Tumorigenesis and Neoplastic Progression

c-Fos-Dependent Induction of the Small Ras-Related GTPase Rab11a in Skin Carcinogenesis

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Malignant transformation of mouse skin by tumor promoters and chemical carcinogens, such as the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), is a multistage process leading to the formation of squamous cell carcinomas. It has been shown that mice lacking the AP-1 family member c-Fos exhibit an impaired transition from benign to malignant skin tumors. Here, we demonstrate enhanced expression of the small Ras-related GTPase Rab11a after short-term TPA treatment of mouse back skin. Expression of Rab11a *in vivo* and *in vitro* critically depended on c-Fos, because TPA application to the back skin of c-Fos-deficient mice and to mouse embryonic fibroblasts did not induce Rab11a mRNA or protein expression. Moreover, dexamethasone, which is a potent inhibitor of AP-1-mediated transactivation that exhibits anti-inflammatory and anti-tumor promoting activities, inhibited TPA-induced expression of Rab11a. Within the *Rab11a* gene promoter, we identified a functional AP-1 binding element that exhibited elevated c-Fos binding activity after TPA treatment of keratinocytes. Enhanced expression was not restricted to chemically induced mouse skin tumors but was also found in tumor specimens derived from patients with epithelial skin tumors. These data identify Rab11a as a novel, tumor-associated c-Fos/AP-1 target and may point to an as yet unrecognized function of Rab11a in the development of skin cancer. (*Am J Pathol* 2005, 167:243–253)

proliferation, differentiation, genome integrity, DNA repair, and induction of apoptosis.¹ Understanding of the molecular basis of tumorigenesis will be essential to provide reliable diagnostic markers and eventually develop novel therapeutic targets for cancer prevention and treatment. In particular, the identification and functional characterization of cellular genes that are targeted by oncogenic stimuli represents a promising experimental approach. The mouse model of chemically induced skin carcinogenesis is one of the best-defined experimental *in vivo* models of carcinogenesis. It represents an important tool for the understanding of current concepts regarding human neoplasia, including the multistage nature of tumor development.^{2,3} Using this model, the development of squamous cell malignancy of the skin can be subdivided into three phases: initiation, promotion, and progression. Although genetic events are crucial for initiation and progression, the tumor promotion is predominantly characterized by epigenetic events. Co-treatment with glucocorticoids, which are in widespread medical use to inhibit inflammatory processes, interferes with both edema formation and the development of papillomas and carcinomas.⁴

Using this carcinogenesis model the central contribution of the transcription factor AP-1 family members such as c-Fos and c-Jun to premalignant conversion and malignant progression of epidermal cells was well described.^{3,5} In mice lacking c-Fos the development of malignant skin tumors is blocked at the transition from benign to malignancy.⁶ Moreover, papillomas of c-Fos-deficient mice quickly hyperkeratinize. Transgenic mice expressing a transdominant-negative version of c-Jun

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Tumorigenesis is a complex multistage process, in which a series of genetic changes is thought to deregulate cell

(TAM67) are resistant to experimental skin tumorigenesis,⁷ which is in line with the observation that overexpression of a dominant-negative c-Jun mutant interferes with 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced AP-1 activity, cell transformation, and invasion.⁸ Mice expressing a c-Jun mutant, which lacks the phosphorylation-acceptor sites for Jun N-terminal kinase (JNK), were impaired in tumor formation.⁹ This block of tumorigenesis is accompanied by a loss of TPA-induced expression of AP-1 target genes such as the matrix-metalloproteinases MMP3 and MMP13.⁸

To identify novel tumor-associated genes with similar expression characteristics as the AP-1 target genes MMP3 and MMP13,¹⁰ we combined the mouse model of chemically induced skin carcinogenesis with suppression subtraction hybridization to create a library enriched for TPA-inducible genes in mouse epithelial cells.^{11–13} One newly identified TPA-inducible cDNA clone was identical to the 3'-end of the Ras-related small GTPase *Rab11a*.¹⁴ Rab11a is a member of the large superfamily of small Ras-related GTP-binding Rab proteins. The members of the Rab11 subgroup, Rab11a, Rab11b, and Rab25, act as master signal components that specifically regulate many aspects of vesicular transport along the recycling, endocytotic, and exocytotic pathways.^{14,15} Although ubiquitously expressed in polarized and nonpolarized cells, Rab11a is more abundant in tissues with a high level of secretion.¹⁶

Here, we show that TPA mediated activation of *Rab11a* mRNA and its encoded protein in mouse back skin and *in vitro* cultivated keratinocytes and fibroblasts in a c-Fos-dependent manner. Expression of Rab11a varies in the process of multistage epithelial carcinogenesis implicating a critical role of this Ras-related small GTPase in the establishment of skin cancer in mouse and man.

Materials and Methods

Animals

Female C57BL/6 mice aged 7 to 9 weeks, female NMRI mice (RCC, Füllinsdorf, Switzerland), as well as *c-fos*^{-/-} mice and their wild-type littermates¹⁷ were housed in specific pathogen-free and in light-, temperature (21°C)-, and humidity (50 to 60% relative humidity)-controlled conditions. Food and water were available *ad libitum*. The procedures for performing animal experiments were in accordance with the principles and guidelines of the ATBW (authority for animal welfare) and were approved by the Regierungspräsidium Karlsruhe, Germany.

Cell Culture

The keratinocyte cell line PMK-R3 was generated by transfection of primary mouse keratinocytes with a SV40 T-antigen-expressing vector.¹⁸ Cells were cultivated in minimal essential medium with Hanks' salt mixture supplemented with a fourfold concentration of amino acids and vitamins, 10% fetal bovine serum, 2 mmol/L glu-

tamine, 2.4 g NaHCO₃/L, 100 IU penicillin, and 100 µg streptomycin/ml at 34°C in a humidified atmosphere of 5% CO₂. Mouse embryonic fibroblasts (MEFs) were established from wild-type and *c-fos*-deficient embryos and described previously.¹⁹ Serum-starved cells, 24 hours with 0.5% fetal bovine serum, were treated for 2 or 6 hours with 100 ng/µl TPA dissolved in acetone.

