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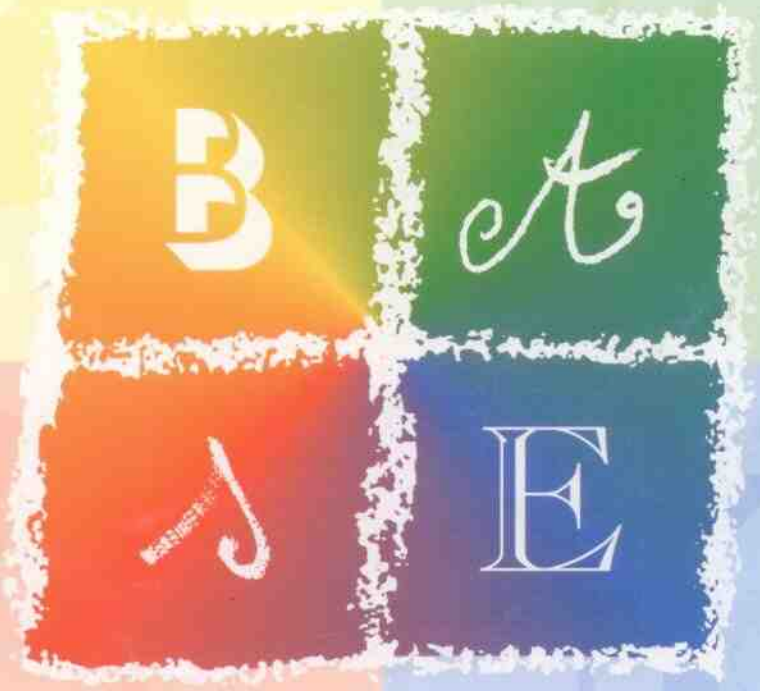

Biotechnology, Agronomy Society and Environment

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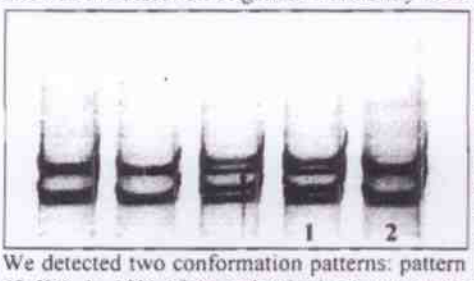
L/M3 ARGUMENTS FOR DIRECT ACTIONS OF GROWTH HORMONE ON MAMMARY EPITHELIAL CELLS. H. Jammes, L. Belair, J. Djiane. *Unité d'Endocrinologie Moléculaire, INRA, 78352 Jouy en Josas, France*

Growth hormone (GH) plays an important role in the mammary gland development and the direct action of GH on epithelial cells is currently under consideration. The presence of mRNA coding for GH receptor has been evidenced in mammary gland (Hauser et al., 1990 ; Jammes et al., 1991). Using a monoclonal anti GHR (Mab263, a gift from Dr. M. Waters, Australia.) we found a GH receptor-like immunoreactivity in human mammary epithelial cells suggesting the ability of GH to act directly on mammary cells via the activation of endogenous GH receptor. The interaction of GH with its receptor has been shown to lead to the tyrosine phosphorylation of JAK2, the receptor itself, and STAT family of transcription factors (Argetsinger *et al.*, 1993; Ihle *et al.*, 1995). After rabbit mammary acini stimulation with 500 ng/ml bGH for 15 min, lysates were prepared, immunoprecipitated with GHR antibody (Mab 263) and subjected to Western Blot analysis using the monoclonal antibody directed against phosphotyrosine (4G10, UBI). Only one immunoreactive band was visualized using ECL system (Amersham) at 120Kd, indicating that GH was able to induce the tyrosine phosphorylation of its own receptors in mammary cells.
The interactions between nuclear factors induced by GH stimulation and regulatory sequence of Spi 2.1 gene (GHRE) and α casein S1 gene (αS1 Cas) were analysed by standard band shift assays. In presence of labelled oligonucleotide GHRE, a binding activity of nuclear extracts was obtained for epithelial clusters stimulated by GH in dose-dependent manner. In presence of an excess of unlabelled oligonucleotide, the GHRE binding was decreased. In order to determine the nature of nuclear factor involved in the GHRE binding different antibodies anti-STATs were used. For GHRE, nuclear factor involved has been identified as STAT5 (a gift from Dr. Ph. D Groner). In presence of labelled (αSICas oligonucleotide, GH was unable to induce a binding activity. This result was consistent with the absence of GH effect on milk protein gene activation.
In order to find a cellular response to GH stimulation, we have used the mouse mammary epithelial cell line HC11 (Ball *et al.*, 1988). A binding of ^{125}I-human GH (ligand for the both lactogenic and somatogenic receptors), was observed on whole HC11 cells. Increasing concentrations of unlabeled recombinant bovine GH or recombinant human GH were able to decrease the ^{125}I -human GH binding with a similar affinity. At maximal concentration (10^{-7}M), bovine GH inhibited 50% of binding of ^{125}I -human GH, suggesting the presence of specific somatogenic receptors on the HC11 cell surface. But the specific binding was too low to determine the number of GH receptor/ cell. The EGF-induced mitogenesis of HC11 cells was analysed by ^{3}H thymidine incorporation. EGF induced DNA synthesis in a dose-dependent manner with an ED50 of 10^{-10}M and a maximally effective dose of 10^{-9}M. The concomittant presence of GH at maximally effective dose (10^{-8}M), induced a decrease of ^{3}H-thymidine incorporation obtained with lower EGF concentrations. At higher EGF concentrations, GH lack to be effective. This result was probably due to ratio between EGF and GH receptor number at cell surface.

