FEBS Letters 491 (2001) 200–206 FEBS 24631

Inhibition of gap junctional intercellular communication in rat liver epithelial cells with transforming RNA

Tomonori Hayashi[a,1], James E. Trosko[a], Katsutomo Hamada[b,*]

[a] *Department of Pediatrics/Human Development, Institute of Environmental Toxicology and the Cancer Center of MSU, Michigan State University, 246 NFST Bldg., East Lansing, MI 48824, USA*

[b] *Division of Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami Ward, Hiroshima 734-0037, Japan*

Received 23 January 2001; accepted 30 January 2001

First published online 9 February 2001

Edited by Veli-Pekka Lehto

Abstract Previous studies indicated that transforming RNA, derived from the 3′ half of the U5 small nuclear RNA first stem structure, suppressed the secretory protein translation in vitro. Gap junctions facilitate homeostatic control of cell growth and differentiation and their dysfunction has been correlated with carcinogenesis. Here, we reported that transforming RNA directly suppressed the gap junction protein, connexin 43, translation and thereby inhibited functional gap junction function in rat epithelial cells. Together with previous data, this implies that altered expression of transforming RNA itself is a potential mechanism in inhibiting gap junction function during carcinogenesis.

Key words: Gap junctional intercellular communication; Connexin 43; Transforming RNA; Rat liver epithelial cell

1. Introduction

During the evolution of a normal cell to a premalignant cell, then to a invasive, metastatic tumor cell, it goes from a contact-inhibited, diploid cell to one that loses contact inhibition and growth control, becomes unable to terminally differentiate and exhibits genomic instability. These phenotypes, shared by all tumor cells derived from various tissues and organs, have been postulated to be the result of the loss of gap junction function, due to a number of different transcriptional, translational or posttranslational mechanisms [1,2].

We have reported that the 3′ half of the first stem structure of U5 small nuclear RNA was capable of transforming normal rat fibroblasts morphologically and driving them eventually into tumorigenic cells when expressed with a poly(A) tail [3]. By using a pulse-chase labeling and immunoprecipitation method, it was found that expression of the RNA resulted in suppression of the extracellular matrix fibronectin protein synthesis [3]. In reticulocyte lysate translation, this transforming RNA had an ability to affect, at the level of elongation, the translation of preβ-lactamase used as a model secretory protein with a signal peptide, blocking a physiological regulatory function (probably elongation arresting function) played by a signal recognition particle (SRP) and the ribosome [4]. A majority of secretory and membrane proteins and part of lysosomal proteins are synthesized through the SRP-mediated translocation system [5–7]. Thus, these findings suggest that the RNA might affect exclusively the synthesis of those proteins in a cell.

Gap junctions are one type of junctional complex that form protein channels between adjacent cells and have a role in maintaining cellular homeostasis by allowing direct transfer of small cytoplasmic constituents [8–10]. They are composed of transmembrane connexin (Cx) proteins [11]. The gap junction-mediated intercellular communication (GJIC) plays an important role in the cellular responsiveness to the environment. Normal, contact-inhibited cells are characterized by having functional GJIC. On the other hand, normal cells, exposed to tumor promoting agents, and malignant tumor cells are frequently characterized by reversible- or stable-disrupted GJIC, respectively [1,2]. Thus, the disruption of GJIC has been hypothesized to overcome the suppression of initiated cells by the surrounding normal cells and to allow the clonal expansion of preneoplastic cells, leading to an idea that the Cx genes belong to a family of tumor suppressor genes [12–15]. Combined with these observations, we expect that the transforming RNA3S$^+$ would also suppress the translation of gap junction proteins which would lead to the inhibition of GJIC. Here, we report that expression of the RNA in rat liver epithelial WB-F344 cells, that have a high level expression of Cx43, numerous gap junctions, and a high percentage of communicating cells, results in a very low incidence of communication with a remarkably decreased amount of Cx43 proteins.

2. Materials and methods

2.1. Cell culture

The rat liver epithelial cell line WB-F344 [16] was cultivated in a

*Corresponding author. Fax: (81)-82-257 5880.
E-mail: khamada@hiroshima-u.ac.jp

[1] Present address: Department of Radiobiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami Ward, Hiroshima 732-0815, Japan.

Abbreviations: Cx43, connexin 43; FRAP, fluorescence redistribution after photobleaching; GJIC, gap junctional intercellular communication; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse transcription; SDS, sodium dodecyl sulfate; SRP, signal recognition particle

0014-5793/01/$20.00
PII: S0014-5793(01)02185-8

modified Eagle's medium supplemented with 7% fetal calf serum and 50 μg/ml gentamicin. The cells were grown at 37°C in an atmosphere of 95% air/5% CO_2.

