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S. Vigneron, E. Brioudes, A. Burgess, J.-C. Labbé, Thierry Lorca, et al.. RSK2 is a kinetochore-associated protein that participates in the spindle assembly checkpoint.. *Oncogene*, 2010, 29 (24), pp.3566-74. 10.1038/onc.2010.105 . hal-00492408

HAL Id: hal-00492408

<https://hal.science/hal-00492408>

Submitted on 23 Jun 2021

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SHORT COMMUNICATION

RSK2 is a kinetochore-associated protein that participates in the spindle assembly checkpointS Vigneron¹, E Brioudes¹, A Burgess, J-C Labbé, T Lorca and A Castro*Universités Montpellier 2 et 1, Centre de Recherche de Biochimie Macromoléculaire, CNRS UMR 5237, Montpellier cedex 5, France*

The spindle assembly checkpoint (SAC) prevents anaphase onset until all the chromosomes have successfully attached to the spindle microtubules. The MAP kinase (MAPK) is an important player in this pathway, however its exact role is not fully understood. One major target of MAPK is the p90 ribosomal protein S6 kinase (RSKs) family. In this study, we analyse whether Rsk2 could participate in the activation of the SAC. Our data indicate that this protein is localized at the kinetochores under checkpoint conditions. Moreover, it is essential for the SAC activity in *Xenopus* egg extracts as its depletion prevents metaphase arrest as well as the kinetochore localization of the other SAC components. We also show that this kinase might also participate in the maintenance of the SAC in mammalian cells as Rsk2 knockdown in these cells prevents the kinetochore localization of Mad1, Mad2 and CENP-E under checkpoint conditions.

Oncogene (2010) 29, 3566–3574; doi:10.1038/onc.2010.105; published online 12 April 2010

Keywords: Rsk2; SAC; kinetochore; Mad2; CENP-E

Introduction

The spindle assembly checkpoint (SAC) is a cell-cycle surveillance system that monitors kinetochore–microtubule attachment and guards against chromosome missegregation (Musacchio and Salmon, 2007). This checkpoint delays anaphase onset until all chromosomes are correctly attached to the spindle microtubules. Unattached kinetochores activate the SAC by recruiting to these chromosome structures the SAC proteins Bub1, Bub3, BubR1, Mps1, Mad1, Mad2 and the passenger proteins Aurora B, INCENP, Survivin and Borealin. In vertebrate cells, MAP kinase (MAPK) is also recruited to the kinetochores after microtubule disruption (Shapiro *et al.*, 1998) and it is also required for SAC activity (Minshull *et al.*, 1994; Takenaka *et al.*, 1997; Wang *et al.*, 1997).

The MAPK is a key signalling pathway that is involved in the regulation of normal cell proliferation,

survival, growth and differentiation. This pathway consists of the MAPK, the MAP kinase kinases and the MAP kinase kinase kinases. An important family of cytoplasmic targets activated by the MAPKs is ribosomal protein S6 kinase (RSKs) (Anjum and Blenis, 2008). In *Xenopus*, the MAPK-dependent activation of RSK is required to promote oocyte maturation through the inhibition of Myt1, an inhibitory kinase of cyclin B/Cdk1, and to prevent DNA replication between meiosis I and II (Nebreda and Ferby, 2000). In these oocytes, both the Rsk1 and 2 isoforms mediate the MAPK-dependent activity of the cytostatic factor required for metaphase II arrest through the phosphorylation of the APC inhibitor Erp1 (Nishiyama *et al.*, 2007; Wu *et al.*, 2007) and of the spindle checkpoint protein Bub1 (Tunquist and Maller, 2003).

The fact that MAPK is required for SAC activity, with RSK being one of the major targets of this kinase and that RSK can modulate the cytostatic activity of metaphase II-arrested oocytes through the phosphorylation of the spindle checkpoint protein Bub1, prompted us to ask whether RSK could also be required for the SAC. In this study, we analysed the involvement of Rsk2 in the activation and maintenance of the SAC in metaphase II-arrested *Xenopus* egg extracts and in human cells.

Results and discussion*Rsk2 localizes at the kinetochores under spindle checkpoint conditions*

Inactive Rsk2 was originally reported to be localized in both the cytoplasm and the nucleus during interphase. The addition of growth factors to quiescent cells induces the activation of this kinase at the plasma membrane and the subsequent nuclear translocation where it induced gene expression (Chen *et al.*, 1992; Murphy *et al.*, 2002). During mitosis, Rsk2 is associated with the centrosomes, the spindle and at the mid-body (Willard and Crouch, 2001); however, the localization of this protein under spindle checkpoint conditions has never been analysed. To study whether Rsk2 could participate in the SAC, we analysed whether this protein localizes at the kinetochores in metaphase II-arrested *Xenopus* egg extracts (CSF extracts) in which the SAC was activated by the addition of Nocodazole and sperm nuclei (9000/μl). Chromosomes were isolated from this mix

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Received 20 July 2009; revised 26 February 2010; accepted 1 March 2010; published online 12 April 2010

