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Comparison of Calmodulin Gene Expression in Human Neonatal Melanocytes and Metastatic Melanoma Cell Lines

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The qualitative and quantitative expression of three calmodulin genes (CAM I, CAM II and CAM III) was characterized in human neonatal melanocytes and metastatic melanoma cell lines in the absence and presence of serum, other growth modulators, and/or 12-0-tetradecanoyl-phorbol-13-acetate (TPA). Results indicated that the qualitative expression in melanocytes was the same as that of melanomas, that is, CAM I gene expressed two transcripts, 4.4 kb and 2.1 kb, whereas CAM II and CAM III expressed one transcript each, 1.95 kb and 2.37 kb, respectively. Differential quantitative expression was seen particularly with CAM I. The average levels of both CAM I transcripts in melanomas were less than one-half those of melanocytes. Serum and other growth modulators (including Ca^{++}, isobutyl methyl xanthine, bovine pituitary extract, and insulin) enhanced CAM I and CAM II gene expression in melanocytes; in contrast, the net effect of serum in melanomas was to decrease expression of CAM I and CAM III. This effect was most prominent in melanoma C81-46C. TPA markedly inhibited expression of all three CaM genes in melanocytes; however, in melanomas the net effect of TPA was to increase their expression. CAM I in melanoma C81-46C was the most sensitive to TPA stimulation. *J Invest Dermatol 99:764–773, 1992*

Calmodulin (CaM) is a small monomeric protein (molecular weight 17,000 daltons) consisting of 148 amino acids and four Ca^{++}-binding domains. This protein has been highly conserved throughout evolution [1–3] and is present in virtually all eukaryotes examined. CaM modulates the activity of several key enzymes and cell components which are involved in numerous fundamental cell processes including cell growth regulation [4–7]. The involvement of CaM in growth regulation and the cell cycle is of particular interest because there is considerable evidence to suggest that diseases characterized by abnormal cell growth, such as cancer, are associated with increased levels of cellular CaM [5,6,8,9]. As a result, the potential value of CaM antagonists as anticancer agents has also been investigated [4,10–13].

In recent years much information has accumulated on the nature of CaM genes in non-malignant cells that range, on the evolutionary scale, from lower eukaryotes having a single CaM gene to higher eukaryotes possessing two or more CaM genes [14–25]. Three distinct bona fide genes have been cloned to date from rats [22,24] and humans [3,17,24,26] and are commonly designated CAM I, CAM II, and CAM III. Recent studies indicate that even though these three genes are located on different chromosomes [27], they encode a single CaM protein [28]. In addition, four retropseudogenes of CaM have been found in rats and humans [22,28–30]. One of these pseudogenes is related to CAM I whereas the other three resemble CAM II. In contrast to bona fide genes, these pseudogenes lack introns and are unable to code for functional CaM protein.

The molecular mechanisms by which CaM influences cell growth in neoplastic and non-neoplastic cells are not well known. The melanocyte/melanoma system has become a useful model for comparative studies in non-malignant versus malignant cells. During the past decade, reliable methods have been developed for growing neonatal melanocytes and human melanoma cells in tissue culture [31–37]. For optimal growth, melanocytes require the presence of a phorbol ester (e.g., TPA), fetal calf serum, Ca^{++}, isobutyl methyl xanthine (IBMX), insulin, bovine pituitary extract (BPE), and glutamine. In contrast, melanoma cells require only the addition of serum and glutamine.

To compare the expression and regulation of CaM genes in human neonatal melanocytes and metastatic melanoma cell lines, northern blot analyses were carried out on total RNA isolated from these cells after growing them in the appropriate complete medium or in medium modified by deleting serum and other growth factors or by adding TPA. The results showed that CAM I transcripts were differentially expressed in melanomas compared to melanocytes. This CaM gene was also the most responsive to regulation by serum and TPA; however, the response of melanomas to growth modulators was generally opposite to that of melanocytes, suggesting that the regulation of CaM gene transcription differs in melanocytes and most melanomas.

MATERIALS AND METHODS

Cell Cultures

Melanocytes: The basic method used in this study [38] was origi-

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Abbreviations:
BPE: bovine pituitary extract
CaM: calmodulin
CAM I, CAM II, CAM III: calmodulin genes
IBMX: isobutyl methyl xanthine
PBS: phosphate-buffered saline
SDS: sodium dodecyl sulfate
TPA: 12-0-tetradecanoyl phorbol-13-acetate