2.2. Transfection of plasmid

The plasmids used in this study were p3S-PA, p3A-PA, and pSH-PA that expressed RNA3S$^+$, RNA3A$^+$ (antisense RNA3S), and RNAPL$^+$ (polylinker region of pSVneoHMTIIA-PA), respectively, as described previously [3]. WB-F344 cells were plated at 5×10^4 cells/7 cm^2 well in 2 ml of the medium. 24 h after plating, the cells were transfected with 1 μg of plasmid DNA as a lipofection (Gibco/BRL, Gaithersburg, MD, USA). The cells were incubated with the precipitate for 12 h and grown with fresh medium for 24 h. They were thereafter selected in the medium containing 500 μg/ml G418 (Gibco/BRL) for 12 days by changing the medium every 3 days. The drug-resistant cells were named WB-3S, WB-3A, and WB-PL, and employed for the following experiments at 3–4 weeks after transfection. Several clones with p3S-PA were also established by using the ring-cloning method until 3 weeks after transfection, taking advantage of very rapid proliferation of WB-F344 cells (doubling time of approximately 22 h).

2.3. Reverse transcription (RT)-polymerase chain reaction (PCR) of Cx43, GAPDH and RNA3S

RNA was extracted with TRI reagent (molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's protocol. RT and PCR were carried out as described previously [17]. Detection of the cDNA of RNA3S$^+$ was performed by using the primers as described previously [3]. Preliminary experiments demonstrated the submaximal 23 cycles of PCR appropriated to avoid an amplification plateau. The PCR products were analyzed on a 8% polyacrylamide gel in 1× Tris–borate/EDTA buffer. Gels were stained with ethidium bromide and photographed using Polaroid type 665 film.

2.4. Western blotting

Western blot analysis of Cx43 was performed as described previously [18]. Proteins were extracted from the cells by treatment with 2% sodium dodecyl sulfate (SDS) containing 2 mM phenylmethylsulfonyl fluoride, 10 mM iodoacetoamide, 1 μM leupeptin, 1 μM antipain, 0.1 mM sodium orthovanadate, 5 mM sodium fluoride and then sonicated for three 10 s pulses using a probe sonicator (Branson, Danbury, CT, USA). Proteins were separated on 12.5% polyacrylamide gels and transferred to PVDF membranes at 20 V for 16 h. Cx43 was detected using anti-Cx43 rabbit polyclonal antibody (Zymed Co., San Francisco, CA, USA), followed by incubation with horseradish peroxidase-conjugated secondary antibody and detection with the ECL chemiluminescent detection reagent (Amersham Co., Arlington Heights, IL, USA). As an internal control, the same extracts were also probed with an antibody for actin (Sigma Chemical Co., St. Louis, MO, USA). The intensity of the Cx43 protein bands was normalized for that of actin.

2.5. Pulse-chase labeling and immunoprecipitation

Pulse-chase labeling and immunoprecipitation were carried out as described previously [3]. Briefly, WB-F344 cells, grown to 80% confluence in a 25 cm^2 well, were incubated for 30 min in methionine-free medium without serum. They were then pulse-labeled for 2 h in 1 ml of the same medium containing 100 mCi of ^{35}S-protein labeling mix (1175 Ci/mmol; New England Nuclear, Boston, MA, USA). At the indicated times, the monolayer was lysed with 500 μl of phosphate-buffered saline (PBS) containing 1% Triton X-100, 0.6% SDS, 100 U/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 0.5 mg/ml EDTA, 50 μg/ml antipain, 0.5 μg/ml leupeptin, 10 mM sodium fluoride, and 1 mM sodium orthovanadate. An aliquot of lysate (2×10^6 cpm) was incubated with 2 μl each rabbit anti-Cx43 antibody (Zymed Co.) or rabbit anti-actin antibody for 8 h at 4°C. 20 μl of a 50% protein A-Sepharose suspension (Sigma Chemical Co.) was added and incubated for 2 h at 4°C with gentle agitation. The beads were washed six times in the washing buffer and incubated in 20 μl of 2-fold concentrated electrophoresis sample buffer (100 mM Tris–HCl, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, and 20% glycerol) at 100°C for 5 min. After removal of the beads by centrifugation at 15 000 rpm for 1 min, the samples were analyzed in 12.5% SDS–polyacrylamide gels. The gels were fixed with 40% methanol and 10% acetic acid, treated with Enlightening (New England Nuclear), dried, and exposed to X-ray film.

2.6. Fluorescence redistribution after photobleaching (FRAP) assay

The quantitative FRAP assay for GJIC was performed as previously reported [19] with an ACAS Ultima laser cytometer (Meridian Instruments, Inc., Okemos, MI, USA). Cultured cells in 35 mm dishes were incubated with 5,6-carboxyfluorescein diacetate (7 μg/ml in Ca^{2+}/Mg^{2+}–PBS; Molecular Probes, Inc., Eugene, OR, USA) at room temperature for 15 min. Cells were randomly selected under a microscope with a 40× objective lens and photobleached to 20–40% of their original fluorescence intensity. They were then examined for recovery of fluorescence after 4 min to obtain the rates of recovery, which were reported as %/min, where % = % of prebleaching fluorescence. Fluorescence recovery was corrected for fluorescence lost in unbleached controls.

