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E.E. Just's ''Independent Irritability'' Revisited: The Activated Egg as Excitable Soft Matter

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SUMMARY

Ernest Everett Just's experimental work on post-fertilization events in invertebrate eggs led him to posit a dynamic and directive role for the zygotic ''ectoplasm'' (cortical cytoplasm), in subsequent development. His perspective was neglected during the years that followed his early death not only because of his well-documented marginalization as an African-American in U.S. science, but because his ideas were at odds with the growing gene-centrism of developmental biology in the latter half of the 20th century. This essay reviews experimental work that shows that the egg cortex in many animal groups is a chemically and mechanically active medium that sustains both spatiotemporal calcium ion transients and periodic deformations in the time leading up to cleavage. These wave phenomena are seen to play regulatory roles in germ plasm localization and gene expression, and influence the reliability and success of developmental outcomes. Just resisted vitalistic explanations for the active processes he observed and inferred regarding the egg cortical cytoplasm, but recognized that the physics and chemistry of his time were inadequate to account for these phenomena and anticipated that expansions of these fields would be necessary to explain them. Here again he proved prescient. Late 20th century developments in the physics of ''excitable media'' and ''soft matter'' have provided the bases for models, some of which are described here, of chemical and mechanochemical wave propagation in the activated egg cortex. Lastly, the implications of these post-fertilization phenomena for animal evolution, a problem also addressed by Just, are discussed.

Just was correct in his estimation of the "informational" role of the ectoplasm's dynamics.

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INTRODUCTION

The embryologist Ernest Everett Just, active in the first half of the 20th century, was a staunch critic of a gene-centered view of living systems (Manning, 1983; Gilbert, 1988; Byrnes and Eckberg, 2006). He argued against the notion, increasingly put forward during his time by geneticists, and later to become the conventional wisdom even among developmental biologists, that genes somehow orchestrate the production of living properties. Rather than the nucleus, he considered the ''ectoplasm,'' the cytoplasm just beneath the surface, to be the cell's dynamic heart. He was convinced that the egg cell, his chosen experimental system, ''like many another living cell—nerve or muscle, for example—possesses *independent irritability*,'' that is, the ability to initiate processes and respond in an active fashion to its microenvironment (Just, 1939; p. 237), and described the egg as ''self-acting, self-regulating and self-realizing'' (Just, 1939; p. 237).

Despite such formulations, Just also rejected vitalism, a then fashionable biological philosophy (Sinnott, 1950). He asserted that living things are ''physico-chemical systems'' which ''obey the laws of physics and chemistry'' (Just, 1939; p. 1). He noted, however, that ''physics has grown beyond 'classical physics'" (Just, 1939; p. 14), and speculated that since ''the state of being alive is confined to that organization

which is peculiar to it,'' what might be needed is ''a physics and chemistry in a new dimension . . . superimposed upon the now known physics and chemistry'' (Just, 1939; p. 3).

Indeed, physical scientists during Just's lifetime (he died in 1941) had only a primitive understanding of ''mesoscopic'' systems, that is, materials on a scale between that of atoms and molecules to one side and billiard balls and planets on the other,[1] but that was to change over the next half century. While most mesoscopic materials are nonliving, including those referred to as ''soft matter'' (liquids, foams, gels, colloids and so forth; de Gennes, 1992), animal egg cells and multicellular aggregates, including embryos and organ primordia, are unequivocally soft, mesoscopic materials.

Another type of physical system in which great advances, experimentally, theoretically and computationally, were made over the second half of the 20th century, is known as ''excitable media'' (Mikhailov, 1990; Mikhailov and Loskutov, 1991). Such systems typically store chemical or elastic energy. When stimulated, therefore, they will often respond in an active, nonlinear fashion, ''giving back'' more than they receive. Excitable media can propagate waves of chemical concentration or mechanical deformation, and some can sustain prolonged trains of temporal oscillations in their chemical or mechanical state. Egg cells and multicellular aggregates have the distinction, virtually unique in the physical world, of being simultaneously soft matter and excitable media.

In the next section I summarize the current understanding of chemical and mechanical excitability in the postfertilization eggs of animal species. As Just observed and inferred, the relevant excitable medium is the ectoplasm or what is now referred to as the cortical cytoplasm. The relevant mechanisms, as Just surmised, can be encompassed within the ambit of chemistry and physics, but of a more sophisticated type than was available to scientists of his time. I then present evidence for Just's even more controversial assertions concerning the role of the ectoplasm in determining the course of embryogenesis. It will be seen that Just was equally prescient in this regard: the dynamics of the cortical cytoplasm does indeed impart key information to the egg that influences subsequent development.

