

# Chapter 11

## Diapause and Estivation in Sponges

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### Contents

11.1 Introduction.....	232
11.2 Gemmulation.....	233
11.3 Diapause.....	236
11.4 Breaking of Diapause.....	237
11.5 Quiescence.....	238
11.6 Germination.....	238
11.7 Summary.....	239
References.....	241

**Abstract** Sponges can be found in fresh or saltwater habitats. As part of their lifecycle, many sponges produce gemmules as a means of surviving environmental challenge. In most sponges, the gemmules contain cells that are initially in a state of metabolic arrest that is controlled by endogenous factors. This state is known as diapause. Following a period of exposure to unfavorable conditions, the cells in the gemmule transit from diapause into a state known as quiescence in which metabolic depression is controlled by environmental factors. When favorable conditions return, the gemmules germinate and produce a new sponge. Production of gemmules is triggered by environmental factors such as decreased temperature or desiccation and involves cell aggregation of thesocytes and the laying down of the gemmule coat. Thesocytes contain yolk platelets as an energy store and high concentrations of polyols that maintain high osmotic concentration in the cells of the gemmules. The high osmotic concentration maintains metabolic depression and turns off cell division. It is the inability to reduce the osmotic concentration that maintains the gemmules in diapause. Transition to quiescence requires the ability of the cells in the gemmules to convert the polyols to glycogen, and thus reduce the osmotic concentration. At this stage, the cells are able to reduce osmotic concentration but do not until favorable conditions return. Early in the germination process, the polyols are

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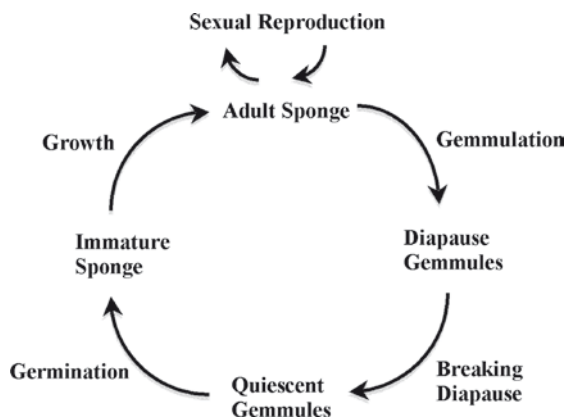
converted to glycogen, reducing the osmotic pressure and releasing the inhibition of cell division and metabolic rate. Both cell division and metabolic rate increase eventually leading to germination of the gemmules and production of a new sponge.

## 11.1 Introduction

Sponges are diploblastic, benthic, filter-feeding organisms that are an important ecological component of a wide variety of habitats world-wide. They can be found in fresh water, estuarine, and marine habitats, and can be present in intertidal, shallow, and deep-sea environments (Worheide et al. 2005; Hooper and van Soest 2002). Marine sponges are common in Antarctica (Dayton et al. 1974; Worheide et al. 2005), temperate regions (Freeman et al. 2007; McDougal 1943; Thakur and Müller 2004), the tropics (Wiedenmayer 1977; Schmahl 1990; Sterrer 1986), and even the Arctic (Plotkin and Boury-Esnault 2004). Likewise, freshwater sponges can be found in ponds, streams, and bogs from the Arctic through the tropics (Frost 1991; Ricciardi and Reiswig 1993; Droscher and Waringer 2007; Manconi et al. 2008). Sponges can represent the predominant benthic component of marine (Freeman et al. 2007) and freshwater ecosystems (Frost 1978). Sponges are important competitors, can provide a food source for other organisms, are known to be involved with a variety of symbiotic relationships (Wulff 2006), play an important role in nutrient cycling and primary production (Frost 1978; Frost and Williamson 1980), and are increasingly known for the presence of bioactive compounds that may be useful in medicine (Thakur and Müller 2004). Recently, sponges have been used to help decipher evolutionary relationships among the metazoans (Maldonado 2004; Worheide et al. 2005).

