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Do Ionic Currents Play a Role in the Control of Development?

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Embryonic and adult cells alike need to control the intracellular concentration of various ions in order to ensure their survival in an otherwise hostile environment. Beyond this basic requirement, however, there is growing evidence that cells and tissues in some embryos can use ion regulation and its by-products (such as the accumulation of transported ions in intraembryonic cavities, or the resulting electrical currents and voltages) to control development directly.

Cells regulate intracellular ion concentrations by transporting them across the cell membrane. However, not all cells in a tissue or organism are the same as one another, and even some regions of a cell differ from other regions of the same cell. Some of these differences affect the rate of transport of some ions. As a result, tissues have characteristic patterns of ionic fluxes, reflecting electrochemical potentials between the different regions. All the electrical laws apply to biological ion fluxes (e.g. Ohm's law), even though the charged molecules carrying the currents are not necessarily electrons; in most cells, the major carriers of current are small inorganic ions: sodium, chloride, potassium, protons, bicarbonate and calcium.

Do Electrical Currents Play a Morphogenetic Role during Early Development?

Very little is known about the control of developmental processes in early embryos. We still have no idea what determines that the correct structures will form at their correct places at the correct time, or what guides cells in their movements to their final destinations. In order for ionic currents to flow between different parts of a tissue, some differences must exist in the transporting properties between different regions of the tissue. At first sight, this implies that the tissue must be organized in some way before the currents appear, and therefore that the currents themselves cannot be the primary agent responsible for setting up positional information. However, it is imaginable that stochastic events within an initially homogeneous, symmetrical field can break the existing symmetry. This break in symmetry could then be amplified and stabilized by autocatalytic mechanisms. For example, I have suggested a model for gastrulation in the early chick embryo,[1] in which a few mesodermal cells arise from the epiblast at a random site; these cells then induce more mesodermal cells to ingress in nearby regions of the epiblast.

If ion homeostasis has a role to play in the control of early morphogenetic processes, two mechanisms, perhaps complementary, can be envisaged: control by intracellular ions, and control by ions in the extracellular environment.

Control by Intracellular Ions

The intracellular level of each ion can be regulated in three main ways (Fig. 1): by controlling the ratio of bound (i.e. electrically inactive) to free ion, by modulating the compartmentalization (i.e. 'packaging') of the ion in intracellular organelles, and by regulating the rate of transport of the ion into and out of the cell via channels, pumps and intercellular junctions. Most cells use all three mechanisms.

Of the early developmental processes where the intracellular level of ions plays a crucial role, fertilization is the best understood and has been reviewed extensively (for example, Ref. 2). Briefly, changes in the intracellular levels of certain ions have been shown to play a role in the activation of the egg in response to fertilization, in triggering cortical granule breakdown, in the prevention of polyspermy and in setting up an initial polarity with respect to the point of sperm entry in some species (e.g. in the fish, *Oryzias*,[3] Fig. 2*i*).

There is considerable morphological and physiological evidence in favour of the existence of intercellular ionic coupling at other early stages of embryonic development in vertebrates. In transporting epithelia, where cells are electrically coupled to one another through intercellular junctions, any regional differences either in ion transport rate or in permeability will generate a current that will flow between the cells, which will in turn produce an intercellular voltage gradient. This voltage gradient might be capable of organizing the distribution of 'morphogens' within the tissue. An impressive experimental demonstration of the feasibility of this type of mechanism has been provided using the *Cecropia* oocyte and its associated nurse cells, where the voltage gradient present is large enough to electrophorese injected charged dyes, through cytoplasmic bridges, to the poles of the cell.[4] The absence of, or other restrictions in intercellular coupling may also play a role in restricting the size of molecule that can pass between cells in different presumptive areas of the embryo, like those present across compartment borders in insects.[5,6] A possible function of these perm-selective intercellular barriers could be to allow essential housekeeping ions to pass through and equilibrate within the tissue, while restricting the passage of more specialized morphogenetic molecules between different territories. In the systems studied so far, molecules larger than 300–500 daltons are prevented from passing between cells at compartment boundaries. Such a mechanism could ensure that each compartment behaves independently from its neighbouring compartments, and thus represents a mechanism that could determine the boundaries of a morphogenetic field. However, it is important to be aware that this observation does not imply that 'informational molecules' are of this size, or indeed that any such informational molecules exist at all.

