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APC^{cdh1} activity in mouse oocytes prevents entry into the first meiotic division

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Summary

Fully-grown mammalian oocytes maintain a prophase I, germinal-vesicle stage arrest in the ovary for extended periods before a mid-cycle luteinizing surge induces entry into the first meiotic division. Cdh1 is an activator of the Anaphase-Promoting Complex (APC), and APC^{cdh1} is normally restricted to late M - early G1 of the cell cycle. Here we find that APC^{cdh1} is active in mouse oocytes and is necessary to maintain prophase arrest.

Fully-grown mammalian oocytes remain arrested at prophase I within antral follicles until stimulated to enter the first meiotic division by a mid-cycle surge in luteinising hormone. An oolemma receptor maintains this arrest by raising protein kinase A activity¹ which inhibits Maturation-Promoting Factor (CDK1-cyclin B1) by affecting the phosphorylation status of CDK12. Oocytes can resume meiosis spontaneously, manifest by germinal vesicle breakdown (GVB), when released into culture media, but remain arrested if agents such as the phosphodiesterase inhibitor milrinone³, are added to maintain protein kinase A.

Raising cyclin B1 levels in milrinone-arrested oocytes by microinjection of its cRNA coupled to GFP induced GVB. Spatially the cyclin B1-GFP expressed in oocytes mirrored the distribution reported in adult cells⁴ (Supplementary Information, Fig S1a). Cytoplasmic cyclin B1 entered the nucleus before GVB and became associated with chromatin afterwards. However, the GVB rate in these oocytes was <15% by 5 h (Fig 1a), and never exceeded 20%, even after 24 h. The proteasomal inhibitor MG132 had a mild stimulatory effect on GVB over 5 h, and when combined with cyclin B1 the rate GVB increased 2-3 fold compared to cyclin B1 alone (Fig 1a and see Supplementary Information, Table S1). The increased rate of GVB was likely caused by increased cyclin B1-GFP since levels doubled with MG132 (Fig 1b).

Cyclin B1 degradation requires polyubiquitination by the Anaphase-Promoting Complex (APC) followed by proteasomal degradation⁵. In mitosis, the APC needs one of two essential co-activators, cdc20 and cdh1, which are both present in mouse eggs⁶. APC^{cdc20} and APC^{cdh1} both degrade substrates such as cyclin B1 that contain a Destruction-(D)-box. Therefore we repeated the above cyclin B1 experiment using Δ 90-cyclin B1, an N-terminal truncation which removes the D-box⁷. Δ 90-cyclin B1 cRNA induced 70% GVB rates by 5 h (Fig 1a), and 80% by 24 h; rates that are 4-5 fold higher than cyclin B1-GFP and with the MG132 data are consistent with cyclin B1 being degraded in GV oocytes.

Cyclin B1 degradation in oocytes, where MPF is low, is likely due to APC^{cdh1} because APC^{cdc20} requires high MPF levels for activity⁸. Oocytes do contain cdh1 (see

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Supplementary Information, Fig S1b) therefore to examine if APC^{cdh1} was active at this time, in addition to cyclin B1, we coupled two further APC^{cdh1} substrates to GFP, injected their cRNA and measured their stability following protein synthesis inhibition. We used *cdc20* itself and a mutant form of securin (securin^{dm}) in which its D-Box has been mutated. Both constructs are degraded only by APC^{cdh1}, and not APC^{cdc20}, by virtue of a KEN-box 7,9.

All three APC^{cdh1} substrates, but not a GFP control, were degraded (Fig 1c and see Supplementary Information, Table S1). We could also observe degradation of endogenous cyclin B1 during the same time period suggesting it is not an artefact of very high protein levels (Supplementary Information, Fig S1e). Consistent with destruction of these proteins through the action of a ubiquitin ligase, *cdc20*-GFP loss was blocked by methylubiquitin, which terminates polyubiquitination, and MG132 (see Supplementary Information, Table S1).

