Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 561-566

REGULATION OF THE CELLULAR ACTIN LEVELS IN RESPONSE TO CHANGES IN THE CELL DENSITY IN ATAXIA TELANGIECTASIA LYMPHOBLASTOID CELLS

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Received January 25, 1984

Summary: The level of actin was found to decrease markedly when ataxia telangiectasia lymphoblastoid cells were stepped from low to high density culture conditions. Additionally, as the actin levels decreased the levels of a protein species of 37K daltons increased by orders of magnitude. Partial proteolytic digestion of the 37K protein and actin revealed that the primary structures of these proteins were not related. This phenomena was observed in three out of four ataxia telangiectasia lymphoblastoid cell lines but not in lymphoblastoid cells derived from normal individuals.

One of the most abundant cellular proteins in eukaryotes is actin. Actin genes are known to be members of a multigene family, and intracellular actin found in nonmuscle cells consists of a heterogeneous population of actin molecules (1, 2). Six different actin molecules have been identified in mammals; β and γ actin are cytoplasmic and α actin is found in muscle. The muscle actin can be further classified into four different subtypes (3). Polymeric actin is found as the main constituent of microfilaments, which are involved in a number of cellular functions including endocytosis, receptor internalisation and cell motility (4). A large number of cellular proteins have been shown to be able to bind to, and regulate the polymerisation and depolymerisation of actin (5, 6).

This paper describes an unusual regulation of actin levels observed in lymphoblastoid cell lines derived from patients with the disorder ataxia telangiectasia (AT), which is characterized by ionising radiation sensitivity and developmental abnormalities.

MATERIALS AND METHODS

Cell culture: Lymphoblastoid cell lines were obtained by Epstein Barr Virus (EBV) transformation. Control lines were C5ABR, C10ABR and the AT

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lines were AT1ABR, AT3ABR, AT4ABR and AT5ABR. All of these cell lines are from different individuals. The AT cell lines were initiated from individuals showing the typical AT phenotype. Details of these lines are described in Lavin et al (7). Cells were grown as suspension cultures in RPMI 1640 media (containing antibiotics) supplemented with 10% foetal calf serum and were routinely split every 3 or 4 days to 0.5×10^6 cells/ml. The AT lines showed the characteristic growth sensitivity to serum quality.

37K Protein induction: Cell cultures at various densities were taken up in Hanks Balanced Salt Solution, and incubated for 2 hours at 37 deg. C in a 5% CO2 humidified incubator. Cells were labeled with [^{35}S]-methionine (Amersham; sp act. 1450 Ci/mmol) in the Hanks Balanced Salt Solution at a final concentration of 2 uCi/ml. Protein samples for electrophoresis were obtained by solubilisation of cold PBS washed cells with 35 mM dodecyl sulphate (tris salt pH = 8.4), followed by a 10 sec. sonication pulse to disrupt the DNA.

One dimensional SDS gel electrophoresis: 10-15% gradient acrylamide gels were used according to the procedure of Laemmli (8). Tris dodecyl sulphate cell extracts were made 10% sucrose, 5% 2-mercaptoethanol and 0.001% bromophenol blue, before being held at 100 deg. C for 30 sec. prior to loading. Molecular weight determinations were done by running standard markers (SDS-6H kit; Sigma). Proteins were visualised by Coomassie blue staining or fluorography according to the method of Bonner and Laskey (9).

Immunoprecipitation of actin: Rabbit antiactin was obtained from Miles-Yeda. Precipitation of [^{35}S]-actin from cell extracts was according to Bartholomew et al (10), using 10 ul of antisera/500 ul of cell extract. Protein A was obtained from the Cowan I strain of S. aureus as described by Kessler (11).

V-8 partial proteolysis: Analysis of the 37K protein and actin primary structure was by partial proteolysis using the V-8 protease from S. aureus (Sigma) as described by Cleveland et al (12). Peptide fragments were subsequently resolved on a 15% SDS-polyacrylamide gel.

RESULTS

Density dependent decrease in a 43K protein (actin) and corresponding induction of a 37K protein:

Figure 1 shows a striking increase in the levels of a 37K dalton protein in an ataxia telangiectasia lymphoblastoid cell line (AT1ABR) in parallel with an increasing cell density. As the level of this protein increased the level of a 43K protein (actin) decreased. No other gross changes in protein species were observed. Trypan blue indicated at least a 95% viability at all cell densities used in this experiment. The control cell lines (in this experiment C5ABR and C10ABR), did not demonstrate this phenomena (Figure 2). Induction of the 37K protein still occurred when foetal calf serum was present in the Hanks Balanced Salt Solution (results not shown), indicating that the induction did not occur as a result of serum deprivation.

