cytoskeletal rearrangements, protein-protein interactions, protein stability, cell movement and apoptosis. About one-third of eukaryotic cellular proteins are phosphorylated (Cohen, 2000), mainly on serine and threonine residues, and their phosphorylation status is regulated by a balance between the kinases and phosphatases for which they are substrates. The regulatory importance of protein kinases has been widely accepted; however, until recently the role of protein phosphatases has largely been considered to be no more than that of counterparts to the key regulatory control exerted by kinases in modulating cellular behavior.

In this Commentary, we focus on the Cdc14 family of dual-specificity phosphatases. We will not discuss in detail the essential role of budding yeast Cdc14 in mitotic exit and its regulation by the Cdc14 early-anaphase release (FEAR) pathway and the mitotic exit network (MEN), because several excellent reviews on these topics were recently published (Amon, 2008; De Wulf et al., 2009; Queralt and Uhlmann, 2008). Instead, we first discuss the role of Cdc14 phosphatases in metazoa versus that in yeast cells and then compare recent findings that suggest the involvement of yeast and vertebrate Cdc14 proteins in DNA replication, DNA damage response and DNA repair. Finally, we emphasize the fact that vertebrate cell lines from which the genes encoding Cdc14A or Cdc14B have been deleted provide an important tool to directly address the cellular functions of these phosphatases.

Protein phosphatases and their regulation

The human genome encodes 518 kinases but only ~147 protein phosphatases (Alonso et al., 2004; Manning et al., 2002). Phosphatases evolved independently from several evolutionary progenitors into distinct families – contrast to protein kinases, which are derived from one common ancestor (Tonks, 2006). Owing to the relatively small number of genes that encode phosphatases and the fact that isolated phosphatases only show low substrate specificity in vitro, phosphatases have been widely regarded as promiscuous enzymes. However, it is now becoming increasingly clear that several mechanisms are likely to contribute to the substrate specificity of phosphatases. For example, members of the serine/threonine phosphatase family are holoenzyme complexes that have various combinations of a limited number of catalytic subunits and a large number of regulatory subunits (Virshup and Shenolikar, 2009), which results in different holoenzymes that control a broad range of biological events. Diversity within the single-subunit protein-tyrosine phosphatase (PTP) superfamily is achieved through the use of alternative promoters to control their expression, the presence of multiple splicing variants and post-translational modifications (Tonks, 2006). Moreover, temporal and spatial regulation of the activity of phosphatases can be obtained through strict control over their localization as it has been observed for members of the Cdc25 family (Davezac et al., 2000; Takizawa and Morgan, 2000) and for protein phosphatase 1 (PP1) (Andreassen et al., 1998; Liu et al., 2010; Trinkle-Mulcahy et al., 2003).

Dual-specificity phosphatases

Dual-specificity phosphatases (DUSPs) are a heterogenous group of phosphatases that belong to the PTP superfamily. The unique feature that characterizes DUSPs is their ability to dephosphorylate

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both phosphotyrosine and phosphoserine/phosphothreonine residues within their substrates. The highly conserved catalytic domain contains the so-called HCx_5R signature motif and its cysteine residue is crucial for catalysis. The catalytic pocket of DUSPs is wider than that found in classic PTPs, explaining why these phosphatases can accommodate phosphorylated serine/threonine or tyrosine residues (Denu and Dixon, 1998). Among the large family of DUSPs (Patterson et al., 2009), the Cdc14 family is one of the most extensively studied, mainly because of the essential role the budding yeast ortholog exerts in regulating late mitotic events and mitotic exit.

Domain structure of Cdc14 phosphatases

All Cdc14 phosphatases share a highly conserved N-terminal core of ~350 amino acids (Fig. 1). Crystal structure analysis of the central core domain revealed an A domain that might contribute to substrate specificity and a catalytic B domain that encompasses the PTP signature motif (Gray et al., 2003). The vertebrate Cdc14B isoform has a unique KKIR motif extending from the N-terminus (Fig. 1) that is responsible for targeting the protein to the nucleolus (Berdougo et al., 2008; Cho et al., 2005; Kaiser et al., 2002; Mailand et al., 2002; Mocciaro et al., 2010; Rosso et al., 2008). However, recent findings indicate that the C-terminal part of the protein is also important for its subcellular localization (Rosso et al., 2008). The length and the sequence conservation of the C-terminus of Cdc14 phosphatases is variable. The C-terminal region of Cdc14 contains a nuclear export sequence (NES), and in the yeast orthologs a nuclear localization sequence (NLS) is also present (Fig. 1), which is subjected to regulation through phosphorylation by nuclear Dbf2-related (NDR) kinases active in the MEN and the septum initiation network (SIN) pathways (Bembenek and Yu, 2001; Chen et al., 2008; Mohl et al., 2009).

An overview of the functions of Cdc14 phosphatases in model organisms

The Cdc14 family of phosphatases is highly conserved, and Cdc14 orthologs have been identified and characterized in several organisms. In the following section we will summarize the most significant findings from studies of Cdc14 proteins in different species, thereby focusing mainly on their localization and putative functions (for a schematic overview, see Fig. 2; for a brief description of the identified Cdc14 substrates, see Table 1).

Budding yeast

Cdc14 of the budding yeast *Saccharomyces cerevisiae*, hereafter referred to as ScCdc14, is the founding member of the family of Cdc14 phosphatases. Extensive genetic and biochemical studies have shown that the subcellular localization of the essential ScCdc14 is regulated initially by the FEAR network, which functions in early anaphase and, subsequently, by the MEN, which is mainly active in late anaphase.

