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Development of Medaka Eggs as Monitored by their Dielectric Behavior

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Embryogenesis, undoubtedly one of the most sparkling facets of life, has now become a favorite target of study from many disciplines (see e.g. ref. 1.)   Among these the morphological approach using microcinematography or conventional histological techniques contributed a great deal to advancing the firsthand concept of embryogenesis because of the rather drastic structural changes involved in it.   For a full description of embryogenesis, however, morphology alone is not amply penetrating and a cross fire from the other lines of approach is desirable.   As such, we would like here to introduce a dielectric dispersion method to monitor and record developmental changes in the organization of fertilized eggs of medaka (Japanese killifish), *Oryzias latipes.*

Dielectric dispersion is a phenomenon that the relative permittivity (ε) and conductivity (κ) of a dielectric material or of a mixture of materials such as biological cell suspensions vary with the frequency of applied alternating electric field.[2,3] Taking advantage of its non-invasive nature and its high sensitivity to electrical as well as morphological properties in particular, we have already utilized the method to analyze several species of living cells in suspension.[4,5,6]   In addition, a recent progress in computerization made it possible to deal with a single, millimeter-sized particle as the test object whose dielectric spectrum can be recorded automatically within a few minutes and also repeatedly at preset intervals.[7]   This same methodology was employed for the present *Oryzias* eggs.

Figure 1 shows a typical set of dielectric dispersion curves extracted from a time-chase experiment on a single egg perifused with 10% Yamamoto's saline.[8] Here the light micrographs of the egg at the respective stages are also included for quick reference.   Curve A represents the initial stages of development, i.e., from the just fertilized (stage 1) to the late blastula (stage 12).   Curves B, C and D refer, in this order, to the egg in passage through stages 20 (3 somite), 23 (12 somite) and 29 (6-day embryo).   As these curves for ε vs. frequency were seriously affected by an

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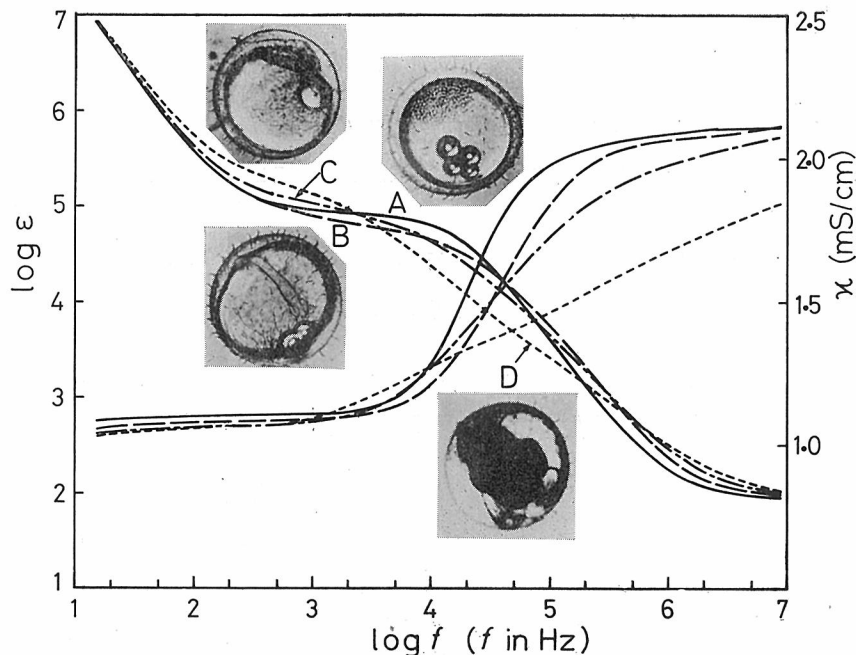K. Asami, A. Irimajiri and T. Hanai