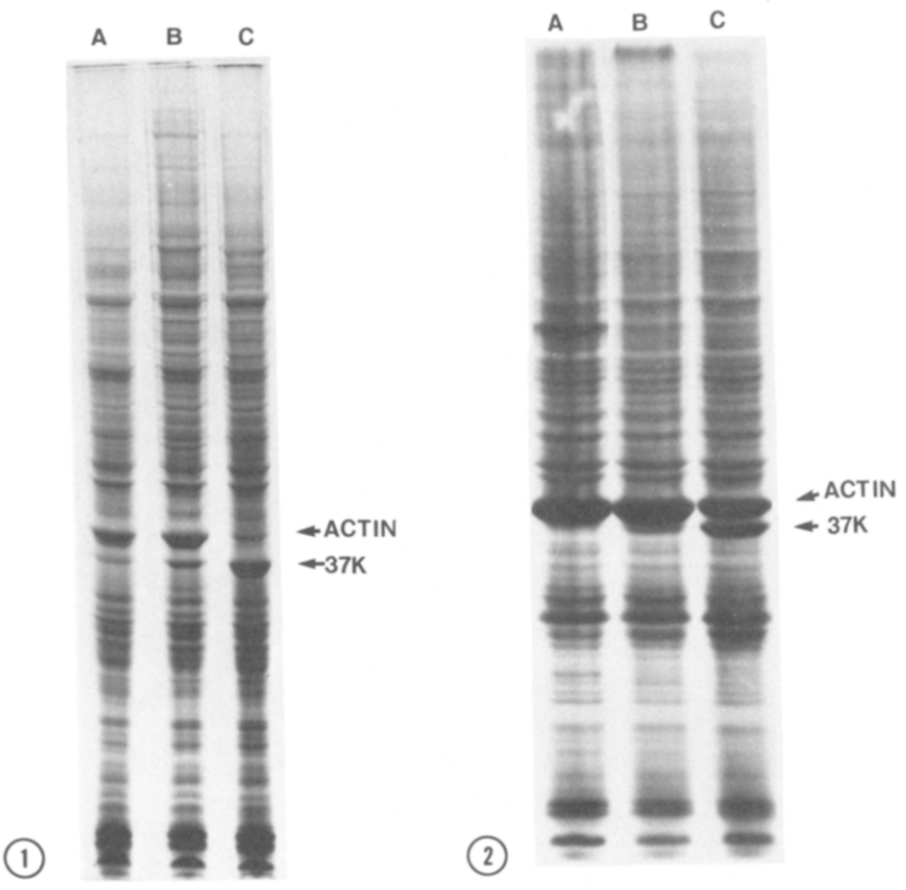


Figure 1. The effect of increasing cell density on the levels of actin and the 37K protein in AT1ABR ataxia telangiectasia cells. Cells at a density of 0.5, 1.0 and 2.0x10^6 cells /ml (total of 10 ml cell suspension) were taken up in 4mls of Hanks Balanced Salt Solution to give a density of 1.25 (track A), 2.5 (track B) and 5.0 (track C) x10^6 cells/ml. 110 ug of protein was loaded for each sample. Proteins were separated on a 10-15% gel and visualized by Coomassie blue staining.

Figure 2. Comparison of AT and control proteins at high density. Control cells C5ABR (track A) and C10ABR (track B) were at 2.0x10^6 cells/ml and the AT1ABR (track C) were at 1.5x10^6 cells/ml. Cells were then resuspended in Hanks Balanced Salt Solution to give 5.0x10^6 cells/ml for the controls and 3.5x10^6 cells/ml for the AT line. Cells were incubated for two hours in the presence of 2 uCi/ml [^{35}S]-methionine. Equal cpm were loaded for each track. Proteins were visualized by fluorography.

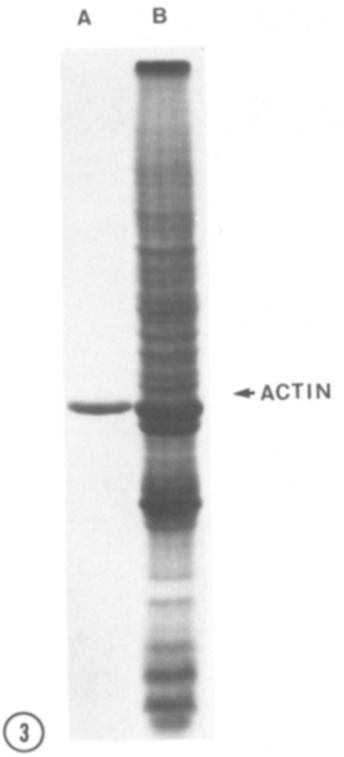
In the experiments reported here, AT1ABR was the ataxia telangiectasia cell line used. However this same increase in the 37K protein and decrease in the 43K protein (actin) has been found for AT3ABR and AT5ABR, but not for AT4ABR.

