Molecular and Cellular Biochemistry **117**: 71–79, 1992.

Transcription induction of c-Ki-ras with the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in normal and transformed liver cells

Sai On Chan, Susanna Siu Chun Wong and Desmond Chak Yew Yeung

Department of Biochemistry, The University of Hong Kong, Hong Kong

Received 22 November 1991; accepted 7 July, 1992

Abstract

Results from nuclear run-off assays show that exposure of hepatocytes and Reuber H35B hepatoma cells to the tumour promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA), leads to enhanced transcription of c-Ki-ras gene. This increase in transcription in turn results in an accumulation of the functionally active c-Ki-ras message. The half life of c-Ki-ras message in both normal and transformed livers cells is not altered by TPA and is determined to be 3.5 hr. The induction of c-Ki-ras message is accompanied by an increase in the level of c-Ki-ras protein. (Mol Cell Biochem **117:** 71–79, 1992)

Key words: c-Ki-ras, 12-O-tetradecanoylphorbol-13-acetate (TPA)

Abbreviations: H-7 – 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine, UTP – Uridine Triphosphate, CTP – Cytidine Triphosphate, ATP – Adenosine Triphosphate, GTP – Guanosine Triphosphate, GTPase – Guanosine Triphosphatase, SDS-PAGE – Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis, TPA – 12-O-tetradecanoylphorbol-13-acetate, MEM – Minimal Essential Medium

Introduction

It is well documented that proto-oncogenes are vital in the control of growth and differentiation [1–5]. Accordingly, immense interests have been directed towards elucidating the mechanisms regulating their expression. The ras gene family which consists of H-ras, Ki-ras and N-ras has been associated with many different types of human tumors [6]. The ras gene products are found to be membrane associated [7] and like G proteins, they are capable of binding to guanine nucleotides and have been shown to possess GTPase activity [8]. In addition, they exhibit a high degree of sequence homology with G proteins [6]. Since several G proteins, such as Gs and Gi, are crucial elements in a number of signal transduction pathways, it is logical to expect that ras proteins may function as signal transducer molecules. Indeed, in the yeast Saccaromyces cerevisiae, the ras related pro-

Address for offprints: D. Chak Yew Yeung, Department of Biochemistry, The University of Hong Kong, Hong Kong

teins, RAS 1 and RAS 2, appear to be involved in the regulation of cAMP levels by coupling to adenylate cyclase [9]. Frank *et al.* [10–13] provided evidence for a similar role of ras proteins in the regulation of adenylate cyclase in rat kidney fibroblasts. In addition, several other studies have implicated the involvement of ras proteins in the activation of the phosphoinositide breakdown pathway [14–17]. If indeed ras proteins were to participate in signal transduction, it is logical to expect that their expression might be regulated by both hormones and growth factors. Recently, a number of growth stimulating agents have been shown to be capable of altering the expression of several proto-oncogenes [18–22]. For example, growth factors such as platelet-derived growth factor, fibroblast growth factor, epidermal growth factor can induce transiently the level of the myc message in fibroblasts [18–22]. Furthermore, in both B and T cells, myb expression may be stimulated during cell cycle progression induced by lymphoid mitogens [23].

Previous work in our laboratory has shown that the inductive effect of glucagon on c-Ki-ras expression is mediated via activation of protein kinase C [24]. This is substantiated by the observations that 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent activator of protein kinase C, can induce the level of c-Ki-ras transcripts, whereas, addition of a specific kinase C inhibitor, H-7, leads to a suppression of the c-Ki-ras message. However, it remains to be established whether the increase in the level of transcript is a consequence of a stimulation of the transcriptional process or a decrease in the rate of degradation of the message. The demonstration of an increase in the level of a message by Northern blot analysis does not necessarily imply an increase of functional ras message. To resolve these uncertainties, nuclear run-off assays were carried out to investigate if the stimulation of c-Ki-ras expression by TPA was a result of an enhancement of transcriptional rate. Furthermore, the stability of the c-Ki-ras message was determined in TPA-treated normal hepatocytes and Reuber H35B hepatoma cells. In addition, the TPA-induced c-Ki-ras message was tested for its functional activity by an *in vitro* translation system and the amount of c-Ki-ras protein in both normal and TPA-induced cells were examined by Western blotting.

Materials and methods

Cell culture

Reuber H35 hepatoma cells [25] adapted to tissue culture (line H4 II E) [26] were obtained from Dr. F.J. Ballard (Commonwealth Scientific & Industrial Research Organization Division of Human Nutrition, Adelaide, South Australia) and were grown as monolayers under sterile condition in minimal essential medium containing 5% fetal calf serum, 100 mg streptomycin sulphate and 60 mg penicillin per litre solution. Cells were incubated at 37° C with the gas phase being 5% CO_2 in air in a CO_2-controlled water-jacketted incubator.

Isolation of hepatocytes from rat liver

Sprague-Dawley rats weighing about 120 g were used. Hepatocytes were isolated by perfusion of the liver according to the method described by Berry and Friend [27] with 0.05% collagenase (mass/vol.) replacing hyaluronidase. Cells were cultured as described above.

