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REVIEW

GSK-3: New Thoughts on an Old Enzyme

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INTRODUCTION

Over the past decade, a serine–threonine kinase first characterized for its role in glycogen metabolism has shown itself to be a key player in numerous processes, in organisms ranging from yeast to mammals (reviewed in Yost *et al.,* 1997). Glycogen synthase kinase-3 was originally identified as a constitutively active kinase that is inactivated in response to extracellular signals. However, it is now clear that extracellular signals can also activate GSK-3 to direct developmental patterning and that GSK-3's function is mediated both by phosphorylation and by its interaction with activating and inhibitory binding partners. In this review we focus on three organisms that highlight the varied roles that GSK-3 plays in embryonic development, *Xenopus,* sea urchin, and *Dictyostelium,* and discuss the molecular mechanisms that regulate GSK-3 activity in each species.

WNT/WG SIGNALING

GSK-3 became of major interest to developmental biologists when genetic analysis in *Drosophila* placed it in the Wingless (Wg) signal transduction pathway (Siegfried *et al.,* 1992). Studies from a variety of vertebrate and invertebrate organisms have now positioned GSK-3 within the canonical Wnt signaling pathway (Fig. 1). In the absence of a Wnt signal, GSK-3 is part of a multiprotein complex that targets the cytoplasmic protein β-catenin for degradation. The backbone of this complex is the scaffolding protein Axin (Zeng *et al.,* 1997) and/or the related protein Conductin/Axil (Behrens *et al.,* 1998; Yamamoto *et al.,* 1998). Axin appears to promote the phosphorylation of β-catenin by GSK-3 by bringing the two proteins together (Hart *et al.,* 1998; Ikeda *et al.,* 1998), leading to the ubiquitination of β-catenin and its subsequent proteasome-dependent degradation (Aberle *et al.,* 1997; Orford *et al.,* 1997). This mechanism maintains a low level of cytoplasmic and nuclear β-catenin, and as a result, the downstream transcriptional targets of β-catenin remain off (see below and reviewed in Miller and Moon, 1996; Behrens, 1999).

A somewhat mysterious member of this complex is APC, the adenomatous poliposis coli tumor suppressor protein (reviewed in Polakis, 1997; Bienz, 1999; Seidensticker and Behrens, 2000). Mutations in APC are found in a majority of human colon cancers and result in elevated cytoplasmic β-catenin levels. APC is a large protein containing interaction sites for Axin and β-catenin. While the function of APC is still not clear, it may function like Axin to bring β-catenin close to GSK-3, although a recent report raises the possibility that APC might cause the degradation of β-catenin in a GSK-3-independent manner (Easwaran *et al.,* 1999). To further complicate the understanding of APC function, both invertebrates and vertebrates have at least two members of the APC family (reviewed in Bienz, 1999). Differences in the function of these proteins, as well as their time and location of expression, may lead to unique developmental outputs. For example, only one APC binds GSK-3 (*Drosophila* E-APC), although the significance of this has not yet been established (Yu *et al.,* 1999).

While GSK-3 is active in the absence of signaling, it becomes inhibited in the presence of a Wnt/Wg signal. Wnt/Wg ligand interacts with seven-pass transmembrane receptors of the Frizzled family, but the events downstream of the receptor are less well understood (Bhanot *et al.,* 1996; Yang-Snyder *et al.,* 1996). Dishevelled (Dsh) is clearly required for the Wnt pathway to be active (Klingensmith *et al.,* 1994; Noordermeer *et al.,* 1994; Theisen *et al.,* 1994; Sokol, 1996) and, at least in vertebrates, so is casein kinase Iε (Peters *et al.,* 1999; Sakanaka *et al.,* 1999). The inhibition of GSK-3 is likely to be a key event in how these factors propagate the Wnt signal (Fig. 1). Since the binding of β-catenin to Axin and APC requires GSK-3 phosphorylation (Rubinfeld *et al.,* 1996; Willert *et al.,* 1999), the inhibition of GSK-3 by the Wnt pathway may cause the degradation complex to at least partially disassemble, as has been observed in cultured cells (Li *et al.,* 1999; Ruel *et al.,* 1999). With GSK-3 inactivated, β-catenin is not phosphorylated and therefore not targeted for degradation (Yost *et al.,* 1996). It accumulates in the cytoplasm and nucleus, where it

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activates the transcription of target genes by binding transcription factors of the Tcf/LEF family and recruiting the transcriptional activators p300/CBP to target promoters (reviewed in Moon and Kimelman, 1998; Willert and Nusse, 1998; Hecht *et al.,* 2000; Takemaru and Moon, 2000).