Despite their biological importance, relatively little is known about the physiology and biochemistry of sponges, especially, as it relates to their ability to enter the states of diapause and quiescence (Casceres 1997; Guppy and Withers 1999; Hand and Podrabsky 2000). Sponge life cycles can vary from species to species; however, a “generalized life cycle” (Fig. 11.1) helps to introduce the concepts of quiescence and diapause and how they relate to the life history of sponges. Adult sponges quickly grow during favorable environmental times when they lay down most of their biomass and undergo sexual reproduction. When the environment becomes less favorable (during times of lake draw-down or as the temperature drops in the fall in temperate regions, for example), many sponges produce asexual reproductive structures called gemmules.

Gemmules contain one type of cell (thesocytes) packaged in collagenous and glass (spicule) capsules that are left behind when the adult sponge dies. In many species, when the gemmules are produced, the cells are in a state of metabolic depression that is endogenously controlled. In this diapause state, gemmules will not germinate and continue development even if conditions become favorable again (Hand and Podrabsky 2000). Instead, the gemmules must go through a period of exposure to unfavorable conditions during which diapause is broken and gemmules



**Fig. 11.1** Generalized life cycle of a sponge

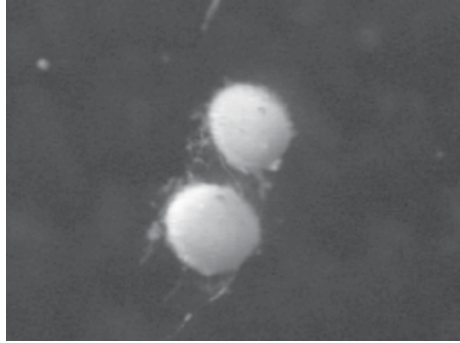
enter a state known as quiescence. In quiescence, metabolic depression is maintained by environmental factors (such as low temperature or desiccation). If favorable conditions return, metabolism and cell division are turned up, the cells will begin to differentiate, and will eventually emerge from the gemmules to develop into an adult sponge (Hand and Podrabsky 2000). Therefore, diapause can be defined as an endogenously controlled state of metabolic depression that is an obligate step in the life cycle of the sponge, whereas, quiescence can be defined as an environmentally controlled state of metabolic depression (See Hand and Podrabsky 2000 and Guppy and Withers 1999 for reviews on diapause and quiescence in animals). It is not necessary for gemmules to go through diapause to reach quiescence and there is a great deal of variability among sponge species and within individual species as to the details of the life cycle.

In this chapter, I will review our current understanding of the cell biology and biochemical mechanisms of each of these steps in the life cycle focusing in particular on (1) gemmulation, (2) diapause, (3) breaking of diapause, (4) quiescence, and (5) germination. Most of what we know about these stages has come from work on freshwater sponges; however, I will mention marine sponges whenever it is appropriate. Opportunities for new directions of study are also highlighted.

## 11.2 Gemmulation

Gemmules are comprised of thesocytes surrounded by a collagen and spicule coat (Fig. 11.2). The thesocytes from freshwater sponges are usually binucleate and are packed with yolk platelets and lipid inclusions along with numerous ribosomes, endoplasmic reticulum, mitochondria, and golgi (Simpson 1984). The yolk platelets are thought to provide energy reserves in the form of basic proteins and glycoproteins (De Vos 1971; Harrison and Cowden 1975; Simons and Muller 1966),

**Fig. 11.2** Gemmules of *Anheteromeyenia ryderi*



polysaccharides (Harrison and Cowden 1975) and lipids (Kauffold and Spannhof 1963) during diapause, quiescence, and, especially, during germination.