Isolation of cell nuclei

Approximately 2×10^7 cells were used. Cells were washed twice with phosphate buffered saline (PBS) containing 0.14 mM NaCl; 3 mM KCl; 8 mM sodium monohydrogen phosphate; 1.5 mM potassium dihydrogen phosphate, pH 7.4. Following centrifugation at $500 \times$ g for 5 min, the cell pellet was resuspended in 2 ml ice cold NP40 lysis buffer. This buffer contains 10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 3 mM $MgCl_2$; 0.5% (vol/vol) NP40. The mixture was incubated on ice for 5 min before it was centrifuged at $500 \times$ g at 4° C for 5 min. The nuclei were washed once with 4 ml NP40 lysis buffer and then resuspended in Hepes buffer and kept at −70° C. This buffer contains 50 mM Hepes, pH 7.5; 4 mM $MnCl_2$; 1 mM $MgCl_2$; 0.1 mM EDTA; 50% glycerol and 5 mM dithiothreitol.

Nuclear run-off assay

Freshly isolated nuclei were incubated with 50 μCi of [^{32}P]-UTP in 200 μl incubation buffer containing 25% glycerol; 2.5 mM $MgCl_2$; 0.05 mM EDTA; 75 mM Hepes, pH 7.5; 100 mM KCl; 4 mM dithiothreitol; 0.5 mM CTP; 0.5 mM GTP; 1.0 mM ATP; 22 units/ml creatine kinase; 8.8 mM creatine phosphate, pH 7.5 for one hour before the reaction was terminated with the addition of 8 units of RNase-free DNase. Labelled RNA was purified and hybridized to a Hybond membrane previously dot blotted with the DNA probe. Hy-

bridization was at 42° C for 48 hr in a buffer containing 50% deionized formamide, 0.5% SDS, 5 × Denhardt's solution (100 × Denhardt's solution contains 2% Ficoll, 2% polyvinylpyrrolidine and 2% bovine serum albumin) and 5 × NaCl/Pi/EDTA (20 × NaCl/Pi/EDTA contains 3.6 M NaCl, 0.1 M sodium dihydrogen phosphate, 0.1 M sodium monohydrogen phosphate and 0.002 M EDTA, pH 7.7). Afterwards, the filter was washed for 15 min twice in 2 × NaCl/Pi/EDTA/0.1% SDS, once in 1 × NaCl/Pi/EDTA/0.1% SDS and twice in 0.2 × NaCl/Pi/EDTA/0.1% SDS at 42° C. The filter was exposed to Kodak XAR film at − 70° C.

Preparation of c-Ki-ras probe

pHiHi-3 which contained the v-Ki-ras probe was obtained originally from Dr Ronald W. Ellis [28]. The plasmid DNA was prepared as described by Maniatis *et al.* [29].

Determination of the half-life of c-Ki-ras mRNA

Cells in 100 mm dish were labelled with 48 μCi [^{3}H]-uridine for 3.0 hr which allowed maximum incorporation into RNA. After thorough washing with MEM containing 10 mM cold uridine, the labelled cells were incubated either in the absence or presence of 1.6×10^{-6} M 12-O-tetradecanoylphorbol-13-acetate (TPA) in MEM containing 10 mM cold uridine. At different time intervals, the cells were washed in MEM containing 10 mM uridine. They were then lysed with the addition of 4 ml guanidinium thiocyanate buffer; this buffer contains 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% (mass/vol.) sodium lauroylsarcosine, 0.1 M 2-mercaptoethanol, pH 7.0. The RNA was prepared by the method Chirgwin *et al.* [30] with minor modifications as described by Chan *et al.* [24].

Hybridization of [^{3}H]-RNA to filter. The [^{3}H]-RNA was dissolved in 0.2 ml hybridization buffer. Filters containing the bound DNA were hybridized with the labelled RNA at 42° C for 24 hr. The filters were then washed twice in 2 × NaCl/Pi/EDTA, 0.1% (mass/vol) SDS, once in 1 × NaCl/Pi/EDTA, 0.1% (mass/vol) SDS and finally twice in 0.2 × NaCl/Pi/EDTA, containing 10 μg/ml ribonuclease A. The filters were air-dried and were then boiled in 0.2 ml of diethylpyrocarbonate-treated water for 5 min. 0.2 ml of the eluted RNA solution was mixed with 1 ml Aquasol II and the mixture was allowed to stand overnight before the radioactivity was determined in a Beckmann Scintillation Counter.

Isolation of poly(A) RNA

Total cellular RNAs were prepared from cell extracts by the guanidium thiocyanate method [30] with slight modifications as described [24]. Poly(A) RNA was isolated by using Hybond-mAP affinity paper (Amersham Co. Ltd).

In vitro *translation of c-Ki-ras message*

mRNA was translated in the presence of [^{3}H]-leucine using an *in vitro* translation system supplied by New England Nuclear Company. The translated c-Ki-ras protein was immunoprecipitated with a specific monoclonal antibody raised against c-Ki-ras (supplied by Oncogene Science Inc.). After thorough washing, the immunoprecipitate was solubilized and separated by SDS polyacrylamide gel electrophoresis. The rod gel was cut into equal slices and solubilized for determination of associated radioactivity by liquid scintillation counting.

Preparation of cell extract for detection of c-Ki-ras protein

Approximately 1×10^7 cells were harvested and washed once in ice cold phosphate buffered saline. Cells were lysed in 1 ml lysis buffer containing 10 mM Tris-HCl; 50 mM NaCl; 1% Triton X-100; 5 mM EDTA; 1 mM phenylmethylsulfonyl flouride; 1 mM iodoacetic acid, pH 7.6. The cell debris was removed by centrifugation at 11,000 × g, 4° C for 30 min and the supernatant was saved for Western blot analysis.

