Role of Protein Phosphatase-2A (PP-2A) in the Control of Mitosis and Meiosis.

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Abstract. A specific form of Protein Phosphatase-2A (PP-2A), namely PP2A-B55 δ was proposed to occupy a central role in the control of mitosis entry and exit, and meiosis in *Xenopus* oocytes [1,3]. It was held that PP2A-B55 δ is responsible for dephosphorylating substrates of cdc2/Cdk1 and that inhibition of PP2A-B55 δ by Arpp-19 phosphorylated at serine 67 by Greatwall kinase triggers entry of both mitosis and meiosis in *Xenopus* oocytes. It was further declared that the phosphorylation of Arpp19 at serine 109 by PKA underlies the blockade of meiotic division and that dephosphorylation of serine 109 of Arpp19 triggers resumption of meiotic division in *Xenopus* oocytes [4]. Recently two groups have stated that PP2A-B55 δ is the protein phosphatase that is responsible for dephosphorylating both serine 67 and serine 109 of Arpp19 [4,5] However, unfortunately for the authors concerned [1-5], no verifiable scientific evidence exists that shows that Arpp19 is a specific inhibitor of PP-2AB55 δ when Arpp19 is phosphorylated at serine 67 by Greatwall kinase and that Arpp-19 phosphorylated at serine 67 and Arpp19 phosphorylated at 109 are both specifically dephosphorylated by PP-2AB55 δ Arpp19. The idea that Arpp-19 phosphorylated at serine 67 is both an inhibitor and a substrate of PP-2AB55 δ has more to do with science fiction than science. The role of other Protein Phosphatases, including, PP-2A-B'56 δ and Protein Phosphatase-1_I (PP-1_I) cannot be ignored.

The role of PP-2A in the control of entry and exit of mitosis and meiosis.

The role of protein phosphatase-2A (PP-2A) in the control of entry and exit of mitosis and meiosis gained much traction as a result of two papers that appeared together in Science [1,2]. Essentially, Mochida et al. [2] stated that a specific form of PP-2A, namely PP2A-B55 δ was responsible for dephosphorylating substrates of cdc2/Cdk-1 and that inhibition of PP2A-B55 δ by Arpp19 phosphorylated on serine 67 by Greatwall kinase underlied in large part entry of mitosis. It was further argued that exit of mitosis was in large part due to the dephosphorylation of serine 67 of Arpp19 and reactivation of PP2A-B55 δ . Through a number of studies that were misleading at best and dishonest at worse, Mochida et al. [3] had previously ruled out the roles of other protein phosphatases, in particular that of PP-1, in the dephosphorylation of cdc2/Cdk-1 substrates. Garbi-Ayashi et al. [2] did not go so far as to suggest that Arpp19 phosphorylated on serine 67 by Greatwall kinase was a specific inhibitor of a specific form of PP-2A but essentially concluded that a form of PP-2A was inhibited by Arpp19 phosphorylated at serine 67 by Greatwall kinase and was responsible for dephosphorylating substrates of cdc2/Cdk1. As it will be shown below, the experimental design, results and conclusions of the papers by Mochida et al. [1] and Garbi-Ayashi et al. [2] are at best misleading and at worse dishonest. Following the publication of the two papers by Mochida et al. and Garbi-Ayashi et al. [1,2], several copycat papers purporting to show that Arpp19 phosphorylated on serine 67 by Greatwall kinase was a specific inhibitor of PP2A-B55 δ were published [4-8]. More recently, a paper/manuscript by Lemmonier et al. [9] proposed that phosphorylation of Arpp19 at serine 109 by PKA and its dephosphorylation by PP2A-B55 δ underlied the molecular mechanism that determined resumption of meiosis by progesterone. Unfortunately, Lemmonier et al. [9] did not provide the requisite scientific evidence that would support their pronouncements and conclusions because their described experimental designs were substandard and faulty, and their results misleading and possibly dishonest. In this paper, the experimental designs, results and conclusions of the above published papers are critically reviewed.

Control of the universal regulator of mitosis.

The universal regulator of mitosis, cdc2/Cdk1 contain two inhibitory phosphorylation sites at threonine 14 and tyrosine 15 that are phosphorylated by Wee1 kinase and Myt1 kinase [10-17]. At the G2/M transition, Cdc25 protein phosphatase becomes activated to dephosphorylate cdc2/Cdk1 at threonine 14 and tyrosine 15 of cdc2/Cdk1 resulting in its activation [18-21]. A role for protein phosphatase-2A (PP-2A) in the control of the molecular events that underlie the activation of cdc2/Cdk1, its activator Cdc25 and the substrates of cdc2/Cdk1 during mitosis have been proposed by various workers [22-33]. PP-2A is not one enzyme but a sub-family of enzymes [34-40]. The exact form(s) of PP-2A that is/are involved in the control of cdc2/Cdk1, its activator, and substrates of cdc2/Cdk1 has/have remained quite controversial. Margolis et al. [27] has proposed a role for PP-2A-B'56d in regulating 14-3-3 release from Cdc25 as a means to control mitosis while Forrester et al. [28] has suggested that failure of PP-2A-B'56d to dephosphorylate Cdc25 at mitosis results in (i) prolonged hyperphosphorylation and activation of Cdc25 causing persistent dephosphorylation and hence activation of Cdc25, (ii) constitutive activation of Cdc25 and Cdk1 leads to delayed exit of mitosis, (iii) knockout of PP-2A-B55d results in constitutively active Cdc25, and (iv) loss of B'56d cannot be compensated by endogenous levels of other B'56 subunits. In contrast to the studies of Margolis et al [27] and Forrester et al. [28], Mochida et al [1] and Gharbi-Ayachi et al. [2] proposed that PP-2A-B55d and a form of PP-2A-B55 was the protein phosphatase that was specifically involved in the dephosphorylation of Cdk1 substrates and the control of entry and exit of mitosis.

Members of the subfamily of PP-2A.

The members of the subfamily of Protein Phosphatase-2A (PP-2A) are key enzymes of the protein phosphorylation/dephosphorylation apparatus of the cell [34-41]. All members of PP-2A contain an A subunit, a C subunit and one of the many B, B' and B'' isotypes. Members of the PP-2A subfamily of enzyme include PP-2A₀-B'-56α, PP-2A₀-B'-56β, PP-2A₀-B'-56γ, PP-2A₀-B'-56d, PP-2A₀-B'-56ε, PP-2A₁-B55α, PP-2A-B55β, PP-2A-B55γ, PP-2A-B55d, PP-2A-B''72, PP-2A-B''130 [35-43]. Because of the multiplicity of PP-2A forms, the reasons for the multiplicity and functions of each member of the PP-2A subfamily of enzymes have been difficult to decipher. It is generally agreed that

members of the PP-2A subfamily of enzymes have key roles in the control of cellular activity, including the control of cellular metabolism [34,37,44-48], cell maintenance [49-54], cell proliferation [55-59], cell cycle [22-28,60-65], cell differentiation [66-70], development [71-76], cell death [77- 84], and cell transformation and tumorigenicity [85-93], and as a tumor suppressor [94-100].

The studies and conclusions of Margolis et al. [27] and of Forrester et al. [28].

Margolis et al. [27] proposed that the activity of Cdc25, a substrate of Cdk1 was regulated by a member of the subfamily of PP-2A, namely PP-2A-B'56d' and that PP-2A-B'56d' was regulated mainly through the phosphorylation of its B'56d' subunit on serine 37 by Chk 1 protein kinase and related protein kinases whereas Mochida et al. [1] and Gharbi-Ayachi et al. [2] stated that a different member of the subfamily of PP-2A, namely PP-2A-B55d' was involved in key steps of mitosis and that PP-2A-B55d' was regulated mainly by α -Endolfin and/or Arpp19 that became a specific inhibitor of PP-2A-B55d' when it/they were phosphorylated by Greatwall kinase. (It must be noted that PP-2A-B55d' and PP-2A-B'56d' are two completely different enzymes in that the B55d' subunit of PP-2A-B55d' and the B'56d' of PP-2A-B'56d' are distinct gene products that are unrelated to one another with different properties and phosphorylation sites). The proposals of Margolis et al. [27] and that of Mochida et al [1] and Gharbi-Ayachi [2] cannot be all correct. In particular, it is submitted that Mochida et al [1] and Gharbi-Ayachi et al. [2] have provided no scientifically reliable evidence to support their proposals. As it will be shown below, the scientific design, results and conclusions of Mochida et al [1] and Gharbi-Ayachi et al. [2] are at best misleading and at worse dishonest.

During interphase. Cdc25 is inactive because it is phosphorylated on a serine residue (serine 287 in *Xenopus*; serine 216 in human) by several DNA responsive check point protein kinases including Chk1, Chk2, C-TAK1, PKA, p38 and MAPKAP kinase-2 [27,101-108]. Phosphorylation of serine 287 (or serine 216 in human) allows it to bind to 14-3-3 protein. It has been proposed that the binding of 14-3-3 prevents the nuclear translocation of Cdc25 [27]. At mitosis, Cdc25 activation is triggered by the

dephosphorylation of serine 287 and the dissociation of 14-3-3. Dissociation, and removal of 14-3-3 from Cdc25 has been proposed to be regulated by phosphorylation of Cdc25 on threonine 138 by Cdk2 which is constitutively active, indicating that the regulation of 14-3-3 dissociation and release from Cdc25 was regulated by a protein dephosphorylation event. Margolis et al. [27] has presented evidence that (i) removal of 14-3-3 preceded and was required for the dephosphorylation of serine 287, (ii) the release of 14-3-3 was regulated by an okadaic sensitive protein phosphatase that had been identified as PP-2A-B'56d. (It must be noted that PP-2A-B55d and PP-2A-B'56d are two completely different enzymes in that the B55d subunit of PP-2A-B55d and the B'56d of PP-2A-B'56d are distinct gene products that are unrelated to one another with different properties and phosphorylation sites), (iii) the binding of PP-2A-B'56d to Cdc25 allows the dephosphorylation of threonine 138 of Cdc25 during interphase, (iv) phosphorylation of the B'56d subunit of PP-2A-B'56d by Chk1 on serine 37 allows the continued dephosphorylation of threonine 138 of Cdc25, and (v) phosphorylation of the B'56d of PP-2A-B'56d at serine 37 enhances the binding of B'56d of PP-2A-B'56d to Cdc25 and ensures that 14-3-3 remains bound to Cdc25.

Forrester et al. [28] extended the studies of Margolis et al. [27] by providing data which showed that (i) Knock Down of B'56d of PP-2A-B'56d resulted in constitutively active Cdc25, (ii) a marked phosphorylation of threonine 138 of Cdc25 (iii) loss of B'56 of PP-2A-B'56d cannot be compensated by endogenous levels of other B'56 subunits, (iv) failure of PP-2A-B'56d to dephosphorylate Cdc25 at mitosis resulted in prolonged hyperphosphorylation and activation of Cdc25, (v) persistent dephosphorylation of Cdc25 causes activation of cdc2/Cdk1, (vi) constitutive activation of cdc2/Cdk1 leads to a delayed exit from mitosis

The papers of Mochida et al. [1,3].

In contrast to the studies of Margolis et al. and Forrester et al. [27,28], Mochida et al. [1] proposed that inhibition of PP-2A-B55d by α -Endoline phosphorylated on serine 67 by Greatwall kinase controlled entry and exit of mitosis. As stated above, PP-2A-B55d and PP-2A-B'56d are two completely different enzymes in that the B55d subunit of PP-2A-

B55d and the B'56d of PP-2A-B'56d are distinct gene products that are unrelated to one another with different properties and phosphorylation sites). In their paper entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis" published in Science [Science (2010) Vol 330, pp1670-1673], the authors, Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T., purported to show that α -Endosulfine (Ensa), a molecule that was homologous and had identity with Arpp19, was an inhibitor of protein phosphatase-2A₁ that contains the B55d subunit (PP-2A-B55d) when Ensa is phosphorylated by Greatwall kinase at serine 67 and that the specific inhibition of PP-2A-B55d by Ensa underlies in large part entry into mitosis in *Xenopus* oocytes. While the proposal appears quite attractive, unfortunately evidence showing that PP-2A-B55d is the major protein phosphatase that dephosphorylates substrates of Cdk1, including Wee1, Myt1 and Cdc25 was lacking. Evidence that Ensa Phosphorylated by Greatwall kinase at serine 67 was a specific inhibitor of PP-2A-B55d was also not very convincing.

In the paper entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis" and published in Science [Science (2010) Vol 330, pp1670-1673], the authors, Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T. started with the assumption that PP-2A-B55d was the major protein protein phosphatase that was responsible for the dephosphorylation of Cdk1 substrates and that depletion of PP-2A-B55d accelerates mitotic progression in *Xenopus* extracts by referencing a previous paper of theirs [3] to bolster their assumption which was not correct (See an Investigative Critique of the paper in [109].

In Figure 2 of the paper entitled "Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in *Xenopus* egg", published in EMBO Journal [EMBO. J. (2009) Vol. 28, pp2777-2785], the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. claimed that: (i) after depleting the protein phosphatase activity in extracts of *Xenopus* oocytes in interphase with anti-PP1C antibody, almost 70% of the protein phosphatase activity remained, (ii) after depleting the protein phosphatase activity with anti-PP2A-A antibody, almost no protein phosphatase activity remained, and (iii) after

depleting the protein phosphatase activity with anti-PP5 antibody almost 85% of the protein phosphatase activity remained. The authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. did not seem to be able to do simple arithmetic because the above results imply that at least ~45% of the total protein phosphatase activity using their model substrate was due to PP-1 and PP-5 and that the rest was due to PP-2A. Simple arithmetic dictates that after depletion with anti-PP2A-A antibody, at least ~45% of the total protein phosphatase activity should still be measurable but somehow mysteriously disappeared. What happened to the ~45% of the total protein phosphatase activity? In Figure 2, The authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. showed that after depletion with anti-PP2A-A antibody, only 5% of the total protein phosphatase activity remained. Only after depletion with anti-PP-1C, anti-PP5 and anti PP-2A-A antibodies together, would one see almost no protein phosphatase activity in extracts of *Xenopus* oocytes at interphase. Perhaps more importantly, the authors of the paper did not seem to know that a large proportion of PP-1 is in an inactive form termed PP-1_I and requires phosphorylation on threonine 72 of its Inhibitor-2 moiety by PP-1_I activating kinase (PP-1_I ACK) to exhibit its full activity [110,111]. Had the authors determined the activity of PP-1_I, they would have seen that more than 50% of the protein phosphatase activity in the extracts of *Xenopus* oocytes would be accounted for by spontaneously active PP-1 and PP-1_I.

The subsequent experiment of the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. purporting to show that PP-2A-B55d is the major protein phosphatase responsible for dephosphorylation of the model substrate in extracts of *Xenopus* oocytes at interphase is quite misleading and possibly dishonest. In Figure 3, the authors Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. claimed that after depleting 65% of the PP-2A with the anti-Bd subunit antibody, they could measure ~35% of the total protein phosphatase activity in extracts of *Xenopus* oocytes at interphase. They should be measuring at least twice that amount. Again, the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. did not appear to be able to do simple arithmetic. In view of the fact that according to the results of Figure 2 which showed that almost ~55% of the total protein phosphatase activity was accounted for by PP-1 and PP-5, only ~45% of the remaining total protein phosphatase

could be accounted for by PP-2A-B55d. According to a simple calculation (~45% of ~45%), the % of total protein phosphatase that could be depleted by anti-PP-2A-Bd should be no more than ~21%. What happened to the almost ~79% of the total protein phosphatase activity that should be measurable after depletion with anti-PP-2A-Bd? Although, the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. did not say how many times they performed the experiments described in Figure 3 (there was no statistics presented!), it can be said that the pronouncement that PP-2A-B55d was the major protein phosphatase that dephosphorylated Cdk1 substrates is misleading at best and dishonest at worse.

In Figure 4 of the paper entitled "Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in *Xenopus* egg", published in EMBO Journal [EMBO. J. (2009) Vol. 28, pp2777-2785], the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. claimed that PP-2A-B55d "controls the proper timing of mitosis and is essential for protein dephosphorylation in exit of mitosis". In Figure 4A, the authors, Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. showed the Coomassie Blue staining of reconstituted PP-2A-B55d that they used to show that following depletion of PP-2A-B55d with anti-B55d subunit of PP-2A, the activity of H1 kinase peaked earlier, remained at near peaked level and that reconstituted PP-2A-B55d readily caused the activity of H1 kinase to decrease to level as seen in mitotic exit. These results contradicted the results described in Figure 1 and Figure 2 of the paper because based on proper and simple arithmetic, PP-2A-B55d accounted for at the most ~20% of total protein phosphatase activity towards Cdk1 substrates, yet in Figure 4D, it was shown that after depletion of PP-2A-B55d with anti-PP-2A-B55d or after adding reconstituted PP-2A-B55d back to PP-2A-B55d depleted *Xenopus* oocytes, the activity of H1 kinase was almost the same as the control.