Gemmule formation is thought to occur in response to a shift from favorable to unfavorable conditions that may include an increase or decrease in temperature and/or desiccation (Fell 1998). The time of year that gemmules form in natural populations can be extremely variable even for the same species. Pronzato et al. (1993) examined the timing of gemmule production by populations of *Ephydatia fluviatilis* along a climatic gradient in Italy. In northern populations, gemmules form early and the sponge spends less time as active adults in the summer. In the southern populations, gemmules are formed during the summer and the adults are active during the winter. The same is true when comparing populations in the United States and Belgium (Harsha et al. 1983; Poirrier 1974; van de Vyver and Willenz 1975). The timing can also be variable for two species in the same body of water. For example, *E. fluviatilis* exists as gemmules from May through August in Lake Pontchartrain, Louisiana while *Spongilla alba* exists as gemmules from late September through early May (Poirrier 1974, 1976).

The extreme variability in the timing of gemmule production leads to the question of what environmental cues are responsible for initiating gemmulation. Rasmont (1963) found that the size and nutritional condition of the sponge influence gemmule formation. Larger sponges that were fed more bacteria formed more gemmules. He suggested that large healthy sponges produced an inducer that initiated gemmule formation. He also proposed that there was an endogenous component controlling gemmulation because gemmules stored at 3°C for different lengths of time form different numbers of gemmules. Pronzato et al. (1993) also argue that there may be an endogenous rhythm controlling the life cycle of *E. fluviatilis* because they have identified a population that undergoes a brief period of gemmule formation in a very stable habitat. Simpson and Gilbert (1973) argue that exogenous and endogenous factors such as prior gametogenesis and larva production may be important in determining when a sponge gemmulates. Water temperature may play a major role in determining the seasonality of the life cycle of freshwater sponges (Harsha et al. 1983; Simpson and Gilbert 1973). Gemmules from *S. alba* are produced in mid fall when the water temperature falls below 22°C (Poirrier 1976) and gemmules of *E. fluviatilis* are

produced when water temperatures rise above 30°C (Poirrier 1974). Light appears to inhibit gemmule formation and may interact with temperature (Simpson 1984). Low levels of silicon dioxide in the incubating medium results in smaller gemmules, with some even lacking spicules (Petermans-Pe et al. 1975; Simpson 1984).

Cyclic nucleotides may be important in initiating gemmule formation in some freshwater sponges. Incubating freshwater sponges in theophylline (an inhibitor of cyclic nucleotide phosphodiesterases) stimulated gemmule formation (Rasmont 1974). Presumably, the increase in cyclic nucleotide concentration resulting from the inhibition of its breakdown increased the production of cell aggregates, the first stage in gemmule formation (see below). Adding cyclic nucleotides along with aminophylline (also an inhibitor of cyclic nucleotide phosphodiesterase) directly to the sponges stimulated gemmule formation in *Spongilla lacustris* but not in *E. fluviatilis*. (Simpson 1984; Simpson and Rodan 1976), so this effect may not be universal.

Supposedly, the cyclic nucleotides would be produced as a result of the presence of an environmental factor stimulating its production in the sponge cells through signal transduction. Very little is known of the possible signal transduction pathways that might link environmental cues to gemmule production. Genes for elements of two signaling pathways have been identified from sponges. The phospholipase C gene, part of the inositol phosphate signaling pathway, was identified in *E. fluviatilis* (Koyanagi et al. 1998) and mitogen-activated protein kinase gene was identified in *Suberites domuncula* (Bohm et al. 2000), but neither has been linked to gemmule production.

