Syngamy and Cell Cycle Control

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Keywords

Actin
A small protein monomer that concatenates to produce thin filaments when it polymerizes.

Allosteric
Modifications to protein structure occur when the binding of a small molecule or another protein to one site in a protein leads to a conformational change in another part of the protein distant from the site of interaction.

Apoptosis
A process of programmed cell death in which cellular components are dismantled in orderly fashion without leakage of cell contents.

Centromeres
Stretches of DNA sequence generally toward the middle of a chromosome that attract accessory proteins that allow mechanical attachment of microtubules in the mitotic spindle and permit the movement of chromosomes into the daughter cells.

Chaperones
Proteins that bind to other proteins to enable them to fold correctly or to remain soluble.

Chromatin
DNA and its accessory proteins, found *condensed* in chromosomes during mitosis and *uncondensed* in the interphase nucleus, where it is surrounded by a double nuclear membrane.

Cyclic AMP
A messenger molecule within cells that activates a protein kinase (protein kinase A); it is made in a single step from ATP by adenylyl cyclase under the control of transmembrane receptors of hormones or growth factors.

Cytokinesis
The process by which the daughter cells separate following mitosis; it involves the actin-based contraction of a purse stringlike contractile ring that generates the cleavage furrow between the daughter cells.

Depolarization
The usually transient loss of the electrical potential that cells maintain across their plasma membranes by setting up asymmetric ionic concentration gradients; it acts as a signal, above all in nerve and muscle.

Disjunction
The separation of the chromosomes at the start of anaphase in mitosis and meiosis; disjunction allows the chromosomes to be pulled into the daughter cells.

Echinoderms
A phylum that sits at the base of the vertebrate lineage to which sea urchins and sea stars belong.

Enzymes
Proteins that act as catalysts in chemical reactions.

Glycoprotein
Protein that has been modified after translation by the addition of sugars, often branching chains of mannose and glucosamine derivatives.

Histones
Very basic (positively charged) proteins that bind to and order negatively charged DNA.

Homologs
The name given to the complementary chromosome pairs that result from recombination.

Hydrophobic
Molecules prefer nonaqueous environments.

Imprinting
An epigenetic modification of key genes that occurs in placental mammals; it regulates embryonic growth.

Interphase
Comprises all stages of the cell division cycle except mitosis and cytokinesis.

Introns
Stretches of DNA within a gene that do not encode amino acids and are cut out before protein sequence is decoded from messenger RNA.

Kinases
Enzymes that attach phosphate groups to other molecules. Protein kinases turn other proteins on and off in this way.

Lipid bilayers
Separate the cell from the extracellular milieu and also separate the cell's internal compartments; they consist of cylindrical lipid molecules with a long hydrophobic tail and a water-loving head group that assemble into planar sheets, lipid tails inwards.

Microtubules
Long cylindrical tubes that assemble from tubulin protein dimers; the dimers pack in a helical array.

Mitosis
The process that precisely segregates the genes between daughter cells after gene duplication; DNA becomes very tightly coiled and packed into chromosomes that are assembled into a disc and then pulled apart into the daughter cells by microtubules.

NADPH
Nicotine-adenine dinucleotide phosphate in its reduced form; it is used by cells as a cofactor to build molecules as this requires a reducing agent.

Phospholipases
Enzymes that cleave phospholipids and remove some or all of the headgroup.

Polyamines
Large linear molecules with many positively charged amine groups.

Polymorphism
The term given to small sequence differences between the same gene in different individuals within a species; many polymorphisms show no obvious phenotype, while at the other end of the spectrum some polymorphisms give rise to debilitating diseases such as cystic fibrosis and muscular dystrophy; the sum of all polymorphisms account for the genetic differences between individuals.

Proteoglycans
Very large proteins found in the extracellular matrix that have an amino acid backbone and very long carbohydrate side chains.

Proteolysis
The process of protein destruction; all proteins eventually undergo proteolysis, but in some cases, proteins are rapidly destroyed as part of a regulatory mechanism rapidly inactivate their activity; proteins can be cleaved into large fragments by proteases and into very short peptides by a cytoplasmic machine known as the *proteasome*.

S-phase
The phase in the cell division cycle during which DNA synthesis occurs and the genome is duplicated.

