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Down-regulation of actin genes precedes microfilament network disruption and actin cleavage during p53-mediated apoptosis

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SUMMARY

Inactivation of Simian Virus 40 large T antigen, in cells immortalized with conditional mutants, leads to activation of p53 and apoptosis. We used the mRNA differential display method to identify genes differentially expressed during this process. We found that steady-state levels of mRNA for cytoplasmic actins decreased early during apoptosis. We also showed that, although the steady-state level of the corresponding proteins is not profoundly affected, they are substrates for an interleukin 1- β converting enzyme (ICE)-like protease activated during the process. However, only a very small fraction of actin is pro-

teolysed during the early stages of apoptosis. The microfilament network is affected and non polymerized actin accumulates in apoptotic bodies after the decrease of mRNA levels, but before a significant amount of actin is cleaved. This suggests that down-regulation of actin genes may be involved in microfilament rearrangements during p53-mediated apoptosis.

Key words: Apoptosis, p53, Simian Virus 40, Microfilament, Actin gene, ICE-like protease

INTRODUCTION

Programmed cell death (PCD) is an active process of cellular self-destruction and in most cases serves a biologically meaningful homeostatic function (Kroemer et al., 1995; Thompson, 1995). Apoptosis, which has been defined from morphological considerations (Kerr et al., 1972), is the most often encountered form of PCD, though not the only form (Clarke, 1990; Schwartz et al., 1993). Apoptosis can be induced by a wide range of stimuli. During the subsequent effector phase, the numerous apoptosis inducing stimuli seem to converge onto a few stereotypical pathways and at a certain point, the cells become irreversibly committed to death. During the successive degradation phase, vital structures and functions are destroyed giving rise to the full-blown phenotype of apoptosis (Kroemer et al., 1995). At the nuclear level, apoptosis is typically accompanied by condensation and fragmentation of chromatin and nucleus. Cytoplasmic boiling, a marked convulsion of the cellular surface and the development of pedunculated protuberances that separate to produce membrane-enclosed apoptotic bodies are also seen. In vivo, apoptotic bodies are phagocytosed and digested by adjacent cells or macrophages. The dramatic changes in cell shape suggest a role for the cytoskeleton in this process. Experiments performed with cytochalasins have suggested that actin polymerization is necessary for the formation of apoptotic bodies in the latter stages of HL60 cell apoptosis (Cotter et al., 1992). Other results suggest that blebbing of the cytoplasmic membrane during apoptosis is associated with a deficiency of filamentous actin (Endresen et al., 1995a,b). In the case of adherent cells,

cytoplasmic condensation begins with loss of cell-cell or cell-substrate contacts. In vitro, rounding up of previously flat substrate-attached cells is easily visible under the light microscope and is rapidly followed by an increase in density and a decrease in volume common to nearly all apoptotic cells that can be monitored by flow cytometry (Nicoletti et al., 1991; Swat et al., 1991; Shi et al., 1992; Vayssière et al., 1994).

Genetic studies of PCD in the nematode *Caenorhabditis elegans* provided evidence that PCD involves a cell-intrinsic death program (Ellis et al., 1991). Among the 14 identified genes participating in *C. elegans* developmental PCD, three were found to act during the effector phase: *ced-9* which is a suppressor of PCD and *ced-3* and *ced-4* which are required for PCD. *Ced-9* is homologous to the mammalian *bcl-2* gene family, *ced-4* has no mammalian homologue identified so far and *ced-3* is homologous to the interleukin-1- β -converting enzyme (ICE) gene family encoding cysteine proteases involved in mammalian apoptosis (Kumar, 1995). A role for such proteases in cytoskeleton rearrangements during apoptosis has been suggested (Martin and Green, 1995). Indeed, cleavage of Gas2 (growth arrest specific protein) by ICE-like proteases can lead to the formation of a Gas2 derivative able to trigger changes in actin cytoskeleton and cell morphology (Brancolini et al., 1995). A calpain-mediated proteolysis of the actin-associated protein fodrin has also been reported (Martin et al., 1995). More recently, actin itself has been shown to be a substrate for an ICE-like protease, different from ICE, activated during U937 leukemia cells apoptosis (Mashima et al., 1995) and for ICE itself in in vitro experiments (Kayalar et al., 1996).

