General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.

- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.

- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.

- This document is paginated as submitted by the original source.

- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

Produced by the NASA Center for Aerospace Information (CASI)
EFFECTS OF GRAVITY ON CONTRACTILE PROTEINS

Final Report, 1 Jan. 1978 - 31 May 1979 (Houston Univ.)

Henry R. Henney, Jr.

University of Houston

Final Report

NAS 9-14857

June 1, 1978 to May 31, 1979

Henry R. Henney, Jr.
Abstract

A method was established for the isolation and purification of nuclei in high yield from the microplasmodia of Physarum flavicomum. Purified nuclei were resistant to breakage by methods commonly employed for isolated plant and animal nuclei. Incubation of nuclei with 5 mM Dithiothreitol at pH 9.2 was found to be the simplest and most effective method for breaking the nuclei. Several methods for the extraction of nuclear protein were compared. Incubation of nuclear lysates with either 2 M NaCl, with or without 5 M urea, or 1 M CaCl₂ resulted in the extraction of nuclear actin together with histones. The histones were chemically fractionated into the 5 basic groups common to other eucaryotic tissue. Amino acid analyses of the total histone were also performed. Nuclear actin was found to have a molecular weight of 41,000 ± 4,000 daltons as determined by SDS polyacrylamide gel electrophoresis. The amino acid composition of the nuclear actin was established.
Introduction

The myxomycete Physarum flavicolum is a eucaryotic organism with a life cycle exhibiting alteration of generations. The diploid phase consists of a motile syncytial plasmodium, characterized by a natural synchronous nuclear division (Cheung et al., 1974; Gray and Alexopoulos, 1968). Large quantities of the plasmodia can be obtained by growth in pure culture in synthetic media (Henney and Henney, 1968). Thus, the organism provides a unique opportunity for the analysis of the replication and expression of the eucaryotic genome.

As the initial step for such studies, we have established a method for the preparation of purified nuclei in high yield from the plasmodia. In addition, nuclear proteins have been extracted and fractionated and some properties of the major protein classes established.
Materials and Methods

Organism

Diploid microplasmodia of Physarum flavecolum variety 1 were grown in our semidefinite medium (Henney and Henney, 1968) in 9 liter glass bottles as previously described (Teague and Henney, 1973). Microplasmodia were subsequently allowed to settle from the growth medium for 10 to 15 min at 4°C, most of the medium was poured off and the cells collected by centrifugation at 2,000 x g for 5 min at 2°C. The cells were washed by suspension and centrifugation in two volumes of 0.01 M Tris-HCl, pH 7.2 containing 0.25 M sucrose and 0.01 M EDTA.

Isolation of nuclei

The washed packed cells were immediately suspended in 0.01 M Tris-HCl, pH 7.2 containing 0.25 M sucrose, 0.01% Triton X-100 and 0.005 M CaCl$_2$ and homogenized for 2 min at high speed in a Waring blender. All procedures were performed at 4°C. The homogenate was filtered through two thicknesses of milk filters (Rapid Flow, single gauze-faced, Johnson and Johnson Co.) previously saturated with homogenization medium. The filtrate was diluted 20 times the original packed cell volume and 1 filtered. The filtrate in 100 ml quantities was dispensed into round-bottom polycarbonate bottles (250 ml) and underlaid with 10 ml quantities of 0.01 M Tris-HCl, pH 7.2 containing 1 M sucrose, 0.01% Triton X-100 and 5 mM CaCl$_2$. The bottles were centrifuged at 1,000 x g for 10 min in a refrigerated swinging bucket rotor. The yellowish sediment was

