

Chapter 10

Sex in Schistosomes – Signaling Mechanisms in the Female Gonads

Svenja Beckmann, Thomas Quack, Cora Burmeister, Christin Buro, Steffen Hahnel, Thavy Long, Colette Dissous, and Christoph G. Grevelding

Abstract Besides their great medical importance as causative agents of schistosomiasis, an infectious disease affecting humans and animals in tropical and subtropical regions worldwide, schistosomes exhibit distinctive biological features. Living in the blood vessels of infected hosts, these blood flukes survive permanent attacks of the immune system over many years. Furthermore, schistosomes represent the only genus of the class trematoda which live doeciously. Their most remarkable attribute, however, is the continuous pairing-contact which is both obligatory for the development of the female reproductive organs and prerequisite for egg production

Over decades great efforts have been undertaken to develop an effective vaccine against schistosomes, but without fundamental success. In addition, due to the upcoming fear for resistance against the commonly used drug praziquantel, there is a pressing need to find new drugs to fight this parasite. In this respect, the understanding of basic processes of schistosome biology, especially its reproductive development, is fundamental. Since egg production is closely associated with the clinical progression of schistosomiasis, elucidating the molecular principles of the male-induced sexual maturation of the female may lead to new strategies intervening in these processes to control parasite spread on the one hand and to limit egg-induced pathology on the other.

S. Beckmann • T. Quack • C. Burmeister • C. Buro •
S. Hahnel • C.G. Grevelding (✉)
Institute of Parasitology, Justus-Liebig-University, D-35392 Giessen, Germany
e-mail: grevelding@vetmed.uni-giessen.de

T. Long • C. Dissous
CIIL – Center for Infection and Immunity of Lille Inserm U1019, CNRS UMR 8204, F-59019
Lille Cedex, France

10.1 Schistosomiasis (Bilharzia) and Its Causative Agent

Second only to malaria, schistosomiasis represents an infectious disease of high global health priority for humans, but also for animals, and is caused by trematode parasites of the genus *Schistosoma* (Chitsulo et al. 2004; Engels et al. 2002; Quack et al. 2006; Ross et al. 2002; Steinmann et al. 2006). In honor of the German physician Theodor Bilharz, who first reported on this disease in 1851, it was initially named Bilharzia. This debilitating disease occurs in 77 countries worldwide affecting a population of about 600 million people. More than 200 million people are infected, 10% of which suffer from severe illness (Chitsulo et al. 2004; Engels et al. 2002; Mayer and Fried 2002; Ross et al. 2002; Xiaonong et al. 2002). About 85% of infected people live in sub-Saharan Africa, where the number of deaths was estimated at 200,000 per year (Engels et al. 2002). In the light of accumulating evidence from experimental and field studies for resistance against the commonly used drug Praziquantel (PZQ) (Botros et al. 2005; Doenhoff et al. 2008; Melman et al. 2009; Messerli et al. 2009; Pica-Mattoccia et al. 2009), and since no vaccine is available yet (McManus and Loukas 2008), great international research efforts have been initiated by governmental and educational institutions, industry, and WHO programs. Their aims are to create innovative ideas with the potential to be translated into safe, effective, affordable and widely utilized interventions to protect against this parasite. Among these efforts are comprehensive approaches to obtaining information on the genome, transcriptome, proteome, and the glycome of schistosomes with the aim of identifying new candidates for vaccine or drug development (Berriman et al. 2009; El-Sayed et al. 2004; Haas et al. 2007; Hokke et al. 2007a, b; Hu et al. 2004; Oliveira 2007; *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium 2009; van Hellemond et al. 2007; Wilson et al. 2007).

10.2 Biology of Male–Female Interaction

Apart from their medical importance, schistosomes exhibit fascinating biological features. Living in the circulation of their hosts, these blood flukes face and withstand immune attacks over years and even decades. Furthermore, as the only family within the trematodes, schistosomes have evolved separate sexes. A nearly unique feature, however, is the sexual maturation of the female, which requires a close and constant pairing contact with the male (Grevelding 2004; Kunz 2001; Popiel and Basch 1984). Pairing induces mitoses and differentiation processes in the female leading to the full development of the ovary and the vitellarium, which is accompanied by a remarkable increase in the size of the females (Den Hollander and Erasmus 1985; Erasmus 1973; Knobloch et al. 2002a; Kunz 2001; Popiel 1986). The development of the female gonads is a prerequisite for egg production, which finally causes the pathological consequences of the disease. About 50–70% of the eggs penetrate the walls of the veins of the intestine or the urinary bladder leaving the body for maintenance of the

life cycle. However, about 30–50% of the eggs remain inside the body of a final host being trapped mainly in the spleen and liver (Moore and Sandground 1956). This provokes hyperimmune reactions resulting in inflammatory processes and chronic progression of the disease that can be lethal (Ross et al. 2002).

