Nuclear Localization of Duplin, a β-Catenin-binding Protein, Is Essential for Its Inhibitory Activity on the Wnt Signaling Pathway*

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Duplin binds to β-catenin and inhibits the Wnt signaling pathway, thereby leading to repression of the β-catenin-mediated transactivation and Xenopus axis formation. To find an additional function of Duplin, yeast two-hybrid screening was carried out. Importin α was isolated as a binding protein of Duplin. Importin α bound directly to basic amino acid clusters of Duplin. Although Duplin was present in the nucleus, deletion of the basic amino acid clusters (Duplin5300–594) retained Duplin in the cytoplasm. Duplin5300–584 bound to β-catenin as efficiently as wild-type Duplin, but it neither repressed Wnt-dependent Tcf transcriptional activation in mammalian cells nor showed ventralization in Xenopus embryos. The Duplin mutant without a β-catenin-binding region lost the ability to inhibit the Wnt-dependent Tcf activation, but retained its ventralizing activity. Furthermore, Duplin not only suppressed β-catenin-dependent axis duplication and expression of siamois, a Wnt-regulated gene, but also inhibited siamois-dependent axis duplication. These results indicate that Duplin is translocated to the nucleus by interacting with importin α, and that nuclear localization is essential for the function of Duplin. Moreover, Duplin has an additional activity of inhibiting the Wnt signaling pathway by affecting the downstream β-catenin target genes.

Wnt proteins constitute a large family of cysteine-rich secreted ligands that control development in organisms ranging from nematode worms to mammals (1). The intracellular signaling pathway of Wnt is also conserved evolutionally and regulates cellular proliferation, morphology, motility, and fate, axis formation, and organ development (1–6). In the current model, the serine/threonine kinase GSK-3β targets cytoplasmic β-catenin for degradation in the absence of Wnt (7, 8). As a result, cytoplasmic β-catenin levels are low. Axin has been shown to be important for the degradation of β-catenin (9). It forms a complex with GSK-3β, β-catenin, APC, and protein phosphatase 2A (8–17), and regulates GSK-3β-dependent phosphorylation of β-catenin, Axin, and APC (8–10, 13, 16, 18, 19). Phosphorylated β-catenin forms a complex with Fbw1, a member of the F-box protein family, resulting in the degradation of β-catenin by the ubiquitin and proteasome pathways (20, 21). Indeed, expression of Axin decreases the protein level of β-catenin (22). Thus, Axin is a negative regulator of the Wnt signaling pathway that keeps cytoplasmic β-catenin level low.

When Wnt acts on its cell-surface receptor Frizzled, the cytoplasmic protein Dvl antagonizes the action of GSK-3β. Although this mechanism has not yet been clarified, it has been suggested that Frat, which was identified as a GSK-3-binding protein (23), forms a complex with Dvl, and that this complex induces the dissociation of GSK-3β from Axin (24, 25). It has also been demonstrated that casein kinase I forms a complex with Dvl and that it enhances the action of Dvl (26, 27). Furthermore, it has been shown that Dvl inhibits GSK-3β-dependent phosphorylation of β-catenin, APC, and Axin in vitro and the phosphorylation of Axin in intact cells (28, 29). Once the phosphorylation of β-catenin is reduced, it dissociates from the Axin complex, and β-catenin is no longer degraded, resulting in its accumulation in the cytoplasm. Accumulated β-catenin is translocated to the nucleus where it binds to Tcf lymphocyte enhancer binding factor, transcription factors, and stimulates the expression of genes including c-myc, fra-1, e-jun, cyclin D1, and puroisome proliferator-activated receptor 6 (4–6). Thus, the Wnt signal stabilizes β-catenin, thereby regulating the expression of various genes.

In addition to the regulation of its stability in the cytoplasm, β-catenin signaling through Tcf is also regulated in the nucleus. It is thought that Tcf may be a transcriptional repressor rather than an activator, because Tcf binds to proteins that can mediate repression. One such repressor is Groucho in Drosophila (30). The binding sites for Armadillo (Drosophila β-catenin) and Groucho on Tcf do not overlap, but whether or not Armadillo and Groucho bind simultaneously to Tcf is not clear. It is possible that expression of Tcf-target genes is regulated by a balance between Armadillo and Groucho. Another Tcf-binding protein is Drosophila CBP (31). Drosophila CBP interacts with the high-mobility group domain of Tcf and acetylates a corepressor; NLS, nuclear localization signal; SV40, simian virus 40; PBS, phosphate-buffered saline; PIAS, protein inhibitor of activated STAT; STAT, signal transducers and activators of transcription.

