



REVIEW

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Distinct and redundant functions of cyclin E1 and cyclin E2 in development and cancer

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Abstract

The highly conserved E-type cyclins are core components of the cell cycle machinery, facilitating the transition into S phase through activation of the cyclin dependent kinases, and assembly of pre-replication complexes on DNA. Cyclin E1 and cyclin E2 are assumed to be functionally redundant, as cyclin E1^{-/-} E2^{-/-} mice are embryonic lethal while cyclin E1^{-/-} and E2^{-/-} single knockout mice have primarily normal phenotypes. However more detailed studies of the functions and regulation of the E-cyclins have unveiled potential additional roles for these proteins, such as in endoreplication and meiosis, which are more closely associated with either cyclin E1 or cyclin E2. Moreover, expression of each E-cyclin can be independently regulated by distinct transcription factors and microRNAs, allowing for context-specific expression. Furthermore, cyclins E1 and E2 are frequently expressed independently of one another in human cancer, with unique associations to signatures of poor prognosis. These data imply an absence of co-regulation of cyclins E1 and E2 during tumorigenesis and possibly different contributions to cancer progression. This is supported by *in vitro* data identifying divergent regulation of the two genes, as well as potentially different roles *in vivo*.

Introduction

Cyclin E1, the prototypic E-cyclin, was first described in 1991 [1], and has since been found to have crucial roles in cell proliferation and oncogenesis [2,3]. The second mammalian E-cyclin, cyclin E2, was identified in 1998 [4,5], and is largely regarded as being functionally redundant with cyclin E1 [2,3,6]. Cyclin E1 and cyclin E2 are encoded by different genes: cyclin E1 by CCNE1 at 19q12, and cyclin E2 by CCNE2 at 8q22.1. The cyclin E1 and cyclin E2 proteins display high sequence similarity (69.3% in *Homo sapiens*), and important functional motifs are conserved. These include domains for Cdk (cyclin dependent kinase) and Cdk inhibitor interaction, a nuclear localisation sequence and a centrosome localisation sequence (Figure 1). This high sequence conservation has supported a hypothesis of complete redundancy between the two proteins.

More recent data have identified instances of specific regulation or function for each E-cyclin. First, animal models hint that we have not fully delineated the roles of the E-cyclins, and cyclin E2^{-/-} mice display subtle phenotypes that may indicate key functional differences

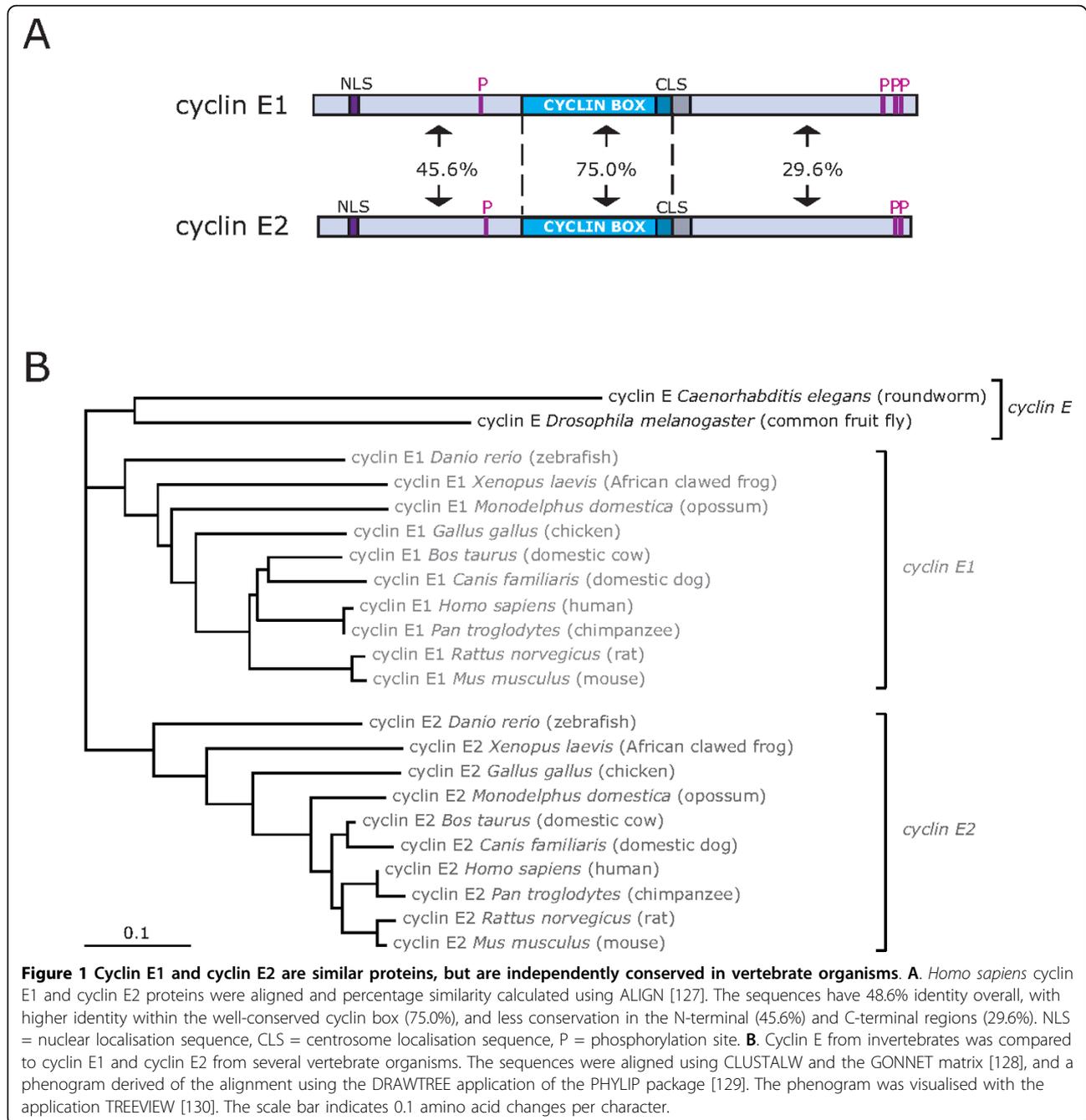
to cyclin E1. A second difference is that cyclins E1 and E2 can be regulated by distinct transcription factors and miRNAs. Finally, the expression of cyclin E1 and E2 is not always linked in cancer, and this discordance confirms that there are likely to be underlying functional and regulatory differences between the two proteins.