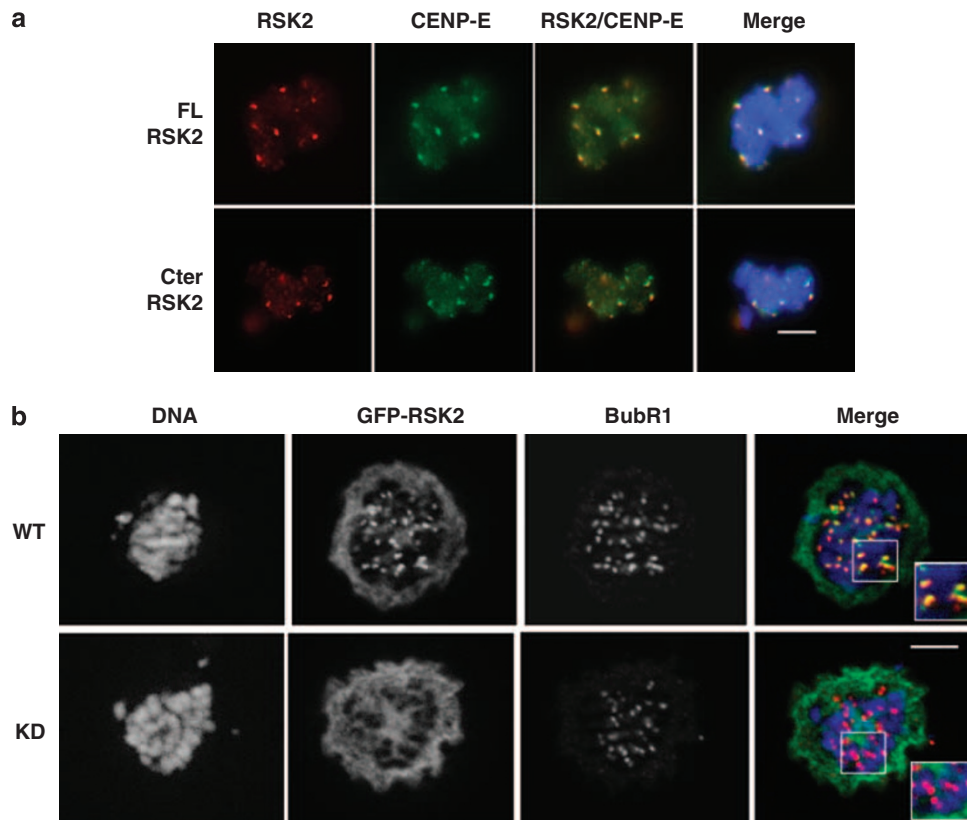


Figure 1 Rsk2 localizes at the kinetochores under spindle checkpoint conditions. **(a)** CSF extracts supplemented with nocodazole and sperm nuclei (9000/ μ l) were incubated for 75 min and used for immunofluorescence staining to analyse the kinetochore localization of Rsk2 by a co-staining with the kinetochore protein CENP-E. Two different antibodies, one against the full-length Rsk2 protein (FL RSK2) and the other against a C-terminal peptide of Rsk2 (Cter RSK2), were used. **(b)** HeLa cells were transfected with either the wild-type (WT) or the kinase-dead (KD) GFP-tagged forms of *Xenopus* Rsk2; 24 h after transfection cells were treated with 50 ng/ml of nocodazole to activate the SAC and the kinetochore localization of GFP-*Xenopus* Rsk2 was analysed by co-staining with the kinetochore protein BubR1. Cells were analysed using a Statif Axioimager 21 (Zeiss) microscope with Structured Illumination (ApoTome). Bar represents 5 μ m.

and the localization of Rsk2 was studied by immunofluorescence with two different antibodies, one against the full-length *Xenopus* Rsk2 protein and the other against the last 12 aminoacids (FL RSK2 and Cter RSK2, respectively). Rsk2 presented a punctuated staining that was identical with the two different antibodies used and that clearly co-localized with CENP-E, indicating that Rsk2 is present at the kinetochores under checkpoint conditions (Figure 1a). We next asked whether this could be also the case in human cells. To determine the localization of Rsk2 in HeLa cells, we overexpressed a GFP-tagged *Xenopus* Rsk2 protein and analysed in the presence or absence of nocodazole (16-h treatment), whether it co-localizes with the SAC protein BubR1. We did not observe a co-localization of GFP-Rsk2 with BubR1 in non-treated cells (data not shown). However, Rsk2 clearly co-localized with BubR1 at the kinetochores when microtubules were depolymerized by the addition of Nocodazole. Interestingly, this localization is dependent on the kinase activity of Rsk2, as co-localization was not observed under checkpoint conditions when a GFP-Rsk2 kinase-dead form was transfected (Figure 1b).

Rsk2 is essential for the establishment of the SAC in CSF extracts

To study the involvement of Rsk2 in the SAC, we first immunodepleted Rsk2 from CSF extracts and subsequently supplemented them with sperm nuclei (9000/ μ l). In these extracts two different activities inhibit the APC. First, the CSF activity mediated by Erp1, and second the SAC activity mediated by the mitotic checkpoint complex. To examine the consequence of Rsk2 depletion in the CSF activity we simply measured H1 kinase activity and assessed chromatin condensation in Rsk2-depleted extracts. To analyse the involvement of Rsk2 in SAC activity, we first depleted this kinase from CSF extracts, then subsequently supplemented these extracts with sperm nuclei and finally we added calcium to inactivate CSF. The H1 activity and chromatin condensation were measured to determinate the mitotic state (Vigneron *et al.*, 2004).

