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REVIEW

A comparison of early molecular fertilization mechanisms in animals and flowering plants

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Abstract Since the first description of the double fertilization process in flowering plants (angiosperms) 110 years ago (Nawaschin in Bull Acad Imp Sci St Petersburg 9:377–382, 1898), little progress has been made during the following 100 years to understand the underlying molecular mechanisms. This seems to be in strong contrast to our steadily increasing knowledge of single fertilization in animals, where a large number of key players and the corresponding molecular mechanisms have been unclosed since the mid-1970s. Are the identified fertilization mechanisms ubiquitously dispersed, occurring also in higher plants? The past few years have seen a number of discoveries indicating that general principles of fertilization mechanisms in animals and flowering plants are more conserved than previously thought. Here, we compare the development and morphology of animal and flowering plant gametes, discuss cell–cell communication events between gametes and gametophytes as well as their physical interaction and fusion during single and double fertilization, respectively.

Keywords Fertilization · Cell-cell communication · Gamete interaction · Chemotaxis · Species-specificity

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Introduction

In comparison to the diplontic life cycle of most animals, where only the gametes are in the haploid state, flowering plants undergo a haplodiplontic life cycle, in which the gametes are not the direct result of a meiotic division. The haplodiplontic life cycle of plants alternates between a multicellular diploid organism, the sporophyte, and a multicellular haploid organism, the gametophyte (Greek *phyton*, "plant"). After meiosis, the sporophytes of higher plants give rise to sexually differentiated types of spores, microspores and megaspores. These spores divide mitotically and develop into haploid gametophytes, whose main function in angiosperms is to produce two male and female gametes, respectively, and to manage the double fertilization process (Drews et al. 1998; Raven et al. 1999; Lord and Russell 2002; Boavida et al. 2005). The male gametophyte (pollen or pollen tube) delivers two sperm cells to fertilize egg and central cell, respectively (double fertilization), which thereafter develop into a diploid embryo and typically triploid endosperm. While plant gametes are derived after meiosis from gametophytic vegetative cells during flower development in adult plants, animal gametes are generated from a founder population of primordial germ cells (PGCs) that are determined early in embryogenesis and set aside for gamete production during adulthood (Aflatoonian and Moore 2006; Gilbert 2006). Thus, flowering plants differ from animals, not only by an alternation of two multicellular bodies (gametophyte and sporophyte), but also by a different sexual life history.

Despite these major differences, mechanisms of cell–cell communication, cell fusion and prevention of polyfusion evolved long before the plant and animal lineage separated more than 1,500 million years ago (MYA), when the first single-celled green algal antecedent to plants

originated (a primitive eukaryote containing simple green plastids; Yoon et al. 2004), and long before the first multicellular organisms appeared, some 1,000 MYA (Kirk 2005). Life existed exclusively in water and it is assumed that haploid cells of the same type fused randomly to form a diploid cell (zygote). After genetic recombination the zygote released haploid offspring that occasionally displayed a higher fitness. Thus, the evolution of sex was disadvantageous for the single cell, but advantageous for the cell population. Taking into consideration that different cell types and species co-existed in the same environment, there also appeared the necessity to evolve species-specific communication and fusion mechanisms as well as the formation of mobility. It is therefore not a surprise that, for example, simulation experiments indicate that evolution of chemotactic cell behavior occurs quickly in evolutionary times, requiring only few proteins (Soyer et al. 2006). As another consequence, lower plants such as algae, mosses and ferns, similarly to animals, use mobile sperm cells for reproduction. The first land plants probably occurred 490–425 MYA, from a single common ancestor (Sanderson 2003) that had to adapt ways to keep from drying out and to reproduce. Under humid environmental conditions, swimming sperm cells still use flagella to arrive at the egg cells. However, the most recent and most successful plant lineage, the angiosperms, which originated some 180–140 MYA (Magallón and Sanderson 2005, Bell et al. 2005) and have captured almost every ecological environment, were forced to evolve new reproduction mechanisms such as precociously forming enclosed male germ cells portable over large distances, highly protected female gametes, as well as a sexual mode to nourish the developing embryo, and a protected, provisioned seedling. The latter need for nutrition is achieved by a novel, second fertilization product, the endosperm.

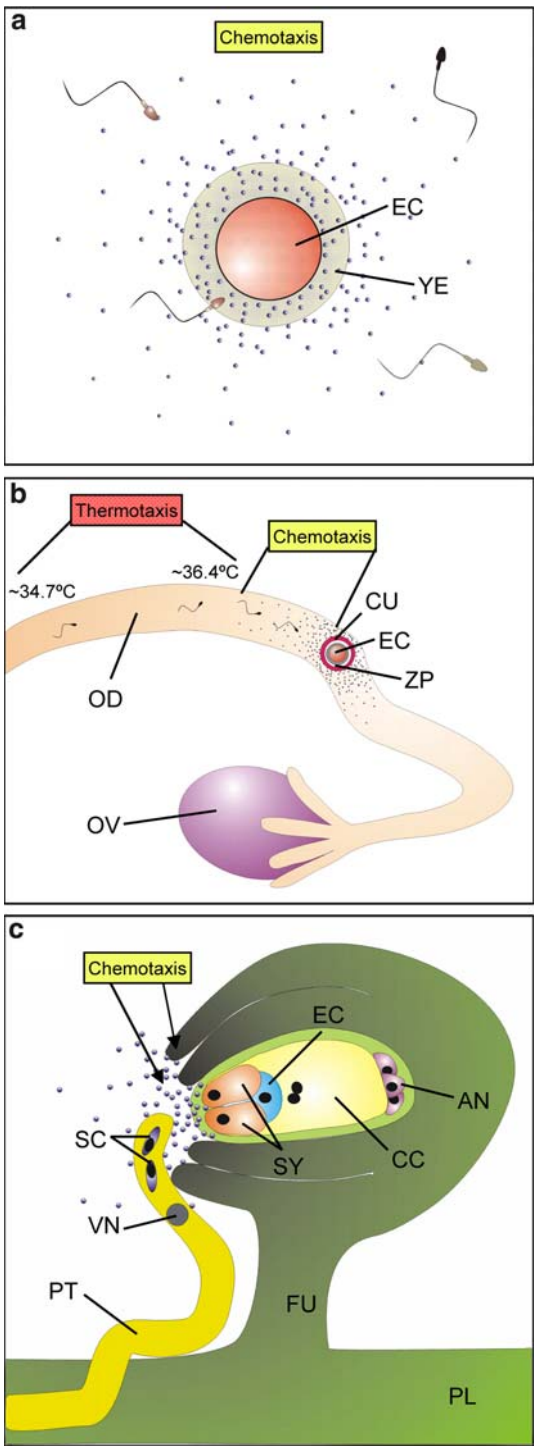
Considering that the sexual life cycle of flowering plants is fundamentally different and arguably more complex than in animals, can we expect that basic fertilization mechanisms and the molecular nature of key regulators are still conserved? If so, can plant reproduction biologists learn from their colleagues working with animal model systems to understand fertilization in plants?