Fig. 1. Changes in the dielectric dispersion curve of a single medaka egg during development. Immediately after fertilization the egg was transferred to a flow cell equipped with Pt electrodes (for details of the cell, see ref. 14) and then subjected to repeated measurements, over the course of 2 weeks, of its admittances with a YHP 4192A impedance analyzer operated at regular (2-hr) intervals. In each operation the paired curves for ε and κ were obtained well within 3 min that was sufficient for scanning 121 frequency points. Of the complete set of dispersion data thus collected, 4 representative curves were selected and identified, from the corresponding photomicrographs, as for stages: 10 (early blastula), curve A————; 20 (3 somite), curve B———; 23 (12 somite), curve C—·—·—; and 29 (6-day embryo), curve D————. Stage Nos., after Matsui.[15]

electrode polarization effect in the lower frequency region, the same data has been replotted in the Cole-Cole[9] diagram (Fig. 2) to help accentuate the details and characteristics of the egg's overall dielectric behavior which is free from the electrode artifact. Numerical results obtained from the Cole-Cole plots are summarized in Table 1. Several immediate points may be extracted from these data: (1) The permittivity increments ($\Delta\varepsilon$) were as large as 10^5; (2) $\Delta\varepsilon$ decreased by about 25% to a minimum of 6×10^4 towards stage 20 and turned up thereafter rather monotonously reaching three times the minimum level; (3) both the conductivity increment ($\Delta\kappa$) and the limiting conductivity at low frequencies (κ_l) remained essentially constant throughout the ongoing stages; and (4) the center for the circular loci became gradually depressed as development proceeded (Fig. 2).

Curve A in Fig. 2 is very near a semicircle (i.e., the Debye type) similar to that obtained with an artificial spherical bilayer membrane of millimeter size.[7] Phenomenologically, this type of dispersion may be characterized by one major relaxation time indicating that the underlying mechanism(s) is virtually single. Since the surface membrane of the killifish egg is known to be highly nonconducting (50–100
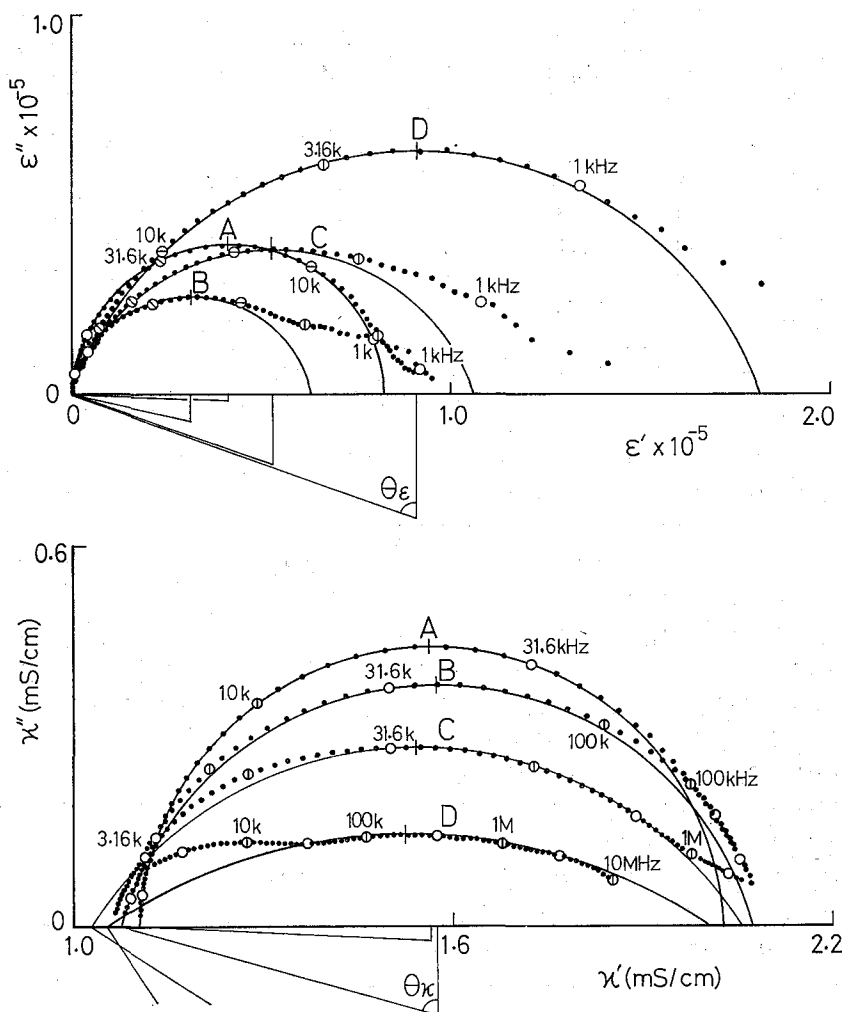
Fig. 2. Complex plane plots of data in Fig. 1. Ordinates, the imaginary part of complex permittivity $\varepsilon^*(=\varepsilon'-j\varepsilon'')$ and that of complex conductivity $\kappa^*(=\kappa'+j\kappa'')$, viz., $\varepsilon''=(\kappa-\kappa_l)/\omega\epsilon_v$ and $\kappa''=(\varepsilon-\varepsilon_h)\omega\epsilon_v$ where subscripts l and h denote respectively the limiting values at low and high frequencies, ϵ_v is absolute permittivity of free space, and $\omega=2\pi f$. Abscissae, the real parts, $\varepsilon'(=\varepsilon)$ and $\kappa'(=\kappa)$. The solid lines represent the best-fit circular arcs.

