Inhibition of Lung Epithelial Cell Proliferation by Hyperoxia

Posttranscriptional Regulation of Proliferation-related Genes

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Abstract

The alveolar surface of the lung is a major target for oxidant injury. After injury, repair of the alveolar epithelium is dependent on the ability of epithelial type 2 (T2) cells to proliferate. The regulation of T2 cell proliferation and the effect of reactive oxygen (O_2) species on this lung cell proliferation have not been well defined. To investigate this process we focused on the regulation of two late cell cycle genes, histone and thymidine kinase, in T2 cells and fibroblasts exposed in vitro to varying periods of hyperoxia (95% O_2). Hyperoxia for 24 to 48 h arrested cell proliferation in a SV40T-immortalized T2 cell line we have developed and in primary and SV40T-immortalized lung fibroblasts. Despite the cessation of proliferation, histone and TK mRNA continued to be expressed at high levels; mRNA half-lives were markedly prolonged but neither protein was translated. Thus proliferation arrest induced by hyperoxia was associated with posttranscriptional control of at least two late cell cycle–related genes. This form of proliferation arrest is also seen when primary and SV40T-T2 cells but not fibroblasts are serum deprived, suggesting that T2 cells in vitro may be uniquely sensitive to alterations in their redox state and that these alterations in turn affect translational control of a subset of proliferation-related genes. (*J. Clin. Invest.* 1992. 90:1812–1818.) Key words: type 2 cells • cell cycle • oxidant injury • histone • SV40T-immortalized cells

Introduction

Reactive (O_2) species are being increasingly implicated in the initiation and progression of a variety of diseases and in the regulation of a number of important biological process (1). The lung is a major target for oxidant injury since, in addition to reactive O_2 species generated by inflammation and by drugs and chemotherapeutic agents that concentrate in the lung, many forms of pulmonary diseases require O_2 therapy, which adds to the oxidant burden of the lung (1, 2).

Numerous animal studies have described the acute and chronic effects of O_2 on the pulmonary alveolus. The two cell types that compose the alveolar epithelium, type 1 (T1)[1] and type 2 (T2) cells, may not be identically sensitive to oxidants. Although it is difficult to assess histological evidence of injury in the elongated T1 cell, ultrastructural changes are evident in T1 cells of rats exposed to 85% O_2 for 7 d, yet T2 cells are able to proliferate (3, 4). The alveolar epithelium, 95% of which is covered by T1 cells, is completely destroyed in monkeys exposed to 100% O_2 for 4 d. (4). Repair of damaged epithelium is dependent on the ability of T2 cells to initiate proliferation, providing additional cells that have the potential to undergo transition into T1 cells, thereby replacing injured alveolar epithelial cells (5). As noted above, T2 cells have been shown to proliferate in 85% O_2 exposure (6), but T2 cell proliferation does not occur during exposure to 100% O_2 in rats (4). It is generally felt that interference with the T2 cell proliferative response delays the alveolar repair process, allowing proliferation of fibroblasts (FIB) in the interstitium, leading to the development of diffuse interstitial fibrosis (7, 8).

Despite the importance of the proliferative response of T2 cells to repair of alveolar injury, the mechanisms that regulate epithelial repair and the possible effects of oxidants on this process have not been well defined. In approaching this question we have investigated the effect of hyperoxia on T2 cell proliferation. Because T2 cells isolated from the adult rat lung proliferate to a limited degree in culture we used a T2 cell line that we have developed by transfection of primary T2 cells with a transformation-defective mutant of simian virus SV40 large T antigen (SV40T-T2) cells (9). We have previously shown that these cells regulate some aspects of proliferation in a fashion similar to primary T2 cells (10). Progression through the cell cycle is normally coupled to a time-dependent induction of proliferation-related genes (11, 12). In this work, we have focused on regulation of expression of two of these genes, histone and thymidine kinase (TK), in SV40T-T2 cells exposed to hyperoxia. These genes are of particular interest as they are induced at the G1/S boundary, and several investigators have reported that inhibition of cell proliferation by reactive O_2 species is characterized by arrest of cells late in the cell cycle (13, 14). For this study, results were compared with a similarly produced SV40T lung fibroblast (SV40T-FIB) cell line and to primary lung FIB.