DIFFERENTIAL REGULATION OF GSK-3

In addition to its role in Wnt signaling, GSK-3 plays a key role in glycogen metabolism (reviewed in Plyte *et al.,* 1992; Yost *et al.,* 1997; Srivastava and Pandey, 1998; Cohen, 1999). In the absence of insulin signaling, GSK-3 phosphorylates and inhibits glycogen synthase. When blood glucose levels are high, insulin levels also rise. Insulin signaling results in the activation of Akt, a kinase that phosphorylates and inhibits GSK-3. With GSK-3 inhibited by Akt, glycogen synthase is free to act on the abundant glucose, converting it into glycogen.

Since GSK-3 inhibition is important for both the Wnt signaling pathway and glycogen synthesis, how is it possible for a cell to regulate GSK-3 activity in one pathway without affecting the other? Several recent studies suggest that the key to differential regulation may lie in different requirements for a substrate prephosphorylation event. Specifically, some GSK-3 substrates, such as β-catenin, do not require prephosphorylation (Yost *et al.,* 1996), whereas others, such as glycogen synthease (GS), require prephosphorylation at a serine–threonine just C-terminal to the GSK-3 site (reviewed in Plyte *et al.,* 1992; Welsh *et al.,* 1996). Thus, different substrate phosphorylation requirements may allow GSK-3 to affect one pathway but not another. For example, addition of a synthetic peptide derived from the GSK-3 binding protein Frat to GSK-3 blocked phosphorylation of β-catenin, but did not prevent phosphorylation of a prephosphorylated peptide derived from GS (Thomas *et al.,* 1999). Conversely, inhibition of GSK-3 by the insulin-stimulated kinase Akt prevented GSK-3 phosphorylation of the GS peptide (Yuan *et al.,* 1999), whereas, Akt had no effect on the downstream readouts of the Wnt pathway in cultured cells: it neither stabilized β-catenin nor activated transcription from a LEF-1 reporter construct (Yuan *et al.,* 1999). Similarly, overexpression of $p90^{rsk}$ in *Xenopus,* which also blocks GSK-3 from phosphorylating prephosphorylated substrates, did not increase cytoplasmic levels of β-catenin (Torres *et al.,* 1999). These results suggest that cells can selectively regulate multiple inputs on GSK-3 to elicit substrate-specific outputs.

GSK-3 IN *Xenopus*

In *Xenopus,* the inhibition of GSK-3 is required to specify the dorsal–ventral axis during the first steps of development (Dominguez *et al.,* 1995; He *et al.,* 1995; Pierce and Kimelman, 1995). Prior to fertilization, GSK-3 was proposed to be constitutively active throughout the embryo (Fisher *et al.,* 1999). Immediately following fertilization, there is a rotation of the outer (cortical) cytoplasm relative to the inner cytoplasm that moves a dorsalizing activity from the vegetal hemisphere to the future dorsal side of the embryo in a microtubule-dependent manner (Fig. 2A) (reviewed in Moon and Kimelman, 1998). This movement results in the enrichment of cytoplasmic and nuclear β-catenin on the future dorsal side of the embryo (Larabell *et al.,* 1997). Though not molecularly defined, the dorsalizing activity has been proposed to cause a localized inhibition of GSK-3 that is required for dorsal axis formation since misexpression of a kinase-dead form of GSK-3, which acts as a dominant negative, dorsalizes embryos (Dominguez *et al.,* 1995; He *et al.,* 1995; Pierce and Kimelman, 1995). However, it is possible that the dorsalizing activity may work on other members of the complex instead of, or in addition to, GSK-3 (Marikawa and Elinson, 1999).