My goal is to provide an update of an area of biology that was of major concern to E.E. Just and to show how an intellectual framework of which he was a relatively isolated expositor has now moved to the center stage of developmental biological research. I will conclude by drawing out a few implications of the dynamical approach to development for the understanding of evolution, a connection that was prominent in Just's thinking and in which he was also ahead of his time.

[1]''Mesoscopic'' refers to phenomena on the ''intermediate'' scale, where average and collective properties of a system's subunits (e.g., density, temperature, viscosity) become more useful descriptors than individual behaviors. Although the term is usually applied to macromolecular systems and liquids and solids in which the subunits are atoms and small molecules, it is increasingly being used for developing multicellular systems (see Merks and Glazier, 2005), where the subunits are cells (linear dimension $\sim 10^{-5}$ m) aggregates of which (linear dimension $> 10^{-4}$ m) tend to behave analogously to viscoelastic liquids, for similar physical reasons (reviewed in Forgacs and Newman, 2005).

CHEMICAL AND MECHANICAL OSCILLATIONS IN POSTFERTILIZATION EGGS

In his experimental work Just studied the fertilization of the eggs of marine echinoderms and annelid worms. He discovered what we now call the slow (structural) block to polyspermy, seen in most animal species, and obtained evidence for the fast block to polyspermy that precedes it in nonmammalian eggs (Just, 1939; discussed in Byrnes and Eckberg, 2006). Just correctly inferred that both these phenomena had electrical aspects, discussing them in relation to the behavior of the nerve fiber (''because among animal cells it is the most highly excitable and the most rapidly conducting''; Just, 1939, p. 114).

The fast block to polyspermy was indeed subsequently found to be an action potential (Jaffe, 1976). Just also speculated that the slow block to polyspermy (''[T]he wave -like process of break-down in the ectoplasm by which the membrane is separated from the egg''; Just, 1939, p. 114), depended on nerve-like electrical transmission. We now know that following fertilization, eggs of many animal phyla (molluscs, annelids, nemerteans, ascidians, mammals, among others) immediately respond in an active fashion, exhibiting one or more spatiotemporal calcium ion transients that alter both the electrical and mechanical properties of their membranes (Jaffe, 1999, 2006; Ducibella et al., 2006; Ducibella and Fissore, 2008).[2]

The following events, though not universal in animal species, are typical: concomitant with the fusion of the egg and the sperm the egg's intracellular calcium ion concentration increases approximately 10-fold. This occurs (depending on the species) in the form of one or more traveling waves of elevated Ca^{2+}, which start at the point of sperm entry. The initial effect of the elevated calcium ion concentration is the triggering of the cortical granule reaction, establishing the slow block to polyspermy (Jaffe et al., 2001). The subsequent waves similarly cause major intracellular restructuring and set the stage for later development (Dumollard et al., 2002).

In mammals, the successive waves of calcium ion concentration induce the completion of meiosis, initiation of mitosis, protein synthesis from stored maternal mRNAs (Runft et al., 2002) and surface waves of cortical contractility which Just observed in annelid species. Visualizations of calcium and surface contraction waves in the mouse egg by Deguchi et al. (2000) are shown in Figure 1. Unlike the ion transients associated with the fast block to polyspermy, which derive from the external medium, the source of ions for the ''organizational'' calcium transients is intracellular, most widely believed to be membranous stores (Bugrim et al., 2003), compartments of the endoplasmic reticulum that are under the control of cytoplasmic signals, most notably inositol trisphosphate (IP_3) (Berridge et al., 2000). An alternative model holds that the Ca^{2+} is instead sequestered by F-actin in the cortical cytoplasm itself (Lange and Gartzke, 2006a,b), with IP_3-dependent effects playing a downstream role (Lim et al., 2002). Once the release of

[2]A special issue of Seminars in Cell and Developmental Biology (Vol. 17, issue 2, April, 2006) is entirely devoted to ''Signals and calcium waves at fertilization'' and contains 10 reviews on the topic.

