FEBS Letters 405 (1997) 148–152 FEBS 18322

Ubiquitin is physiologically induced by interferons in luminal epithelium of porcine uterine endometrium in early pregnancy: global RT-PCR cDNA in place of RNA for differential display screening

Serge Chwetzoff[a,*], Sabine d'Andrea[b]

[a] *Institut National de la Recherche Agronomique, Laboratoire de Virologie et d'Immunologie Moléculaires, F78350 Jouy-en-Josas, France*
[b] *The Babraham Institute, Babraham, Cambridge CB24AJ, UK*

Received 24 January 1997

Abstract Early in the course of pregnancy, at the preimplantation stage, the pig embryo is likely to exert a paracrine effect on the tissue intended to receive it, via the secretion of interferons. Our observations show that trophoblastic interferons induce an increase of some mRNAs in the epithelial cells of the gilt endometrium, which would illustrate this phenomenon. The increase of four mRNAs, whose corresponding cDNAs are dD1, dD2, dD3 and dD4, has been examined in this study. The method used is similar to Northern blot analysis except that mRNAs in the blot are replaced by cDNAs produced from total cellular poly(A)$^+$ mRNAs by global reverse-transcription polymerase chain reaction (RT-PCR). Northern blot hybridization requires a considerable quantity of starting material – which we estimate in this study to be several million porcine endometrium cells – whereas the RT-PCR-based method gives comparable results starting with only a few cells – about 200. Using this method, the differential nature of dD1, dD2, dD3 and dD4 was shown. dD2 and dD3 correspond to genes already identified as interferon-induced: the β_2-microglobulin and Finkel-Biskis-Reilly murine sarcoma virus-associated ubiquitously secreted protein (FAU). dD1 corresponds to a still unidentified gene. dD4 encodes for the porcine UbA_{52} ubiquitin. Up to now, the increase in ubiquitin mRNA as a result of interferon effect has not been reported and is discussed in view of recent publications.

Key words: Interferon; Pregnancy; Ubiquitin; FAU; β_2-Microglobulin; Gilt uterus

1. Introduction

Mammalian placentas secrete interferon during pregnancy [1,2]. Moreover, during the preimplantation stage of early pregnancy, trophoblastic tissue may physiologically produce interferon [3]. The secretion has been established in a number of animals species including ovine, bovine and porcine species [4]. Such an early interferon secretion has been reported, but not confirmed in other species [5,6]. In ruminants, the physiological role of trophoblastic interferon is essential: it allows the maintenance of the corpus luteum. In other species, the role of interferons at this developmental stage remains unknown. Porcine trophoblast physiologically secretes two distinct interferons during early pregnancy: the spI (type I) and the gamma (type II) interferons [7,8]. The secretion of these interferons is detectable in uterine horns from the tenth day of pregnancy and reaches a peak at day 16. Interferon concentrations achieved at this developmental stage are very high and exceed those during immunological processes.

Considerable progress has been made concerning the early action of interferons [9] at the target cell level [10,11] and transcriptional induction of genes has been shown to be an essential part of the cellular response to interferon. Excellent models explain how transcription of certain genes is activated [12,13]. Several induced genes have been characterized but the established activities of corresponding proteins are not sufficient to explain the wide-ranging field of interferons effects. The present work aimed to identify novel interferon-induced genes and especially in target tissues of physiologically secreted interferons.

The technique of differential display [14,15] allows the isolation of cDNAs corresponding to differentially expressed mRNAs from limited amounts of cells. However, these results are usually confirmed by Northern blot hybridizations which requires considerably greater amounts of starting material. We show that global RT-PCR may, from a few cells, generate cDNA for blot hybridization experiments with results comparable to those obtained by Northern blot hybridizations. Using this approach we show that ubiquitin fused to a C-terminal extension encoding for a protein of the large ribosomal subunit is induced by physiological trophoblastic interferons during the preimplantation stage of early pregnancy in the uterine luminal epithelium of gilts.

