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Molecular mechanisms of sex determination and evolution of the Y chromosome: insights from the medakafish (*Oryzias latipes*)

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Summary

Fish exhibit a striking variety of sex determination mechanisms and sex chromosome structures, differing sometimes even between closely related species. Therefore fish are not only interesting objects to study the molecular mechanisms of sex determination operating in a species of interest but also provide models to better understand the evolution of this process. The review will mainly focus on one species, the medaka (*Oryzias latipes*), a small laboratory fish model species originating from freshwater biotopes of Japan and Korea, because the most advanced knowledge of genetic sex determination in a non-mammalian species has been obtained in this species. The master male sex determining factor (*dmrt1bY*) has been isolated and identified as a duplicate of an autosomal gene that is known to function at a most downstream position of the sex determining regulatory gene cascade from worms and flies up to mammals. In medaka, the entire male-specific region of the Y-chromosome and adjacent pseudo-autosomal regions are sequenced. The Y-chromosome is only 5 to 10 million years old. This allows to infer the molecular events that have shaped the medaka Y and to evaluate this against the predictions of the common theory of evolution of sex chromosomes. The molecular mechanisms how *dmrt1bY* initiates male development are only beginning to be understood, but it is apparent that the *dmrt1bY* gene functions by inhibiting male primordial germ cell proliferation at the sex determining stage.

Introduction

Sex determination (SD), the developmental process that determines whether the bipotential gonad anlage will differentiate towards a testes or an ovary is one of the most plastic processes in evolutionary developmental biology. The trigger for this process may be environmental (ESD) or genetic (GSD), and within each type there is a multitude of mechanisms how to spark the process of male or female development. Fish show an amazing variety of sex determination mechanisms and there is hardly anything that cannot be found in this group of organisms. Although some information is emerging about sex determination in lampreys, sharks, rays and sturgeons, by far most of our knowledge stems from studies on teleost fish. Therefore, this review will concentrate on this group of fishes, and especially one species, the medaka, where even the master sex-determining gene is known.

A considerable number of teleost species are hermaphrodites, switching either from first being males (protandrous) to become females, or the other way round (protogynous). There is even one species, *Kryptolebias marmoratus*, which is a simultaneous hermaphrodite with a well-developed ovotestes and where the eggs are fertilized inside the gonad. In the sequential hermaphrodites several mechanism have been described that initiate the switch from one sex to the other and these vary between species. Social factors, size and age are the most common ones.

The majority of teleost fish species are gonochoristic, meaning that they exist as males and females. It is generally believed that temperature-dependent sex determination (TSD) is the most primitive and ancestral mechanism of SD in vertebrates. However, confirmed examples of TSD are rare in fish and appear to be a derived state [1]. Other physiological mechanisms of ESD have not been described. This might point to the fact that most gonochoristic species have GSD. However, one has to confess that only a minor fraction of the approximately 25.000 species of fish has been thoroughly investigated and their sex determination mechanism unequivocally clarified [2].

Within the GSD species all variations of the theme have been found, ranging from male or female heterogamety to polygenic sex determination. Importantly, multiple sex chromosomes, e.g. different Y-chromosomes, or autosomal modifiers that enhance or antagonize the sex determining genes on the gonosomes are quite frequent. This together with the fact that even between closely related fish species the sex determination mechanisms can be different [3,4] demonstrates the high evolutionary plasticity of this fundamental process.

This poses several questions. At first we would like to know if the underlying molecular mechanisms and the major sex determination genes are always the same but just switch their chromosomal location or dosage dependent activity or acquire different responsiveness to external cues. Alternatively the molecular mechanisms may be different and evolving independently in different species. In several studies it was described that many genes that were previously identified in man, mouse or chicken to be involved in sex determination have orthologues in fish and show expression patterns consistent with a comparable role in fish [2]. It should be noted, however, that the master sex determining gene of mammals, *Sry*, has not been found outside the eutherians (see [5] for review). A more general question asks for the reason of this plasticity in fish, which is in stark contrast to the situation in birds and mammals where almost the entire class has a genetic sex determination of the ZZ/WZ or XX/XY type, respectively. There is also some plasticity with respect to *Sry* in mammals (lack or multiple copies in some rodents), this is far from the variability observed in teleost fish. Hence, fish are an attractive group of organisms to study the evolution of sex determination in general and of sex chromosomes in particular.