Simpson and Gilbert (1973) have divided gemmule formation into three stages: early, mid, and late. During early gemmulation, the first visible sign of gemmule production is the formation of aggregates of two or three types of ameoboid cells in the mesenchyme of the sponge. In most freshwater sponges, trophocytes provide the nutrients for development of yolk platelets through cytolysis or phagocytosis by the thesocytes (Simpson and Fell 1974). Harrison and Cowden (1975) identified a third cell type (granular cells) in the aggregates of *Eunapius fragilis* that is involved in gemmule coat formation. Following aggregation, mid gemmulation is marked by the production of a layer of columnar cells around the aggregate. These cells will form the gemmule coat during the late stage of gemmulation (Simpson and Gilbert 1973). The last step in gemmule formation is an increase in the osmotic pressure of the thesocytes (Simpson and Fell 1974). In most freshwater sponges, newly formed gemmules have a relatively high osmotic pressure. Zeuthen (1939) found that gemmules of *S. lacustris* contain thesocytes that are in osmotic equilibrium with a 220 mOsm solution of sodium chloride. Likewise, Schmidt (1970) found that thesocytes from the gemmules of *E. fluviatilis* lysed when placed in distilled water but when released into a 115 mOsm solution of sodium chloride they remained stable. The development of high osmotic pressure in these cells is most likely due to the synthesis of polyols from glycogen. Loomis et al. (1996b) identified sorbitol as the major component of the ethanol-soluble extract of gemmules of *E. fragilis* and Loomis et al. (2009) found that myo-inositol is the major ethanol-soluble component in gemmules of *Anheteromeyana ryderi*. The amount of polyol present in the gemmules can account for the increased osmotic pressure observed in the thesocytes of these sponges (Loomis et al. 2009)

### 11.3 Diapause

Newly formed gemmules can be in the states of diapause or quiescence, and the distinction between these states is not always clear (Fell 1998). Fell and others have measured the depth of diapause by determining the time of exposure to low temperature and/or desiccation that will break diapause (Bazer and Fell 1986; Fell 1995; Fell 1994). Fell (1995) defines gemmules undergoing shallow diapause as those gemmules that will hatch after only a few days of exposure to low temperature (5°C) and gemmules undergoing deep diapause as those gemmules that must be exposed to low temperatures for a month or more before they will hatch. The depth of diapause varies greatly both within and among species. For example, *S. lacustris* produces three types of gemmules: thick-capsuled gemmules, green thin-capsuled gemmules and yellow thin-capsuled gemmules (Fell 1995). When produced, the green thin-capsuled gemmules are in quiescence, the thick-capsuled gemmules are in shallow diapause and the yellow thin-capsuled gemmules are in deep diapause (Fell 1995). *Eunapius fragilis* (Fell 1995) and *Anheteromeyenia ryderi* (Bazer and Fell 1986) undergo deep diapause, whereas, in *Heteromeyenia tubisperma* diapause is much more shallow (Bazer and Fell 1986).

Depth of diapause has also been determined in a few species of sponges by measuring the metabolic rate of diapausing gemmules and comparing that with the metabolic rate of quiescent gemmules and the metabolic rate at the time of emergence of the first cells from germinating gemmules. Rasmont (1962) measured the rate of oxygen consumption of both diapausing and postdiapausing gemmules of *Ephydatia mulleri* and found that the metabolic rate of diapause gemmules was depressed by a factor of 3 when compared with quiescent gemmules. Brondsted and Lovtrup (1953) measured oxygen consumption of gemmules of *S. lacustris* during germination. *S. lacustris* produces two types of gemmules, green and brown. The quiescent green gemmules had an initial rate of oxygen consumption five times greater than that of postdiapause, brown gemmules. Further, the respiration rate of brown gemmules increased by a factor of 7 during germination whereas the respiration rate of green gemmules only increased 1.5-fold. Loomis et al. (1996a) measured both the oxygen consumption rate and heat production of gemmules of *E. fragilis* during diapause, quiescence, and germination. The metabolic rate of quiescent gemmules was twice that of diapause gemmules and the rate of oxygen consumption and heat production steadily increased to six times that of quiescent gemmules. Clearly, the metabolic rate of diapause gemmules is significantly depressed and stays depressed until diapause is broken at which time it increases a small amount.