2. Materials and methods

2.1. Interferons

Recombinant interferon spI was a gift from Dr. F. Lefevre (INRA, France) and recombinant porcine interferon-γ was purchased from Genentech (San Francisco, CA).

2.2. Pregnancy

Gilts were observed daily for estrus and the first day of estrus was designated day 0. Mating occurs at 12 h after detection of estrus.

2.3. Pseudopregnancy

Pseudopregnancy was induced by subcutaneous administration of 5 mg per day estradiol valerate (Sigma) on days 11–15 after the onset of estrus [16].

2.4. Tissues

Endometrium (luminal epithelium+stroma) was aseptically collected

*Corresponding author. Fax: (33) (1) 34 65 26 21.
E-mail: chwetzof@biotec.jouy.inra.fr

Abbreviations: FAU, Finkel-Biskis-Reilly murine sarcoma virus-associated ubiquitously secreted protein; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; SSC, saline sodium citrate; SSPE, saline sodium phosphate EDTA; STAT, signal transducer and activator of transcription.

The sequences reported in this work have been deposited in the GenBank data base (accession no. U72496; U72543).

PII S0014-5793(97)00177-4

from gilts on day 15 either of pregnancy (physiologically interferon-induced) or pseudopregnancy (non-induced), placed in incomplete Hanks' buffered salt solution containing antibiotics, washed, then digested for 20 min at room temperature by dispase (2.4 U/ml, Boehringer) in the culture medium. After addition of pancreatin from porcine pancreas (1.25 g/100 ml, Sigma) tissues were further digested for 3 h at room temperature. The cell suspension was washed three times and pelleted by centrifugation. For primary cultures, cells were cultured in RPMI 1640 supplemented with 2% Ultroser SF (Sepracor, France) and antibiotics. This cell preparation contains a mixture of epithelial and fibroblastic cells. The use of Ultroser in place of fetal bovine serum permits selective adhesion of epithelial cells. To eliminate fibroblastic cells, the wells containing 16 h old culture were gently washed.

2.5. Global RT-PCR

Cells from primary cultures originating from pseudopregnant gilts were used. Immediately after layering, half of the cells were treated with both spI (1000 IU/ml) and porcine γ (2000 IU/ml) interferons for 16 h. About 2000 cells were lysed using the acid guanidinium thiocyanate-phenol-chloroform method [17]. Total RNA was further isolated by CsTFA gradient centrifugation (Pharmacia). The RNA pellet was resuspended in water and poly(A)$^+$ RNA was isolated by adsorption to magnetic beads covalently attached to oligo d(T)$_{25}$ (Dynal). One-tenth of the poly(A)$^+$ RNA was submitted to reverse transcription and tailing by poly(A) addition according to Brady and Iscove [18] with modifications to the PCR cycles: 94°C for 20 s, 42°C for 2 min and 72°C for 4 min with 5 s extension per cycle for 25 cycles. Amplified material was then purified using QIAquick columns (QIAgen) before performing 25 more cycles. For radiolabeling, PCR was carried out as above, but with a second PCR run of 12 cycles only in the presence of [α-^{32}P]dCTP in place of the cold dCTP.

2.6. Dot-blotting

40 ng of cDNA obtained by RT-PCR were applied per spot to nitrocellulose filters (Schleicher and Schuell). Hybridizations were carried out for 16 h at 42°C using 10 kBq/ml radiolabeled probe in a solution containing 5×saline sodium phosphate EDTA (SSPE), 5×Denhardt's reagent, 0.5% (w/v) SDS and 100 μg/ml herring sperm DNA [19]. Post-hybridization washings were carried out at 60°C in solutions of 2×saline sodium citrate (SSC) and 0.1% SDS [19], then in solutions of 0.1×SSC and 0.1% SDS.

