Regulation of the Protein Kinase Activity of ShaggyZeste-white3 by Components of the Wingless Pathway in Drosophila Cells and Embryos

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The protein-serine kinase ShaggyZeste-white3 (SggZw3) is the Drosophila homolog of mammalian glycogen synthase kinase-3 and has been genetically implicated in signal transduction pathways necessary for the establishment of patterning. SggZw3 is a putative component of the Wingless (Wg) pathway, and epistasis analyses suggest that SggZw3 function is repressed by Wg signaling. Here, we have investigated the biochemical consequences of Wg signaling with respect to the SggZw3 protein kinase in two types of Drosophila cell lines used for biochemical experiments, the present inactivation of phosphorylation modulates Armadillo signaling in cellular interactions. The protein-serine kinase ShaggyZeste-white3 (SggZw3) is the Drosophila homolog of mammalian glycogen synthase kinase-3 and has been genetically implicated in signal transduction pathways necessary for the establishment of patterning. SggZw3 is a putative component of the Wingless (Wg) pathway, and epistasis analyses suggest that SggZw3 function is repressed by Wg signaling. Here, we have investigated the biochemical consequences of Wg signaling with respect to the SggZw3 protein kinase in two types of Drosophila cell lines used for biochemical experiments, the present inactivation of phosphorylation modulates Armadillo signaling in cellular interactions.

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and Dsh) on SggZw3 activity was investigated in cultured cells and embryos. We used an imaginal disc cell line (cl-8 (clone 8)) that responds to Wg signals and Schneider (S2) cells, which are unresponsive to Wg (15, 24). Using Wg-conditioned medium, we show that the activity of SggZw3 protein kinase is inhibited by Wg in cl-8 cells and that overexpression of Dfs2 or Dsh in cells reconstitutes Wg signaling in the absence of Wg as judged by inhibition of the kinase and accumulation of Arm protein. We also demonstrate that the regulation of SggZw3 activity, in turn, controls the stability of Arm protein by modulating the level of phosphorylation of D-Axin and Arm. These results provide direct biochemical evidence in support of previous genetic analyses.

**EXPERIMENTAL PROCEDURES**

**Antiserum—**Rabbit antisera to Arm and Dsh were raised against glutathione S-transferase (GST) fusion proteins. GST-Dsh was constructed by cloning a 1256-base pair Xhol-NotI fragment of the dishevelled coding region, corresponding to amino acids 395–624, into Xhol-NotI sites in pGEX-4T-1 (Amersham Pharmacia Biotech). cDNA fragments encoding amino acids 1–367 of Arm protein and 1–514 of SggZw3 protein were cloned into pGEX-4T-1 and pET15b (Novagen), respectively. Fusion proteins were produced in Escherichia coli strain BL21(DE3) and purified from bacterial lysates before immunization.

**Transfections and Cell Culture—**Drosophila Schneider line-2 and wing imaginal disc cl-8 cells were maintained as described (24). Wg protein assays were performed essentially as published (24, 28). Selection of stably transformed cl-8 cell lines was performed using methotrexate (29). The expression vector pRhMHa-1 is designed to express proteins under control of the metallothionein promoter. The 2.8-kilobase pair BamHI-HindIII fragment of sggZw3 cDNA in pBluescript SK- (Stratagene) corresponding to the entire coding region was cloned into the BamHI-HindIII sites of pRhMHa-1. The dsh/pRhMHa-1-HApRmHa-1 vector was introduced into cl-8 cells by cotransfection with a second vector, pHGCO, carrying a selectable dhfr resistance to methotrexate (0.5 μg/ml). Transfected cl-8 cells were maintained between 1 × 10^6 and 1 × 10^7 cells/ml and examined for metallothionein expression by adherent cell immunoblotting.

