Application of androgenesis process to induce haploid and doubled haploid development in several salmonid fish (Salmonidae) species

Oliwia Michalik

Sexual reproduction is the most common type of reproduction among animals. It is based on males and females producing haploid gametes which fuse and form a diploid zygote. The resulting organism carries genes of both parents. Atypical forms of sexual reproduction have been also described. Gynogenesis is a reproductive mode whereby offspring inherit only maternal chromosomes. Gynogenesis has been reported in small number of invertebrates, fish and amphibians. In turn, exclusively paternal inheritance of the nuclear genome is known as androgenesis. Although less frequently observed than gynogenesis, androgenesis has been confirmed in the interspecies hybrids of stick insects from genus *Bacillus*, fruit fly (*Drosophila melanogaster*) and several species of *Corbicula* clams. The first report describing a spontaneous androgenesis in fish (*Squalius alburnoides*) was published in 2017.

Both, androgenetic and gynogenetic development, can be induced artificially under controlled conditions. In fish, such processes require genetic inactivation of gametes achieved by their exposition to high doses of ionizing or UV radiation which destroys the nuclear DNA. Androgenetic development is induced by insemination of the irradiated eggs with untreated spermatozoa whereas gynogenetic individuals develop as a result of egg activation performed with UV-treated sperm. The haploid set of paternal or maternal chromosomes is doubled by means of interrupting the first cleavage of the zygote. For this purpose, haploid embryos are exposed to environmental shock (e.g. high hydrostatic pressure or sublethal temperatures) at a precisely defined point after DNA replication but before karyokinesis. Environmental shock results in the spindle depolymerisation, prevents chromosome segregation and leads to duplication of the maternal or paternal set of chromosomes. Provided specimens are fully homozygous androgenetic or gynogenetic Doubled Haploids (DHs). Haploid androgenetic and gynogenetic fish embryos are useful in research regarding phenotypic consequences of the recessive alleles, functions of particular genes and tumorigenesis. Such embryos are also the excellent source of haploid embryonic stem cells vastly used in the biomedical studies. Haploids and Doubled Haploids are useful in studies concerning gene mapping and genome sequencing. Gynogenesis and androgenesis processes have been applied in selective breeding programs in order to produce all-female or all-male stocks of fish and specimens with the desired breeding traits. Moreover, androgenesis utilizing cryopreserved sperm enables recovery of the gene pools of valuable fish lineages, populations or even species (interspecies androgenesis). Unfortunately, high mortality of the androgenetic fish is a limiting issue for application of androgenesis in practice. Only a small number of androgenetic specimens hatch and survive till the swim-up stage (c.a. 5%), and only few of such individuals reach sexual maturity. Increased mortality of the androgenetic fish results mostly from expression of the recessive alleles. However, side effects of the drastic treatments applied to gametes (irradiation, high hydrostatic pressure/temperature shock) and influence of sex chromosomes may also affect survival of the androgenetic individuals.

The main goal of this work has been to obtain viable androgenetic offspring of chosen salmonid fish species (Salmonidae). Specific objectives of performed studies included: application of gametes from closely related species and their hybrids to induce androgenesis [1], assessment of the biological consequences of the egg exposition to ionizing radiation and application of high hydrostatic pressure to the haploid embryos [2], examination of the sex chromosomes influence on survival rate of haploid [3] and diploid [4] androgenotes and analysis of the sex differentiation and gonadal development processes in androgenetic females (XX) and males (YY) [4].

The choice of salmonid fish for the research was not arbitrary. This family is of the autotetraploid origin and it mainly includes predatory species of a great economic value. Unfortunately, overexploitation of the salmonids combined with their susceptibility to anthropogenic factors endanger their populations. Hence, salmonids are a common subject of research concerning breeding and conservations of gene pools that involve studies related to population genetics, reproduction and embryonic development.

Gametes used for the research were obtained from the fish bred in the aquaculture facilities of Department of Salmonid Research (Rutki), The Stanislaw Sakowicz Inland Fisheries Institute, in Olsztyn. The roe intended for inactivation of the nuclear genome was transported to the Clinic of Oncology and Radiotherapy of the University Clinical Centre in Gdańsk, Medical University of Gdańsk, where it was exposed to X-ray using the linear accelerator Clinac 600. The irradiated eggs were then transported back to the hatchery in Rutki to carry on the further steps of the experiments. Irradiated and non-irradiated eggs were inseminated in order to obtain haploid androgenotes and normal diploid specimens (control groups). During one of the experiments, sperm intended for the gynogenesis induction was treated with UV light, and then used to activate the non-irradiated eggs.