It must be noted that the cloning of A and C subunits of PP-2A of *Xenopus* oocytes was performed by Bosch et al. [112] and these cDNA clones were used by the authors of the paper reviewed here. In the paper by Bosch et al. [112], the apparent molecular mass of the A subunit of PP-2A is described as 65 kDa on SDS PAGE whereas in the paper by

Mochida et al. [3], the apparent molecular mass A subunit of PP-2A is described as less than 52 kDa. In the paper by Bosch et al. [112], the A subunit of PP-2A gave a doublet by Western Immunoblotting of extracts of *Xenopus* oocytes. The authors of the paper entitled "Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in *Xenopus* egg", published in EMBO Journal [EMBO. J. (2009) Vol. 28, pp2777-2785], did not give any information about the specific activity of reconstituted PP-2A-B55d nor did they characterize the molecular mass of the trimeric enzyme. It is therefore not possible to determine whether the reconstituted PP-2A-B55d described in the paper was indeed a trimeric holoenzyme complex and not just a mixture of the three subunits.

In Figure 5 of the paper entitled "Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in *Xenopus* egg" and published in EMBO Journal [EMBO. J. (2009) Vol. 28, pp2777-2785], the authors stated that adding extra PP-2A-B55d delays mitotic progression in a dose dependent manner. This experiment is meaningless in that no bona fide control experiment was performed. It is submitted that very similar results will be obtained if large amounts of PP-1_C and PP-2A_C and alkaline phosphatase were used instead of PP-2A-B55d [60].

In summary, in the paper, "Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in *Xenopus* egg" that was published in EMBO Journal [EMBO. J. (2009) Vol. 28, pp2777-2785], the authors Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. provided no convincing evidence that PP-2A-B55d was the major protein phosphatase that was responsible for dephosphorylating cdc2/Cdk1 substrates and was the only protein phosphatase that was essential for entry and exit of mitosis in *Xenopus* oocytes. It can be surmised that the experimental procedures, results and conclusions associated with the paper are at best misleading and at worse dishonest.

Let us get back to the paper of the authors Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T. entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", authored By Moshida, S., Maslen, S.L., Skehel, M. and Hunt,

T., and published in Science [Science (2010) Vol 330, pp1670-1673] in which they purported to show that α -Endolfine (Ensa) was an inhibitor of protein phosphatase-2A₁ that contains the B55 δ subunit (PP-2A-B55 δ) when Ensa is phosphorylated by Greatwall kinase at serine 67 and that the specific inhibition of PP-2A-B55 δ by Ensa underlied in large part entry into mitosis in *Xenopus* oocytes. As stated above, the authors of the paper [1] started with the assumption that PP-2A-B55 δ was the major protein phosphatase that was responsible for the dephosphorylation of cdc2/Cdk1 substrates and that depletion of PP-2A-B55 δ accelerates mitotic progression in *Xenopus* extracts by referencing to their previous paper [3] which was quite misleading and could be dishonest (See above).

In Figure 2 of the paper [1], the authors, used reconstituted PP-2A-B55 δ that was presumably prepared as described in [3] and showed that using a model substrate, ³²P-labeled MBP-Fizzy-Ser50), Ensa phosphorylated at serine 67 with Greatwall kinase and ATP- γ -S inhibited reconstituted PP-2A-B55 δ but not PP-2A-C and PP-2A-AC. The authors of the paper [1] did not state the concentration of phospho ser 67- α -Endolfine (Ensa) nor did they state the activities and absolute amount of PP-2A-C, PP-2A-AC and PP-2A-B55 δ they used. To prove that phospho ser 67- α -Endolfine (Ensa) is a specific inhibitor of PP-2A-B55 δ , the authors must (i) show a proper dose response inhibitory curve of % Activity v. Concentration of phospho ser 67- α -Endolfine (Ensa) phosphorylated with Greatwall kinase and ATP and not ATP- γ -S because the authors need to determine the stoichiometry of phosphorylation and whether thiophospho ser 67- and phospho ser 67- α -Endolfine (Ensa) have the same effects, and determine the IC₅₀ of the Inhibitory Effect. (In view of the fact that α -Endolfine (Ensa) is a relatively abundant acid and heat stable protein, it should not be too difficult to obtain sufficient α -Endolfine (Ensa) to prepare a proper dose response inhibitor curve), (ii) show a proper dose response inhibitory curve of % Activity v. Concentration of dephospho ser 67- α -Endolfine (Ensa) and determine the IC₅₀ of the Inhibitory Effect if any, (iii) must use different substrates to prove that the effect of α -Endolfine (Ensa) is not substrate directed but enzyme directed (iv) must show that phospho ser 67- α -Endolfine (Ensa).does not inhibit the activities of at least PP-1C, PP-2A-B55 α , PP-2A-B55 β , PP-2A-B55 γ , PP-2B,

PP-2C, PP-4 and PP-6, (iv) must determine which protein phosphatase(s) dephosphorylate serine 67 of α -Endorfine (Ensa).

In Figure 2A of the paper [1], the authors showed that dephospho α -Endorfine (Ensa) had no effect on the activity of PP-2A-B55d which is contradictory to the results of William et al. [7] which showed that dephospho α -Endorfine (Ensa) inhibited PP-2A-B55d with an IC_{50} of ~500 nM. The authors of the paper [1] stated without reservation that thiophospho ser 67- α -Endorfine (Ensa) is a specific inhibitor of PP-2A-B55d in contradiction to Andrade, E.C. et al. [113] who stated that ARPP16 interacted with the A subunit of PP-2A₁ and that ARPP16 phosphorylated by microtubule-associated serine/threonine kinase 3 (MAST3 kinase) on serine 46 becomes an inhibitor of PP-2A containing the B55 α and B'56d subunits (Although, it must be noted that only 70% inhibition was achieved with 200 nM of thio-phospho-ARPP16. It is not clear why a full dose response inhibitory curve of % Control v. Concentration of thio-phospho-ARPP16 and phospho-ARPP16 to determine the IC_{50} of the Inhibition of PP-2As could not be performed. One of the authors of the paper, Angus C. Nairn ought to know better).

Figure 2A of the paper [1] that showed that thiophospho ser 67-- α -Endorfine (Ensa) inhibited PP-2A-B55d by almost 90% is in contrast to the results of Gharbi-Ayachi, A. et al. [2] which showed that PP-2A complex obtained by immunoprecipitation from interphase extracts was inhibited by thio-phospho-GST- α -Endorfine (Ensa) by less than ~45% after 120 mins incubation (it is not clear why Gharbi-Ayachi, A. et al. [2] had to incubate the PP-2A complex with thio-phospho-GST- α -Endorfine (Ensa) for 120 mins. If thio-phospho-GST- α -Endorfine (Ensa) is a specific inhibitor of PP-2A complex and the immunoprecipitated protein phosphatase is active, incubation of 10 mins or less should be sufficient) .

The authors of the paper [1] stated that thiophospho ser 67- α -Endorfine (Ensa) is a specific inhibitor of PP-2A-B55d but did not study the effect of thiophospho ser 67-- α -Endorfine (Ensa) and phospho ser 67- α -Endorfine (Ensa) on PP-2A-B55 α , PP-2A-B55 β and PP-2A-B55 γ , PP-2A-B' enzymes, PP-2A-B'' enzymes, PP-1_C, PP1_I, PP-2B, PP-2C,

PP-4 and PP-6. The results described in Figure 2 contradicted the results of Williams, C.B., et al [7] which showed that thiophospho ser 67- α -Endorfine (Ensa) inhibited PP-2A-B55 α with IC₅₀ of ~.2 nM whereas phospho ser 67- α -Endorfine (Ensa) inhibited PP-2A-B55 α with IC₅₀ of ~ 1 nM under their assay condition. In the paper by Williams et al [7], it was also stated that dephospho- α -Endorfine (Ensa) could inhibit PP-2A-B55 α with an IC₅₀ of ~500 nM casting doubt on the phosphorylation dependent inhibition of PP-2A-B55 α by α -Endorfine (Ensa) (Whether these values are to be believed is another matter which needs to be resolved. Also the idea that that PP-2A-B55 α dephosphorylates serine 67 of α -Endorfine (Ensa) with a Km value of 0.0009–0.0017 μ M is more in the realm of science fiction than science. Based on the fact that 0.5 nM of PP-2A-B55 α was used per assay and the results of Figure 7B and 7C, it can be estimated that the specific activity of PP-2A-B55 α was ~0.00007 nmol phosphate released/min/mg of enzyme when phospho ser 67- α -Endorfine (Ensa) was used as substrate, indicating that the latter is a very poor substrate of PP-2A-B55 α .. The value of ~0.00007 nmol phosphate released/min/mg of enzyme is 1.4 million lower than what one would observe with the dephosphorylation of an Inhibitor of PP-1 such as DARPP-32. Indeed phospho-thr35-DARPP-32 is a very poor substrate of the protein phosphatase (PP-1) that it inhibits. How can one measure PP-2A-B55 α that had a specific activity of ~0.00007 nmol phosphate released/min/mg is beyond normal comprehension and in the realm of science fiction. There are too many problematic data and statements in the paper by Williams, C.B. et al [7] to enumerate here.). As the concentration of α -Endorfine (Ensa) has been estimated be present at ~150 to 300 nM in *Xenopus* oocyte extracts compared to that of ~50 to 70 nM for PP-2A-B55 α , it can be surmised that PP-2A-B55 α would be inhibited by α -Endorfine (Ensa) in the absence of its phosphorylation by Greatwall kinase. It is indeed quite odd to use thiophospho ser 67- α -Endorfine (Ensa) when phospho ser 67- α -Endorfine (Ensa) inhibited PP-2A-B55 α with IC₅₀ of ~ 1 nM!.

The paper by Mochida, S. [113] also showed that dephospho- α -Endorfine can inhibit PP-2A-B55 α with an IC₅₀ of 1800 nM (If the data is to be believed as it appears that the author does not seem to know how to perform a protein phosphatase properly. In the paper by Mochida, S. [113], the author stated that in each protein phosphatase assay, he

used 0.8 unit of enzyme (i.e that amount of enzyme that caused the release of 0.8 nmol phosphate per min and that the assay lasted 120 min. Simple arithmetic indicated that in his control assay, he was measuring the release of 96 nmol ^{32}P -Phosphate. The author of the paper must ask the question whether it is possible to measure the release of 96 nmol ^{32}P -Phosphate and still remain in the linear part of the protein phosphatase assay. To be able to measure 96 nM ^{32}P -Phosphate is more in the realm of science fiction than science. As the author did not state the specific radioactivity of the ^{32}P -labeled substrate, it is impossible to determine what he was measuring and how he was able to measure 96 nmol of ^{32}P -Phosphate).

The paper by Kim, M.-Y. et al [4] showed and stated in Figure 5C that "fly endos suppresses PP-2A/B55 phosphatase activity in *Xenopus* extract and *C. elegans*". It is not clear what activity in the presence of PBS was being measured by the authors and what % Input exactly meant. How did the authors determine that the protein phosphatase activity was in fact PP-2A/B55. The authors referred the reader to the publication of Mochida et al. [3] which is misleading at best and dishonest at worse [3,109]. Can one assay for PP-2A in the presence of 15 μM PBS and was the effect of Endos on PP-2A activity also performed in the presence of 15 μM PBS. More importantly, does D WT Endos mean phospho-Endos or dephospho-Endos. If D WT Endos meant dephospho-Endos, then the whole idea that PP-2A inhibition by Endos is phosphorylation dependent falls to pieces. The apparent inhibition of PP-2A from *Xenopus* extracts by either D WT and D 68A appeared similar in Figure 5C. What is quite remarkable is that there is almost no inhibition of PP-2A from *C. elegans* by neither D WT and D 68A. In Figure 5D of the paper by Kim, M.-Y. et al [4], it was shown and stated that a protein phosphatase activity in *Xenopus* extract was apparently inhibited by Dm Endos S68D with $\text{IC}_{50} \sim 1,5 \mu\text{M}$. First, the result did not show that the protein phosphatase being inhibited was a form of PP-2A. Second, with an IC_{50} of $\sim 1.5 \mu\text{M}$, there is no difference between inhibition by so called phospho-Endos mimetic (Dm Endos S68D) and dephospho-Endos that apparently inhibited PP-2A-B α and PP-2A-B β with IC_{50} of 0.5 μM and 0.8 μM respectively [7,113] casting doubt on the phosphorylation dependent inhibition of PP-2A by Endos. Surprisingly, phospho-Endos mimetic (Dm Endos S68D) had no apparent effect on a

protein phosphatase in *C. elegans* extract. It is not clear why the authors did not determine the effect of phospho-Endos and dephospho-Endos on the activity of purified PP-2A and determine the IC_{50} of the inhibitions if any. The paper by Kim, M.-Y. et al. [4] is informative and significant because it showed and stated that "Despite the importance and evolutionary conservation of this pathway (i.e Gwl-Endos-PP2A pathway), it can be bypassed in two situations. "First, heterozygosity for loss-of-function mutation by twins which encode the *Drosophila* B55 protein suppressed the effects of Endos or gwl mutants. Several types of cell division occur normally in twins heterozygotes in the complete absence of Endos or the near absence of Gwl. Second, this module was non essential in the nematode *Caenorhadlis elegans*. The worm genome does not contain an obvious ortholog of gwl1, although it encodes a single Endos protein with a surprisingly well conserved Gwl target site. Deletion of this site had no obvious effects on cell divisions involved in viability or reproduction under normal laboratory condition".

The work described in the paper [1] is in contradiction with the work of Rangone et al. [114] in which the authors stated that they were unable to demonstrate a substantial inhibitory effect of phospho-Endos upon the ability of PP-2A to dephosphorylate histone H1 phosphorylated by Cdk1. One of the authors's of the paper entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", and published in Science [Science (2010) Vol 330, pp1670-1673] was quoted as stating that "the use of other substrates to assess for the inhibition of PP-2A by phospho-Endos was apparently less effective". These results indicated that any effect that phospho-Endos might be having on a form of PP-2A was most probably substrate directed and not enzyme directed, and that phospho-Endos could not be a specific inhibitor of PP-2A-B55 α or PP-2A-B55 δ . It is significant that Rangone et al. [114] stated that "From a genetic perspective, it is noteworthy that we observed only weak zygotic interactions between Endos and twins mutant. For example, adding either tws allele to either Endos allele (as endos+/endos tws) rescues the eclosion delay and the mild cuticular phenotype but not the female sterility . . . ".

The results described in the above paper and other papers that subscribed to a key role of PP-2A-B55 δ in the control of dephosphorylation reactions in mitotic entry and exit do not take into account the results of (A) Margolis et al. [115] which showed that (i) PP-1 was required to dephosphorylate serine 287 of Cdc25, (ii) the effect of PP-1 on cdc25 was antagonized by a specific inhibitor of PP-1, I-1, (iii) PP-1 forms a complex with cdc25, and (iv) dephosphorylation of serine 287 of cdc2 is masked by 14-3-3 during interphase, (B) Margolis et al. [116] which showed that (i) the dissociation of 14-3-3 and dephosphorylation of serine 287 of Cdc25 by PP-1 was regulated by phosphorylation of Cdc25 on serine 285 by Cdk1, (ii) phosphorylation of serine 285 of Cdc25 by Cdk1 greatly enhances the recruitment of PP1 to Cdc25 thereby accelerating S287 dephosphorylation, (iii) phosphorylation of serine 285 of Cdc25 by Cdk1 required prior phosphorylation of threonine 138 which preceded 14-3-3 removal and dephosphorylation of serine 287 of Cdc25, (iv) dephosphorylation of threonine 138 and serine 285 must be protected for the dephosphorylation of serine 287 to occur, (v) Phosphorylation of threonines 48 and 67 regulated the activity of Cdc25 independently of the dephosphorylation of serine 287, (vi) Cdc25 activation during entry of mitosis involved a dephosphorylation reaction catalyzed by PP-1 and several phosphorylation reactions which must be protected, (C) Wu et al. [117] which reasoned that (i) the activity of PP-1 is also "the main catalyst of mitotic phosphoprotein dephosphorylation", (ii) suppression of PP1 during early mitosis is maintained through dual inhibition by cdc2/Cdk1 phosphorylation of threonine 320 of PP-1 and the binding of inhibitor-1 phosphorylated by PKA, and (iii) as Cdc2/Cdk1 levels drop after cyclin B degradation, activation of PP-1 occurs through auto-dephosphorylation of threonine 320 and dephosphorylation of threonine 35 of Inhibitor-1, and (D) Hsu et al. [118] which provided evidence that mitotic phosphorylation of histone H3 was controlled by PP-1 in opposition to aurora kinase.

The paper "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", authored By Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T., and published in Science [Science (2010) Vol 330, pp1670-1673] is at variance with (i) The paper of Manchado et al. [118] which showed that suppression of B55 α and B55 δ prevented 60% and 40 % respectively of mitotic exit in Cdc20 null cells treated with

Mast1 (mammalian Greatwall kinase) RNAi, suggesting that "both PP-2A-B55 α and PP-2AB-55 δ participated in the PP-2A functional complexes required for mitotic exit in Cdc20 null fibroblasts" and that there was no selectivity for PP-2AB-55 δ as far as Greatwall kinase was concerned. (ii) The work of Schmitz et al. [119] which showed that while many forms of PP-2A were required at all stages of mitosis, PP-2A-B55 α was involved in the exit of mitosis and rate limiting only for post-anaphase progression. (iii) The work of Hegarat et al. [120] which showed that while B55 and B55 depletions did cause an increase in metaphase Cdk substrates phosphorylation, they did not affect the relative amount of Cdk1 substrates dephosphorylation following Cdk1 inhibition, that neither PP-2A, PP-1 nor Fcp1 appeared to be sufficient to dephosphorylate the bulk of mitotic Cdk1 substrates if the Gwl feedback loop was bypassed by Cdk1 inhibition and that the nature of the protein phosphatases that directly counteracts Cdk1 during mitotic exit in mammalian cells remains elusive. (iv) The work of Kim et al. [4] which showed that "many cell types in *Drosophila* can divide normally in the absence of Dwl or Endos if the dosage of twins, encoding the B55 regulatory subunit is halved", that "Endos may have function independent of Gwl or PP2a/B55 during female meiosis", that Endos targeted by Gwl in other organisms is dispensable for cell division in *C. elegans*" and that dephospho-Endos could inhibit PP-2A as well as a mimetic of Phospho-Endos casting doubt on the assertion that the inhibition of PP-2A by Endos was dependent on its phosphorylation by Greatwall kinase.

