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Deep Ancestry of Programmed Genome Rearrangement in Lampreys

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Abstract

In most multicellular organisms, the structure and content of the genome is rigorously maintained over the course of development. However some species have evolved genome biologies that permit, or require, developmentally regulated changes in the physical structure and content of the genome (programmed genome rearrangement: PGR). Relatively few vertebrates are known to undergo PGR, although all agnathans surveyed to date (several hagfish and one lamprey: Petromyzon marinus) show evidence of large scale PGR. To further resolve the ancestry of PGR within vertebrates, we developed probes that allow simultaneous tracking of nearly all sequences eliminated by PGR in P. marinus and a second lamprey species (Entosphenus tridentatus). These comparative analyses reveal conserved subcellular structures (lagging chromatin and micronuclei) associated with PGR and provide the first comparative embryological evidence in support of the idea that PGR represents an ancient and evolutionarily stable strategy for regulating inherent developmental/genetic conflicts between germline and soma.

Keywords

Vertebrate; Genome; Evolution; Lamprey; Embryogenesis; Programmed Genome Rearrangement

INTRODUCTION

Programmed genome rearrangement has been observed in several vertebrate and invertebrate taxa and appears to have arisen multiple times over the evolutionary history of eukaryotes [1]. Notably, PGR has been previously observed within two deeply diverged vertebrate groups (jawless vertebrates). Reproducible differences in the structure and content of germline and somatic cells have been reported for all hagfish species surveyed to date [2]. More recently, PGR was also discovered and characterized in the sea lamprey (Petromyzon marinus) [3–6].

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The lineage leading to P. marinus diverged from the ancestral lineage of all other extant (jawed) vertebrates and the lineage leading to hagfish approximately 500 million years ago (MYA) [7], raising questions as to the ancestry and evolutionary significance of PGR in P. marinus, ancestral gnathostome lineages and the ancestral vertebrate lineages.

Recent efforts have made progress in identifying the gene targets and cellular mechanisms of PGR in P. marinus [4–6]. These analyses indicate that PGR contributes to early differentiation events in the embryo that define somatic and germline lineages, wherein DNA elimination acts to permanently silence “germline” genes in somatic cell lineages. Notably, lamprey PGR parallels differentiation events that occur early in gnathostome development with respect to the genes that are relegated to the germline [4,6] and with respect to the earliest epigenetic modification events that mediate these events [5]. Given the available data, it is possible that observation of PGR in hagfish and sea lamprey reflects the inheritance and maintenance of PGR from their common ancestor, inheritance from the common ancestor of all extant vertebrates, or the independent evolution of superficially similar genome biologies in two deeply diverged vertebrate lineages similar to that observed for closely related finch species [7,8]. While several embryological and genetic details of PGR have been characterized in P. marinus, analogous datasets do not currently exist for any other rearranging vertebrate, which has precluded direct comparative analyses that are necessary to begin evaluating these alternate evolutionary scenarios.

Here we describe the development of new hybridization-based approaches for performing comparative embryological studies of PGR and use these methods to further resolve the ancestry of PGR in the lamprey lineage. These studies reveal several conserved cellular/developmental features of PGR in two divergent lamprey species (lagging chromatin and micronuclei), observations that are interpreted as strong evidence that PGR has occurred in lampreys for at least the last 40 million years.

RESULTS and DISCUSSION

To shed light on the deeper evolutionary history of PGR in the lamprey lineage, we obtained early-stage embryos from the Pacific lamprey (Entosphenus tridentatus). The species E. tridentatus was selected as a representative of a clade of lampreys (genera Entosphenus, Lethenteron and Lampetra) that diverged from the sea lamprey’s lineage ~40 MYA, corresponding to the deepest divergence within the family Petromyzontidae (Northern Hemisphere lampreys) [7]. Presumably features shared between P. marinus and E. tridentatus reflect aspects of their biology that were inherited from the common ancestor of all petromyzontids. Pacific lamprey embryos were generated using husbandry and in vitro fertilization methods optimized for the species [7]. Embryos were sampled at 1, 2 and 3 days post fertilization (Tahara stages 7, 9/10 and 11, respectively)[9], fixed and cleared according to protocols developed for P. marinus [5]. Examination of E. tridentatus embryos revealed anaphases with lagging chromatin at 2 days post fertilization and interphase cells with micronuclei similar to those observed in P. marinus embryos at the same developmental stages (Figures 1 and 2). Lagging chromatin and micronuclei are considered hallmarks of PGR in P. marinus [5]. We interpret these observations as strong evidence that PGR occurs in both species through similar, highly orchestrated events. Notably, micronuclei and lagging

segments appear to be smaller in E. tridentatus when compared to those in P. marinus. Given the large difference in genome size between P. marinus (<2 Gb) and other petromyzontids (<1.5 Gb)[10] observed size differences may not be particularly surprising. This difference in genome size presumably reflects the recent expansion of repetitive elements within the P. marinus genome, which harbors an exceedingly large number of high-identity repeats[11,12].

