Single Cell Biology
ISSN: 2168-9431

Castro and Lorca, Single Cell Biol 2016, 5:2
DOI: 10.4172/2168-9431.1000141

Commentary Open Access

Protein Phosphatases Triggering the Dephosphorylation and Inactivation of Greatwall

Anna Castro* **and Thierry Lorca***

Equipe Labellisée Ligue Contre le Cancer, Université de Montpellier, Centre de Recherche de Biologie Cellulaire de Montpellier, CNRS UMR 5237, 1919 Route de Mende, 34293, Montpellier cedex 5, France

***Corresponding authors:** Anna Castro and Thierry Lorca, Equipe Labellisée Ligue Contre le Cancer, Université de Montpellier, Centre de Recherche de Biologie Cellulaire de Montpellier, CNRS UMR 5237, 1919 Route de Mende, 34293, Montpellier cedex 5, France, Tel: 33434359556; Fax: 33434359410; E-mail: anna.castro@crbm.cnrs.fr, thierry.lorca@crbm.cnrs.fr

Rec date: May 16, 2016; **Acc date:** Jun 06, 2016; **Pub date:** Jun 08, 2016

Abstract

Entry into mitosis requires the coordinated activation of protein kinases and phosphatases that regulate key cell cycle components allowing entry, progression and exit of mitosis. The limiting step is thought to be the activation of both Cdk1-Cyclin B and Greatwall kinases. The former promoting phosphorylation of mitotic substrates when cells enter into mitosis and the latter inhibiting PP2A-B55, responsible for dephosphorylation of these substrates. Here, we summarize recent data on the regulation of Greatwall activity and reversal of mitotic substrates phosphorylation required for mitotic exit.

Commentary

Entry and progression through mitosis are tightly controlled by several protein kinases (Cdk-Cyclin, Wee1, Plk1, Aurora-A) and phosphatases (PP1, PP2A, Cdc25), which play critical roles in coordinating these processes [1]. Historically, protein phosphorylation/dephosphorylation during mitotic entry, progression and exit was thought to be the direct consequence of Cdk1-CyclinB activation and inactivation [1]. However, during the last decade, several results have indicated that besides control of Cdk1-CyclinB activity, phosphatase regulation is also essential to promote correct cell division. This hypothesis has emerged from pioneer studies showing that inhibition of the phosphatase PP2A-B55 is essential to promote mitotic entry [2-4]. Notably, a new signalling pathway has been identified in the control of mitotic division involving the protein kinase Greatwall and the phosphatase PP2AB55. However, Greatwall indirectly inhibits PP2AB55. Greatwall phosphorylates Arpp19 and ENSA proteins, which in turn bind and inhibit PP2AB55, allowing stable phosphorylation of Cdk1-CyclinB substrates and mitotic entry [5-8].

Greatwall activation is required to promote mitotic entry. Greatwall is a unique AGC kinase since, unlike most AGCs, it is devoid of a hydrophobic motif despite the presence of a functional hydrophobic pocket. The AGC kinases subfamily includes PKA, PKC, PDK1 and RSK. These kinases are involved in cell division, growth and metabolism [9]. Greatwall contains a very long T-loop of about 500 amino acids that separates its N-and C-terminal catalytic domains. Greatwall activation also requires phosphorylations on its N-terminal part (Ser 90), on the T-loop (Thr 194 and Thr 207) and on the tail/linker site or C-terminal part (Ser 875) [10,11]. Because Thr194 and Thr207 lie within the minimal consensus site for Cdk1-CyclinB kinase and both residues are phosphorylated *in vitro* by Cdk1-CyclinB kinase, it has been proposed that Cdk1-CyclinB may be the primary kinase responsible for the phosphorylation of these two sites. Phosphorylation of Ser90 is observed when cells enter into mitosis and mutation of Ser90 by alanine dramatically affects the activity of Greatwall *in vitro* [11]. However, the kinase responsible for the phosphorylation on this site is still unknown. Finally, phosphorylation of Ser875 is essential for Greatwall activity but does not fit with any kinase consensus site [10,11]. So, it has been suggested that Cdk1-CyclinB phosphorylates Thr194 and/or Thr207 and that subsequently an intramolecular Greatwall autophosphorylation occurs at Ser875 to promote its full activity.

