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LETTER

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Giuseppina Ortolani (1951–2009): A "grande dame" in ascidian embryology

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Giuseppina Ortolani, colloquially known as "Pina," was born in Palermo, May 7, 1917 (Figure 1). After completing secondary school, she graduated in Natural Sciences in 1943, in the Zoology and Comparative Anatomy Institute in Palermo, under the direction of Prof Andrea Giardina. In 1948, she obtained a second degree in Pharmacy, following in her father's footsteps, with a very advanced thesis for that time, demonstrating the toxicity of DDT by contact and inhalation. She used to recount that initially she was not particularly attracted by research, but she was much more interested in sport activities, such as basketball, volleyball, and swimming.

Nevertheless, starting from 1951, she left the family activity in the pharmacy and went back to the University, at the Institute of Zoology where Prof Giuseppe Reverberi had set up a Laboratory of Experimental Embryology, one of the most advanced and equipped research laboratories devoted to studies of ascidians.

From 1951 until 1964, first as assistant and then as full professor of Zoology, she was a regular visitor of the Zoological Station in Naples. There she established friendship and collaborations with international colleagues. In 1959, she worked at the Laboratoire Arago of Banyuls sur Mer, France. In 1964–1965 she was at the Station de Zoologie Expérimentale de l'Université de Genève, Switzerland, where she participated in the great season of research on nuclear transplants. She worked with Prof Michel Fischberg, the PhD supervisor of Prof John Gurdon, making nuclear transplants from somatic blastula cells of *Xenopus laevis laevis* in enucleated unfertilized eggs of *Xenopus laevis petersi* (Ortolani et al., 1966).

MAJOR CONTRIBUTIONS TO THE FIELD

From the beginning, Ortolani demonstrated an exceptional ability in manipulating the small ascidian embryos. From 1952 to 1955, she published several papers about the localization of the presumptive territories in early embryos (8–16 cells) of the ascidians *Ascidiella aspersa* and *Phallusia mammillata* using the method of carbon marks (Spratt, 1946). Afterwards, she started to use colored chalk marks on the small blastomeres of the 64-cell embryos and established the definitive cell-lineage of individual blastomeres until this stage. In further experiments, she was able to trace the presumptive territories of brain, sense organs, spinal cord, and palps from eight-cell embryos to the tadpole stage (Figure 2) (Ortolani, 1952, 1955a, 1957a). Then, in collaboration with her contemporary Nunzia Farinella Ferruzza, she established that, at Stage 64, the cells committed to form notochord were six, which was in disagreement with the four cell precursors reported in Conklin's ascidian lineage. They removed all six notochord precursors obtaining incomplete larvae or defective embryos lacking notochords (Figure 3). In further experiments, the isolated single chordal cells were transplanted under an animal quartet and rather complete larvae were obtained. This great bulk of experiments definitely showed that "The formation of the neural system in the ascidians is strictly directed by the same laws that Spemann discovered in the amphibians. Only slight differences are to be noticed between the inductive system in the amphibian and in the ascidian embryos: in the amphibians, the inductor is chorda-mesoderm, in the ascidians it is (part of) the chorda-entoderm." These results were reported in a highly cited paper (Reverberi et al., 1960) and were confirmed 25 years later by Nishida (1997) in *Halocynthia roretzi* embryos.

In papers published in 1955–1957, Ortolani described the effects caused by the treatment of unfertilized eggs with trypsin as "displacements on the surface at fertilization different from the untreated eggs, differences in shapes of the embryos and multiple presence of pigment spots." (Ortolani, 1955b, 1957b). Then she treated the isolated animal quartet at Stage 8 with low trypsin concentration (50 μg/mL) for 15 min. The a-line cells of the animal quartet were induced to differentiate into disordered neural-like cells and into pigmented cells. It was the first hint that an alteration of the membrane components could stimulate differentiation in competent cells. These experiments were completed and published later in collaboration with Eleonora

(a)

Segnatura del fascicolo: ASUPA, Didattica, Scienze, Carriera scolastica degli studenti (1932 - 1954), b. 4668, fasc. Ortolani, Giuseppa; «su concessione dell'Università degli Studi di Palermo, Archivio storico di Ateneo

(b)

FIGURE 1 (a) Notarized photograph of Prof Ortolani, written in Italian. The accompanying description translates as "This photograph, accompanied by the mark of my personal seal, is that of Doctor Ortolani Giuseppa, daughter of Giuseppe and of Margherita Miceli, born on May 7th, 1917 in Palermo, where she resides and is personally known by me. Released for academic use in Palermo, on March 12th, 1944. (From concession of the Historical Archive University of Palermo)." (b) G. Ortolani and F. De Bernardi, 1988.