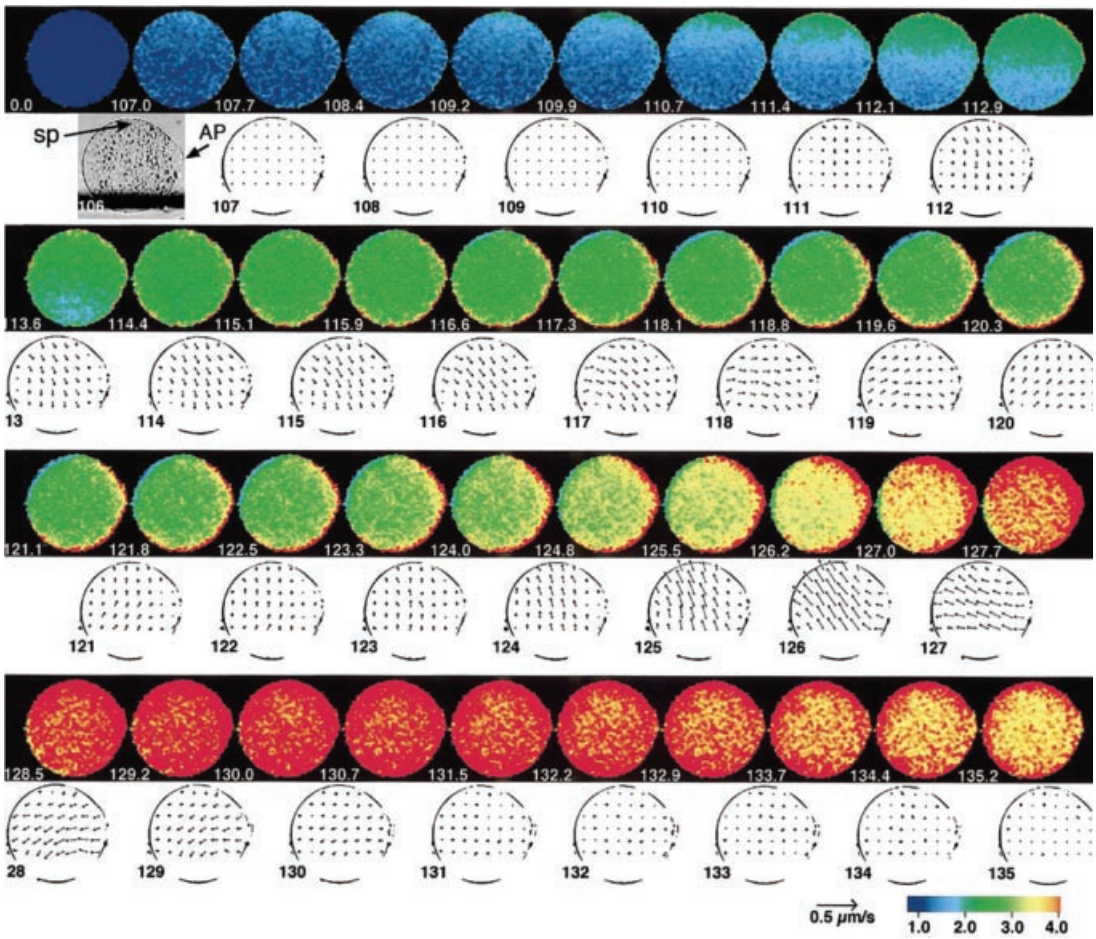

Figure 1. Traveling wave of Ca^{2+} concentration (color) and cytoplasmic movement (black-and-white) during the rising phase of the initial Ca^{2+} wavefront in the mouse egg, following fertilization. The position of the sperm-fusion site and that of the animal pole are indicated by arrows labeled "sp" and "AP," respectively, in the bright-field image (second row, left). The egg was loaded with the Ca^{2+}-sensitive dye calcium green-1 dextran and viewed by fluorescence microscopy. The (pseudo-)colors represent the intensity of the emitted fluorescence relative to a basal value (see bar at the bottom right) obtained from images just before the rise in Ca^{2+} concentration. The number shown in each image is the time (in seconds) of acquisition. The zero of time was when the image for the basal value of fluorescence was taken, before the initial rise in intensity. Arrows in the black-and-white images represent the direction and magnitude of local cytoplasmic velocity detected from the analysis of the time series of bright-field images taken at 0.5 sec intervals (for reference see arrow at the bottom right). After the reference image at 106 sec only the contour of the egg and selected lattice points, approximately 11 μm apart, are shown. (Reprinted from Deguchi et al., 2000, copyright 2000, with permission from Elsevier.)

these ions has been initiated in the fertilized egg, a series of periodic self-sustaining waves of elevated Ca^{2+} concentration travels through the egg's cortical cytoplasm (Kubota et al., 1987; Miyazaki et al., 1993; Eidne et al., 1994; Jones, 1998; Deguchi et al., 2000; Dumollard et al., 2002).