Spindle
The mitotic *spindle* is the array of microtubules that attaches to the chromosomes during mitosis and drags the chromosomes into their respective daughter cells; in its shape, this structure resembles spun thread wrapped around its spindle.

Sympatric
Species are found in the same or overlapping geographical areas.

Vesicles
Spherical compartments bounded by a lipid bilayer membrane; their contents are often released into the extracellular milieu; this is achieved by fusion of the limiting bilayer membrane with the plasma membrane.

The key aspect of sexual reproduction is the generation of genetic variation through the swapping of variable lengths of chromosome arms between pairs of homologous chromosomes during meiotic recombination in the germline. Following recombination, it is the general rule that a single (haploid) set of chromosomes is packaged into each male and female gamete (sperm and egg). These two sets of chromosomes, one from each parent, then come together to form a single nucleus: the process of syngamy. This simple outcome is achieved in a complex way through the process of fertilization. Fertilization comprises the delivery of the male set of chromosomes, tightly packaged in a small and motile sperm, to the female set of chromosomes, contained within a much larger nutrient laden and relatively sessile egg or oocyte; at fertilization, fusion of the two gametes occurs as a prelude to syngamy. In addition, fertilization triggers the onset of the very rapid rounds of cell division that quickly produce a very large number of embryonic cells. The cell division cycle must be coordinated with the events of syngamy. Large intracellular calcium signals occur at fertilization and ensure that the events of the cell cycle are controlled to permit successful melding of the parents' genomes.

1
Formation of Gametes

1.1
Meiosis and Genetic Recombination

Each round of sexual reproduction shuffles the genetic pack giving rise to a unique combination of genetic alleles and thus to a unique individual. The shuffling is brought about by recombination, a form of genetic cutting and pasting. Human genetic information is packaged into 22 pairs of fully recombining chromosomes (the autosomes); the X and Y sex chromosomes recombine only in a limited (pseudoautosomal) region and are ignored in this account. Each of the 22 autosomes differs in the genes it carries and in its shape because chromosomes have two arms, each of which varies in length between autosome pairs. The autosome pairs look identical to one another, like twins. But unlike identical (monozygotic) twins, though they carry the same genes, they are not genetically identical: each member of the pair carries different alleles of the same genes. Recombination cuts one or more of the autosome arms in each member of the autosome pair at the same place (in fact almost always at exactly the same nucleotide position in the DNA on the two chromosomes) and then swaps the fragments, pasting them back on the opposite chromosome (Fig. 1a).

Recombination occurs during meiosis, the specialized form of chromosome separation that is believed to have evolved from the more normal mitosis seen each time a somatic cell divides (It has recently been suggested by Krylov, Nasmyth, and Koonin that meiosis may be the phylogenetically primitive form of chromosome separation.). Meiosis is at the center of the mechanism of sexual reproduction and occurs only once as each gamete is formed. A germ cell destined to become a sperm or egg contains 22 autosomes from the father and 22 from the mother (the 22 autosome pairs). Just before meiosis, the germ cell undergoes a round of DNA synthesis. This generates 44 chromosome pairs, consisting of 44 autosomes with the father's alleles (the paternal homologs) and 44 autosomes with the mother's alleles (the maternal homologs). Each homolog pair is joined at the centromere. The outcome of meiosis is a haploid gamete with 22 nonidentical autosomes, so the 88 autosomes at the outset of meiosis must be packaged into four cells. This requires two rounds of cell division (Fig. 1b). The first meiotic division (meiosis I) is very unusual. As a consequence of recombination, pairs of recombined maternal/paternal homologs are formed, attached at the points on the arms at which recombination has occurred (the chiasmata). Chromosomes are pulled apart during meiosis I by microtubule-based forces, just as in mitosis. As the chromosomes line up in the central spindle under the tension generated by the microtubules of the meiotic spindle attached to the centromeres, they are seen to be arranged as pairs of pairs (tetrads). The first pairing reflects the attachments between sister chromatids of both maternal and paternal homologs established during DNA synthesis at the centromere and along the chromosomal arms; the second pairing is due to the chiasmata that are a consequence of recombination. It is a unique feature of meiosis I that when the spindle microtubules pull the chromosomes apart during anaphase, the chromosomes separate at the arms (synaptonemes), not at the centromeres. This results in detachment of homologs at the chiasmata (a process known as resolution) and disjunction of the homolog pairs, leaving the sister chomatid pairs