A gene family involved in sex differentiation in organisms as phylogenetically divergent as *Caenorhabditis elegans*, *Drosophila*, corals, fish, frogs, reptiles, birds and mammals, are the DM gene orthologs [6]. Members of this family belong to a known putative transcription factor clade sharing a conserved DNA-binding motif, the so-called DM (Doublesex and Mab-3 homologous) domain. In humans, mice, chickens, alligators, and turtles, expression of the

dmrt1 gene was detected in the developing gonads, and at higher levels in testes compared with ovaries, usually in the late sex-determining or early testis-differentiation period [7-13]. Interestingly, it was shown that the DM intertwined zinc finger-like DNA binding module domains from nematodes, fly or mammals select very similar DNA sequences [14].

In *Drosophila* and *C. elegans* the orthologous protein products regulate in a sex specific manner the transcription of genes involved in establishing the male and female phenotype [15,16]. Much less is known for the orthologous *dmrt1* genes from vertebrates. Their importance, however, for sex determination becomes apparent not only from their male development associated expression pattern but also from the facts that in birds *dmrt1* is present on the Z, but absent from the W [17,18], that in humans haploinsufficiency for *dmrt1* is connected to male to female sex reversal [19], that in *Xenopus laevis* a truncated but functional duplicate is encoded by the W chromosome [20] and last not least from findings in medaka, which will be outlined in detail in this review.

Sex determination in medaka

The medaka, *Oryzias latipes*, is a small freshwater fish species that lives in the small rivers and rice fields of East Asia. During the last decade it became a widely used laboratory fish for developmental and biomedical research, comparable to the well-known zebrafish with which the medaka shares many advantages and characteristics [21]. With respect to sex determination, the medaka is certainly the best understood fish species. It has a XX/XY sex determination system with undifferentiated (homomorphic) sex chromosomes [22,23]. Already in the twenties of the last century, the medaka was the first vertebrate, where sex chromosomal inheritance and sex chromosomal crossovers were described [24]. More recently, the male sex determining gene was identified [22,23], making the medaka so far the only vertebrate species outside mammals, where such a gene has been cloned and shown to be necessary and sufficient for directing testes development.

The medaka male sex-determining gene is a duplicated version of the *dmrt1* gene. The duplicated copy was designated *dmrt1bY* or *dmY*. The ancestral copy, which is located on an autosome (linkage groups), consequently is named *dmrt1a*. The duplicated fragment, consisting of *dmrt1* and the three neighbouring genes, was inserted into another chromosome, which became the Y chromosome, while its homologue became the X (Fig. 1). The duplication event occurred approximately 5-10 million years ago in the lineage leading to the medaka and a sister species, *O. curvinotus* [22,25]. Compared to the human Y, which is more than 200 million years old, the medaka Y is very young and allows to study the initial events of Y-chromosome evolution.

Evolution of the medaka Y chromosome

Numerous studies on the heterogametic sex chromosomes of various plant and animal species and theoretical considerations have led to general concepts for a stepwise process of Y chromosome evolution (see [26-30] for reviews). Y-chromosomes, and similarly W-chromosomes as their heterogametic analogs in the ZZ/ZW system, differ from the X and all autosomes because they are generally degenerated. They have much fewer genes and some of them are specific for the Y, meaning that there is no corresponding allele on the X. Those genes usually fulfil male-specific functions, for instance in spermatogenesis. Y-chromosomes are highly enriched for repetitive DNA and transposable elements and often heterochromatic to a large extent. This peculiar genetic organization of the Y is certainly a derived character. It is logical to postulate that the pair of sex chromosomes at the initial stage of its evolution was, like any pair of autosomes, homologous over the entire length – except for the gene determining the development of the embryonic gonad towards either testis or ovary. As the first step in the evolution of a sex chromosome pair, it has to be postulated that the sex-determining gene (SD) has to stay on the one of the two chromosomes, which in the case of a male-determining gene will become the Y. This means that crossing-over around SD has to be