How might the dorsalizing activity regulate GSK-3 activity following cortical rotation? While microinjection of Wnt and Dsh can dorsalize embryos (McMahon and Moon, 1989; Smith and Harland, 1991; Sokol *et al.,* 1991; Rothbacher *et al.,* 1995; Sokol *et al.,* 1995), ectopic expression of dominant-negative mutants of Wnt and Dsh have no effect on endogenous dorsal axis formation, suggesting that they are not involved (Hoppler *et al.,* 1996; Sokol, 1996). It is possible, however, that these constructs failed to have an effect because they were not translated from the injected mRNA early enough to perturb the endogenous axis. To test this, *Xenopus* embryos were injected with RNA encoding a dominant-negative Dsh (Xdd1) at the two-cell stage and then injected at the eight-cell stage with vegetal cortical cytoplasm, which contains the dorsalizing activity. In this experiment Xdd1 also failed to block dorsalization, raising doubts about the role of Dsh in regulating the endogenous dorsal axis (Marikawa and Elinson, 1999). On the other hand, it is possible that there is a unique function of Dsh in forming the dorsal axis that cannot be blocked by Xdd1. Dsh remains attractive as a candidate component of the endogenous dorsalizing activity since it has recently been reported that ectopically expressed Dsh migrates along microtubules from the *Xenopus* vegetal pole to the future dorsal side of the embryo and is later seen to be dorsally enriched (Miller *et al.,* 1999). Dsh is also more highly phosphorylated on the dorsal side of the embryo (Rothbacher *et al.,* 2000). Furthermore, oligo-depletion of a maternal Frizzled (Xfz7) disrupts formation of the endogenous dorsal axis (Sumanas *et al.,* 2000), suggesting that molecules upstream of Dsh may be involved in this process. Therefore, while still controversial, it appears that upstream components of the Wnt signaling pathway could regulate dorsal axis formation.

GSK-3 binding protein (GBP) is required for formation of the endogenous dorsal axis (Yost *et al.,* 1998). It binds to and inhibits GSK-3 (Yost *et al.,* 1998) in a manner that does not inactivate its catalytic cleft (Farr *et al.,* 2000). Instead, GBP appears to inhibit GSK-3 in part by blocking its interaction

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with Axin (Farr *et al.,* 2000), thus keeping GSK-3 from phosphorylating β-catenin.

GBP may also be involved in the degradation of GSK-3. Dominguez and Green (2000) carefully compared dorsal–ventral differences in GSK-3 protein levels in 2- to 32-cell *Xenopus* embryos. Interestingly, they observed an approximately 20% reduction in GSK-3 protein levels in the dorsal half of the embryo. Immunological staining showed that GSK-3 protein is depleted from the thin layer of dorsal cortical cytoplasm, where the *Xenopus* dorsalizing activity is thought to reside after cortical rotation (Fig. 2A) (Dominguez and Green, 2000). This region corresponds to the region of the embryo where β-catenin is stabilized (Larabell *et al.,* 1997).

To further characterize candidate dorsalizing molecules that might be responsible for the observed dorsal decrease in GSK-3 protein levels, Dominguez and Green (2000) examined the roles of Wnt8, Dsh, and GBP. Only ectopic GBP affected GSK-3 protein levels, similar to the decreased dorsal levels seen endogenously. Combining the studies on GBP, we suggest that GBP acts to remove GSK-3 from the Axin complex within the dorsal cortical cytoplasm, thus inhibiting GSK-3 phosphorylation of β-catenin (Fig. 2B). GBP might then target this GSK-3 for degradation, leading to a reduction in GSK-3 levels dorsally.

A key unanswered question is how GBP could inhibit GSK-3 on only the dorsal side of the embryo. GBP might be dorsally localized or posttranslationally modified so that it is active only dorsally. The observations that Dsh is translocated dorsally (Miller *et al.,* 1999), that Dsh interacts with Axin and GBP (Li *et al.,* 1999; Smalley *et al.,* 1999; Salic *et al.,* 2000), and that Dsh and GBP can synergize to inhibit phosphorylation of β-catenin by GSK-3 (Salic *et al.,* 2000) suggest a model wherein Dsh might act to bring GBP to the Axin/APC/GSK-3 complex on the dorsal side of the embryo as part of a "transitional complex" in which GBP could then interact with GSK-3 and remove it (Fig. 2B). Studies with *Xenopus* egg extracts (Salic *et al.,* 2000) and mammalian cell culture using the mammalian orthologue of GBP (Frat) suggest that such a complex could occur (Li *et al.,* 1999).

It should be noted that GBP has not been identified in *Caenorhabditis elegans* or *Drosophila* (Ruvkun and Hobert, 1998, D.M.F. and D.K., unpublished), suggesting that GBP might have evolved as a vertebrate-specific modulator of the Wnt pathway. Whether it is used generally in the vertebrate Wnt signaling pathway or functions only in special circumstances remains to be determined.