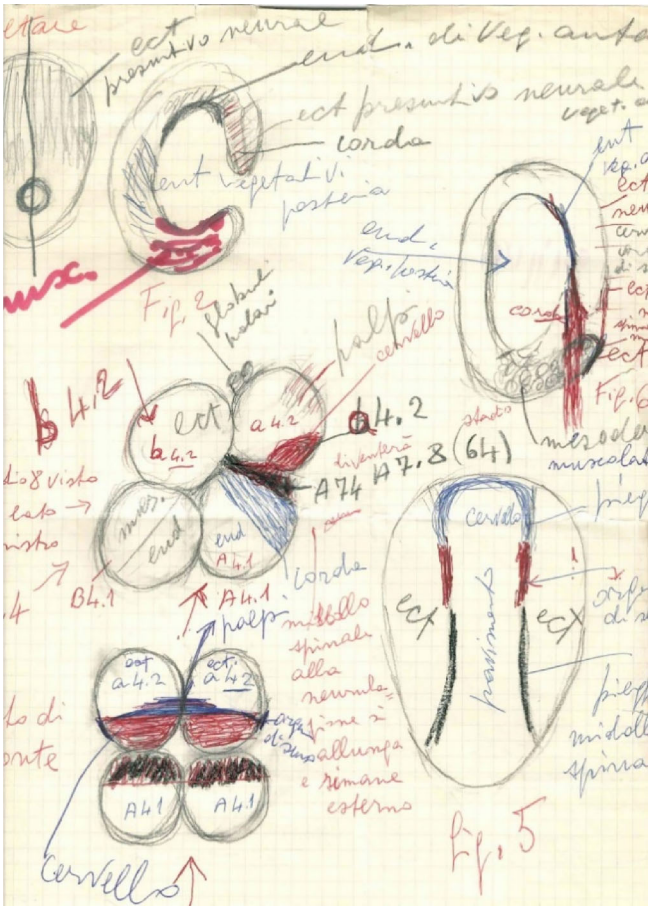

FIGURE 2 Original drawings and notes on ascidian lineage and early development by Prof Ortolani.

Patricolo and Caterina Mansueto, who performed histochemical localization of tyrosinase and electron microscopy analysis of the differentiated cells (Ortolani et al., 1979).

EXPERIMENTAL GYNOGENESIS

After her stint in the Fischberg lab in Geneva, Ortolani conceived a new experimental design to study gynogenesis in ascidians. Gynogenesis is a form of parthenogenesis that requires the presence of sperm without the actual contribution of its DNA for embryo development. Ascidians are normally amphigonic, but experiments by Reverberi (1931) suggested the possibility that gynogenesis could be induced, even though fully developed larvae had never been obtained. To confirm this hypothesis, Ortolani cut off the vegetal pole of *Ascidia malaca* eggs soon after the entry of the sperm, thus eliminating the male pronucleus and endodermal determinants. She obtained vital, small larvae, thus confirming the possibility that gynogenesis could be induced experimentally. Moreover, she fertilized *A. malaca* eggs with heterospecific sperm of *Ciona intestinalis*. The cytological analysis of the resulting larvae revealed that the number of chromosomes was 16, as in *A. malaca*, indicating that the contribution of the sperm to development was limited to the centrosome (Figure 4).

During her additional stays in Naples, Giuseppina started to manipulate the eggs and embryos of Ctenophores. Exploiting the knowledge of the first developmental stages of the ascidian embryos, her ability in separating blastomeres and in marking the blastomeres with chalk granules, she was able to establish a precise cell lineage of the Ctenophore embryo. This research was carried out during the course of many years, and only in 1989, she published a complete review (Ortolani, 1989). In particular, she definitely established the origin of the muscles of the tentacles from the lateral mesodermal micromeres, a deeply debated subject at that time.