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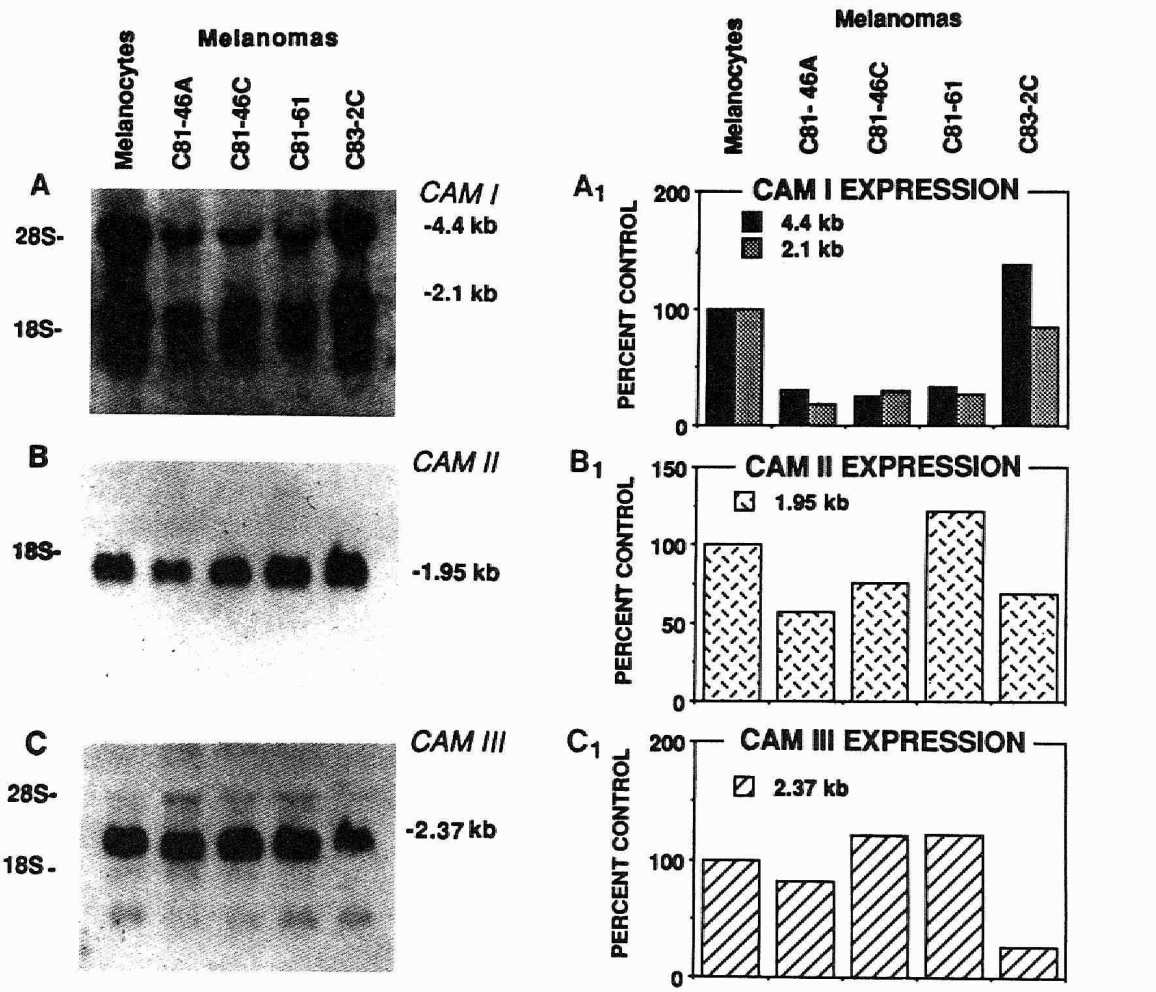

Figure 1. Expression and quantitation of CaM transcripts from human neonatal melanocytes and metastatic melanoma cell lines. *A* and A_1, CAM I gene transcripts (4.4 kb and 2.1 kb); *B* and B_1, CAM II gene transcript (1.95 kb); *C* and C_1, CAM III gene transcript (2.37 kb). Newborn foreskin melanocytes and four melanoma cell lines (C81-46A, C81-46C, C81-61, and C83-2C) were grown in the appropriate complete medium (see *Materials and Methods*) until 70–80% confluent. The cells were then pelleted and total RNA isolated. RNA (10 μg per sample) was separated by electrophoresis on formaldehyde agarose gel, transferred to nylon filters by northern blot transfer, hybridized with ^{32}P-labeled CaM probe, and exposed to film. The same filters were stripped of the probes and reused. RNA ladder standards (BRL, 0.24–9.5 kb) were used to determine RNA sizes. Ribosomal RNA 18S and 28S markers are also shown on the *left*. Preliminary studies indicated that the inherent variability of duplicate experiments was less than 25%.

nally developed from procedures described by Eisinger and Marko [31] and Halaban and Alfano [32]. Briefly, human neonatal foreskin samples were collected, and melanocytes isolated and transferred to a T-75 flask. These cells were grown in MCDB 153 medium (Irvine Scientific, Irvine, CA) supplemented with Ca^{++} (2 mM), TPA (10 ng/ml), fetal calf serum (FCS, 2%), glutamine (1%), IBMX (0.1 mM), BPE (40 μg/ml), insulin (5 μg/ml), penicillin (100 units/ml), and streptomycin (0.1 mg/ml) [33,37]. This medium was designated melanocyte complete medium. Contamination by fibroblasts was suppressed by adding geneticin (250 μg/ml) to the growth medium for 2 d.

Melanoma Cell Lines: Five melanoma cell lines (C81-46A, C81-46C, C81-61, C81-61X, and C83-2C) were cultured in Ham's F-10 medium supplemented with FCS (5%), newborn calf serum (NCS, 5%), glutamine (1%), penicillin (100 units/ml), and streptomycin (0.1 ng/ml) [39]; this medium was referred to as melanoma complete medium. The passage number for the melanoma cell lines used in this study was less than 8. The cell lines C81-46A, C81-46C, C81-61, and C82-2C were described previously [39–41]. C81-46A and C81-46C were developed from two different biopsy sites isolated from the same patient. C81-61X was isolated from a nude mouse following injection of the parental metastatic melanoma cell line C81-61. These cell lines had variable growth rates: C81-46A and C81-46C were slow-growing; C81-61X and C83-2C were rapidly growing; and C81-61 had an intermediate growth rate. All of these cell lines were initially cloned through soft agar and could form tumors in nude mice. All cell lines were free of mycoplasma as determined using a Gen-probe kit (Fisher Scientific, Pittsburgh, PA). Viable cell counts were determined using the trypan blue exclusion method. The melanoma cell lines C81-46A, C81-46C, C81-61, and C83-2C were used for the qualitative and quantitative analysis of CaM gene expression, whereas C81-46C, C81-61, and C81-61X were used in the studies determining the effects of TPA, serum, and other growth modulators on CaM gene expression.