Western blot analysis of c-Ki-ras protein

The c-Ki-ras protein present in the cell extract was immunoprecipitated by addition of a specific monoclonal antibody raised against c-Ki-ras. 1 μg of the monoclonal antibody was added to 500 μl of cell extract. This was followed by the addition of 15 μl of protein A sepharose. The mixture was incubated overnight at 4° C with gentle shaking. The immunoprecipitate was spun down at 12,000 × g and was then washed five times with ice-cold phosphate-buffered saline. The precipitate was analysed by a 7.5%–17.5% gradient SDS-PAGE. After electrophoresis, the protein was then electroblotted onto a Hybond-ECL membrane in an electroblotting buffer at 20 mA current overnight. Non-specific binding sites on the protein blot were blocked by immersing the membrane in 5% (mass/vol) non-fat skimmed milk in Tris-buffered saline-Tween 20 for one hour at room temperature on a roller incubator. This buffer contained the Tris-buffered saline with 0.1% (vol/vol) Tween 20. A monoclonal antibody

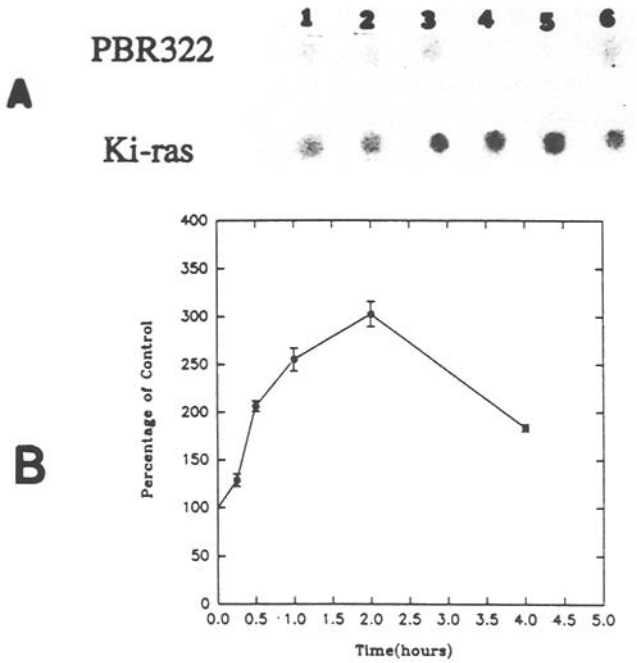

Fig. 1. Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the transcription of Ki-ras gene in normal rat hepatocytes.

Panel A: The figure showed a typical autoradiogram obtained from a nuclear run-off experiment. Cultured hepatocytes were incubated with 1.6×10^{-6} M TPA and were harvested at 0, 1/4, 1/2, 1, 2 and 4 h (columns 1 to 6). Nuclei were isolated and incubated with 50 μCi [^{32}P]UTP for 1 h at 25° C. Labelled RNA was isolated and hybridized to a filter previously dot-blotted with pBR322 and Ki-ras DNA probe. After stringent washing, the filter was exposed to an X-ray film for autoradiography. *Panel B:* The diagram shows a densitometric scan of the autoradiograms obtained. The change in transcription rate of c-Ki-ras was plotted as a per cent of the zero time control versus time of incubation with TPA. Error bars represent the standard error of at least four separate determinations.

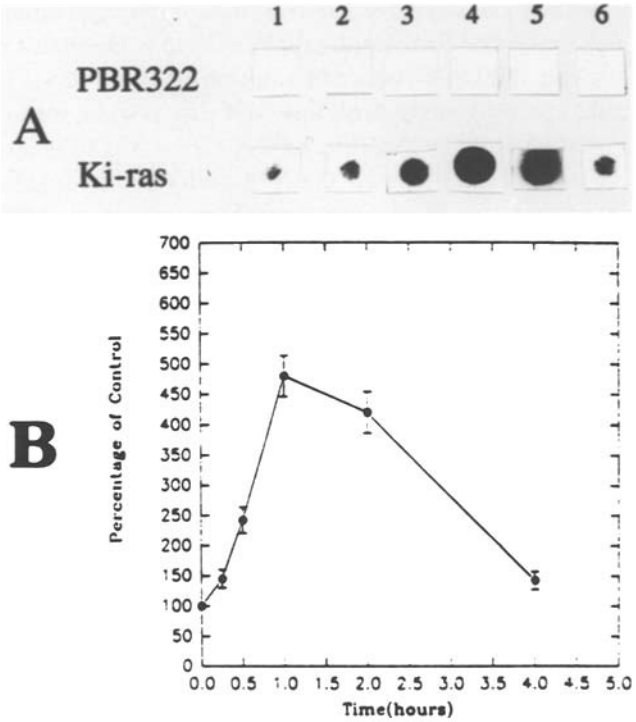

Fig. 2. Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the transcription of c-Ki-ras gene in Reuber H35B hepatoma cells.

Panel A: The figure showed a typical autoradiogram obtained from a nuclear run-off experiment. Cultured Reuber H32B hepatoma cells were incubated with 1.6×10^{-6} M TPA and were harvested at 0, 1/4, 1/2, 1, 2 and 4 h (columns 1 to 6). Nuclei were isolated and incubated with 50 μCi [^{32}P]UTP for 1 h at 25° C. Labelled RNA was isolated and hybridized to a filter previously dot-blotted with pBR322 and Ki-ras DNA probe. After stringent washing, the filter was exposed to an X-ray film for autoradiography. *Panel B:* The diagram showed a densitometric scan of autoradiograms obtained. The change in transcription rate of c-Ki-ras was plotted as a per cent of the zero time control versus time of incubation with TPA. Error bars represent the standard error of at least four separate determinations.

raised against c-Ki-ras diluted in 5% (mass/vol) non-fat skimmed milk in Tris-buffered saline-Tween 20 was added. After an overnight incubation at 4° C, a second antibody coupled with horseradish peroxidase suitably diluted in 5% (mass/vol) non-fat skimmed milk in Tris-buffered saline-Tween 20 was added. The incubation was for 1 h at room temperature before the membrane was washed once for 15 min and four times for 5 min with tris-buffered saline.