suppressed. If recombination is suppressed, this will then lead to deleterious mutations in genes, the expansion of repetitive DNA, and the accumulation of transposons, because there is no way to purge these changes by crossing-over to the non-mutated region of the homologous chromosome. The hypothesis predicts that during evolution this non-recombining region expands, making the part of the X and Y, which are still homologous and do recombine (the so-called pseudo-autosomal region), smaller and smaller. Finally, the Y chromosome should collect those genes, which are beneficial for males or antagonistic to females. Due to the hemizygosity in males, the X is predicted to be minimized in content of genes, whose mutational alteration will cause diseases, for instances tumor suppressor genes. Because the entire male specific region of the Y chromosome (MSY) of medaka has been sequenced [31], this information can now be used to evaluate the predictions from the common theory of Y chromosome evolution.

Prediction #1: Sex chromosome evolve from (different) autosomes. For long time it has been believed that (at least in vertebrates) all sex chromosomes, be they X and Y or Z and W, originated from a common ancestor [32]. Later this changed to a view of a more independent descendance. In the case of the medaka Y the duplicated fragment containing *dmrt1bY* was inserted into linkage group 1 (LG1), which corresponds to the second largest pair of chromosomes. LG 1 is homologous to autosomal linkage groups in other *Oryzias* species, where *dmrt1* is not duplicated [33]. As these other (phylogenetically more basal) species represent the ancestral state it defines medaka LG1 as an autosome derived proto-sex chromosome that became then the sex chromosome. The region where *dmrt1bY* was inserted shows conserved synteny to human chromosome 4 (Hsa4). In stickleback the chromosomal region around MSY is syntenic to Hsa15 [34]. Because human and fish chromosomes evolved from a common ancestral karyotype, which is still recognizable through the traces of such conserved syntenies [35], this confirms that different autosomes can become a sex chromosome.

Prediction #2: Sex chromosome evolution starts with a stepwise allelic diversification of one or two loci on a pair of autosomes. This prediction obviously does not hold true for the medaka case, as *dmrt1bY* was inserted into the proto-Y from elsewhere in the genome and did never have an allele on the proto-X. It diverged considerably from its autosomal ancestor *dmrt1a*, but this process is different from what has been postulated for emergence of sex determining genes [26-30], where one allele becomes a male or female determining gene while the corresponding allele at the same locus retains a non-sex determining function or favours development of the opposite sex.

Prediction #3: Recombination around the SD locus is reduced. The hallmark of evolution of a SD gene is that it becomes shielded from crossing over with the homologous chromosome. Only through lack of recombination the identity of the evolving Y chromosome can be maintained. Because the *dmrt1bY* containing fragment stems from a different chromosome (LG9) and was inserted into one of the LG1 chromosomes (Figure 1), it lacked homologous sequences on the partner chromosomes. Thus, the sex-determining gene of medaka was protected from recombination *ab-initio*. The same holds true for the surrounding, coduplicated area from LG9 creating a MSY being larger than one gene already at the birth of the Y.

Prediction #4: The area of no recombination spreads from the sex-determining region into the pseudoautosomal region. On the medaka Y, due to its relatively young age, the pseudoautosomal region, where crossovers between X and Y occur at regular frequencies, is very large and encompasses almost the entire LG1 [25,31]. A region of 3,5 kb around the non-recombining MSY shows single nucleotide polymorphisms (SNP) in the whole genome sequence of medaka [36], indicative of Y and X specific alleles. This region might therefore have reduced recombination, because regions with free crossing over should not yield SNPs in the whole genome sequence of the highly inbred strain that was used. Anyway, a gradual decrease in sequence similarity between the X and Y should be expected at the MSY-