According to the data reported by other laboratories (Bhatt and Ferrell, 2000; Nishiyama *et al.*, 2007), our data show that Rsk2 depletion does not promote metaphase II exit. Rsk2-depleted CSF extracts presented phosphorylated cyclin B-Cdk1 substrates and condensed

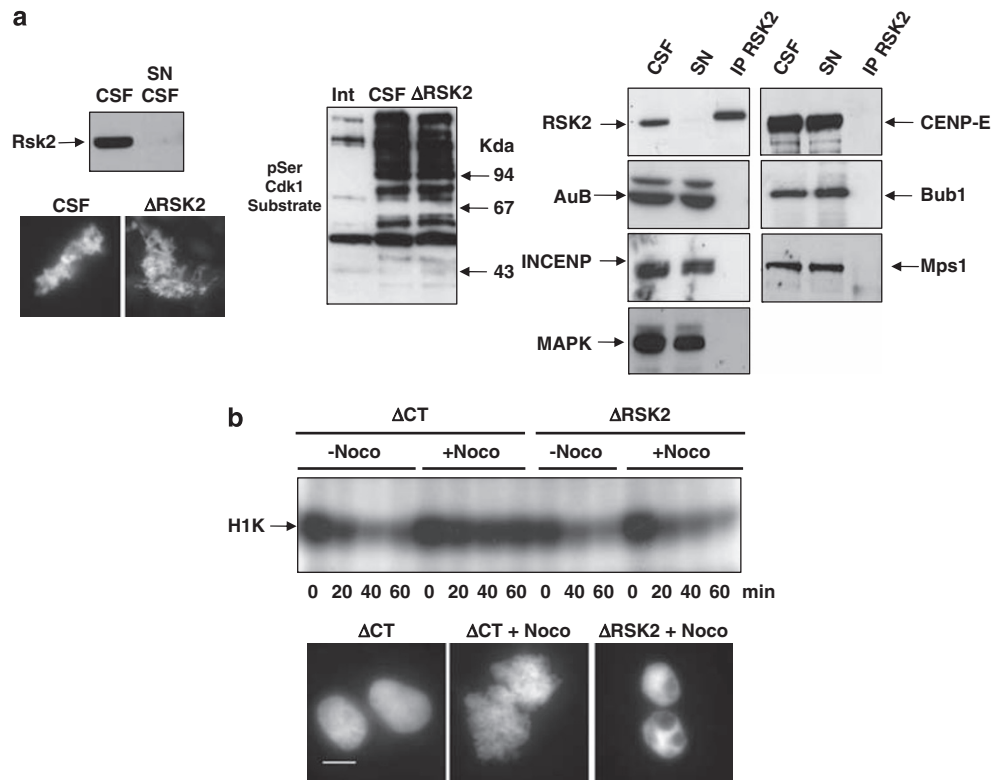


Figure 2 Rsk2 is essential for the establishment of the SAC in CSF extracts. **(a)** CSF extracts were immunodepleted of Rsk2. A sample of CSF extract (CSF) or Rsk2 immunodepleted supernatant (SN) was used as determinate by western blotting the levels of Rsk2 or to analyse chromatin condensation. The phosphorylation of cyclin B-Cdk1 substrates was investigated by using an antibody directed against the phosphorylated Serine of the Cdk consensus motif (Cell Signalling Technology Inc., Danvers, MA, USA) in 1 μ l of CSF and in interphase extracts as well as in Rsk2 immunodepleted supernatants. Finally, the levels of the different spindle checkpoint components (Aurora B, INCENP, MAPK, CENP-E, Bub1 and Mps1) were also analysed by western blotting in 1 μ l of CSF extracts, Rsk2-depleted supernatants and in the immunoprecipitates corresponding to 5 or 30 μ l (for MAPK) of CSF extracts (IP RSK2). **(b)** CSF extracts were immunodepleted by using control (Δ CT) or anti-C-terminal Xenopus Rsk2 (Δ RSK2) antibodies and subsequently supplemented with sperm nuclei (9000/ μ l) and incubated with (+Noco) or without (-Noco) nocodazole (10 μ g/ml). After 75-min incubation, a sample was taken to measure H1 kinase activity (H1K) at 0, 20, 40 and 60 min after calcium addition (0.4 mM) and chromatin condensation at 60 min after calcium addition. Bar, 5 μ m.

DNA (Figure 2a, left). Thus, phosphorylation of Erp1 by Rsk2 must be exclusively required to establish CSF arrest but not to maintain this activity.

Immunodepletion of Rsk2 removed >98% of this kinase but did not deplete either of the other checkpoint proteins. Accordingly, the endogenous levels of these checkpoint proteins were not affected (Figure 2a, right). As shown in Figure 2b, the addition of calcium induced a loss of the H1 kinase activity in both control and Rsk2-depleted CSF extracts in the absence of Nocodazole (Figure 2b, Δ CT and Δ RSK2 -Noco). However, though control-depleted CSF extracts maintained H1 kinase activity and chromatin condensation in the presence of Nocodazole (Figure 2b, Δ CT +Noco), Rsk2-depleted extracts lost H1 kinase activity and decondensed chromatin after calcium addition, indicating Rsk2 is required to maintain the SAC activity in these extracts (Figure 2b, Δ RSK2 +Noco).

We next analysed whether the failure of the spindle checkpoint in Rsk2-depleted CSF extracts was associated with a modification of the localization pattern of the different checkpoint proteins. To do that, we first

depleted CSF extracts of Rsk2 and we subsequently supplemented these extracts with sperm nuclei. Finally, chromosomes were isolated and the kinetochore localization of Aurora B, INCENP, Bub1, Mps1, CENP-E, Mad1, Mad2 and Bub3 was examined by immunofluorescence. The results showed a loss of the kinetochore binding of all these proteins (Figure 3a) indicating that Rsk2 association at the kinetochores is required to maintain the SAC activation by allowing the localization of the other checkpoint proteins to the kinetochores, thus, Rsk2 could be the most upstream protein of the spindle checkpoint pathway. To test whether this was the case, we analysed the kinetochore localization of Rsk2 in CSF extracts in which the most upstream protein of the SAC pathway, Aurora B, was depleted (Vigneron *et al.*, 2004). As depicted in Figure 3b, the depletion of Aurora B from CSF extracts induced a complete disappearance of the immunofluorescence signal of Rsk2 indicating that the kinetochore localizations of Aurora B and Rsk2 are dependent on each other and that they could act together at the same level of the spindle checkpoint pathway.