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1. *Abbreviations used in this paper:* FIB, fibroblasts; GSSG, oxidized glutathione; IRE, iron response element; SV40T-T2, simian virus SV40 large T element; SV40T-FIB, SV40T lung fibroblast antigen cells; T1, type 1 alveolar epithelial cells; T2, type 2 alveolar epithelial cells; TK, thymidine kinase; TMP, thymidine monophosphate.

We have found that exposure of SV40T-T2 cells to hyperoxia (95% O_2) induces arrest of cell proliferation within 24 to 48 h. Despite the block to proliferation, cells exposed to hyperoxia continue to express high levels of mRNA for histone and TK. However, these mRNAs are not translated, as no newly synthesized histone proteins and no TK activity can be detected in hyperoxic cells. In addition, histone and TK mRNAs appear to be more stable than in the control cells. This pattern of histone and TK gene expression in hyperoxic T2 cells is also found in both primary FIB and SV40T-FIB exposed to 95% O_2. It also mimics the pattern we have previously reported in primary T2 cells and SV40T-T2 cells whose proliferation had been arrested by serum deprivation (9, 10).

These data indicate that in both epithelial and mesenchymal lung cells hyperoxia induces arrest of proliferation by a mechanism that involves posttranscriptional regulation of the expression of at least two growth-related genes. Primary and immortalized lung epithelial cells arrest proliferation in response to serum deprivation by a similar posttranscriptional mechanism, whereas neither primary nor SV40-immortalized lung FIB respond to serum deprivation in this fashion. Our results suggest that alveolar T2 cells may control proliferation during hyperoxia and serum deprivation through alterations of their redox state, which affects posttranscriptional regulation of expression of a subset of proliferation-related genes.

Methods

Cells and cell culture conditions. Immortalized T2 cells were derived from isolated neonatal T2 cells by transfection with the SV40-T antigen gene as previously described (9). Primary lung FIB were isolated from 10-d-old rats by collagenase digestion of lung minces, as previously described (10). Immortalized lung FIB were obtained by transfection of primary neonatal lung FIB with the SV40T antigen using the same procedure as described for the establishment of SV40T-T2 cell line (9). Using the PZIPNeoSVXI retroviral vector, freshly isolated lung FIB were infected with the cell-free retroviral supernatants of the psi producer cell line. After 48 h, G418-resistant colonies were selected by growth in medium containing 1 mg/ml of G418. When distinct colonies were visible, they were isolated by trypsinization inside glass cloning cylinders and subcultured. Colonies were expanded into cell lines and one of them was chosen for the studies described here. This cell line will be referred to as SV40T-FIB.

Cells were grown in Earle's MEM supplemented with 4 mM glutamine, 50 U of penicillin/ml, 50 μg of streptomycin/ml, and 10% FBS. For control conditions, cells were cultured in a 5% CO_2/95% air atmosphere at 37°C. For hyperoxic conditions, they were cultured in 5% CO_2/95% O_2 atmosphere at 37°C.

Proliferation studies. Cells were plated at a density of 2×10^4 cells per 35-mm culture dish. 3 d later the medium was changed, and the cells were placed either in control atmosphere or in hyperoxia for various times from 24 to 72 h. Cell number was determined by harvesting the cells with trypsin-EDTA and counting them in triplicate counts using a hemocytometer.

For autoradiography of labeled nuclei, cells were incubated in media with 2 μCi/ml of [^{3}H]methyl-thymidine (60–70 Ci/mmol) (10). After 24 h cells were washed several times with cold PBS, fixed with methanol, air-dried, and coated with NTB-2 liquid emulsion (Eastman Kodak Co., Rochester, NY). 24 h later the plates were developed with Kodak developer D-19 and fixed with Kodak Rapid-Fix. After staining with Giemsa, an average of 300 nuclei in random fields of cells were counted for each experimental condition, at ×400.