The FEAR-pathway and MEN activation direct the release of ScCdc14 from the nucleolus into nucleoplasm and cytoplasm (budding yeast has a closed mitosis) (Fig. 2). This ScCdc14 pool is then involved in a number of different steps – including those of making the metaphase-to-anaphase transition more abrupt, anaphase spindle stabilization, MEN activation and mitotic exit – by reversing phosphorylation of cyclin-dependent kinase 1 (Cdk1) sites (Higuchi and Uhlmann, 2005; Holt et al., 2008; Jaspersen and Morgan, 2000; Khmelinskii et al., 2007; Khmelinskii et al., 2009; Konig et al., 2010; Menssen et al., 2001; Pereira and Schiebel, 2003; Stegmeier et al., 2002; Visintin et al., 1998). Analysis of temperature sensitive Sc*Cdc14ts* mutants showed that the phosphatase is also required for accurate segregation of ribosomal DNA (rDNA) and telomeric regions, through the targeting of condensin to rDNA (D'Amours et al., 2004; Sullivan et al., 2004). Recently, clear evidence was provided that ScCdc14 inhibits transcription of rDNA in anaphase by exclusion of RNA polymerase lI subunits from the nucleolus. Anaphase-specific gene silencing is thought to be a prerequisite for allowing condensin access to rDNA chromatin (Clemente-Blanco et al., 2009).

Consistent with these functions, a broad range of ScCdc14 substrates were identified through genetic and biochemical approaches. Amongst those found are key cell-cycle regulators, spindle and kinetochore-associated proteins, and proteins involved in DNA replication (see Table 1).

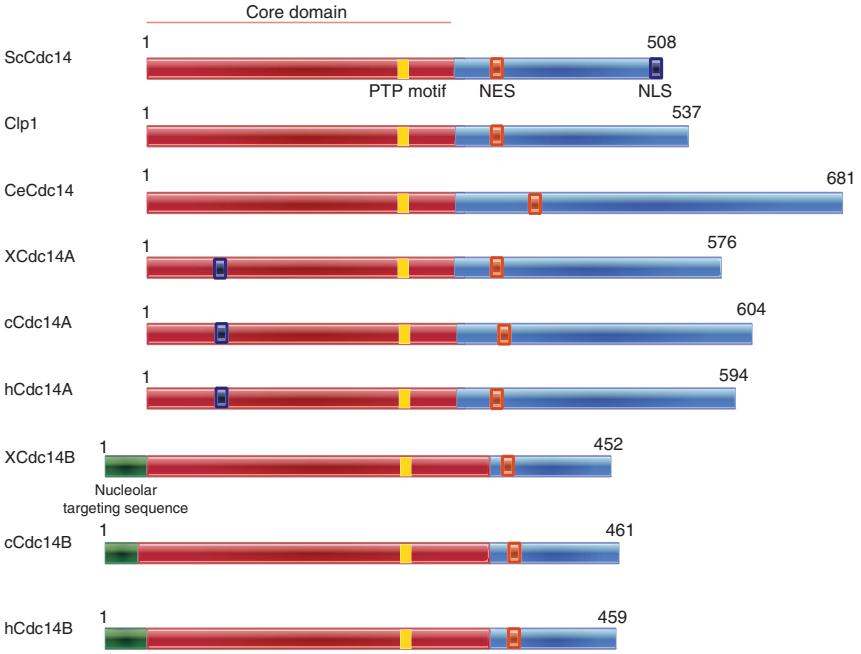


Fig. 1. Schematic representation of Cdc14 primary structure from those species discussed. The conserved domain is shown in red, the variable C-terminus domain is shown in light blue. The nucleolar targeting sequence, green; catalytic motif, yellow; NLS, dark blue; NES, orange.