For expression in cl-8 cells, (Invitrogen) was seeded in 100-mm tissue culture dishes in azide-buffered Dulbecco's phosphate-buffered saline (pH 8) and 100 mM NaCl. For purification of D-Axin-(330–642)-His6, 10 μl of nickel-Sepharose beads were added to each complex. The complexes were washed four times with 20 mM Tris-HCl, 300 mM NaCl, and 10 mM imidazole and resolved by SDS-PAGE, followed by autoradiography of [γ-32P]ATP for 30 min.

**Metabolic Labeling of S2 Cell Lines—**Transfected Dsh S2 cells were treated with CuSO4 to induce Dsh expression and labeled overnight with 1 μCi of [32P]orthophosphate/ml of S2 phosphate-free medium plus 10% diazylated fetal calf serum. Radioimmune precipitation assay buffer cell lysates were normalized for incorporation by Cerenkov counting.

After immunoprecipitation of SggZw3 protein and separation by SDS-PAGE, proteins were transferred to polyvinylidene difluoride membranes. Labeled SggZw3 was subjected to partial acid hydrolysis, and the phosphoamino acids were separated in two dimensions by immunoblotting.

**Preparation of Embryo Lysates—**For overexpression of SggZw3, homozygous HS-SggZw3 Drosophila embryos were collected 3 h after laying, heat-shocked for 8 min at 37 °C, and allowed to recover for an additional 1.5 h at 25 °C. To generate SggZw3 M11-1 mutant embryos, germ line mosaics were produced using the yeast recombinase-base flipase-dominant female sterile system as described by Chou and Perrimon (32). Homozygous mutant embryos can be recognized morphologically by a lack of segmentation. For overexpression of Wg, Drosophila males homozygous for arm-Gal4 were crossed to virgin Drosophila females harboring pUAS-Wg, and their progeny embryos were collected at 3–6 h. Wild-type embryos of the same stage were used as controls. Embryos were lysed in Gentle Soft buffer (28) and subjected to immunoprecipitation analysis as described below.

**Immunoprecipitation and SggZw3 Kinase Assays—**Cells lines were washed with phosphate-buffered saline and lysed in Gentle Soft buffer (28). For SggZw3 immunoprecipitation, 20 μl of protein A-Sepharose or 20 μl of protein G-Sepharose were pre-bound to rabbit polyclonal anti-serum or to monoclonal antibodies (anti-SggZw3, 23265), respectively, and embryos. We used an imaginal disc cell line (cl-8 (clone 8)) that responds to Wg signals and Schneider (S2) cells, which are unresponsive to Wg (15, 24). Using Wg-conditioned medium, we show that the activity of SggZw3 protein kinase is inhibited by Wg in cl-8 cells and that overexpression of Dfs2 or Dsh in cells reconstitutes Wg signaling in the absence of Wg as judged by inhibition of the kinase and accumulation of Arm protein. We also demonstrate that the regulation of SggZw3 activity, in turn, controls the stability of Arm protein by modulating the level of phosphorylation of D-Axin and Arm. These results provide direct biochemical evidence in support of previous genetic analyses.

**RESULTS**

Wingless Protein Represses SggZw3 Activity and Induces Accumulation of Cytoplasmic Armadillo—To analyze the biochemical consequences of Wg signaling, we exploited an imaginal disc cell line (cl-8) that is responsive to Wg (24). To determine the biological effects of Wg, cl-8 cells were exposed to the serum-free conditioned medium from either heat-shocked Schneider HS-wg (Wg-conditioned medium) or Schneider control cells (S2 control medium), and cytoplasmic extracts were prepared and immunoblotted with antibodies to Wg, Arm, and Dsh (Fig. 1A) (15). Wg-containing medium increased Arm levels within 2 h, reaching a maximum after 6 h. By contrast, cellular levels of Dsh did not change in this time period. How-
ever, Wg induced the formation of electrophoretically retarded forms of Dsh. These modifications have been previously observed by Yanagawa et al. (15) and Willert et al. (23) and correspond to hyperphosphorylation of Dsh protein. Exposure of cells to medium conditioned by control S2 cells affected neither Arm levels nor the Dsh electrophoretic pattern.