An enhanced chemiluminescence system supplied by Amersham International Co. was used for the detection of the c-Ki-ras protein. The membrane was drained off of excess buffer and placed in a fresh container and the detection reagents which contain luminol and an enhancer were added. Incubation was carried out for precisely 1 min at room temperature. The blot with the protein side up was put in a film cassette and was exposed to the Hyperfilm-ECL (supplied by Amersham International Co.).

Results

Effect of TPA on the transcriptional activity of c-Ki-ras gene

When normal hepatocytes were incubated with TPA, the transcriptional activity of c-Ki-ras gene showed a very rapid increase reaching a peak value (300% of the control) within two hours, thereafter the rate started to decline (Fig. 1). A similar observation was detected in

Reuber H35B hepatoma cells when they were incubated with TPA (Fig. 2). The increase in transcription rate of c-Ki-ras gene in these transformed cells was equally rapid and the maximal rate of c-Ki-ras gene transcription was greater than four and a half fold.

Effect of TPA on the stability of c-Ki-ras mRNA

The half-life of c-Ki-ras message was 3.5 hr in both hepatocytes and Reuber H35 hepatoma cells. It appears that TPA does not have any appreciable effect on the stability of the c-Ki-ras message in both cell types (Fig. 3A and 3B).

Effect of TPA on the level of functional c-Ki-ras message
We have previously shown that TPA could increase the level of c-Ki-ras transcript as determined by Northern blot analysis [24]. The question of whether or not this increase in c-Ki-ras message was also accompanied by an accumulation of functionally active message was addressed. Using an *in vitro* rabbit recticulocyte lysate system, it was shown that when normal hepatocytes or transformed cells were incubated with TPA, there were marked increases in the functionally active c-Ki-ras transcript (Figs. 4A and 4B).

Effect of TPA on the level of c-Ki-ras products
Results from Western blot analysis showed that when hepatocytes were incubated with TPA, the level of c-Ki-ras protein was increased (Fig. 5a). Several protein bands were shown to react with a monoclonal antibody raised against c-Ki-ras. In addition to the p21 c-Ki-ras protein, a 23 kD protein band which represents the phosphorylated form of the 21 kD c-Ki-ras protein and a 42 kD protein band, which is a dimer of p21, could be readily detected. It is important to note that the monoclonal antibody does not cross react with other proteins (private communication with Dr. Brian Moore, Oncogene Science Inc.). The monoclonal antibody was obtained from a clone derived by fusion of mouse spleen cells with myeloma cells. It was raised to amino acids 66-189 of the authentic c-Ki-ras protein. The antibody reacts by immunoprecipitation, immunoblotting with c-Ki-ras p21 but not with either c-Ha ras or c-N-ras p21's. In transformed rat liver cells, the level of c-Ki-ras protein was also induced upon the addition of TPA (Fig. 5B) and again a similar pattern of protein bands was detected.

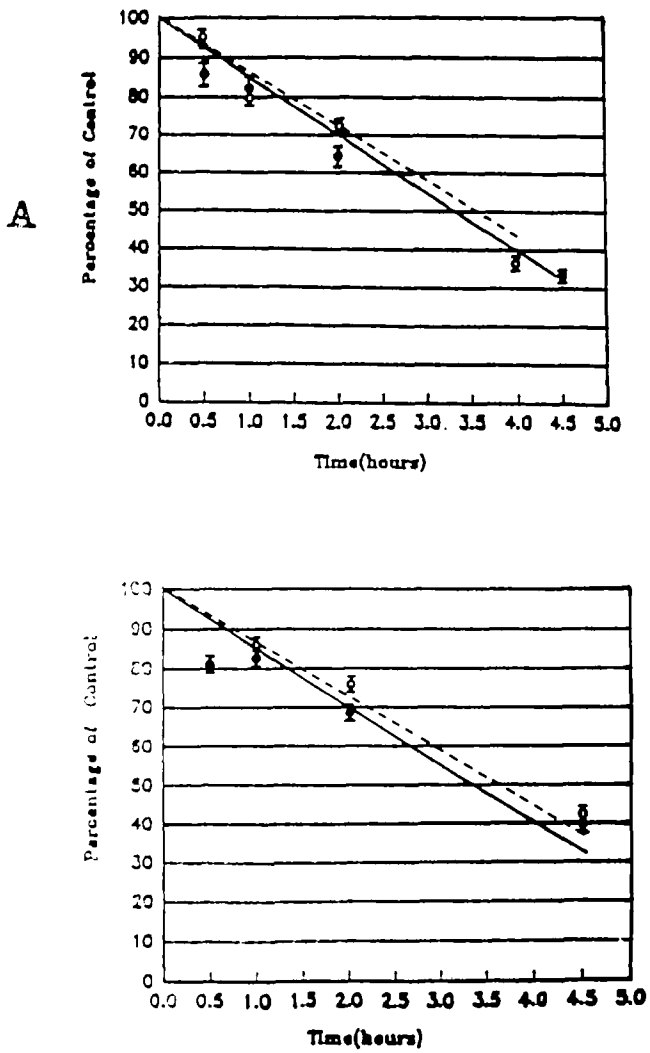

Fig. 3. Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the stability of Ki-ras messages in normal rat hepatocytes and Reuber H35B hepatoma cells.