GSK-3 IN SEA URCHINS

GSK-3 has recently been shown to play a major role in specifying the sea urchin primary body axis, the animal–vegetal (A-V) axis. In the sea urchin, animal cells give rise to ectoderm, while vegetal cells will become endoderm and mesoderm (Fig. 3) (reviewed in Davidson *et al.,* 1998; Angerer and Angerer, 2000). Although there is no cortical rotation that moves or asymmetrically localizes factors in the sea urchin following fertilization as in *Xenopus,* GSK-3 appears to be an important player in establishing the A-V axis as well. Misexpression of kinase-dead GSK-3, which acts as a dominant-negative mutant in *Xenopus* (Dominguez *et al.,* 1995; He *et al.,* 1995; Pierce and Kimelman, 1995), or treatment with the GSK-3 inhibitor lithium (Klein and Melton, 1996; Stambolic *et al.,* 1996; Hedgepeth *et al.,* 1997), elicits a dose-dependent enhancement of vegetal cell fates (Herbst, 1892; von Ubisch, 1929; Nocente-McGrath *et al.,* 1991; Emily-Fenouil *et al.,* 1998). The most striking feature of these embryos is an enlarged exogastrulated digestive tract, indicative of the overdevelopment of endoderm at the expense of ectoderm. Similarly, kinase-dead GSK-3 blocks the expression of ectodermal hatching enzyme (HE). Conversely, overexpression of wild-type GSK-3 results in animalized embryos, often with long cilia, no spicules and few or no cells in the blastocoel, and expanded HE expression (Emily-Fenouil *et al.,* 1998).

As might be predicted from studies in other systems, β-catenin and Tcf also act downstream of GSK-3 to regulate the A-V axis. Specifically, β-catenin promotes vegetal cell fates, whereas animal cells develop in the absence of β-catenin. Embryos overexpressing a stabilized form of β-catenin that cannot be phosphorylated by GSK-3 develop with a highly vegetalized morphology (Wikramanayake *et al.,* 1998). Conversely, blocking β-catenin signaling by overexpression of cadherins, which deplete the cytoplasmic pool of β-catenin by sequestering it to the plasma membrane, has the opposite effect and embryos develop animalized (Wikramanayake *et al.,* 1998; Logan *et al.,* 1999).

Consistent with its role as a transcriptional repressor in *Xenopus* that is activated by stabilized β-catenin, an activated form of Tcf vegetalizes, while a dominant-negative Tcf animalizes (Vonica *et al.,* 2000). Interestingly, the timing of Tcf action is critical. The use of an inducible form of Tcf shows that the degree of vegetalization resulting from activated Tcf is directly related to the time of action. The most severe effects result from activation between fertilization and the 8-cell stage, with a gradual decrease in vegetalization until the 60-cell stage, after which time there is no effect (Vonica *et al.,* 2000). Since Tcf is expressed maternally and ubiquitously in the sea urchin (Huang *et al.,* 2000), its regulation is expected to be through the stabilization of β-catenin in the vegetal cells during the early cleavage stages, most likely through an inhibition of GSK-3. Consistent with this hypothesis, asymmetric accumulation of β-catenin in the nuclei of early cleavage embryos is seen as early as the 16-cell stage, becoming progressively localized to vegetal cells with each subsequent cleavage (Logan *et al.,* 1999). Moreover, inhibition of GSK-3 activity with lithium expands the region of stabilized nuclear β-catenin (Logan *et al.,* 1999). Since GSK-3 transcripts are present in the unfertilized egg and remain at the same level throughout the early cleavage stages (Emily-Fenouil *et al.,* 1998), it will be critical to measure the animal and vegetal levels of