RNA isolation and analysis. Total cellular RNA was isolated as previously described (10). 15 μg of RNA were fractionated by electrophoresis through 1% agarose gels and blotted onto nylon membranes (Stratagene Inc., La Jolla, CA). The blots were prehybridized and hybridized to ^{32}P-labeled probes, washed, and exposed to film as previously described (10). The probes were generated by labeling plasmid inserts with alpha-[^{32}P]dCTP using random oligonucleotide priming. The murine histone H3.2 gene was provided by Dr. W. Marzluff, University of Florida, Tallahassee, FL. The thymidine kinase probe was a 1-kb fragment of the rat TK cDNA provided by Dr. A. Murphy, Stanford University, Stanford, CA. The actin probe was a 1.5-kb cDNA fragment of rat beta-actin gene obtained from Dr. S. Farmer, Boston University School of Medicine, Boston, MA.

Histone protein synthesis. For these experiments, cells were plated as described in four dishes. At the time of the study, three dishes were used for cell counts and one was used for histone protein synthesis. Cells were washed and preincubated in lysine-free MEM for 20 min at 37°C, followed by labeling for 1 h at 37°C with [^{3}H]lysine (100 μCi/mmol) in fresh lysine-free MEM. Labeled cells were washed with cold PBS and lysed in 10 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 0.5% Nonidet P-40, and nuclei were recovered by centrifugation. Nuclear proteins were solubilized in Laemmli buffer; the volume of solubilization buffer used was adjusted to the number of cells determined at the beginning of the labeling experiment. Equal volumes of solubilized proteins were loaded in each lane and analyzed on 18% SDS-polyacrylamide gels, as previously described (9). Labeled proteins were visualized by fluorography.

TK activity. The TK activity was assessed as previously described (9); the only modification was that the results were expressed per cell number as for histone protein synthesis. Briefly, after washing with PBS, the cells were scraped in 0.2 ml of extraction buffer (25 mM Tris-HCl, pH 7.8, 1.6×10^{-5} M thymidine, 10 mM KCl, 1 mM $MgCl_2$, 2 mM dithiothreitol, 5 mM ATP), and sonicated twice for 5 s at maximum power. After centrifugation for 15 min in an Eppendorf centrifuge, 60 ml of the supernatant were mixed with 20 ml of TK reaction buffer (600 mM Tris-HCl pH 7.8, 20 mM ATP, 5 mM NaF, 30 mM creatine phosphate, 12 U creatine kinase/ml, 0.1 mCi [^{3}H]thymidine/ml), and incubated at 37°C after intervals from 10 to 30 min; 25 μl samples of the reaction were mixed with 7.5 μl of 25 mM EDTA and spotted onto DEAE filters. After drying, the filters were washed three times with 1 mM ammonium formate, once with water, and once with ethanol. Filters were then digested in Soluene and the radioactivity bound to the filters was determined in a liquid scintillation counter.

Results

Effects of hyperoxia on cell proliferation. Exposure of SV40T-T2 cells to hyperoxia led to a rapid inhibition of cell proliferation. As shown in Fig. 1 *A*, no increase in cell number was observed after 24 h in 95% O_2. Proliferation arrest was reversible since cells removed from hyperoxia resumed proliferation, although the rate at which recovery occurred depended on the length of prior exposure. After 24 h of hyperoxia, cells resumed proliferation within 24 h. After 48 h in 95% O_2, there was a 48-h delay before the cell number began to increase. The hyperoxia-induced block of proliferation was associated with a progressive decrease in the percent of labeled nuclei (Fig. 2 *A*). The labeling index returned to normal levels within 24–48 h of return to normoxia. SV40T-FIB displayed a similar pattern of proliferation arrest in response to hyperoxia, which was reversed on removal from hyperoxia (see Figs. 1 *B* and 2 *B*). However, lung FIB appeared more resistant to hyperoxia, requiring a longer exposure than SV40T-T2 cells before proliferation was arrested. Similar results were obtained in primary lung FIB (data not shown).

Effect of hyperoxia on proliferation-related gene expression in SV40T-T2 cells. As shown in Fig. 3, hyperoxic cells continued to express histone and TK mRNA actually at a level higher

