LABELLING OF CHICKEN MICROCHROMOSOMES BY MOLECULAR MARKERS USING TWO-COLOR FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

MARCAJE DE MICROCROMOSOMAS DE POLLO MEDIANTE MARCADORES MOLECULARES UTILIZANDO HIBRIDACIÓN *IN SITU* FLUORESCENTE (FISH) BICOLOR

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Palabras clave adicionales

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SUMMARY

The chicken standard karyotype (2n= 78) is constituted of 8 pairs of macrochromosomes plus the W and Z sexual chromosomes, and 30 pairs of indistinguishable microchromosomes usually ordered arbitrarily by decreasing size. This presence of microchromosomes is typical of avian genomes. They represent one third of the chicken genome and have been shown to carry genes. In order to characterize each microchromosome pair, we are developing a cytogenetic map. Single color FISH experiments using biotin labelled probes were carried out with clones from PAC, BAC and cosmid libraries. Out of 50 clones tested, 16 hybridize to microchromosome pairs. Fourtheen are anonymous clones, 7 of which containing microsatellites, and two correspond to the *MHC* and *Fatty acid synthase* genes. In order to distinguish each microchromosome, we realized two-color FISH experiments using simultaneously pools of digoxigenin and biotin labelled probes. Presently, 14 clones provide signals on different microchromosomes and thus enable to individualize nearly half of them. The microsatellite containing clones can be used for genetic mapping on international reference families and thus enable the assignment of linkage groups to the microchromosomes. This will help to assess the degree of coverage of the chicken genetic map.

RESUMEN

El cariotipo estándar de la gallina (2n= 78) está constituido por 8 pares de macrocromosomas, además de los cromosomas sexuales W y Z, y 30 pares de microcromosomas indistinguibles que habitualmente se ordenan arbitrariamente por tamaños decrecientes. Esta presencia de microcromosomas es típica de los genomas aviares. Representan un tercio del genoma de la gallina y se ha demostrado que portan genes. Para caracterizar cada par de microcromosomas, estamos desarrollando un mapa citogenético. Se llevaron a cabo experimentos de

FISH monocolor utilizando sondas marcadas con biotina procedentes de clones de genotecas PAC, BAC y cosmídicas. De los 50 clones testados, 16 hibridaron con pares de microcromosomas. Catorce son clones anónimos, de los que 7 contienen microsatélites y dos corresponden a los genes del MHC y sintetasa de ácidos grasos. Para distinguir cada microcromosoma, realizamos experimentos de FISH bicolor utilizando simultáneamente mezclas de sondas marcadas con biotina y con digoxigenina. En la actualidad, 14 clones proporcionan señales en diferentes microcromosomas, por lo que nos permiten identificar casi la mitad de éstos. Los clones que contienen microsatélites pueden usarse para la cartografía génica en familias internacionales de referencia y permitir así la asignación de grupos de ligamiento a los microcromosomas. De esta forma contribuirán a mejorar el grado de cobertura del mapa génico del pollo.

INTRODUCTION

The chicken karyotype is composed of 2n=78 chromosomes, including the WZ (female) or ZZ (male) sexual chromosomes. A typical feature of avian genomes is the presence of microchromosomes, causing major problems for cytogenetic studies. Indeed, the chicken standard karyotype for the 8 large macrochromosomes has been established after chromosome banding studies (Ladajali *et al.*, 1993), but the sixty microchromosomes remain indistinguishable and are ordered arbitrarly by approximate decreasing size. In spite of their small size, microchromosomes represent one third of the chicken genome and carry a high density of CpG islands probably correlated with a high gene concentration (Mac Queen *et al.*, 1996).

So as to be able to recognize each microchromosome pair, we are developing a collection of large-insert containing clones which are mapped using two-color fluorescence *in situ* hybridization (FISH).

METHODS

PREPARATION OF METAPHASE CHROMOSOMES

Metaphase spreads were obtained from embryo fibroblast cultures, double synchronized with 0.8 mg/ml thymidine (Sigma), arrested with 0.06 μg/ml colcemid (Gibco) and fixed by standard procedures.

SINGLE-COLOR FISH

According to Yerle *et al.* (1992), 100 ng per probes were biotin labeled by random priming using biotin-16-dUTP (Boehringer Mannheim). Probes were hybridized *in situ* for 24 hours and detected with avidin-fluorescein isothiocyanate (FITC) (Vector). Chromosomes were counterstained with propidium iodide (Sigma) in antifade solution (Vector).