To determine whether Wg modulates SggZw3 activity, SggZw3 was immunoprecipitated from lysates of cl-8 cells treated with Wg-conditioned medium or S2 control medium. Protein kinase activity was measured using a peptide substrate specific for the GSK-3 family of protein kinases (GS-1 peptide (33)). Incubation of cl-8 cells with Wg-conditioned medium caused a time-dependent inhibition of SggZw3 protein kinase activity (Fig. 1B). After 2–4 h of treatment with Wg-conditioned medium, total GS-1 peptide kinase activity was suppressed by 40–50% compared with the activity observed in cells treated with S2 control medium. Wg did not affect the expression of SggZw3 as judged by immunoblotting (Fig. 1A).

To confirm the effect of Wg protein on the activity of SggZw3, we investigated how SggZw3 functions in Wg signaling during embryogenesis, analyzing SggZw3 activity in embryos with a wild-type or sggzw3 mutant genotype, embryos overexpressing sggzw3, and embryos expressing wg ubiquitously. sggzw3 embryos were made homozygous for the sggzw3 M11-1 allele, and SggZw3 immunoprecipitates from these mutant embryos contained no detectable SggZw3 activity, which verified the specificity of the assay (Fig. 2B). Furthermore, SggZw3 immunoprecipitates from embryos overexpressing SggZw3 from a heat-shock-inducible transgene (HS-SggZw3) exhibited 2–3-fold higher activity than immunoprecipitates from wild-type embryos (Fig. 2B).

To determine the effect of Wg overexpression on SggZw3 activity, Wg was ectopically expressed in the line that carries a GAL4-driver (arm-GAL4). The activity of SggZw3 was determined to be ~30% lower in these embryos (Fig. 2B). Immunoblotting of the extracts revealed equivalent SggZw3 levels from wild-type or sggzw3 M11-1 allele and in the pUAS-Wg-expressing line, as expected (Fig. 2A). Armadillo immunoblots revealed accumulation of Arm protein in the SggZw3 M11-1 and pUAS-Wg extracts.

Overexpression of Dsh Reduces SggZw3 Protein Kinase Activity—Overexpression of Dsh protein in cl-8 and S2 cells bypasses the need for Wg and mimics Wg signaling (15). To investigate the effect of overexpression of Dsh on SggZw3 activity, we used S2 and cl-8 cell lines expressing Dsh under the control of an inducible metallothionein promoter. Treatment of these cell lines with CuSO4 led to a time-dependent increase in protein kinase activity upon induction of SggZw3 activity (Fig. 3A). Dsh overexpression in cl-8 cells induced an increase in the levels of Dsh protein, as well as induction of forms of the protein with reduced electrophoretic mobility similar to the forms observed in untransfected cl-8 cells exposed to Wg protein (Fig. 3, A and C). Concomitant with the increase in Dsh protein levels was an increase in Arm levels (Fig. 3, A and C), indicating that overexpression of Dsh in S2 and cl-8 cells mimics Wg signaling.

To determine whether Dsh protein inhibits SggZw3 activity, we examined SggZw3 protein kinase activity in the Dsh-inducible cl-8 and S2 cell lines (Fig. 3, B and D). Dsh overexpression in cl-8 and S2 cells revealed similar inhibition curves in both lines and induced a rapid decrease in SggZw3 activity that was detectable after 2 h and reached a maximum (70%) after 4–6 h, whereas SggZw3 expression levels were not affected (Fig. 3, A and C). The decrease in SggZw3 activity observed in the Dsh experiments in cl-8 cells coincided with the effects of Wg on SggZw3 activity in cl-8 cells and supports the genetic model in which Wg repression of SggZw3 is mediated via Dsh.

