Some Immunochemical Studies on the Mitotic Apparatus of the Sea Urchin.*

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The report by Mazia and Dan (1) of the successful mass isolation of the mitotic apparatus (MA) from sea urchin embryos opened the door to a more direct investigation into its molecular origin. The MA, which is built up largely of proteins (2–5), can originate from either of two basically different sources. The first of these is the synthesis of the MA from very small non-specific units, *i.e.*, amino acids or small polypeptides. The second envisages its formation as the result of the assembly of preexisting molecular subunits present in the unfertilized egg (and synthesized between divisions in growing cells) which undergo little or no modi-

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fication in structure during their incorporation into the definitive MA. One can describe the subunits as the precursor protein to the MA. If the precursor protein can be identified immunochemically, the question of the origin of the MA may be resolved by searching for the corresponding antigen in cells that have not yet entered division. For this purpose antisera were prepared against two antigens. A calcium-insoluble fraction (CIF) (6) of cytoplasmic proteins was obtained from unfertilized eggs and the other antigen was obtained by dissolving isolated MA.

The eggs of the local sea urchin *Strongylocentrotus purpuratus* were used in this study. Both unfertilized and dividing eggs from which the MA were to be isolated were preserved in a 10- to 20-fold excess of 30 per cent ethanol (*v/v*) at −10°C. The unfertilized eggs were then extracted in 0.1 M KCl, and the CIF antigen appeared as a fibrous precipitate when $CaCl_2$ was added to a final concentration of 0.05 M. This antigen was put into solution by dialysis against distilled water at about neutrality. The properties of this fraction are described by Kane and Hersch (6). The reasons for selecting the CIF as an antigen were its highly fibrous nature and its sensitivity to small variations in calcium ion concentration. It was used as an antigen representing the unfertilized eggs and when injected gave rise to the antiserum to unfertilized egg antigens. MA were isolated by the digitonin method (4), which relies upon the digitonin to solubilize selectively the cytoplasmic material enveloping the MA. The MA so liberated were washed by centrifuging them lightly and resuspending several times in distilled water at room temperature. They were dissolved immediately by bringing a concentrated suspension to pH 10.5 with dilute NaOH. The solution was

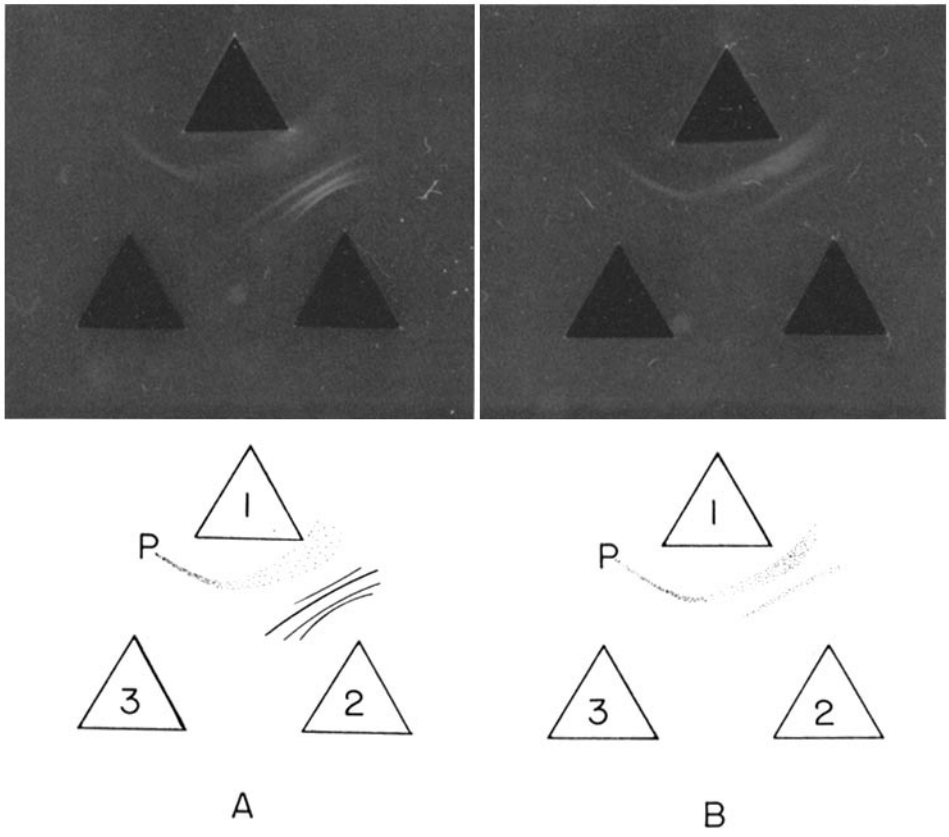

FIG. 1. The behavior of antiserum to unfertilized egg antigens and anti-MA serum toward the same antigens. Beneath each photograph is a line drawing giving the author's interpretation of the corresponding photograph. See the article for details concerning reactants and interpretation of the band patterns.