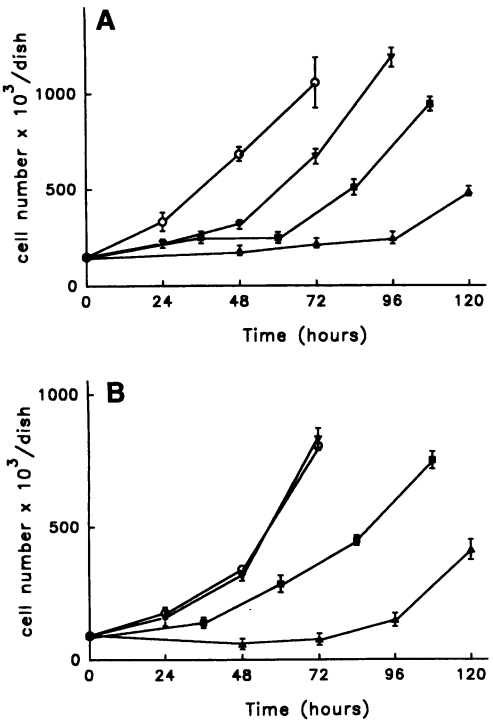


Figure 1. Effect of hyperoxia on cell proliferation. (*A*) SV40T-T2 cells; (*B*) SV40T-FIB. Cell number measured at 24-h intervals in cells exposed to 21% O_2 (control) (○) or 95% O_2 for 24 (▼), 36 (■), or 48 h (▲), and then returned to control conditions.

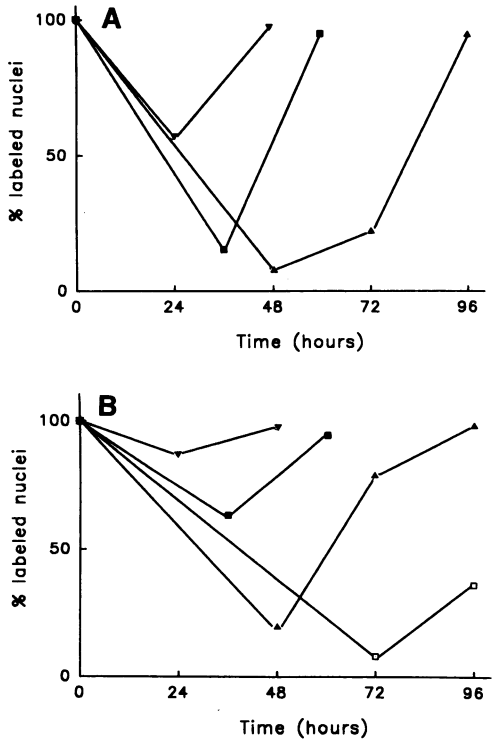


Figure 2. Effect of hyperoxia on labeling index. (*A*) SV40T-T2 cells; (*B*) SV40T-FIB. [^{3}H]Thymidine labeling index in cells exposed to 95% O_2 for 24 (▼), 36 (■), 48 (▲) or 72 h (□), and then returned to control conditions (21% O_2).

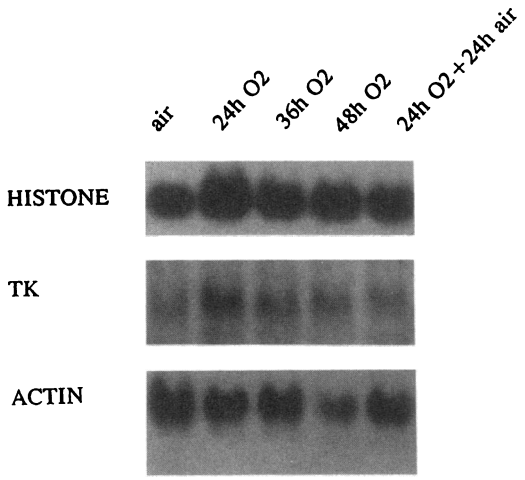
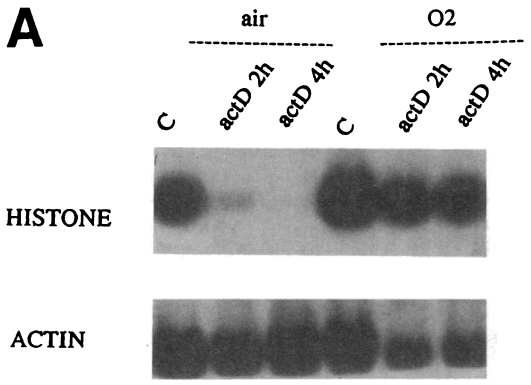


