Volume 211, number 1, 27–30 FEB 04358 January 1987

Oestrogen-induced expression of oncogenes in the immature rat uterus

Maureen T. Travers* and John T. Knowler

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

Received 27 October 1986

4 h after a single precocious administration of oestrogen there was a considerable but short-lived surge in the uterine levels of *myc*-encoded polyadenylated mRNA. This was followed by a further peak 28 h after hormone administration. The expression of ras^{Ha} showed a totally different time course with a build up of hybridizable message that peaked 8 h after oestrogen administration.

c-myc gene; $c\text{-}ras^{Ha}$ gene; Northern blotting

1. INTRODUCTION

Evidence is accumulating that the proteins encoded by some of the cellular equivalents of the viral oncogenes play a role in cellular proliferation and its control.

The *c-myc* gene encodes a nuclear chromatin-associated protein [1] the production of which is strongly correlated with cell proliferation [2] and is inversely correlated with cellular differentiation [3]. In a number of systems, including lymphocytes responding to concanavalin A and fibroblasts responding to platelet-derived growth factor, there are indications that the *myc* gene is regulated by specific growth signals [4].

The ras^{Ha} protein is believed to play a role in transducing signals from growth factors acting at the cell surface to the rest of the cell [5] and may thus represent another level at which the proteins encoded by the cellular equivalent of oncogenes influence cell proliferation. The gene is expressed in many normal tissues as well as in tumours, in which it is often expressed at much higher levels [6]. Müller et al. [7] showed that it was expressed at all stages of pre-natal development of mice and in various tissues of newborn and 10-day-old animals.

Oestrogen stimulates uterine cells initially by inducing hypertrophy and subsequently causing cellular proliferation [8]. It thus provides a non-malignant system with which to study the normal expression of *c-myc* and $c\text{-}ras^{Ha}$.

2. MATERIALS AND METHODS

The source and maintenance of immature female rats and the administration of oestradiol-17β at 1 μg per 30 g rat have previously been described. RNA was isolated by phenol extraction at 60°C and precipitation by ethanol in the presence of 0.3 M sodium acetate, pH 5.8. DNA contamination in the resulting pellet was removed by dissolving it in 1%, w/v, sodium lauryl sarkosinate, 10 mM EDTA, pH 7.0, to which was added CsCl to 1.0 g/ml and centrifugation through high density CsCl (1.4 g/ml in 10 mM EDTA, pH 7.0, at 17000 rpm for 17 h in the SW40 rotor of the Beckman LC5 ultracentrifuge (35900 $\times$ g_{av}). Polyadenylated RNA was isolated by one

Correspondence address: J.T. Knowler, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

* Present address: Ludwig Institute for Cancer Research, St. Georges Hospital, Blackshaw Road, London SW17 0QT, England