Cultured normal rat hepatocytes (--●--●--●--) and Reuber H35B hepatoma cells (—○—○—○—) were pretreated with 48 μCi/ml [^{3}H]uridine in MEM for 3 hr. After thorough washing with MEM containing 10 mM cold uridine, the labelled cells were incubated in the absence (panel A) or presence of 1.6×10^{-6} M TPA (panel B) in MEM containing 10 mM cold uridine. Cells were harvested at different time intervals. The specific radioactivity of labelled c-Ki-ras RNA was measured by liquid scintillation counting and was plotted as a percent of the zero time control versus time of cell harvest. Error bars represent the standard error of at least four separate determinations.

Discussion

Previous studies from our laboratory have shown that c-Ki-ras expression is induced by low concentrations of glucagon and that we have also provided evidence sug-

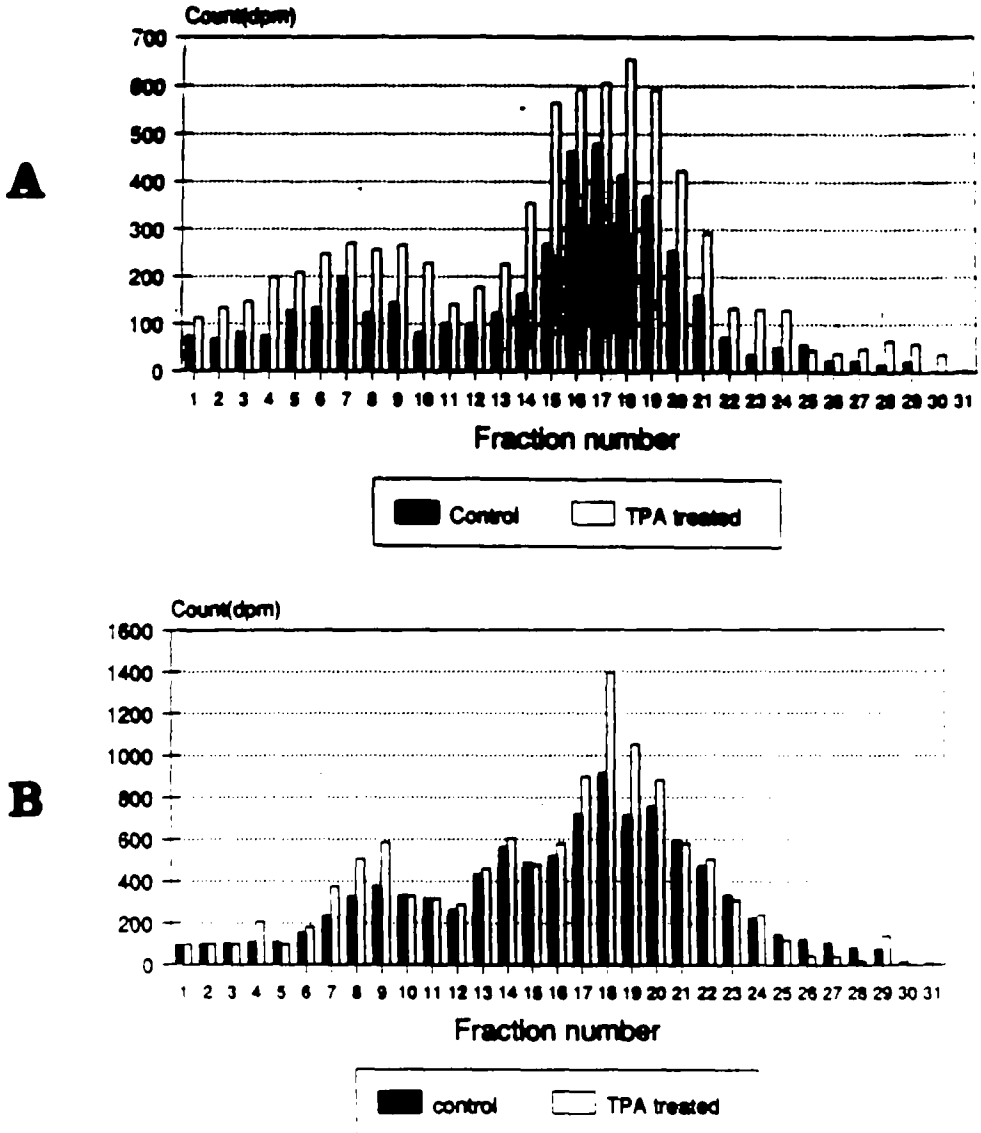

Fig. 4. Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the accumulation c-Ki-ras functional message in normal rat hepatocytes and Reuber H35B hepatoma cells.