Overexpression of Drosophila Frizzled 2, a Putative Wg Receptor, Mimics Wg Signaling—Unlike cl-8 cells, S2 cells do not respond to extracellular Wg as judged by Dsh modification and Arm stabilization (data not shown) (15, 24). Transfection of the transmembrane protein Drosophila Frizzled 2 (Dfz2) into S2 cells enables the cells to accumulate Arm in response to Wg, suggesting that Dfz2 acts as a receptor for Wg and that the reason for the lack of responsiveness of these cells to Wg is simply due to lack of Dfz2 expression (7). To investigate whether Dfz2 expression affected SggZw3 activity, we used S2 cell lines expressing Dfz2 under the control of an inducible metallothionein promoter. Addition of CuSO4 to the medium of these cells induced an increase in the levels of Dfz2 RNA (Fig. 4A), leading to the appearance of slower migrating forms of Dsh and an increase in cytoplasmic Arm levels within 2 h, whereas SggZw3 protein levels were unaffected (Fig. 4A). However, immunoprecipitates of SggZw3 exhibited a time-dependent decrease in protein kinase activity upon induction of Dfz2 expression, similar to the effects of overexpression of Dsh in S2 cells (Fig. 4B). Together, these data demonstrate that overexpression of Dfz2 in S2 cells is sufficient to trigger the Wg pathway, including modification of Dsh, repression of SggZw3, and stabilization of Arm.

Dishevelled Induces Serine Phosphorylation of SggZw3—To probe the mechanism via which Wg, Dfz2, and Dsh inactivate...
SggZw3, S2 cell lines harboring inducible Dsh were metaboli-
cally labeled with [32P]phosphate, and SggZw3 was immunopre-
cipitated and resolved by SDS-PAGE. Induction of Dsh expres-
sion caused a 2–2.5-fold increase in [32P]phosphate associated
with SggZw3 (Fig. 5A). Subsequent phosphoamino acid analysis
revealed the presence of only phosphoserine in the S2 cell
sample (Fig. 5B). These data suggest that Dsh induces a spe-
cific increase in serine phosphorylation of SggZw3, which may
mediate the reduction in protein kinase activity. Surprisingly,
SggZw3 in S2 cells does not contain detectable phosphotyrosine
(34). SggZw3 contained both phosphotyrosine and phospho-
serin in cl-8 cells. Since induction of the Wg pathway resulted
in equal -fold inhibition in both S2 and cl-8 cells, we conclude
that Wg-mediated regulation of SggZw3 is independent of tyro-
sine phosphorylation.

Phosphorylation of Arm and D-Axin by SggZw3.—We have
shown that negative regulation of SggZw3 activity leads to Arm
accumulation in Drosophila embryos and cells. Biochemical
analysis has indicated that D-Axin/Axin negatively regulates
β-catenin/Arm by interacting with GSK-3β/SggZw3 (19).2 D-
Axin is structurally related to vertebrate Axins, with the re-
gions of highest identity corresponding to previously defined
binding domains of Axin.2

Armadillo contains “consensus” phosphorylation site se-
quences for GSK-3/SggZw3 (35). D-Axin also contains such se-
quences (19). However, it has been reported that mammalian
GSK-3 phosphorylates β-catenin significantly only in the pres-
ence of the Axin protein (19). Therefore, we examined whether
SggZw3 could phosphorylate Arm and D-Axin under conditions
in which these proteins formed a complex. To determine
whether D-Axin and Arm are substrates for SggZw3, we purified

D-Axin or various deletion mutants of D-Axin and Arm from E.
coli as histidine fusion proteins (Fig. 6). Baculovirus-expressed
GST-SggZw3 (36) phosphorylated D-Axin, D-Axin-(302–746),
D-Axin-(356–565), and D-Axin-(356–746), but not D-Axin-
(383–565) and D-Axin-(34–356) (Fig. 6). In the absence of D-
Axin, no significant phosphorylation of Armadillo was ob-
served, whereas in its presence, the phosphorylation was
greatly increased (Fig. 6). These data indicate that Sgg phos-
phorylation of Armadillo is directed via D-Axin.