As head of the Zoological Institute of Palermo after the retirement of Prof Reverberi in 1972, she supported and generously promoted other research fields, even different from her own interest, from immunobiology to marine ecology and entomology.

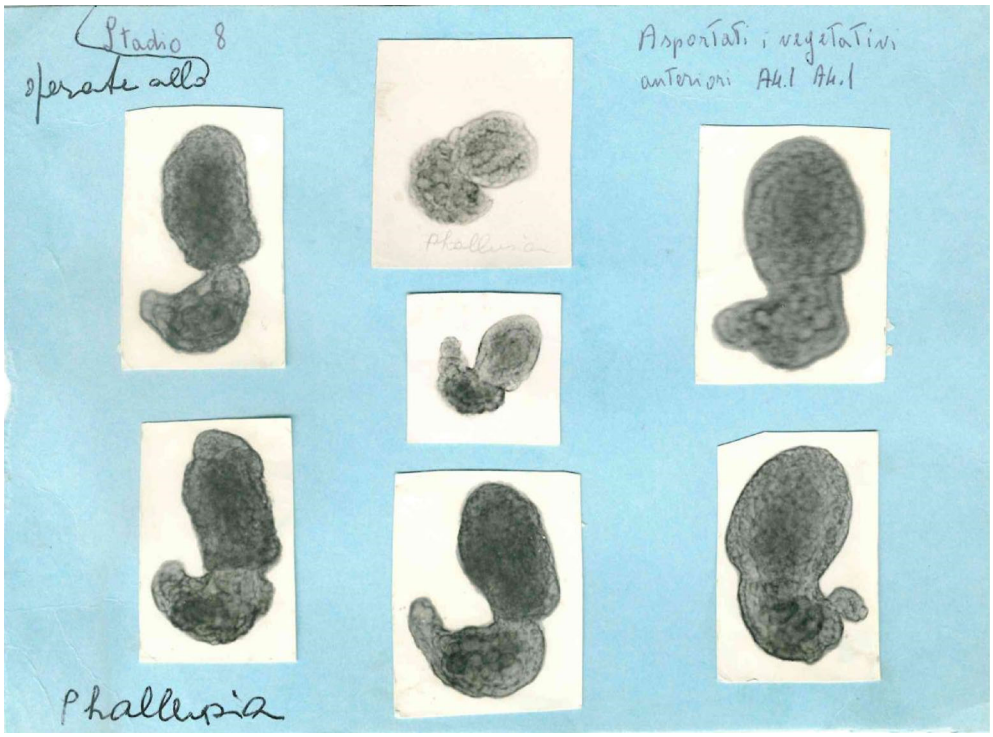

FIGURE 3 Original photos of incomplete *Phallusia* larvae developed after removal of the notochord precursors at the eight-cell stage.