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† The abbreviations used are: GSK-3β, glycogen synthase kinase-3β; APC, adenomatous polyposis coli protein; Tcf, T cell factor; CBP, cAMP response-binding protein-binding protein; CtBP, C-terminal-binding protein; Xwnt-8, Xenopus wnt-8; MBP, maltose-binding protein; GST, glutathione S-transferase; HA, hemagglutinin; GFP, green fluorescent protein; NLS, nuclear localization signal; SV40, simian virus 40; PBS, phosphate-buffered saline; PIAS, protein inhibitor of activated STAT; STAT, signal transducers and activators of transcription.
served lysine in the Armadillo-binding domain of Tcf. This acetylation lowers the affinity of Tcf for Armadillo. Interestingly, mammalian CBP and its related protein p300 (CBP/p300) synergize with β-catenin to stimulate gene expression, and Xenopus CBP positively regulates the axis formation (32, 33). The reasons for the apparent discrepancy between the function of vertebrate CBP/p300 and Drosophila CBP are not known. The other Tcf-binding protein is Xenopus CtBP family that is homologous to the transcriptional co-repressor human CtBP (34). Xenopus CtBP binds to the C-terminal region of Xenopus β-catenin-Tcf-3 and represses its transcriptional activity (34). Furthermore, NEMO-like kinase binds directly to phosphorylates Tcf, which then inhibits the binding of the β-catenin-Tcf complex to DNA (35). These Tcf-binding proteins appear to regulate complex formation among β-catenin, Tcf, and DNA. It has been reported that there are several proteins that bind to β-catenin and inhibit its function. Pontin52 is a nuclear protein that binds to β-catenin and the TATA-box binding protein (36). Recently it has been shown that Pontin52 and Reptin52, a newly identified Pontin52 homologue, antagonistically influence the transactivation potential of the β-catenin-Tcf complex, and that they are essential genes that act antagonistically in the control of Wingless signaling in Drosophila. These results indicate that the opposing actions of Pontin52 and Reptin52 on β-catenin-mediated transactivation constitute an additional mechanism for the control of the canonical Wingless/Wnt pathway (37). X Sox17 is a Xenopus high-mobility group b-box containing protein that activates transcription of endodermal gene and represses β-catenin-stimulated expression of dorsal genes (38). ICAT also binds to β-catenin and represses β-catenin-mediated transactivation, thereby inhibiting Xenopus axis formation (39). Thus, it is likely that β-catenin signaling through Tcf is inhibited by several mechanisms at the level of Tcf and β-catenin in the nucleus.

We have recently identified a novel protein that binds to β-catenin in the nucleus and designated it Duplin (40). Duplin inhibits the binding of β-catenin to Tcf-4, thereby inhibiting Wnt-3a- and β-catenin-dependent Tcf-4 activation in mammalian cells. Duplin inhibits expression of siamois, a Wnt-responsive gene (41, 42), and suppresses Xwnt-8 and Xβ-catenin-induced axis duplication in Xenopus embryos. Based on these functions of Duplin, we proposed that Duplin forms a complex with β-catenin in the nucleus and represses the β-catenin-dependent Tcf activation. Although it is likely that Duplin inhibits the β-catenin signaling in a manner different from Groucho, Xenopus CtBP, Drosophila CBP, NEMO-like kinase, and ICAT, the mechanisms by which the nuclear localization and action of Duplin are regulated are still unclear. To clarify these mechanisms of Duplin, we identified a Duplin-binding protein by the yeast two-hybrid screening. Here we show that Duplin binds to importin α directly through its basic amino acid clusters. We also demonstrate that nuclear localization of Duplin is necessary for its inhibition of Wnt-dependent activation of Tcf in mammalian cells and ventralization in Xenopus embryos. Moreover, we show that Duplin also functions to inhibit the Wnt signaling pathway downstream of β-catenin target genes.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—MBP and GST fusion proteins were purified from Escherichia coli according to the manufacturer’s instructions. The anti-importin α-P, α-Q, and α-S antibodies were generated as described (43). The anti-Myc antibody was prepared from 9E10 cells. The anti-MBP and GST antibodies were prepared in rabbit by immunization with recombiant MBP and GST, respectively. L cells (mouse fibroblasts) stably expressing HA-Duplin500–584 and HA-Duplin(1–668) were generated as described (22, 44). Wnt-3a-conditioned medium was generated as described (45). The anti-β-catenin antibody was purchased from Transduction Laboratories (Lexington, KY). Other materials were obtained from commercial sources.