The fertilization Ca^{2+} waves in sea urchins and *Xenopus* are continuous and of well-defined amplitude, rapidly sweeping through the egg (around 30 sec in sea urchin (Hafner et al., 1988) and 2.5 min in *Xenopus* (Fontanilla and Nuccitelli, 1998)). In the ascidian egg, three different Ca^{2+} wave pacemakers exist in a single egg cell (Dumollard et al., 2004b), and in mammals the train of Ca^{2+} waves is even more complex (see Swann and Yu, 2008 for a detailed review). The generation and propagation of these waves occurs when the cytosolic concentration of calcium ions reaches a threshold value, inducing the release of calcium ions, a phenomenon known as Ca^{2+}-induced-Ca^{2+} release. At the threshold, according to the most widely accepted model, IP_3 responsive receptor-channels in the intracellular compartments are opened. These channels are inactivated as the local Ca^{2+} concentration rises further, and subsequently remain closed during a refractory period. Finally, cytosolic Ca^{2+} is resequestered into the endoplasmic reticulum via specialized pumps (Kline and Kline, 1992).

The fertilization calcium waves in mammals are initiated by a sperm-specific enzyme, phospholipase C zeta (PLCζ), which is activated at low Ca^{2+} levels (Malcuit et al., 2006). The enzyme mediates the production of IP_3, which, by releasing Ca^{2+} from stores in the fashion described above, leads to inhibition of its own production (Swann et al., 2006;

Saunders et al., 2007). The resulting activation of Ca^{2+} releasing channels by a threshold concentration of cytosolic Ca^{2+}, followed by the return of Ca^{2+} to intracellular stores, is a manifestation of the egg as a chemically excitable medium (Lechleiter et al., 1991, 1998).

Several theoretical models have been constructed to describe the generation and propagation of calcium oscillations (for a review see Schuster et al., 2002). A relatively simple one that stays close to the known biochemistry is the fire-diffuse-fire (FDF) model of Dawson et al. (1999). (See also Bugrim et al., 1997 for a similar, earlier model and Coombes et al., 2004, for a more recent version.)

In the FDF model the release of calcium from the intracellular stores (or the formally equivalent F-actin depots) is assumed to take place through an array of release sites represented by point sources formally representing storage vesicles with regulated membrane channels. These sites are spaced at a distance d from one another and are embedded in a continuum (the cytosol) in which calcium ions are assumed to diffuse. Release of calcium takes place while a channel is open, which defines the chemical time scale τ. Another time scale (the intersite diffusion time) is defined by d^2/D, where D is the Ca^{2+} diffusion constant. Whenever the cytosolic Ca^{2+} concentration in the vicinity of a release site reaches a threshold value $[Ca^{2+}]_T$ (above the basal concentration $[Ca^{2+}]_B$), the site starts releasing calcium ions at a rate σ/τ. Here σ is the total number of ions released in time τ by a single site. The system can be represented by one or a set of nonlinear reaction–diffusion diffusion equations (see Forgacs and Newman, 2005, for a detailed description of such models) and exhibits (as do the postfertilization eggs of different species) both sweeping and saltatory dynamics, depending on parameter choices.

Accompanying the postfertilization calcium waves in most of the species studied is a set of deformations of the cell surface, including the subjacent cortical cytoplasm. In sea urchins, for example, successive waves of microvilli elongation and stiffening (Cline et al., 1983) propagate with the same directionality and speed as the initial calcium wave (Suzuki et al., 1995). At about 9 min post-fertilization, actin filaments detach from the cortex and translocate away from the surface into the deeper regions of the cytoplasm (Terasaki, 1996). Within 15 min, the egg cortex is transformed from a fairly flat, soft layer studded with short micropapillae (broad microvilli) into a stiff layer containing numerous surface microvilli and a new set of cortical vesicles, replacing the exocytosed cortical granules (Sardet et al., 2002). In *Xenopus* eggs, within minutes after fertilization dynamic actin ''comet tails'' accumulate around intracellular vesicles, which then begin to move through the cytoplasm (Taunton et al., 2000).