Treatment of Mouse Skin

Wild-type and *c-fos*^{-/-} mice were shaved on the dorsal skin and treated 3 days later topically with 100 µl of acetone as control, 10 nmol of TPA, which is the dose used for the promotion of skin tumors,² or 50 µg of dexamethasone, each dissolved in 100 µl of acetone, or 10 nmol of TPA in combination with 50 µg of dexamethasone in 100 µl of acetone as described previously.^{10,12} The animals were sacrificed at the indicated time points after topical application. Skin tumors derived from female NMRI mice used in this study were generated according to the two-stage carcinogenesis protocol using 100 nmol of 7,12-dimethylbenz[α]anthracene as initiator and 10 nmol TPA as promoter as described previously.^{2,20} For RNA extraction tissues and tumors were immediately frozen in liquid nitrogen after isolation. For *in situ* hybridization and immunofluorescence staining tissues were immersed, fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS), and embedded in paraffin and subsequently cut in 6-µm sections as described previously.²¹

Northern Blot Analysis and Quantification

Total RNA was isolated from cell lines and from 6 hours of acetone-, TPA-, as well as TPA plus dexamethasone-treated mouse skin, as described previously.¹⁰ Fifteen µg of total RNA were fractionated on 1.4% formaldehyde-agarose gels and subjected to Northern blot analysis using an [α -³²P] dCTP-labeled *Rab11a* cDNA insert (nucleotides 352 to 1194 of the published sequence, accession no. NM_017382¹⁴). The fragment was isolated by restriction enzyme digest of the appropriate pCRII.1 plasmid.¹¹ A specific probe for 18S rRNA was used as loading control. Quantification was performed on two experiments with RNA from at least three independent mice per genotype and treatment. Intensities of radioactive signals for the smaller Rab11a variant and 18S rRNA were measured with a Phosphorimager (BAS-1500; Molecular Dynamics, Krefeld, Germany) using the BAS Reader 2.9 software and quantified with the software TINA 2.09. Relative signal intensity for control treated *c-fos*^{+/+} and *c-fos*^{-/-} animals was set to one.

Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR) and Reverse Quantitative (RQ)-PCR Analysis

cDNA synthesis of total RNA was performed as described previously.¹³ One to two µl of the RT reaction were used for PCR detection using the described primers

(see Supplemental Table 1 at <http://ajp.amjpathol.org>). *Hprt* served as internal control for the quality and quantity of the cDNA. Amplified probes were separated on 2% agarose gels in the presence of ethidium bromide and visualized by using the eagle-eye system (Stratagene, La Jolla, CA). Real-time quantitative RT-PCR was performed using a MyiQ single-color real-time PCR detection system (Bio-Rad, Munich, Germany) according to the manufacturer's instructions. The Absolute SYBR Green fluorescein kit (ABgene, Surrey, UK) was used according to the manufacturer's instructions. For thermal cycling, the following conditions were applied: consecutive steps of 15 minutes at 95°C, then 45 cycles of 30 seconds at 95°C, 45 seconds at 55°C, and 1 minute at 72°C, followed by a melting curve program (denaturation throughout 1 minute at 95°C, cooling and holding at 60°C for 10 seconds, and heating at a speed of 0.5°C/10 seconds to 100°C with reading at every 0.5°C). To obtain a calibration graph, the PCR product from *Hprt* was serially diluted in ddH₂O (five 10-fold dilutions from 0.0001 ng to 1 ng). To standardize the amount of sample cDNA, an endogenous control amplicon of *Hprt* was used.

Electrophoretic Mobility Shift Assay

Nuclear extracts of PMK-R3 cells treated for 2 hours with acetone or 100 ng/ μ l TPA were prepared according the protocol described previously,¹⁹ and protein concentration was determined using the protein bioassay solution (Bio-Rad). Electrophoretic mobility shift assays were performed with 4 μ g of nuclear protein and 50,000 cpm of ³²P-radiolabeled probes as described.¹⁹ The TPA-responsive element (TRE) oligonucleotides were described by Porte and colleagues²² and oligonucleotides for the Oct probe were kindly provided by T. Wirth, University of Ulm, Germany. Sequences for the TRE oligonucleotides of the mouse *Rab11a* promoter are described in Supplemental Table 2, which can be found at <http://ajp.amjpathol.org>.

ChIP Analysis

For ChIP assay the ChIP assay kit (Upstate Biotechnology, Lake Placid, NY) was used following the manufacturer's instructions with minor modifications. The ChIP experiment was performed with PMK-R3 mouse keratinocytes treated for 2 hours with acetone or 100 ng/ μ l of TPA. Crosslinked c-Fos protein-DNA complexes were immunoprecipitated by a c-Fos-specific antibody (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA). PCR amplification of the immunoprecipitated samples was performed by using specific primers that flank the three putative TRE sites within the *Rab11a* promoter (see Supplemental Table 1 at <http://ajp.amjpathol.org>). Immunoprecipitates in the absence of antibody and a portion of the sonicated chromatin before immunoprecipitation were used as controls. A PCR using the primer-pair specific for a 3'-coding region of *Rab11a* (see Supplemental Table 1) on ChIP DNA served as an additional control. The PCR product amplified by the *Rab11a* TRE1 primer-

pair was additionally confirmed by sequencing using an ABI Prism 7700 sequencer following the manufacturer's recommendations (Applied Biosciences, Frankfurt, Germany).