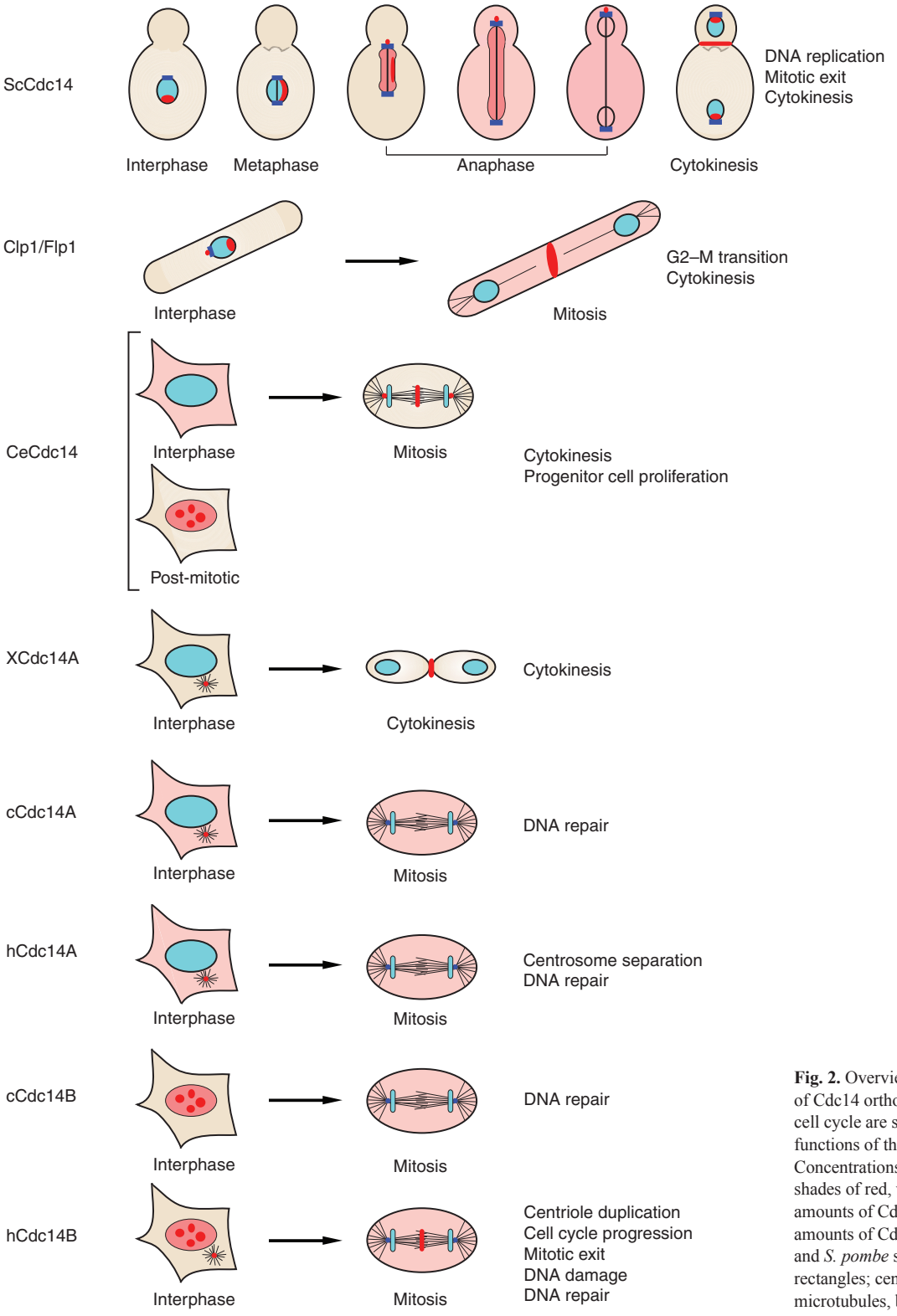


Fig. 2. Overview of Cdc14 orthologs. Localization of Cdc14 orthologs during different stages of the cell cycle are shown together with possible functions of the phosphatase in each organism. Concentrations of Cdc14 are shown in different shades of red, where dark red represents high amounts of Cdc14 and light red represents low amounts of Cdc14. DNA, light blue; *S. cerevisiae* and *S. pombe* spindle pole bodies, dark blue rectangles; centrosomes, dark blue circles; microtubules, black lines.

ScCdc14 and the MEN might also have a role in cytokinesis, as suggested by the transient localization of ScCdc14 to the site of cytokinesis at the time of mitotic exit (Fig. 2) (Bembenek and Yu, 2001; Lippincott et al., 2001; Meitinger et al., 2010). However, so far none of the ScCdc14 substrates identified have been linked to a possible role in cytokinesis.

Fission yeast

In *Saccharomyces pombe* Cdc14-like phosphatase (Clp1; also known as Flp1), a non-essential ortholog of ScCdc14 has been identified (Cueille et al., 2001; Trautmann et al., 2001) (Fig. 3). Although Clp1 and ScCdc14 are well-conserved – with 36% sequence identity over the entire length of the protein – and both

Table 1. Cdc14 substrates

Species (phosphatase)	Substrates	References
S. cerevisiae (ScCdc14)	Cell-cycle regulators Acm1[c], Sic1[a], Swi5[a], Cdh1/Hct1[c], Pds1[a], Mob1[c], Bfa1[c], Cdc15[c] Spindle- and KT-associated proteins Fin1[c], Ase1[c], Ask1[c], Sli15[c] DNA replication factors Sld2[c], Dbp2[c], Pol12[b]	(Bloom and Cross, 2007; Hall et al., 2008; Higuchi and Uhlmann, 2005; Holt et al., 2008; Jaspersen et al., 1999; Jaspersen and Morgan, 2000; Jin et al., 2008; Khmelinskii et al., 2007; Konig et al. 2010; Pereira et al., 2002; Pereira and Schiebel, 2003; Visintin et al., 1998; Woodbury and Morgan, 2007)
S. pombe (Clp1)	Cell-cycle regulators Clp1[c], Cdc25[c] Spindle- and KT-associated proteins Ase1[a], Klp9[a] Contractile-ring components Cdc15[b], Myo2[a]	(Clifford et al., 2008; Esteban et al., 2004; Fu et al., 2009; Trautmann et al., 2004; Trautmann et al., 2001; Wolfe and Gould, 2004; Wolfe et al., 2006)
C. elegans (CeCdc14)	Spindle-associated proteins Zen-4[a]	(Mishima et al., 2004)
X.laevis (XCdc14A)	Not identified	
(XCdc14B)	Not identified	
G. gallus (cCdc14A)	Not identified	
(cCdc14B)	Not identified	
H. sapiens hCdc14A	p53[a], INCENP[a], Cdh1[a], p27[a],Cdc25A[a], MKLP1[a], SIRT2[b], Erk3[c], RN-tre[c]	(Bassermann et al., 2008; Bembenek and Yu, 2001; Dryden et al., 2003; Esteban et al., 2006; Hansen et al., 2008; Lanzetti et al., 2007; Li et al., 2000; Mailand et al., 2002; Mishima et al., 2004; Rodier et al., 2008; Tanguay et al., 2010)
hCdc14B	p53[a], Skp2[a], SIRT2[b], Cdh1[b], Erk3[c], RN-tre[c]	
hCdc14C	Not identified	

[a]Only tested in vitro, [b]only tested in vivo, [c]tested in vitro and in vivo.

antagonize Cdk1 activity in their respective systems, the *S. pombe* ortholog functions differently in cell-cycle control. Clp1 is not important for mitotic exit, but rather mainly regulates mitotic entry and, in addition, coordinates cytokinesis with the initiation of the next cell cycle (Fig. 2) (Cueille et al., 2001; Trautmann et al., 2001).