Known functions of the E-cyclins

The E-type cyclins activate the kinase Cdk2 that phosphorylates substrates including the retinoblastoma protein (Rb). Rb phosphorylation leads to the release of E2F transcription factors and initiation of S phase and DNA synthesis, by induction of expression of S phase proteins including histone proteins and cyclin A. Cyclin E-Cdk2 also directly phosphorylates proteins involved in centrosome duplication (NPM, CP110, Mps1), DNA synthesis (Cdt1), DNA repair (Brca1, Ku70), histone gene transcription (p220/NPAT, CBP/p300, HIRA) and Cdk inhibitors p21^{Waf1/Cip1} or p27^{Kip1} (reviewed in [2,3,7]). The specificity of cyclin-Cdk activity towards particular substrates is predominantly mediated via differences in cyclin sequence and periodic expression of cyclins during cell cycle phases, along with specific sub-cellular localisation [8,9]. Given that cyclins E1 and E2 are very similar in sequence and are both nuclear proteins [5,10],

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it seems probable that cyclin E1-Cdk2 and cyclin E2-Cdk2 phosphorylate a very similar subset of proteins so long as they exhibit the same periodicity of expression. There is considerable overlap even between cyclin E1-Cdk2 and cyclin A-Cdk2 targets [8]. Cyclin E1 can also activate Cdc2/Cdk1. In Cdc2 knockout mice, cyclin E1-Cdk2 kinase activity compensates for the absence of cyclin E1-Cdk2 activity to promote S phase entry [11]. Cyclin E1 also interacts with Cdc2 in the presence of Cdk2, which possibly contributes to S-phase entry in

mitotic cell cycles [11]. Both E-cyclins can also complex with Cdk3, although it is not known if this interaction is significant *in vivo* [5,12].

While a predominant function of the E-cyclins is to activate Cdk2, it has become apparent that there are crucial Cdk-independent roles (Figure 2). Cdk2^{-/-} mice are viable whereas cyclin E1^{-/-} E2^{-/-} mice are embryonic lethal [13], implying an essential Cdk-independent function for the E-cyclins. Furthermore, truncated variants of cyclin E1 that cannot bind Cdk2 are able to induce

malignant transformation [12], and oncogenesis has been associated with increased cyclin E1 in the absence of increased Cdk2 activity [12,14-16]. Subsequent to these studies, additional major roles for cyclin E1 have been established in the formation of pre-replication complexes on DNA, endocycling and centrosome duplication (Figure 2).

In order for DNA synthesis to occur after the transition from quiescence (G_0) into the cell cycle, it is necessary for the DNA replication complexes to be assembled *de novo* at the origins of replication. These pre-initiation replication complexes (pre-RC) consist of the pre-licensing factors Cdt1 and Cdc6 that associate with the origin-binding protein ORC, and the DNA replicative helicase components, MCM 2-7 [17]. Cyclin E1, independent of Cdk2 activity, associates with DNA near replication origins, and facilitates MCM loading at origins through direct interactions with MCM proteins and Cdt1 [18]. This process may also be important in endoreplication, where DNA is replicated without cell division. Endoreplication results in polyploid cells that have essential functions in development, cell differentiation

and as an energy reserve [19]. E-cyclins are crucial for endocycling, with the absence of E-cyclins leading to reduced DNA copy number and mortality due to failures in the polyploid giant trophoblast cells of the placenta [13,20]. Like diploid cells, endocycling cells require pre-RC assembly at origins of replication. While the precise function of the E-cyclins in endocycling is not established, in *Drosophila* cyclin E recruits MCM2 to the DNA during early endocycles of polytene cells of the salivary gland [21], implying that E-cyclins also function in pre-RC formation in these cells.

A 20 amino acid centrosome localisation sequence (CLS) in cyclin E1 targets this protein to the centrosome [22]. Cyclin E1 overexpression increases the proportion of cells in S phase by a mechanism that is dependent upon this region, but independent of Cdk2-binding [22]. The increase in the proportion of S phase cells may reflect a lengthening of S phase, rather than an increase in proliferation *per se* [23]. In fact, the cyclin E1 CLS is responsible for co-localising MCM5 to the centrosome, where its presence inhibits centrosome over-duplication and proliferation [24]. Consequently the CLS may