A complete comparison of all fertilization mechanisms between plants and animals including also algae, mosses, ferns and gymnosperms as well as the numerous evolutionary aspects would go far beyond the scope of this review. We therefore restrict our comparative review to early molecular fertilization mechanisms in flowering plants and animals, reporting mainly about gamete morphology and function, as well as gamete cross-talk and fusion. Late fertilization events that include prevention of polyspermy, nuclear migration and karyogamy as well as egg activation are compared in the corresponding reviews elsewhere in this issue (Spielman and Scott 2008; Kranz and Scholten 2008; Curtis and Grossniklaus 2008, respectively).

Gamete development and morphology

Male and female gametes are unique from all other cells and are also different from each other. **Animal male gametes**, known formally as sperm or spermatozoa, comprise a head, containing the acrosome and the haploid nucleus, and the flagellum, consisting of the neck, the midpiece, the tail and the end piece (Clermont and Leblond 1955; Gilbert 2006; Fig. 1a, b). The acrosome plays a critical role in facilitating sperm fusion during fertilization and is derived from the Golgi apparatus. It contains enzymes needed to digest extracellular coats surrounding the egg or oocyte and thus enables the sperm nucleus to enter the egg cytoplasm. In sea urchins and several other species, recognition between sperm and egg involves molecules of the acrosomal process. Animal sperm are able to swim actively towards the female gamete via chemotropism (see below). The ways sperm cells are propelled vary according to how the species has adapted to its own environmental conditions. In most species, each sperm is able to travel long distances relative to its size by whipping its flagellum. Microtubules represent the structural basis of the flagellum, driven with energy for flagellar motion derived from mitochondrial ATP and powered by a dynein motor ATPase (Shinyoji et al. 1998).

In contrast to animal sperm, **male gametes of flowering plants** are non-motile and are transported towards the female gametes via the pollen tube (Fig. 1c). Here, we describe male gamete development in flowering plants only briefly, as excellent reviews can be found elsewhere that describe also the evolution of aflagellate sperm cells (for an overview see Renzaglia and Garbary 2001; Morrow 2004; Boavida et al. 2005). Angiosperms contain two male gametes (sperm cells) that play a central role in double fertilization. The mature male gametophyte, also referred to as the pollen grain or microgametophyte, develops within the anther locules and is composed of two or three haploid cells: a vegetative cell that encloses either one generative cell (bicellular pollen grain) or two sperm cells (tricellular pollen grain). In the case of the tricellular pollen grain, sperm cells are formed after the second mitotic division within the anther (for example in grasses and crucifers), whereas in the bicellular pollen grain the second mitotic division occurs during pollen tube growth, as in many Solanaceae species (McCormick 2004). The vegetative cell coordinates pollen tube growth and thus the delivery of the two male gametes to the female gametophyte (Weterings and Russell 2004).



◄ **Fig. 1** Models comparing sperm attraction in marine invertebrates, mammals and flowering plants. **a** Eggs (ovums) of marine invertebrates such as sea-urchins secrete species-specific peptides (*dots*) to attract sperm cells. Short-range sperm guidance occurs along a concentration gradient. Sperm of the same species swim towards the egg via chemotaxis (*left*, one sperm penetrates the jelly egg coat), while sperm of other marine animals are not attracted (*right*). **b** Chemotaxis and probably also thermotaxis takes place inside the mammalian oviduct once sperm have passed the uterus. *Dots* indicate a concentration gradient of signaling ligand molecules secreted by the ovulated egg and/or surrounding cumulus cells. **c** Pollen tube guidance precedes double fertilization in flowering plants. A pollen tube harboring two sperm cells and a large vegetative nucleus has left the placenta to grow along the placental surface and the funiculus into the micropyle following gradients generated by the maternal tissues of the ovule as well as by the female gametophyte. A Polygonum-type embryo sac containing the egg apparatus (egg cell and two synergids), a central cell with two polar nuclei and three antipodal cells is surrounded by nucellus cells (*light green*) and the two layers of the inner and outer integuments. The nucellus is disintegrated in some plant species generating a naked egg apparatus at the micropylar region. Abbreviations: *AN* antipodal cells, *CC* central cell, *CU* cumulus cells, *EC* egg cell or ovum, *FU* funiculus, *OD* oviduct, *OV* ovary, *PL* placenta, *PT* pollen tube, *SC* sperm cell, *SY* synergid, *VN* vegetative nucleus, *YE* egg jelly, *ZP zona pellucida*

The **animal female gamete**, called the ovum (a mature egg with a haploid nucleus, as in sea urchins) or the oocyte (an egg at an earlier stage of development arrested at meiosis II, as in mammals), is non-motile and huge in comparison to the male gamete. While the sperm has eliminated most of its cytoplasm during maturation, the egg or oocyte contains a large mass of cytoplasm storing nutritive and regulatory proteins, ribosomes, tRNAs and mRNAs needed to accomplish protein synthesis during embryo development. In many species, morphogenetic factors are also present in different regions in the egg cytoplasm. Furthermore, many egg cells contain protective agents (e.g. ultraviolet filters, DNA repair enzymes) that are needed for their survival and, later on, for embryo survival in particular environments. Each egg contains, within this huge mass of cytoplasm, a large nucleus, which in some species (like sea urchins) is already haploid at the time of fertilization, whereas in other species (like many worms and most mammals) is still in meiosis. The egg cell membrane plays the most important role during the fertilization process by regulating the secretion of sperm attractants, the flow of certain ions and fusing with the sperm cell membrane. Outside of the cell membrane is an extracellular layer that is often involved in sperm–egg recognition (Correia and Carroll 1997). In most animals, this structure is usually called the vitelline envelope, whereas the surrounding layer in mammals is a separate and thick extracellular matrix called the *zona pellucida* (Fig. 1a, b). Many types of egg cells also possess outside the vitelline envelope a layer of egg jelly, which contains glycoproteins and which is most commonly used either to attract or to activate sperm. Mammalian egg cells are additionally surrounded by a layer of cells called the cumulus, with this innermost layer adjacent to the *zona pellucida* called the *corona radiata*. Directly beneath the egg plasma membrane lies the cortex, a thin shell of gel-like cytoplasm containing high concentrations of globular actin molecules. These actin molecules polymerize during

fertilization and form microfilaments that are necessary for cell division and are used as well to extend the egg surface into small projections called microvilli, which may aid in sperm entry into the cell. The final structural feature of the egg cell that serves as a critical function during fertilization is a set of membrane-bound structures, called cortical granules that accumulate within the cortex. These are homologous to the acrosomal vesicle of the sperm and are Golgi-derived organelles that contain proteolytic enzymes and mucopolysaccharides, needed to prevent polyspermy (Yanagimachi 1994; Gilbert 2006).