More recently, Guthrie[7] showed that the blastomeres of early *Xenopus* embryos are electrically coupled according to a very ordered pattern. Warner, Guthrie and Gilula[8] then injected an antibody against the gap junction protein into single *Xenopus* blastomeres at the 8- or 32-cell stage, and were able to show that this treatment blocked dye transfer and electrical coupling and led to some developmental anomalies. It could be argued that this is not an entirely surprising result since shutting down
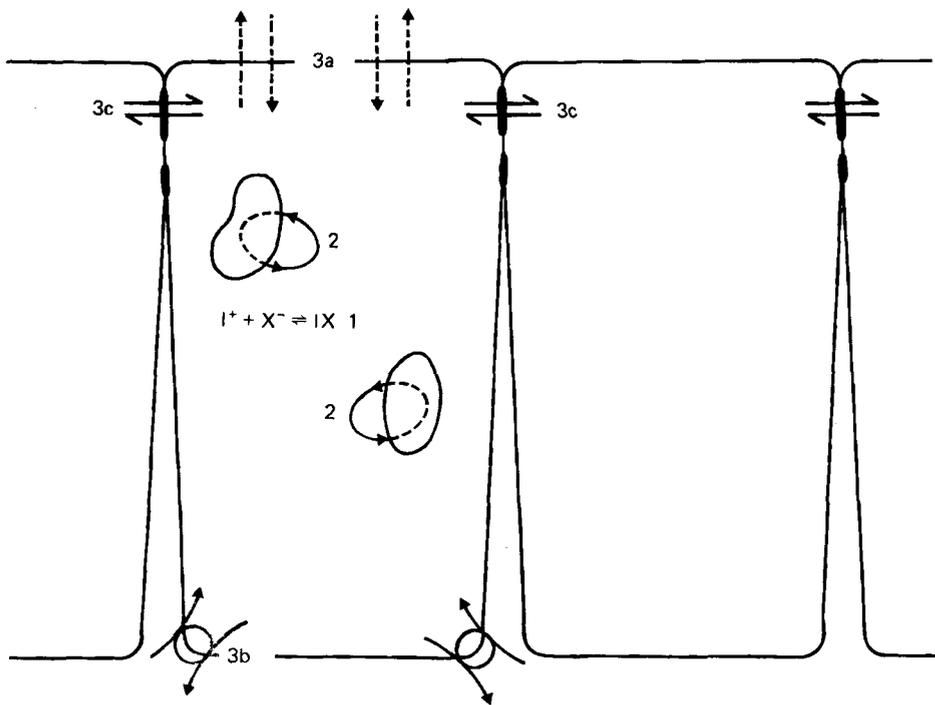
Fig. 1. *Diagram showing the main ways in which the intracellular concentration of a typical ion may be regulated in an idealized epithelial cell. (1). The concentration of electrically active, free cation (I^+) depends on the equilibrium of its binding to anions (X^- in the example) to form electrically inactive salts, IX. X might also represent a macromolecule capable of binding I^+. (2). The intracellular packing of the cation into organelles and its release from these organelles regulates the total cytosolic level of the cation. Further control of the intracellular ion levels can be achieved by transport of the ion into and out of the cell via passive channels (3a), active pumps (3b) and through intercellular (gap) junctions (3c).*

Cecropia[9] and others) oocytes (Fig. 2*f*), amphibian eggs (Fig. 2*g*) and embryos[12] (Fig. 2*h*) and chick embryos[13, 14] (Fig. 2*j*).

Can cells respond to these currents in a developmentally significant way? Often, if currents are inhibited by pharmacological agents that interfere with the transport of specific ions, development is impaired grossly. However, extracellular electrical currents are inseparable from the normal physiological activity of the cell or organism under study. Therefore, it is difficult to separate an effect of the inhibitor on general vital functions of the cells from a more specific effect on a developmentally important mechanism. An alternative approach that can be followed is to interfere with the pathway of the extracellular currents without affecting the homeostasis of the ions within the cells. For example, extracellular currents along the surface of an epithelium could be short-circuited by placing a communicating, low resistance saline bridge between the two aspects of the epithelium. This approach has not yet been pursued extensively.