RESULTS

Identification of Importin α as a Duplin-binding Protein—Various constructs in this study are shown in Fig. 1. To identify a protein that is involved in the functions of Duplin DNA, we screened a mouse brain cDNA library with the yeast two-hybrid method using the C-terminal half of Duplin as bait. Among 2.6 × 10⁷ clones, four clones were found to confer both His⁺ and LacZ⁺ phenotypes, and one of them was importin α-Q2. Importin α recognizes NLSs and mediates the selective transport of karyophilic proteins to the nuclei (50). Mouse

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importin α can be classified into three subfamilies, α-P, α-Q, and α-S families, which have ~50% amino acid identity to one another (51). The α-Q family is composed of the closely related members α-Q1 and α-Q2, and they share more than 80% amino acid sequence identity. The α-S family also has two members, α-S1 and α-S2. To examine whether Duplin forms a complex with importin α in intact cells, Myc-Duplin was expressed in COS cells, and three different antibodies that detect distinct importin α subfamilies were used to probe endogenous importin α (Fig. 2A). When the lysates expressing Myc-Duplin were immunoprecipitated with the anti-Myc antibody, endogenous importin α-P was detected in the Myc-Duplin immune complex (Fig. 2A). Importin α-P was not immunoprecipitated from the same lysates with non-immune immunoglobulin (data not shown). Endogenous importin α-Q and importin α-S were also detected in the Myc-Duplin immunoprecipitates (Fig. 2A). These results indicate that Duplin forms a complex with three subfamilies of importin α.

To determine which region of Duplin forms a complex with importin α, various deletion mutants of Duplin were expressed in COS cells (Fig. 2B). When the lysates expressing Myc-Duplin mutants were immunoprecipitated with the anti-Myc antibody, importin α-P was co-precipitated with Myc-Duplin (full-length) and Myc-Duplin(482–749) but not with Myc-Duplin(1–482) (Fig. 2B). These results are consistent with the previous observations that Duplin(482–749) was present in the nucleus and that Duplin(1–482) was localized in the cytoplasm (40). The region containing amino acids 500–584 had basic amino acid clusters, KKKRK505, KPKK518, KKKRK546, KKR547, and KRK584 (40). One or two clusters of basic amino acids are classical NLSs recognized by importin α (52). By deleting these basic amino acid clusters (Duplin500–584), Duplin did not form a complex with importin α-P (Fig. 2B), indicating that the basic amino acid clusters of Duplin are necessary for its interaction with importin α. To examine whether the interaction of Duplin with importin α is direct, GST-fused Duplin and MBP-fused importin α were purified from E. coli. GST-Duplin(500–584), but not GST-GFP, was co-precipitated with MBP-importin α-P, indicating that Duplin binds directly to importin α (Fig. 2C). Duplin(500–584) and Duplin(565–668) contain three and two basic amino acid clusters, respectively. Both GST-Duplin(500–584) and GST-Duplin(565–668) bound to MBP-importin α-P (data not shown).