It has been previously observed that rodent embryo fibroblasts conditionally immortalized by temperature sensitive mutants of SV40 large T antigen undergo apoptosis at the restrictive temperature (Yanai and Obinata, 1994; Zheng et al., 1994). Apoptosis of these cells in restrictive conditions appears to be mediated by the release of wild-type p53 from the large T antigen as judged by coprecipitation experiments and its ability to induce *mdm-2* (Zheng et al., 1994), *waf1/cip1* (Guénal and Mignotte, 1995) and *bax* (I. G., unpublished results) expression. We also observed that *clusterin* is induced late during the process and that some other genes identified during thymocyte and lymphocyte apoptosis are not induced, showing that the pattern of gene induction is in part specific to the system studied (Guénal and Mignotte, 1995).

In this report we have used the mRNA differential display method to identify novel genes whose expression is modulated during apoptosis. We find that actin genes are down-regulated during apoptosis before any cleavage of actin can be detected. This suggests that gene repression could also be involved in microfilament reorganization during apoptosis.

MATERIALS AND METHODS

Cell lines and cell culture

The REtsAF cell line was isolated at low cell density from a rat embryo fibroblast culture infected with a temperature sensitive mutant (tsA58) of SV40 (Petit et al., 1983). It is immortal at 33°C and undergoes apoptosis upon shift up to 39.5°C (Zheng et al., 1994). REtsAF-Rev1 was derived from REtsAF by selection for proliferation at 39.5°C (Mignotte et al., 1990) and is immortal at both 33 and 39.5°C. Cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum or DMEM/F12 supplemented with 2% Ultrosor G, plus penicillin (100 µg/ml) and streptomycin (100 i.u./ml) under 5% CO₂. The cultures were screened regularly for the absence of mycoplasma.

mRNA differential display

Total cellular RNA was isolated from cells by the method of Chirgwin et al. (1979). The differential display was performed essentially as described by Liang and Pardee (1992). Briefly, total RNA from REtsAF, REtsAF-Rev1 and RELPB grown at 33°C or shifted up to 39.5°C for various times was reverse transcribed, amplified by PCR using T₁₂VC as a 3'-primer and GGGTAACGCC as a 5'-primer and electrophoresed on a sequencing gel. The size of the fragments was estimated by comparison with the end-labeled *MspI* fragment of pBR322 and the band of interest was isolated from the gel as described by Liang et al. (1995), reamplified in the same PCR conditions and cloned using the pCR-Script™ SK(+) cloning kit (Stratagene) according to the manufacturer's instructions.

Northern blotting

Gene expression was assayed by northern blotting analysis. Total RNA (10 µg) was fractionated on a 1% agarose-formamide gel and transferred to nylon membrane (HybondN, Amersham) according to the procedure of Reed and Mann (1985). Probes for β-actin and γ-actin were obtained, respectively, by labeling of the insert of plasmid pSP64-HFβA-3'UT (Kocher and Gabbiani, 1986) and pCR-γA-3'UT, respectively, with [³²P]dCTP by random primers using the Megaprime™ labeling system (Amersham). The amount of RNA blotted for each sample was normalized by hybridization of 18 S rDNA probes to the northern blots. The probe for 18 S rRNA was

labeled with [³²P]dCTP by PCR with 5'ATGCGGCGGCGGT-TATTC3' and 5'GCGACGGGCGGTGTGTA3' as primers. PCR radiolabeling reactions (100 µl) contained 10 ng of an 18 S rDNA genomic PCR product, 100 pmol of primers, 20 nmol each of three nucleotides dATP, dGTP, and dTTP, 2 nmol dCTP, 5 µl of [³²P]dCTP (3,000 Ci/mmol) and 2.5 U of Hi-Taq DNA polymerase (Bioprobe). PCR was carried out for 30 cycles as follows: 94°C for 30 seconds for denaturing; 60°C for 1 minute for annealing; and 72°C for 1 minute for extension, followed by one extension cycle at 72°C for 5 minutes. Hybridization was carried out in 5× SSPE, 0.5% SDS, 5× Denhardt's at 65°C and 20 µg/ml sonicated salmon sperm DNA. Washes were done twice in 2× SSPE, 0.1% SDS at room temperature, once for 15 minutes at 65°C in 1× SSPE, 0.1% SDS and once for 10 minutes at 65°C in 0.1× SSPE, 0.1% SDS.