As typical for trematodes, schistosome eggs consist of one oocyte produced within the ovary and 30–40 vitelline (nurse) cells synthesized within the vitellarium. Both cell types are combined within the ootype to form eggs. Viable eggs are exclusively produced by paired, mature females, but not by virgin-like, immature ones (Shaw 1987). Mitoses and differentiation processes of stem cell-like precursor cells are induced in the ovary and vitellarium of the female upon pairing (reviewed in Kunz 2001). Furthermore, the stimulus of the male is not only necessary for the initiation of the reproductive development of the female, but also for the maintenance of her mature state (Clough 1981). After separation from its partner, the female regresses to an immature state. Mitotic activity and differentiation decline, and egg production stops (Popiel et al. 1984). However, upon re-pairing with the male the female resumes normal reproductive activity. Although many experimental approaches have been performed to identify the male stimulus in the past, its nature still remains unknown. Classical studies had demonstrated that female growth and development are independent of sperm development, sperm transfer or fertilization (Armstrong 1965; Popiel et al. 1984). Tactile stimulation or chemical communications, which is the favored hypothesis, are still discussed today (Kunz 2001; LoVerde 2002). Hormones, growth factors, or lipids were also proposed as being secreted from the male to induce the initial events in female maturation (Kunz 2001; Popiel 1986; Shaw et al. 1977; Silveira et al. 1986). The gynecophoral canal protein (GCP), an 80 kDa cell surface glycoprotein which had been located in the gynecophoral canal of male schistosomes, was discussed to play a key role in the male–female interaction. In unpaired males, GCP was only found in low amounts, which significantly increased following pairing (Bostic and Strand 1996; Jin et al. 2004). In paired females, GCP was found on the entire surface, whereas GCP expression was not observed in immature females. Since the maturation of vitellarium and ovary is limited to the regions of the female lying within the gynecophoral canal of the adult male (Popiel and Basch 1984), it was speculated that this surface protein may be associated with the male-induced maturation of the female during pairing (Cheng et al. 2009). RNAi approaches knocking down GCP in adult *Schistosoma japonicum* led in vitro to an abrogation of worm pairing and in vivo, using infected mice, to an inhibition of early parasite pairing, indicating that GCP might play an important role in pairing and subsequent development (Cheng et al. 2009). Furthermore, it was speculated that the female transmits a signal to the male during pairing which is presumably mediated by a TGF β pathway within the male resulting in the expression and the release of GCP into the gynecophoric canal of male schistosomes (LoVerde et al. 2009). However, no conclusive evidence for the male signaling responsible for the induction of the female's maturation has been found yet.

Early studies on the regulation of female-specifically expressed genes had demonstrated that the male even exerts its influence at the transcriptional level of

the female. For genes expressed in the reproductive organs of paired females such as the eggshell precursor protein genes p14 (Koster et al. 1988) and p19 (Michel et al. 2003), the iron storage protein gene ferritin I (Schussler et al. 1995), and a mucin-like protein gene (Menrath et al. 1995) an influence of the male on transcription was documented. After separation from the male partner, the transcriptional activity of these genes declined, but was restored after re-pairing (Chen et al. 1992; Grevelding et al. 1997; Michel et al. 2003). Recently, male–female interaction was also studied using a microarray approach for global transcriptional analyses (Fitzpatrick et al. 2004; Fitzpatrick et al. 2005). Comparison of the transcriptomes of females at different stages of maturation showed that immature females transcribe a broader spectrum of genes than mature females. In immature females about 180 genes were found to be expressed more abundantly than in mature females. In these females about three times less genes were up-regulated after pairings, which included genes involved in egg formation or red blood cell consumption (Fitzpatrick and Hoffmann 2006). From these results it was speculated that the contact to the male streamlines the gene transcription profile of females on reproductive activities such as egg production.

10.3 Morphological Differences Between Mature and Immature *S. mansoni*

According to the physiological differences, former and recent studies have revealed significant morphological differences in the ovary and vitellarium between mature (paired) and immature (virgin-like) schistosome females (Erasmus 1973; Kunz 2001; Neves et al. 2005; Popiel et al. 1984; Popiel and Basch 1984; Shaw 1987).

In a mature schistosome female the ovary consists of oocytes at different stages of maturation – small, immature oogonial cells in the anterior part and bigger germ cells in the posterior part (Fig. 10.1a, b). The latter represent primary oocytes. They originate from mitoses of oogonial cells, accumulate cytoplasm and grow filling the center and the posterior portion of the ovary (Gresson 1964; Nollen 1997). In some trematode species, meiosis is still ongoing when the oocytes move into the oviduct. Fertilization finally induces meiotic progression. In other species meiosis is not initiated until fertilization has happened (Nollen 1997). Primary oocytes of schistosomes are hexagonal containing large amounts of cytoplasm. They are the biggest cells in the ovary but can also be found within the oviduct where fertilization takes place. Following carmine red staining and confocal laser scanning microscopy (CLSM), the big central nucleus appears dark and the nucleolus bright (Fig. 10.1a–c). Primary oocytes leave the ovary and enter into the oviduct, which forms close to the ovary the *receptaculum seminis* by local wall expansion. Here, mature sperms are stored (Fig. 10.1a) ready to fertilize primary oocytes. The oviduct finally leads to the ootype (Fig. 10.1c) which is lined in its periphery with elongated cells representing the Mehlis' glands (Neves et al. 2005). The ootype is the egg-forming organ. To make one composite egg, one fertilized oocyte and