**Determination of NLS of Duplin**—To determine whether the basic amino acid clusters actually function as NLS, we expressed Myc-Duplin and Myc-Duplin(500–584) in L cells. Myc-Duplin was present in the nucleus, while Myc-Duplin(500–584) was observed in the cytoplasm (Fig. 3A). These results indicate that the region of amino acids 500–584 of Duplin is necessary...
for its nuclear localization. To examine whether the basic amino acid clusters of Duplin are sufficient for its nuclear localization, Duplin-(500–584), Duplin-(500–565), and Duplin-(565–668) were fused to GST-GFP and these fusion proteins were microinjected into the cytoplasm of L cells. The NLS of the simian virus 40 large T antigen (GST-SV40NLS-GFP) was translocated to the nucleus within 30 min, while GST-GFP was retained in the cytoplasm (Fig. 3B, f and g). Duplin-(500–584) and Duplin-(500–565) efficiently directed GST-GFP into the nucleus, but Duplin-(565–668) did so to a lesser extent (Fig. 3B, a–c). Among amino acids 500–565, both GST-Duplin-(500–521)-GFP and GST-Duplin-(542–546)-GFP were translocated to the nucleus with the same efficiency (Fig. 3B, d and e). These results suggest that any of the basic amino acid clusters of Duplin can function as NLS and that they are responsible for the nuclear localization of Duplin.

**Effect of Nuclear Localization of Duplin on Its Function**—In a prior study (40), we demonstrated that Duplin binds to β-catenin and inhibits the binding of β-catenin to Tcf, thereby repressing β-catenin-dependent Tcf transcriptional activation. We examined whether nuclear localization is necessary for these functions of Duplin. Myc-Duplin or Myc-Duplin500–584 was expressed in COS cells, and the lysates were immunoprecipitated with the anti-Myc antibody. β-Catenin associated with Myc-Duplin and Myc-Duplin500–584 with similar efficiency (Fig. 4A), suggesting that Duplin binds to β-catenin irrespective of its subcellular distribution. As reported previously (40), expression of Duplin in L cells did not affect Wnt-3a-dependent accumulation of β-catenin, but it did inhibit Wnt-3a-dependent Tcf activation (Fig. 4, B and C). Expression of HA-Duplin500–584 inhibited neither Wnt-3a-dependent accumulation of β-catenin nor Tcf activation (Fig. 4, B and C). These results suggest that nuclear localization is important for Duplin inhibition of Wnt-dependent Tcf activation.

The Wnt signaling pathway regulates axis formation in Xenopus embryos (53). Dorsal injection of Duplin mRNA into four-cell stage embryos resulted in ventralizing phenotypes such as loss of the head (40). However, dorsal expression of Duplin500–584 did not affect the axis formation, and its ventral expression did not, either (Fig. 4D). These results indicate that Duplin500–584 does not show the ventralizing activity and that nuclear localization is necessary for the action of Duplin in inhibiting axis formation.

**Functions of Duplin Other Than Inhibition of β-Catenin-dependent Gene Expression**—The C-terminal region of Duplin (Duplin-(668–749)) binds to β-catenin (Fig. 1). Duplin-(1–668), in which the β-catenin-binding region is deleted, lost the ability...
to bind to β-catenin (Fig. 5A). To examine whether this region is essential for Duplin inhibition of the Wnt signaling pathway, we generated L cells stably that express Duplin-(1–668). As expected, expression of Duplin-(1–668) did not suppress the Wnt-3α-dependent activation of Tcf under the conditions that Duplin did (Fig. 5B).

Interestingly, however, expression of Duplin-(1–668) in *Xenopus* embryos still resulted in ventralizing activity with similar efficiency to Duplin (Fig. 6, A, a–c, and B). These results suggest that Duplin has another function of regulating the Wnt signaling pathway in addition to inhibiting the interaction of β-catenin with Tcf. *Siamois* is a homeobox gene that mediates the effects of the Wnt signaling pathway on axis formation, and whose expression is induced by β-catenin and Tcf (41, 42). Indeed, expression of *siamois* mRNA was suppressed by dorsal injection of Duplin (40). The results of Fig. 6, A and B, allowed us to ask whether Duplin functions downstream of *siamois*. Duplin suppressed *siamois*-dependent axis duplication, and Duplin-(1–668) showed the similar activity (Fig. 6, A, d–f, and C). Taken together with the previous observations (40), these results clearly indicate that Duplin has two different modes of action for inhibiting the Wnt signaling pathway; one is to inhibit β-catenin-dependent Tcf activation, and the other is to suppress its further downstream.

