



# Cell Communication-mediated Nonself-Recognition and -Intolerance in Representative Species of the Animal Kingdom

Werner A. Mueller<sup>1</sup> · Baruch Rinkevich<sup>2</sup>

Received: 10 March 2020 / Accepted: 7 June 2020 / Published online: 22 June 2020  
© Springer Science+Business Media, LLC, part of Springer Nature 2020

## Abstract

Why has histo-incompatibility arisen in evolution and can cause self-intolerance? Compatible/incompatible reactions following natural contacts between genetically-different (allogeneic) colonies of marine organisms have inspired the conception that self–nonself discrimination has developed to reduce invasion threats by migratory foreign germ/somatic stem cells, in extreme cases resulting in conquest of the whole body by a foreign genome. Two prominent model species for allogeneic discrimination are the marine invertebrates *Hydractinia* (Cnidaria) and *Botryllus* (Ascidiacea). In *Hydractinia*, self–nonself recognition is based on polymorphic surface markers encoded by two genes (*alr1*, *alr2*), with self recognition enabled by homophilic binding of identical ALR molecules. Variable expression patterns of *alr* alleles presumably account for the first paradigm of autoaggression in an invertebrate. In *Botryllus*, self–nonself recognition is controlled by a single polymorphic gene locus (*BHF*) with hundreds of codominantly expressed alleles. Fusion occurs when both partners share at least one *BHF* allele while rejection develops when no allele is shared. Molecules involved in allorecognition frequently contain immunoglobulin or Ig-like motifs, case-by-case supplemented by additional molecules enabling homophilic interaction, while the mechanisms applied to destroy allogeneic grafts or neighbors include taxon-specific tools besides common facilities of natural immunity. The review encompasses comparison with allorecognition in mammals based on MHC-polymorphism in transplantation and following fetomaternal cell trafficking.

**Keywords** Evolutionary immunology · Allorecognition · Self–nonself discrimination · Immunological intolerance · Autoaggression

## Introduction: Questions to be Answered

Transplantation immunity in humans is based on surface-associated, highly polymorphic MHC molecules and is mediated by cytotoxic T-cells and natural killer cells (Biassoni et al. 2009; Moretta et al. 1997; Paust et al. 2010; Reyburn et al. 2006; Sawicki et al. 2001; Sykes et al. 2008). However, since transplantation in humans is not a natural

event, apart from accidental fetomaternal cell trafficking, the biological meaning and evolutionary origin of recognition of allorecognition remained enigmatic. Clearly, self recognition facilitates discrimination of parasites, pathogenic microbes and virus-infected body's own cells, but the evolutionary origin of intolerance to allogeneic tissues of the same species remains unclear. Not only vertebrates but also most invertebrate metazoans have highly effective self–nonself recognition systems to discriminate between isogeneic and allogeneic tissues, and nonself intolerance is observed even in invertebrates lacking an adaptive immune system such as corals and sponges (Kruse et al. 1999; Nicotra 2019; Rinkevich 2004b).

In the 1980s and following decades several studies put forward the hypothesis that histo-incompatibility machineries may have evolved in sedentary colonial marine species to prevent fusions between conspecifics to avoid threats of somatic and germ cell parasitism (Buss 1982, 1987; Pancer et al. 1995; Rinkevich 1992, 2002, 2005; Rinkevich and

Handling editor: Konstantinos Voskarides.

✉ Baruch Rinkevich  
buki@ocean.org.il

✉ Werner A. Mueller  
muellerwm@t-online.de

<sup>1</sup> COS (Centre for Organismal Studies), University of Heidelberg, INF 230, 69120 Heidelberg, Germany

<sup>2</sup> Israel Oceanography and Limnological Research, National Institute of Oceanography, Tel Shikmona, 31080 Haifa, Israel

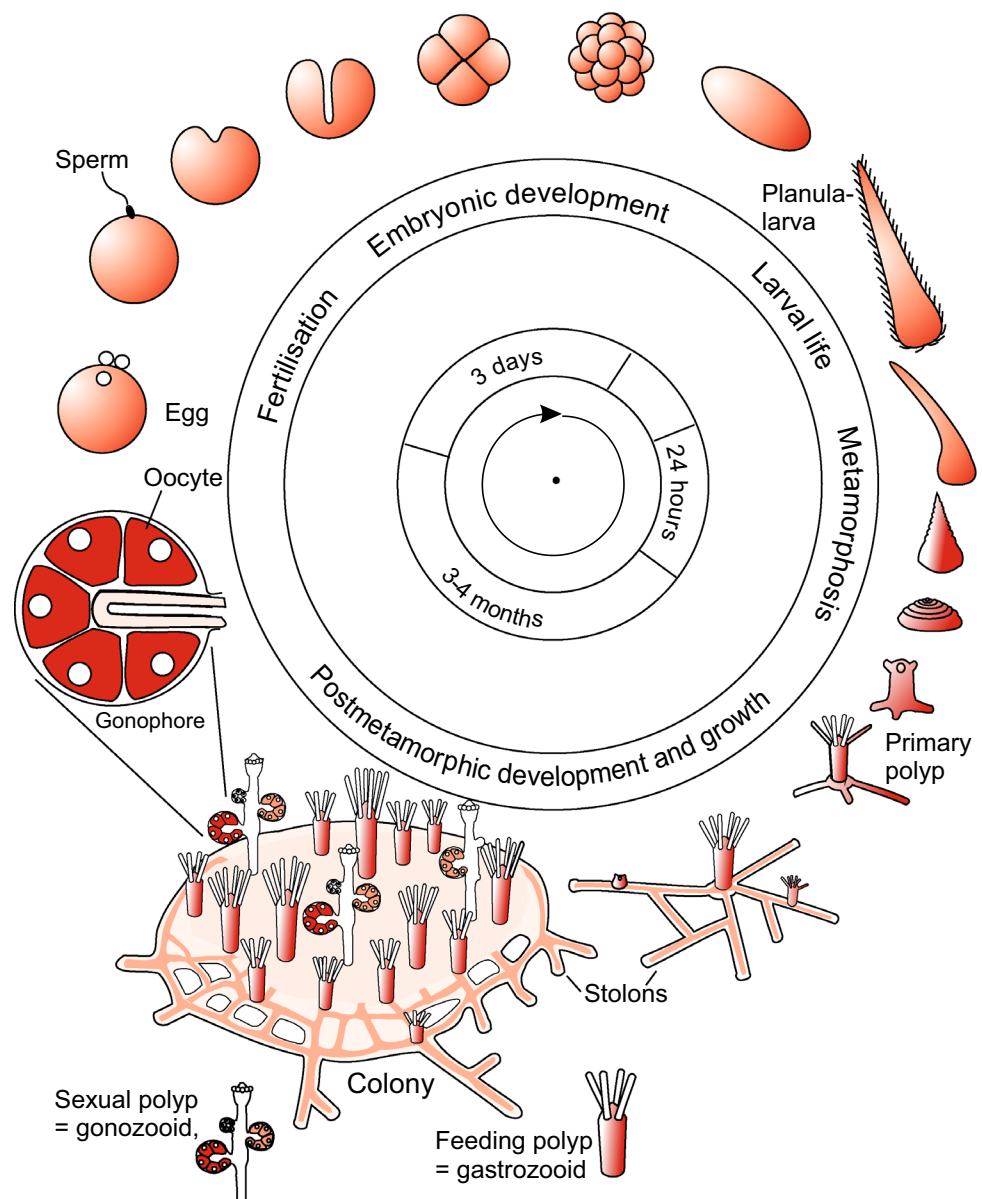
Yankelevich 2004; Simon-Blecher et al. 2004; Stoner et al. 1999; Stoner and Weismann 1996). This proposal does not reveal its reasoning at first sight. However, the studies on allorecognition of some taxa of sedentary marine invertebrates and reflection on their life history patterns disclose interesting substantiation.

Benthic sedentary invertebrates, in particular sponges, colonial ascidians and cnidarians such as sea anemones, colonial corals and hydrozoans, tend to live in close association with conspecifics and are most frequently found in dense clusters. Aggregations of genetically different individuals arise by co-settlement of genetically different planktonic larvae near conspecifics (Amar et al. 2008). Gregarious settlement occurs frequently by itself as a result of preferences of larvae for settling on hard-bottom substrates

covered by bacterial films or crustose coralline algae that provide favorable living conditions and induce settlement of the larvae and their subsequent metamorphosis into sessile individuals (Fig. 1), (Morse and Morse 1996; Mueller and Leitz 2002; Spotorno-Oliveira et al. 2015). Starting condition for isogenic societies is asexual division of a founder settler yielding communities of clones, as in the creation of large coral colonies.

Gregarious settlement, however, has its costs. Sessile colonial species will sooner or later grow into contact with adjacent allogeneic individuals and compete for food and living space. If fused together, they are subjected to the risk of being invaded and occupied by migrating foreign cells including germ cell precursors. In many cases the subsequent interactions are mediated by an array of aggressive

**Fig. 1** CV of *Hydractinia echinata*. The whole life cycle is passed through in the lab in 3–4 months and allows genetics. Spawning is induced by light in the morning, settlement and metamorphosis are naturally induced by films of certain environmental bacteria covering the substratum and can artificially be initiated by depolarizing agents such as cesium ions. From Frank et al. (2009)

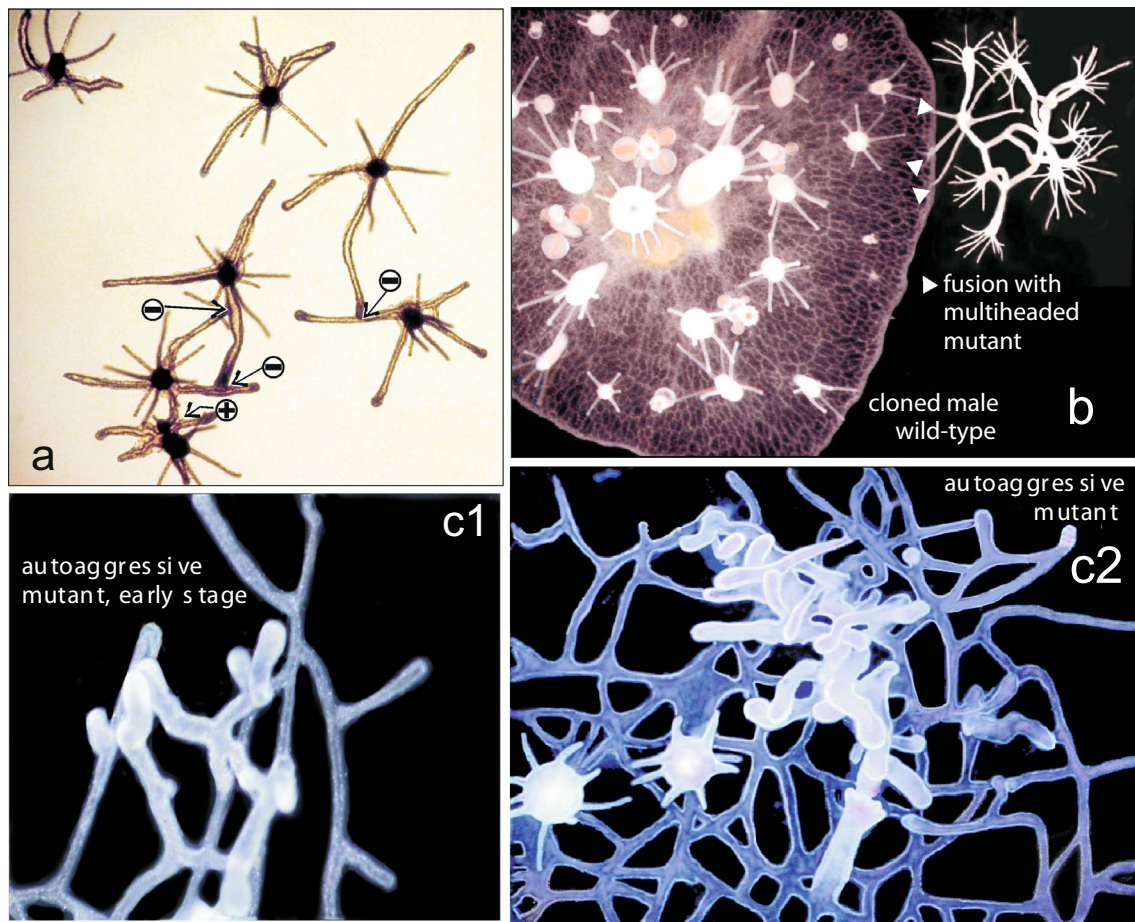


responses, primed to combat nonself conspecifics and prevent fusion with their tissues. Such interactions occur at the molecular level and require precise systems of recognition of self versus nonself upon cell–cell contacts.

