Roles of MAP kinase signaling pathway in oocyte meiosis

FAN Hengyu, TONG Chao, CHEN Dayuan & SUN Qingyuan

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China
Correspondence should be addressed to Sun Qingyuan (e-mail: sunqyl@yahoo.com)

Abstract **Mitogen-activated protein kinase (MAPK) is a family of Ser/Thr protein kinases expressed widely in eukaryotic cells. MAPK is activated by a cascade of protein kinase phosphorylation and plays pivotal roles in regulating meiosis process in oocytes. As an important physical substrate of MAPK, $p90^{rsk}$ mediates numerous MAPK functions. MAPK was activated at G2/M transition during meiosis. Its activity reached the peak at MⅠ stage and maintained at this level until the time before the pronuclear formation after fertilization. There is complex interplay between MAPK and MPF in the meiosis regulation. Furthermore, other intracellular signal transducers, such as cAMP, protein kinase C and protein phosphotase, ect., also regulated the activity of MAPK at different stages during meiosis in oocytes. In the present article, the roles of MAPK signaling pathway in oocyte meiosis are reviewed and discussed.**

Keywords: protein kinase, oocyte, meiosis, signal transduction, fertilization.

Mammalian oocytes enclosed in ovary are arrested at the diplotene stage of the first meiotic prophase, which is also termed GV stage. Following proper stimulation (LH in mammals), the fully-grown oocytes reinitiate meiosis. The resumption of meiotic maturation is characterized by germinal vesicle breakdown (GVBD), followed by chromatin condensation, microtubule organization and emission of first polar body. Then the oocytes arrested again at metaphase Ⅱ[1] which is released by fertilization or parthenogenetic stimulation and followed by the completion of second meiotic division. Protein phosphorylation-dephosphorylation cascades, which are mediated by protein kinases and phosphotases, play pivotal roles in oocyte meiotic cell cycle.

Mitogen-activated protein kinases (MAPK), which are also termed extracellular-regulated kinases (ERK), are Ser/Thr protein kinases that require dual phosphorylation on threonine and tyrosine residues to become fully activated. Two isoforms of MAPKs, ERK1 (p44) and ERK2 (p42) seemed to play a pivotal role in meiosis. In the present article, the roles of MAPK pathway during the oocyte meiosis and fertilization are discussed by reviewing our results obtained in mouse, rat, rabbit, pig and those obtained by others.

1 Mos/MEK/MAPK/$p90^{rsk}$ signaling cascade

The core of MAPK signaling pathway was the cascade of protein kinase phosphorylation. MAPK kinase (MAPKK, otherwise known as MEK), the direct activator of MAPK, is a dual-specificity kinase that phosphorylates MAPK on threonine and tyrosine residues in the catalytic domain. MEK, whose upstream activator is termed MAPKKK, is also activated by phosphorylation. MAPKKK has several members and Mos, a product of the *c-mos* proto-oncogene, was one of them. Mos, a 39 ku Ser/Thr protein kinase, is a germ cell-specific upstream kinase of MAPK. *c-mos* mRNA is stored as a maternal information in the growing oocytes, which translates and intrigues MAPK cascade phosphorylation during oocyte maturation[2]. A total of 10 members of MAPK family have been found in mammalian cells, among which MAPK-1 and -2 have been evidently involved in meiosis regulation. While other family members mainly take part in the signal transduction under stress stimulation, and thus named stimulation-activated protein kinase (SAPK).

The first-found physiological substrates of MAPK in oocytes is a 90 ku protein kinase, $p90^{rsk}$ (ribosome S6 kinase). $p90^{rsk}$ was activated with phosphorylation on a serial of threonine and tyrosine residues. In the oocytes of *Xenopus*, starfish, mouse and rat, the activation of $p90^{rsk}$ is MAPK1/2 activity-dependent[3]. Our results showed that if GVBD and MAPK activation were inhibited, $p90^{rsk}$ could not be phosphorylated in the rat oocyte. Furthermore, following the fertilization or parthenogenetic activation, $p90^{rsk}$ was dephosphorylated accompanying the inactivation of MAPK[4]. All the results suggest that MAPK is critical to the activity of $p90^{rsk}$. However, a recent investigation found that the mouse with the *rsk* gene knockout is fertile, inferring that the lack of $p90^{rsk}$ is not enough to block the maturation and fertilization of oocytes[5]. But it should be careful in evaluating the results come from gene-targeting, since there are always redundant pathways in biochemical processes important for animals. Furthermore, only the studies about the energy metabolism in *rsk*-defected mouse were reported[5]. The detail in oocyte meiosis in these mice is still waiting to be revealed, in considering that "fertile" does not always mean normality in every aspect.