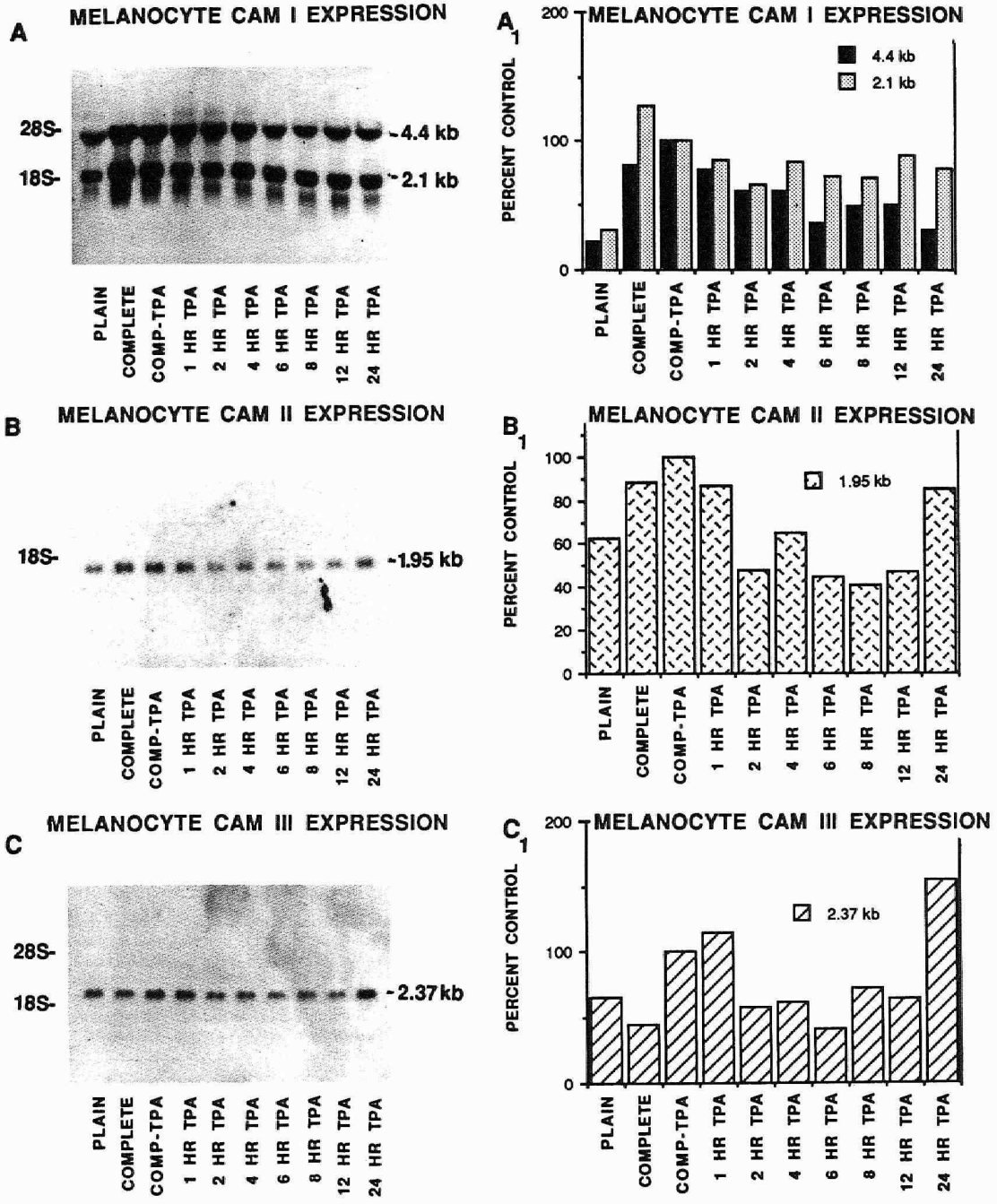

Figure 2. Comparison of CaM gene expression in human neonatal melanocytes in plain medium, complete medium, complete medium minus TPA, and complete medium plus TPA (10 ng/ml) for 1 to 24 h. *A* and A_1, CAM I expression of transcripts 4.4 kb and 2.1 kb in northern blot *(left)* and histogram *(right)*. *B* and B_1, CAM II expression of transcript 1.95 kb in blot *(left)* and histogram *(right)*. *C* and C_1, CAM III expression of transcript 2.37 kb in blot *(left)* and histogram *(right)*. Composition of media was described in *Materials and Methods*. The melanocytes were grown in complete medium until 70–80% confluent and then incubated for an additional 24 h in either plain medium, complete medium, or complete medium minus TPA. For the TPA induction study, melanocytes in the latter medium were isolated and exposed to complete medium (containing 10 ng/ml TPA) for 1–24 h. Total RNA was isolated from pooled cultures and northern blot analyses were carried out as described in *Materials and Methods*. The same filters were stripped of the CaM probes and reused. The RNA transcripts were quantitated by densitometry and corrected for RNA loading using an 18S cDNA probe for rRNA. The comp-TPA transcript values were used as the control and assigned a value of 100%. The other parameters were graphed as percentages of the control values.