## Species Apt for Molecular Analyses and Genetics

The detailed study of self versus nonself interactions in colonial marine invertebrates is only possible under controlled conditions in the laboratory, and presupposes breeding including genetic crossings over several generations. Only very few species showing incompatibility responses have successfully been bred in the laboratory over generations. These are the ascidian *Botryllus schlosseri*, a cosmopolitan, shallow temperate water species (Boyd et al. 1986; Corbo

et al. 2001; Reem et al. 2017; Rinkevich and Shapira 1988; Rinkevich and Weissman 1987a,b), the hydrozoan *Hydractinia echinata* (found along the Atlantic coast of North-Europa, reviewed in refs. Frank et al. (2001, 2020), Plickert et al. (2012), Schnitzler et al. (2016) and the sibling species *Hydractinia symbiolongicarpus* (found along the east coast of North-America, described in refs. Buss et al. (1984), Buss and Yund (1989), Frank et al. (2020). Here, results on both transatlantic sister species are included as they share basic common allorecognition features. Cloning is easily done in either species by means of explants capable of regenerating (Fig. 2a–c), so multiplication of selected strains and of unlimited numbers of isogenic (autologous) colonies can be prepared for standardized allorecognition examinations. Both species harbor stem cells (called i-cells) which in *H. echinata* were shown to be totipotent at the population level (Frank et al. 2009; Mueller et al. 2004) giving rise to soma



**Fig. 2** Lab cultures of *Hydractinia echinata*; **a** Larvae-derived primary polyps. Circles with plus point to compatible, circles with minus to incompatible contacts of growing stolons in encounters with neighbors; **b** Cloned male standard colony deprived of its stem cells in compatible contact to a sprig of a multi-headed mutant; points of fusion indicated by arrow heads; **c1** and **c2** Example of an autoag-

gressive mutant, c1 stolons of the same colony which do not fuse but begin to fight against each other by means of particular nematocytes (see Fig. 5), c2 later stage, zone with stolons striving against each other. **b, c** Hitherto unpublished photos by WAM, contrast electronically enhanced

and germ cells and are the players in the ‘drama of germ cell parasitism’ described below.

## Chimerism and Ontogeny of Histo-Incompatibility

Chimeric corals arise by fusion of coral planulae before settlement (Mizrahi et al. 2014). In soft corals co-settlement of planulae induces spontaneous allogeneic fusions in high frequencies. This is also recorded in hard corals (Jiang et al. 2015; Toh and Chou 2013), and hard corals (Barki et al. 2002). It is further recorded in botryllid ascidians (Rinkevich and Weissman 1987a,b, 1989, 1992a, b). Chimerism frequently results in the subsequent death or morphological resorption of one or more partners (Weissman et al. 1988; Rinkevich et al. 1992b). Emergence of incompatibility in the ontogenetic development has been also studied in *Hydractinia*. Genes mediating tissue incompatibility are expressed not until completion of metamorphosis of the larvae into primary polyps (Fig. 2d). Halves of embryos or larvae of any ancestry can be combined resulting in viable chimerical larvae (a feature that paved the way to the detection of neuropeptides as internal inducers and synchronizing neurohormones of metamorphosis, Fuchs et al. (2002), Plickert et al. (2003), Schwoerer-Boehning et al. (1990). Other than in vertebrates early compatibility of allogeneic tissue does not result in long-term tolerance (Poudyal et al. 2007). When chimeric embryos or larvae were constructed allogeneic parts fell apart during metamorphosis, for instance heads of polyps that arise from the posterior part of a larva separated from the allogeneic lower body that is preprogrammed by the anterior part of the larva (Lange et al. 1992). The mechanisms of rejection and destruction of allogeneic tissue come into play only after metamorphosis. Preceding undisturbed adhesion of embryonic and larval heterogeneous tissues shows that common cell adhesion molecules are not the basis of nonself discrimination.

## A Model System for Self–Nonself Discrimination and Self-Intolerance in Invertebrates: *Hydractinia*

### Genetic background of self–nonself discrimination

In *Hydractinia* compatibility means that two growing colonies fuse upon contact (Figs. 2d, 6), eventually forming a morphologically and physiologically united but genetically chimeric colony. Transplanted tissue is mutually tolerated. Compatibility is not sex-linked so a chimeric colony can consist of a male and a female area (Fig. 6). The specificity of allorecognition is conferred by genetic systems

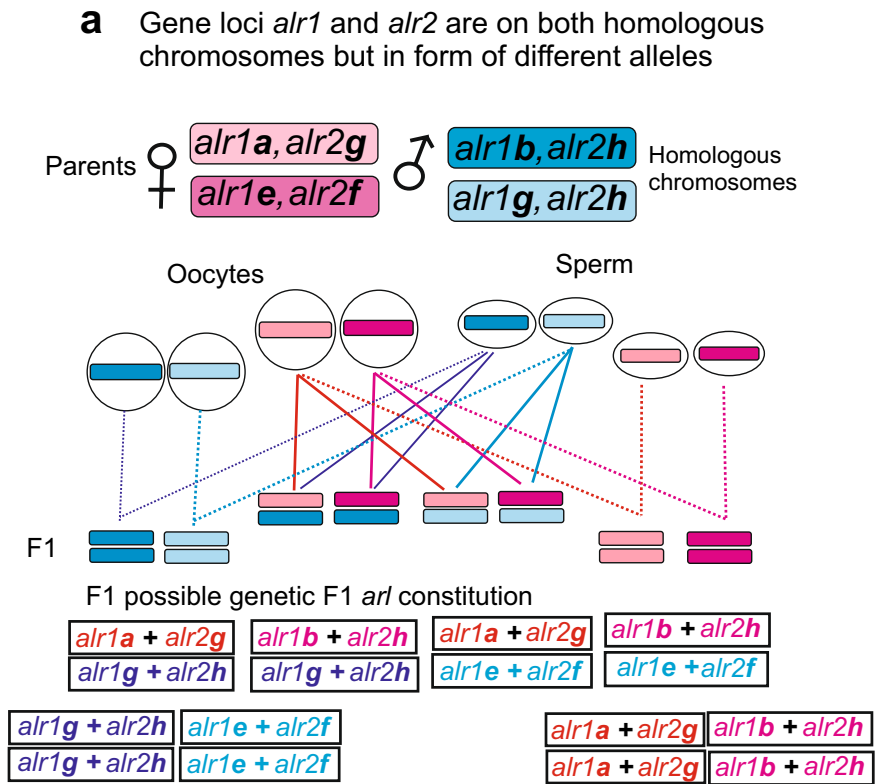
that restrict fusion to self or to a small fraction of close kin (Fig. 3). Among field-collected colonies fusion is an extremely rare event and colonies (and their subclones) do not fuse with other wild-type colonies but remain separate entities, and transplanted tissue is rejected. In *H. symbiolongicarpus* positional cloning of inbred strains over more than 12 generations have revealed two highly polymorphic loci, called *alr1* and *alr2* (Fig. 4a). Both loci are mapped to the same chromosomal region, called the allorecognition complex (ARC). Each *alr* gene encodes a transmembrane receptor protein with two or three hypervariable extracellular regions and including immunoglobulin (Ig) like domains (Cadavid et al. 2004; Grosberg and Hart 2000; Karadge et al. 2015; Mokady and Buss 1996; Nicotra 2019; Nicotra et al. 2004, 2009; Powell et al. 2007; Rosa et al. 2010; Rosengarten et al. 2011; Rosengarten and Nicotra 2011). Since in diplonts genes, here the genes of the ARC region, are present on both the maternal and paternal homologous chromosome and the *alr* genes can occur in form of alleles within an individual. Each cell can potentially have the option of generating four to eight different combinations of ALR proteins (Figs. 4, 5) depending on the degree of heterozygosity of the parents. In populations even more combinations are possible due to many different isoforms of both ARL1 and ARL2.

Colonies are incompatible if they do not share any allele at all four *alr* genes. Mismatching allorecognition alleles at only one locus can lead to transitory fusion of two colonies, followed by their gradual separation along the border of the adjacent genetic identities. Permanent compatibility presupposes perfect homophilic interactions of all ALR proteins exposed on the surface of two neighbors; mismatching and thus incompatibility results from disparate identities of the ALR proteins and, therefore, from deficient fits (Karadge et al. 2015), similar to Fig. 4c. In addition to *alr1* and *alr2*, the ARC region contains an array of additional coding sequences predicted to encode Ig-like and CAM-like proteins of still unexplored function. As many as 315 unique transcripts were predicted as the putative immune recognition repertoire of this species. The predicted sequences included a collection of at least 26 more Ig-related membrane bound proteins (He et al. 2019, Rosengarten et al. 2011, Zarate-Poles et al. 2019).

### Explanation of Unexpected Self Intolerance and Autoaggression in an Invertebrate in Comparison to Mammals

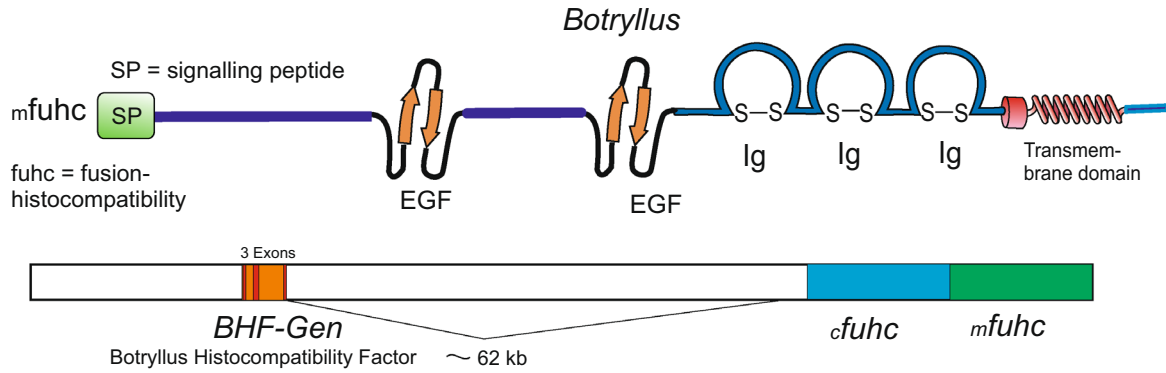
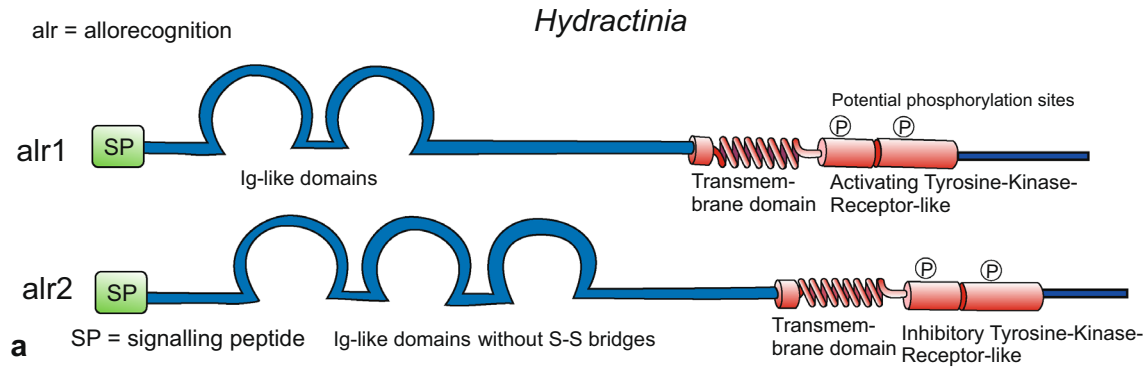
In humans autoimmune diseases based on self-intolerance are known to cause serious problems, and therefore, are intensively studied. In invertebrates only one example of self-intolerance and autoaggressive behavior has been recorded, namely the autoaggressive mutants in *Hydractinia* (Mueller 2002). The partial parallelism of the *alr* loci

**Fig. 3 a** *Hydractinia echinata* allorecognition: Options of expressed surface-exposed ARL proteins in the F1 if parents are completely heterozygous with respect to their *alr* loci as expected from most wild type colonies. Genetic constitution of parents and anticipated ARL proteins encoded by *alr1* and *alr2* alleles in the F1 assuming classic Mendelian genetics without crossover. **b** Anticipated outcome of compatibility tests among F1 siblings. + + permanently compatible; – – permanently incompatible; + – compatibility dependent on the activated chromosome; change of compatibility from plus to minus possible, e.g. by different epigenetic modifications of the alleles