2.7. Immunofluorescent staining of Cx43

Immunostaining of Cx43 in WB-F344 cells was performed with an anti-Cx43 rabbit polyclonal antibody as described previously [16]. Briefly, the cells were incubated with anti-CX43 diluted 1:100 in PBS overnight at 4°C, washed four times with PBS, and then incubated with FITC-conjugated anti-rabbit antibodies (Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA) diluted 1:100 in the bovine serum albumin/Tween/PBS buffer for 1–2 h at room temperature. The cells were washed in the same buffer four times and mounted with poly-aquamount (Polysciences, Niles, IL, USA).

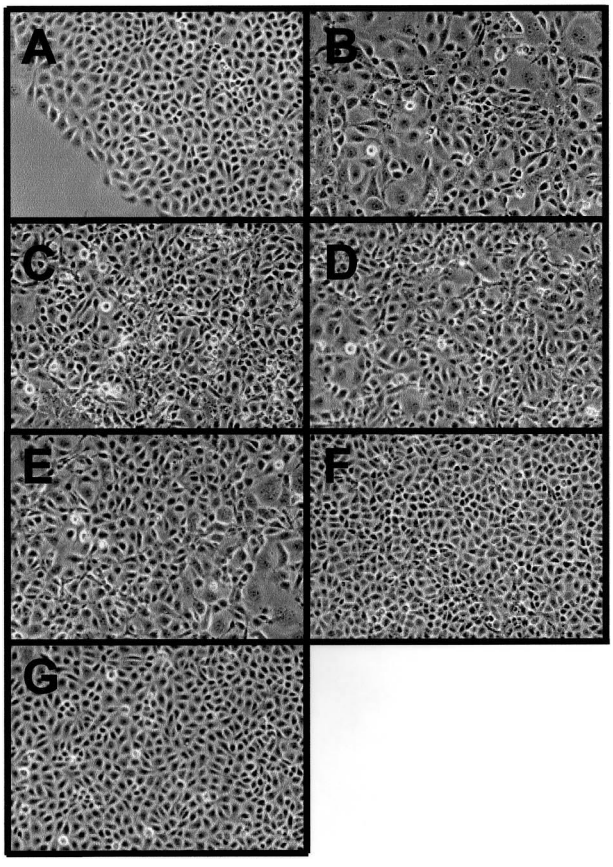

Fig. 1. Morphology of WB-F344 cells with p3S-PA. WB-F344 cells were transfected with p3S-PA, p3A-PA, or pSH-PA and selected with G418 as described in Section 2. (A) Control WB; (B) WB-3S; (C) WB-3S1; (D) WB-3S2; (E) WB-3S3; (F) WB-3A; (G) WB-PL. Photographs were taken with bright-field optics at ×10 magnification at 3–4 weeks after transfection.

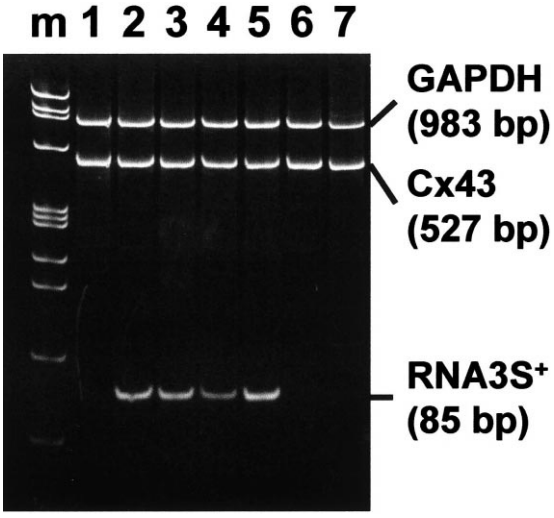

Fig. 2. Detection of Cx43 and RNA3S$^+$ transcripts. RT-PCR assays were carried out to detect Cx43 and RNA3S$^+$ transcripts in WB-F344 cells with p3S-PA. (1) Control WB; (2) WB-3S; (3) WB-3S1; (4) WB-3S2; (5) WB-3S3; (6) WB-3A; (7) WB-PL. The PCR product of GAPDH mRNA was used as an internal marker. Molecular weight marker (ϕX174/HaeIII digest) is also shown in the m lane.

3. Results

3.1. Morphological alteration of WB-F344 cells with p3S-PA

The previous study demonstrated that RNA3S$^+$ expressed from p3S-PA altered rat 3Y1 cells morphologically [3]. To obtain a transforming RNA producing cell line, WB-F344 cells were transfected with p3S-PA and selected with G418. The established line with p3S-PA was named WB-3S. Similarly, those with p3A-PA and pSH-PA were named WB-3A and WB-PL, respectively. Of those lines, WB-3S was morphologically much more spindle-shaped and showed a criss-cross growth pattern at 3–4 weeks after transfection (Fig. 1). Three subclones, WB-3S1, WB-3S2, and WB-3S3, which were derived from parental WB-3S, also exhibited a growth pattern similar to that of WB-3S. On the other hand, control WB, WB-3A, and WB-PL grew in uniform monolayers of polygonal cells and exhibited a clear contact-inhibited growth pattern. This result suggests that RNA3S$^+$ has an ability to alter normal rat epithelial cells morphologically.

