EXPRESSION OF HEAT SHOCK-REGULATED HUMAN GROWTH HORMONE GENES CONTAINING OR LACKING INTRONS BY NIH-3T3 AND WISH CELL LINES

SAMI ALOUANI*, PHILIPPE L'HOTE*, JEAN-BAPTISTE MARQ*, LOUIS-MARIE HOUDEBINE**, FRÉDÉRIC MONTANDON*, MARTINE CHESSEBEUF-PADIEU+, AND MICHEL DREANO*

*Department of Genetic Engineering
IntraCell S.A., Geneva-Carouge, Switzerland
**Unité de Différenciation Cellulaire, INRA, Jouy-en-Josas, France
+Laboratoire de Biochimie Médicale, Faculté de Médecine, Dijon, France

A plasmid containing the complete genomic DNA of the human growth hormone (ghGH) comprising four introns and driven by the human promoter of the human gene of the 70 kDa heat shock protein (hsp70) has been used to transfect mouse NIH-3T3 and human Wish cells. Selected cell lines were characterized for stable hGH secretion. Similarly in the same NIH-3T3 cells, the stable expression of the same plasmid construct, but containing the complementary DNA of the hGH gene (chGH), was compared in terms of the effect of introns on heterologous protein synthesis. Genomic hGH recombinant cells synthetized, in a heat regulated fashion, matured hsp70/hGH hybrid mRNA able to drive the secretion of a 22 kDa polypeptide. Like the natural hGH, this polypeptide expressed the functional hormonal activity of prolactin on casein secretion by mammary cells. The time course of hGH secretion was prolonged in ghGH transcripts, while that of mRNA degradation appeared delayed, especially in Wish cells, as compared to chGH expression. In the human Wish cells the decay of endogenous hsp mRNA has been compared to that of recombinant hsp mRNA, demonstrating that this human hsp70/hGH hybrid mRNA was present in the cytoplasm during a longer period than the human endogenous hsp70 mRNA. In conclusion, similar levels of expression and resulting gene products were expressed from the chGH or the ghGH gene in an inducible manner.

INTRODUCTION

The interactions of introns in the course of gene expression in mammalian cells remain a controversial subject. Indeed, early studies using a series of recombinant Simian virus 40/mouse

1. Address all correspondence to: Martine Chessebeuf-Padieu, Laboratoire de Biochimie Médicale, Faculté de Médecine, 7, bd. Jean D’Arc, F-21033, Dijon, France.

2. Key words: hGH cDNA gene, hGH genomic gene, heat shock promoter, recombinant cell, mRNA expression, hGH secretion.

3. Abbreviations: chGH, hGH cDNA gene; ghGH, hGH, genomic gene; hGH, human growth hormone; hsp70, 70 kDa heat shock protein gene.
β-globin hybrid gene clearly showed that splice sites played a role in the stability of recombinant transcripts and thus in mRNA accumulation (Hamer and Leder, 1979; Hamer et al., 1979; Gruss et al., 1979; Gruss and Khoury, 1980).

It has also been observed that differential splices from identical primary transcripts resulted in the synthesis of protein variants with different properties (Breitbart et al., 1985). Furthermore, some introns contain regulatory sequences, for example, a glucocorticoid responsive element located in the first intron of genomic sequence of the human growth hormone (ghGH) (Moore et al., 1985; Slater et al., 1985) or a tissue-specific cellular enhancer within an intron of the immunoglobulin heavy chain gene (Banerji et al., 1983; Gillies et al., 1983; Picard and Schaffner, 1984). One pivotal function of introns is their capability to be alternatively spliced, leading from one single nucleotide sequence to the synthesis of proteins which differ in both cellular compartmentalization and function, as illustrated by transmembrane or soluble luteinizing hormone receptors (Tsai-Morris et al., 1990). In contrast, a series of cellular genes such as bean phasexolin (Gross et al., 1987), yeast actin (Ng et al., 1985), and chicken thymidine kinase (Chee et al., 1986) function without introns. On the other hand upon exposure to stress, an elevation of temperature, cells, from bacteria to man, respond by a sudden activation of a small set of genes directing the synthesis of heat shock proteins, accompanied by the suppression of much protein synthesis, active prior to the stress (Ashburner and Bonner, 1979; Schlesinger et al., 1982). However, most hsp genes in all organisms, including eucaryotic cells, are free of introns, although there are few exceptions, such as the Drosophila melanogaster hsp83 gene (Yost and Lindquist, 1986), the ubiquitin gene in chicken embryo fibroblasts (Bond and Schlesinger, 1986), the human hsp27 gene (Hickey et al., 1986) and the Caenorhabditis elegans small hsp genes (Kai et al., 1987) contain introns. Intervening sequences are also present in Drosophila hsp cognate genes that encode proteins homologous to Drosophila HSP70, but which are not heat inducible (Ingolia and Craig, 1982). Hsp promoters have been used for the expression of foreign recombinant genes (Nover, 1987) including complex genes such as human B hepatitis virus surface antigen (HBsAg) (Dreano et al., 1987), flounder anti-freeze proteins (Rancourt et al., 1987), tissue-plasminogen activator (Sanzo et al., 1988), or chick neuronal nicotinic acetylcholine receptor (Ballivet et al., 1988). In addition, hsp70 control elements have also been used to express genes containing intervening sequences such as the alcohol deshydrogenase gene (Bonner et al., 1984), a c-myc gene (Wurm et al., 1986), or an immunoglobulin heavy chain gene (Cattaneo and Neuberger, 1987). It was therefore of interest to observe the expression of the human growth hormone gene, hGH, by comparing hGH that contained (ghGH) with hGH that lacked introns (chGH) under the control of the promoter of the human gene of the 70 kDa heat shock protein (hsp70) using cloned recombinant mouse and human cell lines.

This paper compares: 1) the expression of the hGH genomic gene (ghGH) driven by the human hsp70 promoter in human Wish and mouse NIH-3T3 cells to that of the hGH cDNA gene (chGH) as a similar hsp70 hybrid gene in the same mouse cell line, 2) the time course synthesis of the two hybrid hsp70/hGH mRNAs, 3) endogenous and recombinant hsp mRNAs in the human cell line, and 4) the hGH protein secreted by both cell lines.