The **female gametophyte of flowering plants**, also referred to as the embryo sac or megagametophyte, constitutes the structural setting for double fertilization (Nawaschin 1898). More than 15 different patterns of female gametophyte development have been described. The most frequently exhibited pattern, occurring in about 70% of the angiosperms, is known as the Polygonum type (Fig. 1c), which was first described in *Polygonum divaricatum* (Strasburger 1879). The development of the Polygonum-type female gametophyte occurs over two phases referred to as megasporogenesis (megaspore formation during meiosis when three of the four megaspores undergo programmed cell death) and megagametogenesis (embryo sac development) (Huang and Russell 1992; Yadegari and Drews 2004). After megasporogenesis, the functional megaspore undergoes three cycles of mitosis, producing an eight-nucleate syncytium. Phragmoplasts and cell plates form between sister and non-sister nuclei after the third mitosis, and the female gametophyte cells become partly surrounded by cell walls (Webb and Gunning 1994). The three cells at the micropylar pole differentiate into the egg apparatus, which consists of an egg cell that is flanked by two synergids (Punwani and Drews 2008, this issue). During cellularization, two nuclei, one from each pole (referred to as polar nuclei), migrate towards the egg apparatus and fuse before or after fertilization to generate the diploid secondary nucleus of the central cell (Diboll 1968; Webb and Gunning 1994; Christensen et al. 1997; Kranz et al. 1998). The synergids form the filliform apparatus, a complex consisting mainly of cell wall invaginations, at their micropylar end. The three cells opposite to the egg apparatus form antipodal cells. In some species, like Arabidopsis, antipodal cells undergo programmed cell death before fertilization (Murgia et al. 1993; Christensen et al. 1997), whereas in other species, such as maize, they proliferate, generating a cell cluster of up to 60 cells (Diboll and Larson 1966; Huang and Sheridan 1994; Kiesselbach 1999). Thus, in most angiosperms, the haploid mature Polygonum-type female gametophyte is embedded in several maternal diploid cell layers of the ovule and consists of four distinct types of cells, including the two female gametes, the egg cell and the central cell. During double fertilization, the egg cell gets fertilized by one of the male gametes and develops into the diploid embryo, while the central cell is fertilized by the second sperm giving rise to a triploid endosperm.

Despite these obvious differences between plant and animal gametes, there also exist a number of common features. Heteromorphism, for example, the formation of at least two different types of sperm or pollen grains, occurs in both animal and flowering plant species. In animals, sperm heteromorphism is typically related to the generation of one fertile morph and one (or more) sterile morph(s), whereas in plants two or more pollen morphs (one of which can be either sterile or fertile) are produced in all flowers but sometimes in different anthers (Till-Bottraud et al. 2005). This example of convergence suggests a general evolutionary response to sexual selection, either to increase the success of one male's sperm or pollen in competition with others, or directly mediate interactions between male and female gametes. Female gametes of both kingdoms are generally very large and immobile polar cells containing not only a store of RNAs and protein for subsequent development, but also morphogen gradients, generating daughter cells with a different fate after asymmetric cell divisions. In animals, this is generally achieved by polar anchoring of transcripts encoding transcriptional regulators, components of signal transduction chains or RNA-binding proteins and encoded proteins, respectively, in cytoplasmic regions of the egg cytoplasm (for review, see Gilbert 2006). The first examples indicating that similar mechanisms exist in plants are, for example, transcripts for the Homeobox containing transcriptional regulators WOX2 and WOX8, which are expressed in the egg cell, but are differentially distributed to apical and basal daughter cells after the asymmetric division of the zygote (Haecker et al. 2004). Direct communication pathways between male and female gametes of marine invertebrate species have been known already for more than 40 years (Miller 1985); however, higher animals, including mammals, as well as plants, seem to employ helper cells (cumulus cells in mammals and synergids in flowering plants) to attract and guide the male gametes and gametophytes, respectively (for review, see Eisenbach and Giojalas 2006; Punwani and Drews 2008, this issue). The next chapter therefore discusses cell–cell communication and chemotaxis between gametes in animals and flowering plants.

Cross-talk between gametes

Cell–cell communication plays an elementary role in various developmental processes either between neighboring cells or between cells that are separated at some distance. At fertilization, cell–cell communication between male and

female gametes is critical both in animal and plant systems. Although there are several **marine invertebrates** that accomplish internal cross-fertilization via copulation or pseudocopulation or spermcast mating (Bishop and Pemberton 2006), external fertilization is a more widely recognized mode of animal mating in invertebrates species, and this review will only refer to the marine broadcast spawners with external fertilization. In these animals, the encounter between their male and female gametes takes place in a tumultuous, aqueous environment shared with other species that may shed their sex cells at the same time. Hence, such organisms have to solve two problems: in such a dilute concentration, sperm must find a way to meet the eggs from their own species and not to fertilize eggs of another species. In **mammals**, where fertilization is internal, semen is placed inside the female genital tract, requiring less specificity. However, despite the ingenious design, in fact, only few of the ejaculated sperm (in humans, only $\sim$1 of every million sperm enter the Fallopian tubes) ever arrive near enough to the egg to be contenders for fertilization (Williams et al. 1993; Eisenbach and Tur-Kaspa 1999). The number of sperms capable of fertilizing the egg is even smaller. Many do not possess the capacity to bind and fertilize the egg cell and, hence, unlike spermatozoa of marine species, mammalian spermatozoa must first undergo a process of ripening, known as capacitation, which takes place in the female reproductive tract. The percentage of capacitated sperm is low ($\sim$10% in humans) and, therefore, the chances that such low numbers of sperm will successfully reach the egg by chance, without a guidance mechanism, are very small (Cohen-Dayag et al. 1995; Giojalas et al. 2004; Eisenbach and Giojalas 2006). A ripening process has also been observed in **flowering plants.** Pollen tubes of *Torenia fournieri* and *Arabidopsis thaliana* grown in vitro are unable to reach the female gametophyte, whereas pollen tubes emerging from a cut style precisely find their way to the female gametophyte, indicating the existence of competence mediation by the stigma and/or style (Higashiyama et al. 1998; Palanivelu and Preuss 2006; Higashiyama and Hamamura 2008, this issue). However, in contrast to animals, fertilization in plants is more efficient and complex, involving more interaction partners (Dresselhaus 2006). Male gametophytes (pollen tubes) in compatible pollination systems are guided to the female gametophyte (embryo sac) by a multistep process that includes promoting of tube growth and guidance by the sporophytic tissues to verify that almost every ovule is reached by at least one pollen tube. It is therefore not surprising that transcriptome analysis of Arabidopsis pollen grains reveal a very high percentage of transcripts encoding proteins involved in signaling processes (Pina et al. 2005), indicating that communication is a major task of the growing pollen tube. Moreover, communication at different levels also exists between the male and female gametophytes and has been distinguished as funicular and micropylar guidance (Higashiyama et al. 2003). Additionally, it can also be expected that direct communication between male and female gametes takes place during sperm delivery in the receptive synergid to verify that both female gametes are fertilized. Preferential fertilization in *Plumbago* supports this hypothesis (Russell 1985), and first transcriptome analyses of isolated female gametophytes and egg cells suggest the presence of a high number of proteins required for signaling processes (Márton et al. 2005; Sprunck et al. 2005; Yang et al. 2006).