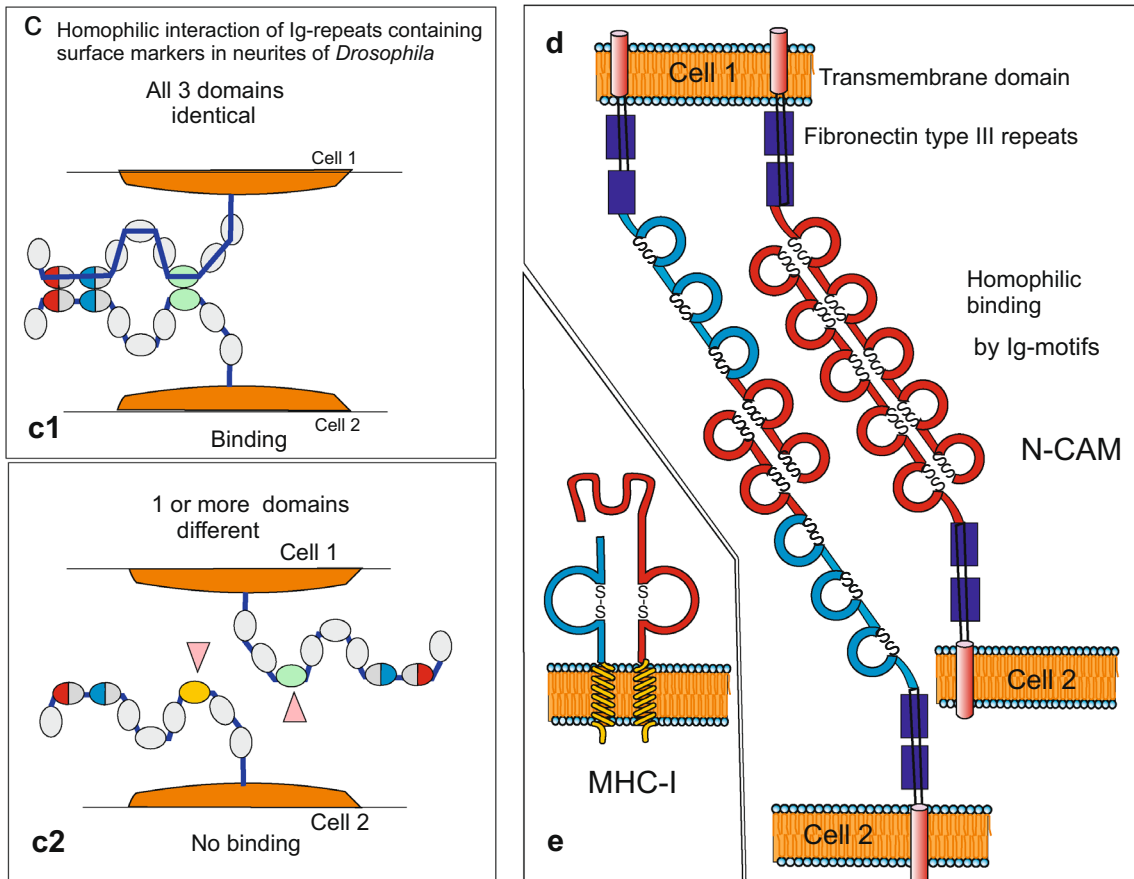


**b** Array of histocompatibility among F1 siblings

| Expressed ARL proteins        | ♀ <i>1a+2g</i> | ♀ <i>1b+2h</i> | ♀ <i>1a+2g</i> | ♀ <i>1b+2h</i> | ♀ <i>1a+2g</i> | ♀ <i>1b+2h</i> | ♀ <i>1g+2h</i> | ♀ <i>1e+2f</i> |
|-------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| sister →                      | <i>1a+2g</i>   | <i>1b+2h</i>   | <i>1a+2g</i>   | <i>1b+2h</i>   | <i>1a+2g</i>   | <i>1b+2h</i>   | <i>1g+2h</i>   | <i>1e+2f</i>   |
| brother ↓                     | <i>1a+2g</i>   | <i>1b+2h</i>   | <i>1g+2h</i>   | <i>1b+2h</i>   | <i>1e+2f</i>   | <i>1e+2f</i>   | <i>1g+2h</i>   | <i>1e+2f</i>   |
| ♂ <i>1a+2g</i> / <i>1a+2g</i> | ++             | --             | +-             | --             | +-             | --             | --             | --             |
| ♂ <i>1b+2h</i> / <i>1b+2h</i> | --             | ++             | --             | +-             | --             | +-             | --             | --             |
| ♂ <i>1a+2g</i> / <i>1g+2h</i> | +-             | --             | ++             | +-             | +-             | --             | +-             | --             |
| ♂ <i>1b+2h</i> / <i>1g+2h</i> | --             | +-             | +-             | ++             | --             | +-             | +-             | --             |
| ♂ <i>1a+2g</i> / <i>1e+2f</i> | +-             | --             | +-             | --             | ++             | +-             | --             | +-             |
| ♂ <i>1b+2h</i> / <i>1e+2f</i> | --             | +-             | --             | +-             | +-             | ++             | --             | +-             |
| ♂ <i>1g+2h</i> / <i>1g+2h</i> | --             | --             | +-             | +-             | --             | --             | ++             | --             |
| ♂ <i>1e+2f</i> / <i>1e+2f</i> | --             | --             | --             | --             | +-             | +-             | --             | ++             |



**b**



**Fig. 4** Surface markers involved in histocompatibility in the hydrozoan *Hydractinia symbiolongicarpus*. **a** *alr* genes after Rosengarten and Nicotra (2011), Nicotra (2019), figures here graphically refined as compared to the previously published figures; **b** Histocompatibility-mediating genes in the tunicate *Botryllus schlosseri*. Both *fuhc* genes, the genes for the secreted sfuhc and the membrane-bound mfuhc molecule, are involved in recognition/rejection responses but only the *BHF* gene matches perfectly with the Mendelian genetics and predicts exactly the outcome of crosses. The internal structure of the polymorphic *BHF* gene is not yet analyzed in detail but apparently it does not contain Ig-repeats. After Voskoboinik et al. (2013). **c, d** Immunoglobulins and cell adhesion molecules in developing animals used as internal recognition markers. **c** Surfaces markers Dscam1 composed of Ig-domains and exposed on neurites and their branches in the developing nervous system of *Drosophila*. Like in the *alr* system of *Hydractinia* discrimination of self versus non-self is based on homophilic interaction of surface markers. Only if all alleles encoding the subunits are identical will neurites recognize each other as being branches of the same neuron. After Hattori et al. (2009). **d** Examples of cell adhesion molecules of the N-CAM class. Proposed models suggest that homophilic binding between two N-CAM molecules occurs via adhesion of two or more antiparallel Ig domains. For more proposed models see Colombo and Meldolesi (2015), Johnson et al. (2004); **e** Heterodimeric MHC-I which mediates self recognition in the adaptive immune system of jawed vertebrates

with sequences coding for the immunoglobulin-superfamily (IgSF) in vertebrates encourages the following hypothesis: Both *alr* genes code for membrane-associated molecules expressed on the surface of somatic cells including the surface of the stolon compartment. They even may constitute two moieties of a dimeric or multimeric molecule similar to most vertebrate members of immunoglobulin superfamily such as the major histocompatibility complex MHC, the B-cell and T-Cell receptors BCR and TCR, and several Ig-repeats containing cell adhesion molecules anchored in the cell membranes of adjacent cells (Fig. 4d). In vertebrates the dominant MHC (Figs. 4e, 8) which mediates self versus non-self recognition is coded by 200 genes with varying exons, and is supplemented by other genes outside the complex (Candon and Margulies 2004; Hirano et al. 2011; Petersdorf and O’huigin 2019). In parallel it is expected that also in *Hydractinia* more than two genes are involved in recognition of the genetic identity of neighbors (He et al. 2019; Rosengarten and Nicotra 2011; Zarate-Poles et al. 2019).

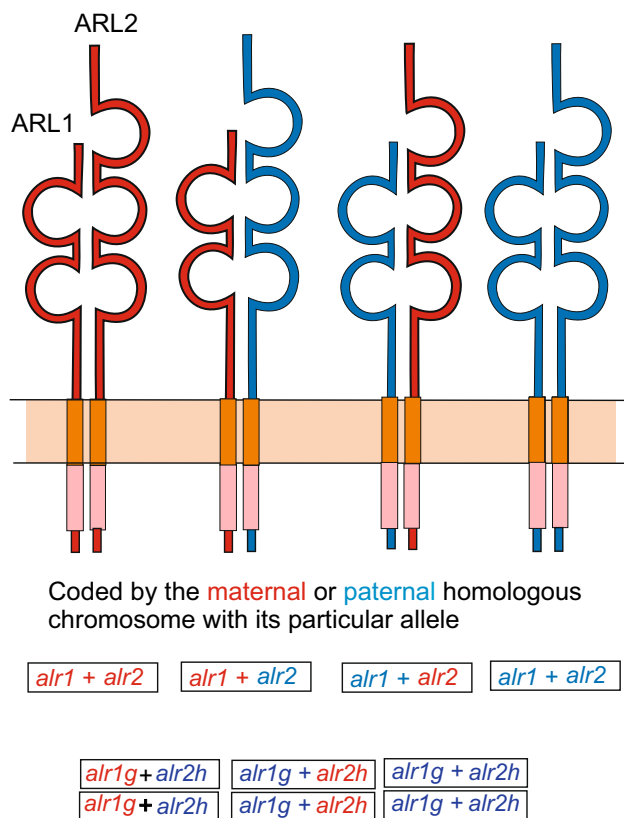
Regardless of possible intricacies by additional genes, the following interpretation focuses on the two apparently momentous *alr* genes.

Wild-type colonies of *Hydractinia* are likely heterozygous in both *alr* genes on both the maternal and the paternal chromosome. After crossing wild-type colonies each larva-derived primary polyp of the F1 has the option to produce one pair out of four to eight different pairs of transmembrane ALR proteins as their identity card ID (four if each gene is present in only one allelic version, eight if parents are heterozygous at all four *arl* loci, Figs. 4, 5), whether these are exposed on cell surfaces as separate entities or together as

paired heterodimers (Fig. 5). The situation would be simplified, and the occurrence of transitory fusion and of auto-aggressive behavior more readily understood, if each individual expressed their *alr1* + *alr2* alleles of only one of the two homologous chromosomes simultaneously in analogy to the expression of one particular combination of MHC allelic variants (HLA-combination) in various mammalian cell types (Candon and Margulies 2004; Petersdorf and O’huigin 2011) or heavy and light chain genes in each single-precursor B cell. In fact, rare observations speak against permanent codominant expression of the *alr* alleles of both homologous chromosomes: To find compatible colonies, a set of cloned male colonies was established and tested for their ability to fuse with an array of genetically akin colonies including mutants (Mueller 2002). Occasionally, large colonies being in contact with the stolons of an adjacent mutant colony at distant points X and Y, fused with the opponent at point X but did not merge but rejected it at point Y. Thus the pattern of *alr* expression can occasionally change in space and time, presumably as result of changing over of *alr* expression from one to the other homologous chromosome. Likewise, transitory fusion may be the result of such a change-over of expression. Actually, in heterozygous colonies the strength of expression of different *alr* alleles might be differently modified in the course of colonial development.

The existence of autoaggressive mutants (Fig. 2c) now finds a similar interpretation. The mutant phenotype emerges in older expanding colonies. Whereas in some areas of the growing colonies the ALR-proteins (e.g. ALR1a + ALR2d coded by the maternal chromosome) remain unchanged, in adjacent areas eventually the proteins coded by the other chromosome prevail (e.g. ALR1b + ALR2c, coded by the paternal chromosome). The change could be due to different susceptibility of different *alr* alleles to subsequent epigenetic modifications. Consequently, newly formed stolons expose surface proteins that are inapt to establish perfect homophilic contacts with neighbors that adhere to their previous expression pattern. Therefore, a locally changed expression pattern initiates aggressive attack.

The suggested instability of *alr* exposition finds its parallel in the variability of MHC exposition in vertebrates. Membrane associated ubiquitin ligases control the stability, trafficking and function of important immunoreceptors, including MHC molecules (Burr et al. 2019; Castel et al. 2018; Liu and Mintern 2019; Luo et al. 2018; Miyadera et al. 2015). Moreover, epigenetic modification of the MHC-I alleles by methyltransferases (Luo et al. 2018) or polycomb proteins (Burr et al. 2019) can reduce or terminate MHC-I expression (and thus promote cancer). Monoallelic prevalence (Vigneau et al. 2018) and changing the kind of expressed alleles with reference and relevance to autoimmune responses were recently shown for the activation of T-cells (Gutierrez-Arcelus et al. 2020).