To further test the assumption that lagging chromatin in divergent lamprey species reflects a shared evolutionary origin of PGR, we developed probes that specifically label eliminated chromatin in P. marinus throughout embryogenesis. These probes were generated by first isolating lagging chromatin from individual anaphases via laser capture microdissection, then amplifying and labeling captured sequences. Hybridization to P. marinus embryos confirmed that these probes specifically label eliminated chromatin before and during PGR (Figure 1). These probes yielded strong and specific hybridization to lagging chromosomes within elimination anaphases and to micronuclei within interphase cells that had recently undergone PGR (Figures 1, 2A, 2C). Notably, these hybridization signals mark several segments of lagging chromatin that also contain Germ1, a multicopy sequence known to be eliminated via PGR [3]. These same probes were also found to hybridize to lagging chromatin and micronuclei in E. tridentatus embryos (Figure 2B, D). These cross-hybridization experiments reveal that phenotypically similar structures associated with PGR in P. marinus and E. tridentatus (lagging chromosomes and micronuclei) also contain similar sequences, lending further support to the idea that PGR is a shared ancestral feature of these two lamprey lineages.

Performing hybridizations in the presence of labeled somatic repetitive DNA (liver CoT_2 DNA) allowed us to competitively suppress cross-hybridization with retained chromosomes and visualize the differential expansion of repetitive elements in E. tridentatus. Hybridization with conspecific CoT_2 yielded strong signals on retained chromosomes and the centromeres of lagging chromatin in P. marinus and E. tridentatus. Notably, hybridization of P. marinus CoT_2 to E. tridentatus yielded signals that were more broadly distributed, including punctate signals on retained and eliminated chromatin as well as signals that overlapped fainter regions of cross-hybridization to our P. marinus germline-specific probe (Figure 2E–H). We interpret these patterns as evidence for the movement of repetitive sequences between germline-specific and retained chromatin and differential expansion within these genomic compartments. These comparative hybridizations reveal broad evolutionary changes in the repeat content of both retained and eliminated chromatin over the last ~40 Million years of lamprey evolution and provide the first evidence for movement of sequences between germline-specific and somatically-retained regions.

Taken together, we interpret these findings as strong evidence that PGR existed in the ancestral lineage that gave rise to all extant species within the family Petromyzontidae, which includes 6 genera and 29 described species that are distributed across the Northern Hemisphere. It therefore appears that PGR occurs in the vast majority of, if not all, agnathan species. While these studies do not necessarily indicate that PGR occurred in the common ancestor of all agnathans (or all vertebrates), they do provide further evidence that PGR represents an ancient and evolutionarily stable strategy for regulating inherent genetic conflicts between germline and soma.

METHODS

PACT Clearing

Lamprey embryos were fixed, embedded in hydrogel and cleared according a modified PACT (passive clarity technique) protocol recently developed for lamprey embryos [5,13]. Briefly, embryos were fixed in 4% paraformaldehyde and gradually transferred to 100% methanol and stored at −20°C [14]. Prior to clearing embryos were gradually rehydrated in 1X PBS then perfused with hydrogel monomer solution (5% acrylamide supplemented 0.5% VA-044) by incubating overnight at 4°C. Hydrogel polymerization was performed at 37°C for 2.5 hours. After brief washes with 1X PBS, embryos were transferred in 50 ml screw-cup tube and incubated in stripping solution (8%SDS in 1X PBS) for 3–5 days at 37°C with gentle rotation. Upon reaching transparency samples were washed in 1X PBS with 5 buffer changes over the course of a day and transferred into staining solution (1X PBS, pH=7.5, 0.1 Triton X-100, 0.01% sodium azide).