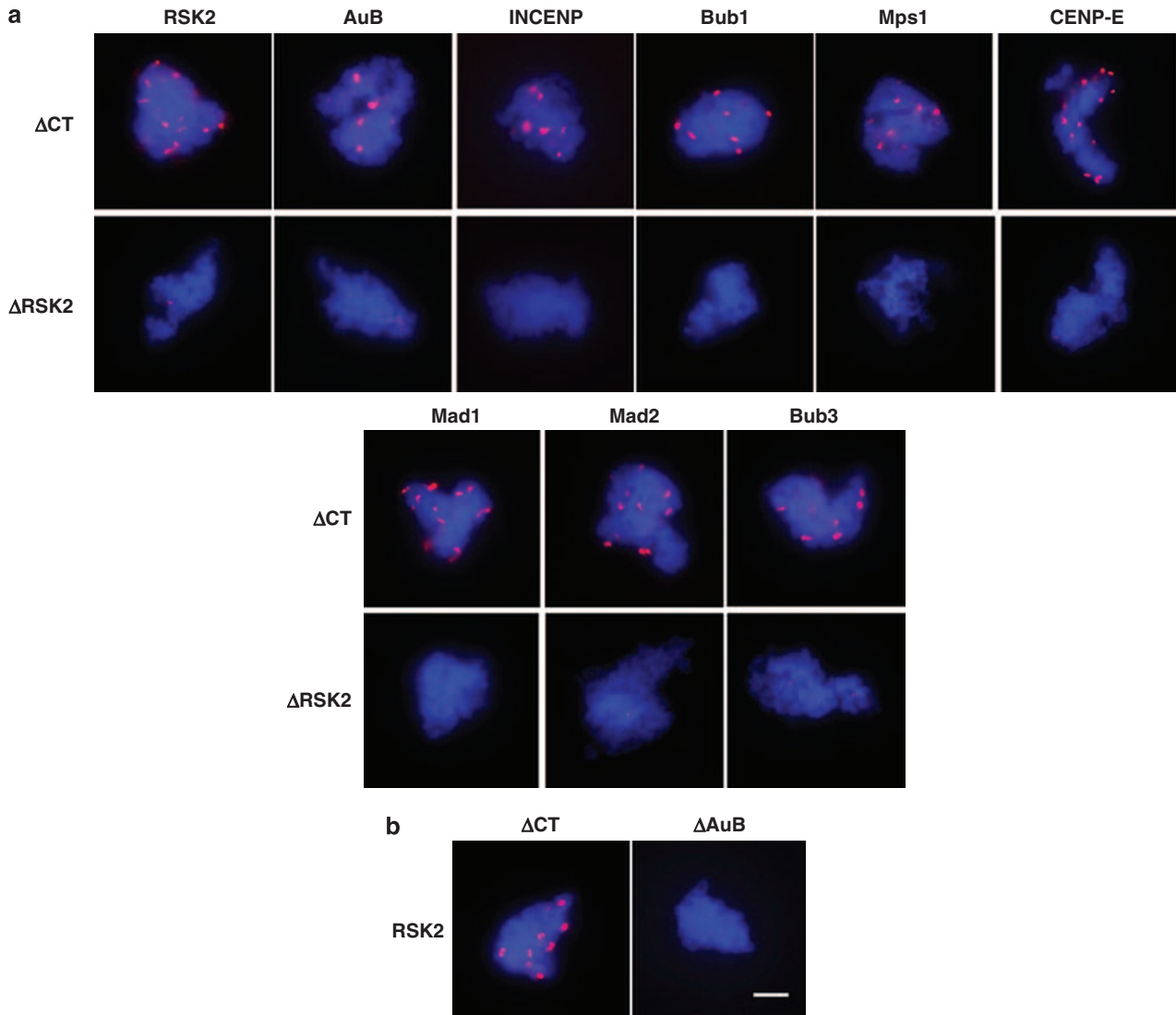


Figure 3 Rsk2 regulates the kinetochore localization of all the other SAC proteins in *Xenopus* egg extracts. (a) Control (Δ CT) or anti-C-terminal *Xenopus* Rsk2-depleted CSF extracts (Δ RSK2) were supplemented with sperm nuclei and nocodazole and after a 75-min incubation used for immunofluorescence staining to analyse the kinetochore localization of Rsk2, Aurora B, INCENP, Bub1, Mps1, CENP-E, Mad1, Mad2 and Bub3. (b) Similar to (a) except for the immunodepletion of Aurora B instead of Rsk2. Bar, 5 μ m.

Rsk2 knockdown in HeLa cells induces a loss of Mad1, Mad2 and CENP-E from the kinetochores under spindle checkpoint conditions

To extend our findings to human cells we knocked down Rsk2 in HeLa cells by siRNA. As shown in Figure 4a, Rsk2 siRNA induced a >80% decrease in endogenous Rsk2, 32 h after transfection. To analyse the role of Rsk2 in the maintenance of the SAC activity in these cells we treated Rsk2-knockdown cells with 50 ng/ml of nocodazole for 16 h and analysed the localization of the kinetochore protein CENP-A, and of the spindle checkpoint proteins BubR1, Bub1, INCENP, Mad1, Mad2 and CENP-E at the kinetochores. The knockdown of Rsk2 did not affect the basic structure of the kinetochores, as no difference in the localization of CENP-A was observed between control and Rsk2 siRNA-treated cells. We neither observed a difference in the localization of the spindle checkpoint proteins