To summarize, the paper "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", authored By Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T., and published in Science [Science (2010) Vol 330, pp1670-1673] has provided no scientifically verifiable evidence to be able to state that phospho ser 67- α -Endoline (Ensa) is a specific inhibitor of PP-2A-B55 δ and that the latter is the major protein phosphatase that controls entry and exit of mitosis in *Xenopus* oocytes. The question as to whether PP-1 or PP-2A or both is/are responsible for the dephosphorylation reactions that are necessary for entry and exit of mitosis remains an open one. The available evidence points to the fact that the form of PP-1 that is involved in the control of entry of mitosis is most probably PP-1_I. The form(s) of PP-2A that is/are involved in the control

of entry and exit of mitosis remain(s) to be fully characterized. Conceptually and logically, the idea that PP-2A is inhibited by α -Endosulfine phosphorylated at serine 67 by Greatwall kinase is incompatible with the known enzymatic properties of the subfamily of PP-2A enzymes because they have low basal activities in the cell, are already in an inhibited state, and require activating molecules [36,120,121] or phosphorylation of the B, B' or B'' subunits [27,66,121-123] to exhibit full activity. The most compelling evidence against inhibition of the PP-2A subfamily of enzymes as a normal form of cellular control during mitosis is the fact that multiple studies have shown that marked inhibition of PP2A would automatically lead to cell transformation and tumorigenesis [85-100].

The paper of Gharbi-Ayachi et al [2].

In the paper entitled "The substrate of Greatwall kinase, Arpp19 controls mitosis by inhibiting protein phosphatase-2A" that was published in Science [Science (2010) Vol. 330, pp 3916-3926], the authors Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioude, E., Van Dorsselaer, A., Castro, A. and Lorca, T. [2] stated that the substrate of Greatwall kinase, Arpp19 controls the entry of mitosis by inhibiting protein phosphatase-2A (PP-2A) and that "we identified cyclic adenosine monophosphate regulated phosphoprotein 19 (Arpp19) and α -Endosulfine as two substrates of Gwl that when phosphorylated by this kinase associated with and inhibited PP-2A thus promoting mitotic entry. Conversely, in the absence of Gwl activity, Arpp19 and α -Endosulfine are dephosphorylated and lose their capacity to bind and inhibit PP2A".

Unfortunately, the pronouncements of the authors, Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioude, E., Van Dorsselaer, A., Castro, A. and Lorca, T., are not based on verifiable scientific evidence. That Arpp19 and α -Endosulfine could act as specific inhibitors of PP-2A are nowhere to be found in the paper. The only circumstantial evidence that Arpp19 and α -Endosulfine could be inhibitors of PP-2A was presented in Figure 3 of the paper. In Figure 3A, it was shown that the A and C subunits of PP-2A were bound to Arpp19 and α -Endosulfine in Interphase extracts and not Mitotic extracts of *Xenopus* oocytes. The rationale for analyzing only the A and C

subunits of PP-2A in the pull-down of protein complexes with GST-Arpp19-Sepharose and GST- α -Endolsulfine-Sepharose was quite puzzling. The authors were presumably referring to the studies of Mochida et al. [3] in which the authors stated that "Gwl mediates entry into mitosis through the inhibition of PP2A". However, the reliance of the studies of Mochida et al. [3] was misplaced as the work of Mochida et al. can be construed as misleading at best and dishonest at worse [1,3,109]. Specifically, the authors of the paper by Mochida et al. [3] were unable to do simple arithmetic with respect to the contribution of PP-2A in extracts of *Xenopus* oocytes at interphase and did not prove that PP-2A-B55d is the major protein phosphatase that dephosphorylates the key substrates of Cdk1. The paper by Mochida et al. [1] also started by assuming that PP-2A-B55d was the major protein phosphatase that was responsible for dephosphorylating "model CDK substrates" and that depletion of PP-2A-B55d accelerates mitotic progression in *Xenopus* extracts". As reviewed in [109], the paper by Mochida et al. [3] suffers from many anomalies and inconsistencies.

In order to prove that α -Endolfine and Arpp19 phosphorylated at serine 67 by Greatwall kinase are specific inhibitors of PP-2A₁, the authors, Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioude, E., Van Dorsselaer, A., Castro, A. and Lorca, T. must (i) show a proper dose response inhibitory curve of % Activity v. Concentration of phospho ser 67- α -Endolfine and ser 67-Arpp19 phosphorylated with Greatwall kinase and ATP and not ATP- γ -S because the authors need to determine the stoichiometry of phosphorylation and whether thiophospho ser 67- and phospho ser 67- α -Endolfine and Arpp19 have the same effects, and determine the IC₅₀ of the Inhibitory Effect. (In view of the fact that α -Endolfine and Arpp19 are a relatively abundant and heat stable proteins, it should not be too difficult to obtain sufficient amount of α -Endolfine and Arpp19 to prepare a proper dose response inhibitor curve), (ii) show a proper dose response inhibitory curve of % Activity v. Concentration of dephospho ser 67- α -Endolfine and Arpp19, and determine the IC₅₀ of the Inhibitory Effects if any, (iii) must use different substrates to prove that the effect of α -Endolfine (Ensa) and Arpp19 are not substrate(s) directed but enzyme directed (iv) must show that phospho ser 67- α -Endolfine and Arpp19 do not inhibit the activities of PP-1C, PP-1I, PP-2B, PP-2C, PP-4

and PP-5. (iv) must determine which protein phosphatase(s) dephosphorylate(s) serine 67 of α -Endolfine and Arpp19.

Unfortunately, in Figure 3 of the paper entitled "The substrate of Greatwall kinase, Arpp19 controls mitosis by inhibiting protein phosphatase-2A" that was published in Science [Science (2010) Vol. 330, pp 3916-3926], the authors, Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioudes, E., Van Dorsselaer, A., Castro, A. and Lorca, T., provided some very badly designed experiments, resulting in substandard and meaningless results that should have been flagged by the editors of Science but were not. In Figure 3D, the authors showed and stated that PP2A complex was obtained by immunoprecipitation of proteins in interphase extracts by antibodies against PP-2A (The authors did not say what antibodies they use and how they did the immunoprecipitation? Can one even obtain fully active PP-2A following immunoprecipitation with anti-PP-2A antibodies.) and then incubated the "immunoprecipitated PP-2A" with 5.3 μ g of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 for 10 mins, and then 32 P-labeled myelin basic protein-cMos fusion protein. The concentration of the substrate used was not stated and were added and further incubated for up to 120 mins. The following are some of the anomalies associated with Figure 3D which is the only figure that the authors provided to show that thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 were specific inhibitors of PP-2A: (i) The activity of immunoprecipitated PP-2A was not provided. It is therefore impossible to estimate the absolute amount PP-2A that is immunoprecipitated. As the amount and concentration of 32 P-labeled myelin basic protein-cMos fusion protein was not provided, it is impossible to determine if any inhibition that could be observed was substrate directed or enzyme directed. Further, it is impossible to determine the IC₅₀ of the inhibition (if any) of PP-2A by thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19.

Without determining the IC₅₀ of any observed inhibition, any data of inhibition is useless and meaningless as the observed inhibition could be substrate directed and therefore non-specific. 5.3 μ g of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 is

equivalent to ~0.3 nmole. From experience, it can be surmised that 5.3 of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 was in large excess of the amount of PP-2A that could be present in the immunoprecipitate. Therefore, if thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 were specific inhibitors of a form of PP-2A, the authors, Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioude, E., Van Dorsselaer, A., Castro, A. and Lorca, T. should be observing near 100% inhibition and not less than 50 % as shown in Figure 3D. (ii) Following incubation of immunoprecipitated PP-2A with 5.3 μ g of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 for 10 mins, the activity of PP-2A was determined for 0, 60 and 120 mins. This indicates that there was very little amount of PP-2A that was immunoprecipitated. After 120 mins of incubation with the substrate, only ~30 % of the bound phosphate was dephosphorylated. A protein phosphatase assay that takes that amount of time to perform is meaningless. It is impossible to determine any kinetics. It is indeed quite odd that after 120 mins incubation, almost ~70 % of the phosphate remains covalently bound to the substrate in the control experiments. Either the enzyme had died at that point. If that was the case, then any effect of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 was meaningless. (iii) Following incubation of immunoprecipitated PP-2A with 5.3 μ g of thio-phospho-GST- Endolfine (D66A) for 10 mins, the activity of PP-2A was determined at 0, 60 and 120 min. The figure showed that there was almost no difference in the amount of phosphate that remained covalently bound to the substrate at the two time points. It is not clear what this experiment tells the reader. If it meant to be another control, it is quite meaningless. The authors should have used dephospho-GST- α -Endolfine or dephospho-GST-Arpp19 to prove that the inhibition of PP-2A by thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 were conditioned upon their phosphorylations by Greatwall kinase. (iv) After 60 mins incubation with the substrate, the authors showed ~25% of the total phosphate was released in the control experiment and that ~10% of the total phosphate was released in the experiments in which 5.3 μ g of thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19 were added and incubated for 10 mins. If one was to correctly plot % of control v. concentration of added thio-phospho-GST- α -Endolfine or thio-phospho-GST-Arpp19, one would obtain a plot which showed that at 60 mins, there was ~40 %

inhibition of the enzyme. After 120 mins incubation with the substrate, the authors showed ~32% of the total phosphate was released in the control experiment and that ~15% of the total phosphate was released in the experiments in which 5.3 µg of thio-phospho-GST-α-Endolfine or thio-phospho-GST-Arpp19 was added and incubated for 10 mins with the enzyme. If one was to correctly plot % of control v. concentration of added thio-phospho-GST-α-Endolfine or thio-phospho-GST-Arpp19, one would obtain a plot which showed that at 120 mins, there was 46% inhibition of the enzyme. The result suggests that the amount of thio-phospho-GST-α-Endolfine or thio-phospho-GST-Arpp19 that were added (5.3 µg) was not enough to saturate all of the PP-2A in the assay as the maximal inhibition that is achieved was ~46% inhibition which is impossible and against the laws of nature. The result also suggests that the effect of thio-phospho-GST-α-Endolfine or thio-phospho-GST-Arpp19 could be substrate directed and not enzyme specific.

Can an experiment to prove specific phosphorylation dependent inhibition of PP-2A by thio-phospho-GST-α-Endolfine and thio-phospho-GST-Arpp19 be taken seriously when the maximal inhibition that could be achieved was less than 50% and one must practice mental acrobatics to actually see any inhibition. (v) In the legend to Figure 3D, the authors stated that Gels were scanned by a Typhoon Scanner and quantified with Image Software. What was exactly 100%. (vi) In the legend to Figure 3D, the authors stated that "Statistical analysis of the results obtained from two different independent experiments was performed with an unpaired Student's t test. The percentage of phosphorylated c-Mos at each time was expressed as the mean ± SD. Can performing an experiment performed twice only provide meaningful mean ± SD and Student's t test? Can $*P < 0.03$ be meaningful when the experiments were performed only twice? (vii) The effects of different concentrations of thio-phospho-GST-α-Endolfine, thio-phospho-GST-Arpp19, phospho-GST-α-Endolfine, phospho-GST-Arpp19, dephospho-GST-α-Endolfine and dephospho-GST-Arpp19 on the activities of PP-2A, PP-1c, PP-1i, PP-2B, PP-2C, PP-4 and PP-5 must be determined and a proper dose response inhibitory curve of % Control v. Concentration of inhibitors must be plotted to determine the specificity of the inhibitory effect (if any) of thio-phospho-GST-α-Endolfine and thio-phospho-GST-

Arpp19 and the IC₅₀ of the inhibition of PP-2A by thio-phospho-GST- α -Endolfine and thio-phospho-GST-Arpp19. Without physiologically relevant IC₅₀ values, it is meaningless and dishonest to talk about specific inhibition of PP-2A by thio-phospho-GST- α -Endolfine and thio-phospho-GST-Arpp19 in view of the fact that Williams et al. [7] have reported that dephospho- α -Endolfine can inhibit PP-2A-B55 α with an IC₅₀ of 500 nM. casting doubt on the phosphorylation dependent inhibition of PP-2A by α -Endolfine (although, it must be admitted that the paper of Williams et al. [7] is itself full of anomalies and results that have more to do with science fiction than science).

To summarize, the experimental design, results and conclusion of the authors of the paper entitled "The substrate of Greatwall kinase, Arpp19 controls mitosis by inhibiting protein phosphatase-2A" that was published in Science [Science (2010) Vol. 330, pp 3916-3926], are at best misleading and at worse dishonest. Until proper studies are performed and scientifically verifiable results are produced that show conclusively that α -Endolfine and Arpp19 can be classified as specific inhibitors of a form of PP-2A when they are phosphorylated by Greatwall kinase, the proposal of Mochida et al. [1] and Gharbi-Ayashi et al. [2] cannot be taken seriously.

Role of PP-2A in the control of mitosis.

The idea that PP-2A is inhibited by α -Endolfine phosphorylated at serine 67 by Greatwall kinase does not account for the fact that the PP-2A subfamily of enzymes have low basal activities in the cell and that they only expressed their full activities following stimulation by activating molecules [36,120,121] or phosphorylation of the B, B' or B'' subunits [27,66,121-123]. This understanding of the regulation of PP-2A subfamily of enzymes through activation is consistent with the fact that in the cell, there are many inhibitors of PP-2A so that most of PP-2A present in the cell would be permanently inhibited and that PP-2A would be undergoing periodic moderate activation instead of periodic inhibition. One or more form(s) of PP-2A in the cell has/have low basal activity upon entry into mitosis and becomes moderately activated prior to and exit of mitosis. As countless studies have shown, logically, extensive inhibition of PP2A would lead to cell transformation and tumorigenesis [85-100].

The following model for the role of PP-1 and PP-2A in the control of mitosis that does not rest upon the conclusions of Mochida et al. [1] and Gharbi-Ayashi et al. [2] are a lot more realistic. The model takes in account the works of Mayer-Jackel et al. [24,25], Hutchins et al. [26], Peng et al. [101,102], Margolis et al. [27,115,116], Forrester et al. [28], Wu et al. [117], Tung et al. [110,111,156], Puntoni and Villa-Moruzzi [152,153], Dohadwala et al. [161], Kwon et al. [162], Lamb et al. [165], Brautigan et al. [166], Fernandez et al. [167], and Li et al [160],

Figure 1. Model for the role of PP-2A in the control of meiosis.

Role of PP-2A in the control of meiosis in *Xenopus* oocytes.

Meiosis consists of the generation of haploid cells from diploid parental cells and is characterized by a single round of replication followed by two rounds of consecutive nuclear breakdown. In most metazoans, the meiotic cell cycle is arrested at two junctions, at prophase of metaphase I and metaphase II following resumption of meiosis or meiotic maturation as a result of hormonal stimulation. The hormones 1-methyladenine, progesterone and 4-4-demethyl-5 α -cholest-8-14-25-triene-3 β -ol have been shown to trigger the resumption of meiosis in Starfish oocytes, *Xenopus* oocytes and Mouse oocytes respectively [124-126] resulting in activation of the universal Maturation Promotion Factor (MPF) that is essentially Cdk1 (Cdc2 complexed to Cyclin B) [127-133], followed by pleiotropic phosphorylation of large number of direct or indirect substrates of Cdk1, including Cdc25, the activator of Cdk1, PP-1 α , Histone H1, Histone H3, Lamin, and many other substrates [127,128,134-138]. Many studies have suggested that a form of PP-2A was involved in the control of meiotic division [60-65,141,142]. Following the publication of the papers by Mochida et al. [1] and Gharbi-Ayashi et al. [2], several copycat papers appeared to show that PP-2A-B55 δ was also the protein phosphatase that was responsible for the control of meiosis in *Xenopus* oocytes [5,9,143]. These paper purported to show that the stimulation of *Xenopus* oocytes arrested at prophase by progesterone resulted in or was accompanied by the inhibition of PP-2A-B55 δ by Arpp19 phosphorylated by Greatwall kinase [5,9,143]. Since these copycat papers were based on the papers of Mochida et al. [1] and Gharbi-Ayashi et al. [2], they did not even bother to do the requisite experiments to show and prove that Arpp19 phosphorylated on serine 67 by Greatwall kinase was a specific inhibitor of PP-2A-B55 δ . They bypassed the issue of reproducibility of Scientific Data and came up with fantastic results, conclusions and erroneous models of the role of PP-2A-B55 δ as a key controller of meiosis.

The paper by Dupre et al [5].

