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This Week's Citation Classic

Lazarides E & Weber K. Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. Nat. Acad. Sci. US* **71:**2268-72, 1974. [Cold Spring Harbor Lab., Cold Spring Harbor, NY]

This paper describes a technique for the production of antibodies against cytoplasmic actin using as an antigen actin purified by sodium dodecyl sulfate (SDS) gel electrophoresis. The antibodies were then used in indirect immunofluorescence to reveal for the first time the cytoplasmic distribution of actin filaments in cells grown in tissue culture. [The *SCI*® indicates that this paper has been cited in over 605 publications since 1974.]

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"When Klaus Weber and I moved to Cold Spring Harbor from Harvard University, where I was a graduate student, we were fortunate to fall into the company of two scientists well versed in the problems of cell biology: Bob Pollack and Bob Goldman. Through numerous discussions with both of them, it became evident that what was needed in cell biology was a new way of studying cell structure and cell motility.

"From earlier work, it was evident that non-muscle cells contained actin and myosin, but the big question was where. Of course the choice would have been localization through antibodies, except that in the past a number of investigators had tried to raise antibodies against actin purified by conventional techniques but had failed, resulting only in the generation of antibodies to other antigens contaminating the actin preparation. This was presumably because actin was highly conserved and hence poorly immunogenic in its native state. I began to characterize what I thought was a new structural cytoplasmic protein from cells grown in tissue culture, trying to meticulously avoid actin. The protein was finally purified and characterized but I didn't know what it was or where it was in the cell.

"I got busy and started to purify enough of this protein to raise antibodies against it, but I realized that it was an uphill battle. I decided to purify enough of this protein from mouse 3T3 cells by sodium dodecyl sulfate (SDS) gel electrophoresis and in the process to also purify some actin from these cells by the same technique to use as a negative control, since actin was so poorly immunogenic. Fred Miller, at the State University of New York in Stony Brook, was kind enough to do the immunizations for me. Several weeks later, we tested the sera using microcomplement fixation. The serum of the unknown protein I had purified unfortunately had no activity, but to our surprise the actin antiserum did. We then tested the actin antibodies by immunodiffusion and got a weak, but nonetheless real, precipitin line. Encouraged, we decided it would be worthwhile to do some immunofluorescence with these antibodies to see if we could detect any fluorescence.

"On December 24, 1973, Art Vogel, then a graduate student with Pollack, and I found ourselves late at night in the lab. Art was about to do some immunofluorescence for the SV40 T antigen and I asked him whether he could spare two coverslips to test my two antibodies. Two hours later, around midnight, we had a look in the fluorescent microscope. The antibody of the unknown protein gave essentially no fluorescence, but what the actin antibody revealed was indeed unbelievable. What a unique Christmas present. In the days to come, important suggestions on how to improve the immunofluorescence technique came from Pollack and Goldman and within two months Jim Watson communicated for us the first paper to the *Proceedings of the National Academy of Sciences.* This was soon followed by a set of papers showing that the filament bundles revealed by the actin antibodies coincided with the classically known microfilament bundles,[1] and contained also other known muscle proteins such as myosin,[2] tropomyosin,[3] and α-actinin,[4] further establishing the reality of the images revealed by the antibodies.

"The combination of SDS gel electrophoresis as a means of purifying structural proteins to be used subsequently as antigens, and the use of immunofluorescence as an assay for the cytoplasmic localization of these proteins provided a new methodological and conceptual approach to cell structure and motility. I think that this paper is quoted more often as being the first to demonstrate the distribution of actin using the immunofluorescence technique. For a recent review see reference 5."

1. **Goldman R D, Lazarides E, Pollack R & Weber K.** The distribution of actin in non-muscle cells. The use of actin antibody in the localization of actin within the microfilament bundles of mouse 3T3 cells. *Exp. Cell Res.* **90**:333-44, 1975.
[The *SCI* indicates that this paper has been cited in over 205 publications since 1975.]
2. **Weber K & Groeschel-Stewart U**. Myosin antibody: the specific visualization of myosin containing filaments in non-muscle cells. *Proc. Nat. Acad. Sci. US* **71**:4561-5, 1974.
[The *SCI* indicates that this paper has been cited in over 270 publications since 1974.]
3. **Lazarides E.** Tropomyosin antibody: the specific localization of tropomyosin in non-muscle cells. *J. Cell Biol.* **65**:549-61, 1975.
[The *SCI* indicates that this paper has been cited in over 185 publications since 1975.]
4. **Lazarides E & Burridge U**. α-Actinin: immunofluorescent localization of a muscle structural protein in non-muscle cells. *Cell* **6**:289-98, 1975.
[The *SCI* indicates that this paper has been cited in over 195 publications since 1975.]
5. **Weeds A.** Actin-binding proteins—regulators of cell architecture and motility. *Nature* **296**:811-16, 1982.