2.7. Northern blot analysis

Total RNA was isolated from porcine endometrium cells of either pregnant or pseudopregnant gilts. Cells were prepared as described in Section 2.4. Cellular RNA was extracted using the acid guanidinium thiocyanate-phenol-chloroform extraction method [17], then poly(A)$^+$ RNA was isolated by using of oligo(dT)-cellulose columns (Pharmacia). Each sample containing 2 μg poly(A)$^+$ RNA – corresponding to ∼4×10^6 cells – was loaded onto an agarose gel under denaturing conditions in the presence of formaldehyde. After electrophoresis, the gel was transferred to nylon membranes (Hybond-N+, Amersham). Hybridizations were carried out for 16 h at 65°C using 40 kBq/ml radiolabeled probe in a solution containing 0.5 sodium phosphate pH 7.2, 7% SDS (w/v), 1 mM EDTA and 1% non-fat dried milk. Washings were carried out as described in Section 2.6.

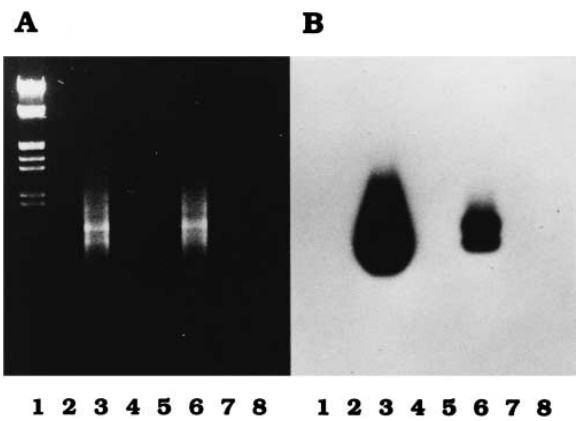

Fig. 1. Analysis of cDNAs produced by global RT-PCR from mRNA extracted from interferon-treated or untreated luminal epithelium cells. (A) Electrophoretic analysis of global RT-PCR products from interferon-treated (lanes 3–5) or untreated cells (lanes 6–8). Lanes: 1, λ digested by *Eco*RI and *Hin*dIII; 3,6, 200 ng of global RT-PCR from poly(A)$^+$ RNA; 4,7, idem, but omitting the reverse transcriptase enzyme; 5,8, control global RT-PCR without poly(A)$^+$ RNA. (B) Hybridization analysis of nucleic acids from the agarose gel (A) transferred to a nitrocellulose membrane. The blot was hybridized with the ^{32}P-labeled dD2 DNA probe originating from interferon-induced cells.

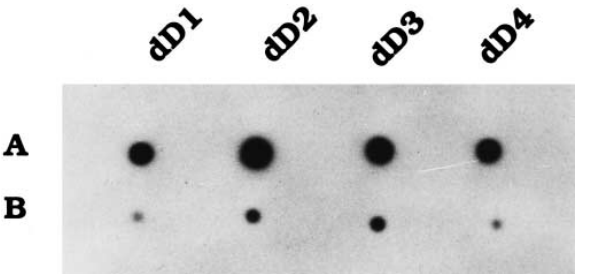

Fig. 2. Dot-blot analysis of cDNA produced by global RT-PCR from mRNA extracted from interferon-treated or untreated luminal epithelium cells. 40 ng of RT-PCR products originating from interferon-treated (A) or untreated (B) cells were blotted per spot and probed with four ^{32}P-labeled DNA probes originating from interferon-induced cells. Lanes: 1, dD1; 2, dD2; dD3, 3; 4, dD4.

2.8. Radiolabeling

Labeling of cDNAs with [α-^{32}P]dCTP was performed by oligonucleotide-primed synthesis [19].

2.9. Differential cDNAs

cDNA isolation and cloning were performed according to standard procedures [19,20].

3. Results

To mimic hormonal conditions of early pregnancy, gilts were injected with estradiol which is the primary determinant initiating maternal cell impregnation and recognition of pregnancy [21]. Luminal epithelium cells from uterine horns of pseudopregnant gilt were isolated and layered as primary cultures. Immediately after layering, half of the cells were treated with both spI and porcine gamma interferons. Poly(A)$^+$ RNA from treated or untreated cells was extracted. cDNAs corresponding to the 3′ ends of putative differentially expressed mRNA were isolated.