Inhibition of SggZw3 Activity by Wingless Medicates Its Phosphorylation and Interaction with D-Axin Protein.—We found that D-
Axin is phosphorylated by SggZw3 and binds to both SggZw3 and
Arm.2 We therefore examined whether the inhibition of SggZw3
activity by Wg affects its interaction with D-Axin and moni-
tored the level of phosphorylation of D-Axin. To test this pos-
sibility, in vitro binding and phosphorylation assays were car-
rried out using a D-Axin-(330–642) fusion protein containing
SggZw3-binding sites and consensus sites of phosphorylation for
SggZw3. D-Axin-(330–642)-His was transfected as a histidine
fusion protein into cl-8 cells, cl-8 cells treated with Wg, and cl-8
cells expressing SggZw3. The histidine-tagged complexes from

[FIG. 3. Overexpression of Dsh in Drosophila cell lines mimics
Wg signaling and leads to inhibition of SggZw3. A, cl-8 cells ex-
pressing Dsh under the control of the metallothionein promoter were
induced for varying times (from 0 to 6 h) with CuSO4. Immunoblotting
revealed time-dependent overexpression and modification of Dsh and
accumulation of Arm. Expression of SggZw3 was unchanged. B,
are the results from assay of SggZw3 activity in immunoprecipitates
from lysates in A. C, Schneider S2 cell lines inducibly expressing
were treated for 0–6 h with CuSO4. Lysates were

[FIG. 4. Overexpression of Dfz2 in Drosophila S2 cells mimics
Wingless signaling and leads to the inhibition of SggZw3 activity.
A, Schneider S2 cells engineered to inducibly express Dfz2 (7) were
treated with CuSO4 for 0–6 h. Immunoblotting analysis revealed Arm
accumulation and electrophoretic retardation of Dsh. Measurement of
Dfz2 induction was determined by cytoplasmic RNA slot hybridization
with a 32P-labeled Dfz2-specific probe. Dfz2 RNA was undetectable in
wild-type S2 cells (data not shown). B, shown is the time course of
SggZw3 activity in response to induction of Dfz2 expression in S2 cells
(average of two experiments).]
the cl-8 cell lysates were purified using nickel-Sepharose beads, and the amount of Sgg Zw3 captured on the beads was determined by immunoblotting. In addition, the phosphorylation of D-Axin-(330–642)-His6 by SggZw3 was determined by addition of \[^{\gamma-32P}]ATP.

In the lysates from cells treated with Wg, Sgg Zw3 was found in association with D-Axin-(330–642)-His6. However, the degree of binding was reduced; 2-fold compared with the amount of SggZw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7). The negative effect of Wg signal on the binding of Sgg Zw3 associated with Axin in lysates of untreated cl-8 cells (Fig. 7).

### DISCUSSION

Previous studies have shown that treatment of cl-8 cell lines with Wg leads to hyperphosphorylation of Dsh protein and to cytoplasmic accumulation of Armadillo (15, 23, 24). Here, we report that Wg signaling as initiated by Wg, Dfz2, or Dsh expression causes enzymatic inactivation of SggZw3 activity in concert with stabilization of Arm. These data indicate that Wg signaling mimics Wingless signaling by specifically inhibiting the activity of Sgg Zw3.