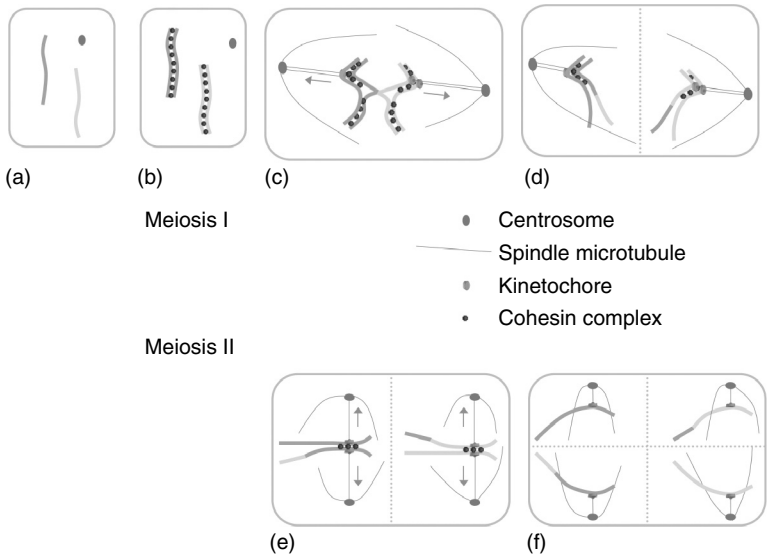

Fig. 1 Recombination and chromatid separation during meiosis. Before meiosis begins, the diploid germ cell contains pairs of homologous chromosomes; for simplicity, only one chromosome pair is illustrated (a). Both chromosomes in each pair undergo DNA synthesis (b); the newly replicated chromosomes are held together along their length by the protein cohesin. Then a complex series of events occurs during recombination that results in the swapping of chromosome arms between the replicated pairs (not shown). The outcome of these recombination events is revealed during the first meiotic division (c): chromosomes aligned on the meiotic spindle can be seen as tetrads, linked by the chiasm that is formed at the site of recombination. At anaphase cohesin falls off the chromosome arms as the chromosomes are pulled toward the centrosomes that organize the meiotic spindle (d); cohesin persists around the kinetochore, maintaining the association between sister chromatids. The meiotic spindle reforms during second meiosis (e). This time cohesin is lost at the kinetochore during anaphase (f). The result is four haploid cells, two of which have swapped chromosome arms in this illustration. With thanks to Neil Hunter for the building blocks of this illustration.

attached at the centrosome. The second meiotic division takes place without any further genome duplication through DNA synthesis. It involves a conventional chromosomal separation at the centromeres, the outcome being four haploid cells.

1.2 Male Gametes

In males, each of the four haploid outcomes of meiosis becomes a gamete. Driven presumably by a strong selection pressure (an unsuccessful gamete has a wasted life and an individual who produces unsuccessful gametes will have no offspring), male gametes have evolved as small, higher energetic cells. As they mature, they lose most of their cytoplasm, develop very highly condensed chromatin due to the replacement of histones with polyamines and grow a very motile tail (flagellum). They also retain a secretory vesicle (the acrosome) whose purpose is