In essentially all species the post-fertilization cortical microfilament cytoskeleton (comprised of actin) is reorganized and contracts in a wave-like manner starting from the site of sperm entry. This mechanical motion of the egg cortex is based on its ability to undergo calcium-dependent contraction–relaxation (Roegiers et al., 1995, 1999; Sardet et al., 1998; Benink et al., 2000). The cortical reorganizations and cytoplasmic flows that occur between fertilization and first cleavage—primarily translocation of cortical and subcortical materials parallel to the plane of the plasma membrane (Eidne et al., 1994)—appear to be driven by interactions between the microfilament and microtubule cortical cytokeletons (Canman and Bement, 1997; Benink et al., 2000).

The cortex of the fertilized egg has mechanical and viscoelastic properties different from those of the unfertilized cortex. In the sea urchin, for example, the cores of the newly arising microvilli have microfilament bundles that extend and intermingle with the microfilament meshwork underlying the membrane, thickening the fertilized cortex (Wong et al., 1997). The cortical microfilament network contracts and relaxes during specific phases of the meiotic and mitotic cell cycles, a process which appears to be regulated by microtubules (Mandato et al., 2000). In the starfish, the cortical actin cytoskeleton modulates the generation and propagation of calcium waves (Kyozuka et al., 2008).

Theoreticians have modeled the Ca^{2+} wave-generated shape changes on the egg surface by considering the coupling of chemical and mechanical waves (Cheer et al., 1987; Ballaro and Reas, 2000). Since cellular shape changes are driven by the dynamical rearrangement of the cytoskeleton (e.g., polymerization–depolymerization of the actomyosin network), the mechanochemical model must incorporate not only the changing concentration of Ca^{2+} and the molecules that control its release, sequestration and resequestration (e.g., IP_3, cAMP), but also actin-containing cytoskeletal filaments along with motor proteins that can move these filaments and factors that can cause them to disassemble.

In their model for the coupling of the chemical and mechanical aspects of cortical wave generation, Cheer et al. introduced the additional hypothesis of involvement of osmotic forces. They proposed that at low Ca^{2+} levels actin remains in a gel state, but as the Ca^{2+} level increase actin filaments are broken down and the gel changes into a sol. It is this Ca^{2+}-driven sol-gel transition that they suggested to be responsible for the expansion-contraction waves in the cortex (Cheer et al., 1987; Fig. 2).

The starting point for the dynamical modeling of cortical mechanochemical wave propagation is a reaction–diffusion framework similar to that used for the FDF model. Here the variables in the model denote the concentrations of Ca^{2+}, IP_3, cAMP, the actomyosin network and solation factors. The reaction–diffusion equations are then coupled to expressions for the mechanical properties of the cortex, including the osmotic pressure-driven expansion and contraction of the viscoelastic cortical actomyosin gel as the state of gelation changes with time. The solation factors are activated by Ca^{2+}, the concentration of which changes periodically due to the fertilization wave, causing periodic swelling. Calcium also activates the contractile machinery of the actomyosin system which causes the gel to contract. Solation and swelling being a more rapid process than contraction, the contraction will lag behind the swelling, leading to propagation of a mechanical wave which, because of its coupling to Ca^{2+} oscillations, will be periodic. Simulations confirm that this mechanism can generate spatiotemporal ripples on the surface of a sphere (Cheer