pseudoautosomal border indicating the spread of recombination suppression. However, there is a sharp border that can be nailed down to the single nucleotide level where the non-recombining MSY (which still corresponds and can be recognized by its similarity to the duplicated region from LG9) with no homology to the X ends and an almost 100% homology region of X and Y begins. The reason for this is the presence of a direct repeat flanking MSY, which was created as a “target sequence duplication” when the autosomal fragment was inserted. Consequently there is a homologous region to this repeat on the X, which represents the ancestral situation. Alternating pairings of the left and right copy of the Y chromosomal flanking elements with the single X copy during successive meioses allows for almost regular crossover rates outside the MSY (Fig. 2). This may also explain the unexpected small size of the MSY in medaka, which spans only 250 kb.

Prediction #5: Due to reduced recombination in the MSY genes degenerate. If there is no crossover newly acquired deleterious mutations cannot be corrected by substituting the fragment with the mutation by its non-mutated allelic counterpart. In the medaka MSY, except for *dmrt1bY*, which by all means is a fully functional gene, all other coduplicated genes from the ancestral autosomal locus acquired point mutations and deletions that impair their functions. The same has happened to another gene derived as a duplicate from a different autosome and that was later inserted into the MSY [31].

Prediction #6: Repetitive DNA and transposable elements accumulate. Due to the reduced recombination in the MSY inserted pieces of DNA from elsewhere in the genome, most importantly transposons and repetitive DNA, cannot be eliminated anymore. Also their local expansion cannot be purged. The MSY in medaka contains a lot of repetitive DNA, mostly low complexity and simple repeats, which are mainly responsible for the expansion of the duplicated fragment from 40 kb to 250 kb.

Prediction #7: Male beneficial and sexually antagonistic genes accumulate on the Y. Due to its evolution as an isolated part of the genome the Y chromosome is prone to collect those

genes, which are needed only for the male phenotype, for instance spermatogenesis genes. In an extrapolation of this argument it appears also reasonable that genes, which benefit the male but may be even detrimental to the female, would home one the Y. In medaka sex reversals can be produced for instance by treatments with androgens or estrogens during a sensitive period. By this method XX males, XY females and even YY females can be obtained (the latter being produced by estrogen treatment of the YY offspring from crossing XY females with XY males) [37]. These are not only viable and show no defects, but are even fully fertile, at least under laboratory conditions. This shows that – besides *dmrt1bY* - no essential genes are solely present on the Y. Such fish also demonstrate that due to the degeneration process in the absence of recombination no important genes have been lost from the Y.

Biological functions of *dmrt1bY* as the male determining gene in the medakafish

*1.) The critical role of *dmrt1bY* for normal testicular differentiation*

Dmrt1bY was initially identified as a candidate for the master sex-determining gene due to the fact that it is the only functional gene of the MSY. Its role as being necessary for male development was confirmed through analysis of naturally occurring sex-reverting mutants [22]. Screening of thousands of fish from wild medaka populations [22,38,39] led to the characterization, among others, of two XY females with distinct mutations in *dmrt1bY* [22]. The first mutant (XY^{wAwr} from Awara) has a single nucleotide insertion in exon 3 of *dmrt1bY*, which causes a frame shift at residue 110 and subsequent premature termination at residue 139. It presumably encodes a non-functional messenger RNA. This mRNA appears to undergo nonsense-mediated mRNA decay [40]. The absence of the *dmrt1bY* gene product causes the XY^{wAwr} sex reversal.

The second mutant, due to an unknown transcriptional abnormality, also shows a severe (up to complete) repression of *dmrt1bY* expression in embryos, resulting in the same phenotypic XY^{wSm} sex reversal. This suggests that a fine-tuned threshold level of *dmrt1bY* expression is

required for male gonad development [22]. Taken together, these mutants point to a necessary role of *dmrt1bY* for normal testicular development, although these mutants come from natural populations and might also carry mutations in other genes as well. A spontaneous inactivation of *dmrt1bY* in a genetically defined laboratory strain has not been obtained so far and gene knockout technologies are still not available routinely in fish.

