Mercola, D. A., Morris, J. W. S., & Arquilla, E. R. (1972) *Biochemistry 11*, 3860–3874.
Miller, D. L., & Weissbach, H. (1974) *Methods Enzymol. 30*, 219–232.
Miller, D. L., & Weissbach, H. (1977) in *Nucleic Acid-Protein Recognition* (Vogel, H. J., Ed.) pp 409–440, Academic Press, New York.
Pingoud, A., & Urbanke, C. (1980) *Biochemistry 19*, 2108–2112.
Pingoud, A., Kownatzki, R., & Maass, G. (1977a) *Nucleic Acids Res. 4*, 327–338.
Pingoud, A., Urbanke, C., Krauss, G., Peters, F., & Maass, G. (1977b) *Eur. J. Biochem. 78*, 403–409.
Rigler, R., Nilsson, L., Wintermeyer, W., Pachmann, U., & Zachau, H. G. (1981) *Nucleic Acids Res. 9*, 1031–1044.
Schulman, R. G., Hilbers, C. W., & Miller, D. L. (1974) *J. Mol. Biol. 90*, 601–607.
Schwartz, I., Gordon, E., & Ofengand, J. (1975) *Biochemistry 14*, 2907–2914.
Schwarz, U., Menzel, H. M., & Gassen, H. G. (1976) *Biochemistry 15*, 2484–2490.
Sprinzl, M., & Faulhammer, H. (1978) *Nucleic Acids Res. 5*, 4837–4853.
Tanada, S., Kawakami, M., Yoneda, T., & Takemura, S. (1981) *J. Biochem. (Tokyo) 89*, 1565–1572.
Tao, T., Nelson, J. H., & Cantor, C. R. (1970) *Biochemistry 9*, 3514–3524.
Vacher, J., & Buckingham, R. H. (1979) *J. Mol. Biol. 129*, 287–294.
Wagner, T., & Sprinzl, M. (1979) *Methods Enzymol. 60*, 615–628.
Wagner, T., & Sprinzl, M. (1980) *Eur. J. Biochem. 108*, 213–221.
Wells, B. D., & Cantor, C. R. (1977) *Nucleic Acids Res. 4*, 1667–1680.
Weygand-Durasevic, I., Kruse, T. A., & Clark, B. F. C. (1981) *Eur. J. Biochem. 116*, 59–65.

Altered Protein Synthesis in Ataxia–Telangiectasia Fibroblasts†

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ABSTRACT: Analysis of protein production in various strains of ataxia–telangiectasia (A–T) fibroblasts demonstrated the overproduction of a group of secreted proteins and variations in the protein characteristics of the extracellular matrix. The most prominent differences involved fibronectin, which was identified by immunochemical analysis. One- and two-dimensional gel electrophoresis demonstrated differences in the production, accumulation, and molecular weight of fibronectin on the cell surface and in the culture medium as compared to normal human fibroblasts. Three other secreted proteins with molecular weights of 185 000, 150 000, and 70 000 were also observed to be produced in excess amounts in some strains of A–T. The finding that extracellular matrix alterations are involved in the abnormal DNA synthesis and reduced cell survival in A–T cells in response to X radiation would be additional evidence for a close association between the extracellular and nuclear architecture.

Ataxia–telangiectasia (A–T) is an autosomal recessive human genetic disease characterized by neurological disorders, immune deficiencies, a high incidence of cancer, and sensitivity to ionizing radiation (Kraemer, 1977; Bridges & Harnden, 1982). The biochemical lesions involved in this disease have yet to be determined, but various lines of evidence indicate that abnormalities in coordination of DNA synthesis are somehow involved. A–T cells have a prolonged S phase (Cohen & Simpson, 1980; Murnane & Painter, 1982) despite their normal rate of DNA chain elongation (Kapp & Painter, 1981) and fail to demonstrate the inhibition of replicon initiation normally occurring in the presence of X-ray-induced DNA damage (Edwards & Taylor, 1980; Houldsworth & Lavin, 1980; Painter & Young, 1980). With the use of these observations, A–T strains have been categorized into at least five complementation groups representing separate gene mutations (Murnane & Painter, 1982; Jaspers & Bootsma, 1982b).

The possible role of chromatin conformation in regulation of DNA synthesis (Blumenthal et al., 1973) has led to the proposal that these mutations may involve structural irregularities in packaging of DNA (Painter & Young, 1980). To gain further insight into the possible relationships of various structural proteins to the A–T phenotypes, we compared protein content and production in fibroblasts from several normal, A–T, and other human genetic disease strains. Although initial experiments centered around investigation of nuclear proteins, these studies demonstrated major differences in the extracellular matrix proteins, which were then further characterized.