3.1. Analysis of global RT-PCR products with a radiolabeled probe

The synthesis of a radiolabeled DNA probe corresponding to one of these cDNAs (dD2) was carried out using [α-^{32}P]dCTP. Additionally, a method that combines reverse transcription of all poly(A)$^+$ RNA and the polymerase chain reaction (also termed global RT-PCR) [18], was used to generate cDNA from mRNA originating in interferon-induced and non-induced cultured cells. The material generated by global RT-PCR was subjected to electrophoresis through an agarose gel. Ethidium bromide staining of DNA prior to transfer to nitrocellulose membrane revealed cDNA lengths ranging from 400 to 900 bases with strong fluorescence at 500–600 bases. Autoradiographs of the membrane hybridized with the dD2 probe show accumulation of cDNAs hybridizing with dD2 in

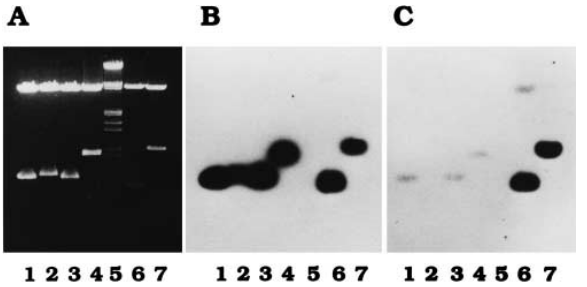

Fig. 3. Analysis of four potentially differential cDNAs with ^{32}P-labeled cDNA probes produced by global RT-PCR from mRNA extracted from interferon-treated or untreated luminal epithelium cells. (A) Electrophoretic analysis of mini-preps of potentially differential cDNAs from interferon-treated cells. Lanes: 1, dD1; 2, dD2; 3, dD3; 4, dD4; 5, λ digested by *Eco*RI and *Hin*dIII; 6, minipreps of stable transcripts from untreated cells, BC; 7, EC. (B) Hybridization analysis of mini-preps from the agarose gel (A) transferred to a nitrocellulose membrane. The blot was hybridized with the ^{32}P global RT-PCR probe from interferon-treated cells. (C) Rehybridization, after washing, of the blot (B) with the ^{32}P global RT-PCR probe from interferon-untreated cells.

the sample corresponding to interferon-induced cells (Fig. 1B, lanes 3,6). The two main strong bands (Fig. 1, lanes 3,6) achieved after global RT-PCR appear to correspond to preferential stops of the reverse transcriptase enzyme because number, position and density are dependent on various parameters including the initial amount of RNA, the total amount of nucleotides present in the reverse transcriptase reaction and the type of the reverse transcriptase. In addition, these bands cannot be resolved by polyacrylamide gel electrophoresis as can differential display bands (data not shown).

3.2. Dot-blot analysis of global RT-PCR products with four radiolabeled probes

Hybridization experiments using dot-blots of global RT-PCR products were carried out to investigate the differential nature of four cDNAs named dD1, dD2, dD3 and dD4 (Fig. 2). After radiolabeling of each of these four cDNAs, probes were separately hybridized to dot-blots of global RT-PCR products from interferon-induced and non-induced cells. The results confirm the experiment involving dD2 and reveal an interferon effect. Moreover, the use of dot-blots reduces by a factor of five the requirements for cDNA generated by RT-PCR and radiolabeled probes in hybridizations.

3.3. Analysis of the four cDNAs with global RT-PCR probes

For easier screening by a similar approach of multiple potentially differential clones, the same hybridizing samples were retained, but the roles were reversed. Cloned cDNAs to be tested were digested with restriction enzymes, the fragments separated by agarose gel electrophoresis then the DNA fragments transferred to a nitrocellulose sheet. In parallel, two global RT-PCR experiments were carried out in the presence of [α-^{32}P]dCTP in order to prepare two global cDNA probes originating from poly(A)$^+$ RNA from interferon-induced or from non-induced cells. Fig. 3 shows that a straightforward mini-prep may be screened after blotting by performing two successive hybridization experiments: first with the global probe originating from induced cells (Fig. 3A), then after removal of the probe from the blot, by checking with the global probe originating from non-induced cells (Fig. 3B). These hybridization experiments were carried out in the presence of two cDNAs designated as BC and EC, corresponding to the 3′ ends of non-induced mRNAs isolated at random from non-induced cells (Fig. 3, lanes 6,7).