The washings were performed in 0.01 M Tris-HCl, pH 7.2 containing 0.01% Triton X-100 and 0.005 M CaCl$_2$ and homogenized for 2 min at high speed in a Waring blender. All procedures were performed at 4°C. The homogenate was filtered through two thicknesses of milk filters (Rapid Flow, single gauze-faced, Johnson and Johnson Co.) previously saturated with homogenization medium. The filtrate was diluted 20 times the original packed cell volume and 1 filtered. The filtrate in 100 ml quantities was dispensed into round-bottom polycarbonate bottles (250 ml) and underlaid with 10 ml quantities of 0.01 M Tris-HCl, pH 7.2 containing 1 M sucrose, 0.01% Triton X-100 and 5 mM CaCl$_2$. The bottles were centrifuged at 1,000 x g for 10 min in a refrigerated swinging bucket rotor. The yellowish sediment was

The washings were performed in 0.01 M Tris-HCl, pH 7.2 containing 0.01% Triton X-100 and 0.005 M CaCl$_2$ and homogenized for 2 min at high speed in a Waring blender. All procedures were performed at 4°C. The homogenate was filtered through two thicknesses of milk filters (Rapid Flow, single gauze-faced, Johnson and Johnson Co.) previously saturated with homogenization medium. The filtrate was diluted 20 times the original packed cell volume and 1 filtered. The filtrate in 100 ml quantities was dispensed into round-bottom polycarbonate bottles (250 ml) and underlaid with 10 ml quantities of 0.01 M Tris-HCl, pH 7.2 containing 1 M sucrose, 0.01% Triton X-100 and 5 mM CaCl$_2$. The bottles were centrifuged at 1,000 x g for 10 min in a refrigerated swinging bucket rotor. The yellowish sediment was
The sedimented nuclei were suspended in twice the original packed cell volume of wash medium containing 0.25 M sucrose, 0.02 M EDTA, 0.5 M KCl and 0.01 M Tris-HCl with a final pH of 6.3. The nuclear suspension was blended at low speed for 1 min and centrifuged at 2,000 x g for 5 min. The washing was repeated about twice more until microscopic examination, after staining with 0.1% Azure C in 0.25 M sucrose, revealed a purity of at least 95%. The concentration of nuclei was determined using a hemacytometer.

Breakage of nuclei

Purified nuclei were virtually unaffected by the following treatments: repeated freezing and thawing; suspension in distilled water or 0.2 mM EDTA, pH 7.2; homogenization in a blender or Dounce homogenizer in hypotonic solutions; or grinding with glass powder or alumina for 30 min. Sonication for 20 min, repeated grinding with dry ice, or passage through a French press at 10,000 psi produced about 70% breakage. A Parr Cell Disruption Bomb at 2,000 psi or incubation with pancreatic lipase for 30 min at pH 8.0 produced about 50% breakage. However, incubation of the nuclei with 5 mM Dithiothreitol (DTT) at pH 9.2 at room temperature for 30 min produced 100% breakage.

Chromatin preparation

The viscous nuclear solution after DTT incubation was dialyzed against 50 volumes of 0.2 mM EDTA, pH 7.2 at 4°C with two additional changes over a period of 24 h. This crude chromatin was centrifuged at 54,000 x g for 15 min at 4°C. The clear viscous material on top was designated as "free chromatin" and the gelatinous opaque material at the bottom was referred to as the "chromatin pellet".
Extraction of chromatin proteins

Three methods of extraction of proteins from chromatin were used. The first method was the extraction of true chromatin with 2 M NaCl or 2 M NaCl and 5 M urea (recrystallized from 95% ethanol). The solution was stirred in the cold for 30 min, centrifuged at 30,000 x g for 18 h at 4°C and the supernatant containing the soluble proteins was dialyzed for 24 h against distilled water and lyophilized.

The second method extracted the chromatin pellet or true chromatin with 1 M CaCl₂ for 24 h at 4°C. After clarification by centrifugation at 50,000 x g for 15 min, a solution of trichloroacetic acid (TCA) was added to produce a 25% concentration, followed by incubation at 4°C for 2 h. After centrifugation at 50,000 x g for 15 min, the precipitate was suspended in cold 0.04 N H₂SO₄ by use of a glass homogenizer. After dialysis against 100 volumes of 0.04 N H₂SO₄ for 24 h at 4°C, the suspension was centrifuged at 50,000 x g for 15 min. The supernatant was dialyzed in the cold against 100 volumes of distilled water and lyophilized.