Animals evolved diverse mechanisms of sperm guidance. In many species, sperm are guided toward eggs by chemotaxis (Table 1), which is the movement of cells up a concentration gradient toward a chemical attractant

Table 1 Signaling ligands and receptors involved in animal sperm chemotaxis

Ligand	Source	Receptor	Species	Reference
Resact	Egg	Resact receptor/mGC	Sea urchin (*A. punctulata*)	Kaupp et al. (2003, 2006); Böhmer et al. (2005)
Asterosap	Egg	Asterosap receptor/mGC	Starfish (*A. amurensis*)	Nishigaki et al. (1996); Böhmer et al. (2005)
Speract	Egg	Speract receptor	Sea urchin (*S. purpuratus*)	Dangott et al. (1989); Kaupp et al. (2006)
RANTES	Follicular fluid	RANTES receptor/mGC	Human	Isobe et al. (2002)
ANP	Follicular fluid	ANP receptor/mGC	Human	Silvestroni et al. (1992); Zamir et al. (1993)
NO	Ovary	NO receptor/mGC	Human	Miraglia et al. (2007)
Bourgeonal, Lyral	NK	G-protein-ass. odorant receptors	Human and mouse	Spehr et al. (2003); Fukuda et al. (2004)
Progesterone	Follicular fluid, Cumulus	NK	Human and rabbit	Villanueva-Diaz et al. (1995); Teves et al. (2006)

NK not known, *mGC* membrane guanylyl cyclase, *ANP* atrial natriuretic peptide, *NO* nitric oxide

(chemoattractant). Several studies have shown that chemoattractant signals which initiate sperm chemotaxis are synthesized and secreted by the egg, or in some cases by somatic cells associated with the egg, and are believed to exert influence over short distance to enhance the efficiency of sperm–egg contact (Miller 1985; Kirkman-Brown et al. 2003; Kaupp et al. 2008).

Sperm chemotaxis differs among animal species and was first discovered in the mid-1960s in **marine invertebrate animals**. Most of our current understanding of sperm guidance originates from those studies, based mainly on sea urchin and starfish as model organisms. In these species, the aim of sperm chemotaxis is to recruit as many spermatozoa as possible to the eggs and to prevent cross-species fertilization, meaning that a chemoattractant for one marine species is usually not recognized by the spermatozoa of another marine species (Miller 1985). Up to now, around 80 peptides have been identified, mainly from cnidaria and echinodermata, that affect sperm motility in a species-specific fashion and are called sperm-activating peptides (SAPs) (Miller 1985; Suzuki 1995). From these, the chemotactic function has been so far clearly proved only for two SAPs. One is resact, a 14 amino-acid peptide that was isolated from the egg jelly of the sea urchin *Arbacia punctulata* and whose gradient extends at least 1 mm around the egg (Ward et al. 1985; Vacquier 1998; Kaupp et al. 2003; Böhmer et al. 2005). The other one is asterosap, a 34 amino-acid peptide from the egg jelly coat of starfish *Asterias amurensis* (Nishigaki et al. 1996; Böhmer et al. 2005). Binding of these peptides to specific sperm receptors belonging to the membrane guanylate cyclase family (localized along the length of the sperm flagellum) stimulates an increase of intracellular cyclic guanosine monophosphate (cGMP) and mediates ion fluxes across the sperm membrane. In turn, this affects flagellar motion and finally determines the direction of movement (Ramarao and Garbers 1985; Bentley et al. 1986; Nishigaki et al. 2000; Matsumoto et al. 2003; Neill and Vacquier 2004; Kaupp et al. 2008). Thus, cGMP is considered to play a pivotal role in the motility response of sea urchin sperm to chemoattractants.

One of the most intensely studied SAPs and the first to be purified and characterized is speract, a decapeptide that is released by eggs of another sea urchin, *Strongylocentrotus purpuratus* (Suzuki 1995). Like resact, speract activates a cGMP-signaling pathway (Cook and Babcock 1993; Matsumoto et al. 2003; Solzin et al. 2004). However, the speract receptor, a 77 kDa plasma membrane receptor localized exclusively in the sperm flagellum, has been reported to be unrelated to guanylyl cyclase (Dangott et al. 1989; Cardullo et al. 1994). The speract-induced motility changes in *S. purpuratus* sperm closely resemble those of other marine species whose sperm undergo chemotaxis, but attempts have failed to demonstrate its chemotactic activity (Solzin et al. 2004; Wood et al. 2007). It was suggested that resact, speract and other SAPs function through a common signal transduction pathway to stimulate and orient sperm motility, and that this signal involves changes in intracellular pH, in the concentrations of cGMP, cAMP as well as Na^+ and Ca^{2+} ions, in membrane potential and in the phosphorylation pattern of several proteins (Ward and Kopf 1993; Cook et al. 1994; Darszon et al. 2005, 2006; Wood et al. 2007). Hence, although the function of most SAPs has not been firmly established, it is tacitly assumed that they are involved in chemotaxis (Table 1). The sequence of events and the specific function of several cellular reactions, however, are still controversial (Kirkman-Brown et al. 2003; Eisenbach 2004; Kaupp et al. 2006, 2008).

In **mammals**, in which fertilization takes place inside the oviducts of the female, two active mechanisms of sperm guidance have been shown: chemotaxis and thermotaxis (the directed movement of cells along a temperature gradient). Mammalian sperm chemotaxis is still surrounded by a great deal of controversy and has been demonstrated in vitro primarily in humans (Ralt et al. 1991; 1994), frogs (Al-Anzi and Chandler 1998), mice (Oliveira et al. 1999) and rabbits (Fabro et al. 2002) (for reviews, see Eisenbach 1999, 2004; Eisenbach and Giojalas 2006). In all of these species, only capacitated spermatozoa, which represents a small fraction of sperm population, are chemotactically responsive (Cohen-Dayag et al. 1995; Eisenbach 1999; Fabro et al. 2002; Giojalas et al. 2004). Up to now, only a few putative chemoattractants are known to satisfy the main criterion for sperm chemotaxis, namely, the accumulation of sperm at the optimal chemoattractant concentration (Eisenbach 1999, 2004). Recent findings indicate that sperm chemoattractants are secreted both prior to ovulation within the follicle and after egg maturation outside the follicle, and that there are two chemoattractant sources: the mature egg and the surrounding cumulus cells (Table 1; Ralt et al. 1991, 1994; Oliveira et al. 1999; Fabro et al. 2002; Sun et al. 2005). The 8-kDa chemokine Regulated on Activation, Normal T Expressed and Secreted Chemokine (RANTES), which is produced in human follicular fluid, prior to ovulation, was shown to be a chemoattractant for human spermatozoa (Isobe et al. 2002). Whether RANTES is also secreted in the female genital tract after ovulation is still not known. In contrast to echinoderms, the molecular mechanisms of sperm chemotaxis in mammals are largely unknown, and the role of cGMP in human sperm functions still needs to be clarified. Atrial natriuretic peptide (ANP), a polypeptide hormone produced in the human follicular fluid and a ligand for mGC, has been observed to attract human spermatozoa in vitro and has been proposed as a chemotactic signal in

mammals (Silvestroni et al. 1992; Zamir et al. 1993). However, this hypothesis is not yet confirmed. Recently, Miraglia et al. (2007) suggested that nitric oxide (NO), known to be synthesized in the ovarian cells of different mammalian species, may behave as a chemoattractant for human spermatozoa and that the signal transduction involved is a cGMP-signaling pathway. In addition, because human sperm are also able to synthesize and release NO, it was hypothesized that during chemotaxis a complex interplay may occur, not only between spermatozoa, oocytes and other cells of the reproductive tract, but also among spermatozoa themselves (Miraglia et al. 2007).