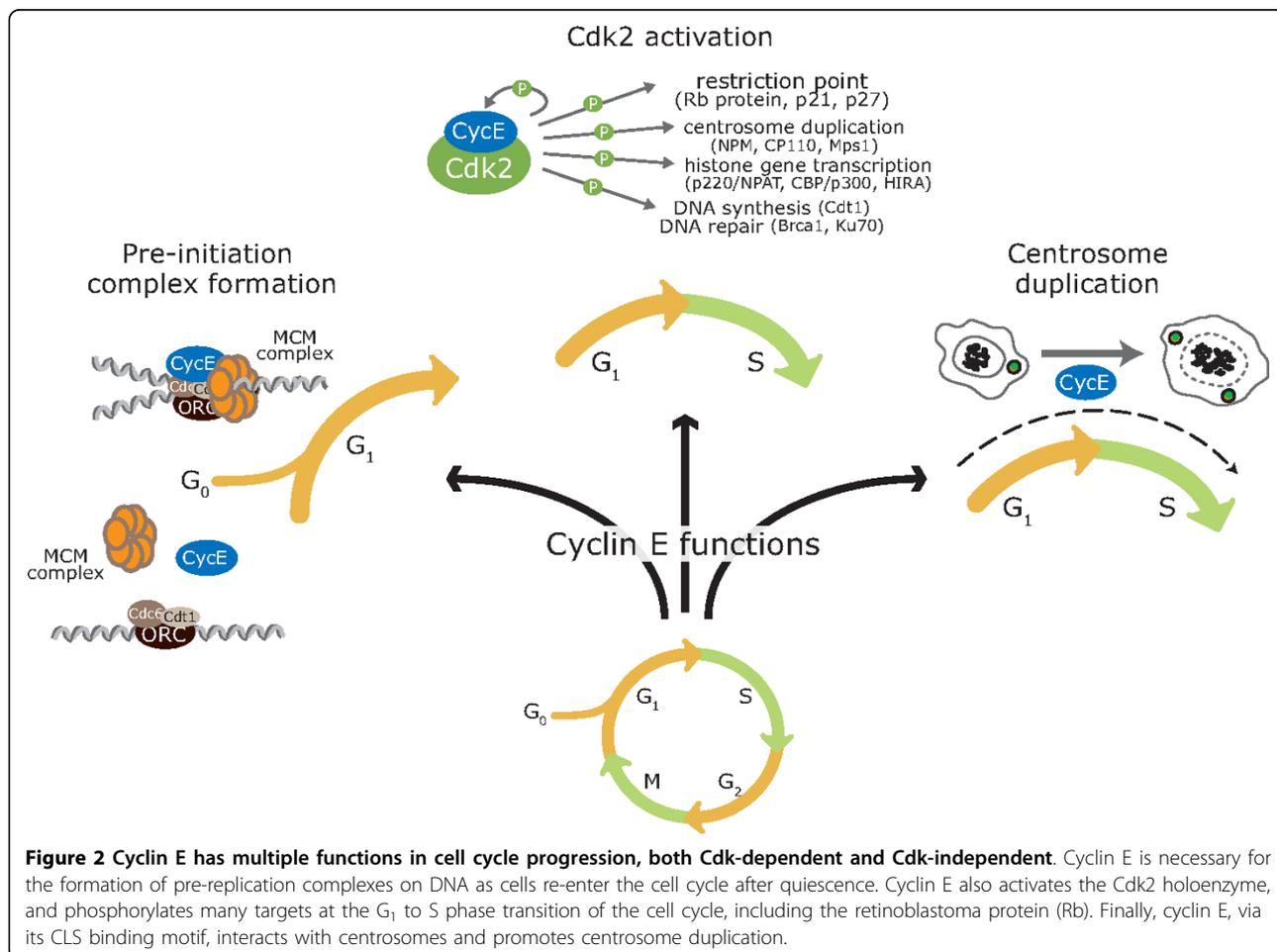


Figure 2 Cyclin E has multiple functions in cell cycle progression, both Cdk-dependent and Cdk-independent. Cyclin E is necessary for the formation of pre-replication complexes on DNA as cells re-enter the cell cycle after quiescence. Cyclin E also activates the Cdk2 holoenzyme, and phosphorylates many targets at the G_1 to S phase transition of the cell cycle, including the retinoblastoma protein (Rb). Finally, cyclin E, via its CLS binding motif, interacts with centrosomes and promotes centrosome duplication.

function as a coordinating link between centrosome duplication and pre-RC formation [24]. Cdk2 activity is also synchronised with these events, as cyclin E1-Cdk2 phosphorylates nucleophosmin, CP110 and Mps1, promoting centrosome duplication [2,3,7].

The non-Cdk functions of the E-cyclins have been investigated using cyclin E1, and have been presumed to be identical for cyclin E2 [18,22]. The CLS motif is well conserved between cyclin E1 and cyclin E2, implying that cyclin E2 would be functionally active at the centrosome [18,22]. Cyclin E1^{-/-} E2^{-/-} mice are embryonic lethal whereas the individual knockout mice have largely normal phenotypes, which supports an assumption that either cyclin E1 or E2 can fulfil all of the functions of the E-cyclins. Despite this apparent redundancy, a careful consideration of animal models leads us to question whether cyclin E1 and cyclin E2 are true homologs.

E-cyclins in animal models

Non-canonical functions of "Cyclin E" in developmental models

Both E-cyclins are expressed in vertebrates, whereas invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans* each have only one "cyclin E", which is essential for viability [25,26]. Cyclin E1, but not cyclin E2, is maternally expressed in *Xenopus laevis* embryogenesis, such that cyclin E1 is the only E-type cyclin during early embryonic cell cycles. The presence of a single E-cyclin has allowed for sophisticated studies of E-cyclins in these organisms that are not confounded by functional compensation between the E-cyclins. Consequently some of the major roles of E-cyclins, including activation of Cdk2 during the G₁ to S phase transition [26], endoreplication [27], and formation of pre-RCs [21,28], were identified very early in these models.