2.) *Dmrt1bY* induces male development in genetically female medaka.

Another line of evidence for *dmrt1bY* as the primary trigger of sex determination would be to show that it is sufficient to initiate male development. This can be investigated by introducing the gene, together with its entire transcriptional control unit, into genetically XX females [41]. To this end, the injection of a *dmrt1bY* genomic fragment (in the form of a BAC clone encompassing 56 kb of the coding, 60 kb of the upstream and 1.4 kb of the downstream regions) into XX individuals was shown to be sufficient to induce male development in more than 20% of these fish reverting the gender phenotype [41]. Similarly, simply over-expressing the *dmrt1bY* ORF under control of a CMV promoter also resulted in male development of 14% of the injected XX individuals. This indicated that the Dmrt1bY protein is sufficient for male development in XX medaka. These reports are intriguing in the light of the fact that the sex reversals were observed in the injected fish (G0), because such fish are generally highly mosaic for any transgene. The situation of genetically mosaic gonads after injection of *dmrt1bY* into embryos is comparable to chimeric gonad transplantation experiments in medaka between blastula embryos using XX recipients and XY donors. Some of the chimeras developed as males despite having only a few XY donor cells in a predominating environment of XX cells [42]. From these experiments it is evident that relatively few XY somatic cells are sufficient to revert the sex of germ cells and somatic cells of the XX recipient gonad. Accordingly *dmrt1bY* likely represents a strong force for testis development in gonads, although fully fertile *dmrt1bY*-lacking XX medaka males have been also described [39,43].

Expression of the medaka *dmrt1* duplicates

As a prerequisite to elucidate the function(s) of medaka *dmrt1* genes, comprehensive and precise information about the temporal and spatial expression profiles of both, *dmrt1bY* and *dmrt1a*, its autosomal ancestor, during embryogenesis and adults as well as characterization of the biochemical properties of their protein products are essential. Interestingly, expression of *dmrt1bY* is detected as early as 1 day post fertilization [23,40]. Then the expression level decreases steadily during the first days of embryonic development to become stable up to 40 dpf (days post fertilization) at around one third of the initial level [40]. In adults, the gene is expressed in testes and, surprisingly, also in spleen. The transcripts in spleen may have no organ specific function as XX animals do not have this expression. Transcripts from the autosomal *dmrt1a* gene are not detected in extracts from whole male fish before 20 days after hatching and at no point in female embryos and larvae [40]. Later on, testis specific *dmrt1a* mRNA expression increases to reach a level that is about 50 fold higher than *dmrt1bY* [40], arguing for a predominant role of *dmrt1a*. This difference is maintained in mature testes [12,40]. The much earlier onset of *dmrt1bY* expression compared to *dmrt1a*, the widespread expression in embryos where *dmrt1a* is not expressed, as well as the significantly more abundant *dmrt1a* levels in adult testes indicate that the sex chromosomal duplicate acquired differences in expression control compared to its autosomal progenitor.

Immunohistochemical analysis using a Dmrt1bY specific antibody revealed the protein to be present primarily in the nuclei of PGC-supporting cells. These are located in the coelomic epithelium under the nephric duct of XY fry during gonadal sex differentiation and testicular differentiation at 1 day before hatching and 5 days after hatching respectively [12]. Noticeably, although the earliest protein expression of *dmrt1bY* is reported from pre-Sertoli cells of the developing testes in hatchlings, *dmrt1bY* transcripts are present from day 1 of development. Thus, the early embryonic mRNA expression may not reflect any function until

a so far unknown mechanism leads to a higher expression and/or stabilization of the protein in the primordial gonad. This is in line with the effect of the mutant for which lower levels of *dmrt1bY* transcripts resulted in sex reversal, supporting the hypothesis of a minimal level of expression for *dmrt1bY* in the primordial gonad at the sex determination stage to be biologically active.