**Fig. 5** Possible combinations of ARL proteins exposed on the cell surface. For simplicity it is assumed that the proteins form heterodimers in analogy to the vertebrate MHC, and preferentially the genes on either the maternal (red) or paternal (blue) homologous chromosome are expressed. In transitory fusion and in autoaggressive mutants expression can change from the maternal to the paternal chromosome or vice versa, again in analogy to the MHC. The actual alleles of both the *alr1* and the *alr2* genes are determined by the genetic constitution of the colonies selected for crossing

### Risk of Germ and Somatic Cell Parasitism with Subsequent Takeover of the Whole Phenotype

Since expression of the *alr* genes is independent of sex, both sisters and brothers of the F1 siblings could potentially present different or, occasionally, identical ALR proteins as their ID cards (Fig. 4). How many of the siblings display identical ALR proteins and will be compatible upon contact depends on the degree of heterozygosity of the parental colonies. If all the *alr* alleles in the parents including their oogonia or spermatogonia are different the numerical fraction of compatible pairs of F1 brothers and sisters is 12.5% (Fig. 4b).

Permanent chimeric colonies were established by bringing compatible male and female sibling colonies in close contact. In most chimeras the male sex transgressed the border of the female area. Intersexual gonophores with both female and male germ cell precursors marked the

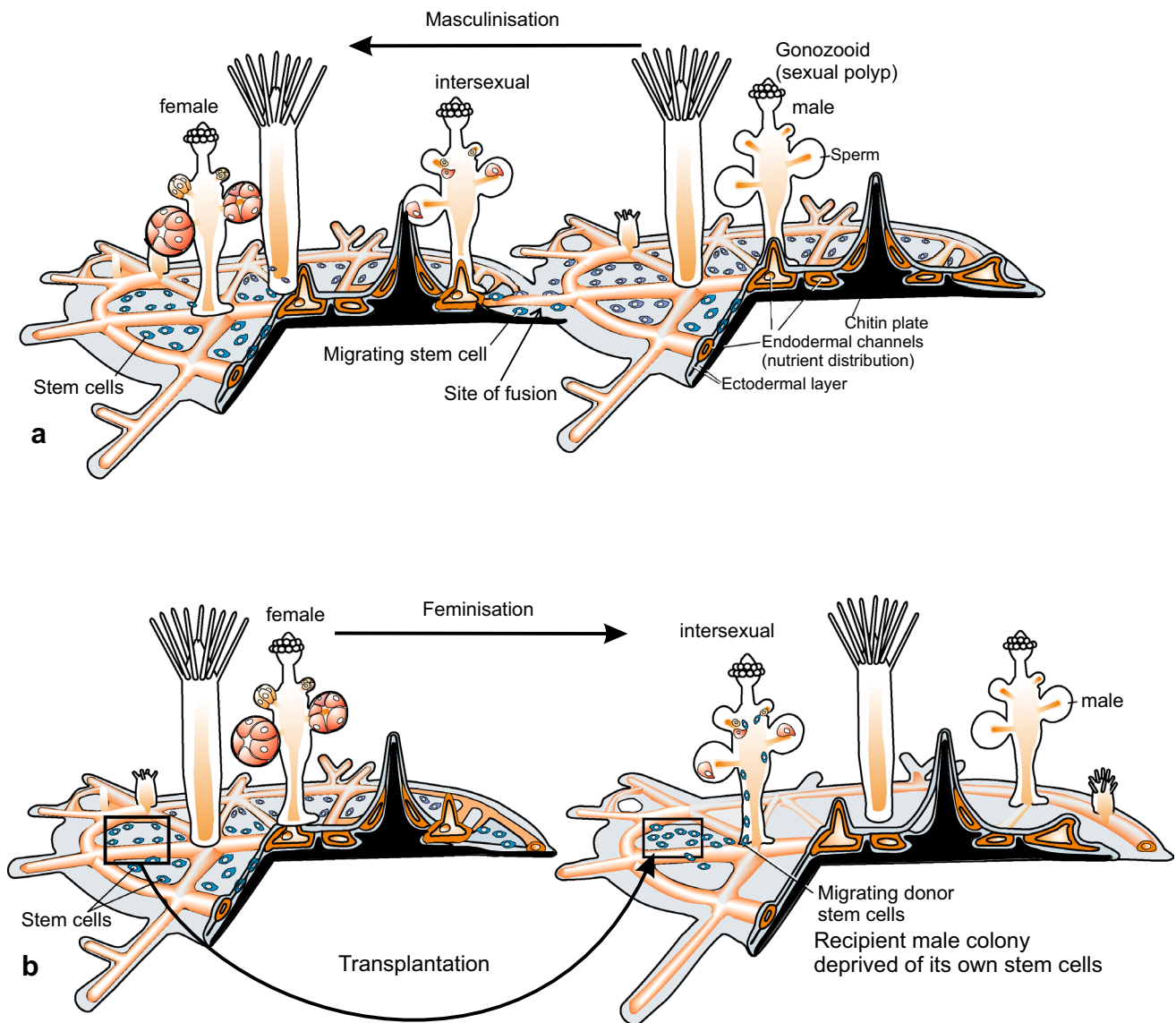
progressing frontline of the takeover of the germ cell production by the male germ line (Fig. 6). This gradual sex reversal over an intermediate phase indicates immigration of male stem cells into the female area. We interpret the dominance of the male sex as the result of sustained proliferation of spermatogonia compared to the lesser proliferating oogonia. In fact, in young intersexual gonophores increasing numbers of spermatogonia simply displaced the few large oogonia (Mueller 1964; Frank et al. 2009). Following invasion of foreign stem cells, the invaded colony produces germ cells bringing along a different genome at the expense of resident germ cells. Thus, fusion can actually lead to germ cell parasitism. Histo-incompatibility averts this imminent risk.

Invasion of male stem cells into female areas can also be initiated by transplantation of stolon tissue harboring stem cells into the stolon compartment of a female colony. The inverse transplantation leads to feminization of male colonies only if the female stem cells enumerate the male cells significantly, experimentally supported by previous deprivation of the male host colony of its own stem cells by treatment with alkylating agents such as mitomycin C (Fig. 6b; Mueller 2002; Mueller et al. 2004). Experiments of this kind demonstrated the totipotency of the invading stem cell cohort. If donor stem cells are introduced from multi-headed or autoaggressive mutants into stem cell-free host colonies (Fig. 2b,c) the recipient colonies transform gradually into colonies with multi-headed polyps or into colonies the stolons of which begin to mutually attack each other at random sites. Thus, in compatible *Hydractinia* colonies invading stem cells have the potential not only to take over the germline but to further conquer the whole soma. This applies also to *Botryllus* chimerism (Sect. 5). The gradually differentiating stem cells build an isogeneic house around the raiding patrol of germ cell precursors.

### Armed Struggle

Physical contact between two neighboring colonies is accomplished by the motile advancing tips of the peripheral, runner-like stolons (Fig. 7). Like the terminal cells of growing blood vessels in vertebrates these motile tips have path-finding functions. Not yet occupied substrata are colonized by these pioneering stolons. If the moving tip comes into contact with stolon tissue of any origin, several steps of reactions can be observed: (1) Whenever an advancing stolon tip approaches the flank of another stolon being in its way, the tip releases a factor that induces the local formation of another tip in the opposing stolon (Fig. 7a). The induced tip can potentially be the origin of a stolon branch. However, provided the neighbouring stolon tissue is part of the same colony or of its cloned progeny (isogeneic encounters) both tips move towards each other, fuse





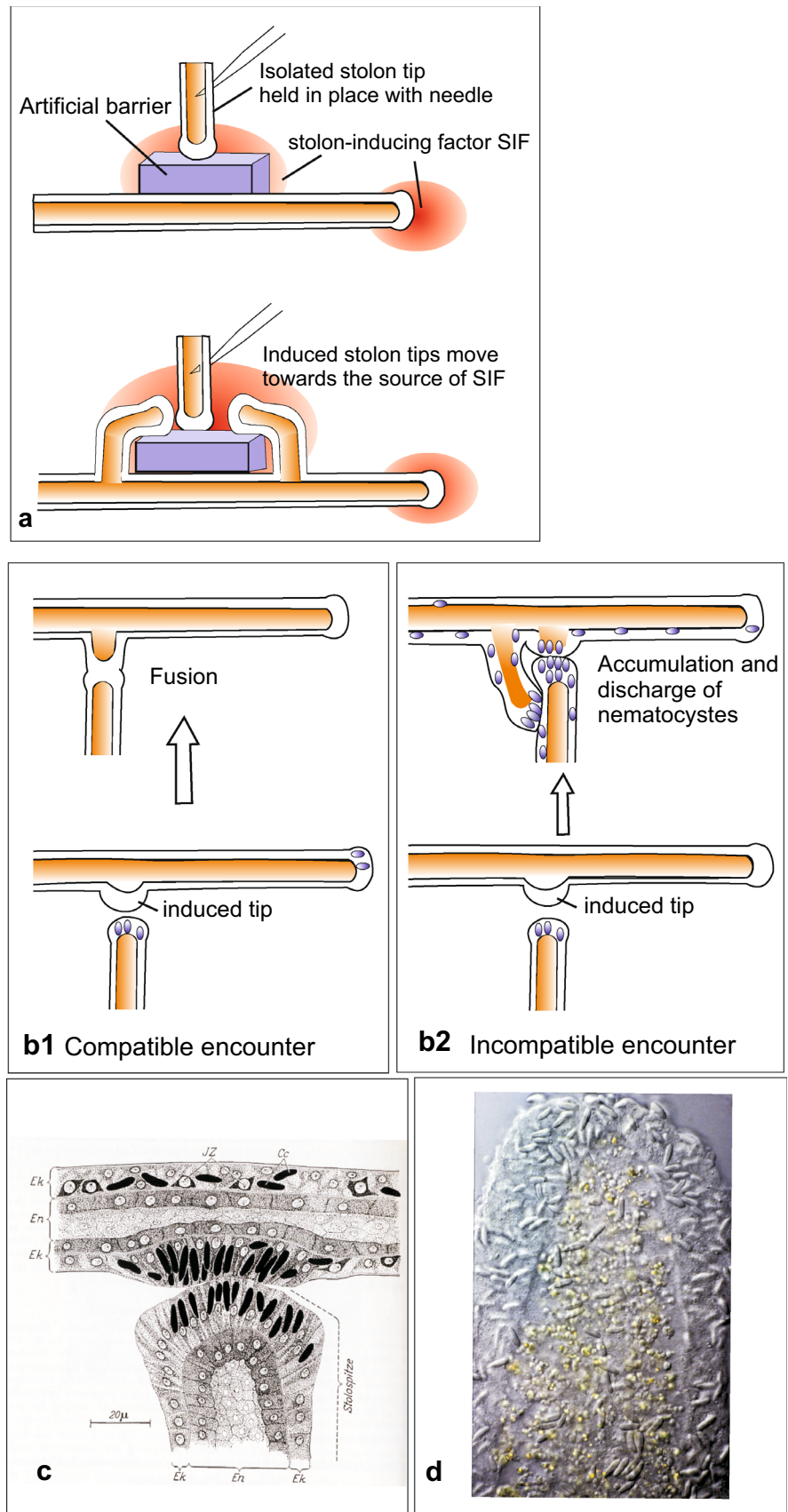
**Fig. 6** *Hydractinia echinata*—Germ cell parasitism in rare encounters of compatible male and female colonies. The male sex gradually dominates as indicated by a proceeding front of transitory intersexual polyps followed by sheer male polyps. Female germ cell precursors prevail if the male recipient is previously deprived of its own stem

cells. Likewise stem cells of mutants assert themselves in recipients freed from own stem cells and gradually replace also somatic cells and dominate the whole phenotype. After Mueller (2002), Mueller et al. (2004)

and form an anastomosis. This event is followed by subsequent complete growing together of the two colonies. (2) By contrast, incompatible tips continue to move and to induce stolon branching in the opponent. The opponent responds by doing the same mutually. Thus along the border of incompatible colonies a border fence consisting of tangled stolons is formed. In autoaggressive mutants such tangled bundles of stolons occur randomly in a colony without contact to an allogeneic neighbor (Fig. 2c). (3) In allogeneic encounters the opposing colonies mount deadly tools of defense and attack at the stolon tips and other contact sites. Fast moving stinging cells of a peculiar type (basic mastigophores)

are recruited from distant areas of the stolon compartment and migrate to, and accumulate at, contact sites (Fig. 7b,c). Here, the stinging cells turn around, directing their sensory cnidocil to the encountered neighbour, and suddenly and synchronously discharge their toxins into its tissue. In autoaggressive mutants the emerging and growing tumor-like masses of stolons kill their own colony if these masses are not removed repeatedly. Longer contact between allogeneic colonies causes both opponents to produce increasing amounts of nematocytes and to delegate them to contact sites. Mutual attacks are repeated until only one competitor for the substrate survives (Buss et al. 1984; Lange et al.