The third method was the direct extraction of true chromatin or the chromatin pellet with 0.4 N H₂SO₄ for 20 min at 4°C. The sample was centrifuged at 35,000 x g for 15 min, the supernatant saved and the sediment re-extracted. The combined supernatants were dialyzed against distilled water and lyophilized.

Column chromatography

Sephadex G-100 (fine) and G-75 (medium) columns (9 mm x 220 cm) were used at room temperature in the presence of 0.02% sodium azide. Proteins were eluted with 0.05 M Tris at a constant flow rate 6 ml/h. Fractions were monitored for protein at 230 nm using a spectrophotometer.
Electrophoresis
Polyacrylamide gel electrophoresis according to Bennett, et al., (1968) was used to monitor basic nuclear proteins. The separating gels contained 7.5% acrylamide and 6.25% urea. The buffer was 3.2% β-alanine and 0.08% acetic acid, pH 4.4 and a charge of 4 mA/gel was applied for 1.5 h. Basic proteins were separated from actin by preparative electrophoresis in 2.2 x 3 cm gel tubes for 5 h - the basic proteins were collected in a dialysis bag. SDS polyacrylamide gel electrophoresis with 10% acrylamide was used to determine the molecular weight of actin (Weber and Osborn, 1967).

Gels were stained for protein in 0.1% Amido Black in 7% acetic acid for 30 min, and destained in a solution of 7% acetic acid - 10% ethanol. Densitometric tracings were prepared with a Gilford linear transport apparatus at 575 nm.

Chemical analyses
Analyses for protein and RNA were as described previously (Henney and Jungkind, 1969). DNA was determined with diphenylamine (Burton, 1968).

Amino Acid analyses
Proteins were hydrolysed and amino acid analyses performed as previously described (Henney and Jungkind, 1969). In addition, spectrophotometric analyses for tyrosine and tryptophan were performed according to Benze and Schmid (1967).

Chemical fractionation of whole histone
The procedure of Oliver et al., (1972) was used to fractionate the histones into four major components (I and II, lysine rich), III (slightly lysine rich), III (very arginine rich), IIb (slightly lysine rich), and IV (slightly arginine rich).
Electron microscopy

Nuclei were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, followed by 1% osmium tetroxide in the phosphate buffer. Other procedures were according to Cheung et al. (1974).
Results

Purified nuclei

The purified nuclei were about 5 μ in diameter (4 to 6 μ range) and the nucleoli occupied about half the nuclear volume. About 1% of the nuclei had two nucleoli. The nuclear membrane was quite elastic since many methods attempted to produce breakage were unsuccessful but did produce volume changes and distortions of the nuclei. The yield of purified nuclei was about \(1 \times 10^3\) nuclei/gram wet weight of microplasmadia. The composition of the nuclei was (picogram/nucleus ± standard deviation): protein 10.50 ± 0.50, RNA 1.59 ± 0.21 and DNA 0.84 ± 0.05.

Figures 1 and 2 present photomicrographs and electron micrographs, respectively, of the nuclear preparations, illustrating the purity and integrity of the organelles.

Chromatin preparation

Regardless of the method employed to break the purified nuclei, the resultant true chromatin (the clear gelatinous supernatant after the 54,000 x g centrifugation) had a typical \(\frac{A_{260}}{A_{280}}\) of about 1.72 with negligible turbidity (i.e., \(A_{260}\) of about 0.04). In addition, the true chromatin represented about 15% of the total nuclear protein.