3.2. Transcription and translation of Cx43 in WB-F344 cells with p3S-PA

The levels of Cx43 and RNA3S$^+$ mRNAs were estimated by using RT-PCR assays. The PCR products of Cx43 exhibited almost the same band intensities in all lines tested and those of GAPDH used as an internal marker the same (Fig. 2), when the intensities were determined by densitometric scanning of the DNA bands (data not shown). The PCR products of RNA3S$^+$ were clearly detected in the cells with p3S-PA, displaying the same DNA length as shown previously [3]. It is evident that expression of RNA3S$^+$ does not affect the transcription of Cx43.

Previously, RNA3S$^+$ blocked elongation of the secretory protein translation in reticulocyte lysate, resulting in suppression of the translation [4]. It is thus expected that the Cx43 translation would be affected by the RNA, because the preprotein of Cx43 has a secretory signal peptide [20]. To ask this, we first performed Western blot analysis to monitor the Cx43 protein levels. Cx proteins are able to be phosphorylated and the degree of phosphorylation can be examined by virtue of the mobilities exhibited by the different phosphorylated forms of the proteins on SDS–polyacrylamide gels [16]. As shown in Fig. 3 (upper panel), three major Cx-immunoreactive protein bands were clearly detectable in control WB, WB-3A, and WB-PL. These bands correspond to the following three forms of Cx43: the fastest migrating unphosphorylated form (P_0), the slowest migrating hyperphosphorylated form (P_2), and phosphorylated form (P_1) migrating between P_0 and P_2. Each protein level of P_0, P_1, and P_2 was markedly lower in parental WB-3S and its subclones, WB-3S1, WB-3S2, and WB-3S3, than in the other lines, exhibiting about 60–70% reduction of P_1 appeared most prominently when compared to that of control WB (lower panel in Fig. 3). On the other hand, there was no significant difference in the actin protein levels in all lines tested. In addition, it appeared that expression of RNA3S$^+$ did not alter the Cx phosphorylation patterns.

Next, we measured the levels of Cx43 and actin synthesis by using a pulse-chase labeling and immunoprecipitation method. As shown in Fig. 4, the level of Cx43 synthesis decreased markedly in all the RNA3S$^+$-expressing lines, whereas the level of actin synthesis was not affected. These results indicate that expression of RNA3S$^+$ results in direct inhibition of the Cx43 translation.

3.3. GJIC in the RNA3S$^+$-expressing lines

To examine the ability of WB-3S to communicate via gap junctions, we carried out the FRAP assay that yielded a quan-

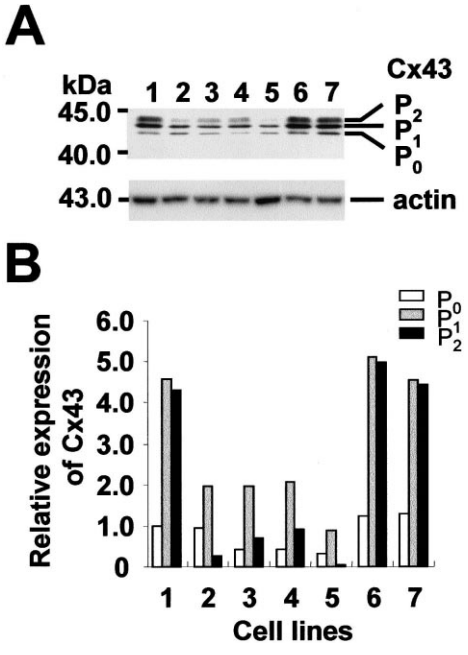

Fig. 3. Detection of Cx43 protein. Western blot analysis (upper panel) and intensities of Cx43 protein bands (lower panel). (1) Control WB; (2) WB-3S; (3) WB-3S1; (4) WB-3S2; (5) WB-3S3; (6) WB-3A; (7) WB-PL. P_0, P_1, and P_2 indicate unphosphorylated, phosphorylated, and hyperphosphorylated Cx43 protein, respectively. As an internal control, the same extracts were also probed with an antibody for actin. The intensities of Cx43 bands were quantitated in help of the Desk scan II and software of NIH image 1.55f.