One avenue followed by several investigators has been to examine the behaviour of embryos, tissues or individual cells cultured in a chamber through which a current is passed. Developmentally important aspects of cell behaviour were investigated: the direction of cell movement, cell elongation and cell and tissue polarity. One of the more interesting experiments of this type was done by Jaffe and Poo,[15] who found that when neural explants were cultured in an electric field the outgrowing neurites were extended preferentially towards the cathode. In a different type of experiment, Stern and Mackenzie[14] found that the apical-basal polarity of the epiblast of the early chick embryo can be reversed in a stable manner if a small voltage is applied across the thickness of the epithelium. Other authors have studied the direction of cell locomotion in electrical fields. Different authors have come up with different results depending on the cell type and on the extent of the field applied to them. Nevertheless, there are several major problems associated with such an approach. First of all, most experiments designed to look at cell movement use an *in vitro* system, where cell adhesion, the composition of the medium and the contact relations between the cells are different from the conditions in the embryo. Second, all experiments done so far that have succeeded in showing an effect of the

intercellular junctions is likely to do more than just prevent the passage of morphogens from cell to cell, and is likely to have deleterious effects on other vital functions of the cell. However, what is striking about their results is that although the antibody is applied very early in development, the anomalies produced are relatively minor (defects in eye and brain morphogenesis) and local.

Thus, the evidence indicating that intracellular ion concentrations play a role in controlling early developmental events is suggestive albeit indirect.

Control by Extracellular Ions

The evidence suggesting a role for extracellular ions in the control of early development is even less strong than that concerning intracellular ions.

Do extracellular ionic currents exist in developing embryos? We owe the answer to this question to the work of Professor L. F. Jaffe, now at Woods Hole. Jaffe started by investigating pattern formation in the eggs of certain brown algae (*Fucus* and *Pelvetia*, Fig. 2*d*). First cleavage in these eggs is asymmetric, and is accompanied by the formation of a rhyzoid at one pole of the egg. Jaffe was able to show that the eggs can be polarized experimentally using directional light or an electrical potential.