Studies in these organisms have also hinted that E-type cyclins may have roles in addition to those described in pre-RC formation, Cdk activation and centrosome biology. In embryonic cell cycles that lack G₁ and G₂ phases, E-cyclins are expressed throughout the cell cycle and particularly during S phase [29], and this is associated with high Cdk2 activity [30], and normal progression through S phase. By contrast, in the somatic cell cycles of mammalian cells, the expression of the E-cyclins is confined to a window between late G₁ and early S phase [4,5,10], and the degradation of cyclin E1 during S phase is a pre-requisite for mitosis and entry into the next cell cycle [23,31]. Cyclin E1-Cdk2 activity is particularly high on the mitotic chromosomes of cells in early *Xenopus* embryos, which suggests that cyclin E may be promoting pre-RC assembly directly after mitosis [32]. An alternative explanation is that cyclin E may facilitate DNA replication fork movement in certain circumstances. A *Drosophila* mutant has been identified in

which a mutation in the cyclin E gene increases replication fork movement in polytene chromosomes [33]. Cyclin E1 accumulates on chromatin during S phase in *Xenopus* extracts [28], potentially with a role in Cdk2-mediated chromatin decondensation for replication fork movement [34].

Sustained expression of cyclin E appears to be associated with mitosis rather than meiosis of embryonic cells. Cyclin E expression is translationally repressed during prophase of *C. elegans* gonadal cells [35]. The maintenance of cyclin E expression in these cells actively promotes mitotic division and embryonic gene expression rather than meiosis, and leads to the development of teratomas [35]. A possible explanation for this phenotype is that high cyclin E leads to precocious centrosome assembly, causing exit from meiosis [35].

Cyclin E is also essential in cell fate determination during *Drosophila* neurogenesis, where its expression drives the asymmetric division of neuroblasts into two lineages of glial and neuronal cells [36]. In the absence of cyclin E expression, neuroblasts only produce glial cells rather than the neuronal precursor [36]. This function does not require the interaction of cyclin E with Cdk2, and is mediated via binding and inhibiting the homeobox transcription factor, Prospero [37]. Likewise, in *C. elegans*, cyclin E functions in maintaining stem cell capacity by suppressing the terminal differentiation of quiescent cells, although in combination with Cdk2 [38].

Cyclins E1 and E2 have distinct roles in *Xenopus* development, where cyclin E2, but not cyclin E1, is necessary for viability [39]. This may reflect a dose requirement for E-type cyclins, as their expression in the developing zygote is sequential, with cyclin E1 being maternally expressed from fertilisation to blastula stage, and cyclin E2 expressed at high levels from blastula to tadpole. However, cyclin E1 knockdown at fertilisation does not affect development [39,40] while cyclin E2 knockdown shows a dose dependent effect on viability [39]. Incidentally, the cell cycles prior to the mid blastula transition in *Xenopus* are rapid embryonic cycles where S and M alternate without variation in cyclin E1 levels, whereas the subsequent cycles, with high cyclin E2 expression, have incorporated G₁ and G₂ phases [41].

Knockout mouse models of cyclins E1 and E2

The *Drosophila* and *C. elegans* cyclin E is phylogenetically equidistant to cyclin E1 and cyclin E2 (Figure 1B) and consequently neither cyclin E1 nor cyclin E2 is likely to be the functionally equivalent ortholog to "cyclin E". It has not been established whether cyclin E1 or cyclin E2 are involved in early embryonic cell divisions or asymmetric differentiation in mammals as described above for *Drosophila*, *C. elegans* and *Xenopus*. Mouse embryonic stem cells proliferate in the absence

of cyclin E1 or E2 [13,20], so perhaps an E-cyclin is required for the post-embryonic cell cycles, but cyclin A can substitute effectively in embryonic cell cycles which lack p21^{Waf1/Cip1} and p27^{Kip1} [20]. These questions can be most effectively addressed by performing cyclin E1 knockin to the cyclin E2 locus, and vice versa.

The phenotype of single knockout mice has yielded clues about E-cyclin function in endoreplication and meiosis (Table 1). Double knockout cyclin E1^{-/-} E2^{-/-} mice die *in utero* due to impairments in endoreplication of the trophoblast giant cells (TSCs) that form the placenta, and perform a crucial role in placental attachment and provision of nutrients to the developing embryo. TSCs normally undergo multiple rounds of DNA replication without mitosis that increase their DNA content to 1000N. TSCs of cyclin E1^{-/-} E2^{-/-} mice barely reach a ploidy of 8N, even with prolonged culture, although they still increase in size and express markers of differentiation consistent with TSC development [20]. Conversely, in Fbw7 knockout mice, which fail to degrade cyclin E1, high cyclin E1 expression is associated with increased DNA synthesis in TSC cells [42]. Another polyploid cell type, the megakaryocyte, similarly fails to reduplicate DNA in cyclin E1^{-/-} E2^{-/-} knockout mice [13]. In the initial studies of this phenomenon no abnormal phenotype of polyploid tissues was detected in single E-cyclin knockout mice [13,20]. However, the E-cyclins are differentially regulated during TSC endoreplication, with cyclin E1 levels declining while cyclin E2 levels remain steady [20]. Cyclin E2 mRNA is also more highly expressed in the polyploid cells of the liver, hepatocytes, where it is found at higher levels in hepatocytes with 8N DNA content than those with 4N DNA content [43]. Together these data suggest that cyclin E1 and cyclin E2 levels may have different roles in endoreplication.

A recent study by Nevzorova *et al* using partial hepatectomy of cyclin E1^{-/-} and cyclin E2^{-/-} knockout mice has shed further light on this subject [44]. Partial hepatectomy of mice leads to a rapid expansion of the polyploid hepatocyte population to regenerate the liver. In cyclin E1^{-/-} mice, this regenerative response was slightly delayed, and associated with a compensatory increase in cyclin A-Cdk2 activity [44]. Surprisingly, cyclin E2^{-/-} mice had accelerated liver regeneration and increased DNA synthesis associated with an upregulation of cyclin E1 expression and cyclin E1-Cdk2 activity [44]. Consequently it appears that cyclin E2 normally acts to repress cyclin E1 expression, thus negatively regulating S phase entry in hepatocytes. The ablation of cyclin E2 leads to increased polyploidy, associated with increased cyclin E1-Cdk2 activity [44]. Thus high cyclin E1 may induce endoreplication, whereas cyclin E2 acts as a brake in this process.