Very little is known of the endogenous control mechanisms that maintain the gemmules in diapause. Loomis et al. (2009), Rozenfeld (1971), Schmidt (1970), Simpson et al. (1973), and Zeuthen (1939), have all demonstrated that the intragemmular osmotic pressure of diapause or quiescent gemmules is high (between 100 and 220 mOsM) and that the osmotic pressure is reduced below 40 mOsM during the early stages of germination. Further, germination is inhibited as long as the osmotic pressure is more than 50 mOsM. Rozenfeld (1974) examined the effects of an

unidentified inhibitor (gemmulostasin) of germination on DNA, RNA, and protein synthesis during germination and found that DNA synthesis was completely inhibited and that RNA and protein synthesis were delayed in the presence of the inhibitor. Since gemmulostasin was never identified, Simpson et al. (1973) and Simpson and Fell (1974) have proposed that gemmulostasin actually represents an increased osmotic pressure. If this is true, cell division, translation, and protein synthesis would be inhibited by high osmotic pressure. Indeed, Loomis et al. (2009) have recently shown that germination, metabolic rate, and cell division of quiescent gemmules of *E. fragilis* and *A. ryderi* are controlled by increased osmotic pressure in the cells. High levels of polyols (sorbitol in *Eunapius* and myo-inositol in *Anheteromeyenia*) maintain the osmotic pressure in the thesocytes above 100 mOsm. These compounds are most likely produced from glycogen during the formation of gemmules (see above) and are present in diapause gemmules.

Cyclic nucleotides such as cAMP may also play a role in inhibition of germination. Simpson and Rodan (1976) found that cAMP levels in *S. lacustris* were high in quiescent gemmules and declined rapidly during the early stages of germination. When cAMP levels were maintained by inhibition of cAMP phosphodiesterase, the thesocytes did not begin cell division.

## 11.4 Breaking of Diapause

The breaking of diapause usually requires exposure of gemmules to adverse environmental condition for a period of time (Fell 1998). For example, diapause can be broken by exposing gemmules to low temperature (5°C) for a few days, (in the case of shallow diapause) or months (in the case of deep diapause). Newly formed gemmules of *E. fragilis* exposed to 3°C for 3 weeks followed by exposure to 20°C for 27 weeks did not germinate. The same gemmules later hatched following further cold treatment of up to 18 weeks (Fell 1995, 1998). Diapause may also be broken by desiccation in the gemmules of some sponges (Fell 1987a, 1987b). Desiccation increased germination of gemmules of *E. fragilis* kept at 20°C and exposure to cold for a few weeks, prior to desiccation greatly reduced the time of exposure to low temperature required for breaking of diapause (Fell 1987b).

One of the first steps in the germination of *E. fragilis* is the reduction of sorbitol levels and concomitant decrease in intracellular osmotic pressure (Loomis et al. 1996b, 2009). Sorbitol appears to be converted to glycogen through the activation or *de novo* synthesis of sorbitol dehydrogenase (SDH) and an unknown mechanism of activation of glycogen synthase (Loomis et al. 1996b). These steps appear to set off the cascade of events that result in emergence of the cells from the gemmules. The molecular switch for breaking of diapause may involve the change in the cell's ability to synthesize or activate the enzymes that convert polyols to glycogen. For example, in *E. fragilis*, the gene coding for SDH may not be capable of being switched on during diapause. During the breaking of diapause, the ability to switch on the SDH gene could occur transitioning the gemmules from diapause into quiescence.

## 11.5 Quiescence

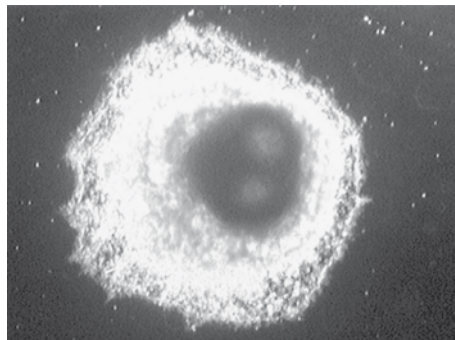
Once diapause is broken, gemmules are kept in depressed metabolic state by continued exposure to adverse conditions. Low temperature, desiccation, oxygen deprivation, osmotic stress, low or high pH, and short day photoperiod have all been shown to inhibit germination in postdiapausing gemmules (Benfey and Reiswig 2005; Loomis et al. 2009; Loomis et al. 1996b; Rasmont 1954, 1963; Reiswig and Miller 1998). Various ions such as  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$  have a long-term effect on inhibition of germination of *E. fragilis* (Fell 1992). Divalent cations (except calcium) also inhibit germination of postdiapausing gemmules from *S. lacustris*; however, calcium appears to overcome this inhibition (Ostrom and Simpson 1979). The effects of ions on germination may be important in those sponges that gemmulate as a result of desiccation.

