cells (determined by the TUNEL method) were visible both in lactating and involuting mammary tissues; however, their relative number (as a percentage of the total number of cells), was higher in the course of drying off, than in early or mid-lactation.

The bax transcript evaluated by the Bax mRNA/GAPDH mRNA ratio (where GAPDH served as a 'housekeeping gene') was down regulated by the administration of EGF (10 ng·mL^{-1}) or prolactin (5 μg·mL^{-1}) to the culture of HC11 mouse mammary epithelial cells. Conversely, withdrawal of EGF and/or prolactin, which can mimic an endocrine pattern at the end of lactation, was associated with increased Bax expression in HC11 cells. Administration of TGF-β_1 (1 ng·mL^{-1}) strongly enhanced Bax transcript both in treated or non-treated cultures with EGF or prolactin, thereby suggesting the involvement of this factor in auto-, or paracrine regulation of apoptosis in mammary epithelial cells.

Communication no. 22

Intraperitoneal injection of EGF stimulates MAP, p90RSK and p70S6 kinase activities in mouse liver in vivo. J. Ostrowski, L. Trzeciak, M. Woszczynski (Department of Gastroenterology, Medical Center of Postgraduate Education and Department of Animal Genetics, Cancer Center, 02-781 Warsaw, Poland)

The epidermal growth factor (EGF) transmits a mitogenic signal. Inducible protein kinases, some of which are directly linked to gene expression, are key intracellular transducers that allow growth factors to signal their events. Because only a few studies have examined the activation of protein kinases in whole organisms, the current knowledge about kinases is largely based on studies in cell cultures. For that reason, whole animal studies on signal transduction pathways are particularly important.

Aim: In this report, we examined the effect of EGF treatment on MAP, $p70^{S6}$, and $p90^{RSK}$ kinase activities in mouse liver.

Methods: 10, 30 and 60 min after the intraperitoneal injections of male mice with either PBS alone or EGF in PBS (10 mg/L g body weight), hepatocytes were isolated by the two-stage perfusion method. Proteins from cytoplasmic and nuclear extracts were immunoprecipitated with anti-ERK1/2, $p70^{S6}$ or anti-$p90^{RSK}$ antibodies and kinase activities were measured using defined substrates. Proteins were also fractionated by POROS HQ/M chromatography and immunoblots were used to compare defined kinase protein levels.

Results: The systemic administration of EGF into mice stimulated both cytosolic and nuclear kinases studied. A 3–4 fold increase in MAPK and 2–3 fold increase in $p70^{S6}$ or $p90^{RSK}$ were found at 10 min; whereas 60 min after injection the kinase activities, in both cytoplasmic and nuclear extracts, almost returned to the activities observed in unstimulated animals. The fractions containing the peak MAPK, $p70^{S6}$, and p90 activities also revealed the highest amount of kinase protein levels, as detected by western blot analysis.

Conclusion: Cytosolic and nuclear activation of MAPK, $p70^{S6}$ and $p90^{RSK}$ by EGF might reflect the involvement of these enzyme pathways in transmitting mitogenic signals of growth factors in the immediate-early phase of liver cellular proliferation, healing and regeneration.

Communication no. 23

Influence of insulin and triiodothyronine on the proliferation and differentiation of bovine satellite cells in primary culture. I. Cassar-Malek., N. Langlois, A. Delavaud, B. Picard (Équipe croissance musculaire, laboratoire croissance et métabolismes des herbivores, Inra, Theix, 63122 Saint-Genès-Champanelle, France)

We have previously shown that during compensatory growth in bovine, muscle fibres are rapidly oriented towards glycolytic fibres (Brandstetter et al., Livest. Prod. Sci. 53 (1998) 25–36). Moreover, small fibres expressing developmental myosin heavy chain isoforms were observed, suggesting that a new population of muscle fibres is formed from recruitment of satellite cells. As high levels of insulin and triiodothyronine (T3) are restored during compensation, we studied the respective influence of both hormones on satellite cell proliferation and myogenesis.

Primary mononucleated cells, most likely satellite muscle cells, were isolated from cultivated muscle *Semitendinosus* explants: cells migrated out of the explants, proliferated and colonised culture dishes. After 1 week, myogenic cells were recovered by trypsinisation of subconfluent cultures. After preplating, cells were seeded at 10^4 cells/cm^2 in a mixture of DMEM and 10 % fetal calf serum in absence or presence of insulin (5, 10 and 50 µU/mL) or T3 (1, 3 and 5 ng/mL). On day 5, the serum concentration of the medium was lowered to 2 %. Cell proliferation was assessed by scoring the cell nuclei number after Giemsa staining. Differentiation was studied by the fusion index and by cyto-immunofluorescence analysis of myogenin, myosin heavy chains (MHC) and connectin.

Influence of insulin: an increase in cell density was observed in insulin-treated cultures. Stimulation of fusion by insulin was observed over all the time-course of culture, and was clearly apparent on day 11. On day 5, insulin treatment was associated with enhanced accumulation of myogenin, foetal and slow MHC, and connectin. These results indicate that insulin stimulates both satellite cell proliferation and differentiation in a dose-dependent manner. Interestingly enough, proliferation was highest at 50 µU insulin/mL (two-fold increase in nuclei number on day 5) while optimal differentiation was observed at 10 µU insulin/mL (three-fold increase in fusion index on day 11).

Influence of T3: we first tested the influence of a T3-depletion of the culture medium. No effect was apparent on cell density; fusion of satellite cells was delayed and decreased in T3-depleted cultures. In contrast to data reported on quail myoblasts (Marchal et al., Biol. Cell. 78 (1993) 191–197), a physiological dose of T3 (1 ng/mL) did not decrease satellite cell density. A moderate anti-proliferative T3 dose-dependent influence was observed at 3 and 5 ng/mL. Fusion was clearly apparent on day 3 in all T3-treated cultures and a dose-dependent stimulation of fusion was registered on day 11. In agreement with these morphological data, T3-treatment was associated on day 3 with a precocious detection of myogenin and rapid MHC. On day 5, an increase in foetal and slow MHC accumulation was observed in T3-treated cultures. Thus, T3 anticipated and enhanced the differentiation of bovine satellite cells in a dose-dependent manner.

This preliminary study indicates that insulin and T3 influence the proliferation and stimulate the differentiation of bovine satellite cells in primary culture. Both hormones have already been shown to regulate the acquisition of muscle fibre properties and muscle growth. Our data suggest that insulin and T3 could be implicated in the formation of new fibre from satellite cells during compensatory growth.

Communications no. 24

Comparison of in vitro proliferation and differentiation of bovine myoblasts from different genetic types. M.P. Duris, A. Delavaud, B. Picard (Équipe croissance musculaire, laboratoire croissance et métabolismes des herbivores, Inra, Theix, 63122 Saint-Genès-Champanelle, France).

It has been shown that the speed of growth selection of bovines is associated