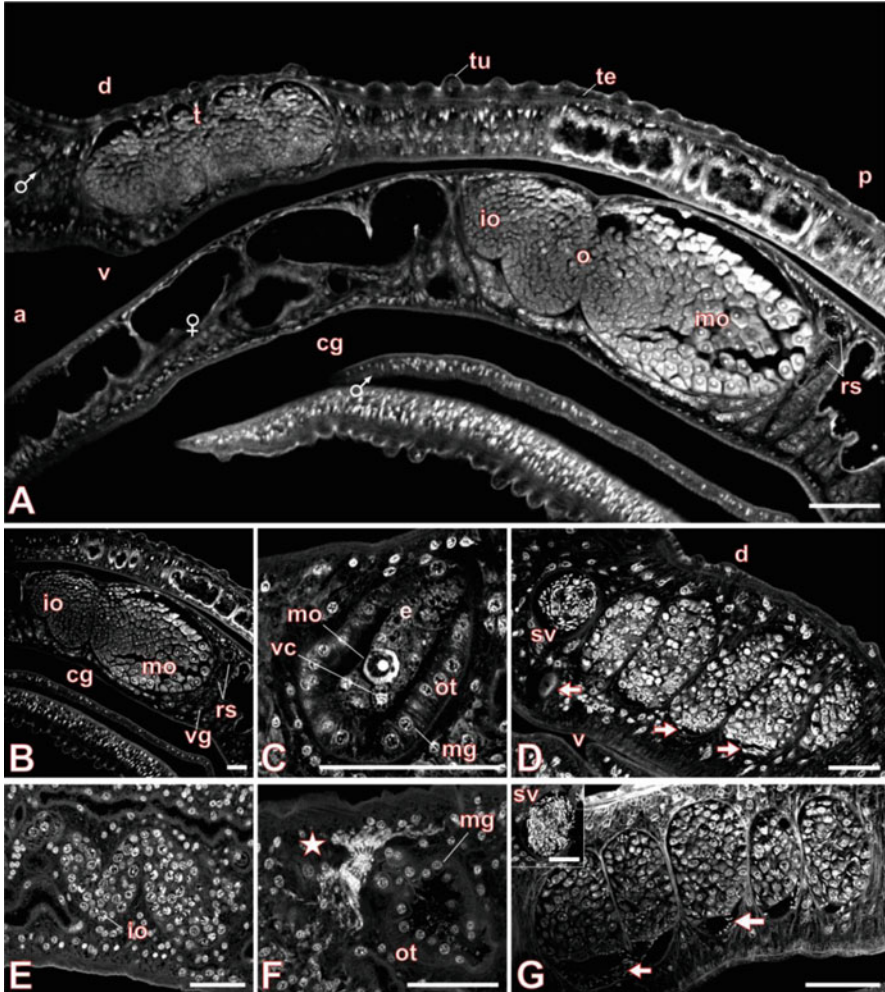


Fig. 10.1 Confocal laser scanning microscope (Leica TSC SP2) images of *S. mansoni* couples from a mixed infection (a–d), of immature *S. mansoni* females (e, f), and pairing-inexperienced puerile males (g) from unisexual infections stained with carmine red and analyzed by reflection mode. (a): Overview, (b): ovary with immature and mature oocytes, (c): ootype with egg and Mehlis’ glands, (d): testicular lobes and sperm vesicle, (e): ovary with immature oocytes, (f): ootype with Mehlis’ glands (asterisk: “hymen”), (g): testicular lobes with spermatocytes (arrows: mature sperms), sperm vesicle filled with mature sperms (Abbreviations: a anterior, c cirrus, cg canalis gynaecophorus, d dorsal, e egg, io immature oocyte, mo mature oocyte, mg Mehlis’ glands, o ovary, od oviduct, ot ootype, p posterior, rs receptaculum seminis, sv sperm vesicle, t testes, te tegument, tu tubercle, v ventral, vc vitelline cell, vg vitelline gland; scale bar (a–d): 60 μ m, scale bar (e–g): 30 μ m) (Figure modified from Beckmann et al. (2010b), with permission from Cambridge University Press)

30–40 vitellocytes are needed. These cells provide egg-shell precursor proteins for egg-shell synthesis and energy resources for the developing miracidium. Vitellocytes are delivered from the vitellarium (Fig. 10.1c) and transported to the ootype via the vitellocyte duct. After formation, the eggs are transported via the uterus to the genital pore below the ventral sucker to be discharged.

Morphology and content of the ovary of an immature female are fundamentally different from that of a mature female. In its immature state, the ovary is small containing only a low number of oocytes, which probably represent stem cell-like oogonia in an undifferentiated stage of development (Fig. 10.1e) (Erasmus 1973; Neves et al. 2005; Popiel et al. 1984). The ootype is also smaller compared to that of mature females and contains no eggs (Fig. 10.1f). During our studies we have discovered a new structure located at the posterior end of the ootype, where oviduct and vitellocyte duct end (Fig. 10.1f). To our knowledge, this filamentous network-like assembly has not been described before. According to its location this structure seems to seal the entrance into the ootype of immature females preventing undifferentiated vitellocytes or oocytes from entering. In the biological sense this filamentous structure may fulfill the role of a hymen (Beckmann et al. 2010b), which corresponds to the observation that there is no egg production in immature females. Since we have never seen this structure in mature females, even if they were separated for 7 days from males, it is tempting to speculate that the hymen disintegrates as a direct consequence of pairing. It may be alternatively possible that the removal of this structure occurs indirectly by contact with mature vitellocytes and/or oocytes. In mature but separated females, undifferentiated vitellocytes and oocytes are able to enter the ootype since the hymen was removed during a former pairing contact. This leads to the production of abnormal eggs, as observed in earlier studies (Neves et al. 2005; Shaw and Erasmus 1981).

