Int. J. Dev. Biol. 46: 699-704 (2002)

Original Article

The ontogeny of allorecognition in a colonial hydroid and the fate of early established chimeras

MARC–AUREL FUCHS[1], OFER MOKADY[2] and URI FRANK*,[1]

[1]*Institute of Zoology, University of Heidelberg, Germany and* [2]*The Institute for Nature Conservation Research, Tel Aviv University, Israel*

ABSTRACT Colonies of the marine hydroid, *Hydractinia*, are able to discriminate between their own tissues and those belonging to unrelated conspecifics. We have studied the ontogeny of this allorecognition system by a series of allogeneic transplantations along a developmental gradient, including two-cell-stage embryos, 8 h morulae, planula larvae and metamorphosed polyps. Allograft acceptance of incompatible tissue was observed in all embryonic and larval stages, whereas metamorphosed polyps rejected incompatible transplanted allografts. Most of the chimeras established at the two-cell-stage, although composed of two allogeneic, incompatible entities with mismatching allorecognition loci, developed normally and remained stable through metamorphosis. The results of post metamorphic transplantation assays among the chimeras and the naive ramets, suggested that both incompatible genotypes were still represented in the chimera despite the onset of alloimmune maturation. The naive colonies always rejected each other. Chimeras established from later embryonic and larval stages did not develop into adult chimeric entities, but rather separated immediately post metamorphosis. We thus show that (1) allorecognition in this species matures during metamorphosis and (2) genetically incompatible entities may coexist in one immunologically mature, chimeric soma, provided that they were grafted early enough in ontogeny.

KEY WORDS: *chimera, histocompatibility, Hydractinia, invertebrate, transplantation*

Introduction

Many colonial/modular marine invertebrates posses genetically controlled allorecognition systems and feature phenomena of histocompatibility between individual genets (Grosberg, 1988; Grosberg and Hart, 2000). These phenomena resemble self/nonself discrimination seen in vertebrates with two main differences. First, vertebrate allorecognition is T cell/MHC mediated and, thus, adaptive. In invertebrates, lacking rearranging antigen receptors (Klein, 1999; Laird *et al.*, 2000), allorecognition is innate. Second, as vertebrates do not come naturally into contact with allogeneic cells and do not become chimeras (with some rare exceptions), vertebrate allorecognition has probably no functional significance. Colonial marine invertebrates, in contrast, may come into direct cell-cell contact with conspecifics during their growth. Such contacts are risky since germ cell progenitors may be exchanged between "donor" and "recipient" following fusion. If one strain proves to be more efficient it may exclude the other from sexual reproduction partly or completely. It is widely accepted that this potential risk of germline parasitism has been the selective pressure under which allorecognition has evolved in modular invertebrates (Buss, 1982, 1999).

Hydractinia, a colonial marine hydroid, has been serving as a model system for studying invertebrate allorecognition and histocompatibility for several decades (Frank *et al.*, 2001). A typical *Hydractinia* colony is composed of a network of gastrovascular canals, termed stolons, from which polyps (or hydranths) arise (Fig. 1A; for a comprehensive description of *Hydractinia* we refer the reader to the *Hydractinia* page on the Internet at http://www.zoo.uni-heidelberg.de/frank/hydractinia/). The colony grows asexually by lateral extension of the stolons. Two stolons within a colony (genet) that come into contact always fuse (Fig 1A). When two colonies of *Hydractinia* come into contact during their growth, they either fuse to form a chimera with a common gastrovascular system or reject each other, a process that may be mediated by several alternative effector mechanisms (Fig. 1B; Buss *et al.,* 1984; Lange *et al.*, 1989). Allorecognition in *Hydractinia* has been proposed to be genetically controlled by a polymorphic, one-locus gene system with codominantly expressed alleles (the allorecognition locus, here below *arl*: *sensu* Cadavid and Buss, 1999). Two individuals sharing at least one allele at the *arl* fuse

Abbreviations used in this paper: arl, allorecognition locus; MHC, major histocompatibility complex.