Actin cleavage assay

Two different protocols were used. In the first experiments, cells were resuspended in PBS-0.5% NP40. Protein concentration of cell extracts was determined by the method of Bradford (1976) and 40 µg of protein were incubated for 30 minutes in this buffer. In subsequent experiments, cells were resuspended in 10 mM Tris-HCl, pH 8.1, and 5 mM DTT and freeze-thawed four times as described by Black et al. (1989). A 100 µg sample of protein was incubated for 2 hours at 37°C in 20 mM Hepes, pH 7.5, 2 mM DTT and 10% glycerol with or without 100 µM YVAD-CMK (N(N-acetyl-tyrosyl-valinyl-alanyl)) (Bachem). For western blotting, after 15% SDS-PAGE electrophoresis, proteins were transferred to 0.45 µm pore size PVDF membrane (Boehringer Mannheim) using a semi-dry blotting apparatus (transfer buffer: 20% ethanol, 48 mM Tris, 39 mM glycine and 0.0375% SDS). After staining with Ponceau S, the membranes were saturated for 1 hour with 5% non-fat dry milk in TBS (50 mM Tris-HCl, pH 7.6, 500 mM NaCl) and incubated overnight at 4°C with the specific anti-actin monoclonal antibody (AC40 or JLA20 from Sigma and Amersham, respectively). Blots were rinsed four times for 7 minutes with TBS-Tween (0.1%) and reacted with peroxidase-conjugated anti-mouse antibody (BioSys) for 1 hour at room temperature. The blots were rinsed for 7 minutes four times with TBS-Tween and revealed with an ECL kit (Amersham). Relative molecular mass was estimated by comparison with the migration of Rainbow™ colored protein markers (Amersham).

Fluorescence microscopy

For indirect fluorescence microscopy, cultured cells were grown on glass coverslips under the conditions described and then fixed with 3.7% paraformaldehyde in PBS for 10 minutes at room temperature, washed twice with PBS and permeabilized with acetone for 3 minutes at -20°C. Fixed cells were washed three times with PBS. The coverslips were then treated with the first antibody diluted in PBS-BSA (3%) for 1 hour at room temperature, washed with PBS-Tween (0.1%) three times and incubated with lissamine rhodamine-conjugated anti-mouse secondary antibody (Jackson Immuno Research) for 40 minutes. DAPI (final concentration: 10 µg/ml, Sigma) was added to the coverslips 5 minutes before the end of incubation and the coverslips were washed twice with PBS-Tween. Experiments in which the first antibody was omitted were also performed as controls.

Alternatively, for detection of actin filaments, cells were fixed and permeabilized as described above and fluorescein-phalloidin (Molecular probe) was used according to the manufacturer's instructions with 2 units/coverslip. Coverslips were then washed twice with PBS-Tween, incubated for 5 minutes in PBS-BSA plus 1 µg/ml propidium iodide (Sigma) or 10 µg/ml DAPI and washed again twice with PBS-Tween.

Coverslips were mounted in glycerol/PBS solution (Citifluor) and cells were examined by epifluorescence with a Leitz DMRB Leica microscope.

RESULTS

The amount of γ -actin mRNA decreases during apoptosis

Although in some systems induction of apoptosis by p53 involves transcriptional activation (Yonish et al., 1995; Attardi et al., 1996), in other cases p53-dependent apoptosis seems to occur in the absence of transcriptional activation of p53-target genes (Caelles et al., 1994; Haupt et al., 1995) which suggests that, in some cells, p53 can mediate apoptosis by repressing certain genes. We have used the mRNA differential display method to isolate genes differentially expressed during apoptosis of cell lines conditionally immortalized by SV40. Among the cDNAs identified on the sequencing gel, one was found in marked lower abundance in apoptotic REtsAF cells but not REtsAF-Rev (Fig. 1). It has been reamplified, cloned and sequenced. The sequence of the insert (211 bp), which was in fact generated by amplification between a T₁₂AC and a T₁₂CC primer (data not shown), was used to search for sequence similarity with the EMBL library. The sequence was found to be identical to the end of the 3'-untranslated region of the rat γ -actin mRNA (nucleotide 1,640 to 1,851 according to Brown et al., 1990) (data not shown). Thus, this plasmid has been named pCR- γ A-3'UT.