We have demonstrated that regulation of kinase activity, rather than protein levels, is the main determinant of the effects of Wg on Sgg Zw3, suggesting post-translational modification of this protein kinase activity. In support of this, induction of Dsh expression increased the levels of Sgg Zw3 phosphorylation 2-fold (Fig. 6), and the presence of phosphoserine in Sgg Zw3 protein from S2 cells suggested that the mechanism of repression of Sgg Zw3 activity is mediated by serine phosphorylation. Previous studies have shown that members of the GSK-3 family are inhibited by phosphorylation at an amino-terminal serine residue (serine 9 in GSK-3[b] and serine 21 in GSK-3[alpha]) (33, 37). Phosphorylation of the Sgg Zw3 residue equivalent to serine 9 does not appear to be the mechanism via which the Wg pathway inhibits Sgg Zw3 for several reasons. In mammals, this site is targeted by agents acting via phosphatidylinositol 3-kinase, and the residue can be phosphorylated in vitro and in transfected cells by protein kinase B/AKT (38). However, Wg inhibition of GSK-3 in 10-T1/2 cells is not sensitive to inhibitors of phosphatidylinositol 3'-kinase, nor is Drosophila protein
activity. However, Arm is a poor in vitro target of Sgg\(^{zw3}\). Phosphorylation of Arm is enormously increased in the presence of D-Axin. We have demonstrated that D-Axin is phosphorylated by Sgg\(^{zw3}\) and that the binding of Sgg\(^{zw3}\) to D-Axin is dependent upon the level of Sgg\(^{zw3}\) activity. Repression of Sgg\(^{zw3}\) activity by Wg signaling induced dissociation of the Sgg\(^{zw3}\)-D-Axin-Arm complex, leading to an accumulation of Arm protein. Together, these data suggest that Sgg binding is dependent upon or stimulated by phosphorylation of Axin. Once bound to Axin, it can access the Arm molecule that is associated with Axin and phosphorylate it. Inactivation of Sgg results in dephosphorylation of Axin and release of the kinase, compartmentalizing it away from Arm.

Mammalian studies have suggested that a more complex mechanism for the regulation of \(\beta\)-catenin levels by GSK-3 involved another player, APC. In this case, Axin forms a complex with GSK-3, \(\beta\)-catenin, and APC (19, 20). APC is directly phosphorylated by GSK-3 via Axin, which increases binding of APC to \(\beta\)-catenin and its subsequent degradation (40, 41). Mutation of a Drosophila APC homolog did not affect Wg function, suggesting either divergence of the molecular mechanisms of Arm stabilization or the existence of additional APC-like molecules in flies (21). Resolution of these mechanisms will require identification of the serine kinase acting to induce Arm hyperphosphorylation and the means by which it is, in turn, controlled.

FIG. 7. Wingless signaling modulates the Sgg\(^{zw3}\)/D-Axin interaction. 10 \(\mu\)g of D-Axin-(330–642)-His\(_6\) were transfected into cl-8 or cl-8 cells expressing Sgg\(^{zw3}\). D-Axin-(330–642)-His\(_6\) contains the phosphorylation site for Sgg\(^{zw3}\) (serines 359, 363, and 377), as well as the Sgg\(^{zw3}\)-binding domain. cl-8 or Sgg\(^{zw3}\)-expressing cl-8 cells were treated with (+) or Wg-conditioned medium (-) or Wg-conditioned medium, with or without (1) or cl-8 conditioned medium (1). This figure shows the decrease in Sgg\(^{zw3}\) activity and accumulation of hypophosphorylated state of Dsh protein and an increase in Arm stability, and the means by which Sgg\(^{zw3}\) induces turnover of Arm. We thank R. Nusse for kindly providing cl-8 cells and A. Martinez Arias for Wg-expressing cl-8 cells for pHGCO and pRmHa-1 vector

