

# A model for detection of early replication bands in cultured fish

J Lobillo, JV Delgado, J Alonso, A Rodero

Facultad de Veterinaria, Universidad de Córdoba,  
Departamento de Genética, Avda Medina Azahara, 9, 14005 Córdoba, Spain

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## INTRODUCTION

Since the first attempts to obtain banding patterns in human chromosomes by means of differential staining after incorporation of bromodeoxyuridine (BrdU) (Latt 1973; Dutrillaux and Lejeune, 1973), other authors have employed this technique to obtain R- and G-bands in mammalian chromosomes.

At present, the BrdU techniques are commonly used because of their high sensitivity in the detection of DNA replication and because of their more resolute banding patterns.

Obtaining such patterns in fish chromosomes using BrdU methodology has not been tried very often. Using trypsin and BrdU incorporation, Lingyun (1986) obtained the G-banding pattern in *Monopterus albus*. Delany and Bloom (1984) established R-banding patterns in *Salmo gairdneri*, and Giles *et al* (1988) did the same in Scorpaenids.

The present paper describes a model for detection of R-bands in *Rutilus alburnoides* (Teleostei, Cyprinidae), using BrdU methodology, and the modified techniques of Delany and Bloom (1984) and Giles *et al* (1988).

The technique we developed has two experimental phases. In the first, the cell cycle duration is estimated by means of the calculation of the half-BrdU treatment time in which the maximum number of M2 metaphase cells (cells that have undergone two rounds of replication in the presence of BrdU) is reached. In the second phase, two different experimental approaches are used to obtain R-bands by means of the incorporation of BrdU during the late period of the DNA synthesis phase.

## MATERIALS AND METHODS

The cell cycle duration was calculated according to Klingerman *et al* (1984), using the following experimental conditions: a dose of 0.8 mg of BrdU per gram of fish and a temperature of 25–27°C.

Two experimental approaches were used to obtain R-bands. First the modified method of Giles *et al* (1988): 48 h before sacrifice, fish were injected ip with 0.5 mg of phytohemagglutinin-M (PHA-M) per 50 g of body weight; 1 injection of 0.8 mg of BrdU/g of fish was administered 5 3/4, 5 or 4 1/4 h before sacrifice (just at the time of the last cell cycle); 2 h before sacrifice fish were injected ip with 0.2 µg of colchicine per gram of fish.

Second, the modified version of the Delany and Bloom (1984) method: one injection of PHA-M 48 h before sacrifice; 3 injections of BrdU were given, 18, 14 and 10 h before sacrifice during the penultimate cell cycle; 1 injection of colchicine 8 h before sacrifice.

After sacrifice, the cephalic kidney was removed, macerated and maintained in a solution of 0.075 M KCl for 90 min. After this time, the cellular suspension was washed and fixed in acetic acid–methanol (1:3), and metaphase plates were prepared using the air-dried technique. Metaphase plates were stained with acridine orange (Dutrillaux and Lejeune, 1973).

## RESULTS

*Rutilus alburnoides*, under the experimental conditions described above showed a cell cycle of 8 h.

With the modified Giles' technique, treatment with BrdU for 5 3/4 h before sacrifice resulted in a maximum percentage of R-banded metaphases (45%). Thus, 5 3/4 hours is the length of time from the mid-DNA synthesis phase to the metaphase, for the majority of the cell population.

The modified technique of Delany and Bloom resulted in 65% banded metaphases.

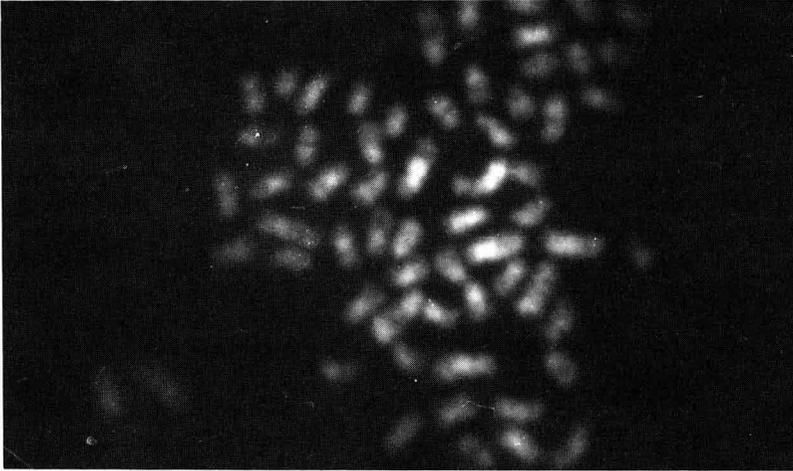
An R-banded metaphase cell, stained with acridine orange, is shown in figure 1.

## DISCUSSION

It is well established that, in mammal chromosomes, there is a clear relation between R-bands (early replicating bands) and G-bands (late replicating bands). In fish, the results obtained by Lingyun (1986) also seem to corroborate this relation between G and R chromosome bands and an order in DNA replication.

The successful preparation of R-banding patterns in *R. alburnoides* could lead us to conclude that, in fish cells (Delany and Bloom, 1984; Lingyun 1986; Giles *et al*, 1988), a temporal organization for replication exists, consisting of alternate early and late replicons, as is observed in other eukaryotic cells (Latt, 1976; Sumner, 1982).

The approach used to obtain R chromosome banding gave positive results. It shows that a high percentage of cells, between 45% (modified technique of Giles *et al*, 1988) and 65% (modified technique of Delany and Bloom, 1984), incorporated BrdU during the mid-to-late S period, which expresses the R-banding pattern.



**Fig 1.** R-banded metaphase cell of *Rutilus alburnoides*.

Dark areas in the chromosome correspond to late-replicating DNA and light ones to early-replicating DNA.

The approach based on the modified method of Delany and Bloom (1984) shows a greater percentage of R-banded metaphases. We suspect that this method, under our experimental conditions, is better adapted to the asynchronic character of the cell population.

The R-banding pattern obtained by the BrdU method will enable progress to be made in the chromosomal characterization and karyotype analysis in fish. R-banding techniques in fish are essential because the attempts to obtain complementary G-bands have been unsuccessful (Delany and Bloom, 1984).

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