In mature testes, Dmrt1bY protein is not only localized in Sertoli cells during spermatogenesis and the epithelial cells of the intratesticular efferent duct, which are derivatives of the Sertoli cell lineage, but also in the nuclei and cytoplasm of A-type spermatogonia-supporting Sertoli cells [12]. This suggests a possible role associated with spermatogonial differentiation. *Dmrt1a in situ* hybridization using XX sex reversed males revealed expression in Sertoli cells and the intratesticular efferent duct, similar to *dmrt1bY* [12] (Fig. 3).

Putative functions of Dmrt1bY during sex determination and differentiation.

1) Dmrt1bY as a pre-Sertoli inducer?

Several lines of evidences make the role of *dmrt1bY* during Sertoli cell lineage differentiation still controversial. While *dmrt1bY* expression, occurring from embryo to adult specifically in the pre-Sertoli/Sertoli cell lineage, would intuitively propose a critical role as pre-Sertoli cell inducer [12]. A functional study of germ cell deficient medaka gonads temperates this conclusion [44]. In such fish, both, germ cell lacking XX and XY gonads develop as gonad tube-like structures and both express male gonad specific markers such as *dmrt1a*, *sox9b* and *amh* [44]. This suggests that although the supporting cells of both sexes here may not be fully differentiated, their lineages are present and have undergone male development regardless of the presence –or absence- of the *dmrt1bY* gene [44]. As a result, it seems that any role for *dmrt1bY* during early induction of pre-Sertoli cells can be excluded. A function of *dmrt1bY* in the Sertoli cells of the adult males (Figure 3) is difficult to predict at the present state of

knowledge because XX males, which do not have *dmrt1bY* have apparently normal testes structure. If in such fish a possible *dmrt1bY* function is taken over by *dmrt1a* reflecting a redundant role for the two co-orthologues, is not known.

2) *Dmrt1bY* plays a role in germ cell proliferation.

In many non-mammalian females, around the time of morphological sex differentiation, the number of germ cells tends to be greater than in males [45]. At the same time, in contrast to the male germ cells, which arrest in mitosis, the germ cells in females continue to proliferate and subsequently enter meiosis [45,46]. In medaka, the first appearance of a sex difference, long before the formation of the male acinous structure around 20 dpf (10 days after hatching), is the difference of germ cells numbers at hatching stage [12,47], just when *dmrt1bY* reaches a reasonable -above background- expression in the somatic cells surrounding the germ cells. Strikingly, in *dmrt1bY* mutants, proliferation and meiosis of germ cells in the XY embryos is seen, just like in XX embryos. Consistent with this idea, *dmrt1bY* knock down experiments using GripNAs or morpholinos (Figure 3) led to a shift of the larval male gonad phenotype towards female [48,49]. Interestingly, the germ cells of the *dmrt1bY* knock-down larvae entered into meiosis precisely at the same stage as in normal XX female gonads [48]. The underlying mechanism explaining these observations is likely a mitotic arrest of the PCGs mediated by *dmrt1bY* [49] (Figure 3). *In-vitro* studies revealed that *dmrt1bY* expression causes a cell cycle arrest in the G2 phase in a cell-autonomous and non-cell autonomous manner [49].

The biochemical events how *dmrt1bY* leads to the proliferation arrest of the PGCs at the sex determination stage and what the Dmrt1 proteins do in the Sertoli cell lineage are totally unknown thus leaving the exact molecular mechanisms of the male determining function still in the dark. Nevertheless, the fact that *dmrt1bY* down-regulates primordial germ cell proliferation already 10 days before somatic gonadal differentiation suggests that in XY

medaka *dmrt1bY* driven PGC number regulation is one of the primary events by which the whole gonad -including germ line and soma- would be specified through a directional cross-talk from pre-Sertoli cells to PGCs. Then, at hatching stage, Sertoli cells would continue to inhibit primordial germ cell proliferation in the male primordial gonad through juxtacrine *dmrt1bY* action.