Poly (A) RNA was isolated from cultured normal rat hepatocytes (panel A) and Reuber H35B hepatoma cells (panel B) incubated with the absence or presence of 1.6×10^{-6} M TPA for 4 h. The mRNA was translated in the presence of [^{3}H]leucine using an *in vitro* translation system supplied by New England Nuclear Company. The translated c-Ki-ras protein was immunoprecipitated with a specific monoclonal antibody raised against the c-Ki-ras protein. After thorough washing, the immunoprecipitate was solubilized and separated by SDS polyacrylamide gel electrophoresis. The rod gel was cut into equal slices and solubilized before the amount of associated radioactivity was measured by liquid scintillation counting. The protein detected in gel fractions 18 and 19 from the origin of the gel corresponded to a molecular weight of 21 kD.

gesting that the inductive effect of glucagon is mediated via protein kinase C [24]. Thus, long term exposure of hepatoma cells to the tumour promoting agent, 12-O-tetradecanoylphorbol 13-acetate (TPA) which leads to down regulation of protein kinase C, results in abolishment of the effect of glucagon. However, when cells are acutely treated with TPA, significant accumulation of c-Ki-ras messages can be demonstrated. The increase in the level of messenger RNA may be a result of either an increase in the rate of transcription or a decrease in the rate of degradation of the message. In the present study, we have shown that both Reuber H35 hepatoma cells and normal hepatocytes when treated with TPA demonstrate significant changes in the rate of transcription of the C-Ki-ras gene as evidenced by the results from nuclear run off assays. The response to TPA is

Fig. 5. Effect of 12-O-tetradecanoylphorbol-13-acetate (TPA) on the accumulation of c-Ki-ras protein in normal rat hepatocytes and Reuber H35B hepatoma cells.

Cultured normal rat hepatocytes (panel A) and Reuber H35B hepatoma cells (panel B) were incubated with 1.6×10^{-6} M TPA and harvested at 0, 2 and 4 h (lanes 1 to 3). The crude cell extract was immunoprecipitated with a monoclonal antibody raised against the c-Ki-ras protein. The immunoprecipitate was analysed by 7.5%–17.5% SDS polyacrylamide gradient gel. The c-Ki-ras protein electroblotted onto the membrane was incubated with the c-Ki-ras specific antibody and detected by a chemilumifluorescence kit supplied by Amersham Co.

much greater in hepatoma cells than that seen in normal hepatocytes. However, we have no evidence to suppose that normal hepatocytes require more TPA than hepatoma cells as the threshold for TPA appears to be the same in both types of cells (result not presented). One possible explanation of this difference probably lies in the fact that there is a larger proportion of protein kinase C translocated from the cytosol to the plasma membrane in rapidly dividing cells [31]. One may therefore expect a higher membrane-associated protein kinase C concentration in the more rapidly dividing Reuber H35 cells and consequently greater induction by TPA. However, one cannot rule out the possibility that the signal transduction pathway downstream to protein kinase C is more sensitive to activation by protein kinase in more rapidly dividing cells than their normal counterparts.

It is well established that TPA exerts numerous biological effects in a wide range of cell types. As well as inducing transcription of several other cellular proto-oncogenes including c-fos [32], c-myc [21], c-jun [33, 34] and c-sis [35]. TPA is also capable of stimulating the expression of collagenase [36] and stromelysin genes [36, 37], both of which have been implicated in tumour invasiveness, angiogenesis [38] and metastasis [39]. In addition, TPA is capable of inducing the synthesis of the transcription factor complex AP-1 via activation of protein kinase C [40]. It has been suggested that the ability of TPA to induce gene transcription is secondary to its inductive effect on AP-1. In this respect, it is interesting to note that several of the TPA-inducible genes studied passess a common sequence TGAGTCAG [41]. The exact nature of the nuclear factors which bind to these TPA responsive elements has not been identified, although they are likely to interact with protein substrates for protein kinase C or proteins modified by the phosphorylation cascade induced by protein kinase C. Baeuerly & Baltimore [42] have demonstrated that in both unstimulated 70Z/3 pre-B cells and Hela cells, the kappa enhancer-binding protein NF-kappa B is present as a cytosolic protein which exists in an inhibited form with no apparent DNA binding activity. However, on stimulation with TPA, the protein is translocated into the nuclei with simultaneous release of the inhibition on DNA binding activity.

In view of the many-faceted actions of TPA, we have also performed experiments to investigate whether or not the stability of the c-Ki-ras message has been altered following TPA treatment. Our results clearly show that the half-life of the C-Ki-ras transcript is essentially similar in both untreated and TPA-treated cells. In addition, the stability of the c-Ki-ras mRNA in H35B hepatoma cells does not differ significantly from that found in normal hepatocytes suggesting that stability of the message is probably not a factor giving rise to the vastly different levels of c-Ki-ras transcripts in the two types of cells under basal conditions. The half-life of c-Ki-ras mRNA as measured in either normal or transformed hepatocytes is 3.5 hr and is quite short in comparison to that of β-globin mRNA which is greater than 24 hr in erythroid cells [43]. The short half-life of RNA transcripts is often a characteristic of those genes involved in growth control as their expression has to be stringently regulated. This group of genes includes proto-oncogenes such as c-fos and c-myc and those coding for lymphokines and cytokines.

We have also carried our an *in vitro* translation study to test the functional activity of the TPA-induced tran-

script. Indeed, the induced message can be efficiently translated into a p21 protein which is recognized by a monoclonal antibody raised against c-Ki-ras. This monoclonal antibody is absolutely specific for c-Ki-ras as it does not cross react with other members of the ras family (see result section). In both normal hepatocytes and Reuber H35 hepatoma cells, the TPA-induced increase in transcription of the c-Ki-ras gene is accompanied by parallel increases in the amount of the c-Ki-ras protein. Three protein species with molecular weights of 42 kD, 23 kD and 21 kD were detected. We have repeatedly demonstrated that the 21 kD ras protein forms a dimer even under the reducing condition employed in SDS gel electrophoresis. The same observation was also made independently in another laboratory (Dr. Brian Moore, Oncogene Science Inc., private communication). The 23 kD protein probably represents the phosphorylated form of the acylated, mature form of c-Ki-ras [40, 44]. The occurrence of post-translational modification of c-Ki-ras has been reported previously [44–49] and its functional significance probably lies in its ability to interact with an integral membrane receptor thus enabling it to play a role in membrane signalling.