BubR1, Bub1 or INCENP. However, Rsk2-knockdown cells presented a clear decrease in the kinetochore localization of Mad1 (Figure 4f) and Mad2 (Figure 4g; Supplementary Figure S1) compared with control cells and a complete disappearance of the kinetochore staining of CENP-E (Figure 4h; Supplementary Figure S2). Thus, Rsk2 is required in HeLa cells for the kinetochore localization of CENP-E, Mad1 and Mad2. However, despite the fact that Rsk2 is clearly required for the kinetochore localization of these checkpoint proteins, we did not observe any difference in the mitotic index of Rsk2-knockdown cells when treated with nocodazole (data not shown) indicating that in these cells the SAC was active enough to maintain a metaphase arrest. In this regard, it is likely that the small amount of Mad2 left at the kinetochores after Rsk2 depletion was sufficient to maintain SAC activity and that this residual kinetochore localization of Mad2 is due to the

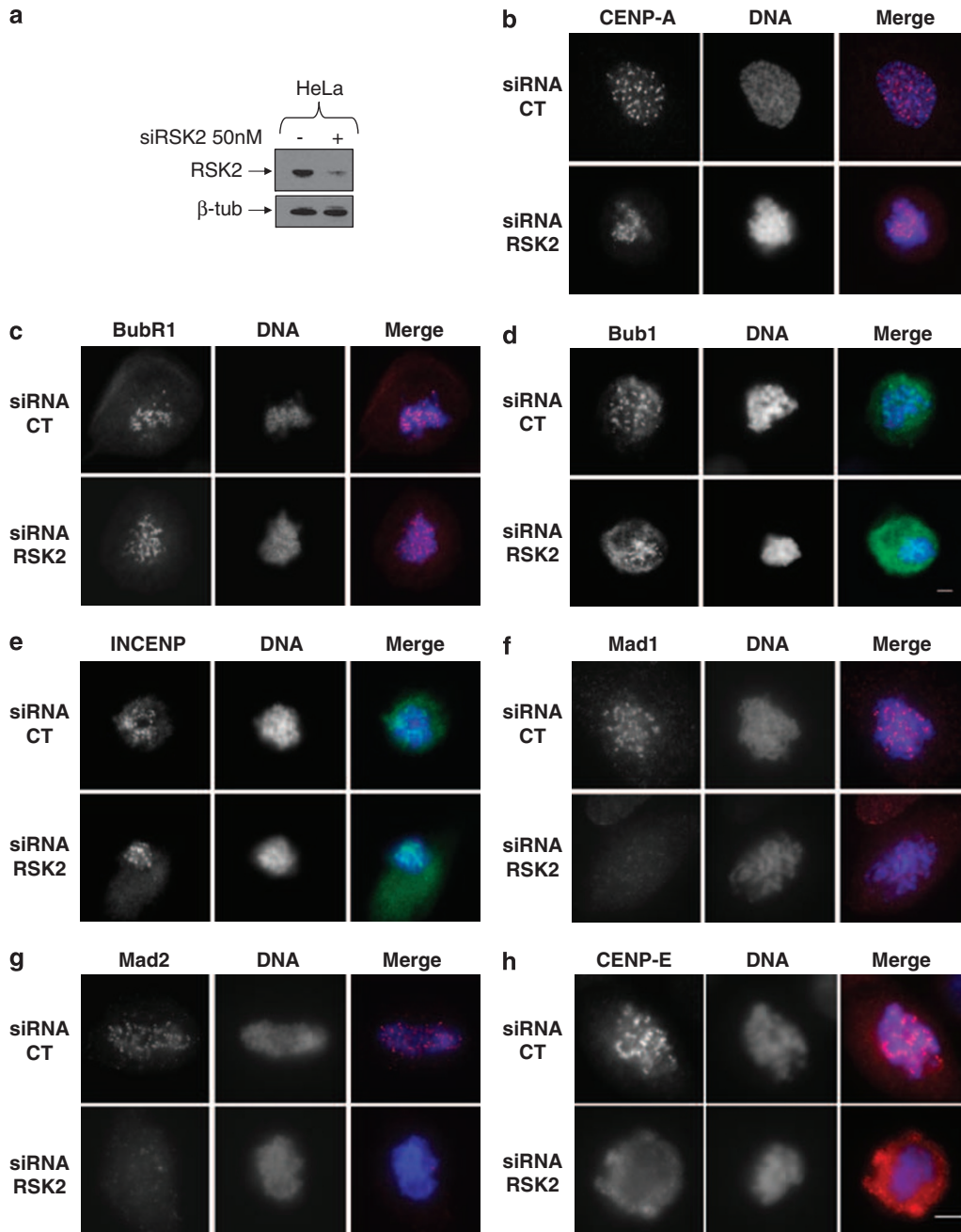


Figure 4 Rsk2 knockdown in HeLa cells induces a loss of Mad1, Mad2 and CENP-E from the kinetochores under SAC conditions. **(a)** HeLa cells were transfected with Rsk2 siRNA and the levels of this kinase were analysed 48 h after transfection by western blotting with anti-C-terminal human Rsk2. **(b)** HeLa cells were transfected with either a scramble siRNA (siRNA CT) or with a siRNA directed to the N-terminal domain of Rsk2 (siRNA RSK2); 24 h later, cells were incubated with nocodazole (50 ng/ml) for 16 h and were subsequently used to analyse the levels of CENP-A by immunofluorescence. **(c–h)** Similar to **(b)** except for the use of anti-BubR1, anti-Bub1, anti-INCENP, anti-Mad1, anti-Mad2 or anti-CENP-E antibodies, respectively, for the immunofluorescence analysis.