Materials and Methods

Cells. Normal human skin fibroblasts HS-1 and HS-27 were established by the Cell Culture Facility, University of California, San Francisco. AT3BI, AT5BI, and AT7BI skin fibroblasts were provided by D. G. Harnden and A. M. R. Taylor, University of Birmingham, England, and AT2SF skin fibroblasts were provided by Diane Wara, Department of Pediatrics, University of California, San Francisco. A–T strains AT3BI and AT5BI represent separate complementation groups (Murnane & Painter, 1982; Jaspers & Bootsma, 1982b); AT5BI and AT7BI have been reported to be proficient

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0006-2960/83/0422-1217$01.50/0

and AT3BI deficient in X-ray-induced excision repair (Paterson, 1978). All cells were grown in minimal essential medium (Gibco) containing 15% fetal calf serum (Gibco) without antibiotics. Experiments were conducted with fibroblasts subcultured 15–25 times in Falcon 75-cm^2 tissue culture flasks. Confluent nongrowing cultures in depleted medium were prepared by subculturing confluent cells one to three, allowing the cells to grow to confluence again, and incubating them for 2 weeks without a change of medium. This medium was then used for subsequent chase incubations. Comparisons between growing and nongrowing cells were made by replating confluent cells at a lower density three days before preparation of samples.

Cell Labeling. Cellular proteins were radiolabeled with 25 μCi/mL [^{35}S]methionine (Amersham; sp act. 1500 Ci/mmol) in methionine-free minimal essential medium without fetal calf serum. All labeling was preceded by two rinses in methionine-free medium and, in the case of short pulses, a 30-min preincubation in this medium.

Cell Sample Preparations. After two rinses in phosphate-buffered saline, cells were scraped off and centrifuged at 200*g*. Samples of whole cells were dissolved directly in sample buffer. After removal of debris by centrifuging at 10000*g* for 20 min, proteins were precipitated from the medium by the method of Peters & Comings (1980). Briefly, after the addition of fresh phenylmethanesulfonyl fluoride and 1% sodium dodecyl sulfate ($NaDodSO_4$), the supernatant was dialyzed first against 5 mM mercaptoethanol and then against 95% ethanol. The protein–$NaDodSO_4$ precipitate was then pelleted at 500*g*, dried, and dissolved in sample buffer.

One-Dimensional Gel Electrophoresis. Samples containing 25 000–35 000 cpm dissolved in $NaDodSO_4$ sample buffer [2.3% $NaDodSO_4$–0.0625 M Tris (pH 6.8)–10% glycerol–5% 2-mercaptoethanol–0.001% bromophenol blue] were solubilized by boiling for 5 min and separated on 5–20% polyacrylamide gradients as previously described (O'Farrell, 1975). After 5–6 h at 30 mA per 120 × 140 × 1.5 mm gel, gels were either stained with Coomassie blue (Peters & Comings, 1980) or prepared for fluorography by swirling in Enhance (New England Nuclear) for 2 h, followed by 1 h in water before drying and exposure on X-Omat AR X-ray film (Kodak) for 1–2 days. Protein half-lives and modifications of molecular weight were then quantified by densitometer scans of the exposed X-ray film.

Two-Dimensional Gel Electrophoresis. Samples containing 500 000 cpm in 20 μL of isoelectric focusing sample buffer (9.5 M urea, 2% Nonidet P40, 5% 2-mercaptoethanol, 1.6% pH range 5–7 ampholytes, and 0.4% pH range 3–10 ampholytes) were first separated by charge in 120-mm isoelectric focusing gels and then separated by size in 5–20% acrylamide gels as previously described (O'Farrell, 1975). Gels were prepared for fluorography as described for one-dimensional gels and exposed for 3–5 days. The charge gradient of the isoelectric focusing gel was determined by placing gel slices in distilled water overnight before measurement of pH.

Immunochemical Labeling of Gel Blots. Proteins were transferred from acrylamide gels to nitrocellulose paper by using procedures described by Towbin et al. (1979) as modified by Matus et al. (1980). Transfers were performed for a minimum of 3 h at 400 mA by using a Bio-Rad Trans-blot cell. Amido black staining and double-antibody labeling with goat anti-human fibronectin (Cappel Laboratories) and ^{125}I-labeled rabbit anti-goat immunoglobulin (Miles-Yeda) were carried out as described by Matus et al. (1980). The anti-human fibronectin was prepared by using cold-insoluble globulin purified from fresh human plasma; immunoelectrophoresis by Cappel Laboratories demonstrated it to be monospecific.