In contrast to females there are no obvious morphological differences between paired and puerile males, which have never been in contact with a female before. Testes of schistosome males consist of several testicular lobes enclosed by a thin albuginea-like capsule, containing germ cells at different stages of maturation. Spermatogenesis occurs from the dorsal to the ventral part in each lobe. In the dorsal part of the lobes, big, round spermatogonial cells are located (Fig. 10.1a, d). They are mitotically active and become primary spermatocytes, which enter meiosis differentiating via primary and secondary spermatocytes (meiosis I) to spermatids (meiosis II). These round cells elongate to become fully differentiated spermatozoa, which are visible in the ventral part of the testicular lobes (Fig. 10.1d). Via the *vas deferens* they move to the seminal vesicle (Machado-Silva et al. 1998), which is anterior of the testicular lobes (Fig. 10.1d). This vesicle is connected to the ejaculatory duct ending in a cirrus posterior of the ventral sucker. From here spermatozoa are continuously released into the gynecophoric canal during pairing (Kitajima et al. 1976).

Compared to pairing-experienced males, puerile males have the same number of testicular lobes containing spermatocytes in different stages of maturation and

a filled sperm vesicle (Fig. 10.1g). Furthermore, the production of sperm seems to occur at a similar level, although Neves et al. (2005) observed a slight size reduction of the testicular lobes of puerile males compared to their experienced counterparts.

10.4 Molecular Aspects of Male–Female Interaction

What we know today with respect to the male–female interaction of schistosomes is:

1. As confirmed by physiological, biochemical and molecular studies, the male influences the sexual development of the schistosome female, probably via transcriptional regulation.
2. Following pairing, mitoses and differentiation processes are initiated in the female.
3. Conserved molecules with functions during signal transduction processes have been identified and characterized in *S. mansoni*. Since homologues of these molecules are known to control mitoses and differentiation processes in other organisms, and since the expression of most of the signaling molecules of *S. mansoni* has been documented in the female reproductive organs, they may contribute to processes involved in male-induced differentiation processes in the female.

If the male factor is indeed of chemical nature, one possibility for its action could be that it diffuses or is actively transported as a ligand across the female tegument and through the parenchyma to find its way to target tissues. Alternatively, the male factor could bind to a surface receptor of the female inducing subsequent signaling events by second messengers (Kunz 2001).

Using molecular methods, various signal transduction molecules have been identified by homology-based cloning strategies or by *in silico* identification. Among those are kinases localized in reproductive organs of the female for example serine-threonine kinases of the transforming growth factor β receptor (TGF β) family (Davies et al. 1998; Forrester et al. 2004), tyrosine kinases of the epidermal growth factor receptor (EGFR) family (Ramachandran et al. 1996), further receptor tyrosine kinases (RTKs) (Vicogne et al. 2003), and cytoplasmatic tyrosine kinases (CTKs) of the Src or Syk families (Beckmann et al. 2010a; Kapp et al. 2001, 2004; Knobloch et al. 2002b). To characterize these molecules in more detail a combination of novel techniques was applied. These included the identification of interaction partners by yeast two/three-hybrid (Y2/3H) analyses, the *in vitro* culture of adult schistosomes and their treatment with chemical inhibitors to block specific enzyme activity, the use of RNAi to silence gene function post-transcriptionally, and CLSM to examine the morphology of adult worms after inhibitor or RNAi treatment. Results of these approaches led us to propose first models of protein networks active in the reproductive organs of the female regulating mitoses and

differentiation processes. Some of these networks are also active in the testes of the male, where they probably exert similar roles as in the female gonads.

10.5 Signaling Cascades in the Vitellarium

The TGF β -signaling cascade is known to play a key role in cell growth and proliferation (Krauss 2008; Massague 1998) and is initiated by transmembrane serine/threonine kinase receptors from type I (T β RI) and II (T β RII). TGF β -ligand binding leads to the formation of a heterodimer composed of a type I and II receptor. This is a prerequisite for further signaling events, which include the activation of Smad proteins. These cytoplasmatic proteins enter the nucleus to regulate the transcription of specific genes in response to the ligand (Derynck et al. 1998). All pivotal TGF β -pathway members like T β RI, T β RII, R-Smad, Co-Smad as well as the T β RI-regulating protein FKBP12 have been identified in schistosomes (Beall et al. 2000; Davies et al. 1998; Knobloch et al. 2004, 2007; Osman et al. 2001, 2004, 2006) so that the TGF β /Smad pathway is one of the best characterized signaling pathways in schistosomes. The expression of the TGF β -pathway members has been detected by in situ-hybridization and/or immunolocalization among other tissues in the vitellarium of *S. mansoni* females suggesting a role in vitellarium development (Fig. 10.2).

To unravel this postulated role of the TGF β pathway, in vitro-cultured schistosome couples were treated with the synthetic TGF β RI kinase inhibitor (TRIKI; Sawyer et al. 2003). TRIKI led to a significant reduction of mitogenic activity in females (over 46%), and in correspondence with its antiproliferative activity also to the decrease of egg production (over 28%) (Knobloch et al. 2007).