***Address correspondence to:** Dr. Uri Frank. INF 230, 69120 Heidelberg, Germany. Fax: +49-6221-544913. e-mail: frank@uni-hd.de

0214-6282/2002/$25.00

Printed in Spain
www.ijdb.ehu.es

upon tissue contact, whereas individuals without a common allele at this locus reject each other's tissue (Mokady and Buss, 1996). The high polymorphism of the *arl* ensures that most wild type animals are heterozygous at this locus, and the probability of two unrelated animals to share one allele, and thus to fuse upon contact, is low. Rejection of an incompatible colony in *Hydractinia* may result in a cytotoxic response, involving specialized stinging cells (microbasic mastigophores: Lange *et al.*, 1989). An alternative, "milder" response involves the formation of an acellular barrier, thus avoiding cell contact between the two allogeneic counterparts (Buss *et al.*, 1984; Shenk, 1991). The "decision" regarding which effector system to mount following contact with an incompatible genet is probably also genetically controlled.

Alloimmunity in several cnidarians is known to mature during ontogeny and the maturation stage is probably species specific (Hidaka, 1985; Frank *et al.*, 1997). In *Hydractinia*, it is known that young polyps already reject *arl*-mismatching conspecifics shortly after metamorphosis (Hauenschild, 1954, 1956; Müller, 1964). It is also known that 8 h embryos (morula stage) are not immunocompetent and may fuse with allogeneic incompatible embryos of the same stage (Lange *et al.*, 1992). The developmental stage at which allorecognition matures, however, has not been characterized yet. Our first aim in this study was to determine the stage between the 8 h morula and the primary polyp at which alloimmunity matures in *Hydractinia.* Furthermore, we intended to study the fate of chimeras established before the maturation stage. We have found that allorecognition in *Hydractinia* matures during, or shortly (< 1 day) post metamorphosis. Interestingly, alloimmune maturation does not necessarily cause the rejection of incompatible allogeneic tissue, providing that it was grafted early enough in ontogeny.

Results

Allorecognition in Embryos and Larvae

The morphological signs of the grafting procedure faded in all embryonic and larval grafts within 24 h post transplantation (Fig. 1 F,G). The resulting chimeras appeared completely normal as compared to non-chimeric embryos and larvae of the same developmental stage or the control isografts, and there was no indication for any incompatibility response as known from grafting of metamorphosed, incompatible animals. Rejection in the latter always occurs within 12-24 h post transplantation (Lange *et al.*, 1992; this study). Out of 37 chimeric embryos and larvae that had been established surgically, 21 (57%) developed normally beyond the first 24 h post transplantation (Table 1). The rest of the chimeric embryos became bifurcated larvae (Fig. 1H) or separated during the larval stage. The control isografts showed similar behavior: Out of the 12 isografts established only 6 developed normally. One showed retarded embryonic development and died before metamorphosis. Five additional isografts became bifurcated embryos and larvae – 3 developed normally after metamorphosis, 1 separated before metamorphosis and 1 died before metamorphosis. These deviations from normal development are probably not related to allorecognition but to polarity conflicts between the grafts (see Discussion). Altogether, 29 chimeric larvae, established at three developmental stages (two-cell-stage, 8 h morula and planula larva; Table 1), were induced to metamorphose. Fifteen of them, all resulting from transplantations at the two-cell-stage, developed to become normal colonies. The others, resulting from 7 two-cell-stage em-