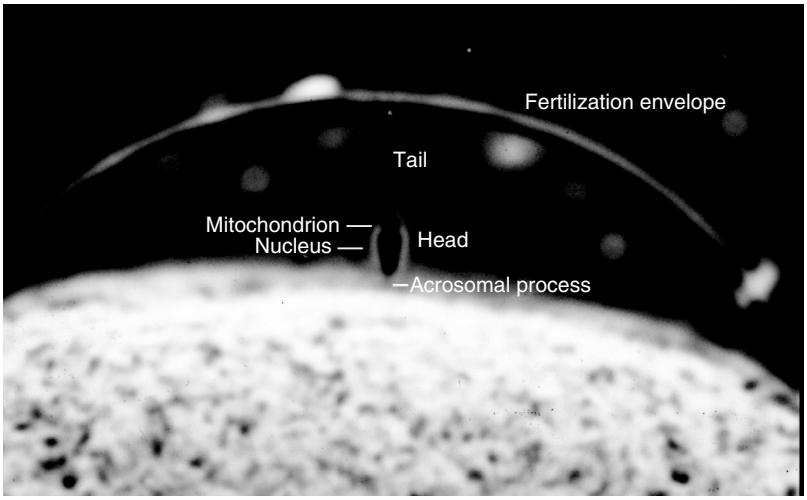

Fig. 2 The spermatozoon as it fertilizes the egg. Light microscope image of a sperm as it fertilizes the sea urchin egg. The sperm has fused with the egg through its acrosomal process and started the calcium wave. The local increase in calcium has caused the local elevation of the fertilization envelope that will exclude other sperm. The sperm head contains the DNA-containing nucleus and the mitochondrion that provides energy to the tail. Once the sperm has fused with the egg, the tail stops beating and stands straight as the sperm enters the egg. The sperm is only 2–3 microns across; the egg is 100 microns in diameter. The disparity in the sizes of the gametes is very obvious in this image.

to secrete digestive enzymes at the time that the sperm is passing through the extracellular matrix surrounding the egg and a mitochondrion or mitochondria to provide the ATP required for motility (Fig. 2).

1.3 Female Gametes

All embryos undergo a period of autonomous development and during this period they require a source of metabolites. As we have seen, sperm offer slim pickings; it is the egg that has specialized to contain a nutrient store (yolk) on which the embryo will live until it can itself feed or undergo uterine implantation. As a consequence, the embryo can grow only a little bigger than the egg itself (by, for example, developing fluid-filled internal cavities). The size of the egg thus determines the size of the autonomous embryo. Chicks undergo autonomous development within an eggshell to a very large size and thus have a very large yolk store. Frog eggs are around 1 mm in diameter and give rise to a macroscopic tadpole. Many eggs, including mammalian eggs are in the 0.1 mm range, with correspondingly smaller microscopic autonomous embryos, but still weigh in at ten times the size and one thousand times the volume of most somatic cells.

Size matters in two ways. First, it sets a constraint when the male and female gametes meet, as the much smaller sperm has had to evolve alongside mechanisms that signal its presence to the relatively enormous egg. We shall return to this.

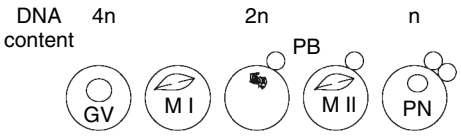

Fig. 3 The female oocyte during meiotic maturation. The immature oocyte is readily identified by its large nucleus (germinal vesicle, GV); by this point, DNA synthesis and recombination have taken place and the oocyte contains four copies of the genome (4n). The first meiotic division (meiosis I, MI) results in the extrusion of a polar body (PB); the oocyte now contains a 2n genomic complement. After second meiosis, a second polar body is formed and the oocyte reforms a haploid (n) pronucleus (PN).

Second, it has made its mark on the way the egg undergoes meiosis.

Strictly speaking, it is an oocyte that undergoes meiosis, the oocyte becoming an egg only once meiosis is complete. In most cases, the oocyte is already a very large cell by the time meiosis occurs, pumped up with yolk by the surrounding nurse cells in the ovary. In the female germline, both meiotic divisions are very asymmetric, both generating a very small cell, budded as it were on the surface of the large oocyte. After meiosis I, the small cell (polar body) contains 46 chromosomes and does not divide further. The polar body extruded during meiosis II contains 23 chromosomes and again has an inconsequential fate (Fig. 3). Thus, of the four possible haploid cells that can be generated by meiosis, only the large oocyte survives in the female germline.

2 Interaction of Sperm and Egg

2.1 Sperm Activation and Chemotaxis

Active sperm have a very limited shelf life. Sperm of external spawners shed into the water column have no ready access to respiratory substrates beyond their own limited stores and sperm of this sort cease to swim within minutes. Mammalian sperm within the uterus are supplied with external substrates; they also have many more mitochondria and can survive as motile and fecund sperm for several days. Mechanisms therefore exist to suppress sperm motility until sperm are delivered to the vicinity of the egg.