Like its budding yeast ortholog, Clp1 localizes to the nucleolus during G1- and S-phase (Fig. 2). But whereas ScCdc14 release occurs in anaphase and depends on activation of MEN or the FEAR pathway, Clp1 is released at the G–M transition without the contribution of fission yeast homologs of the FEAR pathway (Chen et al., 2006), and initially localizes to the mitotic spindle and to kinetochores. It has been suggested that kinetochore-associated Clp1 functions together with Aurora B kinase to correct mono-orientation of the sister kinetochores, thereby ensuring accurate chromosome segregation (Trautmann et al., 2004). In addition, similar to budding yeast ScCdc14, Clp1 regulates functions in the spindle midzone by dephosphorylating the midzone-binding protein Ase1 and the kinesin-6 motor Klp9 (Fu et al., 2009; Khmelinskii et al., 2009). Later in mitosis, Clp1 localizes to the site of cytokinesis, the medial actin-myosin ring at the equator of the cell (Fig. 2). As in budding yeast, Clp1 is controlled in its subcellular localization by the SIN and regulates the formation of the septum during cytokinesis, although it is not essential for either septation or cytokinesis (Clifford et al., 2008; Cueille et al., 2001; Simanis, 2003; Trautmann et al., 2001).

Non-mammalian metazoa

Cdc14 is also highly conserved in metazoa (Fig. 3). However, relatively little is known about the function of Cdc14 in these higher organisms. Early immunofluorescence data has shown that the *Caenorhabditis elegans* Cdc14 (hereafter referred to as CeCdc14) localizes to the spindle midzone in anaphase and to the midbody during telophase (Gruneberg et al., 2002); GFP-tagged CeCdc14 was observed in the cytoplasm in interphase cells, and on centrosomes, on spindle microtubules and at the midbody in mitotic cells (Saito et al., 2004). In post-mitotic cells, the phosphatase localized to the nucleus and the nucleolus (Fig. 2) (Saito et al., 2004). Depletion of CeCDC14 by using RNA interference (RNAi) was found to disrupt cytokinesis, leading to multi-nucleated cells and causing embryonic lethality (Gruneberg et al., 2002). By contrast, another study found that Ce*CDC14*-deficient mutant worms were viable and did not show any mitotic or cytokinetic defects. Instead, these worms showed a specific defect in the duplication of vulval progenitor cells (Saito et al., 2004). The reason for the discrepancy between the findings of these studies is presently unclear; however, it has been suggested that the high concentrations of small interfering RNA (siRNA) oligonucleotides used by Gruneberg et al. might have contributed to or modified the observed RNAi phenotypes (Kipreos, 2004).

The *Xenopus laevis* genome encodes two Cdc14 isoforms, CDC14A and CDC14B (hereafter referred to as XCdc14A and XCdc14B, respectively) (Kaiser et al., 2004; Krasinska et al., 2007) (Fig. 3). Overexpressed GFP-tagged XCdc14A is centrosomal during interphase but is recruited to the midbody at the end of mitosis (Fig. 2). XCdc14A can dephosphorylate Cdc25 in extract of *Xenopus* eggs that had been induced to enter mitosis. In addition, overexpression of X*Cdc14A* inhibits the recruitment of soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE)–exocyst complexes to the site of cytokinesis, where it is required to orchestrate the final step of cytokinesis, known as abscission (Krasinska et al., 2007). There

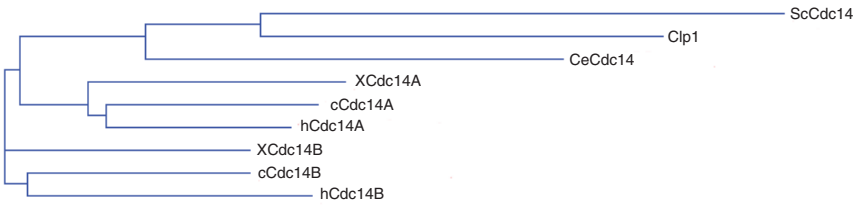


Fig. 3. Cdc14 orthologs in different organisms. Phylogenetic tree on the basis of the discussed Cdc14 coding sequences. The phylogenetic tree was reconstructed by using the ClustalW software.

is currently no information about the physiological role of the XCdc14B isoform.

Recently, we have identified the avian orthologs of Cdc14 (named cCdc14A and cCdc14B) in chicken DT40 cells (Mocciaro et al., 2010) (Fig. 3). cCdc14A is centrosomal in interphase and cCdc14B shows nuclear localization, with enrichment in the nucleolus (Fig. 2). These localization patterns are consistent with those reported for the human Cdc14 orthologs (Mailand et al., 2002). The generation of DT40 cells, from which the genes encoding cCdc14A or cCdc14B have been deleted, have provided a very useful tool to investigate the functions of cCdc14 proteins. One of the most striking findings obtained from the analysis of these c*Cdc14*-knockout cell lines is that none of the avian cCdc14 proteins is essential for cell viability and that the lack of one c*Cdc14* alone does not cause obvious defects in cell-cycle progression, mitotic entry, chromosome segregation, mitotic exit or cytokinesis (Mocciaro et al., 2010) (our unpublished results), which is in contrast to the defects in mitosis and cytokinesis observed in the absence of yeast ScCdc14 function. Moreover, cells that lack c*Cdc14A* or c*Cdc14B* did not show abnormalities in centrosome number and centrosome separation (our unpublished results).