In *E. fragilis* and *A. ryderi*, low temperature is the major factor inhibiting germination in postdiapausing gemmules (Loomis et al. 2009). The primary effect of low temperature is to maintain the high osmotic pressure in the gemmules, inhibiting the early stages of germination.

## 11.6 Germination

When conditions become favorable, the quiescent gemmules begin germination (Fig. 11.3). For example, gemmules from *E. fragilis* remain metabolically depressed as long as the temperature is maintained below 5°C (Fell 1995; Loomis et al. 1996b). When the temperature is raised to 20°C, germination is initiated and cells emerge from the gemmules within 48–72 h (Fell 1995, 1998).

The process of germination begins with the division of thesocytes, gradual breakdown of yolk platelets, and differentiation of thesocytes into other cell types (Simpson and Fell 1974; Fell 1998; Simpson 1984). The foramen or micropyle (the structure through which the cells move to the outside) eventually opens and cells migrate out of the gemmule to differentiate into a small sponge.



**Fig. 11.3** Newly germinated sponge of *Eunapius fragilis*



During germination, the metabolic rate increases slowly and reaches a level six to eight times that of quiescent gemmules at the time of cell emergence (Bronsted and Lovtrup 1953; Loomis et al 1996b). Polyol levels decline dramatically during the early stages of germination and appear to be converted to glycogen (Loomis et al. 1996a; Loomis et al. 2009). In *E. fragilis*, loss of sorbitol is due to the increase in activity of SDH (Loomis et al. 1996b). In *E. fragilis* and *A. ryderi*, initiation of cell division is correlated with the reduction of the osmotic pressure of the thesocytes to below 50 mOsM (Loomis et al. 2009).

At least in some sponges, germination is initiated when the temperature rises and activates the metabolic pathways responsible for conversion of polyols to glycogen (Loomis et al. 2009). Very little is known of the control mechanisms of this pathway during early stages of germination, but in *E. fragilis* it most likely involves upregulation of the gene encoding SDH. The total activity of glycogen synthase does not change during germination of gemmules of *E. fragilis*, but the activity of glucose-6-phosphate dependent glycogen synthase is much higher than that of glucose-6-phosphate independent glycogen synthase (Loomis et al. 1996b). Since glucose-6-phosphate is an intermediate in the pathway from sorbitol to glycogen, it may be that a buildup of glucose-6-phosphate as a result of sorbitol catalysis activates glycogen synthase. Total glycogen phosphorylase activity increases more than double during germination, but the proportion of glycogen phosphorylase declines, attenuating the apparent increase in activity (Loomis et al. 1996b). Since cAMP levels do not change during germination, it is most likely not involved in control of conversion of sorbitol to glycogen (Loomis et al. 1996b).

## 11.7 Summary

Because there is a great deal of variability among sponge species in factors involved in control of gemmulation, diapause, quiescence, and germination, it is difficult to identify the commonalities. Even though our understanding of control mechanisms is rudimentary in most sponges, a story is starting to immerse from studies of *E. fragilis*. Fig. 11.4 summarizes our current state of knowledge along with some “leaps of faith” that still need investigation.