To rule out *cdh1*-independent mechanisms for their degradation we examined the ability of these substrates to be degraded in oocytes knocked-down for *cdh1*. Microinjection of *cdh1* morpholino (^{MO}*cdh1*) for 24 h reduced *cdh1* levels by about 90% (see Supplementary Information, Fig S1c) and inhibited degradation of the APC^{cdh1} substrates (Fig 1c and see Supplementary Information, Table S1). We confirmed the specificity of these observations to *cdh1* knockdown by two approaches. First, we observed that cyclin B1-GFP degradation proceeded normally in oocytes incubated for 24 h with a control morpholino (*inv*^{MO}*cdh1*) which is an inverted base sequence of ^{MO}*cdh1* (see Supplementary Information, Fig S1f and Table S1). Second, we performed a *cdh1* rescue experiment (see Supplementary Information, Fig S1d) and observed degradation of these APC^{cdh1} substrates following addition of *cdh1* cRNA to ^{MO}*cdh1*-treated oocytes (Fig 1c, and see Supplementary Information, Table S1).

We wanted to establish if the measurable APC^{cdh1} activity in GV oocytes had any physiological role in the process of GV arrest, especially since it could degrade cyclin B1. In non-injected oocytes 2.5% of oocytes underwent GVB at 24 h and 5% at 48 h (Fig 2). Similar low levels of GVB also occurred in oocytes microinjected with the control *inv*^{MO}*cdh1* over 48h demonstrating that milrinone provides a very good block to GVB. However, ^{MO}*cdh1* induced GVB with ~30% of oocytes undergoing GVB at 24 h and ~60% at 48 h, an effect that was reversed by *cdh1* rescue. Furthermore ^{MO}*cdh1* dramatically increased the rate of GVB achieved by microinjection of cyclin B1 cRNA (Fig 1a). Finally we could also observe increased endogenous cyclin B1 levels following introduction of ^{MO}*cdh1*, but not *inv*^{MO}*cdh1*, in both oocytes that had remained GV-arrested or had undergone GVB, although as expected cyclin B1 was higher for the latter (Supplementary Information, Fig S1e).

We have described an essential role in prophase I oocytes for APC^{cdh1} in maintaining arrest. Although it is a ubiquitin ligase associated with late M-early G1 of the mitotic cell cycle, such findings are not without precedence since it also functions in the axonal morphogenesis of post-mitotic brain neurones¹⁰. Taken together they suggest that APC^{cdh1} has been adapted to specialised cell functions outside of mitotic exit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Mehlmann LM, et al. *Science*. 2004; 306:1947–50. [PubMed: 15591206]
2. Han SJ, Chen R, Paronetto MP, Conti M. *Curr Biol*. 2005; 15:1670–6. [PubMed: 16169490]
3. Tsafirri A, Chun SY, Zhang R, Hsueh AJ, Conti M. *Dev. Biol.* 1996; 178:393–402. [PubMed: 8812137]
4. Clute P, Pines J. *Nat. Cell Biol.* 1999; 1:82–7. [PubMed: 10559878]
5. Murray AW. *Cell*. 2004; 116:221–34. [PubMed: 14744433]
6. Chang HY, Levasseur M, Jones KT. *J Cell Sci.* 2004; 117:6289–96. [PubMed: 15561765]
7. Madgwick S, et al. *Dev Biol.* 2004; 275:68–81. [PubMed: 15464573]
8. Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters JM. *Mol Biol Cell.* 2000; 11:1555–69. [PubMed: 10793135]
9. Zur A, Brandeis M. *Embo J.* 2001; 20:792–801. [PubMed: 11179223]
10. Konishi Y, Stegmüller J, Matsuda T, Bonni S, Bonni A. *Science*. 2004; 303:1026–30. [PubMed: 14716021]
11. Zernicka-Goetz M, et al. *Development.* 1997; 124:1133–7. [PubMed: 9102300]