residual Rsk2 left in the cell after knockdown. However, we cannot exclude the possibility that this localization is mediated by other Rsk isoform, such as Rsk1. To investigate whether Rsk1 could also participate in the maintenance of the SAC, we depleted this protein by immunoprecipitation in *Xenopus* egg extracts and by siRNA treatment in human cells and analysed the kinetochore localization of the different SAC components. Despite the fact that more than 90% of

the endogenous Rsk1 protein was depleted from CSF extracts, we did not observe any difference in the kinetochore localization of Aurora B, INCENP or CENP-E compared with controls (Figures 5a and b) whereas these proteins clearly disappeared from this localization after Rsk2 depletion. As in CSF extracts, after more than 85% knockdown of Rsk1, both control and siRNA-treated HeLa cells presented identical kinetochore levels of Mad1, Mad2 and CENP-E indicating that

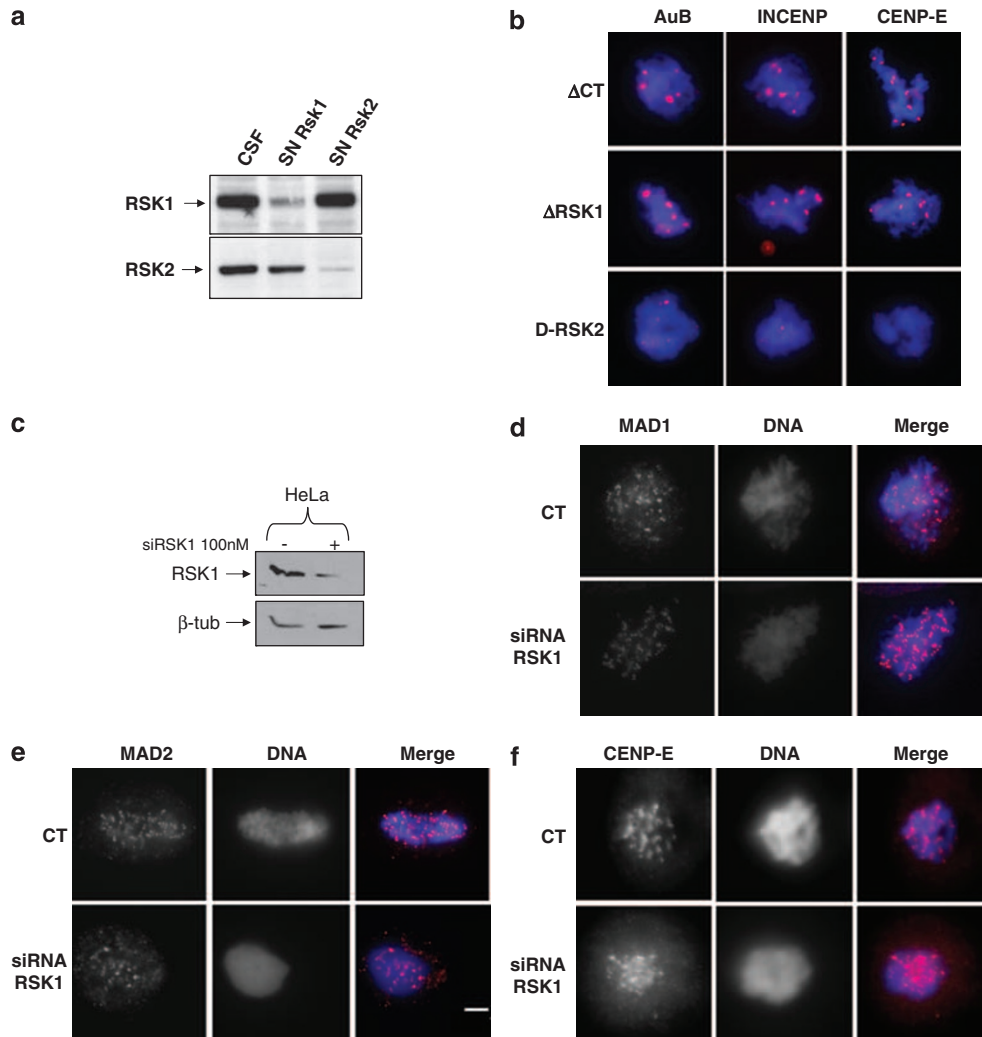


Figure 5 Rsk1 is not required for the maintenance of the SAC in CSF egg extracts and in human cells. (a) A sample of 1 μ l of CSF extract (CSF) and Rsk1 or Rsk2 immunodepleted supernatants (SN) were used to analyse by western blotting the endogenous levels of Rsk1 and Rsk2. (b) Control (Δ CT) or anti-C-terminal Xenopus Rsk1 or Rsk2-depleted CSF extracts (Δ RSK1 and Δ RSK2, respectively) were supplemented with sperm nuclei and nocodazole and after a 75-min incubation used for immunofluorescence staining to analyse the kinetochore localization of Aurora B, INCENP, and CENP-E. (c) HeLa cells were transfected with Rsk1 siRNA and the levels of this kinase were analysed 48 h after transfection by western blotting with anti-C-terminal human Rsk1 antibodies. (d) HeLa cells were transfected (siRNA RSK1) or not (CT) with an siRNA directed to the N-terminal domain of Rsk1; 24 h later, cells were incubated with nocodazole (50 ng/ml) for 16 h and were subsequently used to analyse the kinetochore levels of Mad1 by immunofluorescence. (e, f) Similar to (d) except for the use of anti-human Mad2 or anti-human CENP-E, respectively, for the immunofluorescence analysis.

Rsk1 does not participate in the SAC (Figures 5c–f; Supplementary Figure S2).