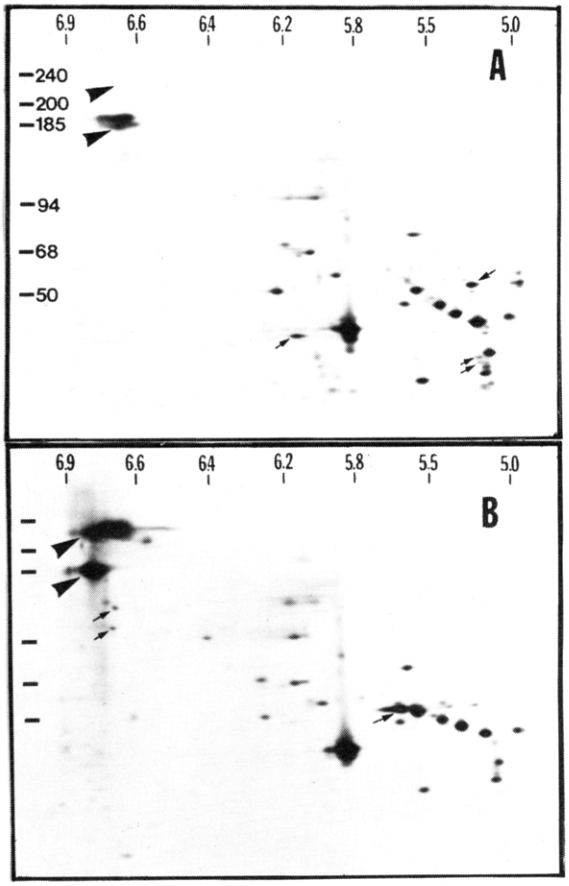

FIGURE 1: Two-dimensional gel electrophoresis of fluorograms of proteins from normal and A–T fibroblasts. HS-27 and AT2SF fibroblasts were incubated for 4 h with 25 μCi/mL [^{35}S]methionine in exponential growth, and proteins were separated by two-dimensional gel electrophoresis. (A) HS-27 whole cells; (B) AT2SF whole cells. The pH range of the isoelectric focusing gel is shown at the top, and the position of molecular weight markers (×10^3) consisting of filamin (240 000), myosin (200 000), macroglobulin (185 000), phosphorylase (94 000), bovine serum albumin (68 000), and globulin subfragment (50 000) are shown on the left. Large arrowheads indicate the major A–T-enhanced proteins, and small arrows indicate other protein differences between these cell types.

Collagen Digestion. After 30-min labeling with [^{35}S]-methionine, fibroblasts were rinsed with phosphate-buffered saline, scraped off, and centrifuged. Cell pellets were then resuspended in 50 mM Tris (pH 7.4)–1 mM $CaCl_2$, followed by boiling for 5 min and brief sonic disruption. Protease-free collagenase (Advance Biofactures, type III) was added to 50 units/mL and the solution was incubated at 37 °C. Digestion was stopped by the addition of an equal volume of 2× electrophoresis buffer and boiling for 5 min.

Results

Two-Dimensional Gel Electrophoresis. Preliminary analysis of proteins from exponentially growing normal human (HS-27) and A–T (AT2SF) skin fibroblasts demonstrated striking differences in the production of two major proteins after 4-h labeling with [^{35}S]methionine (Figure 1, large arrows). These proteins appeared heterogeneous, with molecular weights of 220 000–240 000 and 180 000–190 000 and isoelectric points of 6.8–6.6 and 6.8, respectively. Although these were two of the most intensely labeled regions in whole-cell preparations from A-T cells, only small amounts of label were observed in these regions in identical preparations from normal cells.

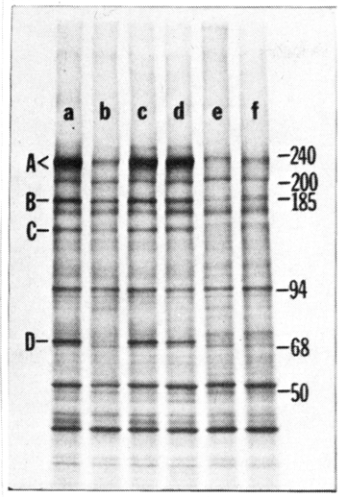

FIGURE 2: One-dimensional gel electrophoresis of fluorograms of proteins from normal and A–T fibroblasts. Whole-cell preparations from exponentially growing cells pulse labeled for 30 min with 25 μCi/mL [^{35}S]methionine are shown for (a) AT2SF, (b) AT3BI, (c) AT5BI, (d) AT7BI, (e) HS-1, and (f) HS-27. Positions of A–T-enhanced proteins A–D are shown along with molecular weight markers ($\times 10^3$), listed in the legend to Figure 1.

Additional studies comparing the two-dimensional gels from whole-cell preparations of AT5BI and AT7BI cells with normal HS-1 cells gave similar results (data not shown). Small arrows indicate other minor differences, some of which varied among different preparations but were not observed on one-dimensional gels.

One-Dimensional Gel Electrophoresis. Figure 2 compares proteins in whole-cell preparations from exponentially growing A–T and normal cells that were labeled with [^{35}S]methionine for 30 min. As shown in Figure 1 for AT2SF, strains AT2SF, AT5BI, and AT7BI all showed a 3–6-fold increase in production of proteins of 220–240 and 185 kilodaltons when compared to normal cells. In addition, one-dimensional gels show that two proteins with molecular weights of 150 000 and 70 000 were also overproduced by 3–6-fold in these cells. These four proteins will be referred to as A, B, C, and D in order of decreasing molecular weight. Some variation in production of these proteins does exist among A–T strains, because the "repair-deficient" strain AT3BI (Paterson, 1978) demonstrated normal production of proteins A and D (Figure 2) and strain AT6BI showed normal production of proteins B and C (data not shown). Neither growth conditions nor cell age was responsible for these differences, because similar results were obtained in confluent cultures and in cultures with as widely varying passage numbers as 6–30.