**Fig. 7** *Hydractinia echinata* ‘civil war’. In encounters of stolons the motile stolon tip approaching any established stolon induces the emergence of another tip in the flank of that stolon (a). The induced tip is similar to autonomously emerging tips which give rise to branches. In encounters of isogenic stolons the two tips fuse forming an anastomosis; subsequently compatible colonies fuse completely (b1). In incompatible encounters of allogeneic stolons the mutual induction of tips and hence of branches continues (b2) and eventually fences of tangled stolons arise (like in autoaggressive mutants Fig. 2c). At the contact sites nematocytes (microbasic mastigophores), produced especially for defense, accumulate (b2, c) and inject their toxin into the neighbors tissue. Upon prolonged contact the weaponry is strongly increased and the inflating stolons become densely filled with weapons (d). After Lange et al. (1989), Mueller (1964, 1996)



1989; Mueller 1996). The phenomenon explains why in nature substrata with *Hydractinia* colonies (usually shells inhabited by hermit crabs) are mostly covered by only one single colony. In only rare occasions field-collected colonies are found on large substrata that display fences of tangled stolons separating two settlers. Such fences are accompanied by a zone of degenerating polyps on both sides. Elimination of allogeneic competitors for living space by means of stinging cells has also been reported from sea anemones (Bigger 1980; Francis 1973).

### Xenogeneic Attacks

Stolons of *Hydractinia echinata* attack not only allogeneic conspecifics with their effector stinging cells but also other, only distantly related hydrozoan species. In interspecific encounters of stolons sprouting from colonies of *Hydractinia echinata* and *Eirene viridula* the presence of allogeneic tissue was recognized, and nematocysts were discharged, over distances of up to 50  $\mu\text{m}$  (Lange et al. 1989). Apparently, some recognition molecules, or fragments of them, are released into the surrounding seawater. Living space is limited and defended also against xenogeneic competitors if their presence is recognized.

### Colonial Ascidians: Like Vertebrates or Different?

A further model organism in the field of self/nonself recognition is the colonial ascidian *Botryllus schlosseri* (Urochordata), of the phylum Chordata (Ben-Shlomo et al. 2008; Corbo et al. 2001; Magor et al. 1999; Rinkevich 2005, 2009; Rinkevich et al. 1995, 1992; Rinkevich and Weissman 1987a, b). Since ascidians and vertebrates are members of the same phylum, in pioneering studies it was suggested that the *Botryllus* allorecognition system would be based on a MHC-like molecule (Scofield et al. 1982; Weissman et al. 1988, 1989, 1990). This expectation appeared to be further supported by the detection of NK-like cells (NK = natural killer) equipped with typical NK-receptors in the blood compartment of *Botryllus* (Khalturin et al. 2003). In vertebrates NK-cells are known to interact with MHC-I molecules exposed on the surface of somatic cells (Parham 2005; Sawicki et al. 2001). However, subsequent work could not (yet) identify dimeric MHC molecules as personal identity card in this species (Khalturin et al. 2005; Khalturin and Bosch 2006). Instead a polymorphic but monomeric molecule, first called fuhc (indicating “fusibility histocompatibility”) was suggested (Rinkevich et al. 2012; Weissman et al. 1990). High-throughput, deep coverage RNA sequencing was employed to elucidate the *Botryllus* histocompatibility determinant from a functional approach: large numbers

of expressed sequences were checked to correlate observed sequence polymorphisms with fusion/rejection outcomes. Eventually the fusibility locus in *Botryllus* was genetically identified and is now termed as *Botryllus histocompatibility factor BHF* (Voskoboynik et al. 2013, Fig. 4b). Analysis of the *B. schlosseri* genome confirmed physical linkage to chromosome 9.

Expressed BHF is a highly charged and partially unstructured protein with no known domains or a signal peptide (Voskoboynik et al. 2013). The gene produces two transcripts: (a) isoform 1 with three exons, encoding a 252-aa protein, (b) isoform 2 with two exons, exon 1 and extended exon 2, encoding a 219-aa protein (Taketa et al. 2015). Attempts to amplify *BHF* from two colonial tunicate species (*Botrylloides* sp. and *Diplosoma* sp.) and recovering highly similar sequences from both species indicated that BHF may represent a general colonial tunicate allorecognition factor. Furthermore, the *BHF*'s expression patterns during fusion or rejection were consistent with known sites of interactions and rejection reactions and were inhibited with translation-blocking morpholinos based on *BHF* (Voskoboynik et al. 2013). As in *Hydractinia* also in *Botryllus* fusion of allogeneic genotypes may lead to germ and somatic cell parasitism. The whole phenotype and genotype of the recipient adopted the feature of the invading cells (Kian Hwa et al. 2011; Pancer et al. 1995; Stoner et al. 1999; Stoner and Weissman 1996; Voskoboynik et al. 2009, 2013).

With respect to the mechanisms of destruction of allogeneic tissue a broad array of responses was described: Aggressive cells, ambiguously called morula cells, infiltrate and aggregate at contact sites and discharge their destructive vacuolar contents. These contain oxidating enzymes such as glutathione peroxidase and phenoloxidase, and toxic low molecular components (Ballarin et al. 1998, 2002; Hirose 2003; Kruse et al. 1999; Weissman et al. 1989).

### Sponges and the Construction of Body Type According to Species-specific Standards

The most ancient, still extant metazoan phylum is represented by Porifera (sponges) and consequently sponges testify to major features of the common metazoan ancestor (Mueller and Mueller 2003). Isogeneic fusion is confronted with allogeneic rejection in postmetamorphic sponges as well as in *Hydractinia* (Gaino et al. 1999). For instance, in the fresh-water sponge *Ephydatia*, a demarcation line occurred at the boundary of the paired partners (Mukai 1992). On the other hand, by bringing sibling larvae in contact stable chimeras of the marine demosponge *Tedania ignis* could be established (Maldonado 1998).

A parallelism between *Hydractinia* and some sponges is the early window of ontogeny at which incompatibility

emerges. In *Amphimedon queenslandica* genetically different individuals can fuse during early development, but, in most instances, not as adults (Gauthier and Degnan 2008).

Migratory cells are common in sponges and include sex cell precursors but since most sponges are hermaphroditic, germ cell parasitism cannot simply be ascertained on the basis of the sex of germ cells but would be recognizable only using individual genetic markers (a technique apparently not yet established in sponges). As germ cell parasitism is a real but at present unexplored risk in sponges, it has been proposed that self cell adhesion in these organisms, as in other colonial marine invertebrates, ensures integrity of genetically uniform animals (Fernandez-Busquets et al. 2009; Weissman et al. 1988). Non-integration of cells from different species ensures a body according to species-specific standards.

Sponges not only possess effective defense systems against microbes and parasites (Mueller et al. 1999) but also mount repelling systems against allografts (Mueller and Mueller 2003). In mixed aggregates of cells from different individuals putative cytotoxic cells that accumulate at the border of interacting partners have been described (Yin and Humphreys 1996). One of the molecules involved in histoincompatibility responses of sponges is the allograft inflammatory factor 1 (ALF1), a highly evolutionarily conserved protein. In the human genome the *AIF1* gene is located within a segment of the major histocompatibility complex class III region and is highly expressed in macrophages and neutrophils (Deininger et al. 2002; Utans et al. 1995). A surprising finding is that *Geodonia cydonium* is equipped with several proteins containing polymorphic Ig-like domains of the variable type, similar to those used to produce specific antibodies in the mammalian adaptive immune system (Kruse et al. 1999). However, the biological significance of these proteins in sponges is still unexplored. In spite of all efforts the actual molecules mediating self recognition in sponges have not yet been identified.

### Role of Self versus Nonself Recognition in the Embryonic Development of Individuals

Self–nonself discrimination is being employed not only in allogeneic interaction of individuals but also within an individual during development. Cells that build a distinct organ or tissue must aggregate and separate themselves from cells belonging to other organs or tissues. Thus, molecules mediating self–nonself recognition are also involved in embryonic development. As examples: In the amphibian embryos different cell adhesion molecules, including varieties that contain Ig motifs, are expressed in various developing organs (Karaulov et al. 2006).

Similarly, the proteins encoded by *Dscam* (*Down syndrome cell adhesion*) alleles in *Drosophila* are expressed in the developing nervous system with 19,008 different ectodomains, enabling self versus nonself recognition in growing and navigating neurites (Johnson et al. 2004). The proteins are composed of a series of Ig motifs and exhibit homophilic binding among each other if all expressed alleles are identical (Hattori et al. 2009, Fig. 4c). The system corresponds to the ALR system in *Hydractinia* but, unlike in *Hydractinia*, here perfect homophilic congruency causes self-avoidance of branches from the same neuron (self-branches). To ensure that repulsion is restricted to self-branches, different neurons express different sets of isoforms. *Dscam1* diversity has a profound role in wiring the fly brain.

### Mammals, Feto-maternal Cell Transfer and Nonself Tolerance

In mammals, adaptive cellular immunity develops late in gestation or after birth only. Yet, immunological tolerance is already of significance in the interaction of the fetus with its mother. The site where tolerance is demanded is the placenta, particularly when the placenta barrier is leaky. Chimeras with a true mosaic of genetic information arise when fetal and maternal cells cross the placental barrier bidirectionally. Male (XY) fetal cells that transgressed the placental barrier were identified in the mother several decades after pregnancy and maternal (XX) cells could be identified in boys (Bianchi 2007, 2012; Bianchi et al. 1996; Kallenbach et al. 2011; Khosrotehrani and Bianchi 2005). Actually, there are many cases of different types of chimerism in mammals, including humans, associated with a wide range of autoimmune diseases, cancer cases and other syndromes (Rinkevich 2001; Voskoboynik et al. 2009, 2013).

In some New World primates such as marmoset twins (small New World monkeys), fetal cells transgress the placenta barrier naturally, leading to chimerism of many tissues in the mother and their children, including germ cells (Sweeney et al. 2012). According to prevailing views long-term postnatal immune tolerance is established in the perinatal period when the adaptive immune system is activated. During this period all cells and macromolecules accessible to the awakened adaptive immune system are accepted as body's own cells or molecules. Postnatal task of the ancestral nonself recognition systems remains the identification of microbial and viral infections, of parasites and of foreign molecules that invade through wounds or the gastro-intestinal tract.

## Mammals. MHC and the Belonging to One's Own or Different Family and Gender

In the postnatal life of mammals one more task associated with self versus nonself discrimination arises. Nonself-recognition systems, like the MHC, further play a key role in social communication as found on over 20 vertebrate species so far. MHC-based communication has repeatedly found to influence individual recognition, odor preferences, mate choice and various forms of cryptic female choice that may reflect maternal reproductive strategies after mating, including mice, horses and raccoons (Chamero et al. 2012; Leinders-Zufall et al. 2014). Primarily, all these ways of behavior are governed by hormones and pheromones. How are they linked to the MHC?