In vitro studies in **flowering plants** have shown that NO has a negative chemotropic effect on pollen tube guidance (Prado et al. 2004) indicating that plant male gametophytes are also sensitive to NO. Other general candidate attractants include Ca^{2+} ions, of which high concentrations have been measured in synergids and pollen tube tips and which were shown to be able to reorient pollen tube growth. The role of Ca^{2+} ions during fertilization has been discussed excellently in reviews elsewhere (Malhó et al. 2000; Dumas and Gaude 2006). Gamma-amino butyric acid (GABA), known as the most important inhibitory neurotransmitter in the nervous system of animals (Boehm et al. 2006), has been shown recently to be involved in long-range pollen tube guidance (Palanivelu et al. 2003). Until now, nothing is known in plants about the involvement of such general molecules in gamete cross-talk.

A general chemoattractant secreted by the cumulus cells in **mammals** is progesterone, whose release does not involve a cGMP-signaling pathway. Interestingly, progesterone has been identified as being a chemoattractant for both human and rabbit spermatozoa (Villanueva-Diaz et al. 1995; Jaiswal et al. 1999; Teves et al. 2006). This finding corroborates well with the lack of chemotaxis-related species-specificity previously reported for follicular fluids and conditioned media of several mammals and suggests that at least some of the mammalian sperm chemoattractants, which originate in the female genital tract, are common or very similar (Sun et al. 2003, 2005). Hence, in mammals, in contrast to marine species, sperm competition, if it exists, is limited to semen from different individuals of the same species (Gomendio et al. 1998; Eisenbach and Giojalas 2006). Bourgeonal and lyral are two ligands of G-protein-associated odorant receptors recently found to be chemoattractants for human and mouse spermatozoa and that like progesterone do not involve a cGMP-signaling pathway. However, it seems that they are not the physiological sperm chemoattractants, because they are probably not secreted in the female genital tract (Spehr et al. 2003; Fukuda et al. 2004). The chemoattractant secreted from the egg is not yet known but it is presumed to be different and more potent or more concentrated than those secreted from the cumulus cells. Thus, mammalian sperm chemotaxis in vivo seems to be a two-step process: first chemoattraction to the cumulus followed by chemoattraction to the oocyte (Sun et al. 2005). However, it is not yet known whether the cumulus cells secrete other chemoattractants besides progesterone or whether the chemoattractant secreted from the egg is species-specific or common. Sperm guidance seems to be much more elaborate than originally thought and chemotaxis seems to extend over a much longer distance. One of the reasons why there are so many chemoattractants is that spermatozoa might undergo a multistep chemotaxis as they travel through the female genital tract, and each step might sequentially guide them to the next chemoattractant source. Another reason is that different spermatozoa might respond to different chemoattractants, resulting in sperm selection. Thus, chemoattractant-specific sperm selection, if it exists in mammals, might be involved in sperm competition or might enable the female to choose sperm (Eisenbach and Giojalas 2006).

A similar multistep process seems to exist also in **flowering plants**: general molecules (see earlier) might be involved in long-range pollen tube chemotaxis that involves the secretion of specific promoting factors from the style (for review, see Higashiyama and Hamamura 2008, this issue), while short-range chemotaxis is mediated by the synergids, which are the most active secretory cells of the female gametophyte, and probably also by the other cells of the female gametophyte. The last step is likely to be controlled by the two female gametes themselves. However, there is no evidence that egg or central cell secreted molecules exist as would be required to attract either one or the other of the two sperm cells or both. Nevertheless, the synergids contain a large number of secretory vesicles and masses of rER (Diboll and Larson 1966; Mansfield et al. 1991) and have been shown to be involved in short-range and probably ovular attraction of pollen tubes (Higashiyama and Hamamura 2008, this issue). Thus, plant synergids might be functionally equivalent to mammalian cumulus cells. Several studies have been performed to demonstrate the existence of species-specific guidance signal(s) in flowering plants. Crosses of *A. thaliana*, for example, with pollen of other Brassicaceae species showed germination and growth of pollen tubes through the transmitting tissue, but not towards the female gametophyte, indicating that it secretes species-specific signaling molecule(s) (Shimizu and Okada 2000). Interspecific cross-pollination of *T. fournieri* with related species showed similar results, supporting the hypothesis that species-specific signaling of the female gametophyte exist (Higashiyama et al. 2006). Until now, only one small secreted protein (EA1) has been identified in maize that fulfils all of the criteria of a species-specific signaling

molecule, as it is secreted by the egg apparatus into the micropylar region of the ovule, is degraded after fertilization and RNA silencing experiments resulted in the loss of short-range micropylar pollen tube guidance (Márton et al. 2005; Table 3). Interestingly, Arabidopsis and other dicotyledonous plant species do not possess EA1-homologs. Homologs occurring in grass species display amino acid variation in the conserved C-terminal region of the peptide that is predicted to contain the mature peptide ligand (M. Márton and T. Dresselhaus, unpublished results) supporting the hypothesis that short-range pollen tube guidance is species-specific in plants. Biotests using the mature EA1 peptide of maize and other grasses should help in answering this question.

In **mammals**, a second active mechanism of sperm guidance exists called thermotaxis (Fig. 1b), which appears to be an essential mechanism in guiding spermatozoa released from the cooler reservoir site toward the warmer fertilization site. Bahat et al. (2003) showed that rabbit and human spermatozoa can sense small temperature differences (0.5°C and, perhaps, even lower) and respond to it by thermotaxis. The temperature difference between the site of the sperm reservoir and the fertilization site is generated at ovulation by a temperature drop at the former. Similar to mammalian chemotaxis, only capacitated spermatozoa are thermotactically responsive (Bahat et al. 2003). Several in vitro studies indicate that capacitated spermatozoa are guided from the storage site to the egg primarily by a combination of chemotaxis and thermotaxis, assisted perhaps by oviductal contractions (Cohen-Dayag et al. 1995; Bahat et al. 2003). Thus, thermotaxis appears to be a long-range guidance mechanism, in addition to chemotaxis, which seems to be short-range and likely occurs at close proximity to the oocyte and within the cumulus mass. Both mechanisms probably have a similar function—to guide capacitated, ready-to-fertilize spermatozoa towards the oocyte, and they may complement each other, meaning that each mechanism is functional in a region where the other mechanism is ineffective. The molecular mechanism of sperm thermotaxis is not yet known, but if it shares some of the chemotaxis signaling pathway, it may involve, for example, cGMP-mediated transient elevation of intracellular Ca^{2+} (Bahat and Eisenbach 2006).