Key words: GH, Mammary epithelial cells, Transducing signal.

L/M4 SINGLE STRAND CONFORMATION POLYMORPHISM DETECTION OF EXON 7 OF ß-CASEIN GENE IN "CHURRA DA TERRA QUENTE" PORTUGUESE INDIGENOUS OVINE BREED. E. Bastos*, A. Cravador**, C. Varejão*, R. Chaves*, H. Guedes-Pinto*. **Universidade de Trás-os-Montes e Alto Douro, Dept. Genética e Biotecnologia, Ap. 202, Vila Real, Portugal. ***Universidade do Algarve, UCTA, Campus de Gambelas, Faro, Portugal.*

Milk protein polymorphisms provide a powerful tool for the knowledge of their molecular biology and physicochemical properties. It is becoming increasingly recognised that genetic variation may offer a source of differentiation that can improve milk technology, including cheesemaking. "Churra da Terra Quente" is an important ovine breed of North Portugal used for milk and meat production. The milk is almost entirely processed into cheese, so it is important to define milk characteristics, especially the protein polymorphisms and their influence on cheesemaking properties. In the present study we used a non-radioactive SSCP protocol that allowed the detection of genetic variability at the exon 7 of ß-casein gene in "Churra da Terra Quente" animals.



Genomic DNA was isolated from blood samples of animals by a phenol-cloroform-proteinase K protocol. The choice of the direct and reverse primers for the amplification of exon 7 of ß-Cn was based on ovine ß-Cn gene sequence (1). The SSCP analysis of the 510 bp amplified product was carried out basically by the method described in (2) with some modifications. The electrophoretic run was performed during 16 hours, at 400V and 15°C in a 15% nondenaturing polyacrylamide gel, without glycerol. The DNA bands were visualised through silver staining. Our study showed genetic diversity in the animals studied with this methodology.
We detected two conformation patterns: pattern 1 with four bands (frequency of 37.5%) and pattern 2 with two bands (frequency of 52.5%). In 10% of the animals there was no amplification suggesting a mutation in the primer region. The SSCP analysis is a very reliable and simple method of detecting single-base substitutions and other alterations, but it doesn't allow the identification of the exact mutation. Actually, the fragments with the two distinct patterns are being sequenced in order to inform us what specific mutation occurred. We think that this preliminary approach could be advantageously used to characterise the genetic variability within this breed. Further studies will be developed to analyse the effect of the polymorphisms detected on milk composition and its technological properties

References: (1) Provot *et al.*, 1995, *Gene*. 154: 259-263; (2) Hongyo *et al.*, 1993, *Nucleic Acids Res.* 21: 3637-3642.

Key words: ß-casein, Milk, Polymorphism, SSCP