These results are distinct from those observed in the other polyploid cell types, TSCs and megakaryocytes, where cyclin E1 and E2 single knockout mice were reported to have “normal” phenotypes. However the morphology and Cdk activity of these tissues has not been explicitly reported in single knockout mice [13,20], so it may be that similar substitutions are occurring in these tissues, where cyclin A is functional in the absence of cyclin E1, and cyclin E1 levels are increased in the absence of cyclin E2.

A further unique phenotype of the cyclin E2 knockout mice is testicular atrophy and reduced male fertility, associated with aberrant meiosis [13]. Could this phenotype be due to low cyclin E2 expression increasing cyclin E1 levels as described for the regenerating liver? This seems unlikely, as cyclin E1 is already expressed at high levels in the mouse and human testes [5,45], but only cyclin E2 deletion results in a phenotype [13,20]. In addition, cyclin E1^{+/-} E2^{-/-} mice display more pronounced testicular hypoplasia and male infertility than cyclin E2^{-/-} mice, indicating that it is unlikely that excess cyclin E1 causes this phenotype, as the phenotype is more severe when a cyclin E1 allele is removed [13]. Meiosis in *C. elegans* specifically requires periodic cyclin E expression [35], so there may be a particular role for cyclin E2 in the meiosis-mitosis switch in mammals. The overexpression of a hyperstable form of cyclin E1 that is not periodically degraded does not lead to altered fertility in mice [46], but this has not been examined in the context of cyclin E2. Of interest, cyclin E2, but not cyclin E1, is upregulated by the p110 isoform of the transcription factor CDP/Cux [47] and CDP/Cux knockout mice also suffer from male infertility, although without testicular atrophy [48].

Transcriptional Regulation of the E-cyclins

The E-cyclins are cell cycle regulated at both the mRNA and protein level, leading to cell cycle phase-specific expression. Cyclin E1 and E2 mRNA (CCNE1 and CCNE2) peak in mid-G₁ to early S phase in multiple models of mitotic division [4,5,10]. During S phase cyclin E1 is rapidly downregulated via proteosomal degradation (for reviews of cyclin E1 proteosomal degradation see [2,49]). In brief, during early S phase cyclin E1 is phosphorylated at conserved residues via Cdk2 and GSK-3 β , leading to recognition and ubiquitination of cyclin E1 by the ubiquitin ligase SCF^{Fbw7}, followed by degradation during S phase [2,49]. The turnover of cyclin E2 is reported to be regulated in a similar manner [50] but has been examined in less detail. Cyclin E1 turnover can also be mediated by ubiquitin ligase components Skp2 [51], Parkin [52] and Cul4 [53], which may contribute to late S-phase degradation of cyclin E1. The combination of transcriptional

Table 1 Attributes of the E-cyclins

	Cyclin E1 (CCNE1)	Cyclin E2 (CCNE2)
Chromosomal location	19q12	8q22.1
Isoforms	2 (Full length and 15 amino acid N-terminal truncation)	1
Transcriptional regulation	<p><i>Upregulated by:</i> E2F1, E2F2, E2F3 [55-57] P300/CBP [58], Src3 [59], Carm1 [60]</p> <p><i>Suppressed by:</i> HDAC1 [58,62,63], SUV39H1/HP1 [64,65], BRG1/hBRM [66], PRMT5/COPR5 [68-71]</p>	<p><i>Upregulated by:</i> E2F1, E2F2, E2F3 [55-57] P300/CBP*, Src3 [61], Carm1 [61]</p> <p>Chd8 [74,75]</p> <p>CDP/Cux p110 [47]</p> <p><i>Suppressed by:</i> HDAC1*, SUV39H1/HP1*, BRG1/hBRM*, PRMT5/COPR5 [68-71]</p>
miRNA regulation	miR15b [86], miRNA 16 family [87]	miR-9, miR-34c and miR200a [88], miR34a [89], miR26a [90]
Post-translational cleavage	Yes [98]	No [99]
Tissue expression	<p><i>Embryonic cells</i></p> <ul style="list-style-type: none"> - Very high in mouse embryonic stem cells [45] - Sole E-cyclin from fertilisation to blastula stage in <i>Xenopus</i> [39] <p><i>Adult tissues</i></p> <ul style="list-style-type: none"> - Mouse: moderate in brain, testes and thymus, low in intestine and spleen [45] - Human: very high in placenta, high in testes, low to moderate in thymus, small intestine and colon [5] <p><i>Polyploid cells</i></p> <ul style="list-style-type: none"> - Low expression in mature trophoblast giant cells [20] - Low expression compared to CCNE2 in hepatocytes [44] 	<p><i>Embryonic cells</i></p> <ul style="list-style-type: none"> - Absent in mouse embryonic stem cells [45] - Not expressed during embryonic cycles of <i>Xenopus</i> [39] <p><i>Adult tissues</i></p> <ul style="list-style-type: none"> - Mouse: high in testes, low to moderate in brain, intestine, muscle and thymus [45] - Human: high in brain, placenta, testes and thymus, low to moderate in spleen, thymus, small intestine and colon [5] <p><i>Polyploid cells</i></p> <ul style="list-style-type: none"> - Sustained expression in mature trophoblast giant cells [20] - High expression compared to CCNE1 in hepatocytes [44], increased expression with increased polyploidy [43]
Knockout mouse	Normal fertility [13,20] Slight delay in liver regeneration following partial hepatectomy [44]	Male infertility and testicular atrophy [13,20] Accelerated liver regeneration and increased hepatocyte polyploidy following partial hepatectomy [44]

* by inference from data on CCNE1

and post-translational regulation results in an intense peak in expression of cyclin E1 in late G₁ and early S phase of the cell cycle. The activity of cyclin E1-Cdk2 and cyclin E2-Cdk2 complexes is further refined through binding of the Cdk inhibitors p21^{Waf1/Cip1} and p27^{Kip1}, whose expression is also cell cycle regulated.