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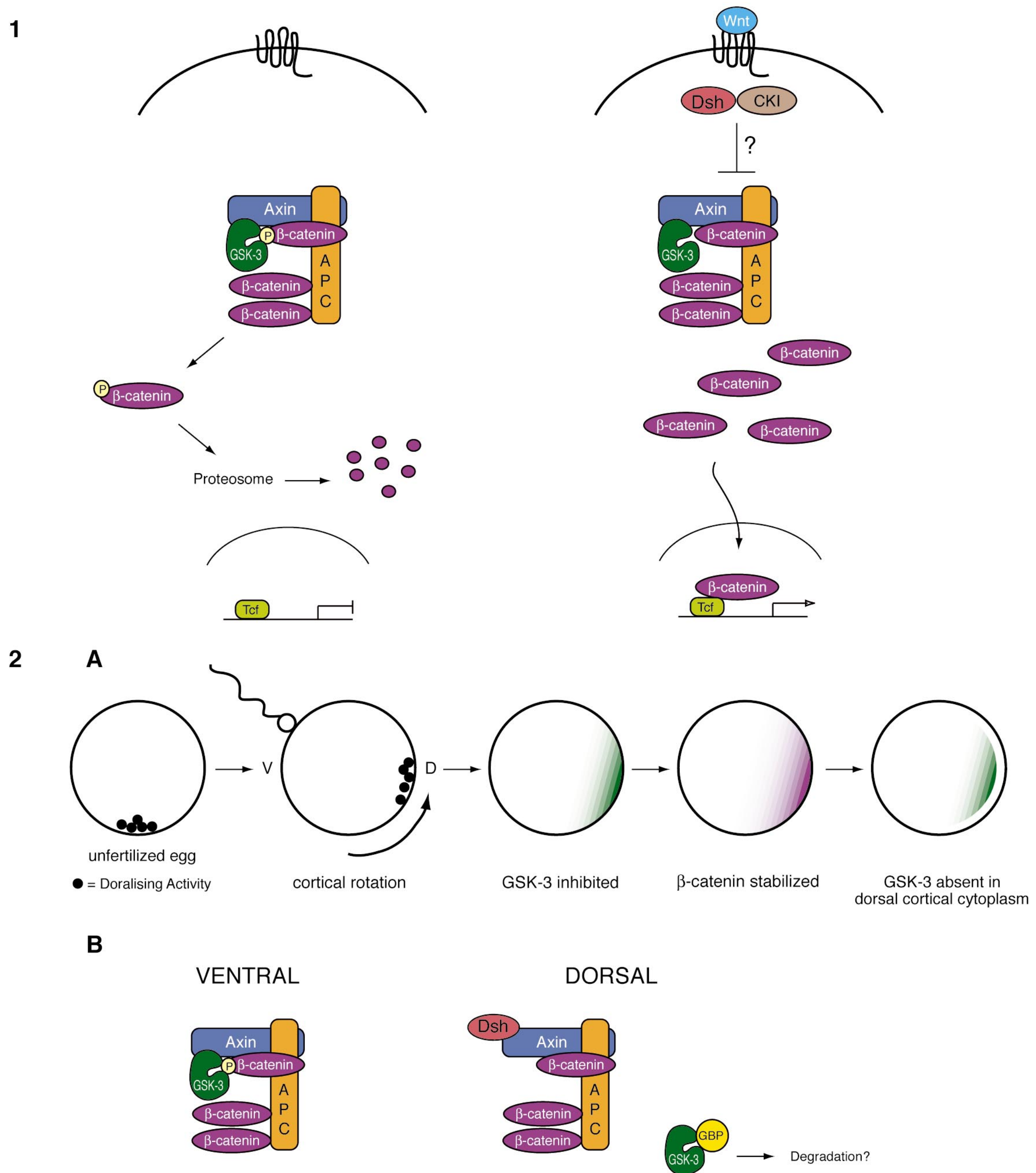

FIG. 1. The canonical Wnt signaling pathway. In the absence of a Wnt signal (left), GSK-3 is part of a multiprotein complex that targets β-catenin for degradation. This complex contains the scaffolding protein Axin, APC, and β-catenin. Axin binds both GSK-3 and β-catenin, presumably bringing them close together so that GSK-3 can phosphorylate β-catenin. Once phosphorylated, β-catenin is targeted for proteasomal degradation. In the presence of a Wnt signal (right), Wnt ligand interacts with receptors of the Frizzled family. Downstream of the receptor, Disheveled (Dsh) and casein kinase Iϵ (CKI) act through an unknown mechanism to inhibit GSK-3. β-Catenin is no longer phosphorylated and targeted for degradation, and it accumulates in the nucleus where it activates target genes by binding transcription factors of the Tcf/LEF family.

FIG. 2. GSK-3 in *Xenopus.* (A) Prior to fertilization, the *Xenopus* dorsalising activity is localized to the vegetal pole of the embryo. Following fertilization, there is a rotation of the outer (cortical) cytoplasm relative to the inner cytoplasm that results in the repositioning of the dorsalizing activity to the side of the embryo opposite the side of sperm entry. The net result of the cortical rotation is GSK-3 inhibition, and thus β-catenin stabilization, on the future dorsal side of the embryo. GSK-3 is depleted from the dorsal cortical cytoplasm, at least as early as the 4- to 8-cell stage. (B) On the ventral side of the embryo GSK-3 is active and part of the multiprotein complex that targets β-catenin for degradation. Dorsally, GSK-3 is inhibited in part through its interaction with GBP, which prevents GSK-3's association with Axin. Dsh may also play a role in dorsalization through its association with Axin. Once removed from the degradation complex, GSK-3 may be targeted for degradation.