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

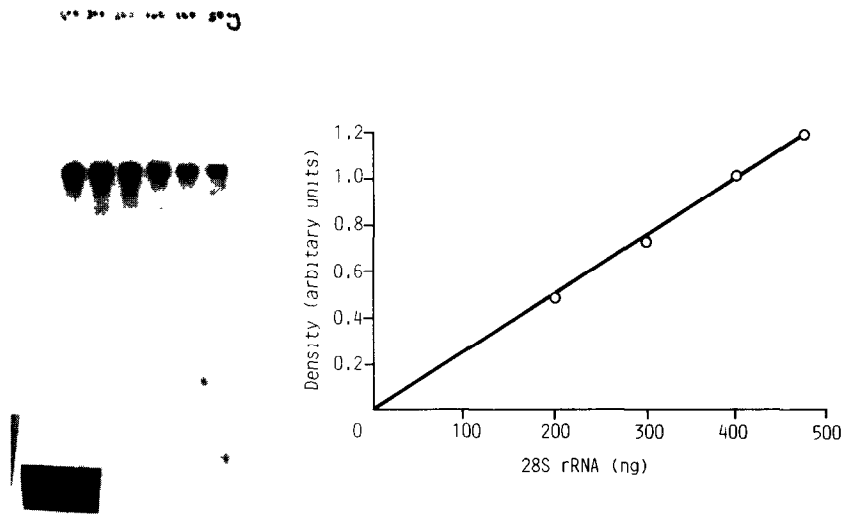

Fig.1. (A) Standard amounts of 28 S rRNA were resolved in a 0.8% agarose/2.2 M formaldehyde gel, transferred to nitrocellulose and hybridized to nick-translated p28S (a 28 S genomic clone in pBR322). Lanes (from left to right) contain 400, 300, 200, 150, 100, 50 ng 28 S rRNA, respectively. (B) Typical standard curve showing densitometric analysis of an assay similar to that in panel A.

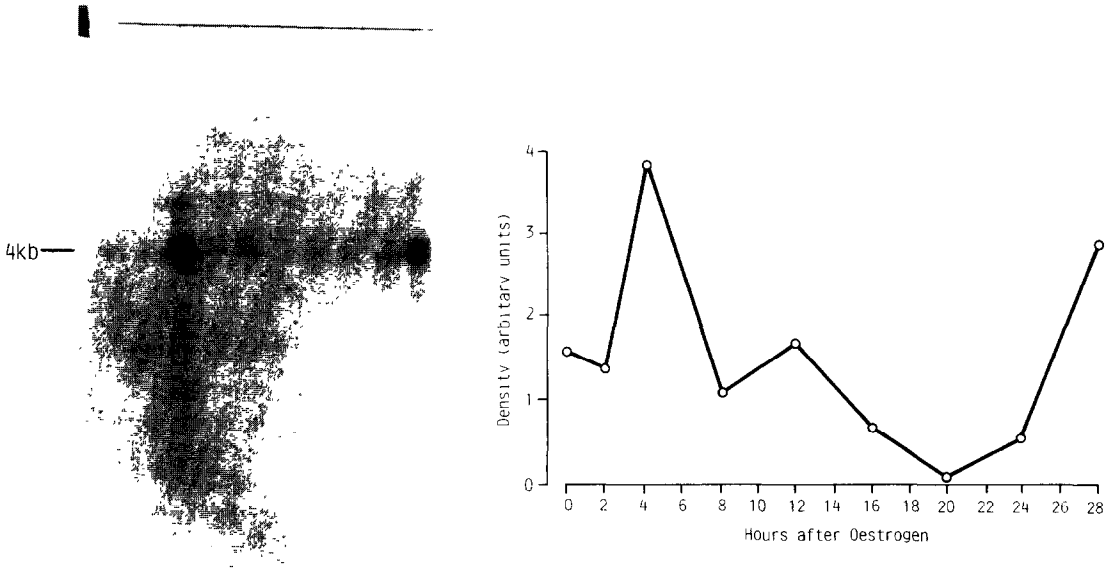

Fig.2. Expression of *c-myc* in immature uterus. (A) The hybridization on Northern blots of nick-translated *v-myc* (clone pmyc-2) to 1 μg polyadenylated mRNA isolated from the uteri of rats which had been given oestradiol-17β, 0, 2, 4, 8, 12, 16, 20, 24, 26 and 28 h before death. Ribosomal RNA contamination of the purified mRNA was assessed by hybridization to a nick-translated cloned 28 S rDNA (fig.1) and its effect corrected for. The quality and quantitation of the mRNA were also checked by cross-hybridization to nick-translated rat skeletal actin cDNA (clone pA-749). (B) Densitometric analysis of the 4 kb band in A.

passage of the RNA through oligo(dT)-cellulose. Such preparations always contained some contamination with rRNA but it was found preferable to quantitate and correct for the effect of this rather than attempt complete removal. Residual rRNA contamination was estimated by hybridization of poly(A)$^+$ RNA to nick-translated p28S, a 28 S rRNA clone in pBR322, followed by comparative densitometric analysis against standard amounts of purified rRNA standards (fig.1). The quality of each mRNA preparation was also checked by cross-hybridization to nick-translated rat skeletal actin cDNA (clone pA-749). Constant amounts of mRNA were analyzed for oncogene sequences on Northern blots by hybridization to nick-translated *v-myc* (clone pMyc-z) and *v-ras*^{Ha} (clone BS-9).