REFERENCES

1. Klingensmith, J., and Nusse, R. (1994) Nature 368–369. 2. Cohen, S. M., and Di Nardo, S., (1993) Trends in Neurobiology 13, 527–540. 3. Diaz-Benjumea, F. J., and Cohen, S. M. (1994) Nature 367, 80–83. 4. Siegfried, E., Wilder, E. L., and Perrimon, N. (1994) Nature 367, 76–80. 5. Yanagawa, S., van Leeuwen, F., Wodarz, A., Klingensmith, J., and Nusse, R. (1994) Nature 367, 1087–1097. 6. Noordermeer, J., Klingensmith, J., Perrimon, N., and Nusse, R. (1994) Nature 367, 80–83. 7. Siegfried, E., Wilder, E. L., and Perrimon, N. (1994) Nature 367, 76–80. 8. Yang, A., van Leeuwen, F., Wodarz, A., Klingensmith, J., and Nusse, R. (1995) Genes Dev. 9, 1087–1097. 9. Noordermeer, J., Johnston, P., Rijswijk, F., Nusse, R., and Lawrence, P. A. (1992) Development 116, 711–719. 10. Peifer, M., Sweeten, B., S. M., and Wieschaus, E. (1994) Development 122, 369–380. 11. Peifer, M., Pai, L. M., and Casey, M. (1994) Dev. Biol. 166, 543–556. 12. Zeng, L., Fagotto, F., Zhang, T., Hsu, W., Vassicek, T. J., Perry, W. L., III, Lee, J. J., Tilghman, S. M., Gumbiner, B. M., and Costantini, F. (1997) Cell 518, 181–192. 13. Behrens, J., Jerchow, B. A., Wurtz, B. M., Grabe, J., and Basler, K. (1996) Science 272, 569–571. 14. Hayashi, S., Rubinfield, B., Souza, B., Polakis, P., Wieschaus, E., and Levine, A. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5121–5124. 15. Sakatani, C., Weiss, J., and Williams, L. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2658–2662. 16. Willert, K., Brink, M., Wadler, A., Varma, H., and Nusse, R. (1997) EMBO J. 16, 3869–3909. 17. van Leeuwen, F., Harryman Samos, C., and Nusse, R. (1994) Nature 368, 342–344. 18. Brunner, R., Peter, O., Schweizer, L., and Basler, K. (1993) Nature 385, 829–833. 19. Beyer, J. J., Embudkar, A., Wurtz, B. M., Redmond, J., and Basler, K. (1996) Nature 386, 519–524. 20. Riese, J., Xu, M., Munnelly, A., Ershe, R., Hsu, S., Grosschedle, R., and Bienz, M. (1997) Cell 89, 777–787. 21. Cook, D., Fry, M. J., Hughes, K., Sumathi, R., Wodarz, J., and Dale, T. C. (1996) EMBO J. 15, 4492–4496. 22. Cumberledge, S., and Krasnow, M. A. (1993) Cell 84, 43–50. 23. Currie, D. A., Milner, M. J., and Evans, C. W. (1988) Development 102, 805–814. 24. Woodgett, J. R. (1992) in Cell Lines in Neurobiology: A Practical Approach, Ed. J. Wood. IRL Press, Oxford, pp133–159.
Biochemical Interactions within the Wingless Pathway

31. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149
32. Chou, T. B., and Perrimon, N. (1992) Genetics 131, 643–653
33. Stambolic, V., and Woodgett, J. R. (1994) Biochem. J. 303, 701–704
34. Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F., and Woodgett, J. R. (1993) EMBO J. 12, 803–808
35. Aberle, H., Bauer A., Slappendel, J., Kispert, A., and Kemler R. (1997) EMBO J. 16, 3797–3804
36. Stambolic, V., Ruel, L., and Woodgett J. R. (1996) Curr. Biol. 6, 1664–1668
37. Sutherland, C., Leighton, I. A., and Cohen, P. (1993) Biochem. J. 296, 15–19
38. Cross, D. A., Alesii, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1995) Nature 378, 785–789
39. Yost, C., Torres, M., Miller, J. R., Huang, E., Kimelman, D., and Moon, R. T. (1996) Genes Dev. 10, 1443–1454
40. Rubinfield, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S., and Polakis, P. (1996) Science 272, 1731–1734
41. Papkoff, J., Rubinfield, B., Schryver, B., and Polakis, P. (1996) Mol. Cell. Biol. 16, 2128–2134
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