Western Immunoblot Analysis

Acetone- and TPA-treated wild-type and c-Fos-deficient MEFs were lysed after 6 hours in RIPA-buffer (50 mmol/L Tris/HCl, pH 8, 150 mmol/L NaCl, 0.1% sodium dodecyl sulfate, 0.5% deoxycholate, 1% Nonidet P-40) with protease inhibitor cocktail (Sigma, Munich, Germany). Equal amounts of protein were separated on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nylon membrane (Schleicher & Schuell, Dassel, Germany). After 30 minutes of incubation in blocking solution (7.5% milk/0.5% Tween 20 in PBS), the membrane was incubated for 2 hours at room temperature with the monoclonal anti-Rab11 antibody (610657; BD Bioscience, Heidelberg, Germany) diluted in blocking solution. After extensive washing with 0.5% Tween 20/PBS, the membrane was incubated for 1 hour at room temperature with a polyclonal goat-anti-mouse-HPRT antibody (DAKO, Hamburg, Germany). After extensive washing with 0.5% Tween 20/PBS, specific signals for the proteins were visualized according to the protocol of the Western Lightning Chemiluminescence Reagent Plus (Perkin-Elmer, Boston, MA). Detection of β -actin using a polyclonal goat anti-actin antibody (Sigma) served as an internal control for quantity and quality of the protein extracts.

In Situ Hybridization

In situ hybridization was performed on 6- μ m paraffin sections as previously described.^{12,21} All tissue samples were fixed in 4% paraformaldehyde, treated with proteinase K (20 μ g/ml) and subsequently washed in 0.1 mol/L triethanolamine buffer containing 0.25% acetic anhydride. The sections were covered with 20 to 100 μ l of hybridization buffer containing 1.5×10^5 cpm/ μ l of ³⁵S-labeled anti-sense or sense RNA probe, and incubated at 53°C for 18 hours in a humidified chamber. After hybridization, the slides were washed under stringent conditions (50% formamide, 65°C), including treatment with RNase A (20 mg/ml) to remove unhybridized probe. After 4 to 10 days of exposure, the NTB2 photographic emulsion (Kodak, Munich, Germany) was developed, and the slides were stained with hematoxylin and eosin. Each sample was hybridized in at least three experiments. *Rab11a* cRNA probe was derived by *in vitro* transcription from the appropriate *Eco*RI linearized recombinant pCRII.1 plasmid described above. As a control for non-specific hybridization, sections were hybridized with ³⁵S-labeled sense RNA from the pCRII.1 plasmid linearized with *Bam*HI.

Immunofluorescence Staining

Four percent paraformaldehyde-fixed and paraffin-embedded 6- μ m tissue sections of mouse skin biopsies and

human skin tumor specimens [keratoacanthoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC)] were incubated for 1 to 2 hours at 25°C with specific primary antibodies or controls diluted in PBS supplemented with 1% bovine serum albumin and 0.2% Tween 20. As primary antibodies we used a monoclonal anti-Rab11 antibody (610657; BD Bioscience). To control for specificity of antibody signals, a blocking peptide (sc-6565 P, Santa Cruz Biotechnology) was added to the primary antibody dilution. After extensive washing with PBS/0.2% Tween 20 the sections were incubated for 30 minutes at 25°C with a secondary goat-anti-mouse-Cy3 antibody (Dianova, Hamburg, Germany) diluted in PBS supplemented with 1% bovine serum albumin and 0.2% Tween 20. For nuclear staining H33342 (Calbiochem, Schwalbach, Germany) was added to a final concentration of 1 μ g/ml to the secondary antibody dilution. After extensive washing with PBS/0.2% Tween 20 sections were mounted with Mowiol and specific signals were visualized by immunofluorescence microscopy.

Human Skin Biopsies

Skin biopsies from patients with keratoacanthoma, well-differentiated SCC, and BCC were obtained from surgical excisions of the affected areas at the Department of Dermatology. One part of the samples was immediately frozen in liquid nitrogen and stored at -80°C until used for histological examination. The patients signed the informed consent from the Department of Dermatology, University of Cologne, approved by the Institutional Commission of Ethics (Az. 9645/96). The diagnoses were confirmed by two experienced dermatopathologists.

Results

In previous studies, we established *in vivo* conditions to induce rapid alterations in gene expression of the well-known AP-1 target genes MMP3 and MMP13 in mouse skin by the phorbol ester TPA.¹⁰ To identify novel TPA-inducible genes with enhanced expression at late stages of skin carcinogenesis, we combined suppression subtraction hybridization with reverse Northern blotting.¹¹ One of the isolated clones was found to be identical to a region within the 3'-end of the small Ras related-GTPase Rab11a. In light of the proposed function and because this gene has not yet been associated with the process of skin carcinogenesis, Rab11a was chosen for detailed analysis.

Elevated Rab11a Expression in TPA-Treated Mouse Skin and in Vitro-Cultured Keratinocytes and Fibroblasts

Expression analysis revealed elevated mRNA levels of *Rab11a* 6 hours after TPA treatment of mouse back skin, whereas in the skin of acetone-treated controls only low basal expression could be detected (Figures

Figure 1. TPA-induced expression of Rab11a mRNA and protein in mouse back skin. **A:** Back skin of wild-type mice was treated with acetone (lane 1, co) or TPA (lane 2, TPA). Animals were sacrificed after 6 hours and total RNA was isolated. The level of Rab11a transcripts was visualized by Northern blot analysis. Rehybridization with an 18S-rRNA-specific probe was performed serving as a loading control for RNA quantity and quality. **B:** PMK-R3 mouse keratinocytes were treated with acetone (lane 1, co) or TPA (lane 2, TPA). Cells were harvested for RNA preparation 6 hours thereafter and analyzed as described in **A**. **C:** Paraformaldehyde-fixed and paraffin-embedded sections (6 μ m) of acetone- and TPA-treated mouse back skin was analyzed by indirect immunofluorescence using a monoclonal antibody raised against Rab11. Staining was performed using a Cy3-labeled secondary antibody and H33342 for nuclear staining (blue signal). Specific signals were visualized by immunofluorescence microscopy. Expression of Rab11a protein (signal in red) was detectable in keratinocytes and dermal fibroblasts 6 hours after TPA application and was even stronger after 12 hours. Only basal expression was visible in skin of acetone-treated animals (0 hours). Blocking of the antibody signal with a blocking peptide served as a control for specificity (12 h co). The dashed line indicates the border between epidermis and dermis. Scale bar, 25 μ m.