The gene encoding β -actin is also down-regulated

In order to determine if the decrease in γ -actin mRNA is specific for this actin isoform or if the other cytoplasmic actin gene displays a similar behavior, we have performed northern blot experiments with β -actin and γ -actin specific probes made, respectively, from the pSP64-HF β A-3'UT and pCR- γ A-3'UT plasmid (Fig. 2). As the mRNAs for β - and γ -

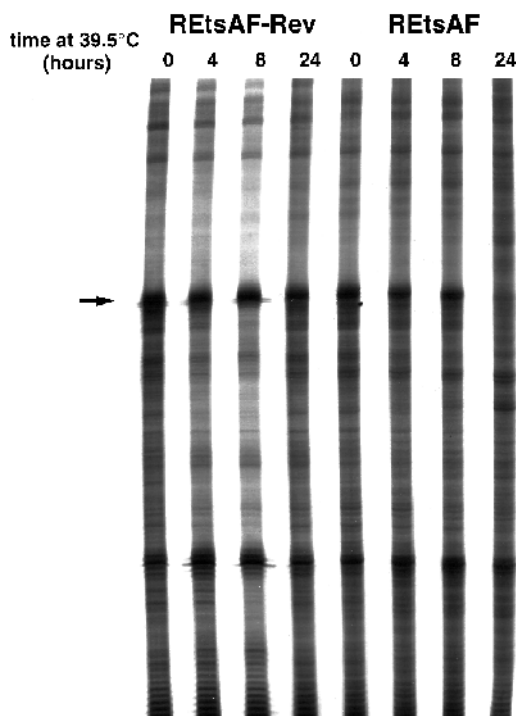


Fig. 1. Differential display of RNA from REtsAF and REtsAF-Rev cell lines at various times after a shift to the restrictive condition.

actin are of the same length (2.1 kb), we used two different blots to measure the change in their respective steady-state levels. The amount of both actin mRNAs decreased during apoptosis of REtsAF cells (Fig. 2A) whereas no difference was observed with control cell lines REtsAF-Rev1 (Fig. 2B) or RELPB (a rat embryo cell line immortalized by wild-type SV40 (Petit et al., 1983; data not shown). Quantification using the Phosphorimager, normalizing to 18 S rDNA rRNA levels in each sample, showed that, although the level of γ -actin mRNA reproducibly diminished slightly faster than that of β -actin, apoptosis of REtsAF is accompanied by a general decrease in levels of mRNA for cytoplasmic actins. After a 24 hour temperature shift, the level of both mRNAs is less than 30% of that observed before the temperature shift (Fig. 2C). Quantification of actin mRNAs isolated from non temperature sensitive cells (REtsAF-Rev) shows that their levels are not affected by the temperature shift (data not shown).