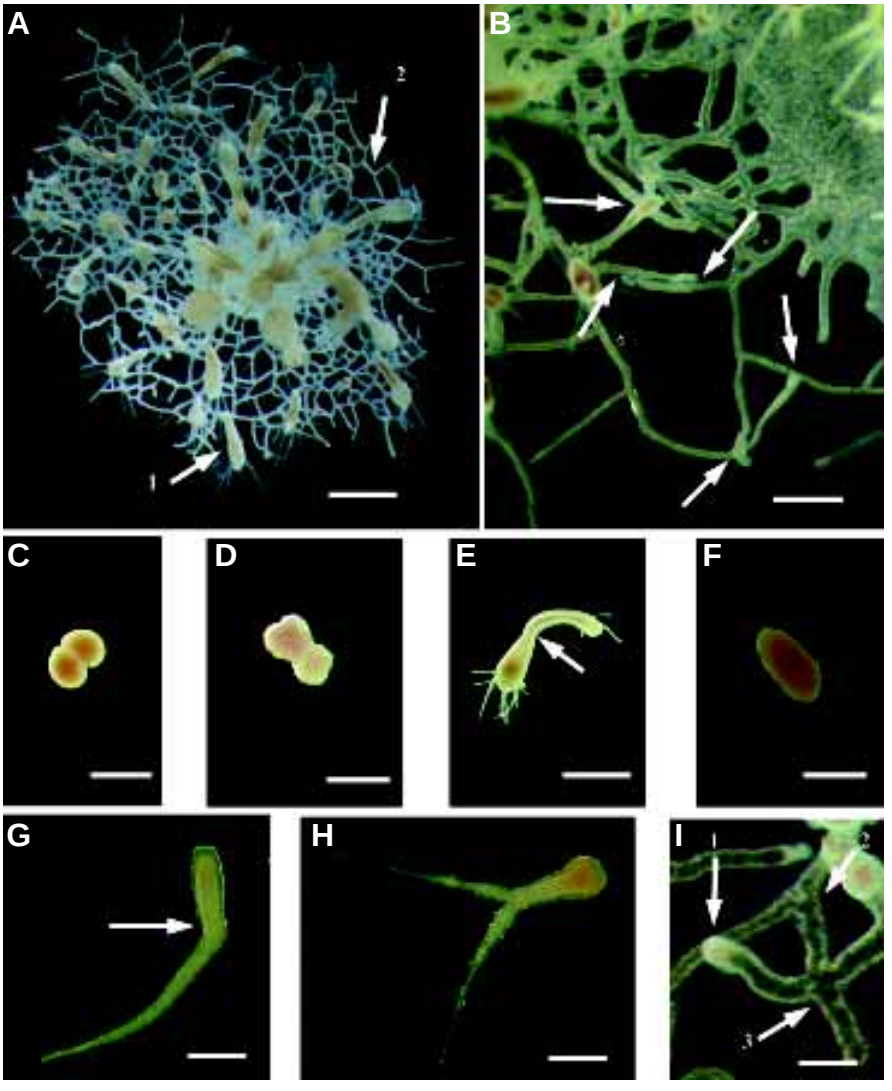

Fig 1. *Hydractinia echinata*. (A) *A juvenile colony. Arrows point to a polyp (1) and a site of stolon fusion (2).* **(B)** *Rejection of incompatible stolons. Arrows point to rejection sites.* **(C)** *A normal two-cell stage embryo.* **(D)** *A chimeric embryo, established from two allogeneic incompatible blastomeres, one hour following grafting.* **(E)** *Two compatible, fused polyps, 24 h post transplantation. Arrow indicates the original contact area.* **(F)** *A chimeric embryo, established at the two-cell stage, 24 h post grafting.* **(G)** *A chimeric larva, established from two, genetically incompatible halves, 24 h post transplantation. The arrow indicates the fusion area.* **(H)** *A bifurcated larva.* **(I)** *Simultaneous fusion and rejection within a chimeric colony. Arrows point to rejection site (1), and fusion sites (2, 3). Scale bars 2 mm in (A); 0.5 mm in (B,C,D,F); 1.5 mm in E; 150 μm in (G-I).*

bryos and all morulae and planula larvae grafts, separated immediately following metamorphosis into two primary polyps, mostly of different sizes, or into one polyp and one stolon system. The resulting animals proved incompatible. Their stolons did not fuse upon renewed contact and one of them eventually died as a result of nematocyst attack by its incompatible counterpart.

Allorecognition Assays Post Metamorphosis

As expected, the two non-sibling naive colonies, which were the offspring of incompatible parent couples, always proved incompatible in each complete experimental set, repeatedly rejecting each other's tissue within 24 h post transplantation in polyp-polyp assays. The outcomes of the transplantation assays involving chimeras established at the two-cell-stage, which remained intact post metamorphosis, were much more variable. In addition to the binary outcomes known from surgical polyp transplantations in *Hydractinia* (*i.e.*, fusion vs. rejection within 24 h), we observed a yet non-described response that we term "delayed-rejection". This type of response was characterized by a prolonged (up to 96 h) adhesion of the grafted tissues, in contrast to the separation within 24 h, characteristic of incompatible grafts, without the formation of a common gastrovascular cavity, characteristic of compatible allografts and isografts. Such outcomes were never obtained in our experiments with non-chimeric, incompatible colonies, where unequivocal rejection (separation of the grafted polyps) was always evident within 12-24 h post transplantation.

