The Journal of Heredity 76:377–378. 1985

High resolution R-bands produced in equine chromosomes after incorporation of bromodeoxyuridine

A. Romagnano and C. L. Richer

ABSTRACT: Cell synchronization was used to obtain an adequate percentage of very long chromosomes in equine mitotic spreads. Reported here is our variation, adapted to horse chromosomes, of a method using excess thymidine followed by bromodeoxyuridine incorporation. This technique routinely yields excellent quality cells, predominantly in prometaphase and prophase. Among other differences with the standard technique, this method does not use Colcemid, which, in addition to inhibiting spindle fiber formation, also increases chromosome contraction resulting in thicker and thus fewer bands. Consequently, horse prometaphase chromosomes, which have incorporated BrdU in the late-S-phase, are very long and display a large number of R-bands after the fluorescence-photolysis-Giemsa method. This technique should definitely be useful for the analysis of structural anomalies and the standardization of equine R-bands.

OUR ADAPTATION to horse chromosomes of R-banding by the fluorescence-photolysis-Giemsa technique after bromodeoxyuridine (BrdU) incorporation (RB-FPG) has produced good quality R-bands[6,7]. Compared to other banding techniques, such as RHG, GTG, and RBA, which have been used for horse chromosomes, the RB-FPG technique gave us the best results. Unlike the RHG and GTG techniques, the RB-FPG method requires no harsh pretreatments such as heat or enzyme digestion, making it useful for detailed analysis of elongated chromosomes. Following the standard whole blood technique[3] a few early mitotic spreads can be found, but the majority of cells are observed in metaphase. In order to obtain a greater percentage of very long chromosomes, it was decided to synchronize the blood cultures with thymidine. This also eliminated the need for Colcemid along with its undesirable chromosome condensing effects.

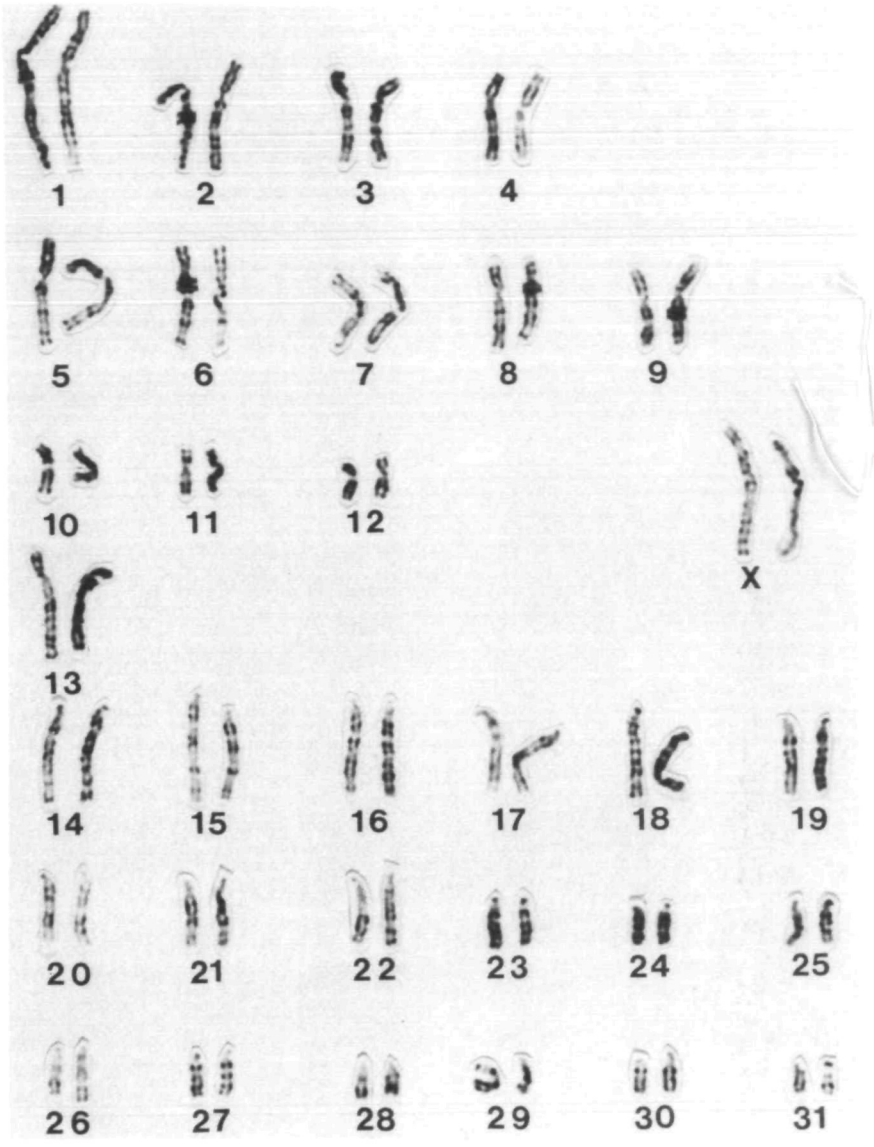

FIGURE 1 Prometaphase RB-FPG karyotype of a female domestic horse.