Mammals

First identified in human cells were the two ScCdc14 orthologs CDC14A and CDC14B (hereafter referred to as hCdc14A and hCdc14B, respectively) (Li et al., 2000), which display 50% amino acid sequence identity (Fig. 3). Recently, it has been shown that in hominoids, a gene retroduplication event gave rise to an additional Cdc14 family member, hCdc14C (also known as Cdc14Bretro or CDC14C), which is very similar to hCdc14B except for the C-terminus. Expression of h*Cdc14C* is predominantly in brain and testis (Rosso et al., 2008). Functional studies have begun to address the roles of hCdc14. For instance, it was found that both hCdc14A and hCdc14B isoforms could rescue Clp1-deficient fission yeast strains and, furthermore, that hCdc14B was able to fulfil all essential functions of ScCdc14, indicating that at least some properties of Cdc14 phosphatases have been conserved throughout evolution (Vazquez-Novelle et al., 2005).

Despite being the subject of intense scrutiny in a number of studies, the exact molecular functions of human Cdc14 phosphatases largely remain to be established; moreover, the little we do know is even somewhat controversial. hCdc14A is found in the cytoplasm and on centrosomes in interphase but it is not on centrosomes in mitotic cells (Fig. 2) (Mailand et al., 2002). Overexpression of h*Cdc14A* in S-phase-arrested human osteosarcoma (U2OS) cells leads to premature centrosome splitting in interphase and erroneous formation of a spindle-like structure (Kaiser et al., 2002; Mailand et al., 2002). Consistent with its presumed role in the centrosome cycle, downregulation of endogenous hCdc14A using RNAi caused impaired centrosome separation and a number of mitotic defects, including failure of chromosome segregation, non-productive cytokinesis and multi-nucleation (Mailand et al., 2002).

hCdc14B localization is predominantly nucleolar (Berdougo et al., 2008; Kaiser et al., 2002; Mailand et al., 2002), although, as described in various studies, it was also detected on centrioles (Wu et al., 2008), long nuclear filaments (Nalepa and Harper, 2004), microtubules (Cho et al., 2005; Rosso et al., 2008), the spindle midzone (Cho et al., 2005) and the midbody (Cho et al., 2005) (Fig. 2). Depending on the particular study and the experiments used, such as depletion by RNAi or overexpression, different roles for hCdc14B have been suggested, including in nuclear organization (Nalepa and Harper, 2004), mitotic-spindle assembly (Cho et al., 2005), centriole duplication (Wu et al., 2008), mitotic exit (Dryden et al., 2003), regulation of the M-to-G1 transition (Rodier et al., 2008), and/or in G2 DNA damage checkpoint activation (Bassermann et al., 2008).

However, the siRNA depletion data are thus far not confirmed by knockout studies because – perhaps surprisingly – cells from which genes encoding either hCdc14A or hCdc14B have been deleted by gene targeting showed no obvious growth and mitotic defects (Berdougo et al., 2008; Mocciaro et al., 2010). For example, analysis of human telomerase-immortalized retinal pigment epithelial (hTERT-RPE) nullizygous cell lines for h*Cdc14A* and human colorectal carcinoma (HCT116) nullizygous cell lines for h*Cdc14B*, showed that neither h*Cdc14A* nor h*Cdc14B* is essential for cell viability or for what appears to be normal cell-cycle progression, similar to what was shown for the avian orthologs of *Cdc14*. Moreover, human somatic cells that lack either h*Cdc14A* or h*Cdc14B* did not show any of the defects associated with RNAi experiments in human cells, such as impaired centrosome separation, centriole overduplication, mitotic exit defects and impaired G2 DNA damage checkpoint (Berdougo et al., 2008; Mocciaro et al., 2010).

Although several putative targets of hCdc14A and hCdc14B have been identified, few have been validated as substrates both in vitro and in vivo (see Table 1 for targets). Therefore, it remains to be established whether these in-vitro-determined substrates, indeed, represent true physiological targets of the hCdc14 phosphatases.

Some information is also available for hCdc14C; it was shown that overexpression of h*Cdc14C* fused to fluorescent reporters led to its localization on microtubules and at the endoplasmic reticulum (ER) (Rosso et al., 2008). The significance of the ER-associated localization is still unclear, and the possible functions of Cdc14 on the ER await further investigation (Rosso et al., 2008).

The road not (yet) taken: new roles for Cdc14 phosphatases

Recent studies of Cdc14 proteins in different species have highlighted possible new functions of these phosphatases, indicating that the members of this family are involved in a much broader range of biological processes than previously considered. It is

becoming increasingly clear that Cdc14 orthologs in some species cannot be strictly classified as 'mitotic phosphatases' simply because of the well-established mitotic functions in budding and fission yeast. Thus, we need to change our conceptual framework and perspectives if we are to gain a more complete understanding of the role of Cdc14 phosphatases in higher eukaryotes. Below, we will summarize the most recent findings for roles of Cdc14 in DNA replication, DNA damage checkpoint and DNA repair, and will then discuss possible new directions for the Cdc14 field.