The cell cycle dependent transcription of the E-cyclins is mediated by E2F transcription factors, which are activated via release from Rb during late G₁ phase. Rb-deficient cells have high expression of cyclin E1 and cyclin E2 [45,54], likely due to constitutive E2F release, and numerous gene expression array studies have confirmed both CCNE1 and CCNE2 as E2F1, E2F2 and E2F3 target genes [55-57]. E2F proteins interact with multiple co-regulators at the E-cyclin promoters, allowing for the input of mitogenic signals. E2F1 recruits the histone acetylase p300/CBP [58] and the co-activator SRC3 [59] to the CCNE1 promoter. This complex further recruits the protein methyltransferase Carm1/PRMT4 to the CCNE1 and CCNE2 promoters [60], leading to increased transcription of at least the CCNE1 gene [60,61]. CCNE1

transcription is actively inhibited by Rb and the other pocket proteins in G₀ and G₁ arrested cells [58,60,62-66] and during late mitosis [67]. Rb, via an interaction with inhibitory E2Fs (E2F4-6) recruits the histone deacetylase HDAC1 [58,62,63], the methylation complex SUV39H1 and HP1 [64,65] and the BRG1/hBRM nucleosome remodeling complex [66] to the CCNE1 promoter, leading to inhibitory deacetylation, methylation and remodelling of the promoter-associated nucleosomes. The methyltransferase PRMT5, via binding partner COPR5 [68,69], also negatively regulates CCNE1 and CCNE2 transcription [70,71], especially during G₀ [60].

Differential transcription of cyclin E1 and E2

CCNE1 regulation has been more closely characterised than CCNE2, with an assumption of similar regulation of the CCNE2 gene [3]. However, CCNE2 appears to be inherently more sensitive to induction by E2F transcription factors than CCNE1, with CCNE2 mRNA showing a 1.5-10 fold greater induction than CCNE1 in 3 separate studies [55-57]. The CCNE2 promoter also shows

greater enrichment for E2F binding by chromatin immunoprecipitation [72]. Furthermore, overexpression of a mutant E2F that derepresses but does not activate transcription, significantly induces CCNE2 but not CCNE1 [57]. Since E2F activity is a core component of cell cycle progression, this suggests that CCNE2 expression may be more strongly amplified than CCNE1 in each cell cycle. Interestingly, cyclin E2 becomes expressed at high levels in immortalised mouse embryonic fibroblasts derived from E2F1 knockout animals, and is also expressed at high levels in chemically-induced tumours derived from the same mice, without concurrent changes to cyclin E1 expression [73]. Consequently cyclin E2 may be targeted independently of E2F factors, or at least E2F1.

Two instances have been identified where CCNE2, independently of CCNE1, is markedly upregulated by E2F binding partners. Chd8, a chromatin remodelling enzyme, facilitates efficient RNA polymerase II transcript elongation of a subset of genes, including E2F1 targets. While Chd8 can interact with E2F at the promoters of both CCNE1 and CCNE2, its presence leads only to the upregulation of cyclin E2 [74]. Chd8 binds constitutively to the CCNE2 promoter throughout the cell cycle, but it is required for the upregulation of cyclin E2 during estrogen rescue from anti-estrogen induced quiescence [75], and as cells pass through the G₁/S-phase transition [74]. Rodriguez-Paredes *et al* propose that Chd8 is recruited to all E2F1-dependent genes, but that only those genes with a specific chromatin structure at the 5' region, such as CCNE2, utilise Chd8 to mobilise RNA polymerase II and nucleosomes during transcript elongation [74]. A distinct chromatin structure may explain why CCNE2 is inherently more sensitive to E2F induction than CCNE1 as described above. Another E2F1 co-activator, CDP/Cux p110, [76] also specifically upregulates CCNE2 without alterations to CCNE1 [47], although binding to the CCNE2 promoter was not demonstrated.

Through their independent regulation by transcription factors, cyclin E1 and cyclin E2 are associated with different networks of genes and thus potentially with distinct biological processes. Cyclin E2 is upregulated via Chd8 downstream of cyclin D1 in estrogen-treated cells [75], and has also been identified as downstream of cyclin D1, or cyclin D1-mediated pathways, in other models [77-79]. Cyclin E1 is often expressed at high levels in the absence of increased cyclin D1 [80], and in fact cyclin E1-Cdk2 activity is increased by estrogen in breast cancer cells primarily through disengagement of p21^{Waf1/Cip1} rather than transcriptional upregulation by cyclin D1 [75]. In this same model, c-Myc is able to induce cell cycle re-entry through the induction of cyclin E1-Cdk2 activity, but without significantly

increasing the expression of cyclin E2 [75]. Thus cyclin E1 and cyclin E2 are distinctly regulated downstream of estrogen through the major regulatory proteins, cyclin D1 and c-Myc [75]. This action may not be confined to estrogen, as cyclin E2 is also induced by androgen, likely downstream of cyclin D1 or D3, although this is not independent of cyclin E1 upregulation [81].

The disparate tissue expression pattern of cyclin E1 and cyclin E2 also suggests that there is differential regulation of the E-cyclins [5,45]. For example, cyclin E2 is expressed at high levels in human brain where cyclin E1 is notably absent [5], whereas cyclin E1 expression is consistently higher than cyclin E2 in the thymus [5,45]. The inhibition of cyclin E1 transcription by cyclin E2 identified in hepatocytes may contribute to the differential expression of the E-cyclins in some tissues [44]. We have made a similar observation in the breast cancer cell line MCF-7 that cyclin E2 knockdown leads to an increase in cyclin E1 protein levels, although this is associated with decreased overall proliferation [75]. However, cyclin E2 modulation does not always lead to changes in cyclin E1 expression, for example in smooth muscle cells the cyclin E2 siRNA treatment leads to downregulation of cyclin E1 [82], and cyclin E1 and E2 are co-expressed in other tissues [5,45], and in some tumours [83].