So, to date the mechanism underlying Greatwall activation and PP2A-B55 inhibition at mitotic entry has been well characterized. However, the inactivation of Greatwall and re-activation of PP2A-B55 during mitotic exit, which are essential for cell integrity, were less well understood. Recently, results published by several independent groups have shown that three protein phosphatases were involved in the dephosphorylation and inactivation of Greatwall and in the re-activation of PP2A-B55. In this line, OA (Okadaic Acid)-mediated PP2A inhibition significantly blocked dephosphorylation of Greatwall on its Thr194 site. Moreover, inhibition of PP2A-B55 by a thiophosphorylated Arpp19 protein resulted in the delay in the dephosphorylation of Greatwall on Thr194 and the maintenance of a partial Greatwall activity and mitotic state [12,13]. On the other hand, depletion of the protein phosphatase 1 (PP1) strongly affects the dephosphorylation of Greatwall on the autophosphorylation site Ser875 and delays the dephosphorylation of Thr194. These results are consistent with data showing that removal of PP1 prevents the dephosphorylation of mitotic substrates, thereby maintaining full Greatwall activity upon Cdk1-CyclinB inactivation [13-15]. Interestingly, re-activation of PP2A -B55 by depletion of Arpp19 promoted dephosphorylation of Greatwall on Thr194 and S875 sites and complete mitotic exit, suggesting that both critical sites are dephosphorylated by PP2A-B55 when it is fully activated [13]. Finally, the protein phosphatase Fcp-1 also known as CTDP1 (Carboxy-Terminal Domain, RNA polymerase II Subunit 1) has been involved in the dephosphorylation of Greatwall. Depletion of Fcp1 maintains cyclin B stability and MPM-2 signals. Moreover, Fcp-1 and Greatwall are transiently associated during mitotic exit and removal of Fcp-1 delayed the dephosphorylation of Greatwall at Ser90 [16].

From these latest results we may propose that proteolysis of CyclinB promotes inactivation of Cdk1 kinase, thereby inducing the re-activation of PP1. Consequently, activation of PP1 initiates dephosphorylation of Greatwall at Ser875 and partial inactivation of Greatwall followed by the re-activation of PP2A-B55. Actually, PP1 and PP2A-B55 in concert with Fcp-1 and other phosphatases could be necessary to complete the dephosphorylation and inactivation of Greatwall thereby promoting mitotic substrates dephosphorylation and efficient mitotic exit (Figure 1).