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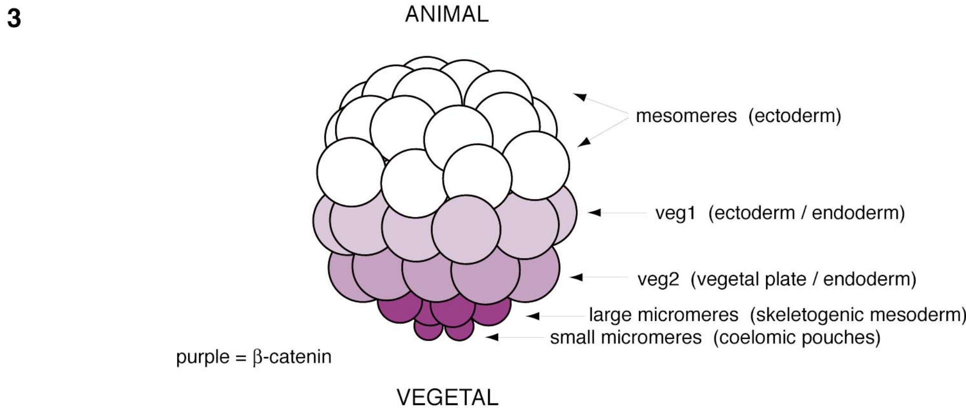

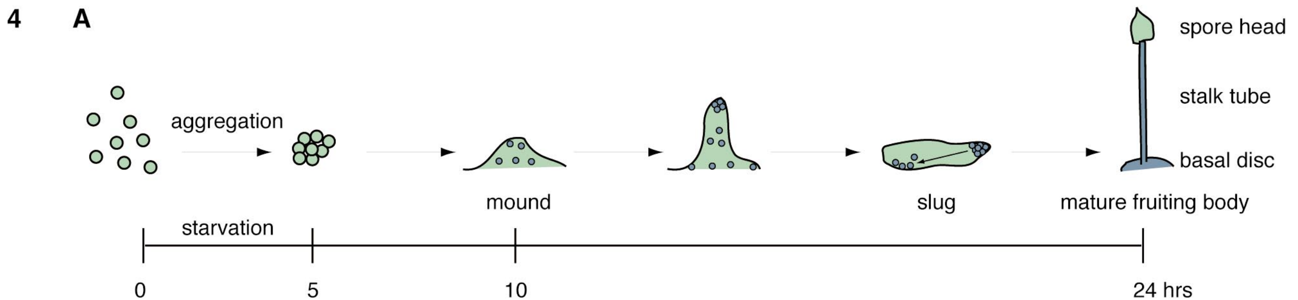

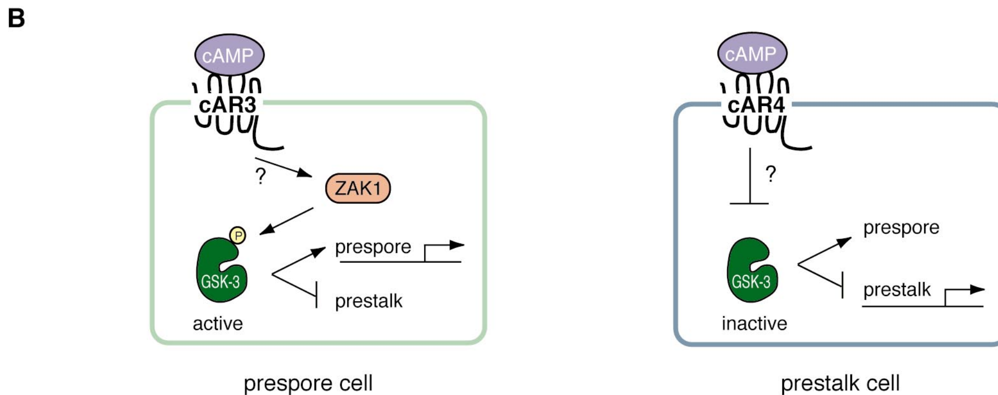

FIG. 3. GSK-3 in sea urchins. Sea urchin embryo following the sixth cleavage. β-Catenin (purple) is stabilized in the vegetal pole of the sea urchin embryo. The highest degree of stabilization is seen in the micromeres and veg2 lineage. β-Catenin is also stabilized in veg1 cells, but to a lesser extent, and becomes restricted to the endodermal derivatives of veg1 following the seventh cleavage. The localized stabilization of β-catenin suggests that GSK-3 is also locally inhibited within the vegetal region.