The above three types of response (fusion, rejection, and the delayed-rejection) were observed in various combinations between the chimeras and their corresponding naive colonies as follows: (a) the chimera fused with both naive colonies (chimeras 1 & 22: Table 1); (b) the chimera fused with one naive colony and displayed delayed rejection with the other (chimera 2); (c) the chimera displayed delayed rejection with both naive colonies (chimeras 8 & 9); (d) the chimera fused with one naive colony and rejected the other (*e.g.* chimera 3); (e) the chimera rejected both naive colonies (chimera 10). Moreover, these observations, carried out in 2-6 replicates each, were not necessarily reproducible when polyps from different regions of a given chimera were grafted. For example, polyp-polyp assays performed with polyps from chimera #1 (which was the product of genotypes A & B) and the corresponding naive colony B resulted in fusion in 3 independent grafting experiments. However, repeated grafting experiments between chimeric polyps and polyps from colony A gave contradictory results: 2 times fusion and 6 times rejection. It has to be noted that this chimera was a single, continuous colony with no signs of internal rejection or separation.

Allorecognition assays performed on separated chimeras also produced variable results, depending on the developmental stage at which separation occurred. In all chimeras, which separated post metamorphosis, each of the chimera-ex-partners fused with only one naive colony and repeatedly rejected both the other naive colony and the ex-chimera-partner (*e.g.* chimera 31: Table 1). The two colonies, which were the result from chimera separations at the larval stage (chimeras 23 & 24), yielded different transplantation outcomes. In chimera 23, the ex-partners rejected each other. Chimera I, however, displayed delayed-rejection with A (in one case) and fused with B. In chimera 24, two ex-partners grew on a glass slide into secondary stolonal contact. We observed some stolons of the two, which were fused while others were in the process of rejection, simultaneously (Fig. 1I). It should be emphasized again that the two naive ramets of the genets composing the chimeras proved to be incompatible, repeatedly rejecting each

TABLE 1

THE FATE OF THE ESTABLISHED CHIMERAS

Chimera No.	Transplantation stage	Chimera history pre-metamorphosis	Chimera history post-metamorphosis
1	Two cell	Normal development	Stable chimera, Chimera rejected A (6), fused with A (2), fused with B (3)
2	Two cell	Normal development	Stable chimera, chimera rejected A (4), DR A (1), fused with B (3)
3	Two cell	Normal development	Stable chimera, chimera fused with A (2), rejected B (6)
4	Two cell	Normal development	Separated PM, chimera I died, chimera II fused with B (3), rejected A (5)
5	Two cell	Normal development	Separated PM, chimera I fused with A (4), rejected B (6), chimera II fused with B (2), rejected A (6)
6	Two cell	Normal development	Separated PM, Chimera I died, chimera II fused with A (2), rejected B (6)
7	Two cell	Normal development	Separated PM, chimera I died, chimera II fused with A (3), rejected B (4)
8	Two cell	Normal development	Stable chimera, chimera fused with B (3), DR B (1), DR A (1), rejected A (6)
9	Two cell	Normal development	Stable chimera, chimera fused with A (2), DR A (1), DR B (3), fused with B (4)
10	Two cell	Normal development	Stable chimera, chimera fused with A (4), rejected A (1), rejected B (6)
11-14	Two cell	Normal development	Stable chimera, no transplantation carried out PM
15-16	Two cell	Bifurcated larva	Stable chimera, no transplantation carried out PM
17-18	Two cell	Bifurcated larva	Separated PM, no transplantations carried out PM
19-20	Two cell	Bifurcated larva	Stable chimera, no transplantation carried out PM
21	Two cell	Bifurcated larvae	Separated PM, chimera I died, chimera II fused with B (2), rejected A (6)
22	Two cell	Bifurcated larva	Stable chimera, chimera fused with A (1), rejected A (6), fused with B (2)
23	Two cell	Separated during larval stage, both halves survived and were induced to metamorphosis	Chimera I rejected chimera II (5), rejected A (4), DR A (1), fused with B (1). Chimera II fused with A (4), rejected B (5)
24	Two cell	Separated during larval stage, both halves survived and were induced to metamorphosis	Chimera I fused and simultaneously rejected chimera II in stolon contact (2), chimera I rejected chimera II (4), chimera II fused with A (1), rejected B (5), chimera I fused with B (2),rejected A (6), DR A (2)
25-30	Two cell	Separated during larval stage and discarded	
31	Morula	Normal development	Separated PM, chimera I fused with A (2), rejected B (5), chimera II fused with B (2), rejected A (4)
32	Morula	Normal development	Separated PM, chimera I fused with B (2), rejected A (6), chimera II fused with A (2), rejected B (6)
33	Morula	Normal development	Separated PM, chimera I died, chimera II fused with A (2), rejected B (4)
34	Morula	Normal development with B (3), rejected A (6)	Separated PM, chimera I fused with A (3), rejected B (5), chimera II fused
35-37	Planula	Fusion and complete regeneration	Separated PM, no transplantations were carried out PM
36-37	Planula	Fusion and complete regeneration	Separated PM, no transplantations were carried out PM