3.4. Analysis of the four cDNAs with Northern blots

For another set of experiments, mRNA extracted from luminal epithelium cells collected at day 15 from pregnant gilts and from pseudopregnant gilts (see Section 2) were analysed by Northern blots with four specific radiolabeled probes corresponding to dD1–dD4. The autoradiographs of Northern blots (Fig. 4) confirm previous results inferred from hybridization experiments carried out with products generated by global RT-PCR.

3.5. Sequence analysis of the four differential cDNAs

Sequence data from the cDNAs corresponding to induced genes allow characterization of three out of four of the isolated cDNAs. Two of them have already been identified as interferon-induced: (i) β_2-microglobulin for dD2 and (ii) Finkel-Biskis-Reilly murine sarcoma virus-associated ubiquitously expressed gene (fau) for dD3. The β_2-microglobulin protein associates with histocompatibility antigens [22] and they concomitantly increase upon interferon stimulation [23], as part of the immune response modulation; the corresponding sequence data is not shown because it is identical to *Sus scrofa* β_2-microglobulin (gb_om:pigmicro2b/rev L13854). FAU is also a previously identified interferon-inducible protein [24] and its biological activity is linked to the presence of a ubiquitin-like motif termed 'monoclonal non-specific suppressor factor β' and responsible for its lymphokine activity [25]. FAU inhibits generation of lipopolysaccharide-induced immunoglobulin-secreting cells and thus also contributes to the modulation of the immune response triggered by interferons.

The two other cDNAs are not related to already identified interferon-induced genes: dD1 corresponds to the 3′ end of an

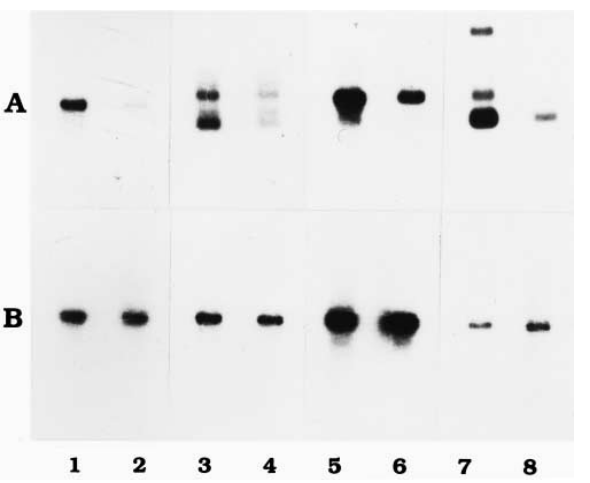

Fig. 4. Northern transfer analysis of poly(A)$^+$ RNA from luminal epithelium cells of pregnant (physiologically interferon-induced) or pseudogestant (non-induced). Lanes 1, 3, 5 and 7 correspond to cells originating from pregnant gilts and lanes 2, 4, 6 and 8 correspond to cells originating from pseudogestant gilts. (A) Blots hybridized with four differential ^{32}P-labeled DNA probes originating from interferon-treated cells. Lanes: 1,2, dD1; 3,4, dD2; 5,6, dD3; 7,8, dD4. (B) Rehybridization, after washing, of the blot (A) with ^{32}P-labeled β-actin.