$k\Omega\cdot cm^2$) and capacitive (somewhat less than 1 $\mu F/cm^2$) at least until stage 22,[10,11] the Debye-type dispersion associated with the early-stage egg can be ascribed solely to the structural relaxation occurring at the outermost membrane-water interface. It has been well established that the fertilized teleostean egg undergoes discoidal cleavage which adds to the cell number but we confirmed that the resulting blastomeres occupied only a few percent in volume of the whole embryonic mass until the late blastula stage was reached (data not shown). This latter fact explains the relative constancy of the egg's dispersion behavior such as represented by curve A.

Curve B, on the other hand, traces a circular arc with a depressed center which

Table 1. Developmental Changes in Dielectric Parameters

Stage No.	Curve	$\Delta\varepsilon/10^5$	f_0	$\Delta\kappa$	κ_l/κ_a	Deg. of Depression	
			(kHz)	(mS/cm)		θ_ε	θ_κ
10	A	0.8	19	0.92	0.71	88	88
20	B	0.6	19	1.00	0.68	78	75
23	C	1.1	7	1.02	0.65	71	58
29	D	1.8	2	0.97	0.67	70	33
Mean				0.98	0.68		

$\kappa_a = 1.56$ mS/cm, Temp.$= 26\text{--}29°C$, Egg diameter$= 1.2$ mm
Stage No. 10, early blastula; 20, 3 somite; 23, 12 somite; 29, 6-day embryo

indicates an involvement of distributed relaxation times. Furthermore, a significant decrease in $\Delta\varepsilon$ took place over the period of epiboly during which the yolk sphere was encompassed with a monolayer of dividing cells that originated from the surface of blastoderm (see, e.g., ref. 12). The decrement of $\Delta\varepsilon$ was roughly proportional to the area of the enveloping cell layer and complete encompassment resulted in the minimum level of $\Delta\varepsilon$. As the embryo passed through stage 20 (3 somite), $\Delta\varepsilon$ began to increase with concomitant lowering of the relaxation frequency (f_0) giving rise to curve C (12 somite) and curve D (6-day embryo) with no further changes until hatching. Both these curves also have depressed centers reflecting some complex organization formed in the egg interior.

Although the relaxation time by itself does not tell us anything definite about the detailed mechanism(s) and architecture of the egg cell interior, the degree of depression in the Cole-Cole diagram has been empirically interpreted in terms of: (1) Heterogeneity in the electrical parameters of the otherwise homogeneous component cells[7] or (2) structural complexity as in the multi-stratified shell model.[13] To the present case, however, a third interpretation would be more pertinent; that is, a large, poorly-conducting shell of surface membrane (or cell monolayer) encloses not only yolk sac but a certain mass of dividing cells whose number and electrical properties vary with the developmental stage. Last but not least, it should be noticed that, judging from the relative constancy of $\Delta\kappa$ and κ_l/κ_a (Table 1), both the egg volume and the embryonic equivalent conductivity were maintained well within a narrow range throughout the course of embryonic development. A more detailed analysis of the dielectric behavior of *Oryzias* eggs in development is now under way in our laboratory.

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