Figure 3. Histone and TK mRNA in SV40T-T2 exposed to 95% O_2. Northern blot of SV40T-T2 total RNA in control cells grown at 21% O_2 and cells in 95% O_2 for 24 to 48 h or cells returned to control conditions (21% O_2) for 24 h. Densitometric analysis of mRNA compared with actin mRNA shows that histone mRNA is 190 and 202% of control at 24 and 48 h, respectively, but returns to control values after 24 h at 21% O_2. TK mRNA is 224 and 234% of control at 24 and 48 h and returns to control values after 24 h at 21% O_2.

than in control conditions despite the fact that after 24 h of hyperoxia they had stopped dividing and the labeling index had decreased. When hyperoxic cells were removed from 95% O_2 after 24 h and cultured at 21% O_2, they rapidly resumed proliferation as indicated in Figs. 1 and 2, but histone and TK mRNA actually decreased. Histone and TK mRNA stability was analyzed by incubating the cells with actinomycin D (5 μg/ml) for periods from 2 to 8 h. In cells cultured under control conditions the amount of histone mRNA was considerably reduced after 2 h and was undetectable after 4 h. In contrast, in cells exposed to hyperoxia for 24 h, the levels of histone mRNA appeared constant in the presence of actinomycin (Fig. 4 *A*) over 4 h, suggesting a marked change in histone mRNA stability. The same results were obtained with the study of TK mRNA; TK mRNA half-life was in the 4–8-h range under control conditions and was markedly prolonged in cells exposed to hyperoxia (Fig. 4 *B*). We next evaluated histone and TK mRNA translation in hyperoxic cells. As indicated in Fig. 5, histone synthesis correlated with the proliferative state of the cells. After 24 h of hyperoxia, newly synthesized histones were barely detectable. This dramatic decrease in histone protein synthesis was not the result of a global shutdown of protein synthesis. Indeed, after this short duration of culture in hyperoxia, the cells synthesized a large number of proteins that were similar to those synthesized in control cultures (data not shown). Similar results were obtained for TK activity. After 36 h of hyperoxia, TK activity fell to a very low level: TK activity of control (normoxic) cells was 33.7 ± 8.4 mmol thymidine monophosphate (TMP)/min per 10^3 cells; TK activity of cells in 95% O_2 for 24 h was 5.1 ± 2.8 mmol TMP/min per 10^3 cells.

Effect of hyperoxia on proliferation-related gene expression in lung FIB. To determine whether this pattern of histone and TK gene expression was present in other lung cells exposed to hyperoxia, we performed similar experiments in both primary and SV40T-FIB. Histone and TK mRNA expression actually



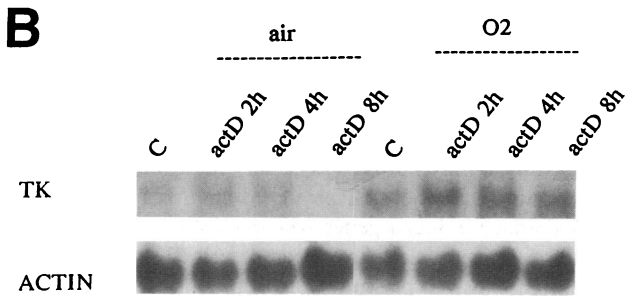


Figure 4. Histone and TK mRNA stability in SV40T-T2 cells. (*A*) Histone mRNA; (*B*) TK mRNA. Northern blots of SV40T-T2 RNA from control cells (21% O_2) and cells cultured in 95% O_2 for 24 h. Cells were incubated with 5 μg/ml actinomycin for time periods indicated. Both histone and TK mRNA half-life was markedly prolonged in 95% O_2.