A similar approach with the Src-kinase-specific inhibitor Herbimycin A also led to a significant reduction of mitogenic activity (down to 27%) as well as egg production (down to 14%) in female schistosomes, indicating that a TGF β pathway as well as a Src-kinase pathway are presumably cooperatively involved in the regulation of cell proliferation in the vitellarium. In correspondence to this hypothesis, the combination of both inhibitors resulted in a higher reduction of mitogenic activity and egg production as with one of the inhibitors alone (Knobloch et al. 2007). At the morphological level, Herbimycin A treatment of adult schistosomes led to a porous structure of the vitellarium of the female, presumably caused by a reduced number of vitelline cells. Additionally, in the ovary the number of big mature oocytes was increased and in the testicular lobes of the male, the number of mature sperms was reduced (Beckmann et al. 2010b). From these results it was concluded that Herbimycin A influences mitogenic activity of cells in the gonads of both sexes.

Besides receptor tyrosine kinases (RTKs), also cytoplasmic tyrosine kinases (CTKs) belong to the superfamily of protein tyrosine kinases. They are activated by transmembrane receptors and transduce signals from the cell surface to a number of different downstream acting molecules, thereby regulating cytoskeleton

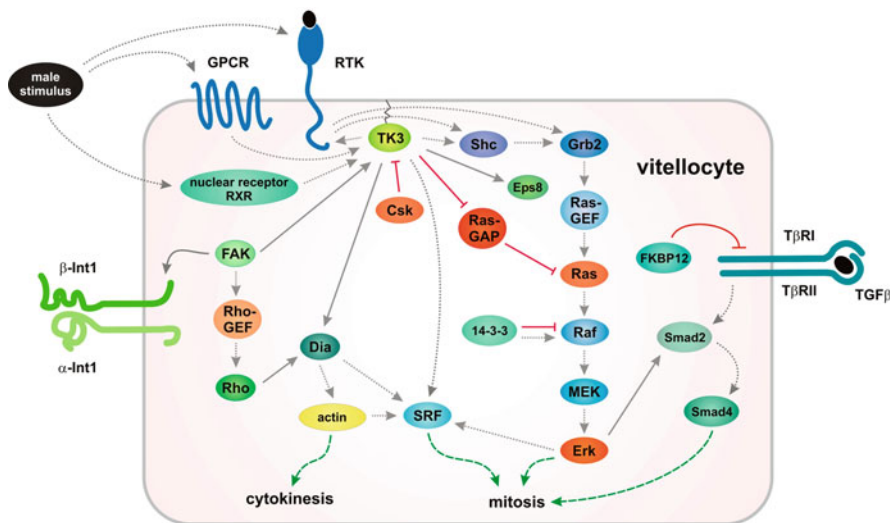


Fig. 10.2 Schematic model of signaling cascades regulating cytoskeletal rearrangements and mitogenic activity in vitellocytes. [Green: cloned molecules localized to vitellocytes; red: cloned molecules more abundantly expressed in mature than in immature females; yellow: cloned molecules not yet localized in vitellocytes; blue: not completely cloned molecules with homologues in the schistosome genome databases; black: molecules not cloned and not identified *in silico* yet; gray continuous lines: direct interactions confirmed by yeast two-hybrid analyses; gray dotted lines: direct interactions known from other organisms, not proven for schistosomes yet; green lines: activating function; red lines: inhibitory function]

rearrangement and cell proliferation (Hubbard and Till 2000; Tatosyan and Mizenina 2000). In schistosomes, several CTKs have been identified and characterized. Among these are a Fes-like kinase (SmFes; Bahia et al. 2007), the Src kinases SmTK3 (Kapp et al. 2004) and SmTK6 (Beckmann et al. 2010a), the Src/Fyn-type kinase SmTK5 (Kapp et al. 2001; Knobloch et al. 2002b), and the Syk kinase SmTK4 (Beckmann et al. 2010a; Knobloch et al. 2002b). With exception of SmFes, transcripts of all these CTKs were localized by *in situ*-hybridizations in the gonads of both sexes including the vitellarium. Only SmTK4 was detected to be expressed exclusively in ovary and testes, but not in the vitellarium (Knobloch et al. 2002b).

Since Src kinases are known to be involved in signaling pathways regulating cell proliferation and differentiation by controlling cell-cycle progression, transcriptional activity of mitogenic genes, and cytoskeleton rearrangements (Barone and Courtneidge 1995; Frame 2002; Ishizawa and Parsons 2004; Tatosyan and Mizenina 2000; Thomas and Brugge 1997), we postulated for Src kinases like SmTK3 a key role in cellular processes during the development of the vitellarium.

To analyze such a potential function, one possibility is the elucidation of the signaling pathway(s) in which these molecules are involved. To this end, yeast two-hybrid (YTH) screenings of a *S. mansoni* adult-stage YTH-library were performed with the aim to identify signaling molecules acting up- or downstream of the Src kinase SmTK3 in a signal transduction cascade. As a potential upstream interaction