2 MAPK and meiotic resumption

MAPK was activated around the G2/M transition of meiosis, which is a phenomenon that is not observed in mitosis. In mammalian oocytes studied so far (mouse, rat, pig, cattle, sheep, and horse), MAPK was activated shortly after or at the same time as the occurance of GVBD, suggesting that MAPK activity is not necessary for maturation-promoting factor (MPF) activation and GVBD. In other vertebrate, such as the *Xenopus*, however, MAPK was activated prior to the GVBD and is necessary for the meiotic resumption. Mos, the upstream kinase of MAPK, is a key factor for the initiation of meiotic maturation in *Xenopus* oocytes. One of the functions of MAPK

in G2/M transition is to prevent the inhibitory phosphorylation of Cdc2. In *Xenopus* oocytes, the inhibitory phosphorylation of Cdc2 is attributed to the activity of Myt1. Injection of active MPF in immature oocytes does not lead to GVBD because of the inhibitory effects of Myt1. But if the MAPK pathway was activated before injection, MPF will initiate the positive feedback of its own activation, and ultimately, the GVBD. It is because that the C-terminal domain of Myt1 could bind to $p90^{rsk}$, the downstream kinase of MAPK. The $p90^{rsk}$ mediates an inhibitory phosphorylation of Myt1, so as to downregulate the inhibitory mechanism on MPF activity in oocytes at G2 stage[6].

In all investigated cases (*Spisula*, starfish, mouse and *Xenopus*), there is a strong correlation between the level of MAPK activity and cyclin B translation. In *Xenopus* oocytes, if the translation or activity of Mos was inhibited around GVBD, the MPF activity remains low after the meiosis Ⅰ and the oocytes are unable to enter meiosis Ⅱ. The increase in the rate of cyclin B translation when oocytes are released from arrest at G2-prophase of the first meiotic cell cycle might be due to the unmasking of its mRNA and depends on a cytoplasmic polyadenylation element (CPE) in the 3′ untranslated region. The CPE is the translation-masking elements in the immature oocyte, and the MAPK activity appears to be required for the unmasking of the CPE under physiological conditions[7].

Throughout the animal kingdom, MAPK exerts a positive feedback control on cyclin B translation in oocytes induced to escape from G2 arrest. This is clearly one mechanism by which MAPK assists in the activation of MPF. Although not required for GVBD in oocytes of all species, this control is very important for the subsequent flow of meiotic events. We found that in pig oocytes that are incompetent to undergo GVBD, the cytoplasmic MAPK activity remained low after maturation culture, suggesting that the ability to activate MAPK is one of the characteristics of cytoplasmic maturation[8].

In discussing the relationship between MAPK and the initiation of meiotic resumption, three important concepts should be kept in mind. Firstly, the functioning time of Mos is different between the two animal models, *Xenopus* and mouse. In mammals, Mos is synthesized after GVBD, and its main function is to keep the MⅡ arrest, instead of the initiation of GVBD in *Xenopus* oocytes. However, in *c-mos* deficient mouse, oocytes usually cannot develop to MⅡ stage, and the release of first polar body is retarded. These facts indicate that although Mos is not necessary absolutely for meiosis Ⅰ, it is involved in the MⅠ/MⅡ transition in physiological conditions[9]. Secondly, although the results obtained by others and us[10] indicate that there is no species-specificity regarding MPF functions, the storage quantities of MPF in oocytes varies among different species, even among different individuals of the same species. Thus the dependence of MPF activation on MAPK may be also different. In some cases, MAPK stimulates G2/M transition by inhibiting Myt1 or by stimulating cyclin B translation, while in other cases, this mechanism is unnecessary. Finally, the activity of MAPK is also regulated by MPF. In oocytes of all animals, MAPK is fully activated only when the Cdc2 is active. Cdc2 may activate Mos, the upstream kinase of MAPK, directly or indirectly. In *Xenopus* oocytes, the phosphorylation of Ser3 is indispensable for Mos activity. This phosphorylation is mediated by MPF. It is possible that MAPK is partly activated through a MPF-independent pathway at the early stage of maturation, whereas MPF induces the full activation of MAPK around GVBD.