1A and 2A). Similar to the induction of Rab11a *in vivo* mRNA expression was efficiently induced in immortalized mouse PMK-R3 keratinocytes (Figure 1B). TPA-induced expression was observed for both splice variants of Rab11a (corresponding human transcripts 1.0 kb and 2.3 kb) that have been reported to encode the same protein.^{14,23} Simultaneous induction is in agreement with the published notion that both variants show primarily coordinated expression levels.^{24–26} Corresponding to enhanced mRNA expression increased Rab11a protein levels were observed in back skin 6 and 12 hours after TPA application but not in acetone-treated controls (Figure 1C). Expression of Rab11a induced by TPA treatment was not only detected for

epidermal cells but was also seen in dermal fibroblasts (Figure 1C) and in *in vitro* cultured MEFs (Figure 3D). These data demonstrate that TPA induces Rab11a expression *in vivo* and *in vitro* similar to the previously described AP-1 target genes MMP3 and MMP13.¹⁰

TPA-Induced Expression of Rab11a Is Blocked by Dexamethasone and Requires c-Fos

Glucocorticoids act as anti-inflammatory and anti-tumor promoting agents in skin⁴ and repress TPA-induced expression of AP-1 target genes.¹⁰ To investigate whether glucocorticoids also affect the expression of Rab11a TPA treatment of mouse back skin was performed in the presence of the synthetic glucocorticoid dexamethasone. As shown in Figure 2A by Northern blot analysis, TPA-induced expression of Rab11a mRNA is repressed by simultaneous glucocorticoid treatment in wild-type skin (from 3.4-fold to 2-fold). Impaired induction of Rab11a by co-treatment with dexamethasone was further demonstrated by quantitative RQ-PCR (Figure 2B) and on protein level using immunofluorescence analysis (Figure 2C). The ability of glucocorticoids to interfere with expression of cellular genes is best explained by negative cross-talk between the glucocorticoid receptor (GR) and other transcription factors, such as AP-1 and nuclear factor (NF)- κ B,^{27,28} opening the possibility that expression of Rab11a might be regulated by AP-1, NF- κ B, and/or other GR-sensitive transcription factors. To address this question *in vivo* by functional means we compared expression of Rab11a in wild-type mice (*fos*^{+/+}) and in mice deficient for c-Fos (*fos*^{-/-}).^{6,17,29} Expression of Rab11a in acetone-treated skin of *fos*^{-/-} mice was not significantly changed compared to wild-type animals indicating that basal expression is primarily independent of the transactivation function of c-Fos/AP-1 (Figure 2). Yet, induction of Rab11a mRNA and protein after TPA treatment was impaired in animals lacking c-Fos expression compared to wild-type skin (Figure 2). Treatment with glucocorticoids, either alone or in combination with TPA, did not significantly affect Rab11a expression. These data identify the small GTPase Rab11a as a c-Fos/AP-1 target gene in keratinocytes and fibroblasts that might contribute to the anti-tumorigenic action of glucocorticoids and impaired progression of skin tumors in c-Fos-deficient animals.

To analyze whether TPA-induced expression is specific for Rab11a or is also evident for the other Rab11

Figure 2. Impaired TPA-induced expression of Rab11a mRNA and protein by co-treatment with dexamethasone and in the absence of c-Fos. Back skin of wild-type (*fos*^{+/+}) or c-Fos-deficient mice (*fos*^{-/-}) was treated with acetone (co), TPA (TPA), or TPA together with dexamethasone (T/D). Animals were sacrificed after 6 hours and back skin was used for total RNA isolation or was fixed in paraformaldehyde and embedded in paraffin. **A:** Expression of Rab11a mRNA and 18S-rRNA was determined as described in Figure 1. Results of two independent experiments were quantified and the relative fold induction is shown. **B:** Expression of Rab11a and Rab11b mRNA was analyzed in wild-type (*fos*^{+/+}) and c-Fos-deficient mice (*fos*^{-/-}) after treatment with acetone (co), TPA (TPA), TPA and dexamethasone (T/D), or dexamethasone alone (Dex). Expression of Hprt served as internal control and relative expression in acetone-treated skin was set to one. The bars represent the mean value of three independent experiments and the SD is indicated. **C:** Sections (6 μ m) of mouse back skins were analyzed by indirect immunofluorescence using a specific monoclonal antibody. Staining was performed using a Cy3-labeled secondary antibody and H33342 for nuclear staining (blue signal). Specific signals were visualized by immunofluorescence microscopy. Expression of Rab11a protein (red signal) was detectable in TPA-treated skin of wild-type animals (*fos*^{+/+} + TPA) but not in skin of TPA-treated mice lacking c-Fos (*fos*^{-/-} + TPA). The dashed line indicates the border between epidermis and dermis. Scale bar, 25 μ m.

Figure 3. *Rab11a* is a direct c-Fos target gene. **A:** Schematic representation of the mouse *Rab11a* promoter region. Three potential TRE elements and their position according to the transcriptional start site are shown. **B:** *In vitro* protein-binding assay with nuclear extract of control (–) and TPA-treated (+) PMK-R3 cells using primers containing the identified TRE elements. The classical TRE element of mouse collagenase-3 (TRE) was used as a positive control for AP-1 complex formation and an octamer probe (Oct) served as control for quality and quantity of nuclear extracts. **C:** ChIP analysis was performed with extracts of control (–) and TPA-treated (+) PMK-R3 cells using a c-Fos-specific antibody. Immunoprecipitated material was amplified with primers located within the mouse *Rab11a* promoter region that were specific for TRE1, TRE2, and TRE3 or with primers of the coding region (*Rab11a*) as a negative control. The material used for co-immunoprecipitation was amplified with primers specific for TRE1 as a control for quality and quantity. **D:** Western immunoblot with protein extracts of acetone-treated (–) and TPA-treated (+) wild-type (*fos*^{+/+}) and c-Fos-deficient (*fos*^{–/–}) MEFs. Expression of β -actin served as control for quality and quantity of protein extracts.

family members such as Rab11b or Rab25, we performed RT-PCR and quantitative RQ-PCR using gene-specific primers (Figure 2B and data not shown). Interestingly, expression of Rab11b in TPA-treated mouse back skin does not differ from acetone-treated controls, both in wild-type and c-Fos-deficient animals. In contrast to Rab11a and Rab11b, no expression of Rab25 was found in mouse skin even after TPA application, although basal expression was detected in RNA from mouse lung and kidney (data not shown).