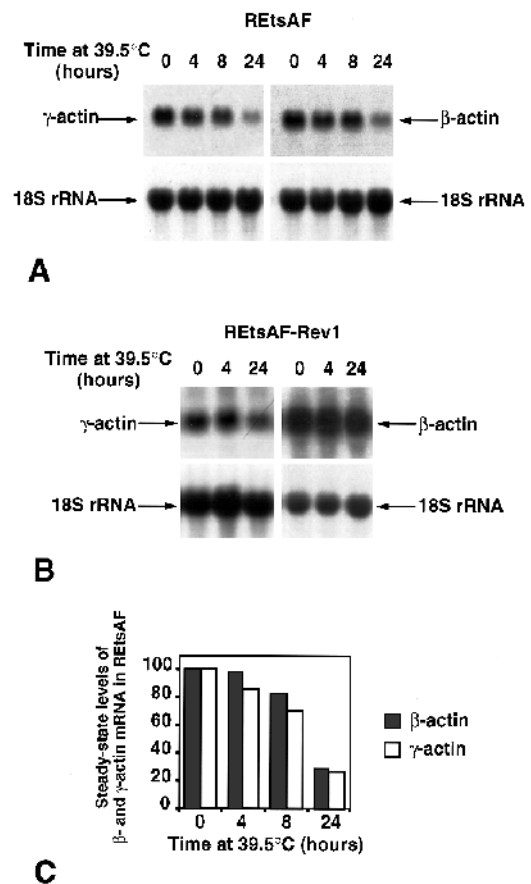


Fig. 2. Steady-state levels of β -actin and γ -actin mRNA decrease during REtsAF apoptosis. (A) Total cellular RNA, prepared from REtsAF at various time after a 39.5°C temperature shift, were analyzed by northern blotting. Nylon membrane was probed successively with γ -actin or β -actin probe and then with an 18 S rRNA probe. (B) The same experiment was performed with REtsAF-Rev1, a cell line that does not undergo apoptosis at 39.5°C. (C) Quantification of the steady-state levels of β - and γ -actin mRNA during REtsAF apoptosis at 39.5°C. Data were standardized according to the relative intensity of the 18 S rRNA band. Results are expressed as a percentage relative to their matched control.

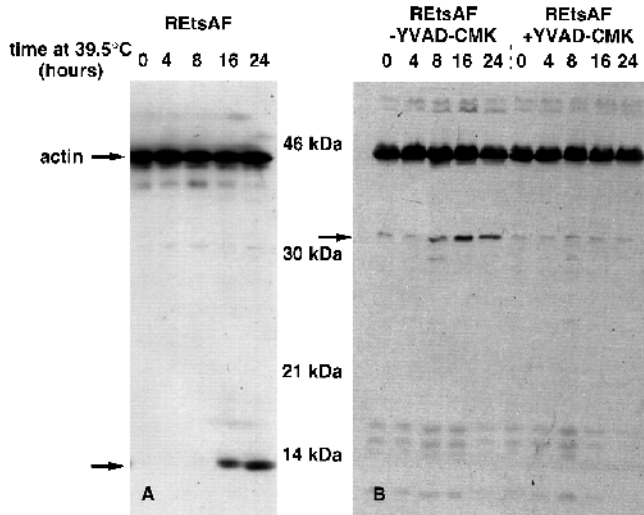


Fig. 3. Actin is a substrate for an ICE-like protease activated during RETsAF apoptosis. (A) extracts of RETsAF cells shifted for various time at 39.5°C were incubated for 30 minutes at 37°C in lysis buffer as described in Materials and Methods, separated by SDS-PAGE electrophoresis and analyzed by immunoblotting with the AC40 monoclonal antibody. (B) extracts of RETsAF cells shifted for various time at 39.5°C were incubated for 2 hours at 37°C in ICE buffer with or without 100 μ M of the YVAD-CMK peptide, separated by SDS-PAGE electrophoresis and analyzed by immunoblotting with the JLA20 monoclonal antibody.

Actin is a substrate of an ICE-like protease activated during apoptosis but only a small amount is proteolysed during the early stages of RETsAF apoptosis

In order to monitor the behavior of actin proteins, we have performed western blotting with two anti-actin antibodies. We found that, although the global amount of actin is approximately constant during apoptosis, an approximately 13 kDa polypeptide that reacts with the AC40 monoclonal antibody is produced in apoptotic cell extracts if the sample is kept for 30 minutes in lysis buffer at 37°C before electrophoresis (Fig. 3A). As the epitope recognized by the antibody is located at the C terminus of the protein, this 13 kDa polypeptide could be an actin C-terminal fragment produced during apoptosis. We used a second monoclonal antibody (JLA20), that recognizes a different epitope, to check if the complementary N-terminal fragment can be detected. For this experiment, protein extracts were prepared as described in Materials and Methods and incubated with and without 100 μ M of the YVAD-CMK

peptide which is a potent inhibitor of ICE-like proteases. Fig. 3B shows that an actin fragment of about 30 kDa is detected when apoptotic extracts are incubated before electrophoresis. Furthermore, this fragment is not produced when the incubation is performed in the presence of YVAD-CMK, showing that an ICE-like protease is responsible for this proteolysis. Thus, as described in U937 leukemia cells (Mashima et al., 1995), actin is a substrate of an ICE-like protease activated during RETsAF apoptosis; however, only a small amount (if any) of actin is proteolysed until advanced stages of the process.