TWO-COLOR FISH

Two-color FISH experiment (Trask *et al.*, 1991) were realized using simultaneously groups of digoxigenin (digoxigenin-11-dUTP, Boehringer Mannheim) and biotin (biotin-16-dUTP, Boehringer Mannheim) random priming labeled probes. Both groups of labeled probes were ethanol precipitated together before hybridization for 24 hours. Biotin probes were detected with avidin-texas red (Vector) and digoxigenin probes with FITC antibodies (Boehringer Mann-heim). Chromosomes were counterstained with

DAPI (4', 6-diamidino-2-phenilin-dole-dihydrochloride, Sigma) in antifade solution (Vector).

SLIDES ANALYSIS

The slides were screened with a Zeiss fluorescence microscope and a minimum of twenty metaphase spreads was analyzed for each experiment. Spot-bearing metaphases were photographed with Ektachrome ASA 400 color slide film.

STRATEGY AND FIRST RESULTS

I- SORTING OF MICROCHROMOSOME PROBES

In order to select microchromosomes probes, single-color FISH experiments were carried out with large-insert clones from PAC and BAC libraries and 3 cosmid clones. Out of 47 PAC and BAC clones tested, 13 hybridize to microchromosome pairs which approximately represents one third of the total localizations. Out of these 13 clones, 6 contain a microsatellite sequence from which a polymorphic marker may be developed **(table I)**.

All clones tested hybridize to one microchromosome pair, except one BAC clone that labels 2 pairs. This particular clone may contain a repeat element, as suggested by the painting signal observed on one of the 2 pairs of microchromosomes. Two cosmid clones gave a painting signal each on one microchromosome pair, suggesting the existence of repeat elements specific to these 2 pairs of microchromosomes.

Usually, BAC clones gave stronger hybridization signals, making them more suitable for the two-color FISH experiments.

II- IDENTIFICATION OF SPECIFIC MICROCHROMOSOME PAIRS

1- Establishment of groups of clones

As it would be tedious to test all combinations of two probes in two-color FISH (120 combinations for 16 clones),

Table I. *Microchromosome probes.* (Sondas con microcromosomas).

Microsatellite containing anonymous clones	PACs	6
Non microsatellite containing anonymous clones	PACs	1
	BACs	5
	Cosmid	1
Anonymous clone labeling two microchromosome pairs	BACs	1
Fatty acid synthase gene (Le Fur *et al.*, 1996)	Cosmid	1
Major Histocompatibility Complex gene (Guillemot *et al.*, 1988)	Cosmid	1
Total		16

we have sized approximately each labeled microchromosome pair in order to constitute 6 groups of 2 or 3 clones corresponding to large, medium, or small microchromosomes. We have tested that all probes of one group hybridized to different microchromosomes in single-color experiments using probe pools.

2- TWO-COLOR HYBRIDIZATION OF GROUPS OF CLONES

Groups of clones were hybridized 2 by 2 in all possible combinations: probes of one group were biotin labeled (red), probes of the other group were digoxigenin labeled (green) and all were hybridized together. Whenever all the spots are independent, the clones are considered as markers for different microchromosome pairs. If red and green hybridization signals are detected together on the same microchromosome, each probe of the 2 groups involved will be then hybridized 2 by 2 in order to identify the clones from the same microchromosome.

Presently, we have labeled 14 different microchromosomes by using the 16 clones as probes. The 4 clones that co-localize to identical microchromosomes will have to be identified and 2 will be removed from the panel.

CONCLUSION

By using two-color FISH, *in situ* localization to microchromosomes will enable to provide more precise localization data for genes, as additional microchromosome probes will be added to the actual panel. Microsatellite containing clones will be used for genetic mapping on international reference families, enabling the assignment of linkage groups and possibly new genes to the microchromosomes. Non microsatellite containing clones may be mapped by SSCP (Single Strand Conformation Polymorphism) for further assignment of linkage groups to microchromosomes. Co-localization of genes to microchromosomes will provide a more precise chicken cytogenetic map, as one third of the genome, hardly mapped up until now, will thus be divided into 30 well defined regions.

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