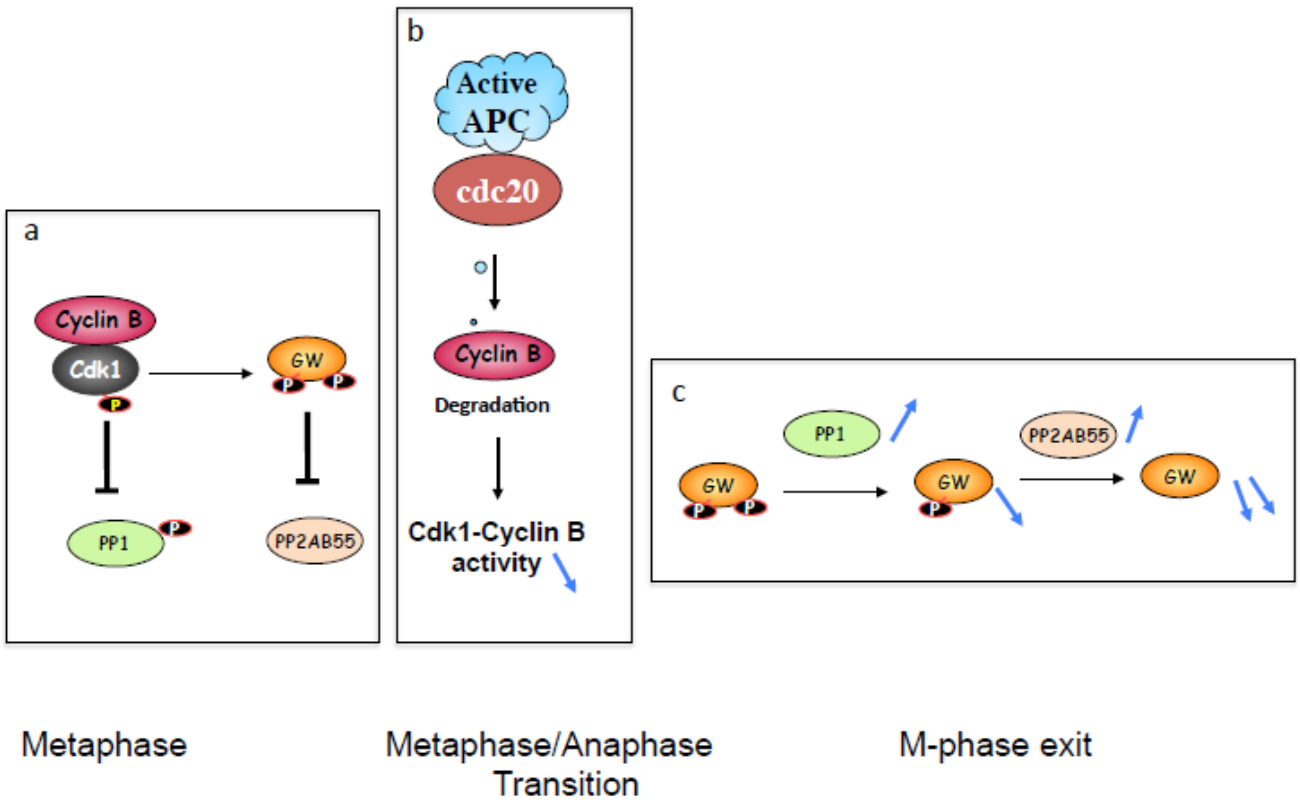

Figure 1: Regulation of mitotic entry and exit by phosphatases. (a)At the G2/M transition, Cdk1-cyclin B kinase regulates both activation of Greatwall kinase and inactivation of PP1 phosphatase. Activation of Greatwall by phosphorylation on Thr194 and Ser875 promotes inhibition of PP2A-B55. PP1 is kept inactive through phosphorylation of Thr320 by Cdk1-cyclin B kinase. (b) At the metaphase/anaphase transition, activation of the APC/Cdc20 leads to the degradation of cyclin B and a decrease of Cdk1 kinase activity. (c) Cdk1-Cyclin B inactivation prevents phosphorylation of PP1 on its inhibitory site. This allows the reactivation of PP1 through autodephosphorylation. Once activated, PP1 phosphatase promotes the dephosphorylation of Greatwall on its Ser875 site. This induces a partial inactivation of Greatwall and reactivation of PP2AB55. The latter phosphatase further dephosphorylates Greatwall at Thr194 which induce its complete inactivation.

References

1. Lindqvist A, Rodríguez-Bravo V, Medema RH (2009) The decision to enter mitosis: feedback and redundancy in the mitotic entry network. J Cell Biol 185: 193-202.
2. Vigneron S, Brioudes E, Burgess A, Labbe JC, Lorca T, et al. (2009) Greatwall maintains mitosis through regulation of PP2A. EMBO J 28: 2786-2793.
3. Mochida S, Ikeo S, Gannon J, Hunt T (2009) Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts. EMBO J 28: 2777-2785.
4. Castilho PV, Williams BC, Mochida S, Zhao Y, Goldberg ML (2009) The M phase kinase Greatwall (Gwl) promotes inactivation of PP2A/B55delta, a phosphatase directed against CDK phosphosites. Mol Biol Cell 20: 4777-4789.
5. Gharbi-Ayachi A, Labbe JC, Burgess A, Vigneron S, Strub JM, et al. (2010) The substrate of Greatwall kinase, Arpp19, controls mitosis by inhibiting protein phosphatase 2A. Science 330: 1673-1677.
6. Mochida S, Maslen SL, Skehel M, Hunt T (2010) Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. Science 330: 1670-1673.
7. Rangone H, Wegel E, Gatt MK, Yeung E, Flowers A, et al. (2011) Suppression of scant identifies Endos as a substrate of greatwall kinase and a negative regulator of protein phosphatase 2A in mitosis. Plos Genetics 7: e1002225.
8. Juanes MA, Khoueiry R, Kupka T, Castro A, Mudrak I, et al. (2013) Budding yeast Greatwall and Endosulfines control activity and spatial regulation of PP2ACdc55 for timely mitotic progression. Plos Genetics 9: e1003575.

9. Arencibia JM, Pastor-Flores D, Bauer AF, Schulze JO, Biondi RM (2013) AGC protein kinases: from structural mechanism of regulation to allosteric drug development for the treatment of human diseases. Biochim Biophys Acta 1834: 1302-21.
10. Vigneron S, Gharbi-Ayachi A, Raymond AA, Burgess A, Labbé JC, et al. (2011) Characterization of the mechanisms controlling Greatwall activity. Mol Cell Biol 31: 2262-2275.
11. Blake-Hodek KA, Williams BC, Zhao Y, Castilho PV, Chen W, et al. (2012) Determinants for activation of the atypical AGC kinase Greatwall during M phase entry. Mol Cell Biol 32: 1337-1353.
12. Hégarat N, Vesely C, Vinod PK, Ocasio C, Peter N, et al. (2014) PP2A/B55 and Fcp1 regulate Greatwall and Ensa dephosphorylation during mitotic exit. PLoS Genet 10: e1004004.
13. Ma S, Vigneron S, Robert P, Strub JM, Cianferani S, et al. (2016) Greatwall dephosphorylation and inactivation upon mitotic exit is triggered by PP1. J Cell Sci 129: 1329-1339.
14. Heim A, Konietzny A, Mayer TU (2015) Protein phosphatase 1 is essential for Greatwall inactivation at mitotic exit. EMBO Rep 16: 1501-1510.
15. Wu JQ, Guo JY, Tang W, Yang CS, Freel CD, et al. (2009) PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. Nat Cell Biol 11: 644-651.
16. Della Monica R, Visconti R, Cervone N, Serpico AF, Grieco D (2015) Fcp1 phosphatase controls Greatwall kinase to promote PP2A-B55 activation and mitotic progression. Elife 14: e10399.