FIG. 4. GSK-3 in *Dictyostelium.* (A) Time course of *Dictyostelium* development. Upon the initiation of starvation conditions, *Dictyostelium* growing as a unicellular population begin to aggregate as extracellular cAMP levels rise, forming a mound of cells within 10 h. This mound grows upward, tips over, and begins migration as a “slug,” looking for a more favorable environment. Approximately 80% of the cells are prespore (green) and are located at the posterior of the migrating slug. The remaining 20% of the cells are prestalk (blue) and are located primarily at the anterior tip; however, a few are interspersed among the prespore cells. When the slug ceases migration, the prestalk cells migrate back through the prespore region to give rise to the basal disc and stalk tube. The prespore cells are carried to the top of the mature fruiting body, where they give rise to the spore head. (B) When cAMP interacts with the cAR3 receptor, ZAK1 is activated. ZAK1 phosphorylates and activates GSK-3, leading to the expression of prespore-specific genes and the inhibition of prestalk determination. When cAMP interacts with the cAR4 receptor, GSK-3 may be inhibited. This would relieve the GSK-3-mediated repression of prestalk cell determination, resulting in the expression of prestalk-specific genes and the inhibition of prespore determination.

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GSK-3 protein, as well as animal–vegetal differences in GSK-3 kinase activity, to examine how GSK-3 is regulated in the sea urchin embryo. Since nuclear accumulation of β-catenin occurs in a cell-autonomous manner (Logan *et al.,* 1999), it appears that in sea urchins there is a localized inhibition of GSK-3 that may not involve a Wnt ligand.

GSK-3 IN *Dictyostelium*

The role of GSK-3 in development extends beyond Wnt/Wg signaling, as it evidenced by its role in the facultative multicellular organism *Dictyostelium,* as was first demonstrated by Harwood *et al.* (1995). When resources are plentiful, *Dictyostelium* cells grow as a unicellular population. Under starvation conditions, approximately 10^5 cells aggregate within 5 h to form a structure known as the mound at about 10 h of development, and initiate a distinct developmental plan (Fig. 4A). Cells of the mound begin to extend upward onto an elongated structure that then tips over and is referred to as the "slug." The slug is able to sense its environment and move toward heat, light, and chemical attractants in order to locate a more favorable environment for later sporulation (reviewed in Fisher, 1984; Brown and Firtel, 1999).

The slug is composed of two general cell types: 80% of the cells are prespore, and are located in the posterior, while 20% are prestalk and located primarily in the anterior tip. When the slug ceases migration, the anterior prestalk cells migrate back through the prespore region toward the base and, along with prestalk cells interspersed through the prespore region, terminally differentiate into the basal disc and stalk tube. During the formation of a growing stalk tube, the prespore cells are concomitantly carried upward and differentiate into the mature spore head. Terminal differentiation of cell types giving rise to the mature fruiting body, consisting of the spore head, stalk, and basal disc, results in stalk cell death and puts the spore cells in stasis, waiting for the return of favorable environmental conditions to initiate sporulation and growth (reviewed in Fisher, 1984; Williams, 1995; Brown and Firtel, 1999).

Cell fates in the mature fruiting body are specified at the very early stages of multicellularization as the mound forms. During mound formation the extracellular level of cAMP rises, inducing the formation of prespore cells, while inhibiting the formation of prestalk cells (Fig. 4B). Much like dorsal–ventral fate decisions in *Xenopus* and animal–vegetal patterning in sea urchin, differential regulation of GSK-3 contributes to prestalk–prespore cell fate decisions in *Dictyostelium.* Exciting new work has linked prespore cell determination to GSK-3 *activation.* Extracellular cAMP acts through the seven-pass transmembrane cAMP receptor cAR3 (with potential input from cAR1) to activate ZAK1, a recently identified novel protein tyrosine kinase (Kim *et al.,* 1999). Genetic and biochemical analyses demonstrate that ZAK1 is required for the activation of GSK-3 in *Dictyostelium.*