Preparation of Genomic-, Cot-, Germ1- Probes

Genomic lamprey DNA was isolated by phenol-chloroform extraction [15] and Germ1 probes were generated from a previously characterized clone [3]. Cot1 and Cot2 fractions were isolated from genomic DNA using S1 nuclease to digest single stranded (low copy) DNA as described previously [16]. Probes for in situ hybridization were labeled by nick-translation using biotin-11-dUTP (Thermo) or direct fluorophores: Cyanine 3-dUTP (Enzo) and Fluorescein-12-dUTP (Thermo) according to previously published protocols [15,16].

Laser Capture Microscopy and Development of Elimination-Specific Probes To aid in laser capture microdissection (LCM) of lagging chromatin, we developed a colorimetric DNA stain that circumvents issues associated with background staining and signal loss following dry mounting to LCM membrane slides (e.g. with DAPI, SYTO and Giemsa) and permits precise visualization of DNA in PACT-cleared lamprey embryos via light microscopy. To robustly label DNA in cleared embryos we developed a biotinylated DNA-probe via nick-translation labeling lamprey germline genomic DNA. Hybridization of the biotinylated probe to whole cleared embryos was performed as described above for 3-D FISH. After washes, samples were incubated in blocking solution (Vector Labs) for 1 hour at 37°C, then in alkaline phosphatase streptavidin (Vector Labs, 1 μg/ml in 1x blocking solution) at the same conditions. Samples were subsequently washed in 100 mM Tris, pH=9 buffer, 30 min at room temperature and treated for color development with BCIP/NBT Substrate kit according to manufacturer instruction (Vector Labs).

After color development embryos were incubated with stripping solution overnight at 37°C and washed as described above. For LCM, 1–2 embryos were transferred to a 1.0 mm PEN-membrane slide (Zeiss) and flattened under HybriSlip™ cover (Sigma). After 10 min of incubation on a 45°C plate, the cover film was carefully removed and slides were dried in sterile box in the presence of dessicant and under slight vacuum. Laser capture was performed using Zeiss PALM Laser Microbeam Microscope. Approximately 10 lagging structures were collected per adhesive cap tube (Zeiss). Samples where re-suspended in 10 μl of sterile water by vortexing the tubes in an inverted position for 30 min and centrifuging

at 6000 g for 5 min. WGA library construction, amplification, reamplification and labeling procedures were performed using GenomePlex® Single Cell Whole Genome Amplification Kit (Sigma) and GenomePlex® WGA Reamplification Kit (Sigma) following the manufacturer instructions. For labeling, Cyanine 3-dUTP (Enzo) or ChromaTide® Alexa Fluor® 594-5-dUTP (Thermo) were used along with 10 mM dATP, dCTP, dGTP, and 3 mM dTTP, replacing dNTP mix from the reamplification step.

3D-FISH

To enhance accessibility of DNA to hybridization, cleared embryos were incubated in 8% sodium thiocyanate overnight at 37°C, then washed in PBS for 30 minutes at room temperature. For each FISH experiment, 4–5 embryos were placed in 2 ml tube with 50% formamide and incubated 1 hour at 37°C. After incubation formamide solution was discarded and 30 μl hybridization mix (50% formamide, 10% dextran sulfate, 200 ng labeled DNA-probe in 1.2xSSC) was added to cover embryos. For competitive hybridizations two or more probes were mixed in equal ratios (200 ng each). Co-hybridized probes should preferentially label their targets and suppress lower-affinity hybridizations, particularly to repetitive sequences. Samples were incubated overnight at 37°C to permit probe penetration, then denatured at 75°C for 5 min. Samples were rapidly cooled in an ice-bath, then left to hybridize at 37°C for three days. After hybridization, samples were washed subsequently in 0.4X SSC, 0.3% IGEPAL CA-630 and 2xSSC, 0.1% IGEPAL CA-630 for 30 min each at 45°C. For visualization, 2–3 embryos were placed on a slide and mounted with DAPI (VectaShield) after removing remaining wash buffer.

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Highlights

- Lagging chromatin is observed in Pacific lamprey (Entosphenus tridentatus) embryos.
- Probes were developed using laser capture of eliminated chromatin from sea lamprey.
- Cross-hybridization reveals homology of eliminated segments in two lamprey species.
- Findings indicate that most lamprey species undergo programmed genome rearrangement.
- Repetitive probes reveal movement of sequences between eliminated and retained DNA.