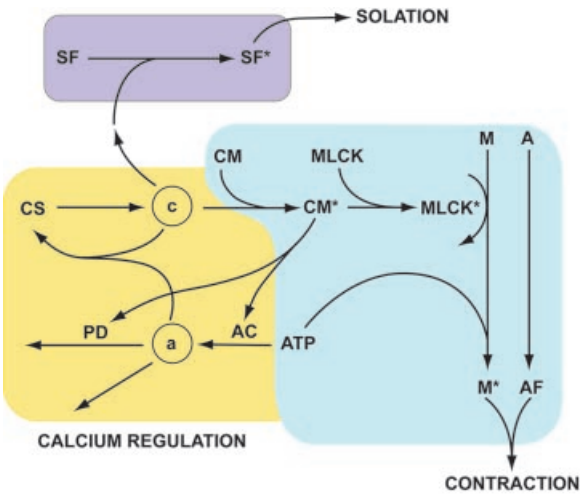

Figure 2. Schematic showing the major chemical steps involved in the solation and contraction kinetics in the model of Cheer et al. (1987). Sequestered calcium (CS) is released autocatalytically from membranous sites. Free calcium (c) follows three pathways: (i) *Resequestration*. The free calcium is resequestered by membranous pumps which require ATP and cAMP (a); the cAMP is produced by adenylate cyclase (AC) and degraded by phosphodiesterase (PD), each of which requires calmodulin/calcium. The cAMP is also utilized in other pathways. (ii) *Solation*. Free calcium binds to solating factors (SF) rendering them active (SF*) which then sever the actin filaments. (iii) *Contraction*. Free calcium binds to calmodulin (CM) inducing a conformational change to its active form (CM*); the activated calmodulin binds to the myosin light chain kinase (MLCK) inducing it to its active form (MLCK*), which then phosphorylates the myosin light chains. Actin subunits (A) assemble to filamentous actin strands (AF) and bind to the activated myosin to form "contractile units." These contractile units shorten to produce traction stresses in the gel.

et al., 1987; also see Forgacs and Newman, 2005 for additional details).

Important elements of the model of Cheer et al. (1987) for fertilization surface waves remain viable, including the essentiality of an actin microfilament cytoskeleton (Benink et al., 2000) and the calcium dependence of contraction (Stack et al. 2006) and disaggregation (Ankenbauer et al., 1988) of the egg's cortical cytoskeleton. While the key role proposed for osmotic pressure has not been confirmed experimentally, related ideas are still under consideration (Charras et al., 2005). An updated model might also incorporate the self-organization of branched actin networks as the generator of surface deformation (Maly and Borisy, 2001; Mogilner, 2009). The approach of Cheer and coworkers nonetheless illustrates the utility of the contemporary physical concepts of soft, excitable materials in generating testable models of the enigmatic cortical phenomena identified by Just.

INFORMATIONAL ASPECTS OF CORTICAL DYNAMICS

Several major postfertilization events in the mouse (cortical granule exocytosis, cell cycle resumption, and recruitment of maternal mRNAs) are initiated by different numbers of Ca^{2+} waves. In mammalian systems the completion of each event requires a greater number of Ca^{2+} waves than its initiation (Ducibella et al., 2002). Moreover, the presence or absence of specific patterns of Ca^{2+} oscillation in fertilized or parthenogenetically activated mouse eggs influence later patterns of zygotic gene expression (Ozil et al., 2006; Rogers et al., 2006).

The mechanisms by which the zygote "interprets" Ca^{2+} oscillations have been relatively elusive. The enzyme Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) appears to play a central role in regulating early events of mouse embryogenesis (i.e., during the first hour) such as cortical granule exocytosis and exit from meiotic metaphase II/resumption of the cell cycle (Abbott and Ducibella, 2001; Tatone et al., 2002; Malcuit et al., 2006). CaMKII oscillates in concert with post-fertilization Ca^{2+} waves (Markoulaki et al., 2003, 2004), but unlike its activity in neurons where it responds to very high frequency Ca^{2+} pulses by remaining active after the signal subsides (De Koninck and Schulman, 1998), in the egg it appears to track the calcium signal with no long-term memory. Several Ca^{2+}-activated protein kinase C isoforms are also involved in the early responses (Eliyahu and Shalgi, 2002; Halet et al., 2004).

Since certain later events such as pronucleus formation and mobilization of maternal messenger RNAs are also dependent on or influenced by the Ca^{2+} oscillations that may have occurred several hours before, the activated egg must exhibit some form of memory (Swann and Yu, 2008). One possible basis for this memory is suggested by the finding that egg mitochondria transform the Ca^{2+} transients into an oscillatory redox cycle with a longer period, thereby representing a persistent record of the earlier events (Dumollard et al., 2004a, 2006, 2007).

Cortical surface waves also impart developmental information. Although the surface contraction waves in embryos of the frog *Xenopus laevis* continue into the cleavage stages (Yoneda et al., 1982; Rankin and Kirschner, 1997), localization of the germ plasm during the first four zygotic cell cycles was shown to depend on post-fertilization surface waves occurring prior to the first cleavage (Quaas and Wylie, 2002).