Although thymidine was used to synchronize cell cultures[9] as early as the 1960s, it was not until the late 70s that its role in producing elongated chromosomes was realized[8]. Here we report our variation, adapted to horse lymphocytes, of this synchronization technique employing the double treatment of thymidine and BrdU.

Materials and Methods

Peripheral blood was collected directly from the jugular vein of 25 horses into green stoppered Vacutainer tubes containing 143 USP units of sodium heparin (Becton Dickinson). Cultures were set up with 0.2 ml of whole blood in 5 ml of HB-103 medium (Hana Media) supplemented with 1 mM glutamin (Flow), 2 percent Pokeweed mitogen (Gibco) and 0.01 mg/ml of Garamycin (Shering). Following a 57-hour incubation period at 37°C, 300 μg/ml of thymidine were added. Eleven hours later cultures were rinsed twice with warmed P.B.S., suspended in fresh medium containing 50 μg/ml of BrdU and reincubated at 37°C for three hours.

At the end of this culture period the suspension was centrifuged and the cell button was suspended in 0.075M KCl and reincubated for 20 minutes. After this hypotonic treatment the cells were fixed, first in methanol:chloroform: acetic acid (3:2:1) and then twice in methanol: acetic acid (3:1). This suspension was spread on cold (0°C) slides and air dried at room temperature. The FPG method[2,5], as previously described for horse chromosomes[6,7], was used to visualize the R-banding thus produced. Prepa-

The authors are affiliated with the Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Québec, Canada; and with the Département d'Anatomie, Faculté de Médecine, Université de Montréal, C. P. 6128, Succ. A., Montréal, Québec, Canada H3C 3J7. Part of this work was presented at the 6th European Colloquium on Cytogenetics of Domestic Animals, Zurich, July 1984. This research was supported by the "Fonds FCAC pour l'aide et le soutien à la recherche", Québec. The authors wish to thank Brigitte L'Hermite-Beugnot, Elise Ménard-Landry, and Louise Laquerre for helpful assistance. Please address reprint requests to Dr. Richer.

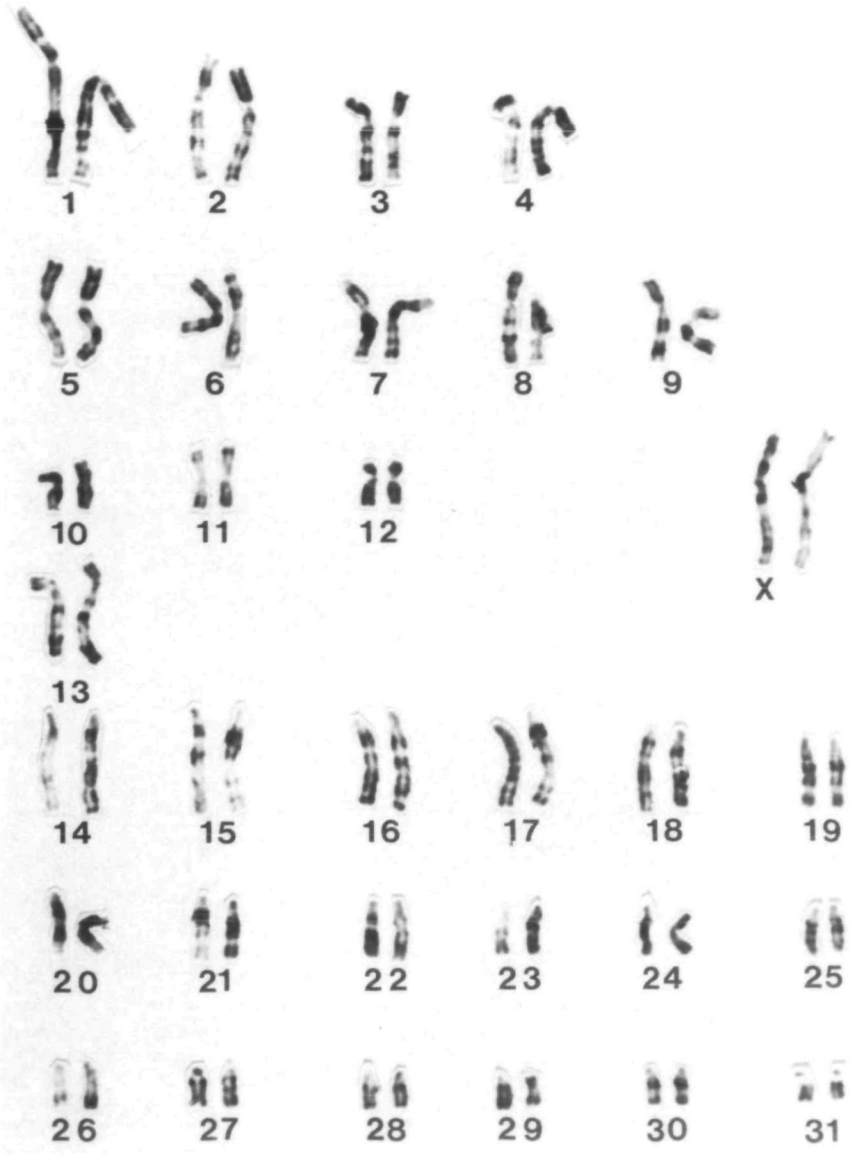

FIGURE 2 Prometaphase RB-FPG karyotype of a female domestic horse.

rations were analyzed and photographed under a light microscope.