DR= delayed rejection, PM= post metamorphosis, Chimera I & Chimera II= products of chimera separation, A & B are the genotypes in each chimera, numbers of replicates in a given grafting assay are given in parentheses, N represents the number of chimeras featuring the described history

other within 24 h post transplantation in the polyp-polyp assay, or within several hours when stolon contact was involved.

Discussion

We have studied here the outcomes of allogeneic tissue transplantations between *arl*-mismatching entities of *Hydractinia echinata* in various developmental stages. Two types of evidence indicated the timing of alloimmune maturation. First, chimeras from genetically incompatible animals could only be established from embryos and larvae while metamorphosed animals always rejected incompatible allografts (These phenomena were also partly observed previously: Lange *et al.*, 1992). Second, most separations of chimeric colonies occurred immediately following metamorphosis. Our results therefore show that alloimmunity in *Hydractinia* matures during metamorphosis or very shortly (<1 day) thereafter. In other cnidarians, specifically scleractinian corals and alcyonaceans, full alloimmune competence is reached later in development, up to several months post metamorphosis (Hidaka, 1985, Frank *et al.*, 1997; Barki *et al.,* 2002). Accordingly, chimeras of two genetically incompatible entities may be established naturally in these animals. This may occur in the field if two planula larvae settle close enough to each other, growing into contact before alloimmune maturation is reached. This is also the case in the sponge *Chalinula* where settled juveniles may fuse to form chimeric entities (Ilan and Loya, 1990). By contrast, in *Hydractinia*, natural chimerism between *arl*-mismatching individuals is impossible, since primary polyps are already immunocompetent and natural transplantation between allogeneic embryos or planula larvae is highly improbable.

The interpreted timing of immune maturation in *Hydractinia* is in line with the theory, which considers the potential costs of germline parasitism as the selective pressure under which allorecognition has evolved in invertebrates (*e.g.*, Buss, 1982, 1999). Following allogeneic fusion and chimera establishment, germ cell progenitors may migrate from one partner to the other, invade its gonads, and contribute disproportionately to the chimera's germline. One partner would be excluded from sexual reproduction partly or completely. According to this theory, allorecognition has evolved in colonial invertebrates to restrict access to the germline and, thus, to the production of gametes, either to self- (Feldgarden and Yund, 1992) or to kin stem cells (Hart and Grosberg, 1999). Either way, allogeneic contacts in *Hydractinia* may only occur naturally following metamorphosis, when stolons develop. Hence, the advantage provided by an allorecognition system, which protects from the invasion of foreign stem cells before metamorphosis, would be negligible.