DNA replication

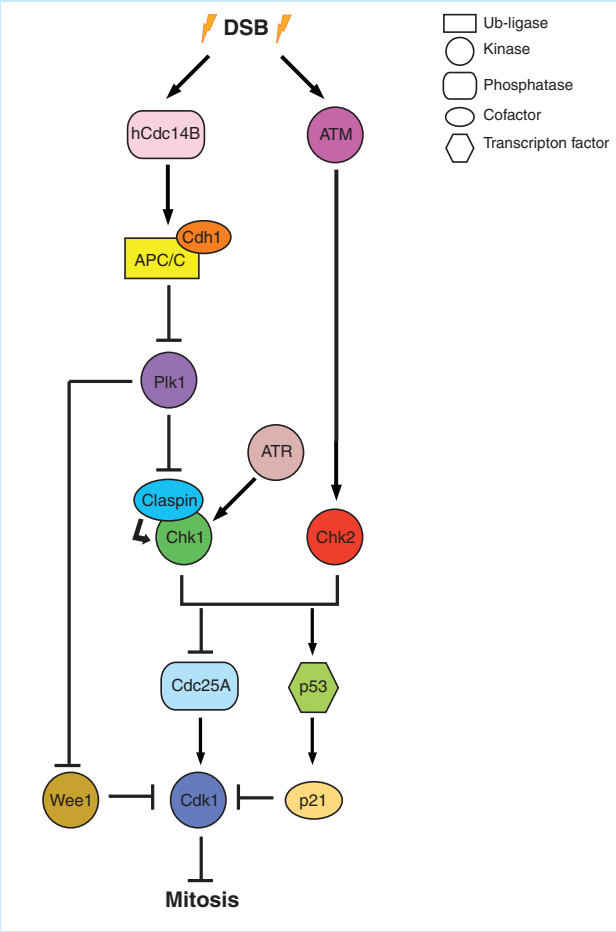
A role for budding yeast ScCdc14 in DNA replication was originally demonstrated by using a genetic approach (Hogan and Koshland, 1992), and replication factors such as Sld2, Dbp2 and Pol12 were identified as possible Cdc14 substrates (Bloom and Cross, 2007; Jin et al., 2008). In addition, a recent study has shown that the delay in chromosome segregation in *S. cerevisiae cdc14(ts)* mutants is not only caused by the inability of these cells to downregulate transcription of rDNA genes during mitosis – which is required for correct chromosome segregation (Clemente-Blanco et al., 2009), but also by a defect in completing DNA replication (Dulev et al., 2009). Such 'underreplication' of DNA is not sensed by the DNA damage checkpoint, which remains active in *cdc14* mutants. The failure to complete replication is probably due to an insufficient availability of the replication machinery arising from impaired G1 transcription and reduced nuclear import of replication factors into the nucleus (Dulev et al., 2009). The question remains whether, in budding yeast, replication factors are substrates of ScCdc14 that are regulated by phosphorylation-dependent nuclear import or whether components of the import machinery themselves are direct targets of ScCdc14; answeres to these questions will require further investigation.

The DNA damage response

Another possible new function of human hCdc14B was highlighted by recent findings from Pagano and co-workers in U2OS cells (Bassermann et al., 2008), who suggested that hCdc14B participates in the regulatory pathway that controls the G2 DNA damage checkpoint (Box 1). According to their model, in response to genotoxic stress at G2 phase, hCdc14B relocalizes from the nucleolus to the nucleus, where it dephosphorylates Cdc20-like protein 1 (Cdh1), a substrate-binding subunit of the anaphase-promoting complex or cytosome (APC/C). The subsequent activation of the resulting APC/C^{Cdh1} then stabilizes the adaptor protein claspin, which facilitates the efficient activation of the checkpoint kinase Chk1. In contrast to a clear change in nucleolar localization of hCdc14B, localization of hCdc14A was not affected by DNA damage, but its potential role in the DNA damage checkpoint has not been addressed (Bassermann et al., 2008).

Perplexingly, we found that avian and human cell lines, in which *Cdc14B* was deleted, retained their G2 DNA damage checkpoint proficiency, although in both cell types relocalization of Cdc14B from the nucleolus to the nucleus upon DNA damage was observed (Mocciaro et al., 2010) (Fig. 4). However, the biological significance of the observed relocalization remains the subject of controversy. Pagano and co-workers initially suggested that release of hCdc14B into the nucleus was necessary for dephosphorylation of Cdh1 and the subsequent checkpoint activation (Bassermann et al., 2008); yet our recent results from human and DT40 cells that lack *Cdc14B* or *Cdh1* but retain their capacity to arrest efficiently in G2 after DNA damage. This clearly indicates that these two

Box 1. The G2 DNA damage checkpoint



Endogenous and exogenous DNA damage are a threat to the integrity of the genome. To ensure that the transfer of genetic information occurs faithfully, eukaryotic cells have evolved checkpoint responses and DNA repair mechanisms that prevent damaged DNA from being converted into heritable mutations.

The G2–M DNA damage checkpoint (see Figure) prevents cells from undergoing mitosis in presence of DNA damage. The sensors of the damage are the kinases ATM and ATR, which phosphorylate the checkpoint kinases Chk2 and Chk1, respectively (Brown and Baltimore, 2003; Zhao et al., 2001). Chk1 activation by ATR requires the mediator protein claspin. Chk1 and Chk2 downregulate members of the Cdc25 phosphatase family and upregulate Wee1, which together control Cdk1–cyclinB activity and mitotic entry (Yarden et al., 2002). Among the three Cdc25 isoforms, Cdc25A is the main effector of the G2–M checkpoint (Zhao et al., 2002). Upon phosphorylation, Cdc25A is degraded by the ubiquitin–proteasome pathway (Furnari et al., 1997; Peng et al., 1997; Sanchez et al., 1997). Inactivation of Cdc25A leads to accumulation of the inhibitory phosphorylation of Cdk1 on Y15 and mitotic arrest. Interestingly, Pagano and colleagues have recently suggested involvement of hCdc14B in the G2–M checkpoint. In their model, in response to genotoxic stress in G2, hCdc14B translocates from the nucleolus to the nucleoplasm and induces the activation of the ubiquitin ligase APC^{Cdh1}, causing the degradation of Plk1, which normally promotes mitotic entry. This process induces the stabilization of claspin and triggers an efficient G2 checkpoint arrest (Bassermann et al., 2008).