He then performed a simple and elegant experiment to show that the eggs did in fact generate extracellular currents: he pushed a number of eggs tightly into a tube, as if electrically 'in series', induced them to polarize towards a source of light shone from one end of the tube, and found that the total potential between the two ends of the experimental tube became measurable with a conventional electrometer, which enabled him to calculate the extent of the axial potential generated by each egg. He then proceeded to study the ionic carriers of the current in the algal eggs (see Ref. 9 for review). Eventually, Jaffe devised a refined measurement tool which he christened the 'vibrating probe', able to detect extracellular currents of the order of a few nA and to determine their direction. The first of these devices was built by Jaffe and R. Nuccitelli in 1973,[10] and now several such probes are in operation in the USA, as well as in England and Germany. The vibrating probe made it possible for Jaffe and his collaborators to collect data from many other developing organisms, which were found to generate asymmetric electrical fields around themselves (summarized schematically in Fig. 2): germinating pollen tubes (Fig. 2*a*), growing barley roots[9] (Fig. 2*b*, *c*), insect (*Drosophila*,[11]
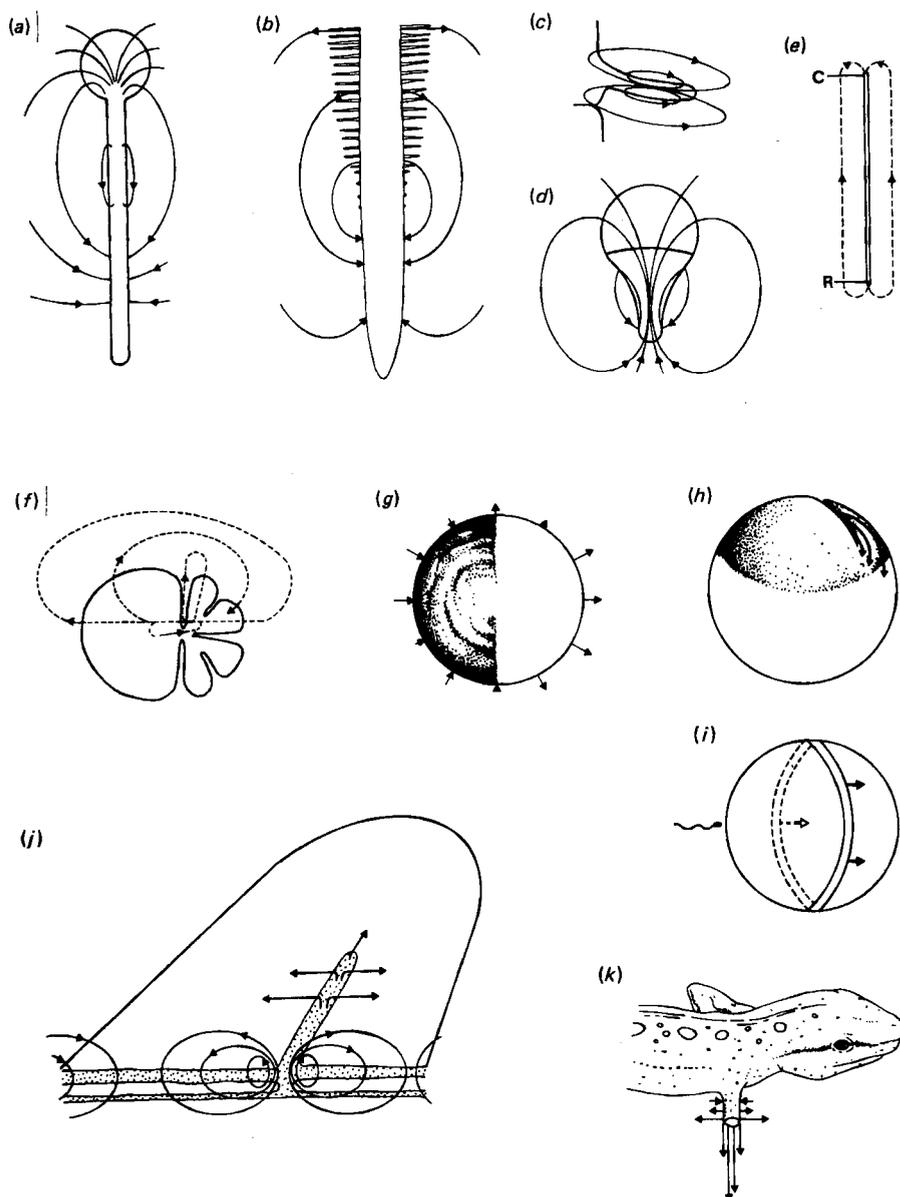
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Fig. 2. *Some of the developing systems in which extracellular electrical currents have been studied. (a) Pollen tube; currents enter the growing tip of the tube; (b) Growing barley root; again, currents enter the tip of the root hair; (c) Detail of barley rootlet; this displays the same pattern as the root hair in minature; (d) Fucus egg; currents enter the growing rhyzoid; (e) Isolated stalk cytoplasm of Acetabularia after removal of cap and rhyzoid; currents enter the original cap end (C) of the plant, and leave from the rhyzoid end (R); (f) Cecropia oocyte and nurse cells; current from the oocyte enters the nurse cells (right). There is also a transcellular component of current flowing between the oocyte and the nurse cells through an intercellular bridge; (g) Xenopus oocyte. An asymmetric pattern of current flow is seen; (h) Xenopus egg at first cleavage. Current leaves the cleavage furrow; (i) Egg of the Medaka (Oryzias latipes) shortly after fertilization; a single wave of free calcium originates at the point of sperm entry and travels within the cortical cytoplasm to the opposite pole of the egg; (j) Chick embryo at gastrulation. Currents, generated by the inwards transport of sodium by the epiblast, leave the primitive streak dorsally in all directions; (k) Regenerating newt stump; currents leave from the end of the stump. (All redrawn from Ref. 9 and various other papers of Professor L. F. Jaffe and collaborators.)*

applied field on the direction of cell movement have required the use of much stronger fields than those measured in embryos. Third, there is a conceptual problem in the design of such experiments. We apply an artificial current (electrons) that cells never see in the embryo and ask the cells to respond *normally* to it. Of course, in order to pass a current we must apply a potential difference across the chamber, and this will cause the ions in the medium to move electrophoretically, but ions with the same electrophoretic mobility (i.e. similar charge/mass ratio) will move in the same way as one another. In other words, the system does not mimic successfully the fine dynamics of, for example, sodium/potassium currents found in embryos. These considerations

imply that negative results do not necessarily show that electrical events are not involved in the process *in situ*, and conversely, that positive results do not show that they are. Back to square one.