partner we identified a schistosome homologue of the epidermal growth factor receptor pathway substrate 8 (Eps8) (Beckmann et al. 2010b; Burmeister et al., unpublished). The expression of Eps8 was localized to the vitellarium, the ovary and the testes of adult schistosomes corresponding to the expression of SmTK3. Eps proteins fulfill diverse functions in signaling pathways leading to cytoskeleton remodeling as well as the proliferation and differentiation of cells. They interact with EGFRs, but also with other transmembrane receptors like integrin receptors (Calderwood et al. 2003; Fazioli et al. 1993). The interaction of Eps8 with schistosome receptors has not been analyzed yet, but it can be speculated that Eps8 may link transmembrane receptors and Src kinases (SmTK3) in signaling pathways controlling mitogenic activity and differentiation of gonadal cells of schistosomes. As a strong downstream interaction partner of SmTK3, we recently identified a schistosome homologue of diaphanous (SmDia) (Quack et al. 2009). Diaphanous proteins often interact with Rho GTPases in signaling pathways regulating cell-cycle progression and cell proliferation. We performed binding experiments of SmDia with the known schistosome Rho-GTPase homologue SmRho1 and showed that SmRho1 can bind GTP-dependently to the Rho-binding domain of SmDia (Quack et al. 2009). The expression of SmRho1 and SmDia1 was localized to the gonads of both sexes, which perfectly corresponds with the expression pattern of SmTK3. Therefore, we postulate a functional trimeric complex of SmRho1, SmTK3 and SmDia, regulating cytoskeleton rearrangement and so differentiation processes in the reproductive organs of schistosomes (Quack et al. 2009).

10.6 Signaling Cascades in the Ovary

Ontogenetically, vitelline cells and oocytes arise from common mother cells (Kunz 2001). Since they are closely related it seems feasible that similar signaling pathways regulate their proliferation and differentiation. Accordingly, most of the signaling molecules expressed in vitelline cells of the female, supposed to be involved in their proliferation and differentiation, were also found to be expressed in oocytes. Among these molecules are SmTK3, SmDia, SmRho1, SmEps8, and TGF β -pathway members. With regard to the distinct function of cells generated in vitellarium and ovary, also differences have to exist. In this respect it was not surprising to find molecules exclusively expressed in one of both organs, like the Syk kinase SmTK4 or the receptor tyrosine kinase SmVKR, which were only expressed in oocytes but not in vitellocytes (Fig. 10.3) (Knobloch et al. 2002b; Vicogne et al. 2003).

Because of the specialized function of Syk kinases in cells of the hematopoietic system of mammals, the presence of the Syk kinase SmTK4 in schistosomes, which is expressed in ovary and testes, was very surprising. To elucidate the function of SmTK4, *in vitro*-cultured worm pairs were treated with the Syk-kinase-specific inhibitor Piceatannol. This inhibitor caused significant physiological and morphological changes. First, Piceatannol reduced the egg production (down to 51%). Second, Piceatannol led to an increased number of big mature oocytes in the ovary

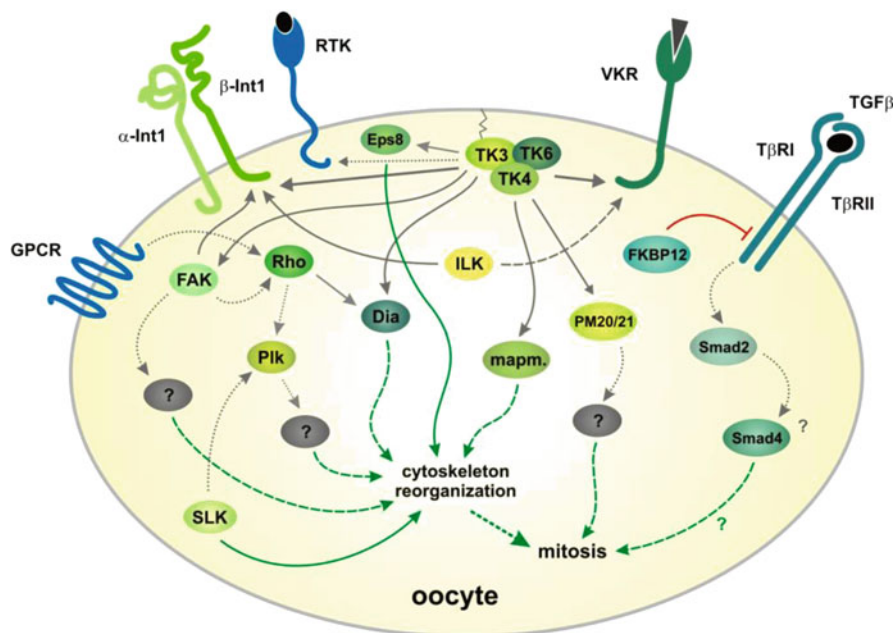


Fig. 10.3 Schematic model of signaling cascades regulating cytoskeletal rearrangements and mitogenic activity in oocytes. [Green: cloned molecules localized to oocytes; yellow: cloned molecules not yet localized in oocytes; blue: not completely cloned molecules with homologues in the schistosome genome databases; black: molecules not cloned and not identified *in silico* yet; gray continuous lines: direct interactions confirmed by yeast two-hybrid analyses; gray dotted lines: direct interactions known from other organisms, not proven for schistosomes yet; gray dashed lines: indirect interactions via adapter molecules known from other organisms; green lines: activating function; red lines: inhibitory function] (Figure modified from Beckmann et al. (2010b), with permission from Cambridge University Press)

of the female and to a lack of mature sperms in the testes of the male. These results were confirmed by SmTK4-specific RNAi approaches, which resulted in similar phenotypes (Beckmann et al. 2010a).