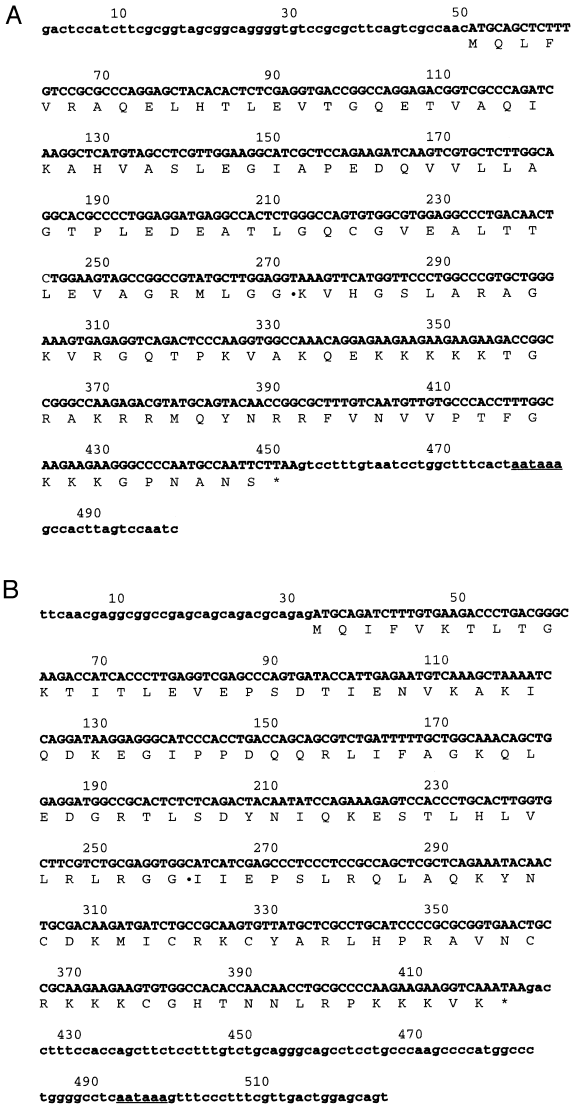

Fig. 5. Nucleotide sequence of porcine fau and ubiquitin UbA_{52} cDNAs. (A) Nucleotide sequence of the cDNA of fau clone dD3. (B) Sequence of nucleotides in the cDNA of UbA_{52} clone dD4. Non-coding regions are shown in lower-case letters. The putative polyadenylation site is underlined. The translation is given underneath the sequence. The asterisk represents the stop codon and the junction between ubiquitin and the C-terminal extension is shown by a filled circle.

unidentified gene and will be published elsewhere, while dD4 corresponds to a fusion gene containing ubiquitin. This fusion gene corresponds to the UbA_{52} subfamily of ubiquitin [26] which encodes a single ubiquitin moiety fused in frame to a carboxyl extension protein: the L40 protein [27] residing in the large ribosomal subunit. Porcine FAU and UbA_{52} share 23.5% throughout the nucleotide sequence and belong to the same protein family [28]. Fig. 5 illustrates this relationship: sequence identity increases to 36.1% after translation.

4. Discussion

As a result of the secretion of physiological interferons during the preimplantation stage of early pregnancy, enhancement of the level of specific mRNAs in cells of the luminal epithelium from endometrium of pseudopregnant gilts was demonstrated. It constitutes evidence that this tissue is a target for interferons and that the embryonic trophoblast has a paracrine effect on the tissue intended for embryo reception.

The present work has been carried out by using endometrium cells treated ex vivo with interferons but not cells taken from pregnant gilt, that is physiologically under the effect of interferons, to avoid the effect of secretions, other than interferons, possibly present in uterine horns at this moment of pregnancy.

This increase of some mRNAs was revealed by a novel screening method, adapted to a limited number of cells, and based on global RT-PCR, a method enabling amplification of the entire poly(A)$^+$ mRNA population from a few cells while preserving their relative representation in the cDNA population. This quantitative aspect of the methodology has been previously postulated by others [29,30], but is demonstrated here by parallel comparisons with Northern blots. After transfer to membranes, the global RT-PCR products provide hybridization signals giving the quantitative profile of the mRNA corresponding to the sample under investigation. The two cDNAs corresponding to genes whose transcription is interferon-induced [23,24] together with the two cDNAs to constitutively expressed mRNAs act as positive and negative controls, respectively.