c-Fos Binds to a Proximal TRE Element within the *Rab11a* Promoter after TPA Treatment

To unequivocally confirm the role of c-Fos in transcriptional control of *Rab11a*, we screened the promoter region for putative AP-1 binding elements using Alibaba2.1 (<http://www.alibaba2.com>) and Genomatix Suite (<http://www.genomatix.de>). Three putative TPA-responsive elements (TRE), named TRE1, TRE2, and TRE3, were identified within the first 1 kb of the 5'-flanking region of the mouse *Rab11a* gene (Figure 3A). Binding of an AP-1 dimer to the identified TRE motifs was analyzed by bandshift experiments with nuclear extracts of control and TPA-treated PMK-R3 cells. Efficient induction of AP-1 binding activity in PMK-R3 cells was monitored by measuring binding to the classical TRE motif of the mouse collagenase-3 promoter (Figure 3B, lanes 1 and 2). We could detect an even stronger complex formation for the proximal TRE1 motif combined with a significant elevation after TPA application (Figure 3B, lanes 3 and 4). In contrast, no AP-1 binding was obvious for the TRE2 motif and only a rather weak binding was observed for the distal TRE3 motif after TPA treatment.

To analyze whether c-Fos is part of the induced AP-1 complex at the TRE1 motif we performed a ChIP assay with formaldehyde-crosslinked extracts of control and TPA-treated PMK-R3 cells and a specific antibody for c-Fos. Co-precipitation of a c-Fos-DNA complex was only detected with extracts of TPA-treated cells and the TRE1 motif (Figure 3C, lane 4), but was not visible with control-treated extracts, in the absence of the c-Fos-specific antibody or with primers located within the *Rab11a* coding region (Figure 3C, lanes 3 and 5 to 7). Corresponding to the bandshift experiment, we could not detect binding of c-Fos to the TRE2 or the TRE3 motif (Figure 3C, lanes 8 to 11), supporting that the proximal TRE1 element is mainly responsible for induced *Rab11a* expression. Finally, we analyzed the expression of *Rab11a* protein in MEFs derived from wild-type and c-Fos-deficient embryos. TPA-induced expression of *Rab11a* protein could be demonstrated for wild-type MEFs but not in cells lacking c-Fos.

Expression of *Rab11a* throughout Skin Carcinogenesis

Following the multistage skin carcinogenesis protocol²⁰ chronic treatment with TPA results in the formation of epithelial tumors, such as papillomas and invasively growing SCCs. To monitor the expression pattern of *Rab11a* throughout carcinogenesis, we first determined its expression in short-term TPA-treated skin, benign papillomas, and malignant SCCs using RT-PCR and quantitative RQ-PCR (Figure 4A and data not shown). In addition to TPA-induced expression, we found significantly enhanced levels of *Rab11a* mRNA in both tumor stages. These data were further confirmed on mouse tumor sections by *in situ* hybridization. In hyperplastic skin (data not shown) and benign

Figure 4. Expression of Rab11a mRNA and protein in chemically induced papillomas and SCC. **A:** Expression of **Rab11a** mRNA was analyzed by RQ-PCR as described in Figure 2B using biopsies taken from mouse skin 6 hours after TPA treatment (TPA) or from chemically induced mouse skin tumors such as papillomas (Pap) and SCC. **B:** 35 S-UTP-labeled anti-sense riboprobe of Rab11a was hybridized to sections of biopsies taken from chemically induced mouse skin tumors. Expression of Rab11a mRNA was detectable in neoplastic keratinocytes of both benign papilloma (Pap) and SCC. Sections are representative for three independent experiments. 35 S-UTP-labeled sense riboprobe served as controls for specificity and are shown as insets in Pap_DF and SCC_DF. Indirect immunofluorescence analysis using a monoclonal antibody raised against Rab11 and parallel sections of the skin tumors revealed protein expression at the same area (SCC_α-Rab11). Staining was performed using a Cy3-labeled secondary antibody (red signal) and H33342 for nuclear staining (blue signal). Incubation without primary antibody served as control for specificity (SCC_co). Specific signals were visualized by immunofluorescence microscopy. Pap_BF and SCC_BF are bright-field images and Pap_DF and SCC_DF are dark-field images. **Arrows** indicate specific signals within the tissue section. Scale bar, 100 μ m.

papillomas expression of Rab11a mRNA was restricted to keratinocytes of the suprabasal compartment (Figure 4B). In contrast, proliferating basal cells, which were identified by proliferating cell nuclear antigen staining (data not shown), did not express detectable levels of Rab11a. At late stages of skin carcinogenesis, in which aggressively growing SCCs appear, elevated levels of Rab11a mRNA and protein were observed (Figure 4B). In analogy to these findings, high levels of Rab11a protein were also found in human keratoacanthoma and SCC (Figure 5) demonstrating the relevance of Rab11a in the development of skin tumors in mice and humans. Interestingly, expression of Rab11a protein was detected in neoplastic keratinocytes of SCCs (Figure 5, C and D) but not BCCs (Figure 5F).

Discussion

In the present study, we used several *in vivo* and *in vitro* experiments to investigate TPA-dependent expression of the small GTPase Rab11a. We demonstrated that TPA-induced expression in keratinocytes can be diminished by dexamethasone and depends on the presence of the AP-1 family member c-Fos. Rab11a is a major regulator of intracellular protein trafficking between recycling endosomes and the plasma membrane, between *trans*-Golgi network and early endosomes and clathrin-independent recycling.³⁰ Therefore, Rab11a might be a promising candidate to link regulators of the cellular trafficking machinery to the process of carcinogenesis.

Figure 5. Expression of Rab11a protein in human skin tumors. Indirect immunofluorescence analysis of Rab11a protein level on human biopsies was done using a specific monoclonal antibody. The specimens represent skin (**A**, **B**), two SCCs (**C**, **D**), one keratoacanthoma (**E**), and one BCC (**F**). Staining was performed using a Cy3-labeled secondary antibody and H333342 for nuclear staining (blue signal). **B**: Treatment with secondary antibody alone served as control. Specific signals were visualized by immunofluorescence microscopy. Scale bar, 50 μ m.