A widespread mechanism for suppression of sperm activation appears to be the acidic environment in testis and seminal fluid, combined with a high partial pressure of carbon dioxide. Once the seminal fluid is diluted, pH rapidly increases and activates motility.

Sperm activation may also be mediated or enhanced by signaling molecules released by the egg or present in the uterus. In echinoderm sperm, small peptides released from the egg coat interact with their receptor, a transmembrane guanylate cyclase located in the sperm tail. Stimulation of the receptor increases both pH and calcium concentrations within the sperm, leading to enhanced motility (Fig. 4). Similarly, progesterone stimulates calcium signals in mammalian sperm and enhances motility.

These agents may also be inducing chemotaxis, the directed movement of sperm toward the egg along a chemical gradient, though this is not yet proven. In other animals, for example, ascidians and siphonophores, sperm chemotaxis has been well demonstrated. Chemotaxis between gametes of the fern can be demonstrated using simple kits designed for high school students. It has recently

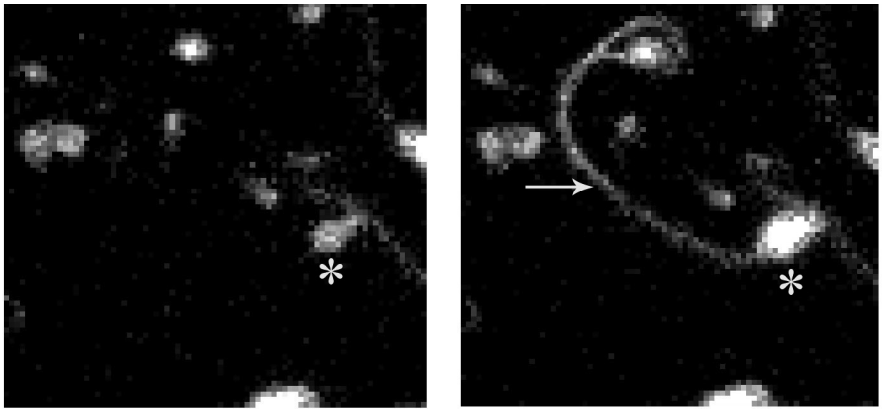

Fig. 4 Calcium signaling and sperm chemotaxis. Calcium ion concentrations can be detected inside cells by using fluorescent dyes whose intensity depends on calcium concentration; sperm head is marked with an asterisk. Sperm have low resting calcium concentrations (left). When the chemotactic peptide speract is added, calcium concentration increases in the tail (arrow); this alters swimming behavior.

been found that human sperm possess receptors equivalent to those found in the olfactory epithelium and that appropriate odorants can induce changes in swimming behavior consistent with chemotaxis.

2.2 The Acrosome Reaction

The naked surface of the egg or oocyte is always well protected, sometimes by thick proteoglycan coats and often in addition with a cloud of accessory cells embedded in this extracellular matrix. Sperm must traverse these barriers in order to reach the plasma membrane and enter the egg and to do so they must dissolve the extracellular matrix. The release of the contents of the acrosomal vesicle is a process akin to the release of neurotransmitters and circulating hormones: a large increase in intracellular calcium concentration triggers fusion of the acrosomal vesicle with the sperm plasma membrane. The acrosomal vesicle contains glycanases and proteases in many cases, capable of hydrolyzing the extracellular matrix.

Fittingly, the release of acrosomal contents is triggered by the proximity of the extracellular coats themselves. In echinoderms, the acrosome reaction can be experimentally triggered by a component of egg jelly in the complete absence of the egg itself. In mammalian sperm, the trigger to the acrosome reaction is a glycoprotein component of the extracellular matrix immediately surrounding the egg, the zona pellucida.

In abalone, the contents of the acrosomal vesicle consist of a protein, lysin, which acts not as an enzyme, but stoichiometrically as a structurally specific chaotropic agent (chaperone) that dissolves the egg vitelline envelope.