Results and Discussion

Most mitotic spreads obtained were either in prophase or prometaphase and were present in adequate numbers when three hours elapsed between the release of the thymidine block and harvesting. During this time BrdU, added at a low concentration, was incorporated into the G-bands and the late replicating R-bands of the inactive X. This resulted, after the FPG technique was applied, in elongated R-banded chromosomes. High resolution RB-FPG banded karyotypes were prepared and two are depicted in Figures 1 and 2. These karyotypes were arranged according to the order proposed at the International Conference for the Standardization of Banded Karyotypes of Domestic Animals[1].

High resolution chromosomes, found earlier in the cell cycle, are longer and contain a greater number of bands than metaphase chromosomes. These bands would eventually have joined together as chromosomes condense to become their shortest at the end of metaphase. Although metaphases are easily accumulated following treatment with Colcemid, this spindle inhibitor also induces chromosome condensation, thus further reducing the potential number of visible bands. Since no inhibitor has yet been found that arrests mitosis at prophase, cell synchronization techniques, such as the one described above, are used to accumulate chromosomes at stages preceding metaphase[8]. This method using an excess of thymidine, as well as another method recently applied successfully to horse chromosomes and using methotrexate[4], blocks the cell cycle at mid-S-phase. Thymidine acts as a competitive inhibitor of 2-deoxycytidine reductase and thus inhibits the production of 2-deoxycytidine; without this important factor synthesis comes to a halt. If this block is released by rinsing to remove the excess thymidine, the vast majority of cells are ready to resume synthesis in unison. When at this point BrdU is added, it is incorporated in the place of thymidine where it inhibits spiralization in late replicating chromosome regions[11]. Thus, incorporated mostly in G-bands, it induces chromosome elongation and also produces R-banding. Moreover, when harvesting is done at the appropriate time after release of the cell block, the chromosomes are further elongated as they are arrested earlier in the cell cycle. In our laboratory we found that this method gave better results than the methotrexate technique[10], as preparations were of superior quality enabling a consistent and precise banding pattern.

The greater number of very long well banded chromosomes is definitely an asset for the analysis of structural anomalies and for the standardization of equine R-bands.

References

1. FORD, C. E., D. L., POLLACK, and I. GUSTAVSSON, Eds. Proceedings of the first international conference for the standardization of banded karyotypes of domestic animals. *Hereditas* 92:145–162. 1980.
2. GAGNÉ, R. Obtention des bandes "R" par incorporation de BrdU et coloration par le Hoescht 33258 et le Giemsa. *Un. Méd. Canada* 109:552–556. 1980.
3. HUNGERFORD, D. A. Leucocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. *Stain Tech.* 40:333–338. 1965.
4. MACIULIS, A., T. D. BUNCH, J. L. SHUPE, and N. C. LEONE. Detailed description and nomenclature of high resolution G-banded horse chromosomes. *J. Hered.* 75:264–268. 1984.
5. PERRY, P. and S. WOLFF. New Giemsa method for the differential staining of sister chromatids. *Nature* 251:156–158. 1974.
6. ROMAGNANO, A., C. L. RICHER, and K. J. BETTERIDGE. R-banded chromosomes of the domestic horse obtained, after bromodeoxyuridine incorporation, by the fluorescence-photolysis-Giemsa technique. *J. Dairy Sci.* 66: suppl. 1:251. 1983.
7. —— and ——. R-banding of horse chromosomes: the fluorescence-photolysis-Giemsa technique after bromodeoxyuridine incorporation. *J. Hered.* 75:269–272. 1984.
8. VIEGAS-PÉQUIGNOT, E. and B. DUTRILLAUX. Une méthode simple pour obtenir des prophases et des prométaphases. *Ann. Génét.* 21:122–125. 1978.
9. XEROS, N. Deoxyriboside control and synchronization of mitosis. *Nature* 194:682–683. 1962.
10. YUNIS, J. J. High resolution of human chromosomes. *Science* 191:1268–1270. 1976.
11. ZAKHAROV, A. F. and N. A. EGOLINA. Differential spiralization along mammalian mitotic chromosomes I. BrdU-revealed differentiation in Chinese hamster chromosomes. *Chromosoma* 38:341—365. 1972.