centrifuged at 100,000 *g* for 5 minutes to remove remaining cytoplasmic debris. The supernatant represented the antigen used for injection. Each antigen was administered intravenously to rabbits in three injections at weekly intervals, and antibody-containing serum was prepared from each animal about a week after the final injection. The CIF antigen solution was injected at a concentration of 5 to 20 mg. protein/injection, while the concentration of the dissolved MA was 2 to 10 mg. protein/injection. The Ouchterlony gel diffusion technique employed was based upon the description by Oudin (7).

In Fig. 1 can be seen the results of an experiment in which the antiserum to unfertilized egg antigens was compared to the anti-MA serum. Wells 2 and 3 of each plate contained, respectively, a 0.1 M KCl extract of unfertilized eggs and a solution of dissolved digitonin-isolated MA. Well 1 of Fig. 1 *A* contained antiserum to unfertilized egg antigens, while that of Fig. 1 *B* contained anti-MA serum. In Fig. 1 *A* can be seen a single band (the P band) assignable to well 3, which contained dissolved MA. This band can be seen to fuse with a broader, more diffuse band associated with well 2. This behavior indicates an immunochemical identity between the antigens responsible for these bands and is strong evidence in support of the precursor concept of the origin of the MA, according to which there is present in the unfertilized sea urchin egg a protein immunochemically identical to a (the) protein present in a solution of dissolved MA. Had the MA been formed from small, non-specific units, one would not expect to find in the unfertilized egg a protein identical to the antigen detected in the solution of dissolved MA. The four remaining bands assignable to well 2 indicate a multiplicity of antigens in the 0.1 M KCl extract of unfertilized eggs, and in the CIF used to prepare the antiserum in well 1. It appears, furthermore, that the dissolved MA solution was lacking these antigens. In Fig. 1 *B* the P band associated with well 3 fuses with a band assignable to well 2. It can also be seen that, as in Fig. 1 *A*, the only band assignable to well 3 is the P band. From this it is apparent that the anti-MA serum contains only a single antibody species directed against dissolved MA, indicating that the dissolved MA solutions used in immunizing the rabbit contained only a single major antigen. This supports earlier indications that the MA were relatively homogeneous in protein composition. The appearance of the second band in Fig. 1 *B* assignable to well 2 does, however, suggest the presence of trace amounts of other antigenic material in the immunizing solutions.

Digitonin-isolated MA dissolved in mersalyl (8) and dissolved MA that had been isolated directly from living material (9) both proved to be immunochemically indistinguishable from the dissolved MA solution used in Figs. 1 *A* and *B*. Occasionally a solution of dissolved MA gave rise to more than one band, but the P band was invariably present and the appearance of the other bands could be correlated to contamination of the isolated MA with cytoplasmic particles as determined by microscopical examination. The multiplicity of bands assignable to well 2 of Fig. 1 *A* suggests that the immunizing CIF antigen solution was not homogeneous. This is not incompatible with the observations of Kane and Hersch (6) on the apparent physical homogeneity of CIF solutions, for the immunochemical criteria are more sensitive than the physical criteria used by them. The properties of two other antisera were also investigated. Antisera obtained after injecting a whole homogenate of unfertilized eggs and the supernatant fraction remaining after precipitating the CIF from a 0.1 M KCl extract of unfertilized eggs each behaved in the same manner toward the precursor protein as did the antisera used in Fig. 1. That is, the precursor band was always present when these antisera were tested against unfertilized egg antigens and dissolved MA. To obtain the four different antisera used, one rabbit each was immunized with a different antigen solution except for the CIF solution which was used to immunize three rabbits. The antisera from the latter rabbits were qualitatively indistinguishable from each other. The band patterns were highly reproducible from one experiment to another.

The MA *in situ* is not soluble in 0.1 M KCl under the conditions used to extract the precursor protein. Therefore we may view the assembly as the polymerization of the precursor protein molecules that does not affect their immunochemical structure.

A more detailed account of this investigation will be published at a later date.

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