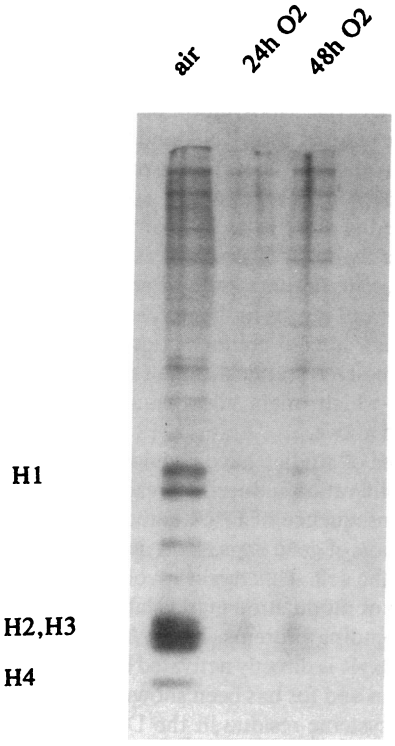


Figure 5. Histone protein synthesis in SV40T-T2 cells. SDS-PAGE gel of [^{3}H]lysine-labeled nuclear proteins from control SV40T-T2 cells and cells exposed to 95% O_2 for 24 or 48 h. Equal volumes of solubilized proteins per cell number were loaded in each lane.

increased in SV40T-FIB (Fig. 6 *A*) and in primary lung FIB during hyperoxia despite the cessation of cell proliferation. This contrasts with mRNA expression in primary FIB (10) and SV40T-FIB (Fig. 6 *B*) during proliferation arrest induced by serum deprivation where histone and TK mRNA were undetectable. No histone protein was synthesized and TK activity was not detected in cells expressing high levels of histone and TK mRNA (data not shown). To confirm the similarities in the mechanisms involved in the posttranscriptional block of histone and TK gene expression in hyperoxic SV40T-T2 cells and SV40T-FIB, we repeated the experiments with actinomycin D in FIB. As shown in Fig. 7 *A*, histone mRNA in control cells was barely detectable after 2 h of treatment and disappeared after 4 h. After 24 h of hyperoxia, histone mRNA half-life was prolonged. TK mRNA stability in SV40T-FIB was similarly increased. Primary FIB behaved in a similar fashion: hyperoxia markedly increased histone mRNA stability (Fig. 7 *B*).

Discussion

We have shown in this study that hyperoxia inhibits proliferation of lung alveolar epithelial cells in a fashion that is associated with altered expression of at least two late cell cycle growth-related genes, histone and TK. This form of proliferation arrest was characterized by constitutive expression of histone and TK mRNAs, stabilization of their mRNAs, and a block to mRNA translation with absence of newly synthesized histone proteins and TK activity. The fact that histone and TK mRNAs actually increased in hyperoxic cells suggests that mRNA degradation slowed in the presence of continued mRNA transcription. Furthermore, we have shown that this form of proliferation arrest is also displayed by both primary and SV40T-immortalized lung FIB whose proliferation had been arrested by hyperoxia.

In most cells, concentrations of histone and TK mRNA are tightly coupled to DNA synthesis (15, 16); they are highest as cells enter S phase and are undetectable when cells leave the cell cycle. In normal conditions, these mRNAs are translated to provide proteins necessary for DNA replication (15, 17–19). The mechanisms involved in the regulation of histone synthesis have been extensively studied. A combination of transcrip-

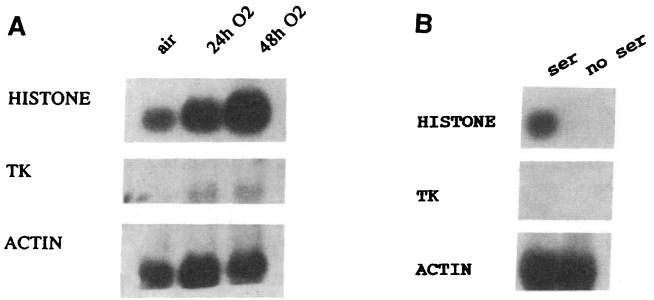


Figure 6. Histone and TK mRNA in SV40T-FIB growth arrested by hyperoxia or by serum deprivation. (*A*) 95% O_2; (*B*) 0% serum. Northern blot of total RNA from SV40T-FIB in control (21% O_2) or 95% O_2 for 24 and 48 h (*A*) and in control (10% FBS) or 0% serum for 24 and 48 h (*B*). Both histone and TK mRNA are expressed at increased levels during proliferation arrest induced by 95% O_2 but are not expressed during proliferation arrest induced by removing serum.