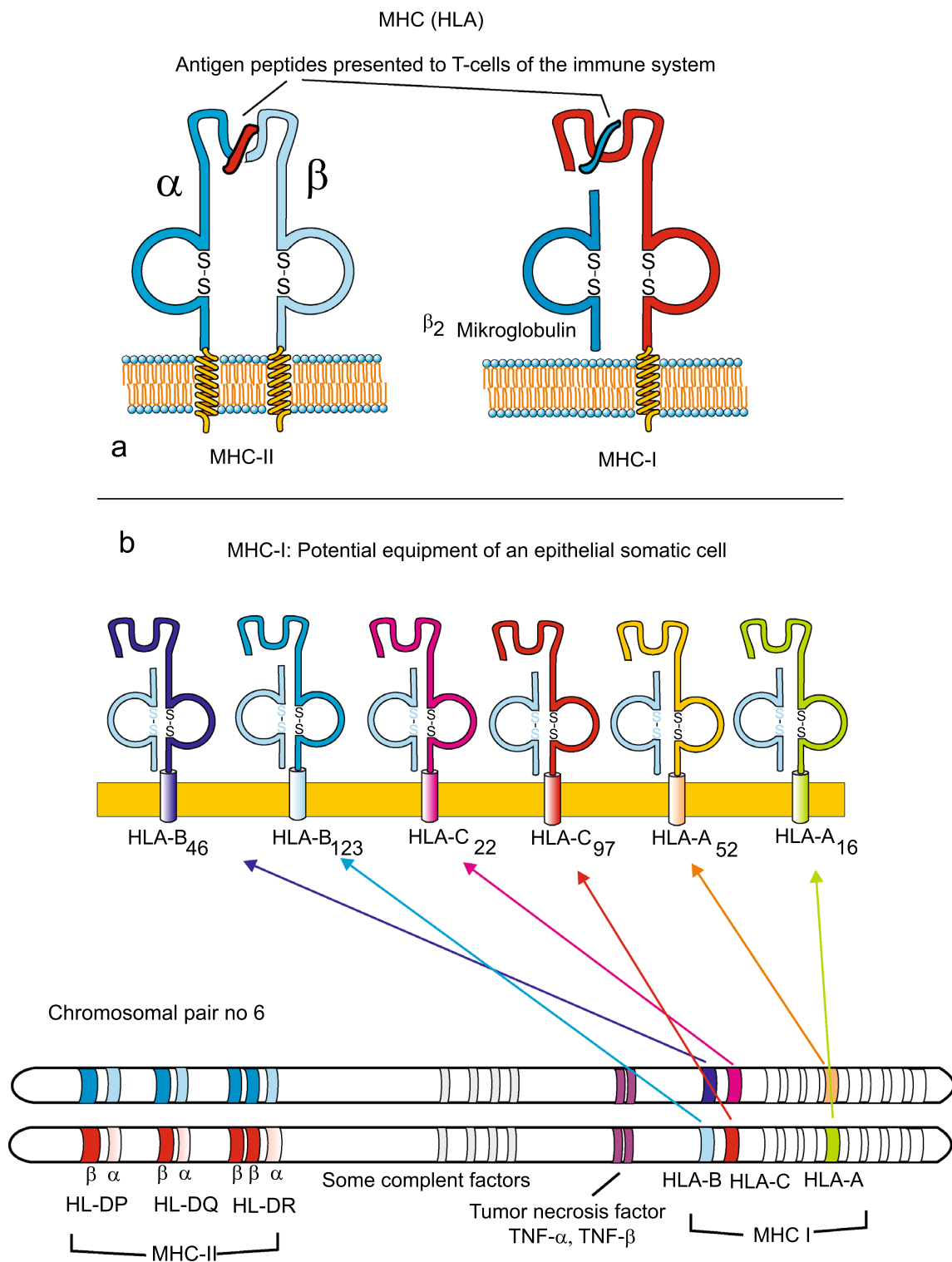
The individual MHC, in humans also known as HLA, is not a single molecule but a complex of dimeric molecules encoded by several DNA sections, in humans found on chromosome 6. There are two dominant complexes MHC-I, expressed in epithelial somatic cells, and MHC-II, expressed in cells of the immune system (Fig. 8a). MHC-I represents the individual identity card (though MHC-II is also involved in recognition and handling of foreign transplants). As mammals are diploid and the various genes/alleles potentially codominantly inherited an individual MHC-I complex can consist of up to six dimeric molecules with six transmembrane sections (Fig. 8b). Such a huge membrane-anchored complex cannot be part of soluble hormones or volatile pheromones. Therefore there must be a peculiar link between the MHC and other individual-specific molecules. Such a link has been found in mice (Chamero et al. 2012). Aerosols of the urine contain individual-specific peptides and are examined by other individuals by means of their vomeronasal organ VNO; this chemosensory organ contains sensory neurons the cell membrane of which is equipped with MHC-I molecules associated with odorant receptors (Fig. 9). In humans who lack a functional VNO such a link is still unknown and a matter of speculation.

There are analogies for allorecognition-based communication in invertebrates (Aanen et al. 2008), further involved in reproductive interactions between mates and/or gametes, in the purpose to reduce inbreeding (Grosberg and Hart 2000). One of such examples is *Botryllus* (Weissman et al. 1990; Cooper et al. 1992), where the hallmark of this recognition system, is their highly defined specificity, conferred by augmented number of alleles on the fusibility locus (Rinkevich 1992; Rinkevich et al. 1995). Watanabe (1962) was the first to propose that allorecognition elements may be involved in self-fertilization in botryllid ascidians, a suggestion that was further tested in follow-up studies (Scofield et al. 1982; Weissman et al., 1990).

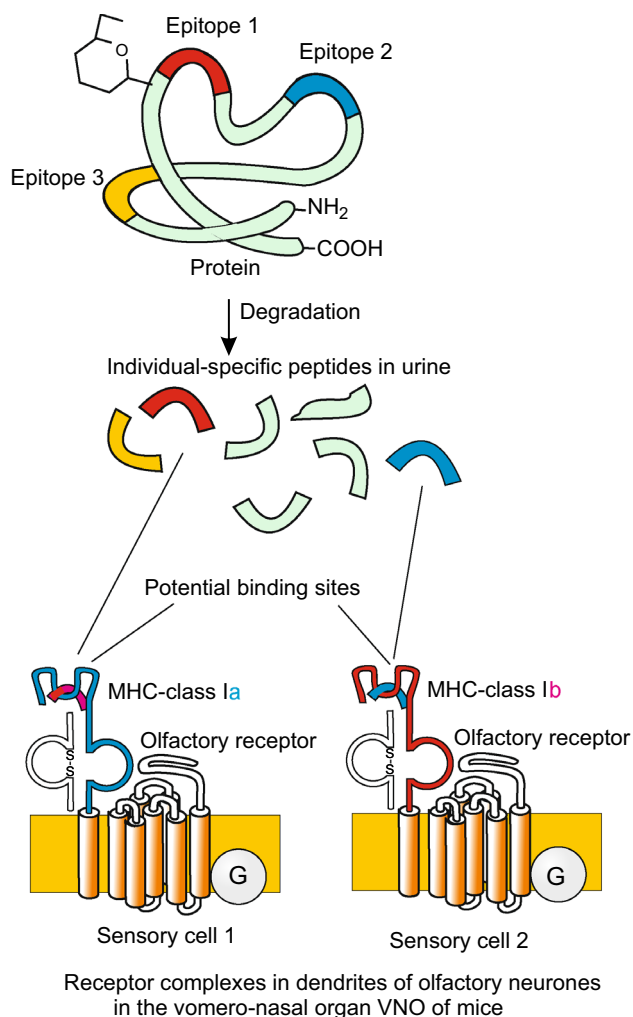
## Interaction of Nonself Identification with Innate Immunity

Several papers that were devoted to self–nonself discrimination discussed the interaction of the recognition systems with innate immunity (Ghosh et al. 2011; Miller et al. 2007; Rinkevich 2004a,b; Weis 2018; Weissman et al. 1989). Many invertebrates, in particular marine species including Cnidaria and Tunicata, are not well protected by physical barriers against invading parasites. In Hydrozoa such as *Hydractinia* the chitinous envelope of the stolons is thin and easily disrupted; polyps even lack such a physical barrier. Tunicates are suspension feeders like sponges and incorporate large quantities of microorganisms including potentially harmful bacteria. Therefore, the members of these phyla must have evolved effective methods of chemical defending like those offered by systems of natural immunity.

The scientific literature on innate immunity speaks of PAMPS (pathogen-associated molecular patterns) and of PPR (pattern recognition receptors) including TLR (Toll-like receptors), of complement like systems, of anti-microbial peptides, of lectins that bind bacterial lipopolysaccharides (LPS), and of aggressive enzymes such as phenoloxidases and chitinases. Such tools preferentially address microbial infections and have been identified in the model species more or less accidentally. The genomes of tunicates code for putative members of innate immunity including a Toll/TLR system, components of the complement system, and an LPS binding protein (Ghosh et al. 2011). By screening for genes that are differentially expressed during allorecognition in *Botryllus schlosseri*, a gene coding for a type II transmembrane protein with a C-type lectin-binding domain has been identified and was shown to be differentially regulated during allorecognition (Khalturin et al. 2003) but apparently as part of the effector mechanism and not as initial recognition element. Comparing two independently developed allogeneic rejection transcriptomes, one from the stony coral *Stylophora pistillata* and the other from the ascidian *B. schlosseri*, revealed common expression patterns of specific immune-related genes and shared functional attributes expressed during allogeneic rejection (Oren et al. 2013). The authors disclosed 74 similar blast matches in both species' libraries, accounting more than one third of the total immune-related matches, of which ca. 60% cases were exact matches. Two highly noticeable genes within this shared list of expressed genes were the immunophilins, Cyclophilin A (CypA) and the FK506-binding protein (FKBP). However, experimental proofs for the participation of these molecules in immunity are still lacking, not to speak of their putative role in the fight against allogeneic conspecifics. This applies also to the chitinases expressed in *Hydractinia echinata* and



**Fig. 8** MHC complexes and their potential composition in humans; textbook figure (Mueller, Frings, Moehrlen: Tier- und Humanphysiologie, Springer-Spektrum 2019, inscripts exchanged)



**Fig. 9** Role of MHC in the vomero-nasal organ of mice. Different MHC-I molecules derived from different alleles are associated with odorant receptors to enable reception of peptides derived from clan- or individual-specific proteins of conspecifics. Adopted from Mueller, Frings Moehrlen: Tier- und Humanphysiologie, Springer Spektrum 2019. Figure constructed after Chamero et al. (2012)

to the thrombospondin-like lectins found in its oocytes and eggs (Mali et al. 2004, 2011).

While the involvement of such molecules in allograft rejection is only sporadically reported in the literature, in nature the application of these tools is expected to be common because rejection is followed by necrosis and hence by the action of destructive enzymes, by infections and inflammation. In several instances the same molecules are employed for a wide range of different tasks such as anti-microbial molecules, as a tool against predators and as the effector mechanisms in allogeneic encounters. Yet, the notion for the customary comparisons made with the mammalian immune systems has been challenged (Rinkevich 2015). While the commonalities of allorecognition phenomena are above dispute (Loker et al. 2004) the evolutionary

basis for the effector mechanisms is not easily understood nor well illustrated. It was further stated that “stemming from the rationale that the early appearance of host defence indicates that same immune constituents are shared by most multicellular organisms; [is] sort of anthropocentrism” (Rinkevich 2011a,b) and that the tendency for combining allorecognition and host parasitic/disease phenomena, elucidates a serious obstacle for the progress in our understanding of invertebrates immunobiology, primarily that cnidarian allorecognition embraces elements that the traditional field of vertebrate immunology has never encountered, i.e., variety of cytotoxic outcomes, different types of effector mechanisms (Rinkevich 2012).

## Conclusion

Inference of the present review is the notion that the system of allorecognition, the innate immune system, and even the adaptive immune system capable of learning, originally arose from membrane-anchored cell adhesion molecules with the immunoglobulin and Ig-like superfamily as conservative, persisting representatives. Although in close-up views we are dealing with themes apparently highly diverse such as cell–cell contacts, transplantation intolerance, combat of rivals for living space, and innate immunology, viewed from a molecular perspective the different facets merge in a common large mosaic picture with cell recognition systems as a central common part. With respect to the evolutionary origin of non-self discrimination the marine invertebrates *Hydractinia* and *Botryllus* played a pioneering role. These and further species including mammals showed that recognition of nonself tissue is based on molecules related to cell adhesion molecules containing repeats of Ig- or Ig-like motifs, as shown for instance by the N-CAMs, MHCs, ALRs and FUHCs (Fig. 4), case-by-case supplemented by new surface-exposed proteins such as BHF in *Botryllus*. Variability and instability of the expression of recognition molecules such as the MHC-I in mammals due to epigenetic modification is causative for several cancer types and autoimmune diseases in mammals, and presumably also for auto-aggression in the invertebrate *Hydractinia*.

On the other hand, the mechanisms of destruction are manifold and highly diverse. Retrospect on *Hydractinia* with its use of stinging cells to kill allogeneic neighbours, on *Botryllus* with its morula cells that release destructive enzymes, and on mammals with their natural killer cells which control MHC-based identity cards of other cells, such comparative retrospect show that frequently particular cell types are entrusted with the task to fight allogeneic tissues but genetic background, morphology and physiology of these cells are highly diverse and taxon-specific.

**Acknowledgements** We thank Prof. Uri Frank, University of Galway, for proof-reading an earlier version of the manuscript and Prof. Matthew Nicotra, University of Pittsburgh, for advice to correct Fig. 5a.