At the proper moment after insemination, part of the activated irradiated eggs was subjected to the high hydrostatic pressure shock in order to restore the diploid state of the embryos under conditions established for each species. Remaining activated eggs were developed as haploid specimens. The survival analysis of progeny was conducted at the eyed stage (pigment appearing in the embryo's eyes), at hatching and at the swim-up stage (after complete resorption of the yolk-sac). Inheritance of only paternal (androgenesis) or maternal (gynogenesis) nuclear DNA and the homozygosity of the androgenotes or gynogenotes were confirmed by the microsatellite DNA analysis. Efficiency of the nuclear DNA inactivation and ploidy of the cells were examined by means of the cytogenetic analysis. Genetic sex of rainbow trout was determined with microscopic identification of sex chromosomes and amplification of Y chromosome linked DNA sequences. Nuclear DNA and metaphase plates were obtained from embryos, larvae, fry and mature fish.

It has been assumed that androgenetic embryo is able to develop in the irradiated egg of another species. Another assumption is that application of eggs originated from hybrid specimens enables successful development of individuals that belong to the parental species. In order to verify these hypotheses, androgenetic development of the brook trout (*Salvelinus fontinalis*) and Arctic charr (*Salvelinus alpinus*) was induced in eggs of both species and in the eggs collected from hybrids of the brook trout and Arctic charr [1]. Unfortunately, almost all embryos developing in the eggs obtained from different species died at an early stage of embryogenesis. Only one brook trout developing in the Arctic charr egg survived until the eyed stage. In the same experiment, proper development of androgenetic brook trout was observed in the eggs from the same species as well as in eggs obtained from hybrids. Some fish from these groups hatched and survived until the fry stage [1].

In order to assess influence of ionizing radiation on the androgenesis efficiency, survival rates of androgenetic and gynogenetic brown trout were compared. In the same experiment, effect of pressure shock on the embryo development was assessed by comparing survivability of haploids and doubled haploids [2]. Significantly higher mortality was observed among the androgenetic progenies. At the eyed stage, considerably higher survivability of the haploid than the diploid larvae was noticed. Furthermore, increased ratio of haploids was observed among androgenetic embryos developing in eggs subjected to the high hydrostatic pressure [2].

Large genetic differences between mammalian X and Y chromosomes result in reduced developmental capacities of haploid embryos with Y chromosome. In several fish species sex chromosomes are morphologically and genetically differentiated which may affect development of androgenetic embryos and reduce survival of those with Y chromosomes. To assess effect of sex chromosomes on the androgenetic development in rainbow trout, androgenesis was induced with the use of sperm obtained from typical, heterogametic males (XY) and from homogametic neomales (hormonally masculinized females) (XX). Groups of androgenotes with the X chromosomes and groups, in which half of the specimens carried X chromosomes while the other half carried only Y chromosomes, were produced. The analysis of sperm by CASA system showed spermatozoa of typical males and neomales exhibited comparable characteristics including motility, morphology and density. No differences in survival rates between rainbow trout androgenetic specimens with X and Y chromosomes were observed [3, 4]. Histological analysis of the diploid androgenotes confirmed proper development of gonads in females (XX) and males (YY). Only one of the eight androgenetic females revealed presence of atypical ovaries with overgrowth of interstitial tissue and oocytes in various stages of maturity [4].

High mortality of androgenotes results mostly from expression of recessive alleles. However, in the present work it has been shown that it is also a side effect of exposure to ionizing radiation and sublethal doses of high hydrostatic pressure. High doses of X and gamma rays destroy maternal nuclear genome but also disregulate cell cycle and delay the 1st mitotic division in embryos developing in the irradiated eggs. High hydrostatic pressure applied after egg activattion enables recovery of diploid state in the androgenetic and gynogenetic embryos. Hydrostatic pressure impairs the spindle apparatus as well as destabilizes and disorganizes other cellular organelles which result in lower survivability of the specimens that were subjected to this treatment. In case of the interspecies androgenesis, mortality of embryos is also the result of the egg cytoplasm and the sperm genome conflict. Utilization of eggs originated from the interspecies hybrids in the process of androgenesis induction reduced effects of this conflict and thus enabled generation of androgenetic offspring. Moreover, it was proved that sex chromosomes do not affect androgenetic development. Provided results exhibited genetic differences between rainbow trout X and Y chromosomes are too small to influence survivability of the androgenetic offspring. Sex differentiation and gonadal development progressed correctly in these fish, regardless of their genetic sex.

References

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