From the methodological point of view, an essential change in the original global RT-PCR protocol [29] was made, concerning the mRNA isolation. In previous work [18], nucleic acids were released from cells by Nonidet P-40 treatment without a further mRNA purification step. When tested as described, this cell breaking method did not lead to consistent results comparable with those from Northern blots and in addition, in controls performed by omitting the reverse transcriptase enzyme prior to the PCR step, DNA amplification was seen by ethidium bromide staining (data not shown). Owing to these observations, mRNA purification steps compatible with a reduced number of cells were carried out (see Section 2). The negative controls in Fig. 1 (lanes 4,7) show the lack of contaminant DNA in the samples.

With the improved protocol including the essential mRNA purification step, the methodology allows one to consider new areas of investigation, such as the search for transcriptional activation in samples containing only a few cells. Until now, such samples were difficult to analyse because the amount of mRNA isolated was markedly insufficient for Northern blots. Moreover, this methodology suits the fast and simultaneous screening of a number of cDNAs isolated by differential display, since both global RT-PCR and differential display generate cDNA corresponding to the 3′ ends of poly(A)$^+$ mRNA.

Recent works concerning ubiquitin may need to be reconsidered with regard to the interferon effect, for example, that concerning the p36 protein [31]. p36 is a 36 kDa interferon-induced protein associated with human lupus inclusions and isolated on two-dimensional gels from the human B lymphoblastoid Raji cell line. On completion of their work, Rich et al. [31] merely indicate the strong homology between the gene encoding p36 and the CELF25B5.4 gene from *Cænorhabditis elegans*, but do not give a name to the gene. In fact, CELF25B5.4 encodes ubiquitin. Nevertheless, because p36 is larger than ubiquitin, and because only half of the peptides isolated and sequenced by Rich et al. correspond to the prod-

uct of the CELF25B5.4 gene (gb_in:u23172), p36 does not correspond to ubiquitin alone. p36 probably corresponds to the covalent association of two proteins, one of which is ubiquitin, i.e. a ubiquitinated protein [32].

The increase of ubiquitin mRNA, as a result of the interferon effect, agrees with recent work on the regulation of the phosphorylated Signal Transducer and Activator of Transcription 1 (STAT) protein by the ubiquitin-proteasome pathway [33]. STAT proteins are transcription factors pre-existing in the cell cytoplasm. The binding of interferons to their specific cell surface receptors induces the phosphorylation and translocation of STAT1 proteins into the nucleus and subsequent activation of a set of genes including ubiquitin. In turn, after the delay necessary for protein synthesis, ubiquitin is likely, by taking part in the proteasome-mediated degradation of the phosphorylated STAT1 protein, to decrease the amount of this factor which controls its transcription. In this way, the chain of events initiated by interferons may exert a negative feedback regulation.

Although the interferon induction of ubiquitin fits well with the previous report on the regulation of the STAT1 protein, it may not be its only function. It was neglected that ubiquitin is a multifunctional polypeptide characterized by other major biological activities: the control of DNA replication by way of binding to H2A and H2B histones [34] and a cytokine function by affecting lymphocytes and platelet differentiation [35,36].

Acknowledgements: Special thanks go to Drs. P. Mollier and F. Lefevre for their collaboration. We also wish to thank Dr. C. LaBonnardiere for support and Drs. J. Leung, I. Small and W. Chen for invaluable advice. We are grateful to B. Hoffman, P. Vende, B. Nicolas, M. Nezonde and C. Bourgeois for their participation in the work.