Expression of a siRNA-resistant Rsk2 plasmid prevents the loss of Mad1, Mad2 and CENP-E from the kinetochores in human cells

To analyse the specificity of the Rsk2 knockdown on the kinetochore localization of Mad1, Mad2 and CENP-E, we performed the knockdown of Rsk2 in HeLa cells and 24 h later, we transfected a siRNA-resistant plasmid encoding a GFP-tagged form of Xenopus Rsk2. Cells were subsequently synchronized with a thymidine block for 24 h and treated with nocodazole for an additional period of 12 h. The kinetochore localization of Mad1,

Mad2 and CENP-E was analysed in GFP-positive and -negative cells.

In this experiment, GFP staining appeared diffuse in most cells and the kinetochore localization of GFP-Rsk2 could not be visualized possibly because of the low permeabilization conditions required for Mad1, Mad2 and CENP-E immunofluorescences (0.1% Triton X100 in anti-human Mad1 and Mad2 versus 0.2% in the rest of the immunofluorescences and 100% methanol for anti-human CENP-E). Moreover, the results show that in GFP-negative cells, Mad1, Mad2 and CENP-E were completely absent from the kinetochores. On the contrary, these proteins clearly stained the kinetochores in GFP-positive cells (Figure 6). These data indicates that Xenopus Rsk2 protein rescues the effect of the

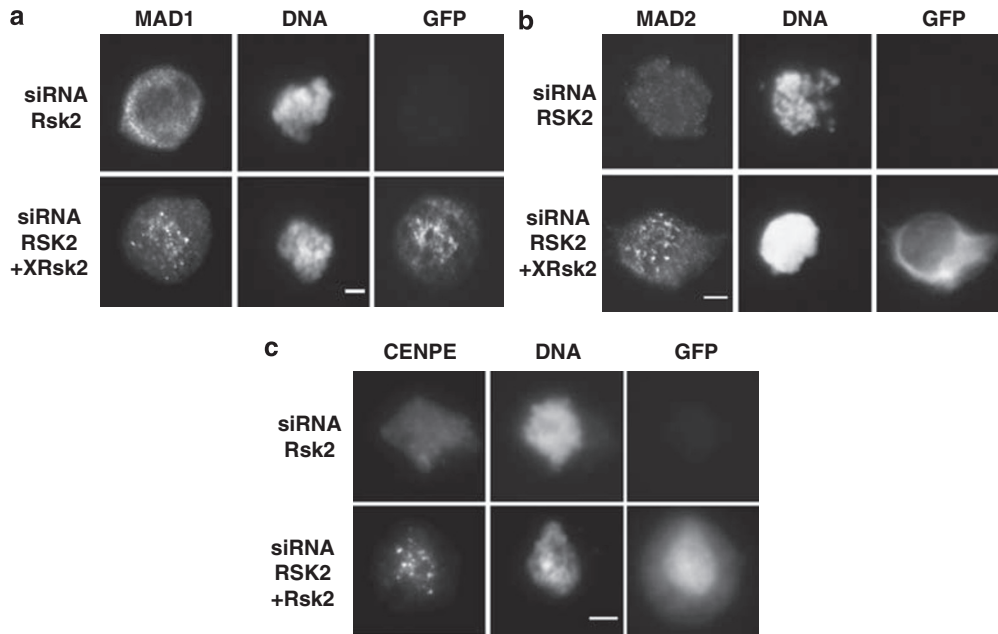


Figure 6 Expression of a siRNA-resistant Rsk2 plasmid prevented the loss of Mad1, Mad2 and CENP-E from the kinetochores in Rsk2-knockdown cells. **(a)** Rsk2-knockdown HeLa cells were transfected with an siRNA-resistant plasmid encoding a GFP-tagged form of Xenopus Rsk2. Cells were subsequently synchronized with a thymidine block for 24 h and treated with nocodazole for an additional period of 12 h. The kinetochore localization of Mad1 was analysed in Rsk2-knockdown cells (siRNA Rsk2) and in Rsk2-knockdown cells co-transfected with GFP-Xenopus Rsk2 (siRNA Rsk2 + XRsk2). **(b, c)** Similar to **(a)** except for the use of anti-human Mad2 or anti-human CENP-E, respectively, for the immunofluorescence analysis.

human Rsk2 siRNA knockdown confirming the specificity of the Rsk2 siRNA knockdown phenotypes.

We also analysed the effect of the add-back of an ectopic Xenopus Rsk2 protein to Rsk2-depleted CSF extracts. Unfortunately, the expression of ectopic Rsk2 did not prevent the phenotypes induced by Rsk2 depletion (data not shown). However, as similar phenotypes are observed in depleted egg extracts and in siRNA-treated cells, and that these phenotypes are clearly rescued in HeLa cells by the expression of an siRNA-resistant Rsk2 protein, we hypothesize that the lack of rescue in Xenopus egg extracts is probably because of the loss of an Rsk2 partner during depletion other than MAPK that forms complex with this protein and that is likely required to mediate Rsk2-dependent SAC activity. Similar results have been shown for the kinase Aurora B in which its depletion in CSF extracts promotes the loss of the Aurora B–INCENP–Survivin–Borealin complex and the inactivation of the SAC. The subsequent addition of Aurora B does not restore SAC activity because of the absence of these other partners that are required to form the active complex (Vigneron *et al.*, 2004).