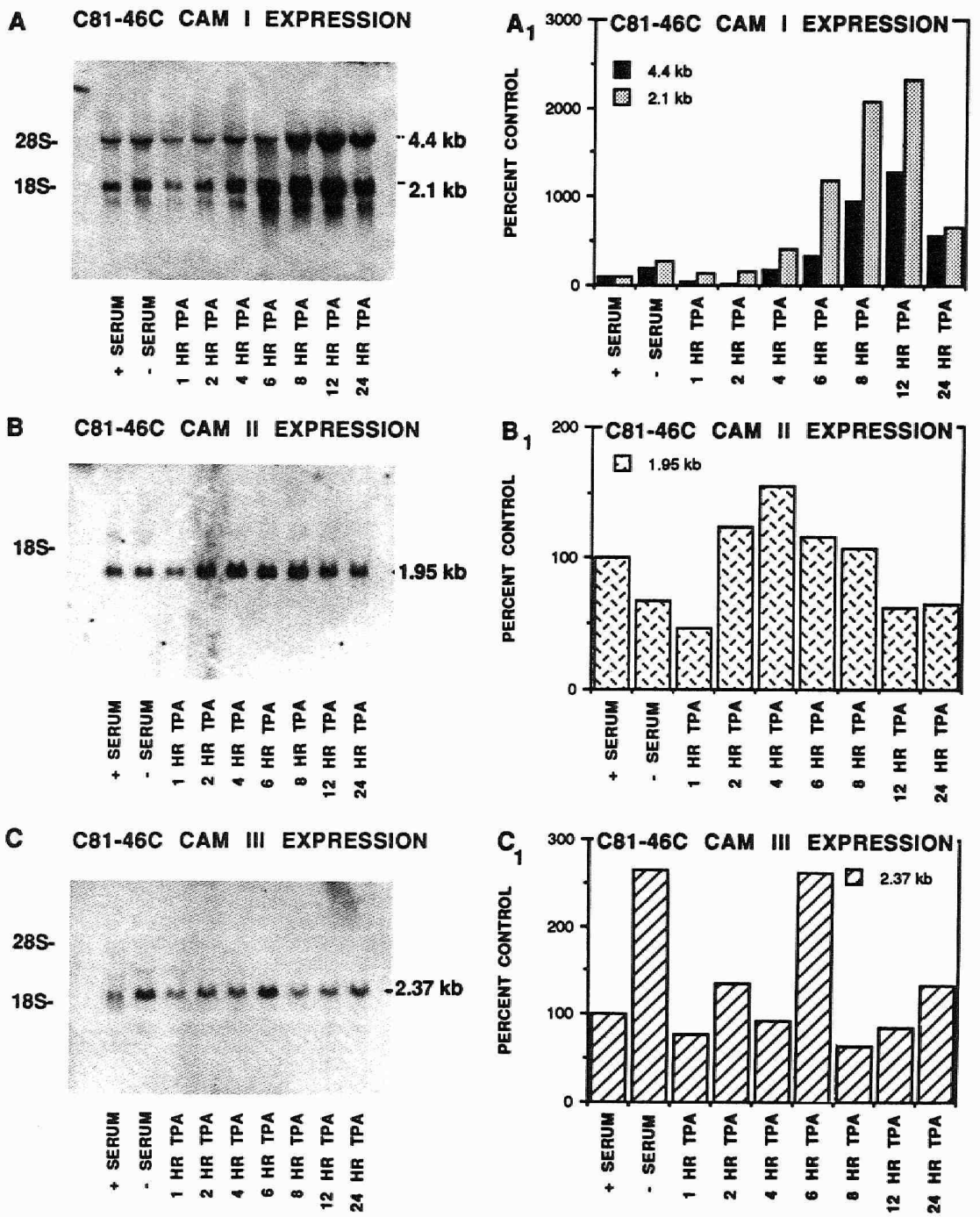

Figure 3. Comparison of CaM gene expression in human melanoma C81-46C cell line in the presence and absence of serum and in the presence of TPA for 1 to 24 h. *A* and A_1, CAM I expression of transcripts 4.4 kb and 2.1 kb in blots *(left)* and histogram *(right)*. *B* and B_1, CAM II expression of transcript 1.95 kb in blot *(left)* and histogram *(right)*. *C* and C_1, CAM III expression of transcript 2.37 kb in blot *(left)* and histogram *(right)*. The C81-46C cells were grown to 70–80% confluency in melanoma complete medium and then incubated for an additional 24 h in the absence or presence of serum. For the TPA exposure part of this experiment, melanoma cells (incubated in complete medium without serum) were then incubated in complete medium (containing 10 ng/ml TPA) for 1 to 24 h. Total RNA was isolated from pooled cultures and northern blot analysis performed as described in *Materials and Methods*. The filters were stripped of the CaM probes and reused. Quantitation of transcripts and correction for RNA loading was carried out as described above. The corrected transcript values for melanoma cells in complete medium (+serum) were used as control and set at 100%. The transcript values for the other parameters were graphed as percentages of the control values.