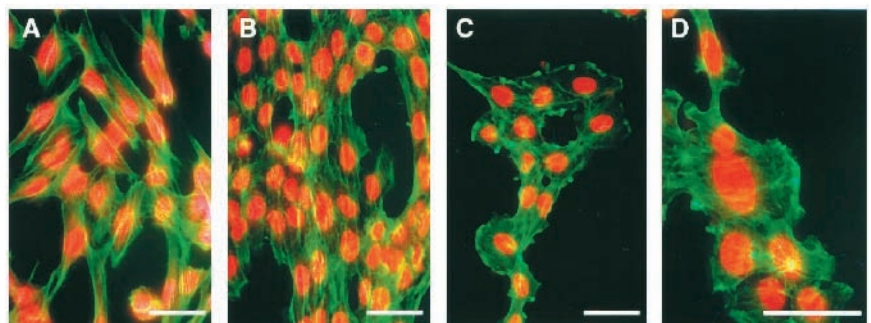
Immunofluorescence analysis of the reorganization of the microfilament network

Changes in the microfilament network have been monitored by immunofluorescence analysis during RETsAF apoptosis by using anti-actin antibodies, and fluorescein-phalloidin (which binds specifically to filamentous actin). The results are shown in Figs 4 and 5: after 24 hours, the microfilament network appeared clearly affected. In particular, an accumulation of fluorescein-phalloidin could be seen near the plasma membrane of apoptotic cells, apparently in regions where cells lose adherence to the plastic dishes, and actin fibers appeared thicker. No such changes were seen in similarly treated RETsAF-Rev1 cells (data not shown). Since these changes were not seen after a 6 hour shift, the down-regulation of cytoplasmic actin genes occurs prior to the microfilament rearrangement. Staining with anti-actin antibody showed that actin accumulated in apoptotic bodies during apoptosis (Fig. 5D). In contrast, a very weak signal was observed in apoptotic bodies when fluorescein-phalloidin is used (Fig. 5B). This result shows that, although unpolymerized actin is found in apoptotic bodies, they are devoid of microfilaments. It can also be seen that, in contrast to the result obtained with phalloidin, a low signal is observed in apoptotic cells when using the anti-actin antibody.

DISCUSSION

Several published observations, some of them apparently contradictory, suggest that microfilament reorganization may be an integral part of the apoptotic process. It has been observed that microfilament-disrupting agents prevent the formation of apoptotic bodies in cells undergoing apoptosis independently of DNA fragmentation (Cotter et al., 1992). However, a microfilament deficiency has been observed during apoptosis which suggests that blebbing of the cytoplasmic membrane during apoptosis is associated with reduced filamentous actin

Fig. 4. Changes in the microfilament network during RETsAF cells apoptosis occur late during the process. RETsAF cells, shifted up to 39.5°C for 0, 6 or 24 hours, were analyzed by fluorescence microscopy after staining with fluorescein-phalloidin (green fluorescence) and propidium iodide (red fluorescence) as described in Materials and Methods. (A) Cells grown at 33°C. (B) Cells shifted to 39.5°C for 6 hours. (C and D) Cells shifted to 39.5°C for 24 hours. Bars, 50 μ m.