To further elucidate the signaling pathway in which SmTK4 is involved, we performed yeast two-hybrid screenings of a *S. mansoni* adult-stage YTH-library with the aim to identify up- or downstream interacting molecules. As strongest upstream interaction partners of SmTK4, the Src kinases SmTK6 and SmTK3 were identified. The expression of both Src kinases co-localized with the expression of SmTK4 in ovary and testes. Also for SmTK6 we showed by yeast two-hybrid analysis an interaction with SmTK3 leading to the postulation of a multi-kinase complex in cells of ovary and testes (Beckmann et al. 2010b; Beckmann et al. in preparation). From mammals it is known that transmembrane receptor-activated Src kinases can phosphorylate downstream-acting Syk kinases leading to the subsequent stimulation of downstream molecules (Geahlen 2007; Kurosaki et al. 1994). As potential downstream effectors of SmTK4 we identified a schistosome

homologue of a MAP kinase-activating protein of the PM20/21 type and a mapmodulin homologue, whose transcripts were localized in ovary and testes (Beckmann et al. 2010a). The MAPK-activating protein is supposed to activate a MAPK (mitogens-activated protein kinase) cascade regulating the proliferation and differentiation of cells in the ovary and testes, whereas the mapmodulin homologue may influence the reorganization of the microtubule-based cytoskeleton of the oocytes and spermatocytes. Taken together, the results of the inhibitor/RNAi studies and the yeast two-hybrid experiments led to the postulation of a key role for SmTK4 in oogenesis and spermatogenesis of schistosomes (Beckmann et al. 2010a).

Potential transmembrane receptors, which are able to activate the postulated kinase complex consisting of SmTK4, SmTK6 and SmTK3, are integrin receptors and/or receptor tyrosine kinases. Integrins are heterodimers composed of one α - and one β -receptor. They bind components of the extracellular matrix (ECM) or cell surface molecules to mediate cell attachment. Integrin receptors also play a role in cell signaling transmitting signals from the ECM to the cell thereby regulating cellular processes such as proliferation, differentiation, migration, cytoskeletal organization, or apoptosis (Giancotti and Ruoslahti 1999; Hynes 2002; van der Flier and Sonnenberg 2001). The intracellular domains of the integrin receptors lack catalytic activity but they can interact with a variety of intracellular signaling molecules like for example Syk and Src kinases (Arias-Salgado et al. 2003; Harrison 2003; Schlaepfer and Hunter 1998; van der Flier and Sonnenberg 2001; Woodside et al. 2002; Zaidel-Bar et al. 2007). Thus, interactions between the schistosome Syk kinase SmTK4 and the Src kinases SmTK3 and SmTK6 with integrin receptor homologues were speculated. In schistosomes, we identified and cloned one α - and one β -integrin receptor (Sm α -Int1, Sm β -Int1), whose transcripts were detected in ovary, ootype, vitellarium, and testes of adults (Beckmann et al. 2010b; Beckmann et al. unpublished). Accordingly, the expression of the integrins co-localizes with the expression of the CTKs, among other tissues, in the ovary of the female. By direct interaction studies in the yeast two-hybrid system we finally showed binding of all three kinases to the intracellular part of the β -integrin receptor. This led to the postulation of the following model. Upon ligand binding, clustering of the integrin receptors Sm α -Int1 and Sm β -Int1 and conformational changes in the intracellular domains occur, which is intracellularly accompanied by an increase in the local Src concentration. Binding to the β -integrin receptor and phosphorylations *in trans* then result in a full activation of the Src kinases SmTK3 and/or SmTK6, a prerequisite for subsequent Syk (SmTK4) activation (Beckmann et al. 2010b).

In signaling cascades downstream of integrin receptors, also focal adhesion kinases (FAKs) can play a role (Mitra and Schlaepfer 2006; Schlaepfer and Hunter 1998). The activation of FAK is facilitated by the β -integrin receptor, leading to the binding of a Src kinase to FAK and finally to Src activation. This is an alternative way of Src activation downstream of integrin receptors. A FAK homolog of schistosomes (SmFAK) was identified in our laboratory, cloned, and transcripts were localized in the ovary, ootype and vitellarium of the female (Beckmann et al. 2010b, Beckmann

et al., unpublished). Finally, we showed binding of the Src kinase SmTK3 to SmFAK as well as binding of SmFAK to the intracellular domain of Sm β -Int1 using direct interaction studies in the yeast two-hybrid system (Beckmann et al. unpublished).

It is well known that integrin receptor-induced signaling cascades “cross talk” with RTK-pathways involving Syk and Src kinases. Furthermore, it has been discussed that this co-operation potentiates downstream signaling (Bromann et al. 2004; Geahlen 2007; Giancotti and Ruoslahti 1999; Jakus et al. 2007; Wu et al. 2008). In *S. mansoni* four RTKs have been characterized (Dissous et al. 2006, 2007), among these is SmVKR1 (SmRTK1), whose expression products were detected in mature oocytes of female schistosomes (Vicogne et al. 2003). Here SmVKR1 co-localizes with the CTKs SmTK4, SmTK6, SmTK3, and the integrin receptors Sm α -Int1 and Sm β -Int1. Yeast two-hybrid analysis performed in our laboratory confirmed binding of SmTK4, SmTK6 and SmTK3 to the intracellular part of SmVKR1 (Beckmann et al. unpublished). Since the three CTKs were also able to interact with Sm β -Int1, a co-operation of the Sm β -Int1 and SmVKR1 pathway via CTKs (SmTK3, SmTK6, SmTK4) and/or SmFAK has been suggested (Beckmann et al. 2010b). Interactions between integrin receptors and RTKs may also be associated with an integrin-linked kinase (ILK; Li et al. 1999). A schistosome homologue, SmILK2, was identified and cloned in our laboratory (Beckmann et al. unpublished). Interaction studies in the yeast two-hybrid system showed binding of SmILK2 to the intracellular part of Sm β -Int1, thus also an involvement of SmILK2 in linking the integrin receptor and RTK pathways may be possible.