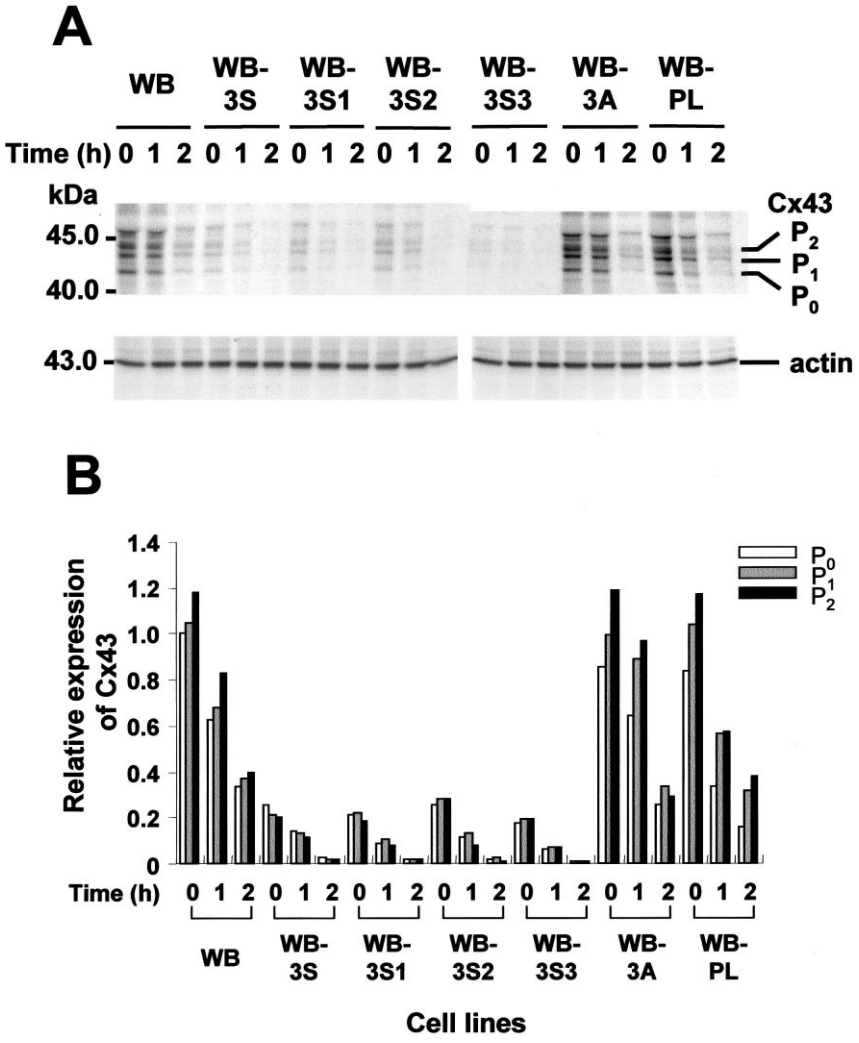

Fig. 4. Pulse-chase analysis of Cx43 turn-over in WB cell lines. Cultures were labeled for 2 h with ^{35}S-protein labeling mix (100 μCi/ml) and chased for 0–2 h in normal media. All cultures were immunoprecipitated with anti-Cx43 antibody or anti-actin antibody and then analyzed by SDS–PAGE and fluorography.

titative estimation of GJIC. Fig. 5A shows the representative of digitalized images performed in control WB and WB-3S. This shows that the fluorescence recovery was markedly inhibited for each selected cell of WB-3S. As summarized in Fig. 5B, GJIC was remarkably affected in all the RNA3S$^+$-expressing cells, reducing by 62–77% of recovery when compared to that in control WB. Additionally, expression of RNA3A$^+$ and RNAPL$^+$ did not inhibit GJIC.

We further examined localization of Cx43 proteins on the plasma membrane by using an immunostaining method. As shown in Fig. 6, the plaques on the membranes were less evident in the RNA3S$^+$-expressing lines, whereas they were readily apparent in the other. Combined with these results, it is evident that the RNA3S$^+$-induced inhibition of GJIC is due to direct suppression of the Cx translation.

4. Discussion

From the fact that transforming RNA blocks the synthesis of a protein with a secretory signal peptide [4], in this study, we have demonstrated that RNA3S$^+$ has an ability to inhibit GJIC by suppressing the synthesis of Cx43 protein. The RNA3S$^+$-expressing WB-F344 cells, as shown in their altered morphology, appeared to grow in a non-contact-inhibited fashion than the non-RNA3S$^+$-expressing cells which exhibited clear contact-inhibited growth. With the normal turn-over of Cx due to a relative short half life, the decrease in GJIC might be the result of a decrease in the replacement of new gap junctions and the altered phosphorylation of Cx43. Expression of Cx43 mRNA was not affected at all by RNA3S$^+$. On the other hand, the decreased levels of Cx43 protein were found to be due to direct suppression of the Cx translation. Additionally, it appeared that Cx protein phosphorylation was not changed by the RNA. Moreover, these results are consistent with the previous observations that expression of RNA3S$^+$ resulted in morphological transformation of rat 3Y1 cells and suppression of the fibronectin protein translation [3]. Taken together, we conclude that transforming RNA suppresses directly the Cx43 translation and thereby inhibits GJIC. Therefore, this is the first demonstration that GJIC is inhibited by the action of a non-coding transcript defined as a novel transforming agent.