In this regard, it seems surprising that kinetochore localization of Aurora B–INCENP–Survivin–Borealin complex is not affected by Rsk2 depletion in HeLa cells (INCENP is normally localized) whereas in Xenopus egg extracts this complex completely disappeared in the absence of Rsk2 kinase (Aurora B and INCENP disappeared from kinetochores after Rsk2 depletion). Similarly, Bub1 is also present in Rsk2-depleted HeLa cells whereas it is completely absent in kinetochores

from Rsk2-depleted extracts. However, these results could be explained taking into account the significant differences described in the regulation of the SAC between eukaryotic cells and egg extracts. Two branches of checkpoint signalling and silencing have been described in eukaryotic cells. One detects microtubule attachment and depends on Mad2 whereas the second detects tension and depends on BubR1 and Aurora B (Pinsky and Biggins, 2005). However, in egg extracts only a single pathway that detects attachment exists and contrary to eukaryotic cells, the attachment is dependent on Aurora B and Mad2 (Vigneron *et al.*, 2004). Our results suggest that Rsk2 in eukaryotic cells participates in the branch regulating kinetochore attachment and thus, depletion of this kinase in HeLa cells induces Mad2 disappearance from the kinetochores without affecting BubR1 or Aurora B. Unlike eukaryotic cells, the same depletion in egg extracts, which contain only one branch, induces a disappearance of the kinetochore localization of all the SAC proteins downstream Rsk2.

CENP-E is completely lost from the kinetochores when Rsk2 is depleted in both human cells and Xenopus egg extracts. In Xenopus egg extracts CENP-E has been shown to be essential for the establishment of the SAC (Abrieu *et al.*, 2000; Vigneron *et al.*, 2004). In contrast, the role of CENP-E in the activation and kinetochore localization of mitotic checkpoint proteins in mammalian cells is not clear (Yao *et al.*, 2000; McEwen *et al.*, 2001). CENP-E is a kinesin-like motor protein that is required for efficient capture and attachment of spindle microtubules by kinetochores, a necessary step in chromosome alignment in the metaphase plate.

Accordingly, functional disruption of CENP-E in cultured cells results in the appearance of unaligned chromosomes. However, some studies report that the presence of unattached chromosomes because of CENP-E knockdown promotes SAC activation and induces a prolonged mitotic arrest (Yao *et al.*, 2000; McEwen *et al.*, 2001), whereas in others mitosis is not delayed suggesting an involvement of CENP-E in the maintenance of this checkpoint (Putkey *et al.*, 2002; Tanudji *et al.*, 2004). Our results show that the inactivation of Rsk2 induces the loss of the recruitment of CENP-E to the kinetochores in both *Xenopus* egg extracts and HeLa cells. However, though in *Xenopus* egg extracts Rsk2 depletion induces SAC inactivation, in HeLa cells the knockdown of Rsk2 does not prevent metaphase arrest after nocodazole treatment despite the complete absence of CENP-E from the kinetochores suggesting that under these conditions CENP-E would not be essential for the maintenance of the spindle checkpoint. In addition, the disappearance of CENP-E from the kinetochores after Rsk2 depletion suggests that this kinase could also be indirectly required for the correct attachment of spindle microtubules to the kinetochores. Accordingly, previous studies have shown that the knockdown of Rsk in HeLa cells induces an increase in chromosome segregation defects (Nam *et al.*, 2008).

Conclusion

Our data shows that Rsk2 is required for the maintenance of the SAC in *Xenopus* egg extracts and probably also participates to maintain SAC activity in mammalian cells, although in the latter model we have been unable to observe a decrease in SAC activity, probably because of a residual level of Rsk2 left in cell after siRNA treatment. However, the knockdown of Rsk2 in these cells induces a clear decrease in the levels of kinetochore Mad1 and Mad2 and completely prevented the recruitment of CENP-E at the kinetochores suggesting that this kinase is required for the correct activation of the SAC in *Xenopus* egg extracts and in mammalian cells.

Materials and methods

Immunization procedures, plasmids and cell transfection and immunofluorescence

Immunization procedures, antibodies, plasmids and conditions of cell transfection and immunofluorescence are described in Supplementary data.

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siRNA knockdown

Rsk2 and Rsk1 knockdown in HeLa cells were performed by using the siRNA sequence 5'-GGAGGAGATTAACCCACAA-3' located at the N-terminal domain of human Rsk2 and 5'-GUGGGCACCUGUAUGCUAU-3' at the N-terminal domain of human Rsk1.

Preparation of Xenopus egg extracts, immunofluorescence and immunoprecipitation

CSF-arrested extracts and demembrated sperm nuclei were prepared as described (Murray, 1991).

For immunofluorescence staining of unreplicated chromosomes, 20 µl of CSF extract were incubated with nocodazole (10 µg/ml) and sperm nuclei (9000/µl) for 75 min. Chromosomes were isolated and processed as described (Abrieu *et al.*, 2001).

For immunodepletion, 50 µg of affinity-purified antibodies (anti-Rsk2 and anti-Aurora B) or non-immune IgG were bound to 250 µl of Dynal beads protein A for 30 min and then added to 250 µl of CSF extract for 1 h at 4 °C. Two successive immunodepletions of 30 min each were performed to remove these two endogenous proteins.

Light microscopy

A DMR A Leica microscope, with a × 63 or × 100 immersion oil objective (HCX PL APO), tube factor 1, was used for epifluorescence imaging (A4, GFP, N2.1 cube filters, excitation HBO light bulb); images were captured with a Roper MicroMax 1300 Y/HS camera; the whole set was driven by MetaMorph (Universal Imaging Corporation, West Chester, PA, USA).

A Statif Axioimager 21 (Zeiss) with Structured Illumination (ApoTome). Images were captured with an AxioCameraMRm (Zeiss, Le Pecq, France).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr JE Ferrell for the generous gift of the pFast-Rsk2 WT and the pFast-Rsk2 KD constructs. We thank Julian Cau from the Montpellier RIO Imaging facility (CRBM). This work was supported by the Ligue Nationale Contre le Cancer (Equipe Labellisée). AB and EB are fellows from the Fondation pour la Recherche Medicale and the Ligue Nationale Contre le Cancer, respectively.

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Supplementary Information accompanies the paper on the Oncogene website (<http://www.nature.com/onc>)