Extrapolating from the misleading and possible dishonest results of Mochida et al. [1] and Gharbi-Ayashi et al [2], the paper entitled "Phosphorylation of ARPP19 by Greatwall

renders the amplification of MPF independent of PKA in *Xenopus* oocytes", and published in Journal of Cell Science [5], the authors, by Dupre, A., Buffin, E., Roustan, C., Nairn, A.C., Jessus, C., and Haccard, O. purported to show that phosphorylation of the ARPP19 by Greatwall Protein Kinase promoted its binding to and inhibited Protein phosphatase-2A (PP-2A) that contains the B55 δ subunit and that this process that was controlled by Cdk1, had an essential role within the cdk1 auto-amplification loop for the meiotic division in *Xenopus* oocytes. The authors of the above paper suggested that "PP-2A-B55 δ , Greatwall and ARPP19 are not only required for entry into meiotic division but are also pivotal effectors with the cdk1 auto-amplification loop responsible with its independence with respect to the PKA-negative control".

The paper entitled "Phosphorylation of ARPP19 by Greatwall renders the amplification of MPF independent of PKA in *Xenopus* oocytes", authored by Dupre, A., Buffin, E., Roustan, C., Nairn, A.C., Jessus, C., and Haccard, O. and published in Journal of Cell Science was flagged in PUBPEER and an anonymous group of concerned French Scientific Researchers for various mischiefs, including Unscientific Image Manipulation with respect to Figure 4D and Figure S4 of the paper. Although, an investigation by an Anonymous Panel of Experts set up by Universite Pierre et Marie Curie and CNRS concluded that there was no Scientific Misconduct involved and recommended a correction of the paper in question [144,144], the corrected paper [146] still had several deficiencies as pointed out in [147], The results presented in the paper entitled "Phosphorylation of ARPP19 by Greatwall renders the amplification of MPF independent of PKA in *Xenopus* oocytes", authored by Dupre, A., Buffin, E., Roustan, C., Nairn, A.C., Jessus, C., and Haccard, O. and published in Journal of Cell Science [5] must be treated as toxic. Moreover, the authors of the paper did not show in any way or form that Arpp19 phosphorylated by Greatwall kinase on serine 67 was specific inhibitor of PP-2A-B55 δ .

The paper by Dupre et al. [143].

Extrapolating from the misleading and possibly dishonest papers of Mochida et al. [1], Gharbi-Ayashi et al. [2] and Dupre et al [5], the authors of the paper entitled

"Phosphorylation of Arpp19 by protein kinase A prevents meiosis resumption in *Xenopus* oocytes" and published in the Journal, Nature Communications [Nature Commun. Vol. 5, Article number: 3318] made many conclusory statements that were not based on verifiable scientific data. Referring to the papers by Mochida et al. [1], Gharbi-Ayashi et al. [2] and Dupre et al [5], the authors stated conclusively in a schematic diagram (Figure 7) that Arpp19 phosphorylated by Greatwall kinase on serine 67 was an inhibitor of PP-2A-B55 in *Xenopus* oocytes. The authors, Dupre et al. [143] have provided not a shred of scientific evidence in their corrected paper that was published in the Journal of Cell Science [5,145] nor in the above paper [143] that Arpp19 phosphorylated by Greatwall kinase on serine 67 was an inhibitor of PP-2A-B55 in *Xenopus* oocytes. In this paper [143], the authors, Dupre et al, stated in Figure 1, that Arpp19 unphosphorylated at serine 109, inhibited GVBD. If the result could be reproduced, then it contradicted the result presented in Figure 1B of their paper that was corrected and published in the Journal of Cell Science [5,145]. Since Greatwall kinase could not phosphorylate S67A-GST-ARPP, the latter should not inhibit GVBD contrary to what is depicted in Figure 1B [5,145]. The results of Figure 1B of the paper by Dupre et al. [143] also contradicted the results published by Mochida [6] and Williams et al [7] if these results can in fact be reproduced. Mochida [6] and Williams et al [7] stated that dephospho- α -Endosulfine (a homologue of Arpp19 with the same phosphorylation sites as Arpp19) could inhibit PP-2A-B55 α and PP-2A-B55 δ with IC₅₀ of ~500 nM and ~1800 nM respectively. In view of the fact dephospho-Arpp19 inhibited PP-2A without being phosphorylated by either PKA or Greatwall kinase on serine 109 or serine 67 respectively, it follows that any amount of dephospho-Arpp19 should inhibit PP-2A in *Xenopus* oocytes and trigger resumption of meiosis. Further, Mochida [6] showed that α -Endosulfine phosphorylated on threonine 28 by Cdk1 was apparently a moderate inhibitor of PP-2A-B55 δ with IC₅₀ of ~130 nM and that prior phosphorylation of α -Endosulfine on serine 109 by PKA affected the inhibition of PP-2A-B55 δ by α -Endosulfine phosphorylated on threonine 28 by Cdk1 causing a shift of the IC₅₀ to ~700 nM. It is not clear why the authors of paper by Dupre et al. [143] did not find α -Endosulfine in their so called acid and heat stable protein preparation from *Xenopus* oocytes. The presence of α -Endosulfine in *Xenopus* oocytes is like sore thumb.

In Figure 2A of the paper by Dupre et al. [143], the authors stated that only one single time point (60 min) showed a difference when compared to no progesterone stimulation with $p < 0.05$. The result is meaningless because one time point difference does not prove anything. The total amount of Arpp19 at the 60 min time point also appeared to be different from no progesterone stimulation. Did the authors determine that there was no significant difference between the total amount of Arpp19 at the 60 min time point and no progesterone stimulation? If not why not? Figure 2C is also problematic because there appeared to be less total Arpp19 at the 30, 60 and 90 min time points when compared with no progesterone stimulation. Again, the authors did not determine whether there were any significant differences between total Arpp19 at the 30, 60 and 90 min times points and no progesterone stimulation. Figure 3A is problematic because it contradicted Figure 1B in that it showed that 775 ng GST-Arpp19 delayed time of GVBD by ~150 min but did not inhibit the extent of GVBD (100 % GVBD was achieved) whereas Figure 1B showed that 775 ng GST-Arpp delayed time of GVBD by ~200 min and inhibited extent of GVBD (only 50% GVBD was achieved). Figure 3A is also problematic because it contradicts the results of Williams et al [7] and Mochida [6] which stated and showed that dephospho- α -Endolfin (a homologue of Arpp19 with same phosphorylation sites) could inhibit PP-2A-B55 α and PP-2A-B55 δ with IC_{50} of ~500 nM and 1800 nM respectively meaning that 775 ng of GST-Arpp-S109D or GST-Arpp-S67A should both inhibit PP-2A-B55 δ and trigger resumption of meiosis if the authors' assumption had nothing to do with science fiction.

A second anomaly with the above paper by Dupre et al. [139] is that the authors claimed that they purified acid and heat stable proteins from *Xenopus* oocytes as described by Boyer et al. [146] but did not show any evidence that they did so. The work of Boyer et al. [146] presented evidence that their acid and heat stable protein preparations consisted of two major proteins of apparent molecular masses 32 kDa and 20 kDa respectively, and that progesterone caused the dephosphorylation of both the 32 kDa and 20 kDa proteins. What happened to the 31 kDa protein? Immunoblotting an extract of *Xenopus* oocytes with anti-Arpp19 antibodies (See Figure 1 of the paper [143] would also revealed a protein of ~20 kDa. To prove that the acid and heat stable protein of apparent molecular

mass ~20 kDa was Arpp19, the authors should purify and determine part of its sequence, a very easy procedure nowadays. Instead of using recombinant GST-ARPP, the authors should use purified acid and heat stable protein of apparent molecular mass ~20 kDa to show that it prevented GVBD and not GST-Arpp19.

What is perhaps more disturbing about the paper by Dupre et al. [143] is that while the author stated that Arpp19 is the long sought substrate of PKA that is responsible for keeping *Xenopus* oocytes arrested at prophase and that Dephosphorylation of Arpp19 triggered resumption of meiosis, they do not account for the seminal works of Huchon et al. [147] and Foulkes and Maller [148] which showed that PP-1-I1 and PP-1-I2 would do exactly what Arpp19 phosphorylated on serine 109 by PKA was proposed to do. PP-1-I-1 is a specific inhibitor of PP-1 when it is phosphorylated threonine 34 by PKA [149,150]. PP-1-I-2 is the regulatory subunit of PP-1_I which has low basal activity and becomes activated when it is phosphorylated on serine 72 by several activating kinases, including PP-1_I.ACK, GSK-3, Cdk1 and Cdk5 [110,111,151-156]. PP-1_I could be the enzyme that dephosphorylates serine 287 of Cdc25 causing its activation and triggering resumption of meiosis [115,116] (See below). The inhibition of PP-1_I by Inhibitor-1 phosphorylated on threonine 35 by PKA could explain the effect of cAMP and PKA on the inhibition of Cdc25 and Cdk1 and resumption of meiosis. [107,157,158]. The authors of the paper by Dupre et al. [143] do not seem to be able to decide what exactly Arpp19 does in *Xenopus* oocytes. On the one hand, they claimed that Arpp19 phosphorylated on serine 67 by Greatwall kinase became a specific inhibitor of PP-2A-B55 (although they have not provided any scientific evidence to show that it was so) and that that reaction controlled resumption of meiosis. On the hand, they claimed that Arpp19 phosphorylated on serine 109 by PKA prevented the resumption of meiosis but do not show how. The authors of the paper by Dupre et al. [143] do not know what Arpp19 phosphorylated on serine 109 by PKA would act on, nor do they know whether its dephosphorylation by a protein phosphatase would also trigger the resumption of meiosis (See below). The difference is that the target PP-1-I1 and PP-1-I2, PP-1 has been shown to regulate and dephosphorylate the inhibitory phosphorylation site (serine 287) of Cdc25, the activator of Cdk1 [115-117].

In another convoluted scheme of ideas that seemed to have more to do with science fiction than science, the authors of the Dupre et al. [143] proposed that the protein phosphatase that dephosphorylates serine 109 of Arpp19 was PP-2A-B55 δ , the same protein phosphatase that Arpp phosphorylates on serine 67 by Greatwall kinase was supposed to inhibit in order to trigger resumption of meiosis in *Xenopus* oocytes. As discussed below, the idea that Arpp19 phosphorylated on serine 109 is phosphorylated by PP-2A-B55 δ is another fantastical proposal of the authors of Dupre et al. [139].

The paper by Lemmonier et al [9].

The paper/manuscript Entitled "The M-phase regulatory phosphatase PP2A-B55 δ opposes protein kinase A on Arpp19 to initiate meiotic division" authored by Tom Lemmonier, Enrico Maria Daldello, Robert Poulhe, Tran Le, Marika Miot, Catherine Jessus and Aude Dupré", and published in bioRxiv [bioRxiv (2019) doi: <http://dx.doi.org/10.1101/810549>] was meant to show that *Xenopus* oocytes arrested at prophase enter meiotic division when Arpp19 was phosphorylated at serine 67 by Greatwall kinase causing Arpp19 to become an inhibitor of PP-2AB55 δ and that the latter is also the main protein phosphatase that can dephosphorylate Arpp19 at serine 109 that was due to PKA, a chemical reaction that is described as underlying progesterone induced dephosphorylation of Arpp19. Unfortunately for the authors concerned, no verifiable scientific evidence exists that shows that Arpp19 is a specific inhibitor of PP-2AB55 δ when Arpp19 was phosphorylated at serine 67 by Greatwall kinase and that Arpp-19 phosphorylated at serine 67 and Arpp19 phosphorylated at 109 are both specifically dephosphorylated by PP-2AB55 δ Arpp19. The idea that Arpp-19 phosphorylated at serine 67 is both an inhibitor and a substrate of PP-2AB55 δ has more to do with science fiction than science

Referring to the papers [1,2], the authors in the paper [9] stated that "PP2A associated with the B55 δ subunit appears to be a major enzyme that dephosphorylates Cdk1 targets (Castilho et al., 2009; Mochida et al., 2009), hence controlling the downstream events of MPF activation: mitotic progression and mitotic exit. The regulation of PP2A-B55 δ depends on the kinase Gwl (Castilho et al., 2009; Vigneron et al., 2009). Once activated,

Gwl directly phosphorylates two small related proteins, α -endosulfine or ARPP19, both of which then become able to interact with and inhibit PP2A-B55 δ (Gharbi-Ayachi et al., 2010; Mochida et al., 2010; Rangone et al., 2011). As a consequence, Cdk1 activity is no longer antagonized by PP2A-B55 δ phosphatase, resulting in an increased and stable level of phosphorylation of the mitotic substrates responsible for cell division (Glover, 2012; Haccard and Jessus, 2011; Lorca and Castro, 2013). Thus, without the contribution of Gwl inhibiting PP2A-B55 δ through ARPP19/ α -endosulfine, active Cyclin-B-Cdk1 can neither promote nor maintain the phosphorylation of its M-phase substrates".

Extrapolating from the papers described by Mochida et al. and Gharbi-Ayashi et al. [1,2], the authors proclaimed that "Here, we investigate for the first time the detailed role of the Gwl/ARPP19/PP2A module in a physiological cellular process, namely meiotic maturation of *Xenopus* oocytes. We show that ARPP19 is expressed in prophase-arrested oocytes and is *in vivo* phosphorylated by Gwl at S67 during meiotic resumption. We reveal that the Gwl/ARPP19/PP2A system plays an essential role during meiotic M-phase entry, not only by counteracting Cdk1 substrate phosphorylation but also by promoting Cdk1 activation. Once activated, it behaves as a major component of the MPF auto-amplification loop, rendering this process independent of PKA activity and independent of protein synthesis provided that a small amount Mos is present. Gwl/ARPP19/PP2A therefore not only regulates the events required for M-phase progression and exit, downstream of MPF, but also MPF activation per se and meiotic M-phase entry" [9].

The obvious question that the authors should have asked was why they ruled out other forms of protein phosphatases, including PP-1 and especially PP-1_I which is an important form of PP-1. The authors of the paper did not do any experiments that showed that PP-2A-B55 δ played "an essential role during meiotic M-phase entry, not only by counteracting Cdk1 substrate phosphorylation but also by promoting Cdk1 activation. Once activated, it behaves as a major component of the MPF auto-amplification loop, rendering this process independent of PKA activity and independent of protein synthesis provided that a small amount Mos is present. Gwl/ARPP19/PP2A therefore not only

regulates the events required for M-phase progression and exit, downstream of MPF, but also MPF activation per se and meiotic M-phase entry".

Ruling out PP-1 contradicts the seminal works of Huchon et al. [149] and Foulkes and Maller [150] which showed that a specific inhibitor of PP-1, namely protein phosphatase-1 inhibitor-1 (PP-1-I1) or PP-1-I2 delays the resumption of meiosis induced by progesterone but not by MPF in *Xenopus* oocytes implying the importance of a form of PP-1 in the resumption of meiosis. In view of the fact that the authors in the paper/manuscript [9] did not do any work to rule out the role of any forms of PP-1 in the initiation, maintenance and ending of meiosis in *Xenopus* oocytes, they cannot scientifically state that no form of PP-1 was not involved. It must be noted that PP-1 exists in many forms and that the activity of PP-1 depends on targeting units [151-153], other modulating proteins including 14-3-3 and whether PP-1 binds directly to its substrates or not [151-161]. A case in point, the PP-1 catalytic subunit binds cdc25 at a RVXM site that is specifically recognized by PP-1 [115,116]. A form of PP-1 termed PP-1 has low basal activity and requires phosphorylation of its regulatory subunit on threonine 72 by PP-1_I activating kinase (PP-1_I ACK) to exhibit its full activity [110,111,154-160]. Both the catalytic subunit and regulatory subunit of PP-1_I have been shown to be phosphorylated by Cdk1 [158,161, 162]. Further work will show that PP-1_I is involved in the direct control of Cdc25. All the evidence currently available supports the view that PP-1_I is involved in the direct control of Cdc25 by dephosphorylating serine 287 of Cdc25. PP-1_I is complexed to 14-3-3 and C-TAK1 [111] which has been shown to be able to phosphorylate serine 287 of Cdc25 and inhibit it [104] and the regulatory subunit of PP-1_I and Cdc25 in a complex of Cdc25-PP-1_I-14-3-3-C-TAK1 resulting in the inhibition of both PP-1_I and Cdc25 (H.Y.L. Tung, unpublished data). Available scientific evidence also shows that the dissociation of 14-3-3 from Cdc25 can also be effected by phosphorylation of threonine 138 of Cdc25 by Cdk2 and that PP-2A-B56' negatively regulates the dissociation of 14-3-3 from Cdc25 by dephosphorylating threonine 138 of Cdc25 [27]. Thus, the activation of Cdc25 may occur after phosphorylation of threonine 138 and release of 14-3-3 followed by dephosphorylation of serine 287 by PP-1_I. Cdc25 is itself phosphorylated on serine 285 and regulated by Cdk1.