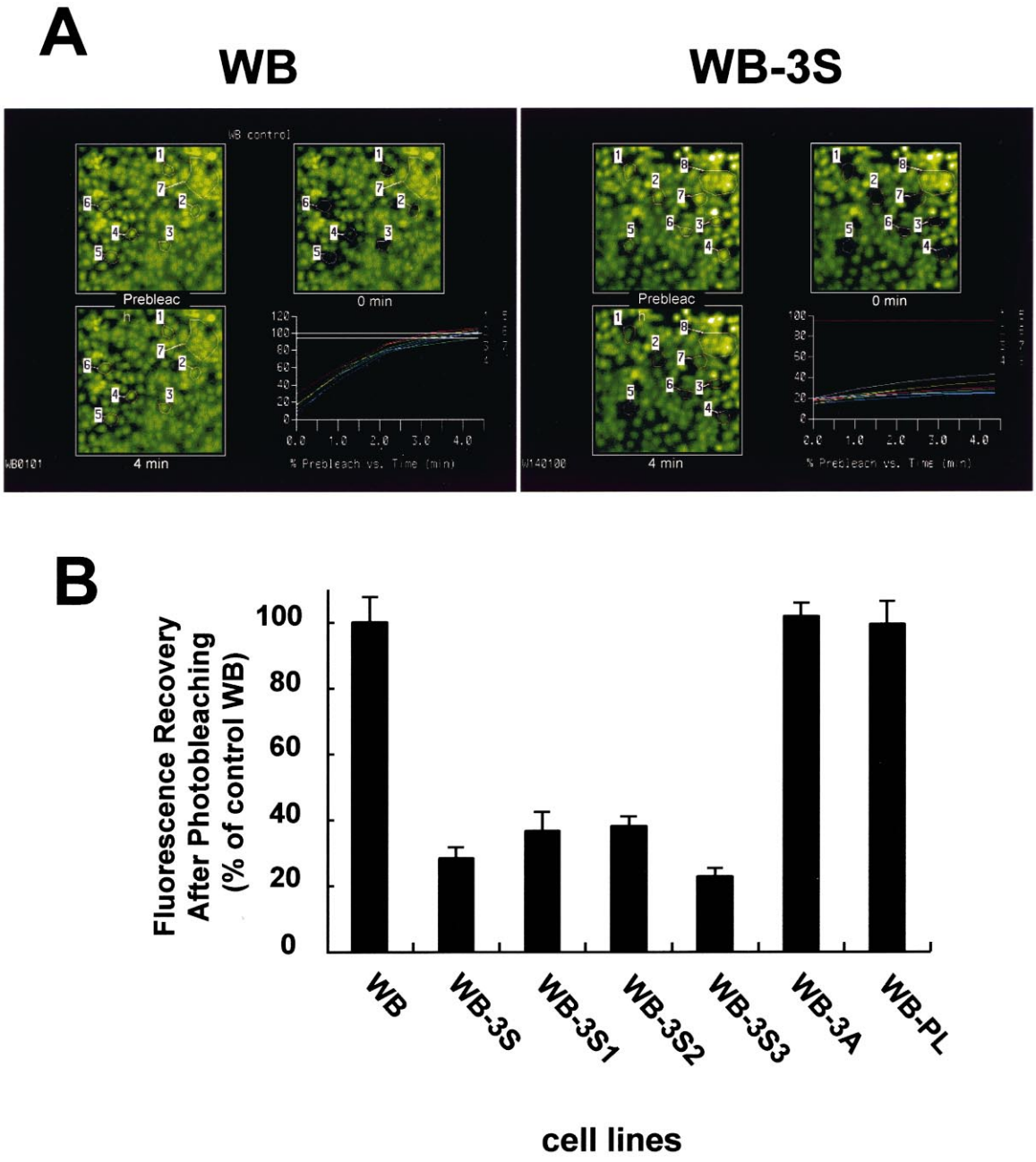

Fig. 5. Quantitative estimation of GJIC. (A) Digitalized images in control WB and WB-3S. GJIC in WB-F344 cells expressing RNA3S^{+} was measured quantitatively by using the FRAP technique as described in Section 2. The images are shown before (prebleach), immediately after (0 min) and 4 min after photobleaching of selected cells. Plots show the percentage of fluorescence recovery vs. time for each selected cell. Data were collected for the background loss of fluorescence in an area with isolated cells (WB, area 7; WB-3S, area 8). (B) Fluorescence recovery. The recovery in WB-F344 cells with each plasmid is compared to that in control WB. Values represent mean ± S.D. ($n = 20$–23).

It is known that during carcinogenesis an initiated cell ultimately loses its ability to perform homologous or heterologous GJIC, having fewer and smaller gap junctions with less Cx proteins [1,21–24]. In addition, many tumor promoting chemicals, transforming agents, including oncogene products, are associated with the disruption of GJIC, though multiple mechanisms [16,17,19,25–33]. Overexpression of the c-Ha-ras oncogene in WB-F344 cells partially reduced GJIC. However, v-myc overexpression did not affect GJIC. Interestingly, co-expression of the two resulted in nearly complete inhibition of GJIC and in the formation of highly malignant cells [33]. Reduction of GJIC is, in and of itself, an apparent necessary but insufficient factor to bring about growth in soft agar or tumorigenesis in vivo. There seems to be a requirement for sufficient reduction of GJIC as shown by a dose-dependent activation of the c-Ha-ras protein and tumorigenesis associated with the rat liver epithelial cells [34,35]. Moreover, even with complete inhibition of GJIC caused by a mutation [36,37], growth in soft agar and tumorigenesis of the rat liver epithelial cells (WB-AB-1) was extremely low. The implication is that it must be accompanied by an induced mitogenic signal transducting system.