3 MAPK and spindle organization

The resumption of oocyte meiotic maturation is linked to the reorganization of interphase microtubules to a metaphase configuration. The components of microtubule organizing center (MTOC) was phosphorylated, which makes microtubules have a higher potential to organize a spindle. MAPK is distributed on the MTOC at both the poles of spindle and cytoplasm in mouse oocytes. It has been found that the actions of microtubule and chromosomes were regulated by MAPK. MAPK can induce the chromosome condensation and spindle organization in mouse oocytes even if the activity of MPF is inhibited[11, 12]. The results obtained by others and us show that in porcine oocytes, MAPK was phosphorylated during GVBD and that the active MAPK was associated with the condensed chromosomes[13]. MAPK was distributed at the poles of spindle during metaphase and migrated to the middle of spindle at anaphase. With the polar body emission, MAPK was associated with the cytokinetic ring. If the activity of MAPK was inhibited by U0126, chromosome separation, first polar body emission and MⅡ spindle formation were also inhibited[14]. We also found that if taxol, a microtubule dissembly inhibitor, was used, many MTOC could be induced in the cytoplasm and MAPK concentrated to them[15]. In bovine oocytes, MAPK migrated to the germinal vesicle (GV) before GVBD. If the mRNA of MKP-1, a MAPK-specific phosphatase, was injected into the GV, MAPK could not be activated as the normal oocytes. Despite the lake of MAPK activity, MKP-1 injected oocytes resume meiosis and progress through meiosis, although the spindle is abnormal and the chromosomes are poorly aligned and the oocytes are unable to arrest at MⅡ stage[16]. All the results suggest that MAPK participates in the organization and maintenance of metaphase spindle in oocytes.

The meiotic divisions are typically asymmetric in oocytes, which was determined by the asymmetric location of the spindle. Spindle movement is often due to the interaction between spindle asters and the cortex. In wild-type mouse oocytes, the meiotic spindle formed in

the center of the cell and migrated to the cortex just before polar body extrusion. The spindle did not elongate during anaphase. In $mos^{-/-}$ oocytes, the spindle formed centrally and elongated but did not migrate to the cortex, resulting in an abnormal large polar body formation[17]. It was known that the migration of the spindle was regulated by network of microfilaments rather than microtubules[18]. These results suggest that the Mos/MAPK pathway may be involved in the regulation of microfilament action as well as the spindle organization.

Although MAPK is a pivotal regulator in the spindle organization, its targets on the spindle are still not known. Polo-like kinases (Plk) are a family of protein kinases that can regulate the spindle construction in eukaryotic cells. Plk was associated with the poles of spindle at early metaphase and migrated to the middle of spindle at metaphase/anaphase transition[19]. We found that in MⅡ mouse oocytes treated with MEK inhibitor U0126, Plk could not migrate from poles to the middle of spindle. This result suggests that there is cross-talk between Plk and MAPK (unpublished results).

Recent studies proved the existence of spindle assembly check point in cell cycle. If the spindle is poorly organized, it can maintain the activity of MPF. As a result, the cell cannot enter anaphase. Since MAPK is a critical regulator in the spindle organization, whether the spindle regulates MAPK activation in a feedback way or not is an interesting question. However, the report in this aspect is very limited. We found that if spindle organization and migration were disturbed by colchicine, taxol and cytochalasin B in porcine oocyte, MAPK activity was not affected[20]. Although this result suggests that the activity of MAPK is not regulated by the spindle organization, the functions of MAPK in the spindle assembly checkpoint need deep examination.