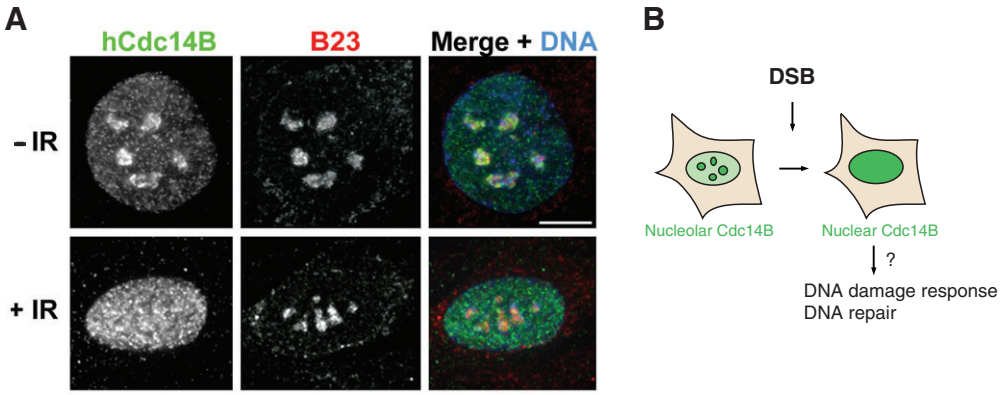


Fig. 4. Relocalization of human Cdc14B upon DNA damage. (**A**) Micrographs of hTERT-RPE cells stably expressing h*Cdc14B-LAP*. Cells were synchronized in G2 and exposed to IR light. Cells were collected and analyzed by indirect immunofluorescence using an anti-GFP antibody (green) and an anti-nucleophosmin antibody (red) as nucleolar marker. Scale bar: 10 µm. Further experimental details can be found elsewhere (Mocciaro et al., 2010). (**B**) Schematic illustration of Cdc14 relocalization upon DNA damage. For a detailed discussion of the possible roles of Cdc14 in DNA damage response and in DNA repair, see Box 1 and main text.

proteins are not essential for an efficient G2 DNA damage checkpoint (Mocciaro et al., 2010).

An alternative interpretation for the relocalization of hCdc14B comes from the fact that the nucleolus is dramatically altered after DNA damage – both in structure and protein composition. In particular, a large number of proteins involved in DNA repair and DNA replication are either recruited to or redistributed from nucleoli in response to genotoxic stress (Dellaire and Bazett-Jones, 2007; Sacher et al., 2006; Torres-Rosell et al., 2007). How hCdc14B is targeted to the nucleolus remains unclear. One trivial explanation for Cdc14B relocalization is that it is an indirect consequence of a general reorganization of the nucleolus upon DNA damage. However, in fission yeast, replication stress also induces changes in the subcellular localization of Clp1 from the nucleolus to the nucleus in a manner dependent on the DNA damage checkpoint. Here, relocalization occurs once Clp1 is phosphorylated by Cds1 (the fission yeast homolog of Chk2), which in turn leads to full activation of Cds1 through a positive-feedback loop (Diaz-Cuervo and Bueno, 2008). These findings are consistent with a conserved role of Cdc14 in DNA-damage management.

Recently, it was shown that RASSF1, MST2 and LATS, components of the Hippo pathway, are activated upon DNA damage, leading to stabilization of the transcriptional complex YAP–p73 (Hamilton et al., 2009). The Hippo pathway was first identified in *Drosophila melanogaster* as a regulator of cell proliferation and apoptosis, but it is conserved from yeast to mammals. Homologs of at least three Hippo pathway proteins are components of the MEN in *S. cerevisiae* and the SIN in *S. pombe* (Harvey and Tapon, 2007). It is tempting to speculate that an MEN-like network can control Cdc14B localization in mammalian cells, and that members of the Hippo pathway can be part of such a network.

DNA repair

Our careful analysis of avian and human somatic cells that were lacking Cdc14 phosphatases also suggest a putative role for Cdc14A and Cdc14B in DNA repair. Cells that lack either Cdc14A or Cdc14B showed a diminished ability to repair endogenous and exogenous DNA damage, which resulted in an increase in γ-H2AX foci – a marker for double-strand breaks – and hypersensitivity to irradiation (Mocciaro et al., 2010). An interesting, but as-yet-unanswered, question arising from our recent results is through which molecular mechanism Cdc14 phosphatases affect DNA repair. One possibility is that Cdc14A and Cdc14B are directly involved in the dephosphorylation of γ-H2AX. However, this seems unlikely, because other phosphatases implicated in γ-H2AX processing, such as PP2A and PP4, colocalize with γ-H2AX at the foci of DNA damage (Chowdhury et al., 2005; Chowdhury et al., 2008; Nakada et al., 2008), whereas Cdc14A or Cdc14B did not associate with these structures (Mocciaro et al., 2010). Moreover, in contrast to what has been shown for PP2A (Chowdhury et al., 2005), we do not find that Cdc14A and Cdc14B co-immunoprecipitate with γ-H2AX after exposure to DNA damage (our unpublished observation).