Our data indicate that the time frame within which stable chimeras may be established by two *arl*-mismatching animals is narrow, consisting of the very early developmental stages, ending before the morula stage. The results obtained in the allorecognition assays, although variable, point to the coexistence of two genetically incompatible entities in one mature chimeric soma, at least in several cases (see below). This observation may imply the acquisition of a state of unresponsiveness by the two partners, which would reject each other if first grafted only post metamorphosis. It resembles the acquisition of allotolerance in vertebrates. If only one genotype survived in a chimera, or, alternatively, the other genotype's cells developed into cells not involved in allorecognition, we would expect the chimera to fuse with only one naive colony and always reject the other. This may have been the case in chimera #3. In many other cases, however, the results of post metamorphic transplantations clearly deviated from this situation, suggesting post metamorphic coexistence of the two incompatible genotypes. This holds true not only for the cases where the chimera fused with both naive colonies (chimeras 1 & 22), but also for the case in which the chimera rejected both naive colonies (chimera 10). In this context, one should keep in mind that even though the partners within the chimera appeared to be unresponsive (tolerant) towards each other and may accordingly be expected to fuse with both naive colonies, the naive colonies are not tolerant. They should accordingly reject the allogeneic component of the chimera. Grafting of two polyps from the naive colonies invariably resulted in rejection within 12-24 h. Thus, in similar grafting assays involving a polyp from the chimera and a polyp from the naive colonies, either one of the naive polyps may reject the allogeneic partner within the chimera. Visually, one cannot discern the 'rejector' and the 'rejected' in a pair of transplanted polyps displaying rejection. Therefore, even if a chimeric polyp is 'prepared' to fuse with the naive polyp, the latter would reject the allogeneic part in the chimeric polyp. The partners' cells in the chimera were probably not uniformly distributed, which could be the interpretation for (1) the variability of the outcomes when transplanting polyps from different areas in the chimera, (2) the delayed-rejection, and (3) the observed simultaneous fusion and rejection of stolons (Fig. 1I). The delayed-rejection probably resulted from contact of the naive colony with a region in the chimera where most cells were allogeneic (incompatible). The few isogeneic cells in the contact zone may have enabled prolonged adhesion. Similarly, in the stolon encounter depicted in Fig. 1I the intermixing of genotypes may have not been uniform. In the fusion region one genotype was locally dominant in both stolon tips enabling fusion. In the rejection area, on the other hand, different genotypes dominated locally, resulting in rejection. The degree of intermixing of allogeneic cells in chimeras is probably stochastic, but may also depend on the timing of grafting. Transplantations carried out at the two-cell-stage allowed the blastomeres and their descendants a longer period to intermix before the onset of alloimmune maturation. The partners may be unable to reject the incompatible counterpart in well-intermixed chimeras following metamorphosis. In chimeras established later, however, intermixing is not complete and a separation is possible when alloimmune competence is established during metamorphosis. No transplantations post metamorphosis were carried out in chimeras 11-20 and, thus, these cases are not informative regarding the coexistance of genotypes in a chimera.

Post-metamorphic separations of chimeras reflected the genotypic identity of the partners in the chimera, and resulted in two genetically-homogeneous colonies, each compatible with only one naive colony (*e.g.* chimera 3). By contrast, the separation of the chimeras, which occurred at the larval stage, is suggested not to be associated with allorecognition. Instead, we interpret this outcome to reflect a 'conflict of polarity' as previously observed in grafts of other hydroids (Teissier, 1931, 1933; Freeman, 1981). The polarity axis (oral/aboral) in *Hydractinia* is already determined in the unfertilized oocyte (Freeman, 1980). Since we did not consider the original polarity of the embryonic-halves dissected and transplanted to create the chimera, it is likely that in some cases the conflict of polarity was too intense, leading to abnormal develop-

ment (*i.e.*, bifurcated larvae) or to separation. Our interpretation is supported by the control isografts, in which tissue incompatibility is excluded. Fifty percent of the isografts displayed a clear deviation from normal development, similar to the allografts (see results). The fact that the colonies, resulting from the separation of chimera 24, demonstrated simultaneous stolon fusion and rejection (Fig. 1I), suggests that the larval separations were not allorecognition-related and resulted in two chimeras in which both incompatible genets were still represented. Finally, most bifurcated chimeras remained stable post metamorphosis, supporting the 'conflict of polarity' hypothesis.

Taken together, our data attest for two main points: (a) allorecognition in *Hydractinia* matures during, or shortly (<1 day) after metamorphosis; and (b) exposure to allogeneic, incompatible tissue in early ontogeny may lead to the acquisition of a state of specific unresponsiveness by individual *Hydractinia*. This observation, which is, to the best of our knowledge, the first to be reported in an invertebrate, calls for further research to address underlying mechanistic aspects. At present, the molecular and cellular background of the documented phenomena is not clear, as are the dynamics of the genotypic composition of the involved partners within the chimera, and possible homology to similar phenomena in vertebrates.

Materials and Methods

Animals

Wild type *Hydractinia echinata* colonies, growing on gastropod shells of the genera *Littorina* or *Buccinum*, inhabited by hermit crabs (*Eupagurus*), were sampled at the Island of Sylt (North Sea) and cultured in the laboratory in artificial seawater at 18°C under a 14/10 h light/dark regime. They were fed five times a week with 4-5 days old brine shrimp nauplii. The animals spawned every other day about one hour following the onset of light. Male and female colonies were put together in one glass beaker shortly after the onset of light and fertilized eggs from different parents were kept separately until used. Planulae were induced to metamorphose by a standard ionic imbalance treatment using CsCl (Müller and Buchal, 1973; Frank *et al.*, 2001).