Nuclear protein extraction

A nuclear protein preparation should have an \(\frac{A_{260}}{A_{280}}\) of less than 1 indicating negligible contamination with nucleic acid. Extraction of true chromatin or the chromatin pellet with the following

- 5 M urea
- 1 M CaCl\(_2\)
- 0.4 M \(\text{H}_2\text{SO}_4\)

\(1 \text{ M HCl}\) followed by TCA precipitation and extraction with
0.04 N \( \text{H}_2\text{SO}_4 \) eliminated the nucleic acid contaminations and resulted in an \( \frac{A_{260}}{A_{280}} \) of about 0.85. Direct extraction of nuclei with 0.04 N \( \text{H}_2\text{SO}_4 \) also produced a clean basic nuclear protein preparation but the protein yield was less than 15. Table 1 summarizes the nuclear protein yields using various extraction reagents. It is clear that the reagents which extracted the most nuclear protein (\( \text{NaCl} + \text{urea} \)) also extracted the most non-histone protein. The latter protein (which we later identified as actin) created a problem in that it collected at the top of the gel during pH 4.4 disc gel electrophoresis. We could eliminate this protein from the histone preparation by preparative electrophoresis and by gel filtration chromatography. Using the latter procedure the actin protein eluted before the histones with a high degree of purity. However, the gel filtration chromatography did not sufficiently separate the histones into their fractions without cross contamination.

Figure 3 presents a tracing of the total histone fraction separated by gel electrophoresis at pH 4.4. The actin protein fraction was eliminated prior to electrophoresis. Table 2 presents the amino acid compositions of the total histone and non-histone (actin) proteins. Tryptophan was not detected in either protein. SDS gel electrophoresis of the nuclear actin revealed it to have a molecular weight of 42,000 + 44,000 Daltons (Figure 4).

The gel electrophoresis set up presented in Figure 5 finally illustrate the separation of the total histone fraction into the

...fractional...
Discussion

P. flavicomon nuclei contain histones which can be fractionated into the same 5 basic groups as are found in all plant and animal tissues (DeLange and Smith, 1971). Amino acid analyses also reveals a similarity in composition to the histones of Tetrahymena pyriformis (Iwai et al., 1965) and P. polycephalum (Bradbury et al., 1973). This is not surprising since histones have been found to be conserved during the course of evolution (DeLange and Smith, 1971, 1972). Some differences in properties and functions of histones can be accounted for by modification of amino acid side chains by acetylation, methylation, oxidation-reduction and phosphorylation. Other differences in histones which can occur between tissues is the relative quantity of each histone fraction. Histones are involved in regulating the conformation as well as the activity of DNA (DeLange and Smith, 1971; Skidmore and Walker, 1973). As organisms become more complex in structure and function, the relative quantities of the various fractions apparently varied rather than their chemical composition since the latter would be less tolerated.

Another essential protein found in all living cells which is also highly conserved is actin (Clarke and Gudich, 1977; Korn, 1976). This study has demonstrated the occurrence of actin as a major protein in the nuclei of P. flavicomon. The molecular weight and amino acid analysis of the nuclear actin are similar to that occurring in the cytoplasm of P. polycephalum plasmodia as well as other cells (Adelman and Taylor, 1969; Adelman, 1977; Nakano and Oosawa, 1968; Nakano and Oosawa, 1970; Goldman et al., 1976). Early studies on cytoplasmic actin of P. polycephalum
(Hatano and Osawa, 1966; Hatano and Ohnuma, 1970) showed that the protein was easily extracted with a neutral salt solution. In this study we found that nuclear actin is also readily extracted together with histones by NaCl₂ (with and without urea) or CaCl₂.

The presence of actin in the nucleus may explain the plasticity, stability and resistance to breakage of isolated diploid nuclei. The contractile protein may be associated with the nuclear membrane, thus giving the membrane a flexible nature to accommodate stress. It is also noteworthy that mitosis in plasmodia is intranuclear in that the nuclear membrane remains intact instead of disintegrating (Gray and Alexopoulos, 1968). Only incubation at pH 9.2 and room temperature with DTT, which has been used to disrupt membranes of other organisms (Epel et al., 1970), was found to achieve complete nuclear and nucleolar breakage.