We could show an abundant overexpression of Rab11a mRNA and protein after TPA treatment in mouse skin. Enhanced Rab11a levels were detected in TPA-treated epidermal keratinocytes and dermal fibroblasts whereas very low basal expression was seen in untreated mouse skin. Preliminary data using specific PKC inhibitors demonstrate that the TPA effect on Rab11a expression, at least in a keratinocyte cell line, depends on the classical phorbol ester receptor PKC- α (data not shown). These data implicate a direct link between PKC- α -mediated signaling, which has been shown to be important for

skin carcinogenesis,³¹ and regulation of intracellular protein trafficking via Rab11a.³² This is supported by the fact that PKC- α is a key regulator of dynamics of plasma membrane/endosomal recycling and function.³³

A well-known downstream target of the PKC-signaling pathway in many tissues including skin is the transcription factor AP-1.^{5,34,35} In fact, putative AP-1 binding sites have been found in mouse and human promoter sequences of Rab11a.^{14,26} In line with these data, transcriptional induction of Rab11a is repressed by dexamethasone, a synthetic glucocorticoid, which is best

explained by negative interference between the activated glucocorticoid receptor and transcription factors involved in the inflammatory response, such as AP-1 and NF- κ B.^{27,28,36} Hence, regulation of Rab11a expression and function in skin may expand our knowledge concerning therapeutic action of glucocorticoids in the context of inflammation and tumor development. The involvement of AP-1 for induced Rab11a expression was further supported by the impaired expression in TPA-treated MEFs lacking c-Fos expression or skin of c-Fos-deficient mice. These mutant mice show remarkable abnormalities in skin carcinogenesis characterized by lack of malignant progression.⁶ We could further demonstrate direct binding of a c-Fos-containing AP-1 complex to a proximal TRE element in the mouse Rab11a promoter supporting that Rab11a is a direct c-Fos target gene. In the past, some genes were described whose expression was found to be dependent on c-Fos-mediated transactivation such as DNMT1, FASL, VEGFD, several MMPs, CD44, Cathepsin L, MTS1, KRP1, TSC36/FRP, Ezrin, Tropomyosin 3, and Tropomyosin 5b.^{5,37} Most of them are known players in a multigenic program that regulates the process of tumor progression and invasion.³⁸ Our work provides *in vivo* evidence that the small Ras-related Rab11a could be an additional player in this scenario.

Induction of Rab11a in keratinocytes was not restricted to the inflammation-linked response of TPA treatment of the skin because elevated expression was also found in chemically induced benign papillomas and malignant SCCs. Additionally, we detected enhanced expression in tumor biopsies of patients with skin cancer. The expression is restricted to more differentiated keratinocytes of keratoacanthoma and SCCs and is not present in tumor cells of BCCs. Therefore, one could ask whether increased expression of Rab11a in tumors with squamous differentiation simply represents an increased number of differentiated cells. However, Rab11a expression in suprabasal cells might illustrate the substantial influence of the differentiation compartment on tumor development via the control of tumor stem cells as has been described for the c-Myc oncogene.³⁹ Moreover, recent work has shown that Rab11a is overexpressed in low-grade dysplastic cells of Barrett's esophagus and marks the transition between benign and low-grade dysplasia.⁴⁰ Abundant expression of Rab11a was also seen in breast ductal adenocarcinoma,⁴¹ and in 5 of 10 samples of esophageal SCC,⁴² which points to a more general function of Rab11a in epithelial carcinogenesis. Most recent data implicate the Rab11 family member Rab25 in aggressiveness of ovarian and breast cancers.⁴³ Overexpression of Rab25 in certain tumor cell lines increased anchorage-independent and anchorage-dependent cell proliferation, prevented apoptosis and anoikis, and increased aggressiveness *in vivo*.⁴³ Our data demonstrate that Rab25 seems not to be involved in skin carcinogenesis whereas Rab11a might substitute for its role as a tumorigenic mediator at least in this tumor type.

Some functions associated with Rab11a may point toward its role in the process of carcinogenesis. It regulates secretion of induced secretory proteins and may also be involved in recycling of induced growth factor

receptors. It is reasonable to assume that exocytosis of soluble factors and recycling of surface-molecules are linked with tumor growth.^{44–46} Overexpression of traffic-regulating proteins such as Rab11a could allow secretion of growth-enhancing factors independent from an otherwise necessary, exocytosis-promoting signal. In light of these findings, it is worthwhile to speculate that TPA-induced expression of Rab11a in dermal fibroblasts might also participate in epidermal tumor growth. Elevated secretion of fibroblast-derived mitogens as a consequence of high Rab11a levels could increase proliferation of initiated keratinocytes, which is in line with recent data demonstrating the importance of dermal-epidermal interaction in skin homeostasis and malignancy.^{47,48} Recent data also describe the role of Rab11a in the recycling kinetics of various cellular surface-receptors, which all have been linked to cancer such as chemokine receptor CXCR2,⁴⁹ protease-activated receptor 2 PAR2,⁵⁰ integrin β 1,⁵¹ and E-Cadherin.⁵² Moreover, Rab proteins participate in trafficking of lysosomal cathepsins B, D, and L that all have been linked to epithelial tumor development.⁵³ Rab11a activation is further involved in TPA-induced reassembly of stress fibers and focal adhesions in an epithelial cell line.⁵⁴ These data support the correlation between Rab11a function and cell motility,^{54,55} which is essential for overall tumor growth and invasive behavior of neoplastic epithelial cells.⁵⁶

Rab protein function is tightly regulated by a variety of specific effector molecules.⁵⁷ PRC17 is one of these Rab effectors regulating the activity of Rab5. Recently, it has been shown that PRC17 has strong oncogenic potential in prostate cancer and that the GAP activity is essential for this process.⁵⁸ Whether or not effector proteins of Rab11a might exhibit similar functions remains to be elucidated.