4 MAPK pathway and MⅡ arrest

The degradation of cyclin B is required for the exit of the metaphase. MPF activated anaphase promoting complex (APC) which makes cyclin to be ubiquitinylated and degraded by 26S proteosome. In vertebrates, maturing oocytes produce a cytostatic factor (CSF), which causes oocyte to arrest at metaphaseⅡ. It has been known that CSF is a complex composed of several types of protein kinase rather than a single molecule. Although the essence of CSF was not clear, Mos-MAPK-$p90^{rsk}$ was known as critical components of it. Microinjection of *mos* mRNA, active-MAPK or active-$p90^{rsk}$ into one blastomere of a two-cell frog embryo induces cleavage arrest at metaphase. The oocytes of *c-mos* knockout mouse cannot arrest at MⅡ, but are activated spontaneously[21]. U0126, an inhibitor of MEK, induced the oocyte to break the MⅡ arrest in porcine oocytes[22]. All the information shows that MAPK pathway can inhibit APC-depended cyclin B degradation and chromosome separation during metaphase Ⅱ in oocytes. However, MAPK pathway cannot be the only factors in CSF and other unknown factors must be involved in the MⅡ arrest. Some unknown factors, which make the oocyte have complete CSF activity, may be synthesized between MⅠ/MⅡ transition, in considering that MAPK pathway has been activated completely before MⅠ, while the meiosis is not arrest at MⅠ.

In oocytes, cyclin B is to some extent continuously degraded and synthesized even at metaphase Ⅱ. One explanation for the CSF-arrested-like state is that the MAPK stimulated the process which prevents active APC from ubiquitinylating its substrates in MⅡ, and/or MAPK-dependent protein synthesis outweighs ubiquitin-dependent degradation. The interesting thing is that although CSF induced the MⅡ arrest, CSF or MAPK inactivation was not necessary for the release of MⅡ arrest after fertilization. Under physiological conditions, releasing from MⅡ arrest is mediated by Ca^{2+}/calmodulin dependent kinaseⅡ (CaMKⅡ), which triggers degradation of M-phase cyclins and sister chromatid segregation[23]. In other words, if MAPK inhibits cyclin degradation, CaMKⅡ can overcome it. CaMKⅡ, as a factor to break the arrest, has been initiated, which can explain why the meiosis does not arrest at MⅡ in the invertebrate oocytes, which are fertilized before MⅡ stage, although MAPK is still activated. However, there are still several puzzles existing: Which factor triggers the metaphase-anaphase transition? What is the relationship between these mechanisms and MAPK as well as MPF?

5 Roles of MAPK after fertilization

Fertilization is the physiological stimulation that releases the oocytes from MⅡ arrest. The fertilized oocytes undergo a series of metabolism transition, including the increase in intracellular Ca^{2+}, reorganization of cytoskeleton, translocation of mitochondria, and so on[24].

The abrupt decrease in MPF activity after fertilization is the prerequisite for oocytes to break MⅡ arrest. But the MAPK activity remains high until the pronucleus formation, i.e. the S phase of the first mitotic cell cycle. And MAPK activity does not appear in the following cell cycle. Apparently the inactivation of MAPK and MPF is mediated by different mechanisms. The knowledge about the roles of MAPK in the first cleavage, especially the significance of the prolonged activation of MAPK after egg activation for the M phase-interphase transition, is limited. Recently, it was reported that in the ocean invertebrate, the inhibition of MAPK activity in oocytes resulted in the pre-formation of sperm aster after fertilization. Maybe it is a ubiquitous mechanism for the MAPK activity to inhibit the sperm aster development after fertilization among animals[25].

In our experiments, the parthenogenetic activation of mouse was induced by various chemicals, including ethanol, calcium ionophore A23187[26], protein kinase inhibitors staurosporine[27], Rp-31-8220, genestein[28], and protein synthesis inhibitor cycloheximide[29]. In these approaches, the MAPK activity decreased abruptly before pronucleus formation, and protein phosphatase inhibitor OA prevents MAPK inactivation and pronuclues formation. These facts suggested that although these stimulations induced the parthenogenetic activation of oocytes through different mechanisms, they led to the same biochemical event, the inactivation of MAPK. Furthermore, considering the function of MAPK during other stages of meiosis, it seems that MAPK is an inhibitor of nuclear membrane assembly, and the inactivation of MAPK is the prerequisite for the nuclear membrane construction. The inactivation of MAPK is due to protein phosphatase activity in the cells. However, the MAPK-specific protein phosphatases in mammalian cells have not been identicifed until now. Although OA, the inhibitor of PP1 and PP2A, can inhibit inactivation of MAPK, it cannot be concluded that PP1 and PP2A are responsible for dephosphorylating MAPK in physiological conditions. Other unknown OA-sensitive protein phosphatases may also play a critical role in the regulation of MAPK activity.