Recently, Nuccitelli and Wiley[16] attempted to use the vibrating probe to detect extracellular ionic currents generated by isolated mouse blastomeres from early cleavage stages. They succeeded in detecting only very small currents in a relatively small proportion of the blastomeres studied. They argued that this was due to the extremely small magnitude of the currents present, and in order to 'overcome' this problem they proceeded to increase the currents by fusing several blastomeres together. In this way, they succeeded in measuring some small extracellular currents, which they concluded, "may actively influence blastomere polarity". In my opinion, this is not a very informative experiment. If any currents that might be present are so small as to be at the limits of detection of the super-sensitive vibrating probe, they are unlikely to play a critical role in the control of development. Altering the basic biology in order to be able to detect these small currents, followed by a discussion of their supposed 'biological role' in controlling cell polarity, is unsatisfactory. In another series of publications by Nuccitelli and his colleagues,[17,18] they argue that because chick lateral plate mesoderm cells do not move in response to large applied fields,[19] while chick neural crest cells do,[17,18] the former cells must have been dead. They claim that because in the former experiments thicker chambers were used, the heat generated by the applied field is greater than in their own experiments using much narrower chambers. However, the heat produced by applying a voltage to a conductor is *directly* proportional to its resistance. If the conductivity is constant, resistance varies directly with length of the conductor but inversely with its cross-sectional area. Thus, the thin, long filament of a tungsten lamp glows with the heat generated as a result of the enormous resistance, while if the filament was made from thicker wire it would be much less efficient.

To summarize this section, we can conclude that the eggs of the algae *Fucus* and *Pelvetia* remain as the only developing system where the development role of extracellular ionic currents has been fully demonstrated, and that further work is required before we can confirm the suggestive pieces of evidence indicating that extracellular ion currents are

involved in early developmental events in other organisms.

Models

One of the reasons why it is so difficult to demonstrate a role for electric currents in the control of development is that there is as yet no consistent model capable of making specific predictions about the currents and development that can be tested experimentally. A few daring attempts have been made, however. Jaffe, for example, suggested that the currents in embryos might be strong enough to move developmentally important molecules to different parts of the field by electrophoresis or electroosmosis.[9, 20] In theory, it is possible to envisage such a mechanism operating in three domains: in the environment surrounding cells, in the plane of the cell membrane and between coupled cells. There is evidence for the latter two mechanisms: Poo and Robinson[21, 22] have shown that cultured muscle cells placed in an electric field showed a re-distribution of their acetylcholine receptors within the cell membrane, while we have already mentioned the *Cecropia* oocyte as an example of electrophoretic redistribution of molecules between cells connected to each other by cytoplasmic bridges. However, there is still no firm evidence that such mechanisms operate in normal development.

Another group of models that several authors have found appealing is described as 'mechano-electrical' (see Ref. 23). These models suggest that there could be a link between local ionic concentrations and activity of the cytoskeleton. The clearest have been put forward by Odell, Oster and their collaborators[24, 25] to explain epithelial morphogenesis, and I have made use of similar ideas to model early morphogenesis in the chick embryo and other vertebrates.[1] The major attraction of mechanical models of morphogenesis is that it is easy to envisage them as operating within a 'field': mechanical interactions between cells in a sheet of tissue can be integrated into a vector field. A field description is attractive because it immediately suggests ways in which the behaviour of cells in different parts of the field is consistent. However, the disadvantage of this sort of model is that because of its intrinsic complexity, the predictions are not always clear and therefore are not readily put to experimental test.