In the future, this study on Rab11a may represent a fruitful start to analyze the role of proteins involved in vesicular trafficking for cancer biology.

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References

1. Hanahan D, Weinberg RA: The hallmarks of cancer. *Cell* 2000, 100:57–70
2. Marks F, Fürstenberger G: The conversion stage of skin carcinogenesis. *Carcinogenesis* 1990, 11:2085–2092
3. Yuspa SH: The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J Dermatol Sci* 1998, 17:1–7
4. Belman S, Troll W: The inhibition of croton oil-promoted mouse skin tumorigenesis by steroid hormones. *Cancer Res* 1972, 32:450–454
5. Angel P, Szabowski A, Schorpp-Kistner M: Function and regulation of AP-1 subunits in skin physiology and pathology. *Oncogene* 2001, 20:2413–2423

6. Saez E, Rutberg SE, Mueller E, Oppenheim H, Smoluk J, Yuspa SH, Spiegelman BM: c-fos is required for malignant progression of skin tumors. *Cell* 1995, 82:721–732
7. Young MR, Li JJ, Rincon M, Flavell RA, Sathyanarayana BK, Hunziker R, Colburn N: Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. *Proc Natl Acad Sci USA* 1999, 96:9827–9832
8. Dong Z, Crawford HC, Lavrovsky V, Taub D, Watts R, Matrisian LM, Colburn NH: A dominant negative mutant of jun blocking 12-O-tetradecanoylphorbol-13-acetate-induced invasion in mouse keratinocytes. *Mol Carcinog* 1997, 19:204–212
9. Behrens A, Jochum W, Sibilia M, Wagner EF: Oncogenic transformation by ras and fos is mediated by c-Jun N-terminal phosphorylation. *Oncogene* 2000, 19:2657–2663
10. Tuckermann JP, Reichardt HM, Arribas R, Richter KH, Schutz G, Angel P: The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1-dependent genes in skin. *J Cell Biol* 1999, 147:1365–1370
11. Breitenbach U, Tuckermann JP, Gebhardt C, Richter KH, Fürstenberger G, Christofori G, Angel P: Keratinocyte-specific onset of serine protease BSSP expression in experimental carcinogenesis. *J Invest Dermatol* 2001, 117:634–640
12. Gebhardt C, Breitenbach U, Tuckermann JP, Dittich BT, Richter KH, Angel P: Calgranulins S100A8 and S100A9 are negatively regulated by glucocorticoids in a c-Fos-dependent manner and overexpressed throughout skin carcinogenesis. *Oncogene* 2002, 21:4266–4276
13. Schlingemann J, Hess J, Wrobel G, Breitenbach U, Gebhardt C, Steinlein P, Kramer H, Fürstenberger G, Hahn M, Angel P, Lichter P: Profile of gene expression induced by the tumor promoter TPA in mouse epithelial cells. *Int J Cancer* 2003, 10:699–708
14. Bhartur SG, Calhoun BC, Woodrum J, Kurkjian J, Iyer S, Lai F, Goldenring JR: Genomic structure of mouse Rab11 family members. *Biochem Biophys Res Commun* 2000, 269:611–617
15. Stenmark H, Olkkonen VM: The Rab GTPase family. *Genome Biol* 2001, 2:1–7
16. Goldenring JR, Smith J, Vaughan HD, Cameron P, Hawkins W, Navarre J: Rab11 is an apically located small GTP-binding protein in epithelial tissues. *Am J Physiol* 1996, 270:515–525
17. Wang ZQ, Ovitt C, Grigoriadis AE, Mohle-Steinlein U, Ruther U, Wagner EF: Bone and haematopoietic defects in mice lacking c-fos. *Nature* 1992, 360:741–745
18. Rennecke J, Richter KH, Haussermann S, Stempka L, Strand S, Stohr M, Marks F: Biphasic effect of protein kinase C activators on spontaneous and glucocorticoid-induced apoptosis in primary mouse thymocytes. *Biochim Biophys Acta* 2000, 1497:289–296
19. Schreiber M, Baumann B, Cotten M, Angel P, Wagner EF: Fos is an essential component of the mammalian UV response. *EMBO J* 1995, 14:5338–5349
20. Fürstenberger G, Kopp-Schneider A: Malignant progression of papillomas induced by the initiation-promotion protocol in NMRI mouse skin. *Carcinogenesis* 1995, 16:61–69
21. Gack S, Vallon R, Schmidt J, Grigoriadis A, Tuckermann J, Schenkel J, Weiher H, Wagner EF, Angel P: Expression of interstitial collagenase during skeletal development of the mouse is restricted to osteoblast-like cells and hypertrophic chondrocytes. *Cell Growth Differ* 1995, 6:759–767
22. Porte D, Tuckermann J, Becker M, Baumann B, Teurich S, Higgins T, Owen MJ, Schorpp-Kistner M, Angel P: Both AP-1 and Cbfa1-like factors are required for the induction of interstitial collagenase by parathyroid hormone. *Oncogene* 1999, 18:667–678
23. Chavrier P, Vingron M, Sander C, Simons K, Zerial M: Molecular cloning of YPT1/SEC4-related cDNAs from an epithelial cell line. *Mol Cell Biol* 1990, 10:6578–6585
24. Drivas GT, Shih A, Coutavas EE, D'Eustachio P, Rush MG: Identification and characterization of a human homolog of the *Schizosaccharomyces pombe* ras-like gene YPT-3. *Oncogene* 1990, 6:3–9
25. Goldenring JR, Shen KR, Vaughan HD, Modlin IM: Identification of a small GTP-binding protein, Rab25, expressed in the gastrointestinal mucosa, kidney, and lung. *J Biol Chem* 1993, 268:18419–18422
26. Gromov PS, Celis JE, Hansen C, Tommerup N, Gromova I, Madsen P: Human rab11a: transcription, chromosome mapping and effect on the expression levels of host GTP-binding proteins. *FEBS Lett* 1998, 429:359–364
27. Karin M: New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? *Cell* 1998, 93:487–490
28. Herrlich P: Cross-talk between glucocorticoid receptor and AP-1. *Oncogene* 2001, 20:2465–2475
29. Gack S, Vallon R, Schaper J, Ruther U, Angel P: Phenotypic alterations in fos-transgenic mice correlate with changes in Fos/Jun-dependent collagenase type I expression. Regulation of mouse metalloproteinases by carcinogens, tumor promoters, cAMP, and Fos oncoprotein. *J Biol Chem* 1994, 269:10363–10369
30. Stein MP, Dong J, Wandinger-Ness A: Rab proteins and endocytic trafficking: potential targets for therapeutic intervention. *Adv Drug Deliv Rev* 2003, 55:1421–1437
31. Rutberg SE, Saez E, Glick A, Dlugosz AA, Spiegelman BM, Yuspa SH: Differentiation of mouse keratinocytes is accompanied by PKC-dependent changes in AP-1 proteins. *Oncogene* 1996, 13:167–176
32. Zerial M, McBride H: Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol* 2001, 2:107–117
33. Becker KP, Hannun YA: cPKC-dependent sequestration of membrane-recycling components in a subset of recycling endosomes. *J Biol Chem* 2003, 278:52747–52754
34. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH: Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 2001, 22:153–183
35. Chang L, Karin M: Mammalian MAP kinase signalling cascades. *Nature* 2001, 410:37–40
36. Nissen RM, Yamamoto KR: The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev* 2000, 14:2314–2329
37. Eferl R, Wagner EF: AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 2003, 3:859–868
38. Ozanne BW, McGarry L, Spence HJ, Johnston I, Winnie J, Meagher L, Stapleton G: Transcriptional regulation of cell invasion: AP-1 regulation of a multigenic invasion programme. *Eur J Cancer* 2000, 36:1640–1648
39. Owens DM, Watt FM: Contribution of stem cells and differentiated cells to epidermal tumours. *Nat Rev Cancer* 2003, 3:444–451
40. Goldenring JR, Ray GS, Lee JR: Rab11 in dysplasia of Barrett's epithelia. *Yale J Biol Med* 1999, 72:113–120
41. Wulfkühle JD, Sgroi DC, Krutzsch H, McLean K, McGarvey K, Knowlton M, Chen S, Shu H, Sahin A, Kurek R, Wallwiener D, Merino MJ, Petricoin EF, Zhao Y, Steeg PS: Proteomics of human breast ductal carcinoma in situ. *Cancer Res* 2002, 62:6740–6749
42. Kazemi-Nouireini S, Colonna-Romano S, Ziaee AA, Malboobi MA, Yazdanboud M, Setayeshgar P, Maresca B: Differential gene expression between squamous cell carcinoma of esophagus and its normal epithelium; altered pattern of mal, akr1c2, and rab11a expression. *World J Gastroenterol* 2004, 10:1716–1721
43. Cheng KW, Lahad JP, Kuo WL, Lapuk A, Yamada K, Auersperg N, Liu J, Smith-McCune K, Lu KH, Fishman D, Gray JW, Mills GB: The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers. *Nat Med* 2004, 10:1251–1256
44. Chan AM, Weber T: A putative link between exocytosis and tumor development. *Cancer Cell* 2002, 2:427–428
45. Polo S, Pece S, Di Fiore PP: Endocytosis and cancer. *Curr Opin Cell Biol* 2004, 16:156–161
46. Aaronson SA: Growth factors and cancer. *Science* 1991, 254:1146–1153
47. Szabowski A, Maas-Szabowski N, Andrecht S, Kolbus A, Schorpp-Kistner M, Fusenig NE, Angel P: c-Jun and JunB antagonistically control cytokine-regulated mesenchymal-epidermal interaction in skin. *Cell* 2000, 103:745–755
48. Mueller MM, Fusenig NE: Friends or foes—bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* 2004, 4:839–849
49. Fan GH, Lapierre LA, Goldenring JR, Richmond A: Differential regulation of CXCR2 trafficking by Rab GTPases. *Blood* 2003, 101:2115–2124
50. Roosterman D, Schmidlin F, Bunnett NW: Rab5a and rab11a mediate agonist-induced trafficking of protease-activated receptor 2. *Am J Physiol* 2003, 284:C1319–C1329