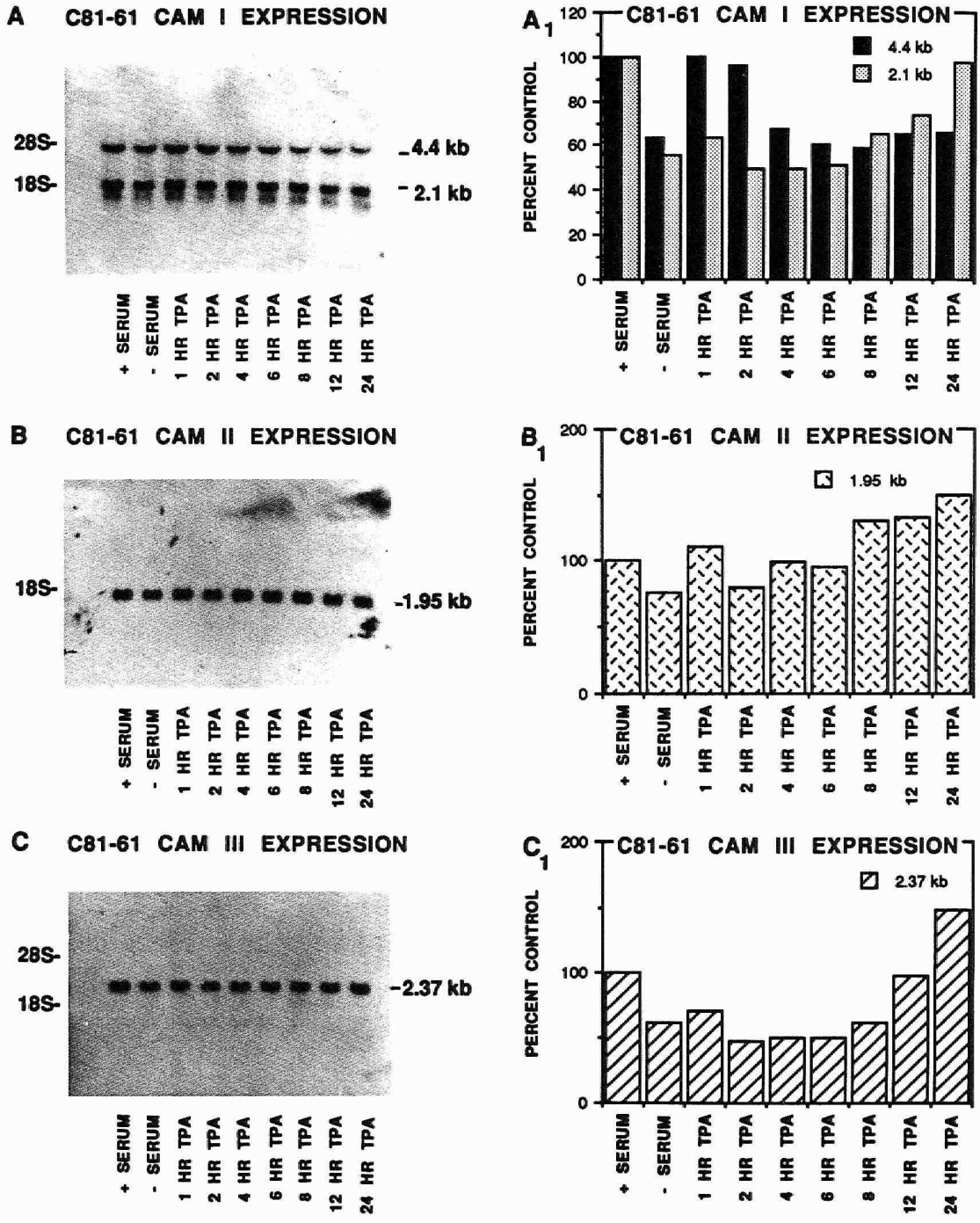

Figure 4. Comparison of CaM expression in human melanoma C81-61 cell line in the presence and absence of serum and in the presence of TPA for 1 to 24 h. *A* and A_1, CAM I expression of transcripts 4.4 kb and 2.1 kb in blot *(left)* and histogram *(right)*. *B* and B_1, CAM II expression of transcript 1.95 kb in blot *(left)* and histogram *(right)*. *C* and C_1, CAM III expression of transcript 2.37 kb in blot *(left)* and histogram *(right)*. Experimental method and data presentation was as described for Fig 3.

Culture Methods Melanocytes and melanoma cells were grown in their respective complete medium (described above) to near-confluency (i.e., 70–80% confluent). For the qualitative and quantitative CaM mRNA studies, the near-confluent cells were harvested (using trypsin as necessary) and pelleted, and total RNA isolated as described below. To determine the effect of media constituents on CaM gene expression the near-confluent melanocytes were incubated for an additional 24 h in either plain medium (i.e., MCDB 153 lacking Ca^{++}, TPA, serum, IBMX, BPE, and insulin); complete medium; or complete medium minus TPA. Near-confluent melanoma cells were incubated for an additional 24 h in F-10 medium either with or without serum. Total RNA was isolated from the melanocytes and melanoma cells as described below. For TPA-induction experiments, near-confluent melanocytes (which had been incubated for 24 h in complete medium minus TPA) were exposed to melanocyte complete medium containing TPA (10 ng/ml) for intervals ranging from 1 to 24 h. Similarly, melanoma cells (which had been incubated for 24 h in complete medium minus serum) were exposed to melanoma complete medium containing TPA for 1 to 24 h. Total RNA was then isolated from pooled cultures of melanocytes and melanomas after various exposure time intervals (i.e., 1, 2, 4, 6, 8, 12, and 24 h) as described below.

Isolation of RNA This procedure was modified from that described by Chirgwin et al [42] as recently updated [43,44]. The melanocytes and melanoma cells grown in T-175 flasks were pelleted, washed once with phosphate-buffered saline (PBS), and lysed using a 4 M guanidine isothiocyanate solution. The lysed homogenate was layered on top of a 5.7 M CsCl cushion and centrifuged at 55,000 rpm for 3 h at 20°C. The RNA pellet was resuspended in Tris-EDTA (TE, pH 8.0) with 0.1% sodium dodecyl sulfate (SDS), extracted, ethanol precipitated, and resuspended in sterile water.

Northern Blot Transfer Analyses The northern blot was prepared as described by Fourney et al [45]. Briefly, total RNA (10 μg/sample) was electrophoresed on a denaturing formaldehyde agarose gel (0.8%), transferred by capillary action overnight to a nylon filter (Nytran, Schleicher and Schuell, Keene, NH), and prehybridized for 2–4 h at 42°C. The probes (see below) were labeled by the method of Feinsberg and Vogelstein [46] using a random priming kit (Promega, Madison, WI). Fresh hybridization solution containing about 1×10^6 cpm/ml of radiolabeled probe was added to the filter as described by the manufacturer's procedures. Hybridization was carried out overnight followed by two stringent washouts at 50–60°C using 0.1% SDS and 0.1 X sodium chloride–sodium citrate solution (SSC). The filters were exposed to Kodak X-OMAT film for 2 to 20 hours at −80°C with intensifying screens. The filters were stripped of probes by using two washes of 0.1% SDS, 0.1 × SSC at 80°C; the same filters were reused with each of the CaM probes. To quantitate individual RNA transcripts, films were exposed for time periods that produced band intensity that was linear with respect to time. The films were then scanned with a densitometer (GS 4300) with software (GS 370) (Hoefer Scientific Instruments, San Francisco, CA) and the quantity of RNA transcript determined from the area of the peak corresponding to each RNA transcript. Values for CaM gene expression were corrected for RNA loading using an 18S cDNA probe for rRNA [47]. RNA standards were used to determine RNA sizes (0.24–9.5-kb RNA ladder, BRL, Gaithersburg, MD). The inherent variability of preliminary duplicate studies was found to be less than 25%.