We are still ignorant of the ways in which cells might be capable of responding to applied extracellular fields. For instance, we do not know whether they can sense the minute differences (about 1% for an average cell where the extracellular voltage gradient is 0.5–1 mV per cell) in trans-membrane ('resting') potential between the cathode-facing and the anode-facing sides, or whether all responses (cell movement, cell polarity) to the applied field are due to electrophoretic or electroosmotic redistribution of plasma membrane components. In addition, it is possible that cells can sense shallow ionic gradients with respect to *time* as well as *space*. However, it then becomes important to understand how such shallow gradients are maintained. Experimentally testable theoretical models for these aspects of cell behaviour are needed before any more useful experiments using extracellular stimulation can be designed. We also do not know whether coupled cells can respond as a single unit to shallow voltage gradients. In order to know whether mechanisms independent of participation by the cell are entirely responsible for its behaviour in electric fields, it becomes important to establish the biological unit to which the magnitude of the field refers, whether 'per cell' or 'per length'.

Electrical Currents in Later Development: Growth and Regeneration, and the Healing of Wounds and Fractures

As early as 1860, DuBois-Reymond[26] observed that when a patient with a cut or graze in the tip of one finger introduced the offended appendage into a beaker of electrolyte solution and a finger of his/her other hand into another, similar container, an electrometer placed between the two vessels showed a potential difference between them. More recently, Borgens, Vanable and Jaffe[27] studied bio-electrical activity associated with the stumps of amputated amphibian limbs. They showed that the stumps were a source of ionic current that entered the rest of the animal (Fig. 2*k*). It is still unclear whether these measured currents have a developmental function and to what extent they are an artefact of the wound, but the same authors have shown that adult frogs, which normally do not regenerate, could be induced to undergo limited regeneration by stimulating the stump with current from a battery implanted under the skin (reviewed in Refs. 9 and 27). Moreover, Smith and Pilla[28] showed that when stumps of amputated newt limbs were stimulated with electromagnetically induced current of various waveforms, regeneration of the limb was either abnormal or accelerated, depending on the waveform used.

Claims by several clinical workers that electromagnetic stimulation was able to heal some chronic skin ulcers and non-unions (bone fractures that have not healed over a long period, often years) have led to considerable interest by some clinicians, and considerable scepticism by others. Conflicting claims have been made (see reviews in Refs. 29–31); at best, in the most reliable studies, the differences between controls and stimulated patients are marginal.

Conclusions

To a reader not involved directly in the field of electrical currents in development, the efforts of workers in this field may seem misdirected. Why is so much time being spent gathering information about a proposition which does not even appear to lend itself to experimental test? It is clear that extracellular ionic currents do exist in virtually all developing (and adult) systems, and that their pathways reflect the pattern of areas actively involved in some obvious developmental event. However, it is more difficult to establish for certain whether the currents play a controlling role in developmental events, or whether they are a consequence of the morphological pattern. Clearly, regions of the embryo that are actively engaged in some process must be different in some way from other regions, and some of these differences may be trivial.

One problem appears to be that the 'electrical current people' and the other mainstream cell physiologists do not read each others' literature. The latter are not generally interested in development and therefore do not follow the developmental literature; furthermore, they do not tend to regard the former as producing scientifically sound work. But why aren't some of the former more aware of the advances made using modern technical developments, such as knowledge on the many ion channels, or epithelial transport? There is a great abyss even in the terminology used by the two groups of workers: physiologists tend towards the more precise though often inhospitable language of biophysics, but often have lost their perspective on major biological questions. The 'current people', on the other hand, have become stuck in the language and concepts of the last century.

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Moreover, the majority of the investigators working on electrical currents continue to concentrate their efforts exclusively on one of two approaches: current 'collecting' (finding yet more organisms with electrical currents) or stimulation, when neither of these approaches are fruitful. The problem, as I see it, lies in that workers are more inclined to work 'on electrical currents' rather than on the developmental problems that we are trying to understand. If we were less technique-orientated and more problem-orientated, we might soon be able to look at development with a better perspective.

There is indeed some strong evidence indicating that ion regulation plays an important part in the control of early developmental processes. A few months ago, Brian Goodwin wrote a lucid essay in these pages (Ref. 23), where he argued that in order to understand morphogenesis we must first understand the *dynamics* of the system. Investigating the mechanisms of ionic regulation and the relationship between changing levels of ions and the mechano-chemical state of the cell may be a way in which we can approach the problem of understanding these dynamics in developmental systems. Indeed, if electrical currents turn out to be important in development, they may well constitute the beginning of a path towards a real understanding of morphogenetic processes.

Acknowledgements

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