It is unlikely however that thermotaxis is involved in long-range guidance in **flowering plants**. Mól et al. (2000) reported that egg cell maturation is essentially accelerated after pollination in maize, but removal of silk directly after pollination did not repress accelerated egg maturation, suggesting an electric nature of the pollen signal. Even wounding using sea sand is able to promote egg maturation (R. Mól, personal communication), indicating the potential existence of long-range signals unrelated to peptides/proteins.

In summary, cross-talk between animal gametes and plant gametophytes is very complex and involves species-specific short-range guidance molecules predominantly consisting of secreted peptides or proteins (Tables 1, 3). The nature of long-range guidance molecules is mostly unclear. Direct mechanisms of cross-talk between plant gametes are unknown to date.

Gamete interaction and fusion

The direct interactions between male and female gametes are among the most fascinating in cell biology and generally first include cell–cell adhesion and then membrane fusion between the two gametes. In **animals**, the interaction between sperm and egg follows, in general, five distinct steps: (1) sperm chemoattraction to the egg (discussed in the previous chapter), (2) the acrosome reaction, which causes acrosomal exocytosis and the release of proteolytic enzymes, (3) sperm binding to the egg extracellular envelope, which is the vitelline layer, in case of marine species, or the *zona pellucida* in case of mammals, (4) penetration of the sperm through this extracellular envelope and (5) sperm adhesion to the egg and fusion of their plasma membranes. However, in mammalian fertilization, steps (2) and (3) are reversed, and the acrosome reaction in mammals occurs upon sperm binding to the *zona pellucida* (Vacquier 1998; Gilbert 2006).

The egg envelope and extracellular matrix of animals play important roles in sperm–egg binding, induction of the sperm acrosome reaction, egg activation and the block to polyspermy. Studies with both mammalian and invertebrate species have shown that carbohydrate moieties of specific egg envelope glycoproteins bind to sperm surface proteins. In **marine invertebrates**, such as sea urchins, activation of spermatozoa triggering the acrosome reaction results from their direct contact with a highly sulfated fucose sulfate polymer in the jelly coat of the fully mature ovum (Vacquier and Moy 1997). This interaction causes adherence of numerous spermatozoa to the jelly coat, and the opening of incurrent Ca^{2+} channels in their cell membranes that permits calcium to enter the sperm head (Hirohashi and Vacquier 2002). Here, calcium-mediated fusion of the acrosomal membrane with the adjacent sperm cell membrane appears to cause acrosomal exocytosis. Interestingly, the ligand that induces the acrosome reaction in sea urchins is a pure polysaccharide, with no associated protein (Vacquier and Moy 1997). Egg jelly sulfated polysaccharides have been isolated and characterized from a number of species, and they are all species-specific inducers of the sperm acrosome reaction (Alves et al. 1997). Such species-specificity seems to be determined by the glycosidic linkage of the polymer and the pattern of

sulfation of the sugar residues (Hirohashi et al. 2002; Vilela-Silva et al. 2002; Biermann et al. 2004). Thus, the activation of the acrosome reaction in sea urchins constitutes a barrier to interspecies fertilizations (Vilela-Silva et al. 2002; Mourão 2007).

In **mammals**, the species-specificity of fertilization is thought to be determined, in large part, at the level of sperm binding to the *zona pellucida* (ZP) of the egg. The mammalian ZP is synthesized and secreted by growing oocytes, and forms an extracellular glycoprotein matrix with a variety of functions. Results obtained by many scientists suggest that species-specific gamete recognition occurs between defined carbohydrate structures of the ZP and their corresponding receptors on the sperm plasma membrane (Tulsiani et al. 1997; Wassarman et al. 2001; Shur et al. 2006). Mouse ZP is composed of three families of glycoproteins, ZP1, ZP2 and ZP3, of which only ZP3 was shown to possess sperm-binding activity with the egg coat (Bleil and Wassarman 1980a, b, 1988). Initial gamete adhesion is mediated by the sperm surface receptor, β1, 4-galactosyltransferase-I (GalT) that binds to specific oligosaccharide chains on the ZP3 (Wassarman et al. 2001; Rodeheffer and Shur 2002). Binding of ZP3 oligosaccharide chains induces aggregation of GalT, thus activating, directly or indirectly, acrosomal exocytosis (Macek et al. 1991; Gong et al. 1995). Recent studies suggest that there are at least two distinct sperm–egg binding events in mouse: a ZP3- and GalT-independent interaction responsible for gametes adhesion, followed by a ZP3- and GalT-dependent interaction that facilitates acrosomal exocytosis (Shur et al. 2006). At least two GalT-ZP3-independent receptors have been identified so far: zonadhesin, originally identified in pig, and mouse isolated SED1 (*S*ecreted protein containing a cleavable signal sequence, N-terminal Notch-like type II *E*GF repeats and C-terminal *D*iscoidin/F5/8 Complement domains). Zonadhesin is a sperm protein that binds to the ZP in a species-specific manner. It is localized in the acrosomal matrix and, thus, may mediate the binding of sperm to the egg coat during early stages of acrosomal exocytosis (Gao and Garbers 1998; Shur et al. 2006). Sperm SED1 seems to be required for the initial sperm adhesion to the egg coat, and in doing so, brings ZP3 oligosaccharides into close enough proximity to bind and aggregate its receptor on the sperm membrane (i.e. GalT among other candidate receptors). ZP3 binding activates specific heterotrimeric G-proteins in the sperm cell membrane and membrane calcium channels that culminate in calcium-mediated acrosomal exocytosis, zona penetration and oocyte activation (Shi et al. 2001; Shur et al. 2006).

The acrosome reaction is a crucial event for successful fertilization and was first discovered in sea urchins, starfish and several other **marine invertebrates** in the early 1950s. In most marine invertebrates, the acrosome reaction is characterized by two major physiological events: the fusion of the acrosomal vesicle membrane with the sperm plasma membrane (an exocytosis that causes the release or secretion of the contents of the acrosomal vesicle) and the extension of the acrosomal process (Colwin and Colwin 1963). In sea urchins, acrosomal exocytosis releases proteolytic enzymes from the acrosomal vesicle that begin to digest constituents of the jelly coat, making a path through it to the egg surface. Once the sea urchin sperm has penetrated the egg jelly and the acrosomal process of the sperm contacts the surface of the egg, the acrosomal protein bindin mediates the species-specific adhesion of sperm to egg (Vacquier and Moy 1977; Vacquier et al. 1995; Gilbert 2006). Sea urchin bindins are not related to any other proteins, but they do contain a central domain of 60 amino acids that has been conserved for more than 150 MY (Biermann 1998). The acrosomal process is formed by the pH-dependent polymerization of globular actin molecules into actin filaments. The process then extends, 1 mm from the tip of the sperm head, projects through the remains of the jelly coat towards the vitelline membrane of the egg, and is covered by the bindin-coated membrane that will finally fuse with the egg plasma membrane (Barre et al. 2003). The interaction between the plasma membranes of sperm and egg is a receptor-mediated event, with the egg receptor for bindin recognizing and binding species-specifically to sperm bindin (Kamei and Glabe 2003; Neill and Vacquier 2004). Thus, in sea urchins, species-specific recognition of gametes occurs at the levels of sperm attraction, sperm activation and sperm adhesion to the egg surface.