Actin probably plays other roles in the cell nucleus. Actin may be necessary for the constriction of the nuclear membrane during telophase in intranuclear mitosis. There is also some evidence for the involvement of actin in the movement of chromosomes during mitosis and meiosis (Goldman et al., 1976). In addition, nuclear actin may be associated with chromatin condensation (Goldstein et al., 1977). An understanding of these complex nuclear events may well depend on further characterization of this universally occurring contractile protein.
References


<table>
<thead>
<tr>
<th>Extraction reagent</th>
<th>Source</th>
<th>Method of nuclear breakage</th>
<th>% of total nuclear protein extracted</th>
<th>% non-histone protein (actin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 N H₂SO₄</td>
<td>True chromatin</td>
<td>CO₂</td>
<td>0.58</td>
<td>0</td>
</tr>
<tr>
<td>0.4 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>CO₂</td>
<td>0.50</td>
<td>0</td>
</tr>
<tr>
<td>0.4 N H₂SO₄</td>
<td>True chromatin</td>
<td>DTT</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>0.4 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>DTT</td>
<td>0.50</td>
<td>0</td>
</tr>
<tr>
<td>0.4 N H₂SO₄</td>
<td>True chromatin</td>
<td>Sonication</td>
<td>0.30</td>
<td>0</td>
</tr>
<tr>
<td>0.4 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>Sonication</td>
<td>0.78</td>
<td>0</td>
</tr>
<tr>
<td>2 M NaCl</td>
<td>True chromatin</td>
<td>DTT</td>
<td>16.00</td>
<td>50</td>
</tr>
<tr>
<td>2 M NaCl + 5 M urea</td>
<td>True chromatin</td>
<td>DTT</td>
<td>30.00</td>
<td>75</td>
</tr>
<tr>
<td>1 M CaCl₂; TCA</td>
<td>True chromatin</td>
<td>DTT</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>0.04 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>DTT</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>1 M CaCl₂; TCA</td>
<td>Chromatin pellet</td>
<td>N₂</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>0.04 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>N₂</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>1 M CaCl₂; TCA</td>
<td>Chromatin pellet</td>
<td>N₂</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>0.04 N H₂SO₄</td>
<td>Chromatin pellet</td>
<td>N₂</td>
<td>5.00</td>
<td>10</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Histone (mole %)</td>
<td>Actin (mole %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid (A)</td>
<td>6.1</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>5.4</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>6.9</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic acid (A)</td>
<td>10.1</td>
<td>11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>3.8</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>13.3</td>
<td>12.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>7.5</td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>6.6</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine/2*</td>
<td>trace</td>
<td>trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.7</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>7.3</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.3</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.9</td>
<td>4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine (B)</td>
<td>12.8</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine (B)</td>
<td>3.2</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine (B)</td>
<td>5.6</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/A</td>
<td>1.4</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined as cysteic acid.
Figure 2. Electron micrographs of purified nuclei fixed with
2.5% glutaraldehyde followed by 1% osmium tetroxide in 0.1 M sodium
phosphate, pH 7.4. (A) 9,000 X, (B) 15,000 X.
Figure 3. Densitometric tracing of histones separated by polyacrylamide gel electrophoresis at pH 4.4. The gels were stained with Amido Black and scanned at 575 nm. The arrow indicates the direction of movement of the proteins during electrophoresis.
Figure 4. Molecular weight determination of nuclear actin by SDS gel electrophoresis. The actin was extracted with 2 M NaCl, isolated by gel filtration chromatography, and electrophoresed on 10% acrylamide gels containing 0.1% SDS (Weber and Osborn, 1969). Standards used were:

(a) Bovine serum albumin \(66,000\) daltons  
(b) Ovalbumin \(43,000\) daltons  
(c) Cytochrome C \(12,000\) daltons

The actin sample(d) had a molecular weight of \(41,000 \pm 4,000\) daltons. Insert (D) is a densitometric tracing of the actin sample gel.
Figure 5. Densitometric tracings of isolated histones fractionated chemically by the method of Oliver et al. (1972). (I) very lysine rich; (IIb2) slightly lysine rich; (III) very arginine rich; (IIb1) slightly lysine rich; and (IV) slightly arginine rich.
Abstract