Transplanting Two-Cell Embryos, 8 h Morulae and Planulae

Animals to be transplanted were the F1 generation of two pairs of wild type parents. By performing pairwise allorecognition assays we confirmed that each of the four parental colonies was incompatible with the other three (data not shown, method described in the next section). We transplanted only non-siblings, which were genetically incompatible (see introduction: genetics of allorecognition). Two-cell embryos (Fig. 1C) and 8 h morulae were cut in half using a fine syringe needle or glass fiber. Two halves from different, genetically incompatible animals were placed in a depression made in a petri dish in 10 µl seawater and forced to contact by a cover slip for up to 2 h without considering their original orientation. The cover slip was then removed and the produced normal sized chimeric embryo was rinsed with seawater (Fig. 1D). Similarly, we also conducted 12 control isogeneic transplantations. Chimeras and isografts were observed every hour during the first 6 h and then every 8-12 h until induced to metamorphose. The two naive halves of the two transplanted genotypes of each chimera were allowed to develop separately without any allogeneic contact to half-sized, but otherwise normal, planula larvae and induced to metamorphose as described above. Chimeras from planula larvae halves (*i.e.*, first allogeneic contact at the planula stage) were established by stringing anterior and posterior incompatible larvae halves on glass needles with the wounded sides facing each other. The allogeneic tissues were forced to contact by two agar blocks for 12 h and were then removed from the needle. Both chimeric- and naive (half sized) planulae were induced to metamorphose, 4-10 days post transplantation, as described above. A chimera and the corresponding (originally half sized) naive colonies are termed hereafter "a complete experimental set".

Allorecognition Assays Post Metamorphosis

About 6-10 weeks post metamorphosis, when the young colonies reached a size of at least 15 polyps, we dissected polyps from each complete experimental set and performed the following transplantation scheme: chimera vs. naive colony A; chimera vs. naive colony B; naive colony A vs. naive colony B; and if applicable (*i.e.* in cases where the chimera separated) chimera I vs. chimera II (the products of a separated chimera, see Table 1). For this we used two alternative procedures that are known to give the same results regarding compatibility (Lange *et al.*, 1992). The first method was the polyp-polyp grafting assay, first described by Lange *et al.*, 1992. Dissected polyps were strung on a glass needle with the wounded sides facing each other and kept in contact with two small agar blocks for 2 h. They were then removed from the glass needles. Outcomes were evaluated at 1, 3, 12 and 24 h, and if applicable, up to 72 h post transplantation. Polyps that remained attached for over 24 h and established a common gastrovascular cavity were scored as fused (Fig. 1E). Those which separated 12-24 h post transplantation were scored as rejected. Alternatively, polyps with stolon tissue were isolated from each of the colonies to be grafted and put in one petri dish, 1-2 mm apart, under small pieces of cover slips to keep them at place. After two days the stolons attached to the plastic and resumed growth. The glass splinters were then removed (Hauenschild, 1954, 1956; Müller, 1964). Allogeneic contact was established within 3-4 days and the outcomes of stolon contact (fusion or rejection) were visible within hours following contact. Each allogeneic combination was assayed at least twice for *arl* phenotype using either one or both of the above methods.

Acknowledgements

We thank Prof. W.A. Müller for fruitful discussions during the course of the study. Ms. H. Greipel is kindly acknowledged for technical assistance. This work was funded by the DFG, the German Science Foundation, to UF and OM (grant No. FR 1346/2-1).

References

BARKI, Y., GATEÑO, D., GRAUR, D. and RINKEVICH, B. (2002). Soft-coral natural chimerism: a window in ontogeny allows the creation of entities comprised of incongruous parts. *Mar. Ecol. Prog. Ser.* 231: 91-99.

BUSS, L.W. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. USA* 79: 5337-5341.

BUSS, L.W. (1999). Slime molds, ascidians, and the utility of evolutionary theory. *Proc. Natl. Acad. Sci. USA* 96: 8801-8803.

BUSS, L.W., MACFADDEN, C.S. and KEENE, D.R., 1984. Biology of hydractiniid hydroids. 2. Histocompatibility effector system/competitive mechanism mediated by nematocyst discharge. *Biol. Bull.* 167: 139-158.

CADAVID, L.F. and BUSS, L.W. (1999). Genetic mapping of the allorecognition locus in *Hydractinia symbiolongicarpus. Proc. 8th Int. Workshop on Hydroid Development*, Tutzing: p. 115

FELDGARDEN, M. and YUND, P.O. (1992). Allorecognition in colonial marine invertebrates: does selection favor fusion with kin, or fusion with self? *Biol. Bull.* 182: 155-158.