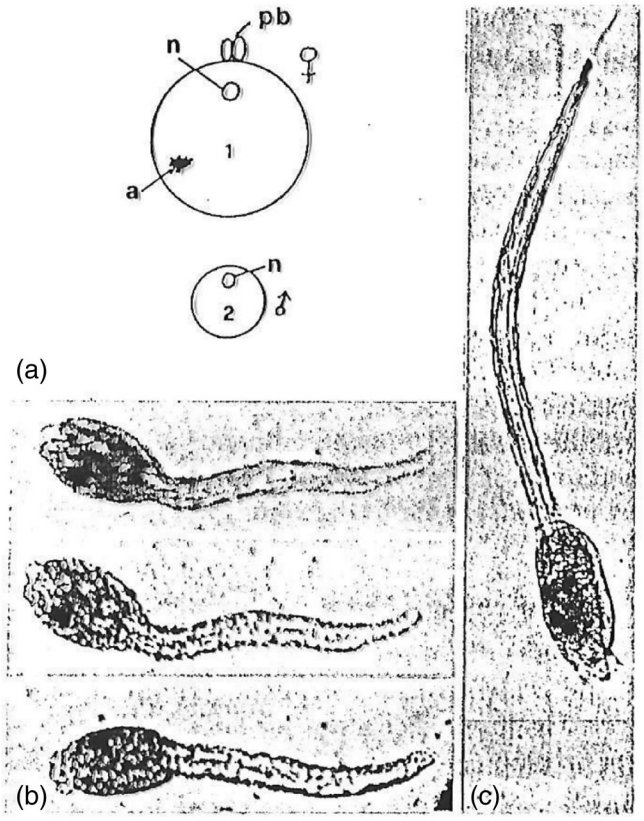

FIGURE 4 Experimental gynogenesis in ascidians. (a) The egg is cut shortly after the sperm entry, to prevent the fusion of the nuclei. n, nucleus; pb, polar bodies. The letter a indicates the sperm entry point. (b) The larger animal fragments containing the centrosome develop normally into complete, albeit smaller, larvae. (c) Normal larva (from Dr. Ortolani's lecture held at the Department of Biology, University of Milano, 1992).

In 2003, during the International Tunicate Meeting, Dr Nori Satoh, in his opening lecture "*Let's move on ascidian biology with new ideas*," made an historical review of the ascidian researches and recalled the major scientists in this field: Chabry, Conklin, Reverberi, and Ortolani. This historic talk was delivered in the presence of some of the most respected ascidiologists, including Hiroki Nishida, William Jeffery, and Christian Sardet, all of which were hosted in Palermo by Pina. Giuseppina Ortolani passed away in September 2009, after a long illness.

A PERSONAL STORY

In 1987, I joined the Zoological Institute of Palermo, attracted by the acquaintance with "Pina" made during many years of common attendance of the meetings of the Italian Embryological Group. She was extremely generous in allowing me to use the facilities of her laboratory, with a fantastic view over the historical Botanical garden. Her enthusiasm for the manipulation of the embryos and eggs of the ascidians was contagious. Very soon, she asked me to study, using immunofluorescence, some endodermal cells containing sulfated acid mucopolysaccharides that in *P. mammillata* and *A. malaca* were located on the sides of the intestine. In fact, first by in situ hybridization with a beta-tubulin probe, and later on with an anti-tubulin antibody and confocal laser microscopy, we noticed the presence of many microtubules in the pseudopodes of these cells, which at metamorphosis reached the base of the adhesive papillae and migrated through the epidermis (De Bernardi et al., 1991, Sotgia et al., 1993). From that moment on, I have been working for more than 20 years with ascidian embryos, focusing on the larval nervous system and adhesive papillae, and I feel like an "unofficial" alumna of "Pina."

DR ORTOLANI'S FEMALE TRAINEES WHO CONTINUED WORKING ON TUNICATES

Nunzia Farinella Ferruzza was a contemporary and first collaborator of Ortolani in the Reverberi's lab. She was a very gifted morphologist whose "magic" fingers allowed her to micromanipulate eggs and embryos.

Together with Ortolani, she studied the neural induction after the repositioning of chordal and endodermal cells, LiCl effect on isolated quartets, fusion of unfertilized eggs and formation of giant, triploid larvae, gynogenesis, and hybrid fertilizations.

Caterina Mansueto participated in the research on the action of trypsin on the membrane and cortex, performing the histochemical localization of tyrosinase. Then she focused on ecotoxicological problems such as the effects of organometallic substances such as tributyltin, a toxic agent contained in antifouling paint, on ascidian embryos and larvae.

Eleonora Patricolo dedicated her studies to the modifications of the egg membrane after fertilization and fine structure of follicle and test cells in ascidians. In 1975–1977, by publishing some papers on the erythrocyte agglutinins and inflammatory-like tunic reaction, she opened a new research field, ascidian immunobiology.

Luisanna Villa was a fine morphologist. Starting in 1992, she applied scanning and transmission electron microscopy to studies of follicle cells in various ascidian species, to the sperm–egg interaction and of the external egg coating during hybrid crosses (*A. malaca* eggs fertilized with *C. intestinalis* sperm). Her last papers were on the polar body formation in *A. malaca*.

ACKNOWLEDGMENTS

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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