Probes CAM I cDNA was obtained from Dr. Richard N. Perham, Cambridge, UK. This probe was originally cloned from human adult liver (clone DD132) by Wawrzynczak and Perham in 1984 [26] and was carried in plasmid pBR322. A 0.8-kb restriction fragment was released by digestion with Pst I. CAM II and CAM III cDNAs were provided by Dr. Emanuel E. Strehler, Zurich, Switzerland. The CAM II cDNA was ligated into pUC18 plasmid DNA at 27-1 and a 1.1-kb insert was released by Hind II digestion. Originally this probe was cloned from human teratoma by Sen Gupta et al in 1987 [24]. The CAM III cDNA insert was carried in plasmid pUC18 at p6-4 and a 2.2-kb restriction fragment was released by digestion with Pst I/Xho I. Fischer et al [17] originally cloned this probe from human teratoma in 1988. The 18S cDNA probe for ribosomal RNA was released by EcoR I digestion and a 5.6-kb insert was isolated from the plasmid pB [47].

RESULTS

Expression of CaM Gene Transcripts in Melanocytes and Melanomas The expression of CaM mRNAs in human neonatal melanocytes and metastatic melanoma cell lines, using northern blot transfer analysis, is shown in Fig 1*A*,*B*,*C*. Densitometric quantitation is shown in Fig 1A_1,B_1,C_1.

The CAM I gene was expressed as two transcripts, 4.4 kb and 2.1 kb in size. The average level of expression of these transcripts in three of four melanomas was 70–75% lower than melanocytes. Only melanoma C83-2C had net CaM transcript levels that were close to the melanocyte levels. The CAM II gene was expressed as a single mRNA (1.95 kb) in melanocytes and melanomas. Three of four melanomas had levels of this transcript that were 24–43% less than melanocytes, whereas melanoma C81-61 levels were 21% higher. With the CAM III gene one main transcript (2.37 kb) was expressed at levels that were similar to melanocytes in three of four melanomas; in contrast, C83-2C had CaM mRNA levels that were 72% lower than melanocytes.

Effect of Serum Factors, Other Growth Modulators, and TPA on CaM Gene Expression in Melanocytes Figure 2 compares the expression of the four main CaM transcripts in melanocytes after growth in various modified media, such as MCDB 153, without the usual supplements (serum, Ca^{++}, TPA, IBMX, BPE, insulin) and referred to in Fig 2 as plain medium; complete medium lacking only TPA (comp-TPA); and complete medium containing 10 ng/ml TPA for various periods of exposure ranging from 1 to 24 h.

When melanocytes were grown in plain medium, the average CAM I level (i.e., both transcripts) was decreased by about 80% in comparison to melanocytes grown in complete medium; in contrast, CAM II and CAM III were only moderately altered.

Deletion of TPA from melanocyte complete medium produced minimal changes in the expression of both CAM I and CAM II genes when compared with corresponding levels in complete medium (with TPA); whereas the CAM III expression levels were more than 50% higher in melanocytes grown in medium lacking TPA.

Exposing melanocytes to TPA for 1 h had a minimal effect on the expression of all three CaM genes. Longer exposure of melanocytes to TPA (e.g., 2–12 h) resulted in a persistent reduction in the expression of all three CaM genes. After 24 h of TPA exposure the CAM I transcripts were still repressed (45%); however, CAM III transcript levels had recovered to a level that was 55% higher than the control (Comp-TPA).

Effect of Serum and TPA on CaM Gene Expression in Melanomas Figures 3 to 5 illustrate the effects of the absence and presence of serum as well as the influence of TPA exposure (1 to 24 h) on the expression of CaM genes in three human metastatic melanoma cell lines.

Deletion of serum from melanoma complete medium (i.e., F-10 medium supplemented with FCS, NCS, and glutamine) increased the average CAM I expression in melanoma C81-46C by more than 140%, whereas the corresponding transcript levels were reduced by 40% in C81-61 and virtually unchanged in C81-61X. CAM II expression was only modestly altered. The CAM III transcript levels were increased 166% in C81-46C, slightly increased in C81-61X, but reduced 39% in C81-61.

Exposure of C81-46C melanoma to TPA markedly induced CAM I gene expression, particularly between 4 and 24 h of TPA exposure. Peak induction was seen after 12 h of TPA when the average increase in the two CAM I transcript levels was 1500% (i.e., fifteenfold) higher than the corresponding controls. Some induction (135% increase) of the CAM I transcripts by TPA was also seen