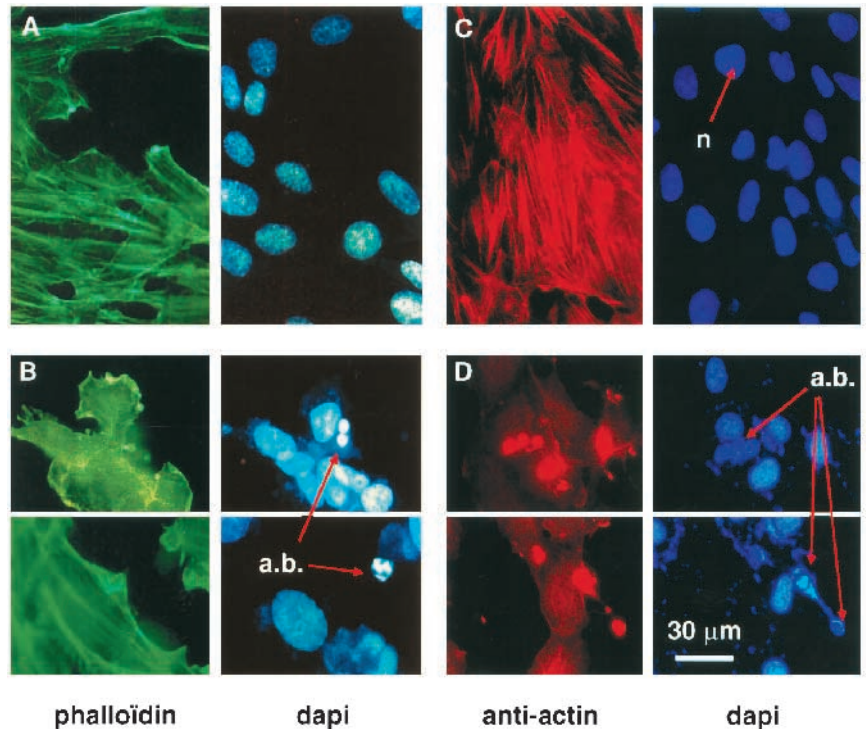


Fig. 5. Status of actin in apoptotic bodies. REtsAF cells kept at 33°C (A and C) or shifted for 24 hours to 39.5°C (B and D) were stained with DAPI and either with fluorescein-phalloidin (A and B) or with the AC40 anti-actin monoclonal antibody (C and D). n, nucleus; ab, apoptotic body.

(Endresen et al., 1995a,b). Expression of thymosine β 10, a globular actin-binding protein that induces depolymerization of microfilaments, increases the ability of NIH3T3 cells to enter apoptosis induced by $\text{TNF}\alpha$ or calcium ionophore (A23187), whereas inhibition of thymosin expression by antisense experiments induces the formation of large actin stress fibers and appears to protect against apoptosis-inducing stimuli (Hall, 1995). It has also been reported that cytochalasin B induces DNA fragmentation similar to that observed during the degradation phase of apoptosis via its effect on the actin filaments (Kolber et al., 1990). However, this effect is not found in all the cell lines tested. One possibility to explain the induction of DNA fragmentation is that the binding of free monomeric actin to DNaseI might inhibit its activity in non-apoptotic cells and that binding of thymosin or cytochalasin to monomeric actin abolishes this effect. In fact, microfilaments could be involved in both the effector phase and the degradation phase. Microfilament depolymerization may be involved in the morphological changes occurring during the effector phase (loss of adherence, cellular shrinkage and blebbing of the plasma membrane) while the polymerization of specific microfilaments or the stabilization of some already polymerized microfilaments could be necessary for the formation of apoptotic bodies occurring latter during the process.

Analysis by fluorescence microscopy of the actin network during apoptosis of REtsAF cells shows that the microfilament network is moderately affected until the late phases of the process. However, analysis of apoptotic cells with anti-actin antibody shows that the microfilament network becomes less accessible to the antibody, which suggests that other components, possibly actin binding proteins, are bound to microfilaments. Alternatively, apoptosis could be associated with changes in actin structure that render it a poor substrate for antibody binding. This experiment also showed that only non-

polymerized actin is present in apoptotic bodies. Thus, microfilaments are needed for the formation of apoptotic bodies (Cotter et al., 1992), but the network is rapidly destroyed once the apoptotic bodies get loose from the cell.