We have demonstrated that transforming RNA was capable of inducing several phenotypes leading to malignancy such as

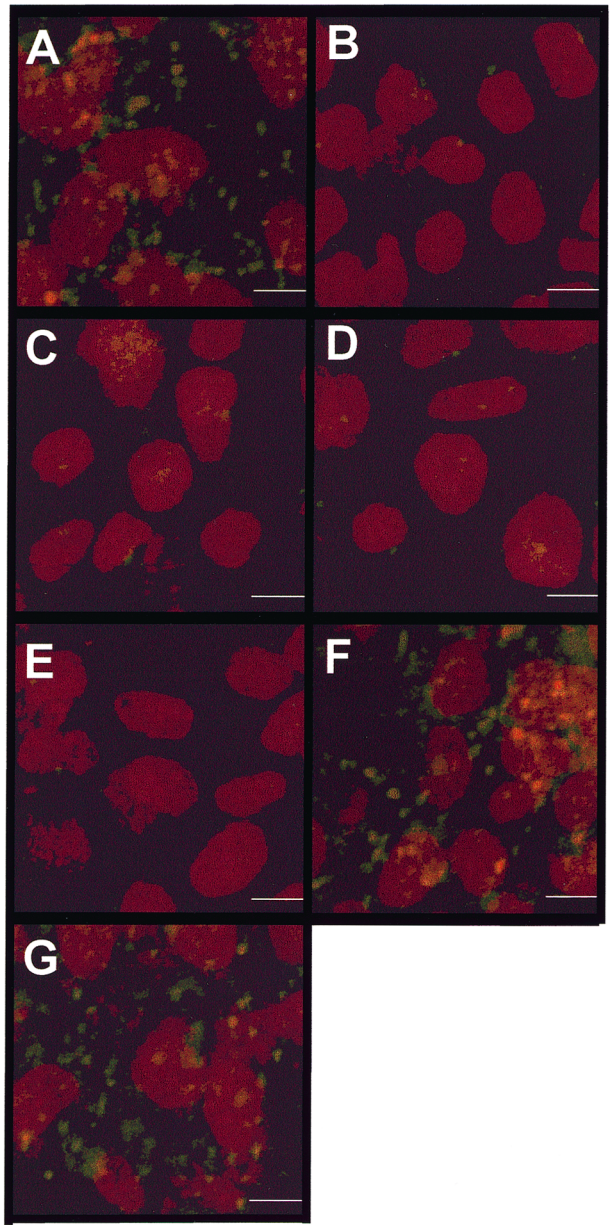

Fig. 6. Immunofluorescent staining of Cx43 proteins. Immunostaining of Cx43 in WB-F344 cells was carried out as described in Section 2. (A) Control WB; (B) WB-3S; (C) WB-3S1; (D) WB-3S2; (E) WB-3S3; (F) WB-3A; (G) WB-PL. Pictures were taken at ×40 magnification. Size bar = 10 μm.

an altered morphology, an enhanced growth rate, a decreased amount of extracellular matrix proteins, and a disrupted GJIC. These alterations are only due to the ability of the RNA to suppress the secretory protein synthesis. These findings suggest that the RNA, by itself, may create a cellular condition prerequisite for malignant transformation. Of these early events in the multistep, multi-mechanism process of carcinogenesis, immortalization is essential [38–42]. It will be of interest to investigate whether transforming RNA might affect a certain oncogene which activates telomerase in normal human cells.

Acknowledgements: This work was supported in part by a Grant-in-Aid for scientific research (08878125) from the Ministry of Education, Science, and Culture, Japan (to K.H.), and by the National Institute of Environmental Health Sciences grant (NIEHS 5 PA 42ES04911-10) (to J.E.T.).