FRANK, U., LEITZ, T. and MÜLLER, W.A. (2001). The hydroid *Hydractinia*: a versatile, informative cnidarian representative. *BioEssays* 23: 963-971.

FRANK, U., OREN, O., LOYA, Y. and RINKEVICH, B. (1997). Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, four months post metamorphosis. *Proc. Roy. Soc. Lond. B* 264 (1378): 99-104.

FREEMAN, G. (1980). The role of cleavage in the establishment of the anterior-posterior axis of the hydrozoan embryo. In: TARDENT, P. and TARDENT, R., (Ed.), *Developmental and cellular biology of coelenterates*. Elsevier, North Holland, Amsterdam, pp. 97-108.

FREEMAN, G. (1981). The role of polarity in the development of the hydrozoan planula larva. *Wilhelm Roux's Arch.* 190: 168-184.

GROSBERG, R.K. (1988). The evolution of allorecognition specificity in colonial invertebrates. *Q. Rev. Biol.* 63: 377-412.

GROSBERG, R.K. and HART, M.W. (2000). Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science* 289: 2111-2114.

HART, M.W. and GROSBERG, R.K. (1999). Kin interactions in a colonial hydrozoan (*Hydractinia symbiolongicarpus*): population structure on a mobile landscape. *Evolution* 53: 793-805.

HAUENSCHILD, C. (1954). Genetische und entwicklungsphysiologische Untersuchungen über Intersexualität und Gewebeverträglichkeit bei *Hydractinia echinata* Flem. (Hydroz. Bougainvill.). *Roux's Arch. Entwicklungsmech.* 147: 1-41.

HAUENSCHILD, C. (1956). Über die Vererbung einer Gewebeverträglichkeits-Eigenschaft bei dem Hydroidpolypen *Hydractinia echinata. Z. Naturforschung* (C) 11b: 132-138.

HIDAKA, M. (1985). Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis. Coral Reefs* 4: 111-116.

ILAN, M. and LOYA, Y. (1990). Ontogenetic variation in sponge histocompatibility responses. *Biol. Bull.* 179: 279-286.

KLEIN, J. (1999). Self-nonself discrimination, histoincompatibility, and the concept of immunology. *Immunogenetics* 50: 116-123.

LAIRD, D.J., DE TOMASO, A.W., COOPER, M.D. and WEISSMAN, I.L. (2000). 50 million years of chordate evolution: Seeking the origins of adaptive immunity. *Proc. Natl. Acad. Sci. USA* 97: 6924-6926.

LANGE, R., PLICKERT, G. and MÜLLER, W.A. (1989). Histoincompatibility in a low invertebrate, *Hydractinia echinata:* Analysis of the mechanisms of rejection. *J. Exp. Zool.* 249: 284-292.

LANGE, R.G., 1992. DICK, M.H. and MÜLLER, W.A. (1992). Specificity and early ontogeny of historecognition in the hydroid *Hydractinia. J. Exp. Zool*. 262: 307-316.

MOKADY, O. and BUSS, L.W. (1996). Transmission genetics of allorecognition in *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). *Genetics* 143: 823-827.

MÜLLER, W.A. (1964). Experimentelle Untersuchungen über Stockentwicklung, Polypendifferenzierung und Sexualchmären bei *Hydractinia echinata. Wilhelm Roux Arch.* 155: 181-268.

MÜLLER, W.A. and BUCHAL, G. (1973). Metamorphoseinduktion bei Planulalarven. II. Induktion durch monovalente Kationen: Die Bedeutung des Gibbs-Donnan-Verhältnisse unter der Na+/K+-ATPase. *Wilhelm Roux Arch.* 173: 122-135.

SHENK, M.A. (1991). Allorecognition in the colonial marine hydroid *Hydractinia* (Cnidaria/Hydrozoa). *Amer. Zool.* 31: 549-557.

TEISSIER, G. (1931). Étude expérimentale du development de quelques hydraires. *Ann. Sci. Nat. Ser. Bot. Zool.* 14: 5-60

TEISSIER, G. (1933). Recherges sur les potentialités de lœuf des hydraires. Polarité des larvas complexes produites par greffe embryonnaire. *C. R. Soc. Biol.* 113: 26-27

Received: April 2002
Reviewed by Referees: May 2002
Modified by Authors and Accepted for Publication: July 2002