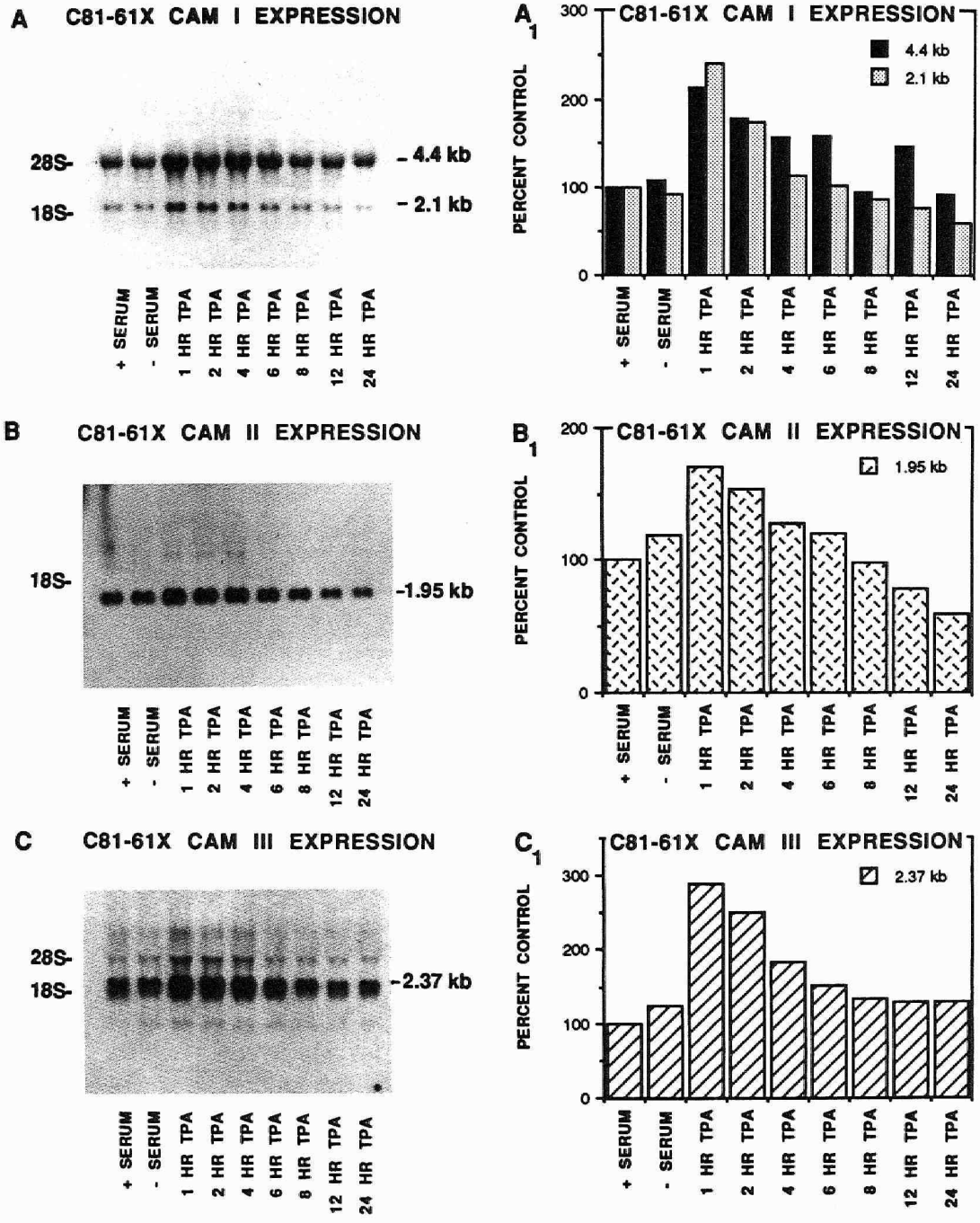

Figure 5. Comparison of CaM gene expression in human melanoma C81-61X cell line in the presence and absence of serum and in the presence of TPA for 1 to 24 h. *A* and A_1, CAM I expression of transcripts 4.4 kb and 2.1 kb in blot *(left)* and histogram *(right)*. *B* and B_1, CAM II expression of transcript 1.95 kb in blot *(left)* and histogram *(right)*. *C* and C_1, CAM III expression of transcript 2.37 kb in blot *(left)* and histogram *(right)*. Experimental method and data presentation was as described for Fig 3.

with C81-61X; however, peak induction occurred 1 h after TPA. In contrast, the average CAM I gene expression in C81-61 was inhibited consistently (35%) between 1 to 24 h of TPA exposure, somewhat similar to the findings seen earlier with melanocytes (Fig 2). With regard to CAM II expression, 1 h exposure to TPA suppressed the C81-46C transcript by more than 50%, followed by an induction period between 2–8 h (35% increase) and suppression of 30% after 12–24 h of TPA. In C81-61, TPA gradually increased CAM II expression (35% between 8 to 24 h). In C81-61X, CAM II expression with TPA followed a pattern similar to CAM I, i.e., initial induction at 1 h (70% increase) followed by a gradual decrease to levels 40% less than the control at 24 h. CAM III expression in C81-46C was increased 161% at 6 h but only minimally altered at the other exposure times. C81-61 transcript levels were generally inhibited (50%) by TPA, particularly between 1 and 8 h; after 24 h expression of this transcript increased to levels that were 50% higher than corresponding controls. In C81-61X, TPA induced CAM III gene expression 188% after 1 h exposure. These transcript levels gradually diminished but were still about 30% higher than control values after 24 h exposure to TPA.

DISCUSSION

The present study has addressed three main objectives: first, to determine whether there were qualitative and/or quantitative differences in the expression of three CaM genes CAM I, CAM II, and CAM III in human melanocytes and metastatic melanoma cell lines; second, to compare the responsiveness of these CaM genes to serum factors and other growth modulators; and third, to examine the effects of TPA on CaM gene expression.