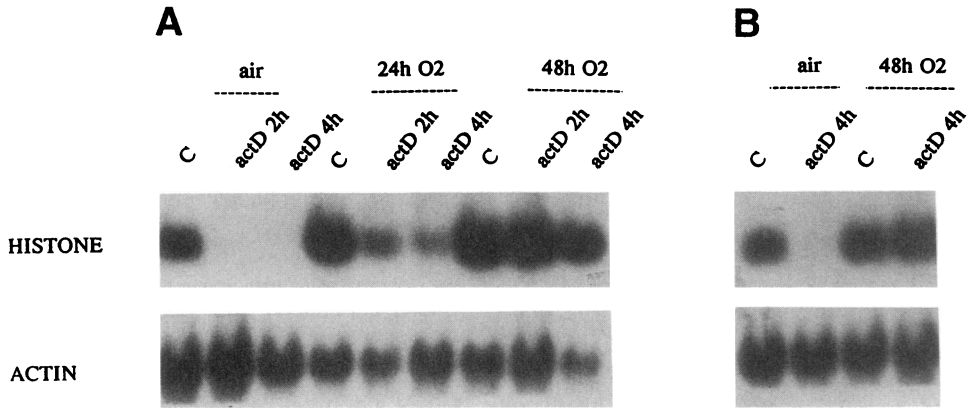


Figure 7. Histone mRNA stability in SV40T-FIB and primary FIB. (*A*) SV40T-FIB; (*B*) primary lung FIB. Northern blots of SV40T-FIB or primary FIB RNA from control cells (21% O_2) and cells grown in 95% O_2 for 24 or 48 h. Cells were incubated with 5 μg/ml actinomycin for time periods indicated. Histone mRNA half-life was markedly prolonged in cells cultured in 95% O_2.

tional and posttranscriptional controls regulate the levels of histone mRNA and ultimately the synthesis of histone proteins. The posttranscriptional control has been shown to occur at two different levels. One acts in the nucleus to process histone mRNA. The second occurs in the cytoplasm to control the half-life of histone mRNA and leads to a degradation of histone mRNA when DNA synthesis is inhibited (15–20).

The results of the experiments described here indicate that hyperoxia initiates a process that alters expression of histone and TK genes at a posttranscriptional level by mechanisms that affect histone and TK mRNA stability but also prevents translation of histone and TK proteins. The effects of hyperoxia mimic the pattern of proliferation inhibition induced when T2 cells are cultured in absence of serum. These similarities and the results of our previous studies exploring the mechanisms of posttranscriptional regulation of histone gene expression in serum-deprived T2 cells lead us to suggest that the translational block of histone and TK genes under hyperoxia may involve mRNA-binding factors that modulate translation and degradation of their mRNA (21). Precedent for this type of gene regulation comes from recent studies showing that proteins which bind to the 5′UTR or 3′UTR of mRNA play an important role in regulation of translation efficiency of genes such as ferritin and transferrin receptor genes (22, 23), protamine gene (24), interferon-beta gene (25), and the creatine kinase gene (26). Furthermore, numerous studies focused on the toxic effects of reactive O_2 species strongly support the involvement of such *trans*-acting factors in hyperoxia-induced growth arrest, these factors being either the products of newly induced genes or modifications of preexisting proteins as discussed below.

There are two major mechanisms by which hyperoxia might affect proliferation-related gene expression; DNA damage and alterations in the redox state of the cell (27, 28). One of the major targets of reactive O_2 species is DNA. It is well known that oxidants can damage DNA, inducing strand breaks, which, if not rapidly and correctly repaired, may interfere directly with the expression of genes involved in the regulation of growth and differentiation (29). The posttranscriptional regulation of histone and TK gene expression we found in both alveolar epithelial and pulmonary FIB cells could have resulted either from induction of new genes coding for mRNA-binding transacting factors or from structural modifications of existing proteins that bind to histone and TK mRNAs. Recently, several DNA damage-inducible transcripts, called growth arrest DNA damage (gadd) genes, have been isolated in mammalian cells (30). These genes are coordinately induced during the growth arrest of cells exposed to DNA-damaging agents, including reactive O_2 species, although their mechanisms of action and the roles they might play in the growth-arrest process have not yet been established. These genes have also been shown to be induced by other growth arrest signals, such as serum deprivation and contact inhibition, suggesting that DNA damage may not be a necessary requirement for hyperoxic-induced arrest of cell proliferation. A number of other DNA damage-induced genes whose role in regulation of cell proliferation is not clear have been described, including metallothioneins, tissue plasminogen activator, and collagenase (31, 32).