Figure 3 demonstrates total Coomassie blue stained protein in whole-cell preparations from both exponentially growing and confluent nongrowing A–T and normal cells. Bands B–D cannot be observed in these gels. A band corresponding to protein A in Figure 2 (220 000–240 000) does appear; it is considerably increased in nongrowing cultures and is substantially greater in all four A–T strains compared to their normal cell counterparts. Differential kinetics of synthesis or breakdown of protein A has therefore been found to be characteristic of all A–T fibroblasts studied, despite its normal rate of production in strain AT3BI (Figure 2). Another band appears to be increased in nondividing A–T strains AT2SF and AT7BI, a 170-kilodalton protein occurring at equivalent amounts in all the strains shown in Figure 2.

Proof of similar effects of confluence on all the strains tested could be shown after [^{35}S]methionine labeling, since all showed an approximate 70% reduction in [^{35}S]methionine incorporation as well as altered patterns of protein labeling, with reduced incorporation in several prominent bands (data not shown).

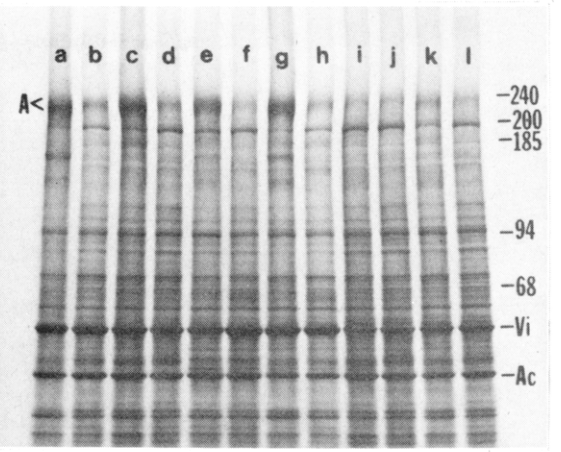

FIGURE 3: One-dimensional gel electrophoresis of Coomassie blue stained proteins from normal and A–T fibroblasts in growing and nongrowing cultures. Whole-cell preparations of confluent nongrowing (a, c, e, g, i, k) and exponentially growing (b, d, f, h, j, l) cultures were alternated for AT2SF (a, b), AT3BI (c, d), AT5BI (e, f), AT7BI (g, h), HS-1 (i, j), and HS-27 (k, l) fibroblasts. Positions of molecular weight markers ($\times 10^3$), actin (Ac), and vimentin (Vi) are shown.

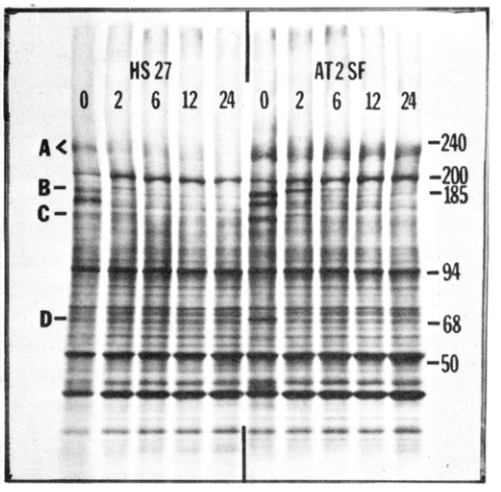

FIGURE 4: One-dimensional gel electrophoresis of fluorograms of proteins from normal and A–T fibroblasts during pulse–chase labeling. Whole-cell preparations of HS-27 and AT2SF fibroblasts pulse labeled for 30 min with 25 μCi/mL [^{35}S]methionine are shown immediately (0) and after 2-, 6-, 12-, and 24-h chase periods. Positions of A–T-enhanced proteins, A–D, and molecular weight markers are shown.

Pulse–Chase Studies. Pulse–chase experiments (Figures 4 and 5) demonstrated the turnover rates of the four proteins of interest and resolved the differences seen in labeled and unlabeled preparations. Because equal amounts of acid-precipitable [^{35}S]methionine were added to each well, these studies demonstrate turnover only in relation to the other observed proteins, which remained nearly constant relative to one another during this time period (Figure 2). Incorporation of [^{35}S]methionine almost ceased after its removal from the medium, with only an additional 20% further incorporation that leveled off by 15 min; the specific activity of total cell protein decreased by 25% after 24 h (data not shown).

The diffuse protein A band had two components, which differed in their kinetics of turnover. The first, which disappeared by 2 h (Figure 4) in both HS-27 and AT2SF, had a half-life of about 60 min (Figure 5a). The second component (approximately 50%) remained nearly constant for up to 24 h.