Unlike cAR3 and ZAK1, which are not expressed until aggregation (Johnson *et al.,* 1993; Kim *et al.,* 1999), GSK-3 is present and active both in growing unicellular populations and during aggregation and multicellular development (Plyte *et al.,* 1999). However, while the levels of GSK-3 protein do not appear to change during development, the level of GSK-3 activity does. GSK-3 activity begins to increase during mound formation and it peaks at about 12 h of development, at a level almost double that of growing unicellular populations (Plyte *et al.,* 1999). This cAMP-dependent increase in GSK-3 activity is dependent upon the presence of cAR3 and ZAK1, placing GSK-3 downstream of both (Kim *et al.,* 1999; Plyte *et al.,* 1999). Cells mutant for cAR3 or ZAK1 display phenotypes similar to GSK-3 mutants, the most significant being reduction in the number of prespore cells and expansion of prestalk cells (Harwood *et al.,* 1995; Kim *et al.,* 1999; Plyte *et al.,* 1999). Tyrosine phosphorylation of GSK-3 by ZAK1 leads to an increase in GSK-3's *in vitro* activity (Kim *et al.,* 1999). Furthermore, overexpression of GSK-3 protein in cAR3 mutant cells is not sufficient to rescue the phenotype, emphasizing the requirement for GSK-3 activation in prespore cell formation (Plyte *et al.,* 1999). Taken together, these results support a cell differentiation mechanism in which cAMP activates the transmembrane receptor cAR3, which activates ZAK1 through an unknown mechanism (Fig. 4B). ZAK1 then activates GSK-3 through direct tyrosine phosphorylation, leading to the specification of prespore cells.

In addition to activation, GSK-3 inhibition is also important for cell fate determination. Two additional cAMP receptors, cAR2 and cAR4, have the opposite effect of cAR3 on *Dictyostelium* development. While cAR3 is expressed in all cell types, cAR2 and cAR4 are expressed primarily in the prestalk cells. Mutations in either cAR2 or cAR4 cause an increase in prespore gene expression (Saxe *et al.,* 1993; Louis *et al.,* 1994; Ginsburg and Kimmel, 1997), while prestalk gene expression is also reduced in cAR4 mutations (Louis *et al.,* 1994; Ginsburg and Kimmel, 1997). Addition of the GSK-3 inhibitor lithium to cAR4 mutants restores normal gene expression (Ginsburg and Kimmel, 1997). Together, these results suggest that cAR4 and cAR2 act as negative regulators of GSK-3 in the induction of prestalk cells. This model awaits the type of biochemical analysis used to study the regulation of GSK-3 by ZAK1. Specifically, it will be of particular interest to identify downstream intracellular modulators of cAR2 and cAR4 to better understand how GSK-3 is regulated in response to cAMP within particular subsets of *Dictyostelium* cells. As suggested by Louis *et al.* (1994), the spatial and temporal expression of cAR1-4, as well as their differential affinities for cAMP, are likely to be critical for cell-type specification. In addition to signaling through cAMP, prespore and prestalk cell determination likely involves other signaling inputs, which may or may not work through GSK-3 (reviewed in Firtel, 1995; Williams, 1995; Aubry and Firtel, 1999; Brown and Firtel, 1999; Thomason *et al.,* 1999). While there is still much to learn about cell fate determination in *Dictyostelium,* the

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experiments analyzing GSK-3 activity in *Dictyostelium* suggests a model wherein prespore cell determination requires GSK-3 activation through cAR3 and ZAK1, and GSK-3 inhibition downstream of cAR2 and cAR4 is important for prestalk cell fates.

FUTURE DIRECTIONS

Once credited only for its role in glycogen synthesis, GSK-3 has now emerged as a major player in at least two important developmental pathways: the Wnt/Wg pathway in vertebrates and invertebrates and the cAMP-responsive pathway in *Dictyostelium.* However, the more we learn, the less we know. The once linear Wnt pathway is becoming increasingly complicated, and questions about the differences in GSK-3 regulation between systems are beginning to emerge where we once saw only similarity. How is GSK-3 regulated in systems like *Xenopus* and sea urchin in which there does not appear to be an involvement of the classic ligand–receptor interaction? Is the activation of GSK-3 seen in *Dictyostelium* a more general phenomenon conserved across phyla? How else might GSK-3 regulation within different signaling pathways be simultaneously coordinated? GSK-3 is now being recognized to have roles in many diverse processes, and as such, we are learning that its regulation is correspondingly complex. There is clearly still much more to learn about this multifunctional kinase and its roles and regulation in insulin signaling, development, and beyond.

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