Antibodies were produced against filamentous proteins from haploid and diploid cells of Physarum flavicolumn, as well as rabbit skeletal muscle actin. The optimum conditions were determined for "Ring tests" and immunodiffusion tests and for photography of immunodiffusion test plates. The data reveal that the proteins exhibit common antigenicity.
Introduction

The purpose of these experiments was to produce antibodies against the filamentous proteins from haploid and diploid cells of Physarum flavicolum and rabbit skeletal muscle actin and to determine the antigenic relatedness of these proteins.

Materials and Methods

Young adult New Zealand white rabbits were obtained for the immunization experiments. The following immunization scheme (a modification of the one for the preparation of actin antibody by Lazarides, 1976) was adopted for the production of antibody against actin: days 1 and 8 (0.25 mg administered subcutaneously in Freund's complete adjuvant); days 15, 17 and 19 (0.1 mg administered intravenously); days 22, 24 and 26 (0.2 mg administered intravenously); day 29 (0.5 mg) and day 33 (0.5 mg administered intravenously).

Actin was prepared from diploid microplasmodia and haploid amoeba-swan cells of Physarum flavicolum as well as rabbit skeletal muscle. Rabbits were bled at day 7, boosted on day 14 with 0.2 mg protein and bled subsequently every 5-7 days. They were boosted every 3 weeks.

Sera was precipitated at ammonium sulfate saturation and fractionated against 0.15 M NaCl. 0.2 M NaCl in 1 pI 7.3 and 7.0 at -20°C. The antibody content of the sera was assayed by immune diffusion tests. (Harlow et al., 1977).
Gel diffusion plates were prepared in sterile 60 x 50 mm plastic petri dishes containing 1% agarose and 0.5% sodium azide in phosphate buffered saline (PBS), pH 7.2-7.4. A center hole and 4 circumferential holes were punched into the agar, the plugs removed and the bottom of the hole sealed with a drop of the above agarose solution. The center well was filled with serum and the 4 peripheral wells with antigen solutions; alternately, antigen was placed in the center well and sera in the peripheral wells. Incubation was at 25°C.

Immunodiffusion assays also were conducted under other conditions including: agarose-merthiolate-borate buffered plates; RM-actin in PBS; Rm-actin in Tris-ATP-mercaptoethanol (TAM); Rm-actin in 0.01% sodium dodecyl sulfate (SDS); Rm-actin in urea; Rm-actin in TAM + 0.5 M KCl + 1% Nonidet P-40 + urea.

Additional precipitin tests were conducted in 6 X 50 mm test tubes. These "Ring tests" were conducted by placing antiserum in the bottom of the tube and layering antigen or buffer (control) on top.

Procedures for the photography of immunodiffusion plates were developed. Darkfield type lighting conditions accentuated the precipitin lines. Plus x film was used to record the results.

Results and Discussion

The immunodiffusion assays demonstrated that antibodies were produced against the filamentous proteins from haploid and diploid cells as well as against RM-actin. The "Ring tests" also confirmed the results of the immunodiffusion assays.
RM-actin was best solubilized in 0.5 M KCl + 1% Nonidet P-40.

Figure 1 presents a photograph of an immunodiffusion plate containing RM-actin in the center well and antisera against RM-actin, haploid cell and diploid cell proteins. The presence of precipitin lines indicate common antigenicity of the proteins from the three different sources. These data confirm the concept that these proteins are highly conserved (Ognevetskaya, 1977).
References


Figure 1. Immunodiffusion test.

The center well contained rabbit skeletal muscle actin in TAM + 0.5 m KCl + 15 Ronidet P - 40.

The peripheral wells contained antisera against the following proteins:

1. haploid cell
2. skeletal muscle
3. diploid cell
4. skeletal muscle
5. haploid cell
6. diploid cell