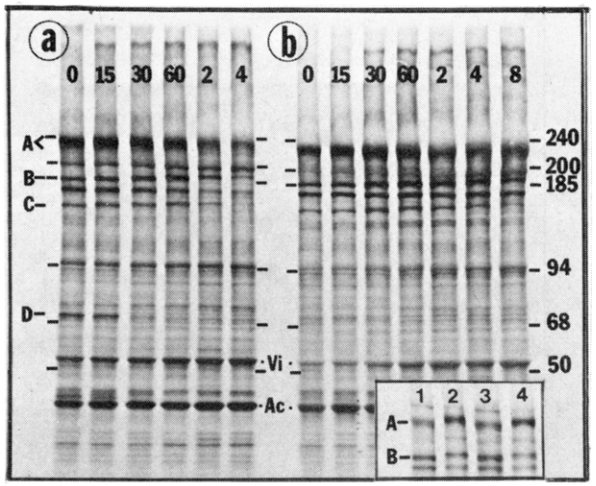

FIGURE 5: One-dimensional gel electrophoresis of fluorograms of proteins from growing and nongrowing A–T fibroblasts during pulse–chase labeling. Whole-cell preparations of exponentially growing (a) and confluent nongrowing (b) AT2SF fibroblasts pulse labeled for 15 min with 25 μCi/mL [^{35}S]methionine are shown immediately (0) and after 15-, 30-, or 60-min or 2-, 4-, or 8-h chase periods. (Inset) Fluorograms of proteins A and B during 4-h [^{35}S]methionine labeling of growing (1, 3) and nongrowing (2, 4) AT2SF fibroblasts. Positions of A–T-enhanced proteins A–D, as well as molecular weight markers ($\times 10^3$), actin (Ac), and vimentin (Vi) are shown.

The 185-kilodalton protein (B) and the 150-kilodalton protein (C) both disappeared by 2 h (Figure 4) and were replaced by slightly larger bands of reduced intensity. The kinetics of this process was similar in both HS-27 and AT2SF cells, with almost complete elimination of these bands by 6 h. Observation of this process at shorter intervals in A–T cells (Figure 5a) and in HS-27 cells (data not shown) showed a gradual increase in molecular weight in both proteins B and C, accompanied by their eventual disappearance with half-lives of approximately 2 h. The heavily labeled band of protein D (70 kilodaltons) seen solely in A–T cells was completely absent by 2 h (Figure 4) and had a half-life of less than 30 min (Figure 5a).

Only two other prominent proteins appeared to be rapidly turning over in these cells. One protein of 170 kilodaltons had a half-life of 1 h (Figure 5a) and was labeled equally in both A–T and normal fibroblasts (Figures 2 and 4). A second protein of 45 kilodaltons had a very short half-life of less than 30 min, similar to protein D.

Absence of cell division affected modification and turnover of these proteins to varying extents. Figure 5b shows the same experiment as Figure 5a but in confluent rather than growing A–T cells. Protein B, protein C, and the 170-kilodalton protein all showed a reduction in this rate of modification by at least 4-fold, as can be seen by the fact that these bands did not begin to disappear until 8 h after labeling (Figure 5b). Similar results were obtained for HS-27 cells. The influence of cell division on protein A, however, differed considerably between normal and A-T fibroblasts. Although growth conditions had no effect on the size of proteins A and B in HS-27 cells, nongrowing A–T cells showed a reduction in molecular weight in protein A equivalent to 10 000. This growth-dependent difference in modification of protein A can be seen clearly in gels that alternate protein preparations from growing and nongrowing A–T cells labeled for 4 h (see inset to Figure 5) and has been seen in all A–T strains tested. The difference in size of protein A does not appear to be a result of enhanced proteolytic degradation, because it was apparent almost immediately after synthesis, with no further change even after 24 h (Figures 4 and 5).

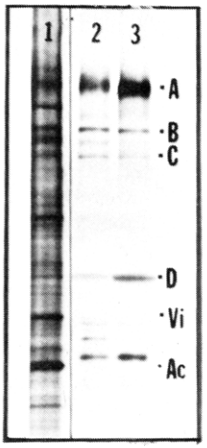

FIGURE 6: One-dimensional gel electrophoresis of fluorograms of proteins isolated from growth medium from normal and A–T fibroblasts. HS-27 and AT6BI fibroblasts were labeled for 4 h with 25 μCi/mL [^{35}S]methionine in exponential growth, and proteins precipitated from the growth medium were separated on one-dimensional gels. (1) Whole-cell preparation from the 4-h-labeled HS-27 cells; (2) secreted proteins from HS-27 cells; (3) secreted proteins from AT6BI cells. Positions of A–T-enhanced proteins A–C as well as actin (Ac) and vimentin (Vi) are shown.

Protein Characterization. Evidence for rapid turnover of these proteins suggested the possibility that they were secreted, especially since fibroblasts are known to secrete a number of different proteins (Hynes, 1981a; Yamada, 1981). Figure 6 demonstrates that all four proteins (A, B, C, and D) were present in the medium isolated from HS-27 and AT6BI cells after a 4-h pulse. As in confluent cells, secreted protein A again appeared approximately 10 kilodaltons smaller in A-T cells than in HS-27 cells. Also consistent with other experiments, the quantity of secreted proteins A and D was greater by 3-fold in A–T cells, since the quantity of sample added was adjusted for the total protein present in the producing cells. The portion of protein A that was retained by the cells (Figures 4 and 5) was demonstrated to be a cell surface component by [^{125}I]lactoperoxidase labeling (data not shown). Proteins B and C were found in identical amounts in the medium of HS-27 and AT6BI fibroblasts, which is consistent with the normal production of these two proteins in AT6BI fibroblasts.