The change in the activity and functions of MAPK and MPF during oocyte maturation and fertilization was shown in fig. 1.

6 MAPK and cAMP/PMA signaling pathway

Mammalian oocytes, arrested at G2 phase of the cell cycle, can reinitiate meiosis spontaneously *in vitro* upon their release from antral follicles. A drop in intracellular cAMP levels followed by inactivation of the cAMP-dependent protein kinase A (PKA) is associated with resumption of meiosis. This process can be blocked by the addition of dibutyryl cyclic AMP (dbcAMP), a membrane-permeable cAMP analogue, isobutyl-methyl-xanthine (IBMX), a phosphodiesterase inhibitor, or forskolin, an adenylate cyclase activator. However, which mechanism initiates GVBD after inactivation of PKA is still not clear. We probed the relationship between MAPK phosphorylation and cAMP pathway in mouse, rat and porcine oocytes (unpublished results of our lab). The results show that MAPK remains inactive in GV oocytes but is activated soon after GVBD. When dbcAMP, IBMX or forskolin was added to increase the intracellular cAMP level, both GVBD and MAPK activations were inhibited. If the drugs were added after GVBD, MAPK activation as well as the following meiotic events such as PB1 emission and MⅡ spindle formation was not inhibited. These effects suggested that perhaps the decrease of cAMP levels in GV oocytes is the prerequisite for MAPK activation[30, 31]. However, cAMP pathway may block GVBD by inhibiting MPF activation rather than MAPK phosphorylation, since MAPK activation is not the premise of GVBD in mammalian oocytes.

How does cAMP/PKA pathway inhibit MAPK activation? We found that if OA was added to the culture me-

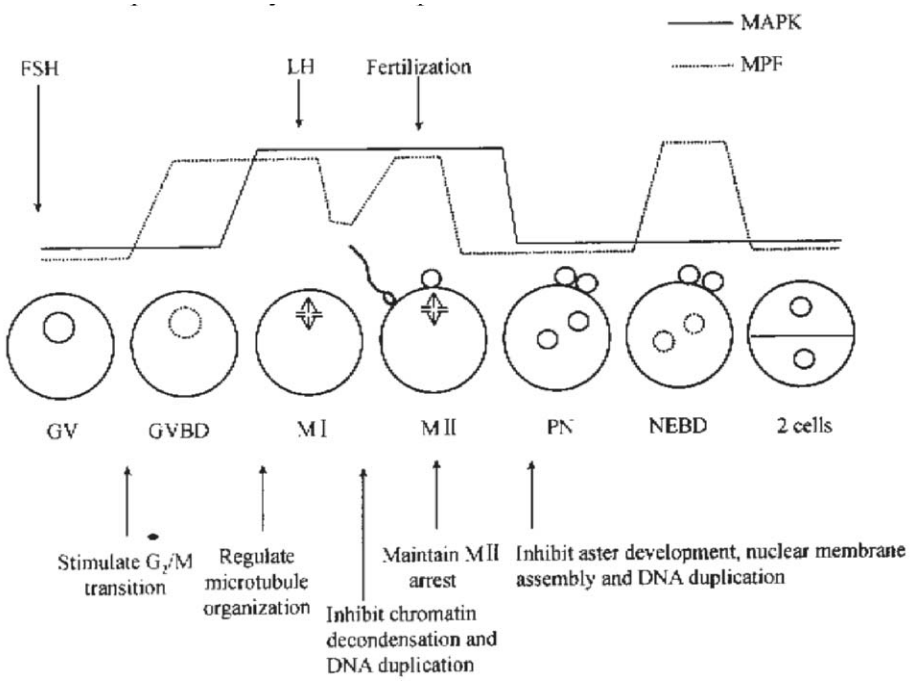

Fig. 1. The change in the activity and functions of MAPK and MPF during oocyte maturation and fertilization.

dium, the block of GVBD and MAPK activation by PKA activation was overcome. Calphostin C, an inhibitor of PKC, could partially reverse the effect of dcbAMP on oocytes. These facts suggest that in cAMP/PKA pathway an OA-sensitive protein phosphatase may mediate the inhibition of MAPK activation. Furthermore, PKC may be involved in the cAMP-mediated MAPK activity regulation[32].