The effects of knocking out *Cdc14A* or *Cdc14B* in cells point to the possible molecular mechanisms of Cdc14 function in DNA repair, as these cells exhibit strikingly similar phenotypes to cells that are defective in the double-strand break (DSB) rejoining pathway, which is dependent upon ataxia telangiectasia mutated (ATM) kinase and the nuclease artemis (Riballo et al., 2004). This function of ATM is independent of its ability to induce the G2–M cell-cycle arrest and requires several proteins that associate with γ-H2AX repair foci (Riballo et al., 2004). For example, cells deficient in artemis and Cdc14 show a delay in DNA repair after DSB-inducing treatments; they enter mitosis with chromosomal lesions after checkpoint arrest and are substantially more sensitive to irradiation than wild-type cells. Thus, another hypothesis to explain the release of Cdc14B from the nucleolus in response to genotoxic stress is that it facilitates the activation of enzymes that repair DSBs.

Concluding remarks

At present, we are far from having a unified view of the functions and regulation of Cdc14 phosphatases in different species. The situation in higher eukaryotes is complicated by the presence of multiple paralogs and the fact that experiments on the basis of RNAi or *Cdc14* gene deletion have thus far come to different – and sometimes controversial – conclusions with respect to the roles of Cdc14 in maintaining cell viability, cell-cycle-progression and -checkpoint control.

Exactly why these different experimental approaches led to radically different outcomes remains unresolved. One possibility is

that the effects of complete and permanent loss of Cdc14 function by gene knockout differ from a more short-term and – in some cases – only partial depletion that is typically achieved using RNAi. Variations in observed phenotypes that arise from different efficiencies of gene depletion have been reported for other proteins. For example, one study has shown that, in HeLa cells, if levels of Cdc20 were reduced by more than 98%, cells arrested in mid-mitosis, and both cyclin A and cyclin B1 were stabilized; however, reducing Cdc20 levels by only 90%, led to a delay in mitosis but not a block (Wolthuis et al., 2008). Similar differences in obtained phenotypes were reported for the mitotic regulator polo-like kinase 1 (Plk1) (Tsou et al., 2009). It is important to note that – in general – phenotypes observed after hCdc14A or hCdc14B depletion by using siRNA were not validated by the expression of RNAi-resistant constructs in order to ensure specificity of the depletion and to exclude possible off-target effects of siRNA oligonucleotids that were used. The relevance of potential off-target effects cannot be dismissed. This is not an unlikely scenario, because Nigg and co-workers have recently demonstrated that off-target effects of oligonucleotids in RNAi of PICH were the reason for the observed effects on spindle checkpoint proficiency (Hubner et al., 2009).

Another explanation for the different phenotypes observed so far could be a redundancy between the vertebrate Cdc14 isoforms, as reported for the two catalytic subunits dis2 and sds21 of fission yeast PP1 (Alvarez-Tabares et al., 2007), or for members of the Cdc25 phosphatase family (Chen et al., 2001; Ferguson et al., 2005). In the case of possible functional redundancy, the lack of obvious phenotypes in cells that lack one of the Cdc14 phosphatases points to partially overlapping and compensating functions – despite the fact that at a least a fraction of the Cdc14 proteins have a different localization pattern in the cell.

Moreover, the possible role of other phosphatases that do not belong to the Cdc14 family should also be taken into account. In budding yeast, ScCdc14 is the main phosphatase responsible for the control of mitotic exit. However, in other organisms Cdc14 orthologs appear to be dispensable for mitotic exit, pointing to other phosphatases that take on the role of reversing the Cdk-mediated phosphorylation events, such as PP1, PP2A, and the Ca^{2+}-dependent calcineurin (De Wulf et al., 2009).

The generation of avian and human cell lines depleted of *Cdc14A* or *Cdc14B* has opened up interesting perspectives for future studies of Cdc14 functions in vertebrates. A key question is to what extent the two isoforms of Cdc14 overlap in function – the generation of a cell line in which both *Cdc14A* and *Cdc14B* are deleted might prove particularly useful.

As mentioned above, only a limited repertoire of Cdc14 substrates have been identified in higher eukaryotes. A comparative proteomic approach of phosphorylated proteins in wild-type versus *Cdc14*-knockout cells would be a powerful mean to address this question. Regarding the possible mechanisms of vertebrate Cdc14A and Cdc14B, they are likely to act similarly to their yeast counterparts by counteracting Cdk1 phosphorylation (Gray et al., 2003), which could be the minimal functional conservation of all Cdc14 phosphatases. As regulation by Cdk1 phosphorylation affects a wide range of biological processes, this might explain why many diverse functions have been attributed to Cdc14 phosphatases. However, it is also important to consider the possibility that Cdc14 is functioning by counteracting phosphorylation events in the MAP kinase signaling pathway. The substrate specificity of Cdk1 and MAPKs overlap at threonine-proline (TP) and/or serine-proline (SP) motifs (Alvarez et al., 1991), and this could account for some of the different functionalities of Cdc14 phosphatases in different organisms, because MAPK signaling is far more complex in higher eukaryotes than in budding or fission yeast (Garrington and Johnson, 1999; Waskiewicz and Cooper, 1995; Widmann et al., 1999).

In conclusion, the analysis of the data available on Cdc14 phosphatases clearly show that for the budding yeast ortholog ScCdc14 a lot of information is available and many of its functions and substrates have been identified and extensively studied. By contrast, the picture remains unclear for Cdc14 orthologs in higher eukaryotes, mainly because there does not seem to be a conserved function of this phosphatase family throughout evolution. Moreover, the presence of multiple Cdc14 isoforms in higher eukaryotes adds a further level of complication, because redundancy mechanisms must be taken into account. Future investigations should focus on a systematic approach to identify relevant Cdc14 substrates in vivo, which might shed some light on the physiological roles of this protein in higher eukaryotes.

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