Post-transcriptional regulation of cyclin E1 and E2

Another layer of complexity is added through the regulation of the E-cyclins by non-coding RNAs. Despite high conservation, the mRNA sequences of CCNE1 and CCNE2 are predicted and validated targets of distinct subsets of microRNAs (miRNAs) [84,85]. For example CCNE1 is targeted by miR15b [86] and the miRNA 16 family [87], and CCNE2 by miR-9, miR-34c, miR-200a [88], and miR34a [89]. miR-26a specifically targets CCNE2 but not CCNE1 [90]. There is an expressed anti-sense transcript which may further modulate CCNE2 expression [91].

miRNAs frequently target gene networks to alter cellular processes such as proliferation, which raises the question why CCNE1 and CCNE2 appear to be targeted as part of discrete regulatory modules if they are functionally redundant proteins. These modules target distinct subsets of cell cycle proteins and may therefore have subtly different effects on proliferation, for example, mir16 co-represses CCNE1, CCND3 and CDC6 [87]. CCNE2 is independently downregulated by the p53-regulated miRNA, miR34a, in colon cancer cells [89]. This may explain why cyclin E2 mRNA and protein, but not cyclin E1, is induced after viral oncoprotein E6 induces degradation of p53 in normal human fibroblasts [5]. Furthermore p53 expression suppresses CCNE2 in prostate cancer cells [92], and the

inactivation of p53 in the mammary gland leads to tumours which are high in CCNE2 [93]. Consequently CCNE2 is frequently suppressed downstream of the p53 tumour suppressor gene, linking CCNE2 to a network of p53 activity independently of CCNE1 [89]. The miRNAs that regulate CCNE1 and CCNE2, as well as the transcription factors discussed above, are often altered in tumorigenesis, which may contribute to the distinct associations of CCNE1 and CCNE2 to different cancer types, as described below.

Associations of cyclin E1 and cyclin E2 with cancer

Cyclin E1 is a well established oncogene, and its overexpression, especially in a hyperstable form, leads to increased incidence of mouse neoplasia [94-96], and increased susceptibility to other oncogenes [96]. Potential oncogenic effects of cyclin E2 have not been examined in mice, except that mouse embryonic fibroblasts from the E-cyclin double knockout mice are not susceptible to transformation [13]. Further evidence from cell culture models indicates that cyclin E2 has similar proliferative effects to cyclin E1 in cancer cells. The overexpression of either cyclin E1 or cyclin E2 leads to a redistribution of cells within cell cycle phases, with a shorter G₁ [5], and a longer S phase at least in cyclin E1 overexpressing cells [23,31]. The siRNA-mediated decrease of either E-cyclin severely attenuates the estrogen-induced proliferation of breast cancer cells [75], and the reduction of either cyclin E1 or E2 leads to reduced colony forming ability in oral squamous cell carcinoma cells, although cyclin E1 ablation is more potent than ablation of cyclin E2 (70% vs 20% reduction) [97]. Cyclin E1 may have higher potency than cyclin E2 as it can be cleaved into low molecular weight fragments with enhanced oncogenic activity [98], and cyclin E2 does not appear to be similarly processed [99].

While cyclin E1-Cdk2 and cyclin E2-Cdk2 kinase activities are increased in breast cancer compared to normal tissue [83], cyclin E1 and E2 expression and kinase activity are not essential for proliferation of all cancer cell types [97]. In some cases increases in cyclin E2 expression are not associated with proliferation, but instead with other oncogenic attributes such as invasion [100] and drug resistance [101,102]. Cyclin E1 may enhance tumorigenesis through increasing genomic instability, an ability which may be derived from promoting premature replication licensing and endoreplication [96,103]. Specific experiments have not been reported that examine whether genomic instability is also induced through cyclin E2 overexpression. Given that recent evidence seems to identify cyclin E1, but not cyclin E2, as a mediator of endoreplication in

hepatocytes [44], cyclin E1 and E2 may not have equivalent roles in the induction of genomic instability.

Due to the limited availability of antibodies suitable for immunohistochemistry, the expression of cyclin E2 has been examined only through mRNA expression in tumour samples, whereas there is a comprehensive array of literature on the expression of both cyclin E1 protein and mRNA in cancer samples [2,104]. CCNE1 and CCNE2 expression has been compared in breast cancer, where these studies are representative of the majority of studies on the relationship of CCNE1 to breast cancer [104]. Both CCNE1 and CCNE2 are expressed at higher levels in breast cancer, with an association of both genes to increased tumour grade [105,106], estrogen receptor (ER) negative status [105,106], progesterone receptor negative status [106], and proliferative index by Ki67 staining [83,105]. High levels of CCNE1 have a more significant relationship than CCNE2 with each of these parameters. Both CCNE1 and CCNE2 have an inverse linear relationship between expression and metastasis-free survival, which is particularly strong for CCNE2 [107], and high expression of each E-cyclin mRNA also predicts poor overall survival [106] and shorter relapse-free interval [105]. One study also found some correlation between the expression of CCNE1 and CCNE2 [83], although each E-cyclin is independently correlated to poor overall and metastasis-free survival [106]. CCNE2 is also a component of three prognostic gene expression signatures that predict shorter metastasis-free survival or relapse-free survival of breast cancer patients, whereas CCNE1 does not feature in any of these signatures [108-110].