The importance of ras proteins in the control of growth and differentiation in eukaryotes is well established. During liver regeneration, the expression of ras gene follows a specific sequential manner which suggests a vital role played by ras proteins in growth-related processes [50, 51]. Recent work has shown that lowering the amount of membraned associated p21 ras protein leads to significant inhibition of growth of hepatocytes [52]. We have recently demonstrated [Chan *et al.*, in press] that c-K-ras shows a specific developmental pattern in liver which is characteristic of a group of enzymes termed as the neonatal cluster which includes phosphoenolpyruvate carboxykinase, tyrosine aminotransferase and serine dehydratase [53]. Like c-Ki-ras, the expression of these postnatal enzymes is induced by glucagon [53, 54]. It will be of interest to investigate if c-Ki-ras protein may be involved in the regulation of the development of these neonatal enzymes.

Acknowledgements

This work was supported by grants from the Committee on Research and Conference Grants and the Medical Research Grants Committee of the University of Hong Kong.

References

1. Studzinski GP: Oncogenes, growth, and the cell cycle: A review. Cell Tissue Kinet 22: 405–424, 1989
2. Reddy DP, Skalka AM, Curran T: The oncogene handbook. Elsevier, Amsterdam, 1988
3. Boettiger D: Interaction of oncogenes with differentiation programs. Curr Top Microbiol Immunol 147: 31–78, 1989
4. Sigal IS, Gibbs JB: Oncogenes Curr Opin Cell Biol 1: 286–290, 1989
5. Zelenka PS: Proto-oncogenes in cell differentiation. Bioessays 12: 22–26, 1990
6. Barbacid M: Ras genes. Annu Rev Biochem 56: 779–827, 1987
7. Willingham MC, Pastan I, Shih TY, Scolnick EM: Localization of the src gene product of the Harvey strain of MSV to the plasma membrane of transformed cells by electron microscope immunocytochemistry. Cell 19: 1005–1014, 1990
8. Sweet RW, Yokoyama S, Kamata T, Feramisco JR, Rosenberg M, Gross M: The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity. Nature 311: 273–275, 1984
9. Toda T, Uno L, Ishikawa T, Powers S, Kataoka T, Broek D, Cameron S, Broach J, Matsumoto K, Wigler M: In yeast, ras proteins are controlling elements of adenylate cyclase. Cell 40: 27–36, 1985
10. Franks DJ, Durkin JP, Whitfield JF: Protein kinase C and a viral K-ras protein cooperatively enhance the response of adenylate cyclase to stimulators. J Cell Physiol 140: 409–417, 1989
11. Franks, DJ, Whitfield JF, Durkin JP: Viral p21 Ki-ras protein: A potent intracellular mitogen that stimulates adenylate cyclase activity in early G_1 phase of cultured rat cells. J Cell Biochem 33: 87–94, 1987
12. Franks, DJ, Whitfield JF, Durkin JP: A viral K-ras protein increases the stimulability of adenylate cyclase by cholera toxin in NRK cells. Biochem Biophys Res Commun 147: 596–601, 1987
13. Franks DJ, Whitfield JF, Durkin JP: The mitogenic/oncogenic p21 Ki-ras protein stimulates adenylate cyclase activity early in the G_1 phase of NRK rat kidney cells. Biochem Biophys Res Commun 127: 318–325, 1985
14. Bar-Sagi D: Ras proteins: Biological effects and biochemical targets. Anticancer Res 9: 1427–1437, 1989
15. Fleischman LF, Chahwala SB, Cantley L: Ras-transformed cells: Altered levels of phosphatidylinositol-4,5-bisphosphate and catabolites. Science 231: 407–410, 1986
16. Wilkison WO, Sandgren EP, Palmiter RD, Brinster RL, Bell RM: Elevation of 1,2-diacylglycerol in ras-transformed neonatal liver and pancreas of transgenic mice. Oncogene 4: 625–628, 1989
17. Wolfman A, Macara IG: Elevated levels of diacylglycerol and decreased phorbol ester sensitivity in ras-transformed fibroblasts. Nature 352: 359–361, 1987
18. Armelin HA, Armelin MCS, Kelly K, Stewart T, Leder P, Cochran BH, Stiles CD: Functional role for c-myc in mitogenic response to platelet-derived growth factor. Nature 310: 655–660, 1984