Surface contraction waves control the onset and duration of cleavage cell cycles in *Xenopus* and the embryos of other organisms, including sea urchin, fruit-fly and mouse, by their role in localizing another cytoplasmic determinant, M-phase promoting factor (MPF) (Beckhelling et al., 2000). This factor has several distinct functions relating to nuclear and chromosomal dynamics, After fertilization it is stored in the perinuclear cytoplasm, placed there in conjunction with, and probably dependent on, early surface contraction waves. In *Xenopus* a relaxation wave and a contraction wave in the cortical cytoplasm precede the first cleavage, accompanied by waves of MPF activation and inactivation. Overall relaxation of the cortex and "rounding up" of the egg is associated with global activation of MPF and the initiation of mitosis (Beckhelling et al., 2000). The mobilization of MPF from pre-localized sites also depends on late-appearing calcium transients in a variety of embryos (Tombes et al., 1992; Wilding et al., 1996).

A series of remarkable cortical and cytoplasmic reorganizations that occur in eggs of ascidians such as *Ciona intestinalis* and *Phallusia mammillata* are circumstantially related to the three Ca^{2+} pacemakers mentioned above and their associated cortical waves (Sardet et al., 2007). During the typical 1 hr between fertilization and the first cleavage a single calcium wave, followed by a microfilament-dependent cortical contraction and a series of repetitive calcium waves, are associated with relocation of several cytoplasmic determinants of body axis organization, gastrulation and organogenesis produced before fertilization. The future dorsoventral axis is determined during this period. Some of the cytoplasmic determinants, which may contain mRNAs and mitochondria in addition to proteins, translocate along the cortex during a subsequent microtubule-dependent phase in which the cortex and egg surface vibrates in a rapid fashion. Finally, during a new microfilament-dependent phase, the cortex relaxes and determinants of the future anteroposterior axis assume their definitive positions (Sardet et al., 2007).

As the preceding example from ascidian development shows, the mechanical excitability of the post-fertilization egg cortex can depend on microtubule dynamics as well as microfilament-mediated contractility. In the nematode *Caenorhabditis elegans*, the establishment of the anteroposterior axis occurs post-fertilization, but prior to the first cleavage. Sperm entry at or near the posterior pole, with the help of several proteins of the PAR family and other factors, destabilizes the cortical actin cytoskeleton, initiating a flow of cortical cytoplasm toward the future anterior pole (Munro et al., 2004). The sperm microtubule organizing center then associates with a different PAR protein in the actin-depleted cortex of the posterior pole (Tsai and Ahringer, 2007). Next, cortical adaptor molecules mobilize microtubule polymerizing energy into pulling forces that produce repetitive contractions and transverse oscillations of the posterior spindle pole (Kozlowski et al., 2007). Although the biological role, if any, of these cortex-associated mechanical periodicities is unknown, they have a plausible explanation in terms of the positive feedback loop formed by motor proteins acting in opposite directions on assembling polymers (microtubules), subject to load-dependent disengagement (Munro, 2007).

CONCLUSION

The use of novel physical theories to account for the ''independent irritability'' of the egg ectoplasm has proceeded as Just anticipated. Though few at the time would have endorsed the call for ''a physics and chemistry in a new dimension'' to deal with complex biological questions, this is exactly what the late 20th century physics of excitable soft matter was.

A more radical proposal by Just, given the ascendancy of the gene during his lifetime, was that the activities of the ectoplasm were determinative of development, not just epiphenomenal to it. As he wrote in *The Biology of the Cell Surface*:

> All these considerations and data indicate that the surface-cytoplasm cannot be thought of as inert or apart from the living cell-substance. The ectoplasm is more than a barrier to stem the rising tide within the active cell-substance; it is more than a dam against the outside world. It is a living mobile part of the cell. It reacts upon and with the inner substance and in turn the inner substance reacts upon and with it. It is not only a series of mouths, gateways. The waves of protoplasmic activity rise to heights and shape the surface anew. Without, the environment plays upon the ectoplasm and its delicate filaments as a player upon the strings of a harp, giving them new forms and calling forth new melodies (Just, 1939, p. 146).

In addition, according to Just, ''without the ectoplasm, fertilization cannot take place, [and] in both fertilization and parthenogenesis the response of the ectoplasm to the inciting means for development is prognostic for the quality of the future development,'' and ''the reactions underlying both differentiation and heredity are shown to be under the domination of cytoplasmic reactions, resulting from an interplay of both ectoplasm and nucleus with the cytoplasm'' (Just, 1939, p. 362).