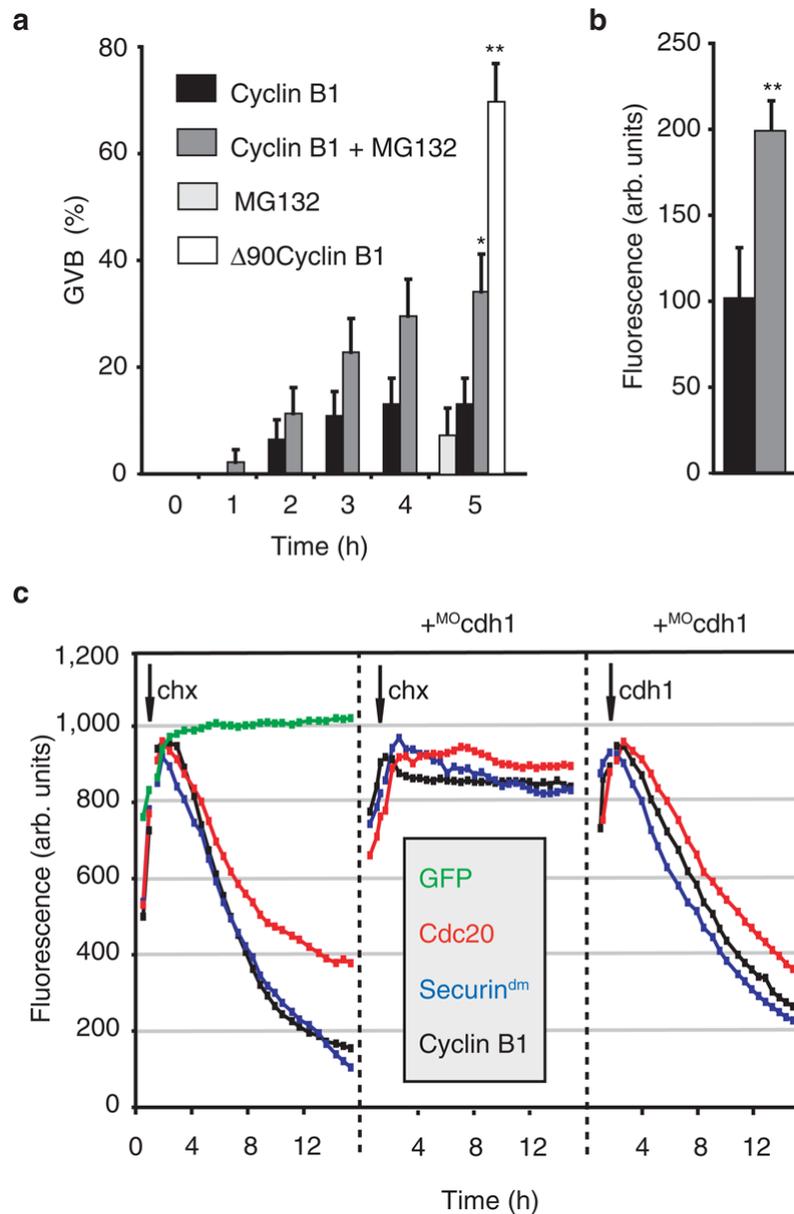


Figure 1. APC^{cdh1} activity in GV oocytes. (a) GVB rates in oocytes from the time of microinjection (0 h) of cyclin B1-GFP cRNA with (n=44) or without 50 μ M MG132 (n=46) or with $\Delta 90$ cyclin B1-GFP (n=50); or incubated with MG132 alone (n=53). Data are means \pm standard error. *p<0.05, **p<0.01, significantly different from cyclin B1 alone (Chi-squared test). (b) GFP levels in oocytes at 5 h from microinjection of cyclin B1-GFP with (grey, n=44) or without (black, n=32) MG132, normalised with respect to oocytes cultured without MG132. (c) GFP levels in oocytes microinjected with cRNA to GFP; GFP-coupled cyclin B1; securin^{dm} or cdc20, following addition of cycloheximide (chx) or cdh1 cRNA as indicated. Where indicated (+^{MO}cdh1) oocytes had been microinjected with cdh1 morpholino 24 h previously. All media contained 1 μ M milrinone. Recordings are representative of between 11-18 oocytes per condition.