Several properties associated with protein A suggested it was fibronectin. These included (1) its molecular weight and diffuse banding in gels, (2) its modification properties and turnover rates, (3) its secretion and presence on the cell surface, and (4) its buildup in confluent cells (Hynes, 1981a; Yamada, 1981). Immunochemical analysis using antiserum prepared against human plasma fibronectin (Figure 7) confirmed that protein A was fibronectin. The identical patterns of stained and radiolabeled proteins demonstrate that A–T fibroblasts have much more fibronectin than normal fibroblasts and that it accumulates on confluent cells.

It was considered likely that protein bands B and C were the α1 and α2 forms of procollagen owing to the similarity in their turnover rates, electrophoresis banding patterns, and secretion from human fibroblasts (Uchida et al., 1979). Digestion studies with collagenase demonstrated that the B and C proteins were selectively degraded by this enzyme (Figure 8) and that, therefore, A–T fibroblasts also overproduce procollagen.

Discussion

A–T is an interesting human mutation for several reasons, including the A–T cell's sensitivity to X rays (Taylor et al., 1975) and abnormalities in DNA synthesis (Painter & Young,

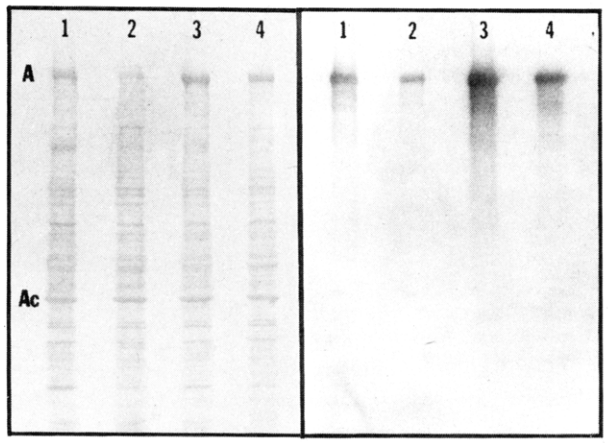

FIGURE 7: Immunochemical analysis of protein A in preparations of normal and A–T fibroblasts. Whole-cell preparations from nongrowing (1) and growing (2) HS-27 fibroblasts and nongrowing (3) and growing (4) AT6BI fibroblasts were separated on one-dimensional acrylamide gels and transferred to nitrocellulose paper; total proteins were stained with amido black (left) or fibronectin was identified by ^{125}I double-antibody labeling (right). Positions of protein A (A) and actin (Ac) are shown.

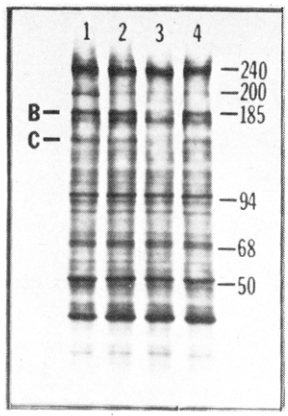

FIGURE 8: One-dimensional gel electrophoresis of fluorograms of proteins from A–T fibroblasts digested with collagenase. Whole-cell preparations of [^{35}S]methionine-labeled cells were either dissolved directly in sample buffer (1), boiled for 5 min and sonically disrupted (2), boiled for 5 min, sonically disrupted, and digested at 37 °C with 50 units/mL collagenase for 5 min (3), or boiled for 5 min, sonically disrupted, and incubated at 37 °C for 15 min without collagenase (4). Positions of proteins B and C are shown on the left, and molecular weight markers ($\times 10^3$) are shown on the right.

1980; Houldsworth & Lavin, 1980; Edwards & Taylor, 1980; Cohen & Simpson, 1980; Murnane & Painter, 1982). Although deficient DNA excision repair is suggested to be directly involved with this disease (Paterson et al., 1976), only about half of A–T isolates demonstrate this property (Paterson, 1978), and recent evidence suggests this phenomenon is not of primary importance (Jaspers & Bootsma, 1982a).

Another hypothesis is that irregularities in chromatin conformation cause abnormalities in DNA synthesis and packaging in the presence of DNA damage (Painter & Young, 1980). This is consistent with other studies suggesting that interference with regulation of progression through the cell cycle in cells with damaged DNA can enhance lethality (Murnane et al., 1980, 1981; Painter, 1980).