Gemmulation is initiated by environmental cues that include decreasing temperature in northern populations and desiccation in southern populations. The result is aggregation of the cells that will become thesocytes and formation of the gemmule coat. During thesocytes formation, platelets are produced and glycogen is converted to sorbitol, increasing the osmotic pressure in the cells. These thesocytes are in a state of diapause because they do not have the ability to convert glycogen to sorbitol most likely because the SDH gene is downregulated. Osmotic pressure above 100 mOsM inhibits cell division and maintains metabolic depression. Following a long exposure to cold or a period of desiccation, the cells gain the ability to convert sorbitol to glycogen and enter quiescence. Cell division and metabolic depression are maintained by high osmotic pressure. An increase in temperature

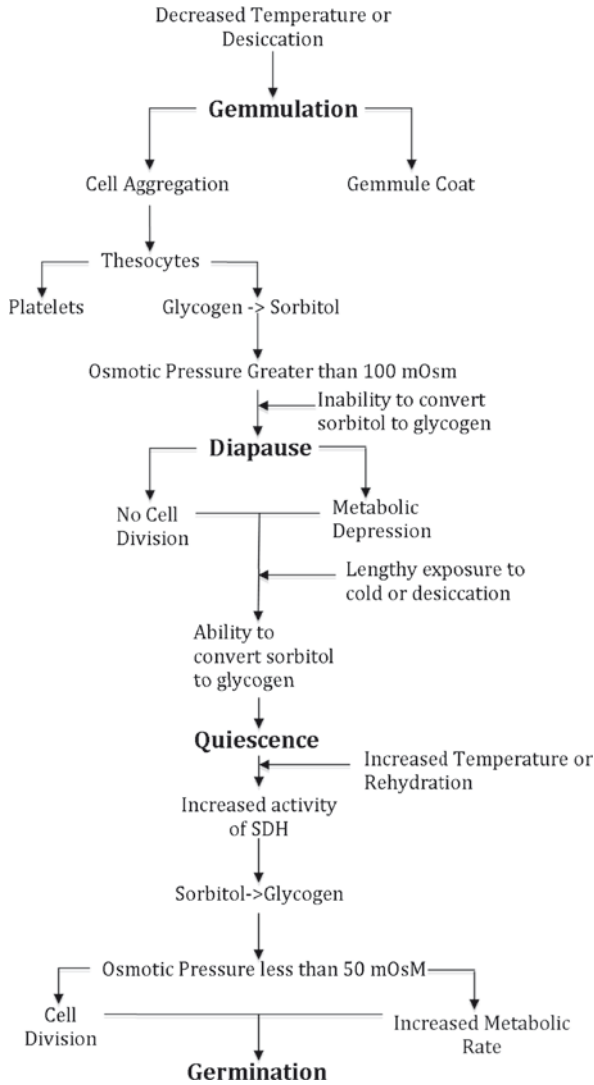


Fig. 11.4 Proposed mechanisms of control of the life cycle of *Eunapius fragilis*

triggers activation of the SDH gene and *de novo* synthesis of SDH. Sorbitol is then converted to glycogen, lowering the osmotic pressure below 50 mOsm. Cell division is initiated and the metabolic rate begins to increase triggering germination.

Obviously, there is a great deal that we still need to understand about the mechanisms of control of the life cycle of *E. fragilis*. We need to confirm that there is inhibition of the SDH gene during diapause and that the gene is activated during germination. We have not identified transcription factors acting as switches between diapause and quiescence. The clock that determines the amount of time a gemmule

must be exposed to adverse conditions before switching to quiescence needs to be identified, and control of the rest of the metabolic pathway leading from sorbitol to glycogen needs to be elucidated. One approach to deciphering these factors may be through the use of microarrays to identify differences in gene activity in diapause, quiescent, and germinating gemmules. Likewise, a proteomics approach may be fruitful in understanding some of these mechanisms. A number of other sponges must be studied to determine commonalities in mechanisms in light of the unusual diversity of responses that different species of sponges or even individuals within a species have to changes in their environment. Only then, will we begin to unravel the complexity of diapause and quiescence in sponges.

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