References

[1] Trosko, J.E. and Ruch, R.J. (1998) Front. Biosci. 3, D208–D236.
[2] Yamasaki, H. and Naus, C.C. (1996) Carcinogenesis 17, 1199–1213.
[3] Hamada, K. (1997) Mol. Carcinog. 20, 175–188.
[4] Hamada, K., Kumazaki, T. and Satoh, S. (1999) J. Biol. Chem. 274, 15786–15796.
[5] Schlenstedt, G., Gudmundsson, G.H., Boman, H.G. and Zimmermann, R. (1990) J. Biol. Chem. 265, 13960–13968.
[6] Wiech, H., Klappa, P. and Zimmerman, R. (1991) FEBS Lett. 285, 182–188.
[7] Walter, P. and Johnson, A.E. (1994) Annu. Rev. Cell. Biol. 10, 87–119.
[8] Loewenstein, W.R. and Kanno, Y. (1966) Nature 209, 1248–1249.
[9] Loewenstein, W.R. (1979) Biochim. Biophys. Acta 560, 1–65.
[10] Watton, S.J. and Downward, J. (1999) Curr. Biol. 9, 433–436.
[11] White, T.W., Bruzzone, R. and Paul, D.L. (1995) Kidney Int. 48, 1148–1157.
[12] Lee, S.W., Tomasetto, C. and Sager, R. (1991) Proc. Natl. Acad. Sci. USA 88, 2825–2829.
[13] Hirschi, K.K., Xu, C.E., Tsukamoto, T. and Sager, R. (1996) Cell. Growth Differ. 7, 861–870.
[14] Mesnil, M., Piccoli, C. and Yamasaki, H. (1997) Cancer Res. 57, 2929–2932.
[15] de Feijter-Rupp, H.L., Hayashi, T., Kalimi, G.H., Edwards, P., Redpath, J.L., Chang, C.C., Stanbridge, E.J. and Trosko, J.E. (1998) Carcinogenesis 19, 747–754.
[16] Matesic, D.F., Rupp, H.L., Bonney, W.J., Ruch, R.J. and Trosko, J.E. (1994) Mol. Carcinog. 10, 226–236.
[17] Hayashi, T., Hasler, C.M., Oh, S.Y., Madhukar, B.V., Chang, C.C. and Trosko, J.E. (1996) In Vitro Toxicol. 9, 3–17.
[18] Nomata, K., Kang, K.S., Hayashi, T., Matesic, D., Lockwood, L., Chang, C.C. and Trosko, J.E. (1996) Cell. Biol. Toxicol. 12, 69–78.
[19] Wade, M.H., Trosko, J.E. and Schindler, M. (1986) Science 232, 525–528.
[20] Yeager, M. and Gilula, N.B. (1992) J. Mol. Biol. 223, 929–948.
[21] Weinstein, R.S., Merk, F.B. and Alroy, J. (1976) Adv. Cancer Res. 23, 23–89.
[22] Loewenstein, W.R. (1981) Physiol. Rev. 61, 829–913.
[23] Yamasaki, H. (1990) Carcinogenesis 11, 1051–1058.
[24] Cesen-Cummings, K., Fernstrom, M.J., Malkinson, A.M. and Ruch, R.J. (1998) Carcinogenesis 19, 61–67.
[25] Trosko, J.E. and Chang, C.C. (1988) in: Banbury Report 31: Carcinogen Risk Assessment: New Directions in the Qualitative and Quantative Aspects (Hart, R.W. and Hoerger, F.G., Eds.), pp. 139–197, Cold Spring Harbor, NY.
[26] Klaunig, J.E., Ruch, R.J., Weghorst, C.M. and Hampton, J.A. (1990) In Vitro Toxicol. 3, 91–107.
[27] Trosko, J.E., Chang, C.C., Madhukar, B.V. and Klaunig, J.E. (1990) Pathobiology 58, 265–278.
[28] Bukauskas, F.F., Kempf, C. and Weingart, R. (1992) Exp. Physiol. 77, 903–911.
[29] Kalimi, G.H., Hampton, L.L., Trosko, J.E., Thorgeirsson, S.S. and Huggett, A.C. (1992) Mol. Carcinog. 5, 301–310.
[30] Ruch, R.J. (1994) Ann. Clin. Lab. Sci. 24, 216–231.
[31] Kang, K.S., Wilson, M.R., Hayashi, T., Chang, C.C. and Trosko, J.E. (1996) Environ. Health Perspect. 104, 192–200.
[32] Shiokawa-sawada, M. et al. (1997) J. Bone Miner. Res. 12, 1165–1173.
[33] Hayashi, T., Nomata, K., Chang, C.C., Ruch, R.J. and Trosko, J.E. (1998) Cancer Lett. 128, 145–154.
[34] de Feijter, A.W., Ray, J.S., Weghorst, C.M., Klaunig, J.E., Goodman, J.I., Chang, C.C., Ruch, R.J. and Trosko, J.E. (1990) Mol. Carcinog. 3, 54–67.

[35] de Feijter, A.W., Trosko, J.E., Krizman, D.B., Lebovitz, R.M. and Lieberman, M.W. (1992) Mol. Carcinog. 5, 205–212.
[36] Jou, Y.S. et al. (1993) Mol. Carcinog. 8, 234–244.
[37] Oh, S.Y., Dupont, E., Madhukar, B.V., Briand, J.P., Chang, C.C., Beyer, E. and Trosko, J.E. (1993) Eur. J. Cell. Biol. 60, 250–255.
[38] Newbold, R.F., Overell, R.W. and Connell, J.R. (1982) Nature 299, 633–635.
[39] Land, H., Chen, A.C., Morgenstern, J.P., Parada, L.F. and Weinberg, R.A. (1986) Mol. Cell. Biol. 6, 1917–1925.
[40] Hurlin, P.J., Maher, V.M. and McCormick, J.J. (1989) Proc. Natl. Acad. Sci. USA 86, 187–191.
[41] Reddel, R.R. (2000) Carcinogenesis 21, 477–484.
[42] Trosko, J.E., Chang, C.C., Wilson, M.R., Upham, B., Hayashi, T. and Wade, M. (2000) Methods 20, 245–264.