An acrosomal reaction seems not to exist in **flowering plants** where the synergids play the key role in double fertilization: synergids are required for short-range pollen tube attraction (see above), pollen tube growth arrest and pollen tube discharge as well as transportation of the two sperm cells towards their targets, the female gametes (see also Punwani and Drews 2008, this issue). Recently, a first key regulator of pollen tube arrest and bursting was identified in Arabidopsis as a plasma membrane-localized receptor-like serine-threonine kinase (Escobar-Restrepo et al. 2007). FERONIA (FER) belongs to the CrRLK1L-1 group of receptor-like kinases and accumulates asymmetrically in the synergid membrane at the filiform apparatus, which is the entry point of the pollen tube. A FER-dependent signaling pathway is proposed involving an unknown ligand from the pollen tube. Activated FER would cause the synergid to send another signal back to the pollen tube, inducing both growth arrest and bursting. Candidate pollen/sperm ligands have been reported by Engel et al. (2003), but none has been shown yet to be involved in pollen tube-synergid signaling.

As in animals, the actin cytoskeleton seems to play also a key role in flowering plants. In the degenerated synergid,

the actin cytoskeleton becomes reorganized during synergid cell death soon after pollen tube discharge and forms two actin coronas. The coronas extend from the middle of the degenerated synergid, one extending to the vicinity of the egg nucleus and the second towards the vicinity of the egg and central cell extending to the polar nuclei (Huang and Sheridan 1998; Huang et al. 1999; Fu et al. 2000; Ye et al. 2002). It is thought that these coronas are involved in actomyosin-mediated sperm transport to approximate male and female gametes. It is still a matter of debate whether sperm cells become activated after delivery, are attracted each by only one female gamete and fuse preferentially either with the egg or central cell. In Plumbago, which contains dimorphic sperm cells, it was shown that the mitochondria-rich sperm preferentially fertilized the central cell and the plastid-enriched sperm the egg cell (Russell 1985). Two distinguishable types of sperm cells have also been observed in tobacco (Yang et al. 2005); however, it is unclear whether they are required for preferential fertilization. Genetic studies in maize involving supernumerary B-chromosomes could not show a difference (Faure et al. 2003). Recently, a mutant of Arabidopsis known to produce pollen containing a single sperm-like cell was used to pollinate embryo sacs. All sperm-like cells fused with egg cells only (Nowack et al. 2006) indicating that either egg cell fertilization occurs first due to the shorter transport distance after delivery or due to the presence of egg-adhesion molecules at the sperm surface. However, a similar Arabidopsis mutant described by Chen et al. (2008) adds more complexity to this process as the single sperm-like cell could not discriminate either female gamete.

In **mammals**, sperm must be acrosome-reacted to penetrate the ZP and fuse with the egg (Florman and Ducibella 2006). As sperm undergo the acrosome reaction, they must remain transiently attached to the ZP prior to initiation of zona penetration. It has been shown that the binding of acrosome-reacted sperm to the ZP seems to depend on ZP2, because acrosome-reacted sperm lose their affinity for ZP3 and gain affinity for ZP2 (Bleil et al. 1988; Mortillo and Wassarman 1991). During the acrosome reaction in mammals, sperm membrane alterations occur that lead to the subsequent relocalization of some membrane proteins (e.g. the protein Izumo) into other membrane regions and are required for fusion (Inoue et al. 2005). Acrosomal exocytosis releases a variety of proteases that lyse the ZP, thus creating a hole through which the sperm can travel toward the egg (Yamagata et al. 1999; Gilbert 2006).

Once a sperm cell has undergone the acrosome reaction and has managed to travel to the egg, fusion of the sperm cell membrane with the egg cell membrane can finally proceed. In sea urchins, sperm–egg fusion causes actin polymerization in the egg to form a fertilization cone (Terasaki 1996). Since the acrosomal process is formed by polymerization of actin as well, a connection is then formed by the actin from both gametes. This connection expands the cytoplasmic bridge between the egg and the sperm, and the sperm nucleus and tail can pass through this bridge (Gilbert 2006). The fusion process, being an active process, is often mediated by specific "fusogenic" proteins. Among these, the sea urchin sperm protein bindin was suggested to play a second role as a fusogenic protein, in addition to its main role to recognize the egg (Ulrich et al. 1999).

In **mammals** only a few cell surface proteins have been identified to date in both gametes as being essential for gamete fusion (example candidates are listed in Table 2). Interestingly, no mammalian homologs of invertebrate sperm–egg fusion proteins have been discovered to date (Primakoff and Myles 2007). Tetraspanins localizing to the microvillar-rich region of the egg membrane are thought to play an important role in mammalian gamete fusion as they function primarily as organizers of networks of

Table 2 Candidate molecules mediating gamete interaction and fusion in animals

Molecule	Source	Species	Function	Reference
Sulfated polysaccharides	Egg jelly	Sea urchins	Induction of acrosome reaction	Alves et al. (1997)
Bindin	Sperm (acrosome)	Sea urchins	Species-specific egg adhesion, fusion	Vacquier and Moy (1977); Ulrich et al. (1999)
GalT/ZP3	Sperm/ZP	Mouse	Initial gamete adhesion	Wassarman et al. (2001)
SED1	Sperm surface	Mouse	Binding to the egg coat[a]	Shur et al. (2006)
Izumo	Sperm surface	Mouse	Gamete fusion[a]	Inoue et al. (2005)
DE / CRISP-1	Sperm surface	Rat	Binding to the egg surface	Cohen et al. (2000b), Ellerman et al. (2006)
Zonadhesin	Sperm surface	Pig	Binding to the egg coat[a]	Gao and Garbers (1998)
Tetraspannins	Egg membrane	Mammals	Gamete fusion[a]	Primakoff and Myles (2007)

ZP zona pellucida

[a] Not yet confirmed

Table 3 Signaling ligands and receptors involved in double fertilization of flowering plants

Ligand/receptor	Source	Species	Function	Reference
GCS1	Sperm	*A. thaliana*, lily	Gamete interaction[a]	Mori et al. (2006)
LGC1	Sperm	Lily	NK	Xu et al. (1999)
EA1	Egg apparatus	Maize	Short-range PT attraction	Márton et al. (2005)
FERONIA	Synergid	*A. thaliana*	PT arrest	Escobar-Restrepo et al. (2007)

