Brief Gene Mapping Report

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Physical mapping[1] of the 5S rRNA genes in the common sea urchin, *Paracentrotus lividus* (Echinodermata: Echinoidea), by in situ hybridization

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[1] To our knowledge this is the first time this gene has been mapped in the common sea urchin.

Rationale and significance

Locations of the 5S rRNA genes (rDNA) are usually considered to be helpful, as generally invariable chromosome markers, especially if chromosome banding is not possible. The genes have been defined in about 90 invertebrate species, and were found linked to other multigene families in genomes of some nematodes and arthropods (Drouin and Moniz de Sa, 1995; Barzotti et al., 2000). They were also found co-localized by FISH with major rDNA clusters (NORs) and telomeric repeats in some annelids and molluscs (Vitturi et al., 2002, 2004). Here we report for the first time the chromosomal localization of 5S rDNA in a species of echinoderms, *Paracentrotus lividus*, which, compared to other echinoderms, has an exceptionally low chromosome number, 2n = 36 (Boveri, 1902). Its karyotype is composed of one pair of large submetacentric chromosomes, a pair of sex-specific heteromorphic chromosomes, and 16 pairs of subtelocentric chromosomes (Lipani et al., 1996).

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Materials and methods

Specimens and chromosome preparations

Adult specimens of *P. lividus* were collected in the gulf of Palermo and kept in aquaria culture for several days. Mature eggs were fertilized and embryos at the stage of 32–64 blastomeres were dissociated in Ca-free sea water, as described by Polyakov et al. (1992). Chromosome preparations were obtained from dissociated blastomeres by standard air-drying technique. Colcemid treatment was omitted.

Hybridization probe

5S rDNA was PCR-amplified from sperm DNA of the sea urchin, using two primers: 5′-GCATGGTATGGTCGTAGGC-3′ and 5′-TATA CCGGTTCTCGTCCGATC-3′, designated from the published sequence (AJ417698). PCR products were cloned, and a clone containing an entire 5S rDNA repeat (coding region + intergenic spacer) was biotin-labelled by Random Priming (Invitrogen), and used as a FISH probe.

Probe name: pPL-5S-2
Probe type: plasmid clone
Insert size: 874 bp
Vector: pGEM-T-easy
Proof of authenticity: DNA sequencing
Gene reference: AJ417698

Fluorescence in situ hybridization

Over-night FISH was performed with 2 μg/ml of the probe in a hybridization mixture (50% formamide, 10% dextran sulphate, 2× SSC, 10 μg/ml salmon sperm DNA) on RNase pre-treated slides. Post-hybridization washes were performed at low-stringency: 3 times in 2× SSC at RT. Signals were detected by avidin DCS-FITC, and amplified by biotinylated anti-avidin (Vector). Chromosomes were counterstained with DAPI and propidium iodide, and mounted in Vectashield medium (Vector). Observations were performed under a Zeiss AxioPhot epifluorescence microscope and the images were digitalized by Sensys CCD camera (Scanalytics) and processed by IPLab (Scanalytics) and Adobe Photoshop 7.0 software.

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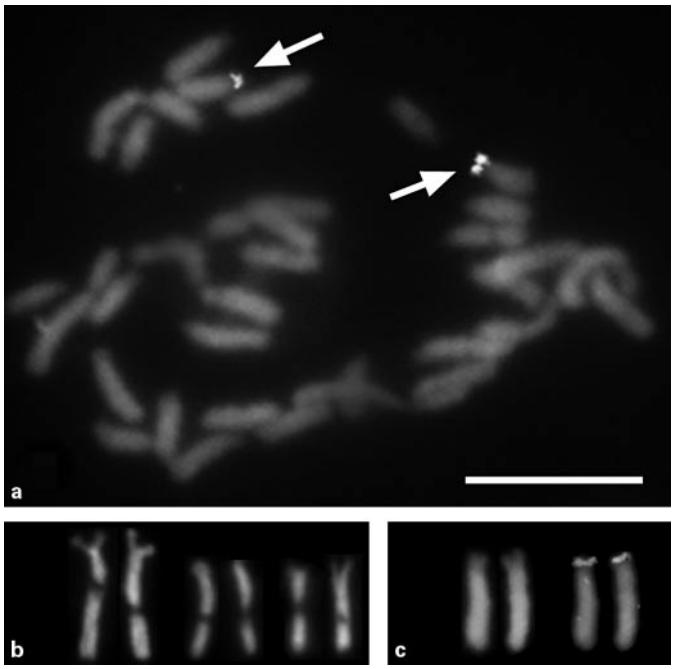

Fig. 1. In situ hybridization of 5S rDNA to (**a**) metaphase chromosomes of *P. lividus*, arrows indicate 5S rDNA sites. (**b**) DAPI-counterstained elongated chromosomes with interstitial negative bands, and (**c**) 5S rDNA-bearing chromosome pair; left: DAPI; right: FISH with 5S rDNA. Scale bar = 5 μm.

Results and discussion

Mapping data:

Most precise location: short arms of a medium-sized subtelocentric chromosome pair, classified as no. 14

No. of cells examined: 30

Number of cells with specific signal: 1 (0), 2 (0), 3 (4), 4 (26) chromatids per cell

The 5S rDNA probe hybridized all-over the short arms of two medium-sized subtelocentric chromosomes (Fig. 1a). In the remaining set, six subtelocentric chromosomes showed distinctive DAPI-negative interstitial bands on the long arms, best evident in the spreads with slightly elongated chromosomes in the prometaphase-early metaphase stage (Fig. 1b), whereas the long arms of the 5S rDNA-bearing chromosomes were uniformly stained with this AT-specific fluorochrome (Fig. 1c). Considering this feature and the chromosome size and morphology, the six chromosomes depicted in Fig. 1b are most likely the NOR-bearing ones. In fact, Lipani et al. (1996) reported Ag-NORs on three pairs of homologs (no. 5, 8 and 12) characterized by G-negative interstitial bands on the long chromosome arms. Thus, we assume that minor (5S) and major rRNA genes are not co-located in *P. lividus* chromosomes.

Except for few species, in which two chromosome pairs (Pelliccia et al., 1998) or two sites in the haploid set (Joffe et al., 1998) were FISH-labelled, invertebrate species studied so far typically show one 5S rDNA-bearing chromosome pair. The same is true for the common sea urchin. No other, minor or variable, 5S rDNA sites were detected in *P. lividus* even under low-stringency conditions.

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