The posttranscriptional regulation of histone and TK gene expression may not imply induction of new genes but may be related to oxidant-induced modifications of proteins involved in gene regulation. DNA fragments have been shown to activate poly-(ADP-ribose)-polymerase (33). The targets of poly-ADP ribosylation include a large variety of proteins (34). Recently, human immunodeficiency virus gene induced by ultraviolet irradiation has been reported to involve poly-ADP ribosylation of proteins that regulate expression of other genes at the posttranscriptional level (35). It is therefore conceivable that, after O_2 exposure, poly-ADP ribosylation of specific proteins may modulate their binding to mRNA and may play a role in translational efficiency of the growth-related genes noted in our experiments. Another important consideration relates to the fact that histone proteins are one of the chromosomal proteins that serve as poly-ADP receptors. Poly-ADP ribosylation of histone proteins occurs rapidly after exposure of cells to reactive O_2 species and results in the release of histones from DNA because of their altered conformation (36). This results not only in impaired DNA replication but makes available DNA-binding sites, which might allow transcription of genes such as those noted above.

An increasing number of studies have provided evidence that inhibition of cell proliferation induced by reactive O_2 species is not simply the consequence of DNA damage but may represent active modulation of gene expression induced by altering the redox state of the cell. This might involve both formation of new transcripts or modulation of translation by modification of preexisting binding proteins. In *Escherichia coli*, the transcription factor OxyR is directly activated by oxidation (37). DNA-binding of jun and fos has been shown to be regulated by oxidation of a cysteine residue in the DNA-binding

domains of these proteins (38). In addition, the activity of several metabolic enzymes and the iron response element (IRE)-binding proteins have been found to be sensitive to oxidation (39). The IRE-binding protein, which controls iron-dependent ferritin translation and translation of transferrin receptor mRNA, must be in a fully reduced state for its specific interaction with IRE. Once oxidized, these proteins no longer bind to DNA and thus allow translation to proceed. These studies illustrate mechanisms by which the redox status of the cells may influence gene expression at several steps, including the level of mRNA translation. Indeed, in a recent paper, Schreck et al. (40) presented evidence that reactive O_2 intermediates may be widely used as physiological regulators of gene expression. They have shown that a number of agents known to activate the ubiquitous transcription factor NF-kB all act through a common mechanism involving synthesis of reactive O_2 intermediates.

We have previously shown that when proliferation of either primary or SV40T-T2 epithelial cells is arrested by serum deprivation, expression of several growth-related genes is regulated at the posttranscriptional level in a fashion similar to that reported here for hyperoxia. In contrast, serum-deprived FIB and SV40T-FIB do not continue to express histone and TK mRNAs after proliferation has ceased, indicating that they regulate expression of these genes, as do most other cells, at the level of transcription. Posttranscriptional regulation of proliferation-related genes in T2 cells might relate to their unique in vivo environment. Unlike most cell types, pulmonary epithelial cells are exposed to high levels of environmental O_2 under normal circumstances. Comparative studies of antioxidant systems in various types of lung cells have indicated that alveolar T2 cells have higher levels of glutathione peroxidase than other lung cells (41). Glutathione peroxidase catalyzes the oxidation of GSH by H_2O_2, which leads to the formation of oxidized glutathione (GSSG). The high ratio of GSH to GSSG in cells is largely responsible for the reducing potential of the cytosol (42). It is likely that, in hyperoxic conditions where large amounts of reactive O_2 species including H_2O_2 are produced, the constitutively high level of glutathione peroxidase in T2 cells may result in an altered T2 cell redox buffer state affecting gene expression by any of the mechanisms described above. The metabolic alterations induced by serum depletion may also alter the GSH/GSSG ratio in T2 cells. In support of this concept, Messina and Lawrence (43) have shown that lymphocytes depleted of glutathione are locked in S phase of the cell cycle.

The fact that T2 cells appear to arrest proliferation in a similar fashion during hyperoxia and serum deprivation but that FIB do not suggests that in vitro T2 cells may be uniquely sensitive to alterations of their redox state and that these alterations in turn affect translational control of a subset of proliferation-related genes. Further experiments must now focus on the potential role of reactive O_2 species in this posttranscriptional regulation.

Acknowledgments

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