51. Powelka AM, Sun J, Li J, Gao M, Shaw LM, Sonnenberg A, Hsu VW: Stimulation-dependent recycling of integrin beta1 regulated by ARF6 and Rab11. *Traffic* 2004, 5:20–36
52. Lock JG, Stow JL: Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol Biol Cell* 2005, 16:1744–1755
53. Sloane BF, Moin K, Sameni M, Tait LR, Rozhin J, Ziegler G: Membrane association of cathepsin B can be induced by transfection of human breast epithelial cells with c-Ha-ras oncogene. *J Cell Sci* 1994, 107:373–384
54. Imamura H, Takaishi K, Nakano K, Kodama A, Oishi H, Shiozaki H, Monden M, Sasaki T, Takai Y: Rho and Rab small G proteins coordinately reorganize stress fibers and focal adhesions in MDCK cells. *Mol Biol Cell* 1998, 9:2561–2575
55. Cheng H, Sugiura R, Wu W, Fujita M, Lu Y, Sio SO, Kawai R, Takegawa K, Shuntoh H, Kuno T: Role of the Rab GTP-binding protein Ypt3 in the fission yeast exocytic pathway and its connection to calcineurin function. *Mol Biol Cell* 2002, 13:2963–2976
56. Oxford G, Theodorescu D: Ras superfamily monomeric G proteins in carcinoma cell motility. *Cancer Lett* 2003, 189:117–128
57. Deneka M, Neeft M, van der Sluijs P: Regulation of membrane transport by Rab GTPases. *Crit Rev Biochem Mol Biol* 2003, 38:121–142
58. Pei L, Peng Y, Yang Y, Ling XB, Van Eyndhoven WG, Nguyen KC, Rubin M, Hoey T, Powers S, Li J: PRC17, a novel oncogene encoding a Rab GTPase-activating protein, is amplified in prostate cancer. *Cancer Res* 2002, 62:5420–5424