References

[1] Fowler, A.K., Reed, C.D. and Giron, D.J. (1980) Nature 286, 266–267.
[2] Duc-Goiran, P., Chany, C. and Doly, J. (1989) J. Biol. Chem. 264, 16507–16511.
[3] Roberts, R.M., Cross, J.C. and Leaman, D.W. (1992) Endocr. Rev. 13, 432–452.
[4] Cross, J.C. and Roberts, R.M. (1991) Proc. Natl. Acad. Sci. USA 88, 3817–3821.
[5] Perez, A., Riego, E., Martinez, R., Castro, F.O., Lleinart, R. and De la Fuente, J. (1991) J. Interferon Res. 11 (Suppl. 1), S214.
[6] Aboagye-Mathiesen, G., Toth, F.D., Juhl, C., Norskov-Lauritsen, N., Petersen, P.M. and Ebbesen, P. (1990) J. Gen. Virol. 71, 3061–3066.
[7] Lefèvre, F. and Boulay, V. (1993) J. Biol. Chem. 268, 19760–19768.
[8] Lefèvre, F., Martinat-Botte, F., Guillomot, M., Zouari, K., Charley, B. and La Bonnardière, C. (1993) Eur. J. Immunol. 20, 2485–2490.
[9] Sen, G.C. and Lengyel, P. (1992) J. Biol. Chem. 267, 5017–5020.
[10] Hannigan, G.E. and Williams, B.R.G. (1991) Science 251, 204–207.
[11] Darnell, J.E., Kerr, I.M. and Stark, G.R. (1994) Science 264, 1415–1421.
[12] Jaramillo, M.L., Abraham, N. and Bell, J.C. (1995) Cancer Invest. 13, 327–338.
[13] David, M. (1995) Pharmacol. Ther. 65, 149–161.
[14] Liang, P. and Pardee, A.B. (1992) Science 257, 967–971.
[15] Zhang, H., Zhang, R. and Liang, P. (1996) Nucleic Acids Res. 24, 2454–2455.
[16] Frank, M., Bazer, F.W., Thattcher, W.W. and Wilcox, C.J. (1977) Prostaglandins 14, 1183–1196.
[17] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[18] Brady, G. and Iscove, N.N. (1993) Methods Enzymol. 225, 611–623.
[19] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[20] Ausubel, I. and Frederick, M. (1994) Current Protocols in Molecular Biology, Wiley, New York.
[21] Perry, J.S., Heap, R.B. and Amoroso, E.C. (1973) Nature 245, 45–47.
[22] Morello, D., Daniel, F., Baldacci, P., Cayre, Y., Gachelin, G. and Kourilsky, P. (1982) Nature 296, 260–262.
[23] Wallach, D., Fellous, M. and Revel, M. (1982) Nature 299, 833–836.
[24] Nakamura, M., Xavier, R.M., Tsunematsu, T. and Tanigawa, Y. (1995) Proc. Natl. Acad. Sci. USA 92, 3463–3467.
[25] Nakamura, M., Xavier, R.M. and Tanigawa, Y. (1996) J. Immunol. 156, 533–538.
[26] Baker, R.T. and Board, P.G. (1991) Nucleic Acids Res. 19, 1035–1040.
[27] Chan, Y.-L., Suzuki, K. and Wool, I.G. (1995) Biochem. Biophys. Res. Commun. 215, 682–690.
[28] Michiels, L., Van der Rauwelaert, E., Van Hasselt, F., Kas, K. and Merregaert, J. (1993) Oncogene 8, 2537–2546.
[29] Brady, G., Barbara, M. and Iscove, N.N. (1990) Methods Mol. Cell. Biol. 2, 17–25.
[30] Varmuza, S. and Tate, P. (1992) Mol. Reprod. Dev. 32, 339–348.
[31] Rich, S.A., Bose, M., Tempst, P. and Rudofsky, U.H. (1996) J. Biol. Chem. 271, 1118–1126.
[32] Finley, D. and Chau, V. (1991) Annu. Rev. Cell Biol. 7, 25–69.
[33] Kim, T.K. and Maniatis, T. (1996) Science 273, 1717–1719.
[34] Davie, J.R. and Murphy, L.C. (1990) Biochemistry 29, 4752–4757.
[35] Goldstein, G., Scheid, M., Hammerling, U., Boyse, E.A., Schlesinger, D.H. and Niall, H.D. (1975) Proc. Natl. Acad. Sci. USA 72, 11–15.
[36] Pancré, V., Pierce, R.J., Fournier, F., Mehtali, M., Delanoye, A., Capron, A. and Auriault, C. (1991) Eur. J. Immunol. 21, 2735–2741.