Studies on the molecular mechanisms underlying the histological gradient of gonadal sex differentiation in Pejerrey

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Studies on the molecular mechanisms underlying the histological gradient of gonadal sex differentiation in pejerrey （ペヘレイの性分化過程における組織学的勾配の分子機構に関する研究）		

The pejerrey *Odontesthes bonariensis* is considered as the most marked case of temperature-dependent sex determination (TSD) among teleosts. In this species, all-female and all-male populations can be obtained by rearing the larvae at low (17°C) and high (29°C) temperatures, respectively, whereas both sexes are formed when larvae develop at intermediate temperatures (around 24-25°C). At this intermediate range, sex is influenced by an genetic factors. The influence of temperature for sex determination in pejerrey is generally observed between 1 and 5 wah (weeks after hatching) and therefore it is conceivable that this period is critical for the sex determination in this species. In spite of its marked TSD, pejerrey belongs to the differentiated type of gonochorists (e.g. ovaries and testes develop directly from an indifferent primordia) and intersexes are extremely rare. Also, histological gonadal differentiation follows a characteristic cephalocaudal, left-to-right gradient regardless of sex, whereas intense apoptosis in the anterior region of the right gonad has been tentatively associated with testis differentiation. The sex-related genes *amha*, *amhy*, and *cyp19a1a* (gonadal aromatase) are thought to play important roles in the sex differentiation process of pejerrey, but their possible interplay and relation with the differentiation gradient are still unclear. The purpose of this study is to clarify the molecular mechanisms underlying the histological gradient of gonadal sex differentiation in pejerrey.

In the first chapter, experiments were conducted to examine the spatiotemporal correlations between amh and cyp19a1a transcript expression and apoptosis during gonadal sex differentiation of pejerrey at a sexually neutral temperature. Progeny from a single XX ($amhy^{-/-}$) and XY ($amhy^{+/-}$) cross was reared for 14 weeks at 25°C and fish were analyzed weekly to examine the degree of histological gonadal differentiation and the location/intensity of gene expression in the gonads by *in situ* hybridization, and at the end of the period to estimate the sex ratio of the progeny. Ovarian and testicular differentiation began at 4 and 7 weeks, respectively and the XX and XY individuals were 36% and 96% male, respectively. *amh* expression was detected from 1 and 2 weeks in the left and right gonads, respectively, of almost all XY fish and about 1/3 of the XX fish; in both cases, *amh*-expressing cells were typically more abundant in the left gonad than in the right gonad and expression expanded from an initial site in the anterior region towards more posterior regions of the gonads. *cyp19a1a* expression was observed from 2 weeks in the anterior region of both gonads regardless of genotype but was maintained only in XX gonads without *amh* expression. Apoptosis appeared first in the anterior region of the right gonad and then expanded to the posterior regions of the same side; it was virtually absent from and when present had limited intensity in the left gonad. Gonadal apoptosis was observed from 2-4 wah and became abundant in almost all larvae reared at 25°C regardless of genotype. The expression profiles of *amhs* and *cyp19a1a* allowed to distinguish the presumptive phenotypic sex at an early stage of development in both the XX and XY genotypes. Thus, presumptive males have intense and faint or inexistent *amhs* and *cyp19a1a* expression, respectively, whereas females have the opposite pattern. Also, both genes showed a gradient of expression that was consistent with the gradient of histological differentiation, from anterior to posterior regions and from the left to the right gonad. The appearance of *cyp19a1a* expression at an early developmental

stage in both genotypes and presumptive sexes suggests that the females may be the default state in pejerrey, at least at this intermediate temperature.

The second chapter examined the effects of feminizing and masculinizing temperatures on the spatiotemporal patterns of expression sex related genes and apoptosis. irst chapter. Sex ratios at 17 and 29ºC were 40% and 100% male, respectively and testicular and ovarian differentiation started at 5 and 8 wah at 29 and 17ºC, respectively. *amhs* (*amha* and *amhy*) transcripts were first detected from 1 and 2 weeks in the anterior region of the left and right gonads, respectively, in individuals reared at 29°C regardless of genotype, whereas *cyp19a1a* expression was completely suppressed at this temperature. XY fish at 17°C could be divided in two subgroups, one with faint *amhs* expression and strong *cyp19a1a* expression (72% occurrence), and another with strong *amhs* expression and faint or negligible *cyp19a1a* expression (28%) that, based on the relative concordance with the sex ratios (60% female: 40% male), were assumed to represent presumptive females and males, respectively. All XX fish at 17°C had complete suppression of *amhs* and, on the contrary, abundant *cyp19a1a* expression from 3 weeks. As 25°C, gonadal apoptosis was observed from 2-4 wah and became abundant in almost all larvae reared at 29°C regardless of genotype. At 17°C, apoptosis was observed in about half of the individuals of both sexes from 6-7 wah and was much less intense than at the other temperatures. Altogether, there was no association between the incidence of apoptosis on any particular week and the resulting phenotypic sex ratios but, interestingly, apoptosis was observed 1-2 weeks earlier in the XY than in the XX at all temperatures studied. The gradients of *amhs* and *cyp19a1a* expression observed in the first chapter were also observed and were not affected by the masculinizing and feminizing temperatures. These results indicate that the high temperature promotes expression of *amhs* and suppresses *cyp19a1a* expression, overriding the default female pathway. On the other hand, the low temperature suppresses *amhs* expression and promotes *cyp19a1a* expression, leading to feminization in some of the XY. Moreover, at all temperatures there was a fairly high correlation between the occurrence of apoptosis in the right gonad and the disappearance or reduction of the expression of both *amhs* and *cyp19a1a* in the same area.

In summary, the spatiotemporal expression of *amhs*, *cyp19a1a*, and apoptosis supports a cephalocaudal, left-to-right gradient of gonadal sex differentiation in pejerrey at the molecular level. The location and timing of *amh*s, *cyp19a1a* and apoptosis seems highly coordinated among themselves and with the time of gonadal sex differentiation. Since all animals show *cyp19a1a* expression at an early stage regardless of the genetic or presumptive phenotypic sex, it is surmised that the female may be the default state in pejerrey. Likewise, the results also suggest that the early expression of *amhs* (conceivably related with the presence of *amhy* in XY fish and of environmentally-induced stress in the XX) antagonizes the expression and effects of *cyp19a1a*, causing to testicular formation. This study also provided a novel role for apoptosis in gonadal differentiation of pejerrey. Apoptosis was shown to occur typically in the anterior region of the right gonad in individuals of both sexes and generally coincided spatiotemporally with the reduction or disappearance of *amhs* and *cyp19a1a*. Thus, rather than a direct role of apoptosis in testicular formation, the results suggest that early apoptosis in the right gonads could be a mechanism to delay differentiation in this area until it is firmly established in the left gonad, and hence prevent gonadal ambiguity (intersexes) that could occur if different areas of the gonads responded differently to environmental signals. According to this view, in putative females there is no conflict with the default sex, so apoptosis is delayed or even unnecessary, leading to the casual observation that apoptosis is more common in putative males. In other words, the molecular data obtained in my thesis supports the notion that gonadal sex differentiation in pejerrey starts in the anterior region of the left gonad and that the right gonad simply follows the direction set by the left side, and that the delay in the right side is probably driven by apoptosis. Further studies are needed to confirm the female-default and molecular gradient/apoptosis-based intersex prevention hypotheses put forth in this work and examine their existence in other TSD species.