Results indicated that the qualitative expression of CaM genes was the same in melanocytes as in all melanomas. With both cell types, CAM I expressed two species of mRNA (4.4 and 2.1 kb in size) whereas CAM II and CAM III each expressed one main transcript (1.95 and 2.37 kb, respectively). However, CaM transcripts did show some distinct quantitative differences between melanocytes and melanomas. For example, the expression of both CAM I transcripts was low in three melanomas (C81-46A, C81-46C, and C81-61), i.e., only one quarter to one half the levels of melanocyte CAM I. In melanoma C83-2C, however, CAM I transcript levels were more than threefold higher than the other melanomas and, in the case of the 4.4-kb transcript, was 37% higher than melanocyte levels. It is concluded that the CAM I gene is differentially expressed in melanomas and melanocytes. CAM II mRNA levels in melanomas were considerably higher, on average, than melanoma CAM I levels, i.e., approached the CAM II levels in melanocytes. The average expression of CAM III mRNA was found to be as high as that of melanocytes in three melanomas (C81-46A, C81-46C, and C81-61). In contrast, C83-2C had CAM III transcript levels that were only about one quarter that of the melanocytes and the other melanomas. These findings suggest that CAM III is constitutively expressed in melanocytes and in all three slow-to-intermediate growth rate melanomas.

Our findings with regard to the number and size of the CaM gene transcripts are in general agreement with previous reports using rodent tissues and other human cell types [22,24,48,49] with the exceptions that the largest CAM I transcript (4.0–4.4 kb) was not observed by some of these workers [48,49]. These investigators also found that the smallest CAM III transcript (0.8–1.0 kb) was more prominently expressed than was seen in the present study. MacManus et al [49] recently examined CaM gene expression in developing rat tissues and rodent neoplastic tissues. Their results suggested that only CAM II expression was increased in developing tissues, whereas CAM I and CAM III remained relatively constant; differential expression was also seen in neoplastic tissues and CAM II was the main gene expressed. It is of interest that these workers also found the level of expression of the CAM II gene in rat hepatomas to be considerably lower than normal liver cells even though the hepatomas had higher levels of CaM protein than liver. They concluded that overproduction of CaM in hepatoma cells, in the face of low mRNA levels, was due to a difference in turnover of CAM II mRNA in hepatomas as compared to liver. This observation is analogous to our finding of low CAM I transcript levels in three of the melanoma cell lines (relative to melanocytes). Although we did not measure the corresponding CaM protein levels in these melanoma cells, other workers reported recently [50] that proliferating melanoma cells contain substantial levels of CaM protein, with cell lines of metastatic origin having the highest levels. There is, however, a need for further comparative studies between CaM levels in melanomas and melanocytes and also to correlate changes in CaM mRNA with changes in CaM protein levels. The recent studies of Van Eldik et al [51] also emphasize the need to examine corresponding changes in CaM-binding proteins when investigating CaM control differences between neoplastic versus non-neoplastic cells.

Deletion of serum and other growth modulators (Ca^{++}, TPA, IBMX, BPE, insulin) from MCDB 153 medium markedly reduced the expression of both CAM I transcripts in melanocytes, with minimal effects on CAM II and CAM III. These results suggest that one or more serum factors and/or growth modulators (excluding TPA) have a significant enhancing effect on CAM I expression with a minimum effect on CAM II and CAM III. When FCS and NCS were deleted from the Ham's F-10 medium a number of interesting quantitative differences were observed between three melanoma cell lines (C81-46C, C81-61, and C81-61X) with regard to expression of the CaM gene transcripts. For example with CAM I, serum deletion brought about a substantial increase in the expression of both transcripts in C81-46C; however, a reduction was seen in C81-61 and virtually no change was seen in C81-61X. CAM II expression was not changed in the melanomas. With CAM III, a substantial increase in expression was seen in C81-46C, whereas in C81-61X the increase was more modest and in C81-61 a decrease resulted. These results indicate that serum factors tend to decrease CaM expression in melanomas, particularly CAM I and CAM III. This effect was opposite to that seen earlier with melanocytes. A common feature of serum depletion on CaM expression in melanomas is the marked variation between the tumor cell lines, suggesting that the CaM gene expression system in melanomas is heterogeneous with regard to responsiveness to serum factors. It should also be noted that CaM expression was significantly different in the parental cell line C81-61 (isolated from soft agar) compared with C81-61X (passaged through a nude mouse). Analogous heterogeneous responses in these melanoma cell lines were reported recently with regard to PKC gene expression [38] as well as the expression of oncogenes c-jun, jun-B, and c-fos [52].

The third objective of this study was to examine the effects of TPA on CaM gene expression in melanocytes and melanoma cell lines. Melanocytes and three metastatic melanomas (C81-46C, C81-61, and C81-61X) were exposed to TPA (10 ng/ml) for various intervals ranging from 1 to 24 h. In melanocytes, TPA markedly suppressed the expression of all three CaM genes particularly between 2-h and 12-h exposures. In contrast, the melanoma C81-46C CAM I gene was found to be remarkably sensitive to induction by TPA resulting in an average increase in both CAM I transcript levels of 1500% (fifteenfold) at the peak (12-h) period. With melanoma C81-61X, substantial induction of CAM I was also seen; however, peak induction occurred much earlier (1 h). It is also of interest that the CAM I gene expression of the parental cell line C81-61 was consistently inhibited throughout the 24-h TPA exposure period, similar to the response seen with melanocytes. The effect of TPA on CAM II and CAM III expression also demonstrated some induction in melanomas C81-46C (at 4–6 h) and C81-61X (1–4 h) but the overall effect was lower than on CAM I expression. Melanoma C81-61 CAM III was consistently inhibited by TPA between 1- and 8-h exposure but CAM II expression was not affected. These results indicate that CaM genes in melanomas and melanocytes appear to contain elements that respond, either

directly or indirectly, to TPA and to regulation by serum and other growth modulators. The CAM I gene seems to be most sensitive to this regulation. Zimmer et al [53] found that the CaM gene in *Chlamydomonas reinhardtii* contains 5′-sequence motifs that resemble binding sites for AP-1/jun. How the expression of CaM genes is modified by transcription regulating factors is a subject for further investigation.

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