Identification of the 43K protein as actin:

The 43K protein was identified as actin by three criteria: Its electrophoretic mobility, its abundance and the fact that rabbit antisera against actin precipitated a protein that comigrated with the 43K band upon gel electrophoresis (Figure 3).

Comparison of protease fingerprints of actin and the 37K protein:

Because the amount of the 37K protein clearly increased as actin decreased it seemed possible that the 37K protein was a type of actin derivative. This was tested by examining the peptide fragments produced following limited proteolysis with the V-8 protease. Figure 4 shows that the 37K protein



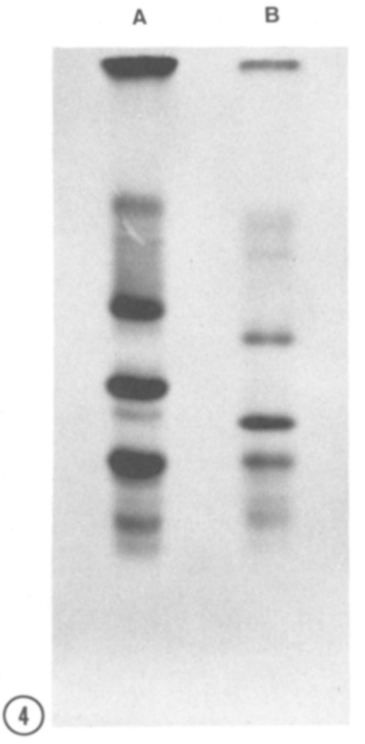


Figure 3. Immunoprecipitation of [^{35}S]-labelled cellular protein extracts. Rabbit antibodies against actin were used for immunoprecipitation from whole cell extracts of AT1ABR cells. Track A is the immunoprecipitate and track B is a run of a whole cell extract on a 10-15% gel. Proteins were visualized by fluorography.

Figure 4. Partial proteolysis of actin and the 37K protein by the V-8 protease. Actin (track A) and the 37K protein (track B) were excised from a 10-15% gel and then digested by 50 ng of the V-8 protease as described in the materials and methods. The peptide fragments were separated in a 15% gel and visualized by fluorography.

produces a peptide pattern which bears no resemblance to that of actin. This strongly indicates disimilarity between the 37K protein and actin.

DISCUSSION

The results presented here have described an unusual regulatory phenomena associated with some aspect of high cell density on the levels of actin in ataxia telangiectasia lymphoblastoid cells. Apart from developmental regulation of actin levels (13), there do not appear to be any reports mentioning the type of actin regulation described in this paper.

Actin levels could be regulated by actin specific proteases and/or by modulation of actin gene expression. The correlation between the decrease in the actin levels and the increase in the 37K protein, superficially suggests that the 37K protein is in some way linked to the regulation of actin. The 37K protein could conceivably be either an actin specific protease or a gene regulator, but the very high levels of the protein make this unlikely. Interestingly, a report in the literature (14) describing the regulation of actin levels during sea urchin development included an SDS-gel of [^{35}S] labelled proteins from various times of sea urchin development (Figure 1A in reference 14). This gel shows that as the synthesis of actin increases, a protein, in an area which appears to approximate the region of 37K daltons, also decreases correspondingly with the increase in the level of actin. However, the identity of the lymphoid 37K protein and the similar sea urchin protein is only conjecture.

The cell lines used in this study were EBV-transformed lines but the 37K protein does not appear to be of viral origin. The EBV viral antigens that are known to be present in EBV-transformed cell lines not undergoing the lytic cycle do not correspond to the 37K protein (15). A 37K protein has been detected (along with many other viral proteins), in cells induced to enter the lytic cycle (16), but the pattern of induction of the 37K protein does not correspond to that reported for transformed cells entering the lytic cycle (17, 18). We cannot rule out the possibility that some unusual expression of the viral genome occurs in the AT cells.

Although no function is known for the 37K protein it seems possible that this protein, which at maximal induction may be one of the most abundant cellular proteins, could have major functional significance.

So far, the response to high density conditions has been observed only in AT cells (three of the four lines we have examined). The AT cells used in this study were derived from different individuals all showing the characteristic AT phenotype. The reason for the absence of the reported response in one AT cell line, (AT4ABR), may be explained by the well documented existence of genetic heterogeniety in AT (19,20). The implication of this phenomena as an aspect of the AT phenotype is thus unclear. However this novel regulation of actin and the increase in synthesis of the 37K protein may aid the study of actin regulation and gene expression control.

ACKNOWLEDGMENTS

The supply of the lymphoblastoid cell lines from Paula Imray and Jane Houldsworth is gratefully acknowledged.

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