How is the disruption of the microfilament network mediated? We observe that actin protein is a putative substrate for an ICE-like protease activated during apoptosis, confirming the study of Mashima et al. (1995) during drug-induced apoptosis of U937 cells. This observation suggests that cleavage of actin might contribute to the cytoskeleton rearrangements seen during apoptosis. However, although we detect this ICE-like activity after 8 to 16 hours in restrictive conditions, cleaved actin is nearly undetectable if the cellular extracts are not pre-incubated before electrophoresis. This is the case even in extracts of cells that have been held for 24 hours in restrictive conditions, i.e. when nearly all the cells were irreversibly committed to death (Vayssi re et al., 1994). Furthermore, we do not observe any significant decrease in the steady-state levels of actin proteins, which would have been expected if protein cleavage operated together with gene down-regulation. Thus, although this ICE-like protease is activated relatively early during the process, proteolysis of actin occurs late and is therefore probably an event of the degradation phase of apoptosis rather than an event of the effector phase. Alternatively, the ICE-like protease could cleave only a minor subpopulation of actin (for example globular actin) during apoptosis.

In contrast, the decrease in the steady-state levels of γ - and β -actin mRNAs is detected as soon as 4 hours and 8 hours, respectively, after the shift up to 39.5°C. Following a 24 hour shift (when only 20% of the cells remain able to grow when shifted back to 33°C; Vayssi re et al., 1994) the levels of γ - and β -actin mRNAs are less than 30% of those observed before the temperature shift, whereas the steady-state levels of most

mRNAs are stable (Guénal and Mignotte, 1995). Thus, this decrease in actin mRNA levels could be related to the morphological changes occurring during apoptosis.

It is well known that regulation of gene expression is one of the levels of control of microfilament network structure. Every mammalian cell contains several actin isoforms encoded by a gene family (Vandekerckhove et al., 1981; Chang et al., 1984). These genes belong to three classes, depending on the isoelectric point of the protein. β -Actin and γ -actin are the main components of microfilaments in non-muscle cells. Although they are generally coexpressed, they exhibit specific regulation (Vandekerckhove et al., 1981). The expression of actin genes is controlled by various external signals, including EGF (Elder et al., 1984), TGF- β (Leof et al., 1986), insulin (Weinstock et al., 1993); cytosolic calcium appears to be involved in at least some of these up-regulations (Weinstock et al., 1993). A feedback regulation has also been detected by experiments where the microfilaments were stabilized by phalloidin. In that situation, the concentration in monomeric actin decreases and actin mRNA levels increase (Serpinskaya et al., 1990). However, β -actin is not simply autoregulated since over-expression of an exogenous β -actin induces the expression of the endogenous gene (Lloyd et al., 1992). In contrast, exogenous γ -actin appears to repress both β - and γ -actin gene expression and this effect can be abolished by cytochalasin B. Untranslated regions also appear to be involved in controlling the microfilament network structure. For example, a 3' untranslated region of the β -actin gene is responsible for its localization to the periphery of the cell and thus affects the cellular morphology (Kislauskis et al., 1994); transfectants expressing the γ -actin gene deleted for intron III are rounded with blebs over the cell surface and show decreased surface area (Lloyd and Gunning, 1993), morphological changes that are reminiscent of apoptosis. Thus, changes in actin mRNA steady-state levels during apoptosis could regulate the microfilament network not only by controlling the quantity of actin in the cell but also by a qualitative effect on spatial distribution of actin message within the cell.

We have found that a decrease in the mRNA for γ -actin precedes that of β -actin by a few hours. To our knowledge, no data concerning the regulation of γ -actin expression during apoptosis are yet available. A specific decrease in steady-state levels of β -actin mRNA have been recently reported to be associated with apoptosis of HL-60 and U-937 cells induced by actinomycin D (Naora and Naora, 1995). However, in response to apoptosis induced by cycloheximide or calcium ionophore (A23187) those authors observe an increase in β -actin mRNA levels, so they suggest that a decrease in β -actin expression may not be related to apoptosis. However, cycloheximide or A23187 are known inducers of actin gene expression (Elder et al., 1984; Weinstock et al., 1993) and apoptosis induced by these agents may not be representative of the general picture. Thus, the repression of actin genes observed during REtsAF apoptosis at 39.5°C could be a widespread phenomena involved in microfilament rearrangements during apoptosis.

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