**DISCUSSION**

We originally identified Duplin as a nuclear protein that binds to β-catenin and prevents its interaction with Tcf, thereby inhibiting the Wnt signaling pathway (40). In this study, we have further characterized Duplin and clarified the following points. (i) Duplin binds to importin α through its basic amino acid clusters, thereby translocating to the nucleus. The basic amino acid clusters of Duplin are necessary and sufficient for its nuclear localization. (ii) Nuclear localization of Duplin is essential for its inhibition of the Wnt signaling pathway, and (iii) Duplin not only prevents the interaction of β-catenin with Tcf but also inhibits downstream events of the β-catenin-dependent gene expression. These results indicate that Duplin functions in the nucleus and that it inhibits the Wnt signaling pathway by at least two distinct mechanisms.

In a prior study (40), we found that the C-terminal region containing the basic amino acid clusters of Duplin is present in the nucleus and that the N-terminal region of Duplin is in
the cytoplasm. Consistent with these results, we here identified importin α as a Duplin-binding protein by the yeast two-hybrid screening with the C-terminal region of Duplin as bait. In the nuclear transport of proteins, the best characterized signals are the classical NLSs that contain one or two clusters of basic amino acids but do not conform to a specific consensus sequence (52). The two major classes of NLSs are the monopartite NLS, exemplified by the SV40 NLS, 126KKKKRKKV132, and the bipartite NLS, exemplified by the NLS of nucleoplasmin, 155KRPAATKKAQAKKKK170. The monopartite type contains 3–5 basic amino acids with the weak consensus K(R/K)(X)K, and the bipartite type contains two clusters of basic regions of 3–4 residues, each separated by about 10 amino acids. The targeting efficiency of NLSs can be altered by modification (phosphorylation) of flanking sequences, the number of NLSs within a protein, and the distances between them. Despite the variability, the classical NLSs are recognized by the same receptor, importin, consisting of a heterodimer of α- and β-subunits (50, 54). Importin α contains the NLS-binding site, and importin β is responsible for the docking of the importin-substrate complex to the cytoplasmic filaments of the nuclear pore complex and its translocation through the pore. Duplin has a region (amino acids 500–584) containing the five basic amino acid clusters where importin α binds directly. The five basic amino acid clusters are KKKRK205, KPKK218, KKKRK246, KRR275, and KRK284. Among them KKKRKSSGERLKEEKKPK218 and KRRSSNRQVKKR284 may be the bipartite type and KKKRK246 may be the monopartite type. All of these basic amino acid clusters can bind to importin α directly, and are sufficient for nuclear translocation. The Duplin mutant without these five basic amino acid clusters loses the ability to bind to importin α and to translocate to the nucleus. Although which basic amino acid cluster is mainly responsible for the nuclear localization of Duplin is not clear, the region containing these basic amino acid clusters is essential for it. Rather, to have several NLSs reflects the importance of nuclear localization for the functions of Duplin. Indeed, the Duplin mutant without the five basic amino acid clusters loses the ability of Duplin to inhibit Wnt-dependent Tcf activation in L cells and to induce ventralization in Xenopus embryos. These results indicate that nuclear localization is essential for the functions of Duplin. Since the Duplin mutant that is not translocated to the nucleus can still bind to β-catenin, it is conceivable that Duplin co-operates with other nuclear proteins that interact with β-catenin and Tcf to inhibit β-catenin signaling. We have previously shown that the β-catenin-binding region (Duplin-(667–749)) without the basic amino acid clusters ventralizes Xenopus embryos, although its activity is weak (40). Since it is known that signaling pathways other than the Wnt pathway are involved in the regulation of axis formation (55, 56), Duplin(667–749) may affect these pathways.