It is first important to consider a number of conclusory statements of the authors of the paper/manuscript [9]. It is stated that: (i) Importantly, Cdk1 activation also requires the inhibition of a specific phosphatase, the PP2A-B55 δ isoform, which counteracts Cdk1-dependent phosphorylations of mitotic/meiotic substrates, including Cdc25 and Myt17. PP2A-B55 δ inhibition is achieved by Arpp19, a specific inhibitor of this phosphatase when phosphorylated at S67 by the kinase Greatwall (Gwl)". As stated above, that phosphorylation of Arpp19 by Greatwall kinase at serine 67 converts it to a specific inhibitor of PP2A-B55 δ is a fallacy that is not based on concrete verifiable scientific data [109]. (ii) "In *Xenopus* oocyte, it is clearly established that S67 phosphorylation of Arpp19 by Gwl promotes its binding to PP2A-B55 δ and the inhibition of the phosphatase". As stated above, that phosphorylation of Arpp19 by Greatwall kinase at serine 67 converts it to a specific inhibitor of PP2A-B55 δ is a fallacy that is not based on concrete scientific data [109,145]. (iii) "The molecular regulation of this last step, involving Gwl, Arpp19 and PP2A, has been well deciphered in mitosis and meiosis". "In *Xenopus* oocyte, it is clearly established that S67 phosphorylation of Arpp19 by Gwl promotes its binding to PP2A-B55 δ and the inhibition of the phosphatase". As stated above, the role of Arpp19 phosphorylated by Gwl at serine 67 as a specific inhibitor of PP-2A B55 δ is a fallacy that is not based on concrete scientific data [109,145]. (iii) "we identify PP2A-B55 δ as the phosphatase that dephosphorylates Arpp19 at S109, thus enabling oocytes to resume meiosis. The level of Arpp19 phosphorylated at S109 in prophase-arrested oocytes results from a balance between PKA and PP2A-B55 δ activities in favor of the kinase. Upon hormonal stimulation, PP2A-B55 δ activity remains unchanged while PKA is downregulated, leading to the partial dephosphorylation of Arpp19 at S109 that unlocks the prophase arrest. Therefore, the timing of meiosis resumption relies on the temporal coordination of S109 and S67 phosphorylations of Arpp19, orchestrated by one single phosphatase, PP2A-B55 δ , opposing two kinases, PKA and Gwl". The authors have not provided any shred of scientific evidence that proved that "PP2A-B55 δ was the protein phosphatase that dephosphorylated serine109 of Arpp19, that the dephosphorylation reaction was connected to resumption of meiosis, that the level of Arpp19 phosphorylated at S109 in prophase-arrested oocytes results from a

balance between PKA and PP2A-B55δ activities in favor of the kinase, that upon hormonal stimulation, PP2A-B55δ activity remains unchanged while PKA is downregulated, leading to the partial dephosphorylation of Arpp19 at S109 that unlocks the prophase arrest and that the timing of meiosis resumption relies on the temporal coordination of S109 and S67 phosphorylations of Arpp19, orchestrated by one single phosphatase, PP2A-B55δ, opposing two kinases, PKA and Gwl".

The authors of the paper [9] proclaimed that they identified PP-2AB55δ as the protein phosphatase that is responsible for dephosphorylating serine 109 of ARPP19. The authors used GST-tagged Arpp19 previously *in vitro* phosphorylated at S109 by PKA (pS109-GST-Arpp19) as substrate to assay for protein phosphatase(s). In Figure 1 of the paper [9], the protein phosphatase that acted on pS109-GST-Arpp19 was determined by Western Immunoblotting of pS109-GST-Arpp19 following incubation with extracts of prophase *Xenopus* oocytes in the presence of 1 or 10 μM okadaic acid. The authors observed dephosphorylation of pS109-GST-Arpp19. However, 1 or 10 μM okadaic acid would not allow them to determine the nature of the protein phosphatase. It is noteworthy that according to Figure 1C, almost ~30 % of total protein phosphatase activity was not inhibited by 10 μM okadaic acid, assuming that the authors knew how to assay for total protein phosphatase activity. A large proportion of PP-1 is in the form of PP-1_i which is inhibited by high concentration of ATP and requires Mg²⁺ and low concentration of ATP to exhibit full activity [110,111,151-156] and a large proportion of PP-2A is in its latent form and requires activating molecules to exhibit full activity [36,121]. The result of Figure 1C showed that ~30% of total protein phosphatase activity was not due to PP-1 and PP-2A, and that the amount of total protein phosphatase activity being measured was incorrect and misleading.

In Figure 2, the authors of the paper [9] used ammonium sulfate precipitation to apparently separate PP-1 and PP-2 activities. The result of Figure 2 was meant to show that PP-2A could be recovered with 40% ammonium sulfate precipitation which contained PP-2A and a small amount of PP-1 was the predominant protein phosphatase that acted upon pS109-GST-Arpp19. This is a misleading statement since the specific

activity of PP-1 is an order of magnitude higher than PP-2A. This is basic enzymology. The fact that one sees less protein does not mean that its activity is lower. The activity of the enzyme depends on its specific activity not on the amount of protein one sees.

In Figure 3B, the authors of the paper [9] used Mono Q column to separate the protein phosphatase activities that acted upon pS109-GST-Arpp19 and stated that PP-2A-B55d was the main protein phosphatase that acted upon pS109-GST-Arpp19. Unfortunately, the statement is misleading because: (i) the peak of activity does not coincide with the peak of protein represented by PP-2A-B55d. Most of the protein phosphatase activity was accounted by PP-2A-B56'-E and also by PP-1, PP-4 and PP-6. It is quite puzzling that the authors did not probe for B55 α , B55 β and other B' and B'' subunits of PP-2A. As stated above, the authors have failed to assay for PP-1_I and PP-2A₀. The results of Figure 3B is therefore meaningless and the statement that PP-2A-B55d was the major protein phosphatase that dephosphorylated pS109-GST-Arpp19 is misleading at best and dishonest at worse.

In Figure 4, the authors of the paper [9] used Fraction 5 from the eluates of the Mono Q column and loaded it onto a Phenyl Superose column followed by gel Filtration on Superose 10. Based on probing with anti-PP-2A-B55d antibodies, the authors concluded that they have isolated the main protein phosphatase that acted upon pS109-GST-Arpp19. It is quite puzzling that the authors did not probe for B55 α , B55 β and other B' and B'' subunits of PP-2A. The results of Figure 4 do not answer the question asked and the statement that PP-2A-B55d was the major protein phosphatase that dephosphorylated pS109-GST-Arpp19 is misleading at best and dishonest at worse. One can observe that PP-1 would be in the Flow Through Fractions of the Phenyl Superose Chromatography. Since the Flow Through Fractions were not analyzed and were discarded, PP-1 would not be observed in the subsequent Superose 10 Gel Filtration Chromatography.

In Figure 5, the authors of the paper [9] used Fractions 7 and 8 from the eluates of the Mono Q column, pooled them and loaded them onto a Phenyl Superose Column followed by Gel Filtration on Superose 10. Based on probing with anti-PP-2A-B55d antibodies, the

authors concluded that they have isolated the main protein phosphatase that acted upon pS109-GST-Arpp19. It is quite puzzling that the authors did not probe for B55 α , B55 β and other B' and B'' subunits of the PP-2A. The results of Figure 5 did not show that PP-2A-B55 δ was the major protein phosphatase that dephosphorylated pS109-GST-Arpp19 and the statement that PP-2A-B55 δ was the major protein phosphatase that dephosphorylated pS109-GST-Arpp19 is misleading at best and dishonest at worse. In fact, Figure 5 showed that a large proportion of the protein phosphatase that acted upon pS109-GST-Arpp19 was in fact a form of PP-1 because PP-1 is recovered in the Flow Through Fractions of the Phenyl Superose Chromatography and would not be observed in the subsequent Superose 10 Gel Filtration Chromatography. In order to prove that a protein phosphatase is responsible for the dephosphorylation of serine 109 of Arpp19, proper Enzymology and Enzyme Kinetics must be performed. The K_m and V_{max} of the dephosphorylation of pS109-GST-Arpp19 by various purified protein phosphatases including, PP-1C, PP-1^I, PP-2A-B55 α , PP-2A-B55 β , PP-2A-B55 γ , PP-2B and PP-2C, PP-4, PP-5 and PP-6 must be determined and compared.

In Figures 6, 7 and 8 of the paper [9], the authors purported to show and claim that they were measuring the activity of PP-2A-B55 δ , an assumption that is based solely on the results of Figures 3,4 and 5 which as indicated above are at best misleading and at worse dishonest. The authors have provided no scientific evidence that PP-2A-B55 δ is the protein phosphatase that dephosphorylated pS109-GST-Arpp19. In fact if the results of Figure 5 were to be believed and interpreted correctly, a form of PP-1 was also a major protein phosphatase that can dephosphorylate pS109-GST-Arpp19.

To then come up with a scheme that depicts Arpp19 as both an inhibitor and a substrate of PP-2A-B55 δ requires a lot of imagination and mental acrobatics that has more to do with science fiction than science. The first part of the scheme depicted in Figure 9 of the paper [9], it was claimed that at prophase, PP-2A-B55 δ is responsible for the dephosphorylation of serines 67 and 109. The authors have provided zero scientific evidence that PP-2A-B55 δ is the enzyme that specifically dephosphorylated serines 67 and 109 of Arpp19 (See above). The only paper that alluded that a form of PP-2A,

namely PP-2A-B55 α and not PP-2A-B55 δ is capable of dephosphorylating serine 67 of Arpp67 is itself riddled with anomalies and unscientific data [7]. As discussed in [109], the paper in question did not show that that PP-2A-B55 α was the protein phosphatase that dephosphorylated serine 67 of Arpp19.

In the second part of the scheme, the authors of the paper [9] showed that upon stimulation with progesterone, PKA is inhibited, resulting in dephosphorylation of serine 109 of Arpp19 by PP-2A-B55 δ . As stated above, the authors have provided zero scientific evidence that PP-2A-B55 δ is the enzyme that specifically dephosphorylates serines 67 and 109 of Arpp19. In order to show that of PP-2A-B55 δ is the protein phosphatase that dephosphorylate serines 67 and 109 of Arpp19, the authors must determine K_m and V_{max} values for the dephosphorylation of pure Arpp19 phosphorylated at serine 67 and pure Arpp19 phosphorylated at serine 109 by PP-1 $_C$, PP-1 $_I$, PP-2A $_C$, PP-2A $_2$, PP-2A-B55 α , PP-2A-B55 β , PP-2A-B55 γ , PP-2A-B55 δ , different forms of PP-2A-B', different forms of PP-2A-B'', PP-2B, PP-2C, PP-4, PP-5 and PP-6, and compare the generated K_m and V_{max} values.

In the third part of the scheme, the authors of the paper stated that Greatwall kinase phosphorylates Arpp19 at serine 67 converting it to a specific inhibitor of PP-2A-B55 δ . As stated above, the evidence that showed that Arpp19 phosphorylated at serine 67 was a specific inhibitor of PP-2A-B55 δ is misleading at best and dishonest at worse. In order to show that Arpp19 phosphorylated at serine 67 is a specific inhibitor of PP-2A-B55 δ , the authors must do proper Enzymology and Enzyme Chemistry work. They must do the following: (i) Show that pure Arpp67 phosphorylated at serine 67 is a specific inhibitor of pure preparations of PP-2A-B55 δ and not of pure preparations of PP-1 $_C$, PP-1 $_I$, PP-2A $_C$, PP-2A $_2$, PP-2A-B55 α , PP-2A-B55 β , PP-2A-B55 γ , different forms of PP-2A-B', different forms of PP-2A-B'', PP-2B, PP-2C, PP-4, PP-5 and PP-6 (ii) Plot proper Dose Response Inhibitory Curve of % of Control v. Concentration of pure Arpp19 phosphorylated at serine 67 and determine the IC_{50} of each inhibition (iii) determine the inhibition of PP-2A-B55 δ by pure Arpp19 phosphorylated at serine 67 using a number of at least 5

different substrates to show that the effect of Arpp19 phosphorylated at serine 67 is not substrate directed but enzyme directed.

The authors of the paper [9] stated that "initiation of meiosis by progesterone in *Xenopus* oocytes occurs via inhibition of PKA but that PP-2A-B55d is not activated". The authors admit that they could not show the binding of Arpp19 phosphorylated at serine 109 to PP-2A-B55d nor can they show any effect of Arpp19 phosphorylated at serine 109 on PP-2A-B55d activity. "Phosphorylation of serine 67 of Arpp19 by Greatwall kinase converts Arpp19 into a specific inhibitor of PP-2A-B55d". The scheme provided by the authors is not founded on verifiable scientific results and do not take into account the seminal work of Huchon et al. [149] which showed that PP-1-I1 which becomes a specific inhibitor of PP-1 when it is phosphorylated on threonine 35 by PKA interferes with Progesterone effect in *Xenopus* oocytes arrested at prophase and the paper by Foulkes and Maller [150] which showed that PP-1-I2 delays the effect of progesterone in *Xenopus* oocytes arrested at prophase, nor does the scheme reconciled itself with the work of Duckworth et al. [107] which showed that (i) Cdc25, the activator of Cdk1 is itself phosphorylated by PKA on serine 287 which inhibited Cdc25, (ii) serine 287 is dephosphorylated in response to progesterone just before cdc2 dephosphorylation and M-phase entry, (iii) high PKA activity maintains phosphorylation of Ser-287 *in vivo*, and (iv) inhibition of PKA by its heat-stable inhibitor (PKI) induces dephosphorylation of Ser-287, and the work of Margolis et al. [115,116] which showed that (i) PP-1 was required to dephosphorylate serine 287 of cdc25, (ii) the effect of PP-1 on cdc25 was antagonized by PP-1-I1, (iii) PP-1 forms a complex with cdc25, and (iv) and that dephosphorylation of serine 287 of cdc25 is masked by 14-3-3 during interphase, Why the authors of the paper [9] ignored PP-1-Inhibitor-1 which is a key substrate of PKA in the cell is beyond comprehension. It is not clear how dephosphorylation of serine 109 of Arpp19 by PP-2A-B55d would affect the activity of Arpp19 as an inhibitor of PP-2A-B55d. Does phosphorylation of serine of 109 of Arpp19 converts Arpp19 to a worse substrate for Greatwall kinase? Does dephosphorylation of serine 109 of Arpp19 converts Arpp19 to a better substrate for Greatwall kinase?. These are the questions that must be answered if a role for the dephosphorylation of serine 109 of Arpp19 is to be found.

These kind of studies will require proper Enzyme Chemistry and Enzyme Kinetics works (tasks that the authors of the paper/manuscript did not seem to know about) and not Western Immunoblotting type work. Mochida [6] showed and stated that phosphorylation of α -Endolfin on serine 109 by PKA antagonized the effect of α -Endolfin phosphorylated on threonine 28 by Cdk1 on PP-2A-B55d by shifting the IC₅₀ from 130 nM to 700 nM (if the results are to be believed as the author of the paper [6] did not seem to know how to perform a protein phosphatase inhibitory assay). If the effect of the phosphorylation of α -Endolfin on serine 109 by PKA can be repeated with Arpp19, it follows that the function of Arpp19 phosphorylated on serine 109 by PKA would only serve to antagonize the inhibition of PP-2A-B55d by Arpp phosphorylated on threonine 28 by Cdk1 and not to keep *Xenopus* oocytes arrested at prophase as suggested in [5,9,143].

The results described by Dupre et al. [5], Lemmonier et al. [9] and Dupre et al. [143] are at variance with those of Hara et al. [163] which showed that "Greatwall kinase is not essential for G2/M transition and full activation of cyclin B-Cdk1 in Starfish oocytes treated with 1-MeAde". Okumura et al. [8] explained the non-essentiality of Greatwall kinase in Starfish oocytes by invoking the phosphorylation of Arpp19 on threonine 28 by Cdk1 converting the former into a moderate inhibitor of reconstituted PP-2A-B55d. However, the inhibition of reconstituted PP-2A-B55 by Arpp19 phosphorylated on threonine 25 by Cdk1 is hardly if at all convincing. In Figure 3C of their paper, Okumura et al. [8] showed that thiophospho-Arpp19 phosphorylated on serine 69 (the equivalent site in *Xenopus* is threonine 28) by Cdk1 inhibited reconstituted PP-2A-B55d by approximately less than 50% whereas thiophospho-Arpp19 phosphorylated on serine 106 (the equivalent site in *Xenopus* is serine 67) by Greatwall kinase inhibited PP-2A-B55d by ~70%. First, without determining the IC₅₀ of the inhibitions of reconstituted PP-2A-B55d, the results are meaningless because it is impossible to tell whether the observed effects are enzyme directed or substrate directed or both. In view of the fact that despite the fact that the concentration of thiophospho-Arpp19 was present in excess of PP-2A-B55d by a factor of at least 4 and that only ~50% inhibition could be observed, it would imply that the effects thiophospho-Arpp19 were most probably substrate directed.

However, since no information with respect to the amount and concentration of the substrate was provided, it is impossible to tell whether the effects of thiophospho-Arpp19 was enzyme or substrate directed or both. This would be significant because, if the effects of thiophospho-Arpp19 was substrate directed, it would imply that there would be virtually no inhibition of PP-2A-B55 δ in the Star fish oocytes. Second, the authors, Okumura et al. [8] simply stated in the Method Section that PP-2A-B55 δ was assayed for its ability to dephosphorylate ^{32}P -labeled Fizzy-pSer50 (5000 cpm) in the presence of thiophospho-Arpp19 phosphorylated by Cdk1 but did not state what the specific radioactivity of the substrate was. Could the authors have accurately measure less than 500 cpm of ^{32}P -labeled phosphate released in the control and less than 250 cpm ^{32}P -labeled phosphate released in the experiment in which thiophospho-Arpp was added. (in order to be in the linear part of the enzyme reaction, it is important to assay less than 10% dephosphorylation of the substrate). As stated above, in order to show that Arpp19 phosphorylated on serine 69 by Cdk1 is a specific inhibitor of PP-2A-B55 δ , the authors must perform the following: The effects of different concentrations of thiophospho-Arpp19, phospho-Arpp19, dephospho-Arpp19 on the activities of PP-2A α , PP-2A-B55 α , PP-2A-B55 β , PP-2A-B55 γ , PP-2A-B55 δ , PP-2A-B'56 α , PP-2A-B'56 β , PP-2A-B'56 γ , PP-2A-B'56 δ , PP-2A-B'56 ϵ , PP-2A-B''70, PP-2A-B''130, PP-1 α , PP-1 β , PP-2B, PP-2C, PP-4 and PP-5 must be determined and a proper dose response inhibitory curve of % Control v. Concentration of inhibitors must be plotted to determine the specificity of the inhibitory effect (if any) of thiophospho-Arpp19 and phospho-Arpp19 and the IC₅₀ of the inhibition of PP-2A by Arpp19 and phospho-Arpp19.