The results of the experiments reported here suggest that A–T fibroblasts differ from other human skin fibroblasts with respect to the biosynthesis of a number of extracellular proteins. In addition to fibronectin and collagen, [^{14}C]glucosamine labeling (Nishimoto et al., 1982) demonstrated the overproduction of proteoglycans in several A–T strains (data not shown). The 70-kilodalton band may be serum-binding protein, an additional protein secreted by fibroblasts that, like fibronectin, is involved in cell adhesion (Hayman et al., 1982). Fibronectin, collagen, and proteoglycans are closely interacting components of the extracellular matrix, which is involved in cell attachment and proper cellular interaction (Hynes, 1981b; Yamada, 1981). Fibronectin is also closely associated with the cytoskeleton, and disruption of either structure interferes with the normal architecture of the other (Hynes, 1981b). Fibronectin is nearly absent from the surface of transformed fibroblasts owing to both decreased production and decreased binding (Vaheri & Ruoslahti, 1975; Olden & Yamada, 1977), and its addition to these cultures reconstitutes normal cell morphology (Yamada et al., 1976; Ali et al., 1977).

Abnormalities in components of the extracellular matrix could explain the defective tissue differentiation suggested to be the basis for this disease (Peterson et al., 1966; Waldmann & McIntire, 1972); however, it is not immediately apparent how to correlate our findings with the abnormal DNA synthesis associated with A–T. Several possibilities could explain these seemingly diverse phenomena: (1) Components of the extracellular matrix could have additional functions in chromatin. This is consistent with the high binding affinity of fibronectin for DNA and its reported presence in the nucleus (Zardi et al., 1979). Glycosaminoglycans, which have been suggested to modulate fibronectin binding on the cell surface (Perkins et al., 1979; Oldberg & Ruoslahti, 1982), have also been reported by several investigators to be located in the nucleus (Stoddart, 1979; Furukawa & Bhavanandan, 1982) and appear to influence the rate of DNA synthesis in isolated nuclei (Coffey et al., 1974; Furukawa & Bhavanandan, 1982). Evidence for the presence of fibronectin or glycosaminoglycans in the nucleus must be reviewed critically, however, because of the difficulty in separating the nucleus from the cytoskeleton and the fibronectin associated with it (Brown et al., 1976; Hynes et al., 1976; Hodge et al., 1977; Staufenbiel & Deppert, 1982). (2) Alterations on the cell surface may influence the cellular response to DNA damage. Numerous reports now demonstrate the importance of cell membranes in determining sensitivity of cells to DNA damage (Szumiel, 1981). In addition, the close association of the extracellular matrix, cytoskeleton, and nuclear matrix has led to the suggestion that the external architecture can lead directly to alterations in the nucleus (Penman et al., 1981; Bissell et al., 1982). (3) Abnormalities in protein secretion and the extracellular matrix may not be directly involved with the defects in DNA synthesis but may be independent secondary consequences of the actual mutation. Our results could solely reflect differences in the types of cell strains isolated from A–T patients and the large variation in production of fibronectin observed in different cell types (Yamada et al., 1976). This may be more consistent with the finding that A–T lymphoblastoid cells are also sensitive to X-rays (Chen et al., 1978), despite the absence of detectable fibronectin in these cells (personal observation; Hynes, 1981a); however, other cell surface components and their interrelationships are critical in proper lymphocyte response and are closely associated with nuclear function (Hume & Weidemann, 1980). Surface changes could also explain the impaired A–T lymphocyte response to phytohemagglutinin activation (Cohen & Simpson, 1982).

Although Fanconi's anemia fibroblasts appear to produce normal amounts of the A–T-enhanced proteins, xeroderma pigmentosum group A fibroblasts also synthesize increased amounts of these proteins, although to a lesser degree than do A–T cells (personal observations). This may reflect the

similarities in these two genetic diseases, as already indicated by the numerous clinical features they have in common (Kraemer, 1977). The situation is further complicated by the heterogeneity among the A–T strains themselves; fibronectin accumulates in all four A–T strains in confluent cultures, but one of them (AT3BI) does not show enhanced production. Because complementation analysis has detected multiple subgroups in A–T (Murnane & Painter, 1982; Jaspers & Bootsma, 1982b), these differences in protein synthesis among cell strains are not unexpected and may further demonstrate the heterogeneity of this syndrome.

Acknowledgments

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References

Ali, I. U., Mautner, V., Lanza, R., & Hynes, R. O. (1977) *Cell (Cambridge, Mass.) 11*, 115–126.

Bissell, M. J., Hall, H. G., & Parry, G. (1982) *J. Theor. Biol.* (in press).

Blumenthal, A. B., Kriegstein, H. J., & Hogness, D. S. (1973) *Cold Spring Harbor Symp. Quant. Biol. 38*, 205–223.

Bridges, B. A., & Harnden, D. G., Eds. (1982) *Ataxia-Telangiectasia*, Wiley, New York.

Brown, S., Levinson, W., & Spudich, J. A. (1976) *J. Supramol. Struct. 5*, 119–130.

Chen, P. C., Lavin, M. F., & Kidson, C. (1978) *Nature (London) 274*, 484–486.

Coffey, D. S., Barrack, E. R., & Heston, W. D. W. (1974) *Adv. Enzyme Regul. 12*, 219–266.

Cohen, M. M., & Simpson, S. J. (1980) *Cytogenet. Cell Genet. 28*, 24–33.

Cohen, M. M., & Simpson, S. J. (1982) in *Ataxia-Telangiectasia* (Bridges, B. A., & Harnden, D. G., Eds.) pp 203–218, Wiley, New York.