The Duplin mutant without the β-catenin-binding region lost the ability to inhibit Wnt-3a-dependent Tcf activation in L cells. This is reasonable from the view of the mode of action of Duplin that we proposed (40). Unexpectedly, however, Duplin(1–668), which does not bind to β-catenin, induced ventralization in Xenopus embryos. Since the effects of Duplin on all stages of axis formation are examined in the assay using Xenopus embryos, it is conceivable that Duplin may affect events other than β-catenin-Tcf-dependent gene expression. In Xenopus, siamois is induced by β-catenin and Tcf, and overexpression of siamois induces axis duplication similar to β-catenin and Tcf (41). We have shown that Duplin not only inhibits siamois mRNA expression and β-catenin-dependent axis duplication but also suppresses siamois-dependent axis duplication. Furthermore, Duplin(1–668) also inhibits siamois-dependent secondary axis formation. From these results, it is clear that Duplin acts downstream of siamois and regulates axis formation. Previously, we showed that the C-terminal half of Duplin, Duplin-(482–749), has ventralizing activity while the N-terminal half, Duplin(1–482), does not (40). Although Duplin(1–668) does not bind to β-catenin, it can be translocated to the nucleus. Deletion of the β-catenin binding region from Duplin(482–749) does not have ventralizing activity (40). Therefore, inhibition of the axis formation by the C-terminal region of Duplin is mediated through the prevention of the binding of β-catenin to Tcf. Taken together, it is possible that Duplin(1–482) acts downstream of siamois when Duplin is in the nucleus. Although the ventralizing activity of Duplin(482–749) is weaker than that of Duplin (40), Duplin(1–668) and Duplin show similar ventralizing activity (Fig. 6). This newly identified function of Duplin may dominate the regulation of axis formation in Xenopus embryos.

The new mechanism by which Duplin inhibits the Wnt signaling pathway is not known in detail. Siamois is a homeobox gene that stimulates gene expression (41). One possibility is that Duplin may interact with siamois and alter the proteins level of siamois. However, it is unlikely, because Duplin did not form a complex with siamois under the same conditions that it interacts with β-catenin, and Duplin did not affect the protein stability of siamois (data not shown), as far as these experiments were done in COS cells. Another possibility is that Duplin may suppress siamois-dependent gene expression by interacting with protein(s) other than siamois since Duplin did not bind DNA directly in our preliminary experiment (data not shown). We have recently isolated PIAS as a Duplin-binding protein by the yeast two-hybrid screening. PIAS has been originally identified as a protein that inhibits STAT-dependent gene expression (57) and has been recently shown to affect steroid receptor-dependent gene expression (58). Furthermore, STAT inhibits Xenopus axis formation (59). Therefore, it is intriguing to speculate that Duplin regulates downstream of β-catenin-Tcf-dependent gene expression by interacting with PIAS.

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REFERENCES

1. Wodarz, A., and Nusse, R. (1998) Annu. Rev. Cell Dev. Biol. 14, 59–88
2. Dale, T. C. (1998) Biochim. J. 329, 209–223
3. Miller, J. R., Hocking, A. M., Brown, J. D., and Moon, R. T. (1999) Oncogene 18, 7860–7872
4. Bientz, M., and Clevers, H. (2000) Cell 103, 311–320
5. Polakis, P. (2000) Genes Dev. 14, 1837–1851
6. Seidensticker, M. J., and Behrens, J. (2000) Biochim. Biophys. Acta 1495, 168–182
7. Yost, C., Torres, M., Miller, J. R., Huang, E., Kimelman, D., and Moon, R. T. (1996) Genes Dev. 10, 1443–1454
8. Ikeda, S., Kishida, S., Yamamoto, H., Murai, H., Koyama, S., and Kikuchi, A. (1998) EMBO J. 17, 1371–1384
9. Kikuchi, A. (1999) Cell. Signal. 11, 777–788
10. Yamamoto, H., Kishida, S., Uchi, T., Ikeda, S., Koyama, S., Asashima, M., and Kikuchi, A. (1998) Mol. Cell. Biol. 18, 2867–2875
11. Kishida, S., Yamamoto, H., Ikeda, S., Kishida, M., Sakamoto, I., Koyama, S., and Kikuchi, A. (1998) J. Biol. Chem. 273, 10823–10826
12. Sakanaka, C., Weise, J. B., and Williams, L. T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3020–3023
13. Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B., and Polakis, P. (1998) Curr. Biol. 8, 573–581
14. Ish, K., Kroupnik, V. B., and Sokol, S. Y. (1998) Curr. Biol. 8, 591–594
15. Hsu, W., Zeng, L., and Costantini, F. (1999) J. Biol. Chem. 274, 3439–3445
16. Ikeda, S., Kishida, M., Matsuzura, Y., Usui, H., and Kikuchi, A. (2000) Oncogene 19, 537–545
17. Yamamoto, H., Hinoi, T., Michiue, T., Fukui, A., Usui, H., Janssens, V., Van Hooft, C., Goris, J., Asashima, M., and Kikuchi, A. (2001) J. Biol. Chem. 276, 26875–26882
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