Beside tyrosine kinases further kinases, like Polo-like kinases (Plks) and Ste20-like kinases (SLKs) have been shown to be expressed in reproductive organs of schistosomes and were supposed to play a role in development. Plks regulate the cell-cycle and thus are essential for progression through mitosis. The schistosome homologue of Plk1, SmPlk1, was shown to be expressed in the vitellarium and the ovary of the female (Long et al. 2010). To elucidate its function, schistosome pairs were incubated with the Plk1-specific inhibitor BI 2536 (Steggmaier et al. 2007). This resulted in an increased number of big mature oocytes in the ovary of the female. Additionally, the morphology of the small immature oocytes was significantly altered exhibiting a more elongated form with a loose arrangement (Beckmann et al. 2010b; Long et al. unpublished). The inhibitor presumably led to a mitotic arrest in the oocytes and the induction of apoptosis, an effect which was described before for this inhibitor (Steggmaier et al. 2007). A similar effect of BI 2536 was observed in the testes of the schistosome male, which showed a decreased size of the testicular lobes and a reduced number of spermatocytes and mature sperms after treatment, indicating that SmPlk1 might also be involved in the mitosis of spermatogonial cells (Beckmann et al. 2010b; Long et al. unpublished).

Plks are activated by phosphorylation. A potential activating kinase is xPlkk1 (polo-like kinase), a member of the Ste20-like kinase (SLK) family (Qian et al. 1998). In schistosomes, such a kinase has been identified and characterized (Yan et al. 2007), which in correspondence with SmPlk1 is also transcribed in the ovary of the female. It could be shown that SmSLK can phosphorylate SmPlk1 and thus is supposed to be involved in the regulation of cell proliferation in schistosomes, too

(Dissous and Long unpublished). Besides this function, an involvement of SmSLK in cytoskeletal rearrangement associated with reproductive organ development and function has been hypothesized (Beckmann et al. 2010b; Dissous and Long unpublished).

10.7 Signaling Cascades in the Testes

Pairing of schistosomes seems not to influence maturation processes in the male. Thus, proliferation and differentiation of spermatocytes and thus the continuous production of mature sperms seem to be independent from the contact with a female (Armstrong 1965; Basch and Basch 1984). Accordingly, the testes of unpaired males are already fully developed, and they produce mature elongated sperms (Fig. 10.1g).

With respect to the evolutionary ancestry from hermaphroditic blood flukes, it seems conceivable that both genders still use similar signaling pathways to regulate cellular processes in the gonads. This is supported by the fact that nearly all molecules expressed in the ovary of the female were also expressed in the testes of the male, indicating a function in both genders and both gonadal cell types. Therefore, we hypothesized that signaling pathways involving the Syk kinase SmTK4 and upstream receptors like integrins and RTKs as well as further signaling molecules such as the Src kinases SmTK3 and SmTK6, are not only involved in the proliferation and differentiation of oogonial cells and oocytes in the female, but also in the proliferation of spermatogonial cells in the males (Beckmann et al. 2010b). Accordingly, the inhibitor treatment of adult schistosomes pairs with Herbimycin A, Piceatannol, and BI 2536 and thus the inhibition of Src, Syk, and Plk negatively influenced not only the differentiation of cells in the ovary, but also the proliferation of spermatogonial cells in the male, leading to a dysfunction in the production of mature sperms (Beckmann et al. 2010b).

10.8 Conclusions

With respect to the worldwide importance of schistosomes as infectious agents and the need to find new ways to fight these parasites, their unusual reproduction biology is one focus of research activities. During the last years comprehensive genome information has been generated for schistosomes (Berriman et al. 2009; *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium 2009) and research on this parasite is now at the beginning of the postgenomic era. A combinatory application of new techniques like yeast two-hybrid analysis (Beckmann et al. 2010a; Quack et al. 2009), transient transfection or RNAi (Beckmann et al. 2007; Correnti and Pearce 2004; Kines et al. 2006; Ndegwa et al. 2007; Wippensteg et al. 2002), inhibitor treatment and morphological analysis using confocal microscopy (Beckmann et al. 2010b; Beckmann et al. 2010a; Long

et al. 2010), as well as laser microdissection for tissue-specific transcriptome analyses (Gobert et al. 2009; Jones et al. 2007) now allows functional analyses of signaling molecules involved in reproductive biology of this parasite, and the elucidation of signal transduction pathways these molecules are involved in. The understanding of the cellular processes during gonad differentiation and the identification of molecules controlling these processes contribute to our knowledge about the unusual biology of this parasite, thus providing a promising starting point for the development of new strategies to fight schistosomes. In the light of first studies describing the emergence of partial resistance to Praziquantel (Botros et al. 2005; Cioli and Pica-Mattocchia 2003; Doenhoff et al. 2008; Kusel and Hagan 1999; Melman et al. 2009; Messerli et al. 2009; Pica-Mattocchia et al. 2009), and due to the fact that there is still no vaccine available ready to use, there is an urgent need for the development of alternative medication.

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