19. Bravo R, Burckhardt J, Curran T, Muller R: Stimulation and inhibition of growth by EGF in different A431 cell clones is accompanied by the rapid induction of c-fos and c-myc proto-oncogenes. EMBO J 4: 1193–1197, 1985
20. Cochran BH, Zullo J, Verma IM, Stiles CD: Expression of the c-fos oncogene and a newly discovered r-fos is stimulated by platelet-derived growth factor. Science 226: 1080–1082, 1984
21. Kelly K, Cochran BH, Stiles CD, Leder P: Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell 35: 603–610, 1983
22. Muller R, Bravo R, Bruckhardt J, Curran T: Induction of c-fos gene and protein by growth factors precedes activation of c-myc. Nature 312: 716–720, 1984
23. Kuehl WM, Bender TP, Stafford J, McClinton, Segal S, Dmitrovsky E: Expression and function of the c-myc oncogene during haematopoietic differentiation. Curr Topics Micro Immuno 141: 318–323, 1988
24. Chan SO, Wong SSC, Yeung DCY: Regulation of Ki-ras gene expression in Reuber H35 cells. Eur J Biochem 193: 681–685, 1990
25. Reuber MD: A transplantable bile-secreting hepatocellular carcinoma in the rat. J Nat Cancer Inst 26: 891–899, 1961
26. Pitot HC, Peraino C, Morse PA: Hepatomas in tissue culture compared with adapting liver *in vivo*. Nat Cancer Inst Monogr 13: 229–245, 1964
27. Berry MN, Friend DS: High-yield preparation of isolated rat liver parenchymal cells. J Cell Biol 43: 502–520, 1969
28. Ellis, RW, Defeo D, Shih TY, Gonda MA, Young HA, Tsuchida N, Lowry DR, Scolnik EM: The p21 ras genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature 292: 506–511, 1981
29. Maniatis T, Fritsch EF, Sambrook J: Molecular cloning. Cold Spring Harbor, Cold Spring Laboratory Press, 1982, pp 86–96
30. Chirgwin JM, Przbyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5249–5299, 1979
31. Miloszewska J, Trawicki W, Janik P, Moraczewski J, Przybyszewska M, Szaniawska B: Protein kinase C translocation in relation to proliferation state of C3H 10T1/2 cells. FEBS Lett 206: 283–286, 1986
32. Greenberg ME, Ziff EB: Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. Nature 311: 433–438, 1984
33. Bohmann D, Bos TJ, Admon A, Nishimura T, Vogt PK, Tjian R: Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science 238: 1386–1392, 1987
34. Lee W, Mitchell P, Tjian R: Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 49: 741–752, 1987
35. Colamonici OR, Trepel JB, Vidal CA, Neckers LM: Phorbol ester induces c-sis gene transcription in stem cell line K562. Mol Cell Biol 6: 1847–1850, 1986
36. Whitham SE, Murphy G, Angel P, Rahmsdorf HJ, Smith BJ, Lyons A, Harris TJR, Reynolds JJ, Herrlich P, Docherty AJP: Comparison of human stromelysin and collagenase by cloning and sequence analysis. Biochem J 240: 913–916, 1986
37. Matrisian LM, Leroy P, Ruhlmann C, Gesnel MC, Breathnach R: Isolation of the oncogene and epidermal growth factor-induced transin gene: Complex control in rat fibroblasts. Mol Cell Biol 6: 1679–1686, 1986
38. Montesano R, Orci L: Phorbol esters induce angiogenesis *in vitro* from large-vessel endothelial cells. Cell 42: 729–739, 1985
39. Mignatti P, Robbins E, Rifkin DB: Tumor invasion through the human amniotic membrane: Requirement for a proteinase cascade. Cell 47: 487–498, 1986
40. Lamph WW, Wamsley P, Sassone-Corsi P, Verma M: Induction of protooncogene jun/AP-1 by serum and TPA. Nature 334: 629–631, 1988
41. Spandidos DA, Nichols RA, Wilkie NM, Pintzas A: Phorbol ester-responsive H-ras 1 gene promoter contains multiple TPA-inducible/AP-1-binding consensus sequence elements. FEBS Lett 240: 191–195, 1988
42. Baeuerle PA, Baltimore D: I Kappa B: A specific inhibitor of the NF-kappa B transcription factor. Science 242: 540–546, 1988
43. Ross J, Pizarro A: Human beta and delta globin messenger RNAs turnover at different rates. J Mol Biol 167: 607–167, 1983
44. Buss JE, Sefton BM: Direct identification of palmitic acid as the lipid attached to p21 ras. Mol Cell Biol 6: 116–122, 1986
45. Willumsen BH, Norris K, Papageorge AG, Hubbert NL, Lowy DR: Harvey murine sarcoma virus p21 ras protein: Biological and biochemical significance of the cysteine nearest the carboxy terminus. EMBO J 3: 2581–2585, 1984
46. Chen ZQ, Ulsh LS, Dubois G, Shih T: Posttranslocational processing of p21 ras proteins involves palmitoylation of the C-terminal tetrapeptide containing Cys 186: J Virol 56: 607–612, 1985
47. Magee AI, Hanley MR: Protein modification: Sticky fingers and CAAX boxes. Nature 335: 114–115, 1988
48. Clarke S, Vogel JP, Deschenes RJ, Stock J: Postranslational modification of the H-ras oncogene protein: Evidence for a third class of protein carboxyl methyltransferanse. Proc Natl Acad Sci USA 85: 4643–4647, 1988
49. Gutierrez L, Magee AI, Marshall CJ, Hancock JF: Post-translational processing of p21 ras is two-step and involves carboxyl-methylation and carboxyl-terminal proteolysis. EMBO J 8: 1093–1098, 1989
50. Fausto N, Shank PR: Oncogene expression in liver regeneration and hepatocarcinogenesis. Hepatology 3: 1016–1023, 1983
51. Fausto N, Mead JE, Braun L, Thompson NL, Panzica M, Goyette M, Bell GL, Shank PR: Proto-oncogene expression and growth factors during liver regeneration. Symp Funda Cancer Res 39: 69–86, 1987
52. Cruise JL, Muga SJ, Lee YS, Michalopoulos GK: Regulation of hepatocyte growth: Alpha-1 adrenergic receptor and ras p21 changes in liver regeneration. J Cell Physiol 140: 195–201, 1989
53. Bohme HJ, Sparmann G, Hofmann E: Biochemistry of liver development in the perinatal period. Experentia 39: 473–483, 1983
54. Kraus-Friedman N: Hormonal regulation of hepatic gluconeogenesis: Physiological Reviews 64: 171–259, 1984