7 MAPK and protein kinase C

The rise in intracellular Ca^{2+} levels is necessary for the oocyte activation. However, the downstream molecule is still not known. Recently, protein kinase C (PKC) drew a lot of attention[33—35] as a possible target of Ca^{2+}. We found that in mouse[32] and rat[36], if PKC was activated by phorbol ester, GVBD would be inhibited strongly in oocytes. We also found that in MⅡ eggs, phorbol ester can induce parthenogenetic activation with pronuclear formation. It seemed that PKC activation always make the eggs arrested at interphase. Since both PKC and MAPK pathways participate in meiosis regulation and MAPK is a cross point of many signaling pathways, there may be cross-talk between MAPK and PKC pathway. Our suggestion was supported by experimental results. PKC activator can inhibit MAPK activation with the block of GVBD in GV oocytes. In MⅡ eggs, PKC activator induced parthenogenetic activation as well as the inactivation of MAPK. These results indicate that PKC can downregulate the activity of MAPK in meiosis.

MAPK may not be the only target of PKC in meiotic regulation since PMA can inhibit PB1 emission without affecting the activity of MAPK when PMA was used after MAPK activation. Furthermore, the GVBD block induced by PMA may not be mediated by MAPK because MAPK activity is not indispensable for GVBD in mouse oocyte. PKC may regulate these processes by interaction with MPF rather than MAPK.

In discussing the interaction between MAPK and cAMP or PKC signaling pathway, it was suspected that the inhibitory effects of cAMP elevators or PKC activators are just the results of their inhibition on GVBD. A recent result argued that the MAP kinase activation is independent of the germinal vesicle materials during porcine oocyte maturation. We found that it is also the case in mouse oocytes, and the MAP kinase activation in enucleated mouse oocytes could be also inhibited by PKA or PKC activation (unpublished results). These results indicate that the interaction between PKA or PKC and MAPK does exist in cytoplasm, instead of being resulted from the inhibition of GVBD.

8 MAPK and protein phosphatase

The importance of protein kinase and protein phosphorylation in the regulation of life activity has been well recognized. However, its reverse process, i.e. the protein dephosphorylation mediated by protein phosphatase (PP) is also an important element of signal transduction in a cell. PP1 and 2A have been identified from mouse oocytes. They are involved in the regulation of MAPK activity and microtubule organization. PPs inactivate MAPK by dephosphorylating the Thr residue of ERK1/2. OA, the inhibitor of PP1 and 2A, could completely reverse the inhibitory effect of PKA on GVBD and MAPK activation. Based on this fact, we hypothesized that the inhibitory effect of OA on MAPK is down stream to cAMP/PKA. OA also leads to the disturbance of microtubule organization in MⅡ mouse oocytes. In the fertilized or parthenogenetically activated oocytes, OA can induce the pronucleus membrane breakdown, chromatin condensation, and the re-activation of MAPK. OA also accelerates GVBD and MAPK activation in GV oocytes. In pig oocytes treated with OA, the MAPK was activated within 5 min, even in the case when MPF was inhibited[37]. We deduced from these results that there exists a mechanism responsible for the downregulation of MAPK activity before the meiotic resumption in oocytes, and OA induces GVBD and pronucleus membrane breakdown by overcoming the effects of this mechanism. Though it is MPF, instead of MAPK, that initiates the G2/M transition in physiological condition, OA treatment leads to the early activation of MAPK, and initiates meiotic resumption by a redundant pathway.

9 Conclusions

MAPK and MPF, the two important protein kinases for oocyte meiosis, interact intimately and perform essential regulating roles in meiotic maturation and fertilization. No matter at what stages of the cell cycle the oocytes from different animals arrest and fertilized, the activation and inactivation of MAPK follow the same rule; that is, its activity emerges and vanishes concurrently with the chromatin condensation and incompetent with the nuclear membrane. MPF is inactivated between the two meioses, but MAPK activity remains high, inferring its roles in the inhibition of chromatin de-condensation and DNA duplication before the completion of meiosis. Although $p90^{rsk}$ appears to be downstream to MAPK in several meiotic processes, we still do not know exactly the substrates of MAPK cascade, which highlights a recurrent problem in signal transduction biology: the difficulty of identifying genuine *in vivo* biochemical targets of protein kinases in biological processes.

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