The results described by Dupre et al. [5], Lemmonier et al. [9] and Dupre et al. [143] are in stark contradiction with the work of Adhikari et al. [164] which showed that (i) Mastl kinase (equivalent of Greatwall kinase) was not required for the resumption of meiosis and progression to metaphase I in mouse oocytes, (ii) Mastl-deficient oocytes did not fail to progress through meiosis I but underwent GVBD with kinetics and efficiencies that were indistinguishable from those of Mastl^{+/+} oocytes, (iii) Mastl has no essential role during meiotic resumption or prometaphase I progression in mouse oocytes, (iii) there might be some slight differences in the timing of completion of meiosis I between control

and mutant oocytes in vivo, (iv) *Mastl* was required for the timely activation of APC/C that was needed for the completion of meiosis I in mouse oocytes, (v) The activation of spindle assembly checkpoint occurred normally in *Mastl* deficient oocytes, (vi) The level of Cdc20 in *Matl* deficient oocytes remained low compared to normal oocytes, (vii) *Mastl* was essential for MII entry, (viii) *Mastl* was not required for the upregulation of Cdk1 activity during prometaphase I but was required for entry in MII, (ix) PP-2A activity in both *Mastl* deficient and normal oocytes were comparable at GV and GVBD stages, implying that mouse oocytes progress through metaphase I without the suppression of PP-2A activity and that *Mastl* did not regulate PP-2A activity during prometaphase I, and (x) *Mastl* likely play an essential role in triggering the activation of APC/C that is required for the down regulation of Cdk1 activity that might mediate the timely onset of anaphase I in mouse oocytes.

To summarize, the papers by Dupre et al. [5], Lemmonier et al. [9] and Dupre et al. [143] are just copycat papers that hinge on the misplaced assumption that Arpp19 is a specific inhibitor of PP-2A-B55 δ when the latter is phosphorylated by Greatwall kinase (recently reviewed in [109,147]. Now, the authors of the paper [9] make further statements that are at best misleading and at worse dishonest as they have provided no scientific verifiable evidence that PP-2A-B55 δ is the protein phosphatase that dephosphorylates both serine 67 and serine 107 of Arpp19. The authors of the papers by Dupre et al. [5], Lemmonier et al. [9] and Dupre et al. [139] do not seem to be able to decide what exactly Arpp19 does in *Xenopus* oocytes. On the one hand, they claimed that Arpp19 phosphorylated on serine 67 by Greatwall kinase became a specific inhibitor of PP-2A-B55 (although they have not provided any scientific evidence to show that it was so) and that that reaction controlled resumption progression of meiosis. On the other hand, they claimed that Arpp19 phosphorylated on serine 109 by PKA prevented the resumption of meiosis but did not state how. The authors of the Dupre et al. [5], Lemmonier et al. [9] and Dupre et al. [139] completely ignored published works that contradicted their work and did not provide any reasons to the readers and Scientific Researchers who are trying to decipher who is/are telling the truth and whether to spend resources in man hours to try to repeat their published works that are at best misleading

and at worse dishonest. The idea that a form of PP-2A could undergo marked inhibition during resumption of meiosis is not compatible with the known function of PP-2A as a key controller of cell transformation and as tumor suppressor [85-100).

The role of PP-2A in the control of meiosis.

The following is a more realistic model for the role of PP-2A in the control of meiosis. The model depicted below takes into account several published works, including those of Huchon et al. [149], Foulkes, G. and Maller, J. [150], Tung et al. [110,111,151], Puntoni and Villa-Moruzzi [152,153], Li et al [160], Margolis et al. [27], Margolis et al. [115,116], Wu et al. [117], Duckworth et al. [107], Brautigan et al. [166], Fernandez et al. [167], Maller and Krebs [167], Huchon et al. [169], and Adhikari et al. [164]. Although some of the works referenced here have more to do with mitosis, there is currently just not enough rigorous scientific research by knowledgeable scientific researchers taking place in the areas of meiotic control by PP-2A. The model below will be updated when more results become available.

Figure 2. Role of PP-2A in the control of meiosis resumption.

References.

1. Mochida, S., Maslen, S.L., Skehel, M. and Hunt, T.(2010) *Science* (2010) Vol 330, pp1670-1673.
Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis.
2. Gharbi-Ayachi, A., Labbe, J.C., Burgess, A., Vigneron, S., Strub, J.M., Brioude, E., Van-Dorsselaer, Castro, A. and Lorca, T. (2010) *Science* (2010) Vol. 330, pp 3916-3926
The substrate of Greatwall kinase, Arpp19 controls mitosis by inhibiting protein phosphatase-2A",
3. Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. (2009) *EMBO J.* Vol. 28, pp2777-2785.
Regulated activity of PP-2A-B55 δ is crucial for controlling entry into and exit from mitosis in *Xenopus* egg",
4. Kim, M.Y., Bucciarelli, E., Morton, D.G., Williams, B.C., Blake-Hodek, K., Pellacani, C., Von Stetina, J.R., Hu, X., Somma, M.P., Drummond-Barbosa, D. and Goldberg, M.L. (2012) *Genetics*, Vol. 191, pp1181-1197.
Bypassing the Greatwall-Endosulfine pathway: plasticity of a pivotal cell-cycle regulatory module in *Drosophila melanogaster* and *Caenorhabditis elegans*.
5. Dupre, A. et al. (2013) *J Cell Sci* **126**, 3916-3926 (2013).
The phosphorylation of ARPP19 by Greatwall renders the autoamplification of MPF independently of PKA in *Xenopus* oocytes.
6. Mochida, S. (2013) *FEBS J.*, Vol. 281, pp1159-1169.
Regulation of α -endosulfine, an inhibitor of protein phosphatase-2A by multisite phosphorylation.
- 7.. Williams, B.C., Filter, J.J., Blake-Hodek, K.A., Fuda, N.J., Shalloway, D. and Goldberg, M.L. (2014) *eLife*, e01695, doi: 10.7554/elife.01695.
Greatwall-phosphorylated Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers.
8. Okumura, E., Morita, A., Wakai, M., Mochida, S., Hara, M. and Kishimoto, T. (2014) *J. Cell Biol.* Vol 204, pp881-889.

- Cyclin B-Cdk1 inhibits protein phosphatase-PP2A-B55 via a Greatwall kinase-independent mechanism.
9. Lemonnier, T. , Maria Daldello, E. , Poulhe, E. , Tran Le, T. , Miot, M. , Jessus, C. and Dupré, A. (2019) bioRxiv (2019) doi: <http://dx.doi.org/10.1101/810549>
The M-phase regulatory phosphatase PP2A-B55 δ opposes protein kinase A on Arpp19 to initiate meiotic division.
 10. Morgan, D. (1995) *Nature*, Vol. 374, pp131-134.
Principles of CDK regulations.
 11. Meijer, L., Guidet, S. and Tung, H.Y.L. (1995) (eds) in *Progress in cell cycle research*, Vol. 1, Plenum Press, New York.
 12. Doree, M. and Hunt, T. (2002) *J. Cell. Sci.* Vol. 115, pp2461-2464
From cdc2 to Cdk1: when did the cell cycle kinase join its cyclin partner.
 13. Nurse, P. (2002) *ChemBioChem*, Vol. 3, pp596-603.
Cyclin dependent kinases and cell cycle control
 14. Hartwell, L.H. (2002) *Biosci. Rep.* Vol 22, pp373-394.
Yeast and Cancer.
 15. Morgan, D.O. (2007) *The cell cycle: Principles of control*, London, New Science Press Ltd.
 16. Harashima, H., Dissmeyerr, N. and Schnittger, A. (2013) *Trends Cell Biol.*, Vol. 3, PP345-356.
Cell cycle Control across the eukaryotic kingdom.
 17. Arata, Y. and Takagi, H. (2019) *Front. Physiol*,
<https://doi.org/10.3389/Phys.2019.0122>.
Quantitative studies for cell division cycle control.
 18. Dunphy, W.G. and Kumagai, A. (1991) *Cell*, Vol 67, pp189-196.
The Cdc25 protein contains an intrinsic protein phosphatase activity.
 19. Gautier, J., Salomon, M.J., Booher, R.N., Bazan, J.F. and Kirshner, M.W. (1991) *ell*, Vol. 67, pp197-211.
Cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2.
 20. Strausfeld, M., Labbe, J.C., Fesquet, D., Cavadore, J.C., Picard, A., Sadhu, K., Russell, P. and Doree, M. (1991) *Nature*, Vol 351, pp242-245.

- Dephosphorylation and activation of a p34^{cdc2}/cyclin B complex in vitro by human CDC25 protein
20. Lee, M.S., Ogg, S., Xu, M., Parker, L.L., Donoghue, D.J., Maller, J.L. and Piwnica-Worms, H. (1992) *Mol. Biol. Cell.* Vol.3, pp73-84.
Cdc25+ encodes a protein phosphatase that dephosphorylates p34cdc2.
21. Sebastian, B., Kakizuka, A. and Hunter, T. (1993) *proc. Natl. Acad. Sci.*, Vol 90, pp3521-3524.
Cdc25M2 activation of cyclin-dependent kinases by dephosphorylation of threonine-14 and tyrosine-15.
22. Ishida, Y., Furukawa, Y., Decaprio, J.A., Saito, M. and Griffin, J.D. (1992) *J. Cell. Physiol.*, Vol. 150, pp484-492.
Treatment of myeloid leukemia cells with the phosphatase inhibitor okadaic acid induces cell cycle arrest at either G1/S or G2/M depending on dose.
23. Tournebise, R., Anderson, S.S., Verde, F., Doree, M., Karsenti, E. and Hyman, A. (1997) *EMBO J.*, Vol. 16, pp5537-5549.
Distinct roles of PP1 and PPA-like phosphatases in control of microtubule dynamics during mitosis.
24. Clark, P.R., Hoffmann, I., Draetta, G. and Karsenti, E. (1993) *Mol. Cell. Biol.* Vol. 4, pp397-411.
Dephosphorylation of Cdc25 by a type 2A protein phosphatase: specific regulation during the cell cycle.
24. Mayer-Jackel, R.E., Phkura, H., Gomes, R., Sunkel, C.E., Baumgartner, S., Hemmings, B.A. and Glover, D.M. (1993) *Cell*, Vol. 73, pp621-633.
The 55 kd regulatory subunit of Drosophila protein phosphatase-2A is required for anaphase.
25. Majer-Jackel, R.E., Ohkura, H., Ferrigno, P. Andjelkovic, N., Shiohu, K., Uemura, T., Glover, D.M. and Hemmings, B.A (1994) *J. Cell. Sci.*, Vol. 107, pp2609-2616.
Drosophila mutants in the 55kDa regulatory subunit of protein phosphatase-2A show strongly reduced ability to dephosphorylate substrates of p34cdc2.

26. Hutchins, J.R., Dikovskaya, D. and Clarke, P.R. (2002) FEBS Lett. Vol 528, pp267-271.
Dephosphorylation of the inhibitory phosphorylation site S287 in Xenopus Cdc25C by protein phosphatase-2A is inhibited by 14-3-3 binding.
27. Margolis, S.S., Perry, J.A., Forrester, C.M., Nutt, L.K., Gup, Y., Jardim, M.J., M.J., Freel, C.D., Darbandj, A., Ahn, J.H., Arroyo, J.D., Wang, X.F., Shenolikar, S., Nairn, A.G., Dunphy, W.G., Virshup, D.M. and Kornbluth, S. (2006) Cell, Vol. 127, pp759-773.
Role for the PP2A/B56 delta phosphatase in regulating 14-3-3 release from Cdc25 to control mitosis.
28. Forrester, C.M., Maddox, J., Louis, J.V., Goris, J. and Virshup, D. (2007) Proc. Natl. Acad. Sci., Vol. 104, pp19867-19872.
Control of mitotic exit by PP2A regulation of Cdc25 and Cdk1.
29. Mochida, S., Ikeo, S., Gannon, J. and Hunt, T. (2009) EMBO J. Vol. 28, pp2777-2785.
Regulated activity of PP-2A-B55d is crucial for controlling entry into and exit from mitosis in Xenopus egg",
30. Castilho, P.V., Williams, B.C., Mochida, S., Zhao, Y. and Goldberg, M.L. (2009) Mol. Biol. Cell., Vol 20, pp4777-4789.
The M phase kinase Greatwall (Gwl) promotes inactivation of PP2A/B55delta, a phosphatase directed against CDK phosphosites.
31. Vigneron, S., Brioudes, E., Burgess, A., Labbe, J.C., Lorca, T. and Castro, A. (2009) EMBO J., Vol 28, pp2786-2793.
Greatwall maintains mitosis through regulation of PP2A
32. Manchado, E., Guillamot, M., de Carcer, G., Euguren, M., Trickey, M., Garcia-Huguera, Moresnos, S., Yamano, H., Canamero, M. and Malumbres, M. (2010) Cancer Cell, Vol 18, pp641-654.
Modulation Cdk1, Mast1 and PP-2A/B55 α , δ phosphatase.
33. Schmit, M.-H.A., Held, M., Janssens, V., Hutchins, J.R.A., Hudercz, O., Ivanova, E., Goris, J., Trinkle-Mulcahy, L., Lamond, A.I., Poser, I., Hyman, A.A.,

- mechtler, K., Peters J.M. and Gerlich, D.W. (2013) *Nat. Cell Biol.* Vol 12, doi: 10.1038/ncb2092.
Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells.
34. Ingebritsen, T.S. and Cohen, P. (1983) *Science*, Vol 221, pp331-338.
Protein phosphatases: properties and role in cellular regulation.
 35. Tung, H.Y.L., Resink, T.J., Hemmings, B.A., Shenolikar, S. and Cohen, P. (1984) *Eur. J. Biochem.*, Vol. 138, pp 635-641.
 36. Tung, H.Y.L., Alemany, S. and Cohen, P. (1985) *Eur. J. Biochem.*, Vol. 148, pp253-263.
The protein phosphatases involved in cellular regulation: Purification, subunit structure and properties of protein phosphatases-2A₀, 2A₁, and 2A₂ from rabbit skeletal muscle.
 37. Cohen, P. (1989) *Annu. Rev. Biochem.* Vol. 58, pp453-508.
The Structure and regulation of protein phosphatases.
 38. Wera, S. and Hemmings, B.A. (1995) *Biochem. J.*, Vol 311, pp17-29.
Serine/threonine protein phosphatases.
 39. Millward, T.A., Zolnierowicz, S. and Hemmings, B.A. (1999) *Trend Biochem. Sci.*, Vol 24, pp186-191.
Regulation of protein kinase cascades by protein phosphatase-2A.
 40. Janssens, V. and Goris, J. (2001) *Biochem. J.*, Vol. 353, pp417-439.
Protein phosphatase-2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling.
 41. Sangodkar, J., Farrington, C.C., McClinch, Galsky, , Kastrinsky, D.B. and Naria, G. (2015) *FEBS J.*, Vol. 283, pp1004-1024.
All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase.
 42. Raman, D. and Pervaiz, S. (2019) *Redox Biol.*, Vol. 27, <https://doi.org/10.1016/J.redox.2019/101105>.
Redox inhibition of protein phosphatase PP2A: potential implications on oncogenesis and its progression.

43. Zolnierowicz, S., Van Hooh, C., Andjelkovic, N., Cron, P., Stevens, I., Merlevede, W., Goris, J. and Hemmings, B.A. (1996) *Biochem. J.* Vol 137, pp187-194.
The variable subunit associated with protein phosphatase 2A₀ defines a novel multimember family of regulatory subunits.
44. Hojlund, K., Poulsen, M., Staehr, P. Brusgaard, K. and beck-Nielsen, H. (2002) *Eur. J. Clin. Invest.*, Vol 32, pp918-923.
Effect of insulin on protein phosphatase 2A expression in muscle in type 2 diabetes.
45. Ugi, S., Imamura, T., Maegawa, T., Shi, K., Obata, T., Ebina, Y., Kashiwagi, A. and Olefsky, J.M. (2004) *Mol. Cell. Biol.*, Vol. 19, pp 8778-8789.
Protein phosphatase-2A negatively regulates insulin's metabolic pathway by inhibiting Akt (protein kinase B) activity in 3T3 L1 adipocytes.
46. Mandavia, C. and Sowers, J. (2012) *Cardiorenal Med.*, Vol. 2, pp308-33.
Phosphoprotein phosphatase PP2A regulation of insulin receptor substrate 1 and insulin metabolic signaling.
47. Galbo, T, Olsen, G.S., Quistorff, B. and Nishimura, E. (2011) *Plos One*, <https://doi.org/10.1371/journal/pone.00274424>.
Free fatty acid-induced PP2A hyperactivity selectively impairs hepatic insulin action on glucose metabolism.
48. Tsuchiya, A., Kanno, T. and Nishizaki, T. (2014) *Cell. Phys. & Biochem.*, Vol 34, pp617-627.
Dipalmitoleoyphosphoethanolamine as a PP2A enhancer obstructs insulin signaling by promoting ser/thr dephosphorylation of Akt.
49. Mulkey, R.M., Herson, C.E. and Malenka, R.C. (1993) *Science*, Vol. 261, pp1051-1056.
An essential role for protein phosphatases in hippocampal longterm depression.
50. Vogelsberg-Ragaglia, V., Schuck, T., Trojanowsky, J.Q. and Lee, V.M. (2001) *Exp. Neurol.*, Vol. 168, pp402-412.
PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus.