## References

- Aanen DK, Debets AJ, de Visser JAG, Hoekstra RF (2008) The social evolution of somatic fusion. *BioEssays* 30:1193–1203
- Amar KO, Chadwick NE, Rinkevich B (2008) Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evol Biol* 8:126
- Ballarin L, Cima F, Sabbadin A (1998) Phenoloxidase and cytotoxicity in the compound ascidian *Botryllus schlosseri*. *Dev Comp Immunol* 22:479–492
- Ballarin L, Cima F, Floreani M, Sabbadin A (2002) Oxidative stress induces cytotoxicity during rejection reaction in the compound ascidian *Botryllus schlosseri*. *Comp Biochem Phys C* 133:411–418
- Barki Y, Gateño D, Graur D, Rinkevich B (2002) Soft-coral natural chimerism: a window in ontogeny allows the creation of entities comprised of incongruous parts. *Mar Ecol Prog Series MEPS* 231:91–99
- Ben-Shlomo R, Motro U, Paz G, Rinkevich B (2008) Pattern of settlement and natural chimerism in the colonial urochordate *Botryllus schlosseri*. *Genetica* 132:51–58
- Bianchi DW (2007) Fetomaternal cell trafficking: a story that begins with prenatal diagnosis and may end with stem cell therapy. *J Pediatr Surg* 42:12–18
- Bianchi DW (2012) Prenatal diagnostics: fetal genes in mother's blood. *Nature* 487(7407):304–305
- Bianchi DW, Zickwolf GJ, Weil S, DeMaria MA (1996) Male fetal progenitor cells persist in maternal blood as long as 27 years postpartum. *Proc Natl Acad Sci USA* 93:705
- Biassoni R, Ugolotti E, Maria De (2009) NK cell receptors and their interactions with MHC. *Curr Pharm Des* 15(28):3301–3310
- Bigger CH (1980) Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones: a recognition of self and not-self. *Biol Bull* 159:117–134
- Boyd HC, Brown SK, Harp JA, Weissman IL (1986) Growth and sexual maturation of laboratory-cultured Monterey *Botryllus schlosseri*. *Biol Bull* 170(1):91–109
- Burr ML, Sparbier CE, Chan KL, Lehner PL, Sutherland K, Dawson MA (2019) An evolutionarily conserved function of polycomb silences the MHC class I antigen presentation pathway and enables immune evasion in cancer. *Cancer Cell* 36(4):385–401e8
- Buss LW (1982) Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc Natl Acad Sci USA* 79(17):5337–5341
- Buss LW (1987) *The evolution of individuality*. Princeton University Press, Princeton
- Buss LW, McFadden CS, Keene DR (1984) Biology of hydractiniid hydroids. 2. Histocompatibility effector system competitive mechanisms mediated by nematocyst discharge. *Biol Bull* 167:139–158
- Buss LW, Yund PO (1989) A sibling species group of *Hydractinia* in the north-eastern United States. *J Mar Biol Assoc UK* 69:857–874
- Cadavid LF, Powell AE, Nicotra ML, Moreno M, Buss LW (2004) An invertebrate histocompatibility complex. *Genetics* 167(1):357–365
- Candon S, Margulies DH (2004) MHC: Structure and Function. In: Wilkes DS, Burlingham WJ (eds) *Immunobiology of organ transplantation*. Kluwer Academic/Plenum Publishing, New York, pp 29–44
- Castel SE, Cervera A, Mohammadi P, Aguet F, Reverter F, Wolman A, Guigo R, Iossifov I, Vasileva A, Lappalainen T (2018) Modified penetrance of coding variants by cis-regulatory variation contributes to disease risk. *Nat Genet* 50(9):327. <https://doi.org/10.1038/s41588-018-0192-y>
- Chamero P, Leinders-Zufall T, Zufall F (2012) From genes to social communication: molecular sensing by the vomeronasal organ. *Trends Neurosci* 35(10):597–606
- Colombo F, Meldolesi J (2015) L1-CAM and N-CAM: from adhesion proteins to pharmacological targets. *Trends Pharmacol Sci* 36(11):69–781
- Cooper EL, Rinkevich B, Uhlenbruck G, Valembos P (1992) Invertebrate immunity: another viewpoint. *Scandinavian J Immunol* 35:247–266
- Corbo JC, Di Gregorio A, Levine M (2001) The ascidian as a model organism in developmental and evolutionary biology. *Cell* 106(5):535–538
- Deininger MH, Meyermann R, Schluesener HJ (2002) The allograft inflammatory factor-1 family of proteins. *FEBS Lett* 514(2–3):115–121
- Fernandez-Busquets X, Koernig A, Bucior A (2009) Self-Recognition and Ca<sup>2+</sup>-dependent carbohydrate-carbohydrate cell adhesion provide clues to the cambrian explosion. *Molec Biol Evol* 26(11):2551
- Francis L (1973) Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol Bull* 144:73
- Frank U, Leitz T, Mueller WA (2001) My favorite model organism: *Hydractinia echinata*. *BioEssays* 23:963–971
- Frank U, Nicotra ML, Schnitzler CE (2020) The colonial cnidarian *Hydractinia*. *EvoDevo* 11:7
- Frank U, Plickert G, Mueller WA (2009) Cnidarian interstitial cells: the dawn of stem cell research. In: Rinkevich B, Matranga V (eds) *Stem cells in marine organisms*. Springer, Dordrecht
- Fuchs MA, Mokady O, Frank U (2002) The ontogeny of allorecognition in a colonial hydroid and the fate of early established chimeras. *Int J Dev Biol* 46(5):699–704
- Gaino E, Bavestrello G, Magnino G (1999) Self/non-self recognition in sponges. *Italian J Zool* 66(4):299
- Gauthier M, Degnan BM (2008) Partitioning of genetically distinct cell populations in chimeric juveniles of the sponge *Amphimedon queenslandica*. *Dev Compar Immunol* 32(11):1270–1280
- Ghosh J, Lun CM, Majeske A et al (2011) Invertebrate immune diversity. *Dev Compar Immunol* 35(9):959–974
- Grosberg RK, Hart MW (2000) Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science* 289(5487):2111–2114
- Gutierrez-Arcelus M, Baglaenko Y, Arora J, Hannes S, Luo Y, Amariuta T, Teslovich N, Rao DA, Ermann J, Jonsson AH et al (2020) Allele-specific expression changes dynamically during T cell activation in HLA and other autoimmune loci. *Nat Genet* 52:247–253
- Hattori D, Chen Y, Matthews BJ, Salwinski L, Sabatti C, Grueber WB, Zipursky SL (2009) Robust discrimination between self and non-self neurites requires thousands of Dscam1 isoforms. *Nature* 461:644–648
- He S, Grasis JA, Nicotra ML, Juliano CE, Schnitzler CE (2019) Cnidofest 2018: the future is bright for cnidarian research. *EvoDevo* 10:20. <https://doi.org/10.1186/s13227-019-0134-5>
- Hirano M, Das S, Guo P, Cooper MD (2011) The evolution of adaptive immunity in vertebrates. *Adv Immunol* 109:125
- Hirose E (2003) Colonial allorecognition, hemolytic rejection, and viviparity in botryllid ascidians. *Zool Sci* 20:387–394
- Jiang L, Xin-Min L, Sheng L, Huang H (2015) Fused embryos and pre-metamorphic conjoined larvae in a broadcast spawning reef coral. *F1000Research* 4:44–55
- Johnson CP, Fujimoto I, Perrin-Tricaud C, Rutishauser U, Leckband D (2004) Mechanism of homophilic adhesion by the neural cell



- adhesion molecule: use of multiple domains and flexibility. *Proc Natl Acad Sci USA* 101(18):6963–6968
- Kallenbach LR, Johnson KL, Bianchi DW (2011) Fetal cell microchimerism and cancer: a nexus of reproduction, immunology, and tumor biology. *Can Res* 71(1):8–12
- Karadge UB, Gosto M, Nicotra ML (2015) Allorecognition proteins in an invertebrate exhibit homophilic interactions. *Current Biol* 25(21):2845–2850
- Karaulov EE, Bottcher RT, Niehrs C (2006) A role for fibronectin-leucine-rich transmembrane cell-surface proteins in homotypic cell adhesion. *EMBO Rep* 7:83–290
- Khalturin K, Becker M, Rinkevich B, Bosch T (2003) Urochordates and the origin of natural killer cells: identification of a CD94/NKR-P1-related receptor in blood cells of *Botryllus*. *Proc Natl Acad Sci USA* 100(2):622–627
- Khalturin K, Bosch TCG (2006) Self/nonself discrimination at the basis of chordate evolution: limits on molecular conservation. *Curr Opin Immunol* 18:1–6
- Khalturin K, Kürn U, Pinnow N, Bosch TCG (2005) Towards a molecular code for individuality in the absence of MHC: screening for individually variable genes in the urochordate *Ciona intestinalis*. *Dev Compar Immunol* 29(9):759
- Khosrotehrani K, Bianchi DW (2005) Multi-lineage potential of fetal cells in maternal tissue: a legacy in reverse. *J Cell Sci* 118(8):1559–1563
- Kruse M, Steffen R, Batel R, Mueller IM, Mueller WEG (1999) Differential expression of allograft inflammatory factor 1 and of glutathione peroxidase during auto- and allograft response in marine sponges. *J Cell Sci* 112(23):4305–4313
- Lange R, Dick M, Mueller WA (1992) Specificity and early ontogeny of historecognition in the hydroid *Hydractinia*. *J Exp Zoology* 262:307–316
- Lange R, Plickert G, Mueller WA (1989) Histoincompatibility in a low invertebrate, *Hydractinia echinata*. Analysis of the mechanism of rejection. *J Exp Zool* 249:284–292
- Leinders-Zufall T, Ishii T, Chamero P, Hendrix P, Oboti L, Schmid A, Kircher S, Pyrski M, Akiyoshi S, Khan M, Vaes E, Zufall F, Mombaerts P (2014) A family of nonclassical class I MHC genes contributes to ultrasensitive chemodetection by mouse vomeronasal sensory neurons. *J Neurosci* 34(15):5121–5133
- Liu H, Mintern JD (2019) MARCH ligases in immunity. *Curr Opin Immunol* 58:38–43
- Loker ES, Adema CM, Zhang SM, Kepler TB (2004) Invertebrate immune systems—not homogeneous, not simple, not well understood. *Immunol Rev* 198:10
- Luo N, Nixon MJ, Gonzalez-Ericsson PI et al (2018) DNA methyltransferase inhibition upregulates MHC-I to potentiate cytotoxic T lymphocyte responses in breast cancer. *Nat Commun* 9:248
- Magor BG, De Tomaso AW, Rinkevich B, Weissman IL (1999) Allorecognition in colonial tunicates: protection against predatory cell lineages? *Immunol Reviews* 167:69
- Maldonado M (1998) Do chimeric sponges have improved chances of survival? *Mar Ecol Prog Ser* 164:301–306
- Mali B, Millane CR, Plickert G, Frohme M, Frank U (2011) A polymorphic, thrombospondin domain-containing lectin is an oocyte marker in *Hydractinia*: implications for germ cell specification and sex determination. *Int J Dev Biol* 55:103–108
- Mali B, Moehrlen F, Frohme M, Frank U (2004) A putative double role of a chitinase in a cnidarian: pattern formation and immunity. *Dev Compar Immunol* 28(10):973–981
- Miller DJ, Hemmrich G, Ball EE, Hayward DC, Khalturin K, Funayama N, Agata K, Bosch TC (2007) The innate immune repertoire in Cnidaria—Ancestral complexity and stochastic gene loss. *Genome Biol* 8:R59
- Miyadera H, Ohashi J, Lernmark A et al (2015) Cell-surface MHC density profiling reveals instability of autoimmunity-associated HLA. *J Clin Invest* 125(1):275–291
- Mizrahi DS, Navarrete A, Flores AAV (2014) Groups travel further: pelagic metamorphosis and polyp clustering allow higher dispersal potential in sun coral propagules. *Coral Reefs* 33(2):443–448
- Mokady O, Buss LW (1996) Transmission genetics of allorecognition in *Hydractinia symbiolongicarpus* (Cnidaria, Hydrozoa). *Genetics* 143:823–827
- Moretta A, Biassoni R, Bottino C, Pende D, Vitale M, Poggi A, Mingari MC, Moretta L (1997) Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev* 155:105–117
- Morse ANC, Morse DE (1996) Flypapers for coral and other planktonic larvae. *Bioscience* 46(4):254–262
- Mueller WA (1964) Experimental investigations on colony development, polyp differentiation and sexual chimeras in *Hydractinia echinata* (Foreign title: Experimentelle Untersuchungen über Stockentwicklung, Polypendifferenzierung und Sexualchimeren bei *Hydractinia echinata*). Wilhelm Roux' Arch für Entwicklungsmechanik 155:181–268
- Mueller WA (1996) Defense of conspecific habitat competitors in *Hydractinia echinata*. *Encyclopaedia Cinematographica VHS-Video C 1907*. Institut für den Wissenschaftlichen Film, Göttingen. Copies available on request from uri.frank@nuigalway.ie or muellerwm@t-online.de
- Mueller WA (2002) Autoaggressive, multi-headed and other mutant phenotypes in *Hydractinia echinata* (Cnidaria: Hydrozoa). *Intern J Dev Biol* 46(8):1023–1033
- Mueller WA, Leitz T (2002) Metamorphosis in the Cnidaria. *Canad J Zool* 80:1755–1771
- Mueller WA, Teo R, Frank U (2004) Totipotency of migratory stem cells in a hydroid. *Dev Biol* 275:215–224
- Mueller WEG, Blumbach B, Mueller IM (1999) Evolution of the innate and adaptive immune systems: relationships between potential immune molecules in the lowest metazoan phylum (Porifera) and those in vertebrates. *Transplantation* 68(9):1215–1227
- Mueller WEG, Mueller I (2003) Origin of the metazoan immune system: Identification of the molecules and their functions in sponges. *Integrat Compar Biol* 43:281–292
- Mukai H (1992) Allogeneic recognition and sex differentiation in chimeras of the freshwater sponge *Ephydatia muelleri*. *J Exp Zool* 264(3):298–311
- Nicotra ML (2019) Invertebrate allorecognition. *Curr Biol* 29(11):R463–R467
- Nicotra ML, Moreno MW, Buss LW (2004) An invertebrate histocompatibility complex. *Genetics* 167(1):357
- Nicotra ML, Powell AE, Rosengarten RD, Moreno M, Grimwood J, Lakkis FG, Dellaporta S, Buss LW (2009) A hypervariable invertebrate allodeterminant. *Curr Biol* 19(7):583–589
- Oren M, Paz G, Douek J, Rosner A, Amar KO, Rinkevich B (2013) Marine invertebrates cross phyla comparisons reveal highly conserved immune machinery. *Immunobiol* 218:484
- Pancer Z, Gershon H, Rinkevich B (1995) Coexistence and possible parasitism of somatic and germ cell lines in chimeras of the colonial urochordate *Botryllus schlosseri*. *Biol Bull* 189:106–112
- Parham P (2005) Immunogenetics of killer cell immunoglobulin-like receptors. *Mol Immunol* 42:459–462
- Paust S, Senman B, Von Andrian UH (2010) Adaptive immune responses mediated by natural killer cells. *Immunol Rev* 235(1):286–296
- Petersdorf EW, O'hUigin C (2019) The MHC in the era of next-generation sequencing: Implications for bridging structure with function. *Human Immunol* 80(1):67–87