Edwards, M. J., & Taylor, A. M. R. (1980) *Nature (London) 287*, 745–747.

Furukawa, K., & Bhavanandan, V. P. (1982) *Biochim. Biophys. Acta 697*, 344–352.

Hayman, E. G., Engvall, E., A'hearn, E., Barnes, D., Pierschbacher, M., & Ruoslahti, E. (1982) *J. Cell Biol. 95*, 20–23.

Hodge, L. D., Mancini, P., Davis, F. M., & Heywood, P. (1977) *J. Cell Biol. 72*, 194–208.

Houldsworth, J., & Lavin, M. F. (1980) *Nucleic Acids Res. 8*, 3709–3720.

Hume, D. A., & Weidemann, M. J. (1980) *Mitogenic Lymphocyte Transformation*, Elsevier/North-Holland, New York.

Hynes, R. O. (1981a) in *Cell Biology of Extracellular Matrix* (Hay, E. D., Ed.) pp 295–334, Plenum Press, New York.

Hynes, R. O. (1981b) *Cell Surf. Rev. 7*, 97–136.

Hynes, R. O., Destree, A. T., & Mautner, V. (1976) in *Membranes and Neoplasia: New Approaches and Strategies* (Marchesi, V. T., Ed.) pp 189–201, Alan R. Liss, New York.

Jaspers, N. G. J., & Bootsma, D. (1982a) *Mutat. Res.* (in press).

Jaspers, N. G. J., & Bootsma, D. (1982b) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2641–2644.

Kapp, L. N., & Painter, R. B. (1981) *Biochim. Biophys. Acta 656*, 36–39.

Kraemer, K. H. (1977) in *DNA Repair Processes* (Nichols, W. W., & Murphy, D. G., Eds.) pp 37–71, Symposia Specialists, Miami, FL.

Matus, A., Pehling, G., Ackermann, M., & Maeder, J. (1980) *J. Cell Biol. 87*, 346–359.

Murnane, J. P., & Painter, R. B. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1960–1963.

Murnane, J. P., Byfield, J. E., Ward, J. F., & Calabro-Jones, P. (1980) *Nature (London) 285*, 326–329.

Murnane, J. P., Byfield, J. E., Chen, C. T., & Wang, C. H. (1981) *Biophys. J. 35*, 665–676.

Nishimoto, S. K., Kajiwara, T., & Tanzer, M. L. (1982) *J. Biol. Chem. 257*, 10558–10561.

O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.

Oldberg, A., & Ruoslahti, E. (1982) *J. Biol. Chem. 257*, 4859–4863.

Olden, K., & Yamada, K. M. (1977) *Cell (Cambridge, Mass.) 11*, 957–969.

Painter, R. B. (1980) *J. Mol. Biol. 143*, 289–301.

Painter, R. B., & Young, B. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7315–7317.

Paterson, M. C. (1978) in *DNA Repair Mechanisms* (Hanawalt, P. C., Friedberg, E. C., & Fox, C. F., Eds.) pp 637–650, Academic Press, New York.

Paterson, M. C., Smith, B. P., Lohman, P. H. M., Anderson, A. K., & Fishman, L. (1976) *Nature (London) 260*, 444–446.

Penman, S., Fulton, A., Capco, D., Ben Ze'ev, A., Wittelsberger, S., & Tse, C. F. (1981) *Cold Spring Harbor Symp. Quant. Biol. 46*, 1013–1028.

Perkins, M. E., Ji, T. H., & Hynes, R. O. (1979) *Cell (Cambridge, Mass.) 16*, 941–952.

Peters, K. E., & Comings, D. E. (1980) *J. Cell Biol. 86*, 135–155.

Peterson, R. D. A., Cooper, M. D., & Good, R. A. (1966) *Am. J. Med. 41*, 342–359.

Staufenbiel, M., & Deppert, W. (1982) *Exp. Cell Res. 138*, 207–214.

Stoddart, R. W. (1979) *Biol. Rev. Cambridge Philos. Soc. 54*, 199–235.

Szumiel, I. (1981) *Adv. Radiat. Biol. 9*, 281–321.

Taylor, A. M. R., Harnden, D. G., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Stevens, S., & Bridges, B. A. (1975) *Nature (London) 258*, 427–429.

Towbin, H., Staehlin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Uchida, N., Smilowitz, H., & Tanzer, M. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1868–1872.

Vaheri, A., & Ruoslahti, E. (1975) *J. Exp. Med. 142*, 530–535.

Waldmann, T. A., & McIntire, K. R. (1972) *Lancet*, 1112–1115.

Yamada, K. M. (1981) in *Cell Biology of Extracellular Matrix* (Hay, E. D., Ed.) pp 95–114, Plenum Press, New York.

Yamada, K. M., Yamada, S. S., & Pastan, I. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1217–1221.

Zardi, L., Siri, A., Carnemolla, B., & Santi, L. (1979) *Cell (Cambridge, Mass.) 18*, 649–657.