Differences become apparent between CCNE1 and CCNE2 in their relationship to survival and response to therapy of ER positive and anti-estrogen (tamoxifen) treated patients. High CCNE1 expression predicts shorter metastasis-free survival in both ER negative and ER positive patients, whereas CCNE2 expression is only predictive for the ER positive patient subset [105,106]. By contrast, in primary tumours high levels of CCNE1, but not CCNE2, predict a shorter relapse-free interval of tamoxifen-treated patients [105]. However CCNE2 has been detected at high levels in recurrent disease after tamoxifen treatment [111], hinting at a functional, if not prognostic, role. In tamoxifen resistant cell lines, CCNE2, but not CCNE1, is induced as part of the agonist response to tamoxifen [112]. Anti-estrogen resistance in MCF-7 breast cancer cells conferred by the TNF α inhibitor, A20, is also associated with increases in cyclin E2 expression [113]. This is consistent with studies in estrogen-responsive breast cancer cell lines where cyclin E2 is a highly estrogen responsive target, whereas cyclin E1 is only marginally increased [75,114].

CCNE1 is expressed at high levels in multiple tumour types other than breast [2]. CCNE2 shows moderate increases in expression in various malignancies, such as lung, ovarian, nasopharyngeal, colorectal, non small cell lung cancer and leukaemia [83,88,115-117], and these increases in expression are frequently not correlated to CCNE1 expression [83]. In two studies the expression of CCNE2 is lower in ovarian and non small cell lung tumours than matched controls, whereas CCNE1 expression remains unchanged or increases [118,119]. Two further studies indicate that CCNE2 is expressed at significant levels in recurrent disease, including therapy-related myeloid leukemia [120] and recurrent non small cell lung carcinoma [121]. These data reflect the more comprehensive data collected with respect to breast cancer, where CCNE2 expression is frequently upregulated in tumours independently of CCNE1, and CCNE2 is often detected in recurrent disease.

Concluding remarks

Cyclin E1 and E2 display largely overlapping functions, and high redundancy. Does the presence of two E-cyclins provide a safety net to ensure proliferative potential, or do they have unique functions? The E-cyclin gene pair has been maintained without significant divergence throughout the evolution of higher eukaryotes, implying a selective pressure for the maintenance of each gene. Genome wide studies in yeast have found that apparently redundant paralogs do in fact have distinct and non-overlapping functions, although these only become evident in circumstances of stress or particular stimuli [122]. This is certainly the case for cyclin E1 and E2, where cyclin E1 promotes hepatocyte endoreplication after liver injury, whereas cyclin E2 suppresses this process [44]. Further differences between cyclin E1 and cyclin E2 are possibly found during meiosis and embryogenesis [13,39]. There are tantalising observations on the activity of cyclin E in *Drosophila* and *C. elegans* which suggest that there may be additional functions for the E-cyclins in DNA replication of embryonic cells and lineage determination of stem cells. Consequently the differences between cyclin E1 and E2 may be more apparent in non-mitotic cell cycles than in normally proliferating cells, which may explain their apparent redundancy in common laboratory model systems.

Cancer cell cycles represent another form of aberrant cell division, often compared to embryonic cell cycles, and cyclins E1 and E2 promote oncogenic transformation and to promote cancer cell proliferation [13]. Data from cancer studies have already identified that cyclin E1 and E2 are frequently not co-expressed in tumours, and may have distinct associations with recurrent

disease. A likely explanation for the discordant expression of cyclin E1 and E2 in cancer is their regulation by distinct subsets of transcription factors and miRNAs. The expression of cyclin E2, independently of cyclin E1, can be induced via cyclin D1, Chd8 and CDP/Cux, all of which are upregulated in cancers [47,123], and cyclin E2 is also independently suppressed by the tumour suppressor p53 [89]. The co-regulation of cyclin E1 and cyclin E2 with distinct gene sets could explain the different relationship of each gene to disease. For example, cyclin E2, which is a target of cyclin D1 and Chd8 in estrogen-responsive cells [75], has been associated with poor outcome only in ER-positive breast cancers [106], which resembles the association of cyclin D1 overexpression to ER-positive but not ER-negative breast cancers [124]. The functional outcome of overexpression of cyclin E1 compared to cyclin E2 is not known. However, given the potential differences in function in endoreplication and other processes, cyclin E1 and cyclin E2 may have distinct attributes that contribute to cancer progression.

The cyclin and Cdk proteins of the cell cycle have considerable redundancy and compensation between members of each family [125], yet careful study reveals unique functions of many of these proteins. For example, cyclin D2 knockin cannot completely substitute for cyclin D1 in mouse development [126]. Recent data on the function and regulation of cyclin E1 and cyclin E2 demonstrates that they are also not redundant homologs (Table 1). The identification of cyclin E1 as promoting, and cyclin E2 as repressing, hepatocyte endoreplication deserves further investigation to unravel the mechanistic difference between the two proteins. Cyclin E1 and E2 should also be investigated as distinct entities in cancer studies, especially in the context of examining relationships with other markers of tumorigenesis such as cyclin D1 and p53. There will be difficulties in ongoing studies on cyclin E1 and cyclin E2 as they do have considerable functional redundancy in their interactions with Cdk2 and Cdk inhibitor proteins, as well as having the potential to regulate one another. The identification of further differences between the E-cyclins is likely to require an appropriate molecular environment that highlights their differences, such as during endoreplication or the proliferation of cancer cells.

Abbreviations

CCNE1: Cyclin E1 mRNA; CCNE2: Cyclin E2 mRNA; Cdk: cyclin dependent kinase; CLS: centrosome localisation sequence; ER: estrogen receptor; miRNA: microRNA; Pre-RC: pre replication complex; TSC: trophoblast giant cell.

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Authors' contributions

CC and EM drafted the manuscript. Both authors read and approved the final manuscript.

Competing interests

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