The autosomal *dmrt1a* gene, due to its specific expression pattern, is implicitly associated with spermatogonial differentiation and generally with the regulation of spermatogenesis [12], but not with the primary sex determination process. Unfortunately, the late *dmrt1a* expression (around two weeks after fertilization) makes any morpholino-based strategies inappropriate. Alternatively, newly emerging fish reverse genetic methods *via* targeted gene disruption using designed zinc-finger nucleases or the generation of loss of function mutants by TILLING [50,51] may be a suitable alternative [52,53].

A comparative view of sex determination molecular mechanisms of medaka and mammals

Other than in medaka where X and Y chromosomes appear to differ mainly by a single gene, the Y-chromosomal *dmrt1bY*, in mammals the Y chromosome differs considerably from the X chromosome. While the number of functional genes is around 1100 for the mammalian X chromosome [54], the male-specific region of the Y chromosome contains only 156 known transcriptional units, which include 78 protein-coding genes that collectively encode only 27 different proteins, including 10 testis-specific genes and *Sry* [55]. In medaka, the X and the Y have the vast majority of genes in common, and the Y has even at least one gene in addition, namely *dmrt1bY*. Interestingly, *Sry* is thought to have arisen from either an autosomal *Sox* gene duplication event followed by subsequent formation of the Y chromosome, or, more probably, from *Sox3* (a X chromosomal gene) after divergence of the sex chromosomes [56].

Hence, if these scenarios suggest an evolutionary origin pattern similar to the one established for *dmrt1a* and *dmrt1bY*, how far a comparison between *dmrt1bY* and *Sry* stands?

Sry expression during mouse gonadogenesis is tightly regulated. *Sry* mRNA and protein expression start at 10.5 dpc in the center of the XY genital ridges, encompass the whole length of the gonad and reaches a peak at 11.5 dpc. Then expression recedes from anterior to posterior at 12 dpc, with the last positive cells detectable around 12.5 dpc at the caudal pole (see [5] for review). *Sry* expression at 11.5 dpc is associated with the somatic cells of the genital ridge and not the germ cells [57] (Fig. 3). Transgenic mouse models expressing GFP [58] under the control of the *Sry* promoter confirmed that *Sry* expression is restricted to the Sertoli cell lineage, or more accurately the pre-Sertoli cells. It is noticeable that both *Sry* and *dmrt1bY* are DNA-binding, putative transcription factors and are expressed in the pre-Sertoli cells of the gonadal primordium although the time of the enclosure of PGCs by the somatic cells is different between mice and medaka (Fig. 3). To conclude, although *dmrt1* would be one of the downstream effectors of *Sry* in most of the mammals, the timing of their expression during the respective sex determination windows in mice and medaka is compatible with a similar conceptualized action on this process as a molecular switch for pre-Sertoli cell induction (Fig. 3).

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Figure legends

Fig. 1. Origin and evolution of the sex chromosomes in medaka.

(a) A segment on linkage group (LG) 9 containing the *dmrt1* gene and neighbouring genes including the paralogs *dmrt3* and 2 is duplicated [31]. (b) The duplicated segment is inserted into one of the chromosomes of LG 1. This chromosome is the proto Y while its homologue becomes the proto X. (c) Due to lack of recombination between the duplicated fragment on the Y and the X genes not involved in sex determination degenerate and transposable elements and repetitive sequences accumulate.

Fig. 2. Restriction of the area of no-recombination by alternate pairing of the MSY flanking direct repeat.

Crossing over can occur in the X and Y homologous regions (pseudoautosomal region of the sex chromosomes) left and right of the MSY. The size of the direct repeat flanking the MSY is approximately 20 kb [31].

Fig. 3. A comparative view of sex determining cascades of medaka and mammals.

Molecular pathways leading to the formation of the gonad in medaka and mice. Conserved *dmrt1* transcription factors are indicated with light gray boxes. A comparative view of *dmrt1*-related factor expression patterns during development, as well as the effects in mutant knockout mice, mutant and morpholino injected medaka, is also provided.

Figure 1

Figure 2