51. Kins, S., Crameri, A., Evans, D.R., Hemmings, B.A., Nitsch, R.M. and Golz, J. (2001) *J. Biol. Chem.*, Vol. 276, pp38193-38200.
Reduced protein phosphatase 2A activity induces hyperphosphorylation and altered compartmentalization of tau in transgenic mice.
51. Sontag, E. (2004) *J. Neuropathol. Exp. Neurol.*, Vol 63, pp287-301.
Altered expression levels of the protein phosphatase 2A.ABC enzyme associated with Alzheimer's disease.
52. Beaulieu, J.-M., Sotnikova, T.D., Marion, S., Lefkowitz, R.J., Gainetdinov, R.P. and Caron, M.G. (2005) Vol. 261-273.
53. Kirchhefer, H., Brekle, C., Eskander, J., isensee, G., Kucerovala, D., Muller, F.U., Pinet, F., Schulte, J.S., Seldi, M.D. and Noknik, P. (2014) *J. Biol. Chem.*, Vol. 289, pp33862-33873.
Cardiac function is regulated by B56 α mediated targeting of protein phosphatase-2A to contractile relevant substrates.
54. Naraki, Y., Watanable, W. and Takeya, K. (2019) *J. Smooth. Mus. Res.*, Vol. 55, pp14-22.
Inhibitory effect of rubratozin A, a potent inhibitor of protein phosphatase 2 on the Ca²⁺-dependent contraction of skinned carotid artery from ginea pig.
55. Ballou, L.M., Geno, P. and Thomas, G. (1988) *J. Biol. Chem.*, Vol 263, pp1188-1194.
Protein phosphatase-2A inactivates the mitogen stimulated S6 kinase from Swiss 3T3 cells.
56. Seeling, J.M., Miller, R., Gil, R., Moon, R.T., White, R. and Virshup, D.M. (1999) *Science*, Vol. 283, pp2089-2091.
Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A.
57. Petritsch, C., Beug, H., Balmain, A. and Oft, M. (2000) *Genes & Dev.*, Vol. 14, pp2093-3101
TGF β - inhibits p70S6 kinase via protein phosphatase-2A.
58. Van Kanegan, M.J., Adams, D.G., Wadzinski, B.E. and Strack, S. (2005) *J. Biol. Chem.*, Vol. 280, pp36029-36036.

- Distinct protein phosphatase 2A heterotrimers modulate growth factor signaling to extracellular signal regulated kinases and Akt.
59. Letourneux, C., Rocher, G. and Porteu, F. (2006) *EMBO J.*, Vol. 25, pp727-738.
B56 containing PP2A dephosphorylates ERK and their activity is controlled by the early gene 1EX1 and ERK.
60. Meijer, L., Pondaven, P., Tung, H.Y.L., Cohen, P. and Wallace, R.W. (1986) *Exp. Cell. Res.*, Vol. 163, pp489-499.
Protein phosphorylation and oocyte maturation: II. Inhibition of starfish oocyte maturation by intracellular microinjection of protein phosphatase 1 and 2A and alkaline phosphatase.
61. Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merleved, W. (1989) *FEBS Lett.*, Vol 245, pp91-94.
Inhibition of PP2A induces oocyte maturation and results in premature activation of cdc2/Cdk1.
62. Picard, A., Capony, J.P., Brautigan, D.L. and Doree, M. (1989) *J. Cell. Biol.*, Vol. 109, pp3347-3354.
Involvement of protein phosphatases 1 and 2A in the control of M phase-promoting factor activity in starfish.
63. Lee, T.H., Solomon, M.J., Mumby, M.J. and Kirschner, M.W. (1991) *Cell*, Vol. 64, pp415-423.
INH, a negative regulator of MPF, is a form of protein phosphatase-2A.
64. Clark, P.R., Hoffmann, I., Draetta, G. and Karsenti, E. (1993) *Mol. Biol. Cell.*, Vol 4, pp397-411.
Dephosphorylation of Cdc25-C by a type 2A protein phosphatase: Specific regulation during cell cycle in *Xenopus* egg extracts.
65. Ferrigno, P., Langan, T.A. and Cohen, P. (1993) *Mol. Biol. Cell.*, Vol 4, pp669-677.
Protein phosphatase-2A₁ is the major enzyme invertebrate cell extracts that dephosphorylates several physiological substrates for cyclin-dependent protein kinases.

66. Mc Cright, B., Rivers, A.M., Audlin, S. and Virshup, D.M. (1996) *J. Biol. Chem.*, Vol. 271, pp22081-22089.
The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation induced phosphoproteins that target PP2A to both nucleus and cytoplasm.
67. Everett, A.D., Kambiyashi, C. and Brautigan, D.L. (2002) *Am. J. Physiol. Lung. Cell Mol. Physiol.*, Vol 282, pp1266-1271.
Transgenic expression of protein phosphatase 2A regulatory subunit B56gamma disrupts distal lung differentiation.
68. Okamura, H., Yoshida, K., Ochai, K. and Haneji, T. (2011) *Bone*, Vol 49, pp368-367.
Reduction of protein phosphatase 2A C enhances bone formation and osteoblast differentiation through expression of bone-specific transcription factor osterix.
69. Okamura, H., Yang, D., Yoshida, K., Teramachi, J. and Hanji, T. (2014) *Biochim. Biophys. Acta.*, Vol. 1843, pp2376-2384.
Reduction of PP2A α stimulates adipogenesis by regulating the Wnt/GSK-3 β / β Catenin pathway and PPAR expression.
70. Xu, D., Jin, X., Zheng, M., Rohila, D., Fu, G., Wen, Z., Lou, J., Wu, S., Sloan, R., Wang, L., Hu, H., Gao, X. and Lu, L. (2019) *Proc. Natl. Acad. Sci.* Vol 115, pp982-987.
Phosphatase PP2A is essential for T_H17 differentiation.
71. Gotz, J., Probst, A., Ehier, E., Hemmings, B.A. and Kues, H. (1996) *Proc. Natl. Acad. Sci.* , Vol 95, pp12370-12375.
Delayed embryonic lethality in mice lacking protein phosphatase 2A catalytic subunit C alpha.
72. Yang, J., Wu, J., Tan, C. and Klein, P.S. (2003) Vol. 130, pp5669-5678.
PP2A:B56 is required for Wnt/ β catenin signaling during embryonic development.
73. Viquez, N.M., Fuger, P., Valaka, T.M. and DiAntonio, A. (2009) Vol 37, pp11484-11494.
PP2A and GSK β act antagonistically to regulate active zone development.

74. O'Shaughnessy, R.F.L., Welte, J.C., Sully, K. and Byrne, C. (2009) *Development*, Vol 136, pp3423-3431.
Akt-dependent PP2a activity is required for epidermal barrier formation during late embryonic development.
75. Li, D, Musante, V., Zhou, W., Picciotti, M.R. and Nairn, A.G. (2018) Vol. 293, pp11179-11194.
Striatin-1 is a B subunit of protein phosphatase PP2 A that regulates dendrite arborization and spine development in striatal neurons.
76. Rajala, A., Wang, Y., Abcomver, S.F., Gardner, T.W. and Rajala, R.V.S. (2018) *Oncotarget*, Vol. 9, pp1505-1523.
Development and light regulation of the tumor suppressor protein PP2A in the retina.
77. Ravulo, P.P., Deng, X., Ito, T., Carr, B.K. and May, W.S. (1999) *J. Biol. Chem.*, Vol. 274, pp20296-20300.
Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A.
78. Chiang, C.W., Harris, G., Ellig, C., Masters, S.C., Subramanian, S., Shenolikar, S., Wadzinski, B.E. and Yang, E. (2001) *Blood*, Vol. 97, pp1289-1297.
Protein phosphatase 2A activates proapoptotic function of BAD in interleukin 3-dependent lymphoid cells by a mechanism requiring 14-3-3 dissociation.
79. Chiang, C.W., Kanles, C., Kim, K.W., Fang, W.B., Parkhurst, C., Xie, M., Henry, T and Yang, E. (2003) *Mol. Cell. Biol.*, Vol 23, pp6350-6362.
Protein phosphatase 2A dephosphorylation of phosphoserine 112 plays the gatekeeper role for BAD-mediated apoptosis.
80. Alvarado-Kristensson, M. and Andersson, T. (2003) *J. Biol. Chem.*, Vol. 280, pp6238-6244.
Protein phosphatase 2A regulates apoptosis in neutrophils by dephosphorylating both p38 MAPK and its substrate.
81. Rey, R.M., Bhattacharya, S. and Johnson, L.R. (2005) *J. Biol. Chem.*, Vol 280, pp31091-31100.
Protein phosphatase-2A regulates apoptosis in intestinal epithelial cells.

82. Janoo, A., Morrow, P.W. and Tung, H.Y.L. (2005) *J. Cell. Biochem.* , Vol. 94, pp816-8125.
Activation of protein phosphatase-2A₁ by HIV-1 cell death causing peptide in intact CD⁴⁺ T cells and in vitro.
83. Boudreau, R.T., Conrad, D.M. and Hoskin, D.W. (2007) *Cell Signal.*, Vol 19, pp139-151.
Apoptosis induced by protein phosphatase 2A (PP2A) in T leukemic cells is negatively regulated by PP-2A associated p38 mitogen activated protein kinase.
84. Xiong, Y., Lan, J., Huang, K., Zhang, Y., Zheng, L., Wang, Y. and Ye, Q. (2019). *Apoptosis*, Vol. 24, pp921-933.
PP2Ac upregulates P13K-Akt signaling and induces apoptosis in liver donor after brain death.
85. Yang, Y.C., Hearing, P. and Rundell, K. (1979) *J. Virol.*, Vol. 32, pp147-152.
Cellular proteins associated with simian virus 40 early gene products in newly infected cells.
86. Rundell, K., Major, E.O. and Lampert, M. (1981) *J. Virol.*, Vol. 37, pp1090-1093.
Association of cellular 56,000- and 32,000- molecular weight protein with BK virus and polyoma virus T-antigens.
87. Pallas, D.C., Shahrik, L.K., Martin, L., Jaspers, S., Miller, D.L., Brautigan, D.L. and Roberts, T.M. (1990) *Cell*. Vol 60, pp167-176.
Polyoma small and middle T antigens and SV40 small T antigen form stable complexes with protein phosphatase 2A.
88. Walter, G., Ruediger, R., Slaughter, C. and Mumby, M. (1990) *Proc. Natl. Acad. Sci.*, Vol 87, pp2521-2525.
Association of protein phosphatase 2A with polyoma virus medium tumor antigens.
89. Chen, W., Possemate, R., Campbell, K.T., Plattner, C.A., Pallas, D.C. and Hahn, W.C. (2004) *Cancer Cell.*, Vol. 5, pp127-136.
Identification of specific PP-2A complexes involved in cell transformation.

90. Yeh, E., Cunningham, M., Arnold, H., Chasse, D., Monteith, J., Ivaldi, G. Hahn, W.C., Stukenberg, P.T., Shenolikar, S., Uchida, T., Counter, C.M., Nevins, J.R., Meams, A.R. and Sears, R. (2004) *Nature Cell Biol.*, Vol. 6, pp308-318.
A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells.
91. Rangarajan, A., Hong, S.J., Gifford, A. and Weinberg, R.A. (2004) *Cancer Cell.*, Vol. 6, pp171-183.
Type-species and cell specific requirements for cellular transformation.
92. Chen, W., Arroyo, J.D., Timmons, J.C., Possemato, R. and Hahn, W.C. (2005) *Cancer Res.*, Vol 65, pp8193-8199.
Cancer-associated PP2A-A α subunits induce functional haploinsufficiency and tumorigenicity.
93. Sabina, A.A., Hector, M., Colpaert, N. and Hahn, W.C (2010) *Cancer Res.* Vol. 70, pp10474-10484.
Identification of PP2A complexes and pathways in cell transformation.
94. Juntilla, M.R. Puustinen, P., Niemala, M., Sears, R., Kahari, V.-M., Westermarck, J., Ahola, R., Arnold, H., Bottzauw, T., Ala-aho, R., Nielsen, C., Ivasta, J., Taya, Y., Lu, S.-L., Lin, S., Chan, E.K.L., Wang, X.-J., Grenman, R., Kast, J. and Kallunki, T. (2007) *Cell*, Vol. 130, pp51-62.
CIP2A inhibits PP2A in human malignancies.
95. Janghorban, M., Farell, A.S., Allen-Petersen, B.L., Pelz, A.S., Daniel, C.J., Oddo, J., Langer, E.M., Christensen, D.J. and Sears, R.C. (2014) *Proc. Natl. Acad. Sci.*, Vol 111, pp9157-9162.
Targeting c-Myc by antagonizing PP2A inhibitors in breast cancer.
96. Janghorban, M., Langer, E.M., Wang, X., Zachman, D., Daniel, C.T., Hooper, J., Flemng, W.H., Agarwal, A., and Sears, R.C. (2017) *Plos One*, <https://doi.org/10.1371/journal.pone.0188910>.
The tumor suppressor phosphatase PP2A-B56 α regulates stemness and promotes initiation of malignancies in a novel murine model.

97. Wang, J., Pkkeri, J., Pavic, K., Wang, Z., Kauko, O., Holonen, T., Sarek, G., Ojala, P.M., Rao, Z., Xu, W. and Westermarck, J. (2017) *EMBO Rep.*, Vol. 18, pp437-450.
Oncoprotein CIP2A is stabilized via interaction with tumor suppressor PP2A/B56.
98. Lamprecht, C., Libbrecht, L., Sagaert, X., Pauwels, P., Hoorne, Y., Crowther, J., Louis, J.V., Sents, W., Sablina, A. and Janssens, V. (2018) *Oncogene*, Vol 37, pp544-552.
Loss of protein phosphatase-2A regulatory subunit B56 promotes spontaneous tumorigenesis.
99. Allen-Petersen, B.L., Risom, T., Feng, Z., Wang, Z., Jenny, Z.P., Thoma, M.C., Pelz, K.R., Morton, J.P., Sansom, O.J., Lopez, C.D., Shepperd, B., Christensen, D.J., Ohlmeyer, M., Naria, G. and Sears, R.C. (2019) *Cancer Res.* Vol 79, pp209-219.
Activation of PP2A and inhibition of mTor synergistically reduce Myc signaling and decrease tumor growth in pancreatic ductal adenocarcinoma.
100. Liu, C.Y., Huang, T.T., Chen, Y.T., Chen, J.L., Chu, P.Y., Huang, C.T., Wang, W.L., lau, K.Y., Dai, M.S., Shau, C.W. and Tseng, L.M. (2019) *E BioMedicine*, Vol 40, pp265-275.
Targeting Set to restore PP2A activity disrupts an oncogen CIP2A feedforward loop and impairs triple negative breast cancer progression.
101. Peng, C.Y., Graves, P.R., Thomas, R.S., Wu, Z., Shaw, A.S. and Piwnica-Worms, H. (1994) *J. Biol. Chem.*, Vol. 269, pp30461-30469.
Purification of serine kinase that associates with and phosphorylates human Cdc25C on serine 216.
102. Peng, C.Y., Graves, P.R., Thomas, R.S., Wu, Z., Shaw, A.S. and Piwnica-Worms, H. (1997) *Science*, Vol. 277, pp1501-1505.
Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25 serine 216.
103. Kumagai, A., Yakower, P. and Dunphy, W.G. (1998) *Mol. Biol. Cell.*, Vol 9, pp345-354.