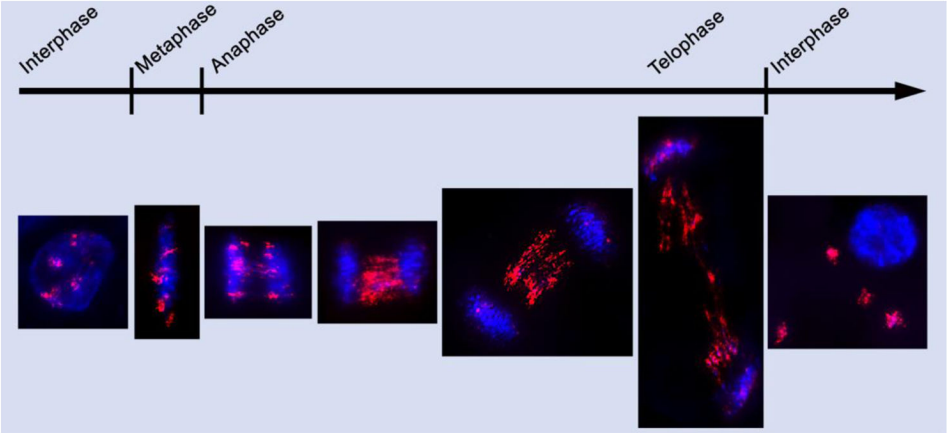

Figure 1.

Tracking germline-specific DNA before and during programmed elimination. Laser-capture FISH probes mark eliminated sequences during all phases of the cell cycle, including those that precede the first cellular events known to be associated with programmed genome rearrangement (lagging of eliminated chromatin). Cells are hybridized with probes generated from amplified lagging chromatin that was isolated by laser capture from P. marinus (eliminated DNA: red) and counterstained with DAPI (blue).

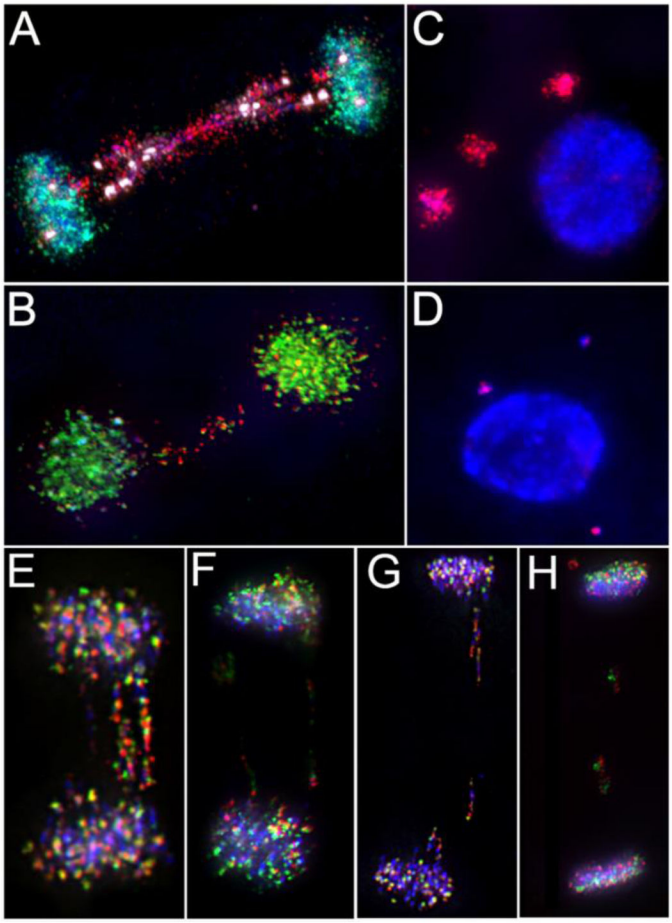

Figure 2.

Evidence for PGR in Northern Hemisphere lampreys. A–H) Cells undergoing DNA elimination in P. marinus and E. tridentatus embryos. Cells are hybridized with probes generated from amplified lagging chromatin that was isolated by laser capture from P. marinus (eliminated DNA: red) and counterstained with DAPI (blue). A) An elimination anaphase from P. marinus. This anaphase is also labeled with somatic CoT_2 DNA (green) and the eliminated element Germ1 (white). B) An elimination anaphase from E. tridentatus, counter-labeled with E. tridentatus CoT_2 DNA. C–D) Post-elimination interphase cells showing localization of eliminated DNA to micronuclei in C) P. marinus and D) E. tridentatus. E - H) Elimination anaphases from E. tridentatus, counter-labeled with P. marinus somatic CoT_2 DNA (green). E) early/mid anaphase F) mid anaphase G,H) late anaphase.