PT pollen tube, *NK* not known

[a] Not yet confirmed

transmembrane and cytoplasmic proteins (Hemler 2003). However, until now, it is not known how exactly tetraspanins facilitate membrane fusion (Primakoff and Myles 2007). GPI-anchored proteins localized on the egg surface are additional candidates mediating sperm–egg fusion; however, a clear role in membrane fusion remains to be demonstrated (Alfieri et al. 2003). The membrane protein Izumo, which localizes to the sperm cell surface, was found to be required for sperm–egg fusion either in in-vitro assays (Okabe et al. 1988) or in knockout lines (Inoue et al. 2005). Izumo is a novel member of the Immunoglobulin super-family (IgSF) proteins, with a C-transmembrane domain, and shows a testis and sperm-specific expression pattern. Like many cell surface IgSF proteins, Izumo could act in cell–cell adhesion, but to date, no specific function for Izumo in the fusion process has been determined. One of the most-studied gamete surface proteins is the sperm-associated glycoprotein DE, a candidate molecule to mediate sperm–egg fusion. DE is known as well as CRISP-1 protein, being the first member of the Cysteine-RIch Secretory Protein (CRISP) family to be described in the rat epididymis (Cameo and Blaquier 1976; Cohen et al. 2000b). Several CRISPs with a high amino acid sequence similarity have been identified in animals, plants and fungi, but their function is still largely unknown. CRISP-1 proteins are candidates to mediate gamete fusion in the rat, mouse and human through their binding to complementary sites on the egg surface (Cohen et al. 2000a, 2001). Deletion mutants of protein DE showed that its binding site resides in an evolutionarily conserved region of the CRISP family (Ellerman et al. 2006).

In summary, our current knowledge of the key players and the molecular mechanisms mediating gamete fusion in mammals is far advanced compared with the current understanding of gamete fusion in **flowering plants** (Table 3). However, it was shown recently in *A. thaliana* that disruption of a sperm-specific gene, referred to as *Generative Cell Specific* 1 (GCS1), results in severe male sterility due to disturbed pollen tube guidance and gamete interaction (von Besser et al. 2006; Mori et al. 2006). The main role of GCS1 seems to be its involvement in sperm–egg attachment or membrane fusion as suggested by the authors (Mori et al. 2006), and its function might be similar to that of mammalian C-terminal transmembrane proteins localized on the sperm surface, like Izumo (discussed above). The encoded GCS1 protein was first identified using generative cells (precursors of the two sperm cells) isolated from *Lilium longiflorum* pollen. Molecular biological and immunological assays indicate that *GCS1* is specifically expressed in the male gamete, accumulating during late gametogenesis and is localized on the plasma membrane of generative cells and the sperm cells, probably anchored by the putative transmembrane domain identified at the C-terminus. *GCS1* homologues are present in various species, including non-angiosperms.

Another transmembrane protein, LGC1, has been reported in *Lilium* generative cells (Xu et al. 1999). LGC1 was identified as a male gamete-specific protein, which due to its male gamete surface expression may have an important function in female gamete recognition. However, no evidence for such a function has still been obtained. Transcriptomics approaches have identified a number of candidate plasma membrane localized proteins in sperm and egg cells of maize and Arabidopsis, respectively, (for review, see Dresselhaus 2006). Although we are still awaiting functional studies, a number of plant sperm and egg marker lines are now available (see also Singh et al. 2008, this issue) that will aid to identify and characterize more key players of early and late fertilization events.

Conclusions and prospects

In conclusion, early fertilization events are complex multistep processes in both animals and flowering plants requiring extensive cross-talk between the gametes and the gametophytes, respectively. Long-range attraction of male gametes/gametophytes involves thermotaxis in mammals and possibly electric signals in plants; however, the majority of known communication events are mediated by chemotaxis. Many chemoattractants have been identified during the past three decades in animals, and first chemoattractant candidates have now been discovered also in plants. Small common molecules such as NO and GABA seem to be involved in long-range attraction/guidance, whereas species-specific proteins/peptides seem to

represent the major mediators of short-range chemotaxis. Whereas marine animals use small secreted peptides directly derived from the egg cell, higher organisms including mammals and flowering plants involve helper cells such as cumulus and synergids as an additional step, probably to enhance attractant concentrations for guidance of male gametes and gametophytes, respectively. In this sense, plant synergids seem to be functionally equivalent to mammalian cumulus cells. In another sense, plant synergids are probably also involved in the prevention of polyspermy not only by blocking secretion of further attraction signals but also by gametophyte arrival induced vesicle exocytosis similar to the cortical reaction in animals (Spielman and Scott 2008, this issue). However, more detailed cellular and molecular data are required to sustain these hypotheses.

A major difference between animals and flowering plants is the absence of flagellar or independent movement in higher plant sperm cells and also, for example, the lack of an acrosome reaction. Nevertheless, male gametophytes of higher plants steadily release vesicles by exocytosis from the pollen tube tip during growth through the female tissues of the ovary (Campanoni and Blatt 2007). Interestingly, both mammalian and flowering plant male gametes/gametophytes undergo a ripening process inside the female sexual organs. Another similarity is the polymerization/reorganization of actin filaments during the fertilization process. In animals, the acrosomal reaction and sperm–egg fusion, respectively, cause actin polymerization in the egg to form a fertilization cone as a prerequisite of gamete fusion. In the degenerated synergid of flowering plants, the actin cytoskeleton becomes reorganized during cell death soon after pollen tube discharge and two actin coronas are formed that are likely to be involved in sperm transport and gamete fusion.

Marine organisms require species-specific attractants and fusogenic molecules in order to assure intra-specific fertilization. This seems to be less important in mammals and flowering plants where sperm of the same species are often either deposited inside or at the surface of female sexual organs of the same species. Additionally a number of molecular mechanisms have been evolved to inactivate incompatible male gametes and gametophytes, respectively, in order to prevent inter-specific fertilization. In invertebrate marine animals, the interaction between the plasma membranes of sperm and egg is a species-specific receptor-mediated event, and it is therefore not a surprise that mammalian homologs of invertebrate sperm–egg fusion proteins have not been discovered. Plant receptors involved in chemotaxis and gamete fusion are not known to date. However, in contrast to animals, flowering plants contain hundreds of predicted membrane bound receptor-like kinases (Shiu et al. 2004) suggesting that these processes are more complex and potentially involve more interacting molecules. Membrane guanylate cyclases that play a major role in animal sperm chemotaxis do not exist in plants (Schaap 2005) indicating once more that although principles of fertilization mechanisms in animals and flowering plants are more similar than previously thought, the precise molecular nature of the key players are completely different. The identification and functional characterization of these players remain a major challenge of plant reproduction biologists for the next decade. The identification of first candidates, already available transcriptomes of male and female gametes and gametophytes, as well as an increasing number of marker lines, will now help in identifying and studying a larger number of fertilization molecules to understand the molecular regulation of fertilization also in flowering plants.

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