The experiments reviewed here show that Just was correct in his estimation of the informational role of the ectoplasm's dynamics. But unlike the dynamical properties themselves, which are now becoming physically comprehensible, the basis of their species-specific incorporation into developmental mechanisms remains obscure, and can only be understood in relation to the evolution of those mechanisms.

The best-characterized developmental dependencies of the chemical and mechanochemical spatiotemporal oscillations of the fertilized egg are single-cell functions, completed before the first zygotic cell division with formation of the pronucleus (Ducibella et al., 2006). But more extended consequences of the early events are indicated by the findings, mentioned above, that the calcium transients and cortical contraction waves necessary for proper localization of germ plasm, determinants of gastrulation, axial organization and organogenesis occur in the zygote, whereas their effects are manifested during cleavage stages and beyond.

It has further been found that when normal patterns of Ca^{2+} oscillations were perturbed, interrupted or circumvented by a variety of different techniques, developmental outcomes in terms of implantation rate, and pre- and post-implantation embryo growth rates were impaired (Kurokawa and Fissore, 2003; Rogers et al., 2006; Ozil et al., 2006). Although a causal connection between calcium oscillations and these deleterious developmental effects has yet to be established, Ducibella et al. (2006) have suggested that the well-known relationship between in utero nutritional impairment and adult cardiovascular disease, hypertension, type II diabetes and other pathologies (Hales and Barker, 2001; Gluckman et al., 2005), may be mediated in part by perturbation of Ca^{2+} transients at periconceptional phases of development.

Although the reorganization of the egg interior, including the cortical cytoplasm, precedes multicellular pattern formation and morphogenesis ontogenetically, it need not have preceded them phylogenetically. In particular, since multicellular organisms likely evolved from free-living single cells, the egg stage could represent a later specialization within which preparative steps for subsequent embryogenesis can be taken.

Animal development originated in and continues to be based on a set of dynamical patterning modules (DPMs) (Newman and Bhat, 2008, 2009). These are morphogenetic elements consisting of one or more products of genes of the so-called ''developmental-genetic toolkit'' (Carroll et al., 2005) in coordination with mesoscopic physical processes or effects that they mobilize only in the context of the multicellular state. All the major morphological motifs of multicellular animals can be traced evolutionarily to the action of the DPMs, examples of which include cadherins/cell–cell adhesion; Notch–Notch ligands/lateral inhibition; Wnt–Wnt receptors/cell surface and shape polarization; Hh, BMP, FGF/molecular gradients.

One of the key DPMs indeed involves intracellular oscillation (of the concentration of the Hes1 transcriptional co-factor), but its multicellular role depends on the synchronization of the oscillations across a broad tissue domain (see, e.g., Giudicelli et al., 2007), where it permits the constituent cells to act coordinately as part of a ''morphogenetic field'' (Gilbert, 2006; Newman and Bhat, 2009). In contrast, the immediate effects of the Ca^{2+} waves and associated cortical contractions described above are confined to single cells, disqualifying them from DPM status. The physics involved is similar to that of the DPMs, however, since animal eggs are of a size (typically $>100\,\mu m$ in diameter) that brings them into the mesoscopic range (Forgacs and Newman, 2005).

It is possible that the moderately fast calcium waves ($\sim 10\,\mu m$/sec, corrected for temperature; Jaffe and Créton, 1998), seen in the activating eggs of many animal species, and the associated cortical waves, represent a special class of egg patterning modules (EPMs). These modules may mediate the rearrangement of molecular factors in the cortical and deeper cytoplasm of animal cells above a certain size, an activity that though possibly not originally adaptive could be harnessed over the course of evolution to lay down the initial and boundary conditions for the action of the multicellular DPMs. In effect, the preservation of ecologically successful forms whose evolutionary origin was in the multicellular dynamics of the DPMs could serve as a criterion for retention of genetic changes affecting the dynamical organization of the zygote.

The ectoplasm of the fertilized egg has thus turned out to be a meeting ground of developmental and evolutionary mechanisms, just as E.E. Just predicted it would be once the appropriate physical theories were in place. It has also, in line with his ideas, emerged as a dynamical regulator of the egg's chromosomal determinants, that is, the genes. In light of the growing role of the new physics of soft excitable materials in developmental biology (reviewed in Forgacs and Newman, 2005) and the increasing recognition of biological information that extends ''beyond the gene'' (Sapp, 1987; Müller and Newman, 2003; Jablonka and Lamb, 2005), it appears that the scientific mainstream may finally be catching up with Just's far-reaching views.

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