3. RESULTS AND DISCUSSION

4 h after a single precocious administration of oestrogen to 3-week-old rats, there was a considerable but short-lived surge in the uterine levels of *myc*-encoded polyadenylated mRNA. This was followed by a further peak 28 h after hormone administration (fig.2). These two peaks corresponded to the periods of stimulated RNA and DNA synthesis, respectively, that occur when oestrogen stimulates first hypertrophy and then hyperplasia in the rat uterus [8]. Similar rapid, short-lived changes in *myc* expression have been reported as a result of DMSO-induced differentiation of mouse erythroleukaemia cells [9] and during liver regeneration [10]. The rapid changes appear to be due to a very short half-life of the mRNA which has been estimated to be approx. 10 min [11].

The expression of *ras*^{Ha} in response to oestrogen showed a totally different time course to the expression of *myc* (fig.3). There was a gradual build up of hybridizable message which peaked at 8 h after hormone administration after which levels tailed off. 8 h post-oestrogen treatment corresponds to the time when the hormone-induced surge in total cellular protein synthesis is approaching its maximum. A similar time course in the stimulation of *ras*^{Ha} expression is seen in regenerating rat liver [12] and this could also result from a change in the hormonal environment.

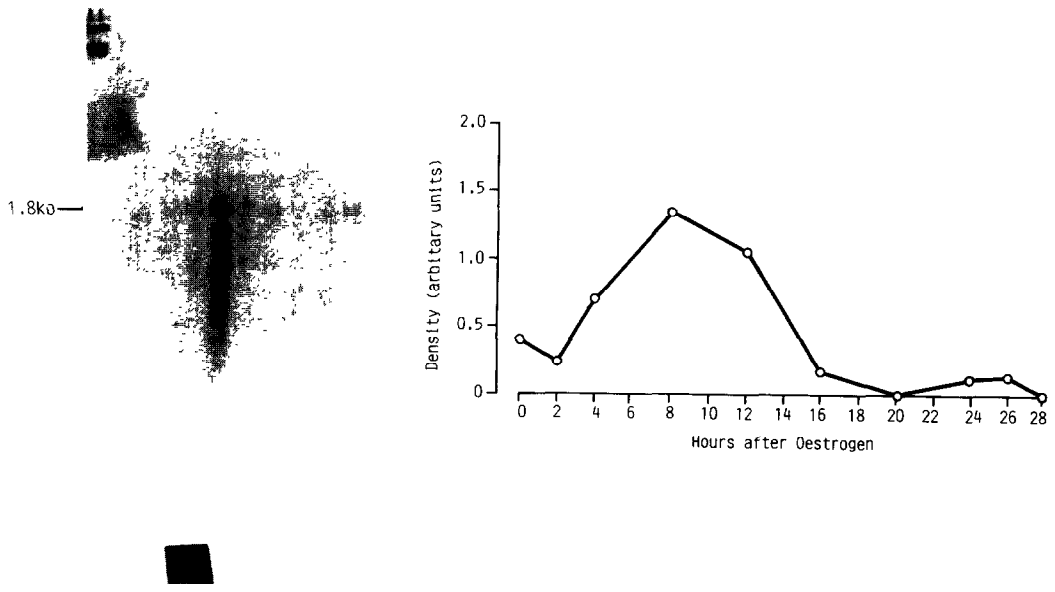

Fig.3. Expression of *c-ras*^{Ha} in immature uterus. (A) The hybridization on Northern blots of nick-translated *ras*^{Ha} (clone BS-9) to 5 μg polyadenylated mRNA isolated from the uteri of rats which had been given oestrogen 0, 2, 4, 8, 12, 16, 20 and 24 h before death. Other details as in the legend to fig.1. (B) Densitometric analysis of 1.8 kb band in A.

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