- 14-3-3 proteins act as a negative regulators of the mitotic inducer Cdc25 in *Xenopus* egg extracts.
104. Peng, C.V., Graves, P.R., Ogg, S., Thoma, R.S., Byrnes, M.I. and Piwnica-Worms, H.C. (1998) *Cell Growth & Differ.*, Vol 9, pp197-208.
C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 binding.
 105. Zeng, Y., Forbes, K.C., Wu, Z., Moreno, S., Piwnica-Worms, H. and Enoch, T. (1998) *Nature*, Vol. 395, pp507-510.
Replication checkpoint requires phosphorylation of the phosphatase Cdc25 by Cds1 and Chk1.
 106. Bulavin, D.V., Higashimoto, Y., Popoff, I.J., Gaarde, W.A., Basrur, V., Potepova, O., Appela, E. and Fornace, Jr., A.J. (2001) *Nature*, Vol. 411, pp102-107.
Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase.
 107. Duckworth, B.C., Weaver, J.S. and Ruderman, J.V. (2002) *Proc. Natl. Acad. Sci.*, Vol. 99, pp16794-16799.
G2 arrest in *Xenopus* oocytes depends on phosphorylation of Cdc25 by protein kinase A.
 108. Manke, I.A., Nguyen, A., Lim, D., Stewart, M.Q., Elia, A.E.H. and Yaffe, M.B. (2004) *Mol. Cell.*, Vol 17, pp37-48.
MAPKAP kinase 2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation.
 109. Tung, H.Y.L. (2019) *J. Invest. Cri. Pub. Sci. Articles.*, Vol 1, pp126-136.
Post Publication Critique of the Paper Entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", authored By Moshida, S., Maslen, S.L., Skehel, M. and Hunt, T., and published in *Science* [Science (2010) Vol 330, pp1670-1673].
 110. Tung, H.Y.L. and Reed, L.J. (1989) *J. Biol. Chem.*, Vol. 264, pp2985-2990.
Purification and characterization of protein phosphatase-1_I activating kinase from bovine brain cytosolic and particulate fractions.
 - 111, Platholi, J., Heerdt, P., Tung, H.Y.L. and Hemmings, Jr., H.C. (2008)

- Activation of brain protein phosphatase-1_I following cardiac arrest and resuscitation involving an interaction with 14-3-3 γ .
112. Bosch, M., Cayla, X., Van Hoof, C., Hemmings, B.A., Ozon, O., Merlevede, W. and Goris, J. (1995) *Eur. J. Biochem.* Vol.230, Pp1037-1045.
The PR55 and PR65 subunits of protein phosphatase 2A from *Xenopus laevis*: Molecular cloning and developmental regulation of expression.
 113. Mochida, S. (2014) *FEBS J.*, Vol. 281, pp1159-1169.
Regulation of κ -endolfin, an inhibitor of protein phosphatase 2A, by multisite phosphorylation.
 114. Rangone, H., Wegel, E., Gatt, M.K., Yeung, E., Flowers, A., Janus, D., Dadlez, M., Janssens, V., Carpenter, A.T. and Golver, D.M. (2011) *Plos. Genet.*, Vol. 7, doi.10371/journal.pgen.1002225.
Suppression of Scant identifies Endos as a substrate of Greatwall kinase and a negative regulator of protein phosphatase 2A in mitosis.
 115. Margolis, S.S., Walsh, S., Weiser, D.C., Yoshida, M., Shenolikar, S., Kornbluth, S. (2003) *EMBO J.*, Vol 22, pp5734-5745.
PP1 control M phase entry exerted through 14-3-3-regulated Cdc25 dephosphorylation.
 116. Margolis, S.S., Perry, J.A., Weltzel, D.H., Freel, C.D., Yoshida, M., Haystead, T.A. and Kornbluth, S. (2006) *Mol. Biol. Cell.*, Vol 17, pp1779-1789.
A role for PP1 as the Cdc2/Cyclin B-mediated positive feedback activation of Cdc25.
 117. Wu, J.Q., Guo, J.Y., Tang, W., Yang, C.S., Freel, C.D., Chen, C., Nairn, A.C. and Kornbluth S. (2009) *Nat. Cell. Biol.*, Vol 11, pp644-651.
PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation.
 118. Hsu, J.-Y., Sun, Z.-W., Li, X., Reuben, M., Tatchell, K., Bishop, D.K., Gruschcow, J.M., Brame, C.J., Caldwell, J.A., Brame, C.J., Caldwell, J.A., Hunt, D.F., Lin, R., Smith, M.M. and Allis, C.D. (2000) *Cell*, Vol. 102, pp279-291.

- Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes.
119. Manchado, E., Guillaumot, M., de Cárcer, G., Eguren, M., Trickey, M., García-Higuera, I., Moreno, S., Yamano, H., Cañamero, M. and Malumbres, M. (2010) *Cancer Cell*, Vol 18, pp641-654.
Targeting mitotic exit leads to tumor regression in vivo: Modulation by Cdk1, Mastl, and the PP2A/B55 α , δ phosphatase.
 119. Schmitz, M.F.A., Held, M., Janssens, V., Hutchins, J.R.A., Hudecz, O., Ivanova, E., Goris, J., Trinkle-Mulcany, L., Lamond, A.I., Poser, I., Hyman, A.A., Mechtler, K., Peters, M. and Gerlich, D.M. (2010) *Nat. Cell. Biol.* Vol. 12, doi: 10.1038/ncb2092
Live-cell imaging RNAi screen identifies PP2A–B55 α and importin- β 1 as key mitotic exit regulators in human cells.
 120. Hegarat, N., Vesely, C., Vinod, P.K., Ocasio, C., Peter, N., Gannon, J., Oliver, A.W., Novák B. and Hochegger H. (2014) *Plos Genet.*, Vol 10, doi: 10.1371/journal.pgen.1004004.
PP2A/B55 and Fcp1 regulate Greatwall and Ensa dephosphorylation during mitotic exit.
 121. Tung, H.Y.L., Pelech, S., Fisher, M.J. and Pogson, C.I. and Cohen, P. (1985) *Eur. J. Biochem.*, Vol. 149, pp305-313.
The protein phosphatases involved in cellular regulation. Influence of polyamines on the activities of protein phosphatase-1 and protein phosphatase-2A.
 122. Usui, H.R., Inoue, O., Tanabe, Y., Nishito, M., Shimizu, H., Hayashi, H. and Kagamiyama, H. (1998) *FEBS Lett.*, Vol. 430, pp312-316.
Activation of protein phosphatase 2A by cAMP-dependent protein kinase-catalyzed phosphorylation of the 74kDa B" regulatory subunit in vitro and identification of the phosphorylation sites.
 122. Xu, Z. and Williams, B.R.G. (2000) *Mol. Biol. Cell.* Vol. 20, pp5285-5299.
The B56 α regulatory subunit of protein phosphatase-2A is a target for regulation by double stranded RNA-dependent protein kinase.

123. Ahn, J.-H., McAvoy, T., Rakhilin, S.V., Nishi, A., Greengard, P. and Nairn, A.C. (2007) *Proc. Natl. Acad. Sci.* Vol. 104, pp2979-2984.
Protein kinase A activates protein phosphatase 2A by phosphorylation of the B56 δ subunit.
124. Kishimoto, T. (2011) *Mol. Repro. Dev. Biol.*, Vol. 78, pp704-707.
A primer on meiotic resumption in starfish oocyte: the proposed signaling pathway triggered by maturation inducing hormone.
125. Maller, J.L. and Krebs, E.G. (1980) *Curr. Top. Cell. Regul.*, Vol. 16, pp271-311.
Regulation of oocyte maturation.
126. Byskov, A., G., Andersen, C.Y. and Leonardsen, L. (2002) *Nol. Cell. Endocrinol.*, Vol. 187, pp189-196.
Role of meiosis activating sterols, MAS in induced oocyte maturation.
127. Arion, D., Meijer, L., Brizuela, L. and Beach, D. (1988) *Cell*, Vol. 55, pp371-378.
Cdc2 is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF.
128. Labbe, J.C., Lee, M.G., Nurse, P., Picard, A. and Doree, M. (1988) *Nature*, Vol. 335, pp251-254.
Activation in M-phase of a protein kinase encoded by starfish homologue of the cell cycle gene *cdc2*⁺.
129. Lohka, M.J., Hayes, M.K. and Maller, J.L. (1988) *Proc. Natl. Acad. Sci.*, Vol. 85, pp3009-3013.
Purification of maturation-promoting factor, an intracellular regulator of early mitotic events.
130. Dunphy, W.G., Brizuela, L., Beach, D. and Newport, J. (1988) *Cell*, Vol. 54, pp423-431.
The *Xenopus cdc2* protein is a component of MPF, a cytoplasmic regulator of mitosis.
131. Labbé J. C., Capony J. P., Caput D., Cavadore J. C., Derancourt J., Kaghad M., Lelias J. M., Picard A., and Dorée M. (1989) *EMBO J.* Vol. 8, pp3053-3058.
MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of *cdc2* and one molecule of cyclin B.

132. Meijer, L., Arion, D., Golsteyn, R., Pines, J., Brizuela, L., Hunt, T. and Beach, D. (1989) *EMBO J.*, Vol. 8, pp2275-2282.
Cyclin is a component of the sea urchin egg M-phase specific histone H1 kinase.
133. Adhikiri, D., Zheng, W., Shen, Y., Gorre, N., Ning, Y., Halet, G., Kaldis, P. and Liu, K. (2012) *Human Mol. Genet.*, Vol 21, pp2476-2484.
Cdk1, but not Cdk2, is the sole Cdk that is essential and sufficient to drive resumption of meiosis in mouse oocytes.
134. Izumi, T., Walker, D.H. and Maller, J.L. (1993) *Mol. Biol. Cell.*, Vol 4, pp1337-1350.
Elimination of cdc2 phosphorylation sites in the Cdc25 phosphatase blocks initiation of M-phase.
135. Nebraska, A.R. and Ferby, I. (2000) *Curr. Opin. Cell. Biol.* Vol 12, pp266-275.
Regulation of meiotic cell cycle in oocytes.
136. Perdiguero, E. and Nebraska, A.R. (2004) *Cell Cycle*, Vol. 3, pp731-735.
Regulation of Cdc25C activity during G2/M transitions.
137. Wang, X., Swain, J.E., Bollen, M., Liu, X.T., Ohi, D.A. and Smith, G.D. (2004) *Reproduction*, Vol. 128, pp493-502.
Endogenous regulators of protein phosphatase 1 during mouse oocyte development and meiosis.
138. Wei, Y., Mizzen, C.A., Cook, R.G., Gorovsky, M.A. and Allis, C.D. (1998) *Proc. Natl. Acad. Sci.*, Vol. 95, pp7480-7484.
Phosphorylation of histone H3 at serine is correlated with chromosome condensation during mitosis and meiosis in *Tetrahymena*.
139. Peter, M., Nakagawa, J. Doree, M., Labbe, J.C. and Nigg, E.A. (1990) *Cell*, Vol 61, pp591-602.
In vitro disassembly of the nuclear lamin and M phase specific phosphorylation of lamins by Cdk kinase.
140. Blethrow, J.D., Glavy, J.S., Morgan, D.O. and Shokat, K.M. (2008) *Proc. Natl. Acad. Sci.*, Vol. 105, pp1442-1447.
Covalent capture of kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates

141. Cormier, P. Osborne, H.B., Bassez, T. Poughle, R., Belle, R. and Mulner-Lorillon, O. (1991) FEBS Lett., Vol. 295, pp185-188.
Protein phosphatase 2A from *Xenopus* oocytes.
142. Winston, N.J. and Maro, B. (1999) Biol. Cell., Vol 91, pp175-183.
Changes in the activity of type 2A protein phosphatases during meiotic maturation and the first mitotic cell cycle in mouse oocytes.
143. Dupre, A., Daldello, E.M., Nairn, A.G., Jesus, C. and Haccard, O. (2014) Nature Commun. Vol. 5, Article number: 3318.
Phosphorylation of Arpp19 by protein kinase A prevents meiosis resumption in *Xenopus* oocytes.
144. http://www2.cnrs.fr/sites/communiquer/fichier/rapport_conclusions.pdf.
145. <http://www.sauvonsluniversite.com/spip.php?article8356>.
146. Journal of Cell Science (2018) 131, jcs222182. doi:10.1242/jcs.222182.
147. Tung, H.Y.L. (2019) J. Invest. Cri. Pub. Sci. Articles., Vol. 1, pp89-92.
Why was the Paper Entitled "Phosphorylation of ARPP19 by Greatwall renders the amplification of MPF independent of PKA in *Xenopus* oocytes" Authored by Dupre, A., Buffin, E., Roustan, C., Nairn, A.C., Jesus, C., and Haccard, O. and published in the Journal of Cell Science [J. Cell. Sci. (2013) Vol. 126, pp 3916-3926.] not Retracted.
148. Boyer, J., Asselin, J. Belle, R. and Ozon, R. (1986) Dev. Biol., Vol. 113, pp420-428.
Progesterone and cAMP-dependent protein kinase regulate in vivo the level of phosphorylation of two proteins (Mr 20,000 and Mr 32000) in *Xenopus* oocytes.
149. Huchon, D, Ozon, R. and Demaille, J.G. (1981) Nature, Vol. 294, pp358-359.
Protein phosphatase-1 is involved in *Xenopus* oocyte maturation.
150. Foulkes, J.G. and Maller, J.L. (1982) FEBS Lett., Vol 150, pp155-160.
In vivo actions of protein phosphatase inhibitor-2 in *Xenopus* oocytes.
151. Hubbard, M.J. and Cohen, P. (1993) Trends Biochem. Sci. Vol. 18, pp172-177.
On target with a new mechanism for the regulation of protein phosphorylation.
152. Egloff, M.P., Johnson, D.F., Moorehead, G., Cohen, P.T., Cohen, P. and Barford, D. (1997) EMBO J., Vol. 16, pp1876-1887.

- Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1
153. Cohen, P.T. (2002) *J. Cell. Sci.*, Vol. 115, pp241-256.
Protein phosphatase-1 targeted in many directions.
 154. Hemmings, B.A., Resink, T.J. and Cohen, P. (1982) *FEBS Lett.*, Vol. 150, pp 319-324.
Reconstitution of a Mg-ATP dependent protein phosphatase.
 155. Resink, T.J., Hemmings, B.A., Tung, H.Y.L. and Cohen, P. (1983/ Vol. 133. pp455-461.
Characterization of a reconstituted Mg-ATP dependent protein phosphatase.
 156. Tung, H.Y.L. and Cohen, P. (1984)
Protein phosphatases involved in cellular regulation. Comparison of native and reconstituted Mg-ATP dependent protein phosphatase.
 157. Villa-Moruzzi, E. (1992) *FEBS lett.* Vol. 304, pp295-315.
Activation of type-1 protein phosphatase by cdc2 kinase.
 158. Puntoni, F. and Villa-Moruzzi, E. (1995) *Biochem. Biophys. Res. Commun.* Vol. 207, pp732-739.
Phosphorylation of the inhibitor 2 of protein phosphatase-1 by cdc2-cyclin B.
 159. Agarwal-Mawal, A. and Paudel, H.K. (2001) *J. Biol. Chem.*, Vol 276, pp23712-23718.
Neuronal cdc2-like protein kinase (Cdk5/p25) is associated with protein phosphatase-1 and phosphorylates inhibitor-2.
 160. Li, M., Stefansson, B., Wang, W., Schaefer, E.M. and Brautigan, D.L. (2005) *Cellular Signalling*, Vol.18, pp1318-1326.
Phosphorylation of the Pro-X-Thr-pro site in protein phosphatase inhibitor-2 by cyclin dependent protein kinase during M-phase of the cell cycle.
 161. Dohadwala, M., Da Cruz e Silva, E.F., Hall, F.L., Williams, R.T., Carbanaro-Hall, D.A., Nairn, A.C., Greengard, P. and Berndt, N. (1994) *Proc. Natl. Acad. Sci.*, Vol. 91, pp6408-6412.
Phosphorylation and inactivation of protein phosphatase-1 by cyclin dependent protein kinase.

162. Kwon, Y.-G., Lee, S.Y., Choi, Y., Greengard, P. and Nairn (1997) *Proc. Natl. Acad. Sci.*, Vol. 94, pp2168-2173.
Cell cycle-dependent phosphorylation of mammalian protein phosphatase-1 by cdc2 kinase.
163. Hara, M., Abe, Y., Tanaka, T., Yamamoto, T., Okumura, E. and Kishimoto, T. (2012) *Nat. Commun.*, doi: 10.1036/comms2062.
Greatwall kinase and cyclin B-Cdk1 are both critical constituents of M-phase-promoting factor.
164. Adhikari, D., Diril, M.K., Busayavalasa, K., Risal, S., Nakagawa, S., Lindkvist, E., Shen, Y., Coppola, V., Tessarollo, L., Kudo, N.R., Kaldis, P. and Liu, K. (2014) *J. Cell. Biol.*, Vol. 206, pp843-853.
Mastl is required for timely activation of APC/C in meiosis and Cdk1 reactivation in meiosis II.
165. Lamb, N.J., Cavadore, J.C., Labbe, J.C., Maurer, R.A. and Fernandez, A. (1991) *MBO J.*, Vol. 91, pp1523-1533.
Inhibition of cAMP-dependent protein kinase plays a key role in the induction of Mitosis and nuclear envelope breakdown in mammalian cells.
166. Brautigan, D.L., Brautigan, D.L., Sunwoo, J., Labbee, J.C., Fernandez, A. and Lamb, N.J. (1990) *Nature*, Vol 344, pp74-78.
Cell cycle oscillation of phosphatase inhibitor-2 in rat fibroblasts coincident with 34cdc2 restriction.
167. Fernandez, A., Brautigan, D.L. and Lamb, N.J. (1992) *J. Cell. Biol.*, Vol. 116, pp1421-1430.
Protein phosphatase type 1 in mammalian cell mitosis: chromosomal localization and involvement in mitotic exit.
168. Maller, J.L. and Krebs E.G. (1977) *J. Biol. Chem.*, Vol 252, pp1712-1718.
Progesterone stimulated meiotic cell division in *Xenopus* oocytes. Induction by regulatory subunit and inhibition by catalytic subunit of adenosine 3',5'-mono phosphate dependent protein kinase.
169. Huchon, D., Ozon, R. Fisher, E.H. and Demaille, J.G. (1981) *Mol. Cell. Endocrinol.*, Vol 22, pp211-222.

The pure inhibitor of cAMP dependent protein kinase initiates *Xenopus laevis* meiotic maturation. A 4 step scheme for meiotic maturation.