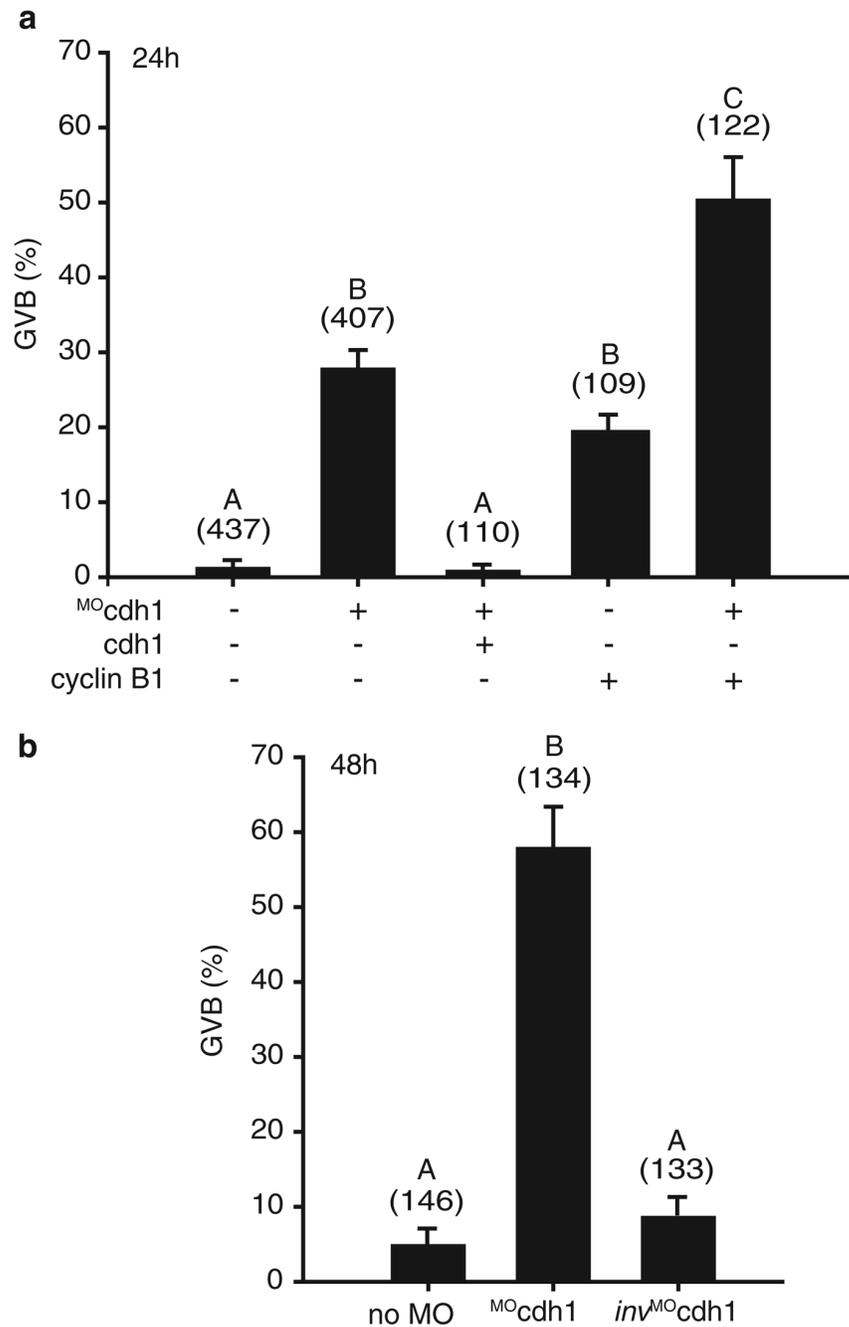


Figure 2. APC^{cdh1} maintains GV arrest. GVB in oocytes 24 h (a) or 48 h (b) after microinjection of MO^{cdh1}, inv^{MO}cdh1, cyclin B1 cRNA or cdh1 cRNA as indicated. The number of oocytes assessed for each condition is in parenthesis. At both timepoints bars with different letters (A, B and C) are significantly different ($p < 0.01$, Chi-squared).