- Plickert G, Frank U, Mueller WA (2012) *Hydractinia*, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. *Int J Dev Biol* 56:519–534
- Plickert G, Schetter E, Verhey-van-Wijk N, Schlossherr J, Steinbuechel M, Gajewski M (2003) The role of alpha-amidated neuropeptides in hydroid development: LWamides and metamorphosis in *Hydractinia echinata*. *Int J Dev Biol* 47(6):439–450
- Poudyal M, Rosa S, Powell AE, Moreno M, Dellaporta SL, Buss LW, Lakkis FG (2007) Embryonic chimerism does not induce tolerance in an invertebrate model organism. *Proc Natl Acad Sci USA* 104(11):4559–4564
- Powell AE, Nicotra ML, Moreno MA, Lakkis FG, Dellaporta SL, Buss LW (2007) Differential effect of allorecognition loci on phenotype in *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). *Genetics* 177:2101–2107
- Reem E, Douek J, Paz G, Katzir G, Rinkevich B (2017) Phylogenetics biogeography and population genetics of the ascidian *Botryllus schlosseri* in the Mediterranean Sea and beyond. *Mol Phylogeny Evol* 107:221–341
- Reyburn H, Mandelboim O, Valés-Goméz M, Sheu EG, Pazmany L, Davis DM, Strominger JL (2006) Human NK cells: their ligands, receptors and functions. *Immunol Reviews* 155(1):119–126
- Rinkevich B (1992) Aspects of the incompatibility nature in botryllid ascidians. *Anim Biol* 1:17–28
- Rinkevich B (2001) Human natural chimerism: An acquired character or a vestige of evolution? *Human Immunol* 62:651–657
- Rinkevich B (2002) Germ cell parasitism as an ecological and evolutionary puzzle: Hitchhiking with positively selected genotypes. *Oikos* 96:25–30
- Rinkevich B (2004a) Primitive immune systems: are your ways my ways? *Immunol Reviews* 198:25–35
- Rinkevich B (2004b) Allorecognition and xenorecognition in reef corals: a decade of interactions. *Hydrobiologia* 530(531):443–450
- Rinkevich B (2005) Natural chimerism in colonial urochordates. *J Exp Mar Biol Ecol* 322:93–109
- Rinkevich B, Matranga V (2009) Stem cells: autonomy interactors that emerge as causal agents and legitimate units of selection. In: Rinkevich B (ed) *Stem cells in marine organisms*. Springer, Dordrecht, pp 1–19
- Rinkevich B (2011a) Quo vadis chimerism? *Chimerism* 2011(2):1–5
- Rinkevich B (2011b) The „immunology trap“ of anthozoans. *Invertebr Surv J (ISJ)* 8:153–181
- Rinkevich B (2012) Neglected biological features in cnidarians self-nonsel self recognition. In: Lopez-Larrea C (ed) *Ancient origin of self recognition systems in nature*. Landes Bioscience, Austin, pp 46–59
- Rinkevich B (2015) Conserved histocompatible machinery in marine invertebrates? *Invertebr Surv J (ISJ)* 12:170–172
- Rinkevich B, Douek J, Rabinowitz C, Paz P (2012) The candidate Fu/HC gene in *Botryllus schlosseri* (Urochordata) and ascidians' historecognition—an oxymoron? *Dev Compar Immunol* 36:718–727
- Rinkevich B, Porat R, Goren M (1995) Allorecognition elements on a urochordate histocompatibility locus indicate unprecedented extensive polymorphism. *Proc R Soc London B* 259:319–324
- Rinkevich B, Shapira M (1988) An improved diet for inland broodstock and the establishment of an inbred line from *Botryllus schlosseri*, a colonial sea squirt (Ascidacea). *Aquat Living Resour* 11(3):163–171
- Rinkevich B, Shapira M, Weissman IL, Saito Y (1992) Allogeneic responses between 3 remote populations of the cosmopolitan ascidian *Botryllus schlosseri*. *Zool Sci* 1992:989–994
- Rinkevich B, Weissman IL (1987a) The fate of *Botryllus* (Ascidacea) larvae cosettled with parental colonies: Beneficial or deleterious consequences? *Biol Bull* 173:474–488
- Rinkevich B, Weissman IL (1987b) A long-term study of fused subclones of a compound ascidian. The resorption phenomenon. *J Zool* 213(4):717–733
- Rinkevich B, Weissman IL (1989) Variation in the outcomes following chimera formation in the colonial tunicate *Botryllus schlosseri*. *Bull Mar Sci* 45:213–227
- Rinkevich B, Weissman IL (1992a) Allogeneic resorption in colonial protochordates—consequences of nonself recognition. *Dev Compar Immunol* 16:275–286
- Rinkevich B, Weissman IL (1992b) Chimeras vs genetically homogeneous individuals: potential fitness costs and benefits. *Oikos* 63:119–124
- Rinkevich B, Yankelevich I (2004) Environmental split between germ cell parasitism and somatic cell synergism in chimeras of a colonial urochordate. *J Exp Biol* 207:3531–3536
- Rosa SFP, Powell AE, Rosengarten RD, Nicotra ML, Moreno MA, Grimwood J, Lakkis FG, Dellaporta S, Buss LW (2010) *Hydractinia* allodeterminant *alr1* resides in an immunoglobulin superfamily-like gene complex. *Curr Biol* 20(12):1122–1127
- Rosengarten RD, Moreno MA, Lakkis FG, Buss LW, Dellaporta SL (2011) Genetic diversity of the allodeterminant *alr2* in *Hydractinia symbiolongicarpus*. *Mol Biol Evol* 28(2):933–947
- Rosengarten RD, Nicotra ML (2011) Model systems of allorecognition. *Curr Biol* 21:R82–R92
- Sawicki MW, Dimasi N, Natarajan K, Wang J, Margulies DH, Mariuzza RA (2001) Structural basis of MHC class I recognition by natural killer cell receptors. *Immunol Rev* 181:52–65
- Schnitzler CE, Nguyen AD, Klasfeld SJ, Bond SR, Plickert G, Buss L, Wolfsberg TG, Mullikin JC, Nicotra ML, Cartwright P et al (2016) Genomics of *Hydractinia*: A cnidarian model for regeneration, allorecognition, and developmental biology. *Integrat Compar Biol* 56(1):E196
- Schworer-Boehning B, Kroiber M, Mueller WA (1990) Signal transmission and covert prepattern in the metamorphosis of *Hydractinia echinata* (Hydrozoa). *Roux's Arch Dev Biol* 198:245–251
- Scofield VL, Schlumpberger JM, West LA, Weissman IL (1982) Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* 295:499–502
- Simon-Blecher N, Achituv Y, Rinkevich B (2004) Protochordate concordant xenotransplantation settings reveal outbreaks of donor cells and divergent life span traits. *Dev Compar Immunol* 28(10):983–991
- Spotorno-Oliveira P, Figueiredo M, Tãmega FT (2015) Coralline algae enhance the settlement of the vermetid gastropod *Dendropoma irregulare* (d'Orbigny) in the southwestern Atlantic. *J Exp Mar Biol Ecol* 471:137–145
- Stoner DS, Rinkevich B, Weissman IL (1999) Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. *Proc Natl Acad Sci USA* 96:9148–9153
- Stoner DS, Weissman IL (1996) Somatic and germ cell parasitism in a colonial ascidian: possible role for a highly polymorphic allorecognition system. *Proc Natl Acad Sci USA* 93:15254–15259
- Sweeney CG, Curran E, Westmoreland SV et al (2012) Quantitative molecular assessment of chimerism across tissues in marmosets and tamarins. *BMC Genomics* 13:98
- Sykes M, Wood K, Sachs DH (2008) Transplantation immunology. In: Paul WE (ed) *Fundamental immunology*, 6th edn. Lippincott Williams & Wilkins, New York, pp 1426–1488
- Taketa DA, Nydam ML, Langenbacher AD, Rodriguez D (2015) Molecular evolution and *in vitro* characterization of *Botryllus* histocompatibility factor. *Immunogenetics* 67:605
- Toh TC, Chou LM (2013) Aggregated settlement of *Pocillopora damicornis* planulae on injury sites may facilitate coral wound healing. *Bull Mar Sci* 89(2):583–584

- Utans U, Arceci RJ, Yamashita Y, Russell ME (1995) Cloning and characterization of allograft inflammatory factor-1: a novel macrophage factor identified in rat cardiac allografts with chronic rejection. *J Clin Invest* 95(6):2954–2962
- Vigneau S, Vinogradova S, Savova V et al (2018) High prevalence of clonal monoallelic expression. *Nat Genet* 50:1198–1199
- Voskoboynik A, Rinkevich B, Weissman IL (2009) Stem cells, chimerism and tolerance: lessons from mammals and ascidians. In: Rinkevich B, Matranga V (eds) *Stem cells in marine organisms*. Springer, Heidelberg, pp 281–308
- Voskoboynik A, Newman AM, Corey DM, Sahoo D, Pushkarev D, Neff NF, Weissman IL (2013) Identification of a colonial chordate histocompatibility gene. *Science* 341(6144):384–388
- Watanabe H (1962) Further studies in the regulation in fused colonies in *Botryllus primigenus* (Ascidiae Compositae). *Sci Rep Tokyo Kyoiku Daiq (B)* 10:253
- Weis RA (2018) Open questions: knowing who's who in multicellular animals is not always as simple as we imagine. *BMC Biol* 16:115
- Weissman IL, Saito Y, Rinkevich B (1989) Allorecognition in colonial tunicates: parallels with and tangents from vertebrate immunity. In: Melchers F (ed) *Progress in immunology*. Springer, Heidelberg, pp 1256–1263
- Weissman IL, Saito Y, Rinkevich B (1990) Allorecognition histocompatibility in a protochordate species: Is the relationship to MHC semantic or structural? *Immunol Rev* 113:227–241
- Weissman IL, Scofield V, Saito Y, Boyd H, Rinkevich B (1988) Speculations on the relationships of two *Botryllus* allorecognition reactions—colony specificity and resorption- to vertebrate histocompatibility. In: Grosberg RK, Hedgecock D, Nelson K (eds) *Invertebrate historecognition*. Plenum Press, New York, pp 67–78
- Yin C, Humphreys T (1996) Acute cytotoxic allogeneic histoincompatibility reactions involving gray cells in the marine sponge *Callyspongia diffusa*. *Biol Bull* 191(2):159–167
- Zarate-Poles A, Ocampo ID, Cadavit LF (2019) The putative immune recognition repertoire of the model cnidarian *Hydractinia symbiolongicarpus* is large and diverse. *Gene (Amsterdam)* 684:104–117