One consequence of the acrosome reaction in many species is the formation of the acrosomal process, a fine protrusion at the tip of the sperm head that is the locus of interaction with the egg membrane. This fact is exemplified by the behavior of *Thyone* sperm: they undergo

an acrosome reaction at the very periphery of the egg jelly, sending a 100-µm process through the jelly coat to reach the egg plasma membrane. In *Thyone*, as in other species, the process grows due to actin polymerization. The growing plus end of the actin filaments is at the tip of the acrosomal process while the reservoir of monomeric actin lies at the base of the filament; the rate of growth decreases exponentially, limited by the increasing diffusion time for actin monomers along the length of the growing process. Actin polymerization is triggered by the increase in pH that accompanies the calcium rise that triggers the acrosome reaction.

2.3 Sperm–Egg Interaction and Speciation

Reproductive isolation is the major mechanism driving the formation of new species. This often occurs as a result of geographic isolation of a population, but can also occur sympatrically. Closely related sympatric species may remain speciated by separation of habitat or by asynchronous spawning, but reproductive isolation is very often due to rapid evolution of the molecules that are the gatekeepers for syngamy.

A striking example is the abalone lysin released during the acrosome reaction. There are five species of abalone living sympatrically on the Pacific coast of North America. Their reproductive isolation is due in large part to the species specificity of the lysin reaction: lysins from other related abalone species are much less potent at dissolving the vitelline envelope. Lysins show very little polymorphism within species. Nonconservative substitutions within the protein are very high relative both to housekeeping proteins and to lysin introns, implying very strong positive selection. Lysin interacts with a protein designated VERL in the vitelline envelope. VERL is a very large (>1 m kDa) protein that consists of well conserved ~100 amino acid repeats. Analysis indicates that it is not under strong positive selection. The hypothesis put forward is that VERL undergoes a process of concerted evolution that puts very strong evolutionary pressure on lysin that can lead to speciation. Concerted evolution is a phenomenon found in proteins with multiple repeats. A mutation in one repeat can propagate through all repeats as a result of preferential recombination events. The finding that lysin shows a very low polymorphism within a population indicates that positive selection produced an optimal lysin in response to concerted evolution in VERL that then swept through a population, creating a new species.

Lysin/VERL is the only known example of how sperm/egg receptor interaction may govern speciation. Very little is known about other sperm/egg receptors. It has been suggested that an 18-kDa protein that coats the acrosomal process in abalone (a close relative of lysin, but with little sequence similarity) may be the sperm receptor for the egg plasma membrane. Similarly, the very hydrophobic protein bindin, which coats the echinoderm acrosomal process, may interact with a receptor on the egg plasma membrane. In mammalian eggs, it has been suggested that integrin/disintegrin ligand receptor pairs that are known to be involved in cell–matrix interaction and signaling may be the sperm/egg ligand/receptor at fertilization, but the evidence is contradictory.

2.4 Sperm–Egg Fusion and Sperm Incorporation

Once the sperm and egg plasma membranes are in close proximity, they fuse

to establish continuity of egg and sperm cytoplasm. Cell–cell fusion in general is not well understood and gamete fusion is no exception. The protein bindin that coats the acrosomal process in echinoderms is very hydrophobic, as are the cores of lipid bilayers: it is thought that bindin may promote membrane fusion, not least because it has been found to promote fusion of lipid vesicles *in vitro*. The 18-kDa protein of abalone has been ascribed a similar function. In mammalian fertilization, the integrin/disintegrin interaction may be fusogenic, but there is no sound evidence for this.

It is humbling that we do not yet understand the mechanism of sperm–egg fusion: it represents the moment of fertilization. A variety of observations isolate the event of sperm–egg fusion as the key event at fertilization:

- In echinoderms, sperm–egg fusion causes depolarization of the egg, the first response observed at fertilization that, significantly, much reduces the probability of other sperm fusing with the egg (an electrical block to polyspermy).
- In echinoderms, under certain conditions, sperm–egg fusion is reversible; when it is reversed, eggs may or may not undergo activation and the longer the sperm cytoplasm is in continuity with the egg, the greater the chances of egg activation.
- Sperm–egg fusion is the earliest detectable event at fertilization, as measured by dye transfer between egg and sperm.
- Mammalian sperm carry an enzyme in their cytoplasm that can activate the egg and is delivered to the egg when the sperm fuses with it.

Once the sperm fuses with the egg, it has fulfilled its purpose of finding the egg and fusing with in local competition with tens (mammals) or millions (external spawners) of other sperm. Incorporation

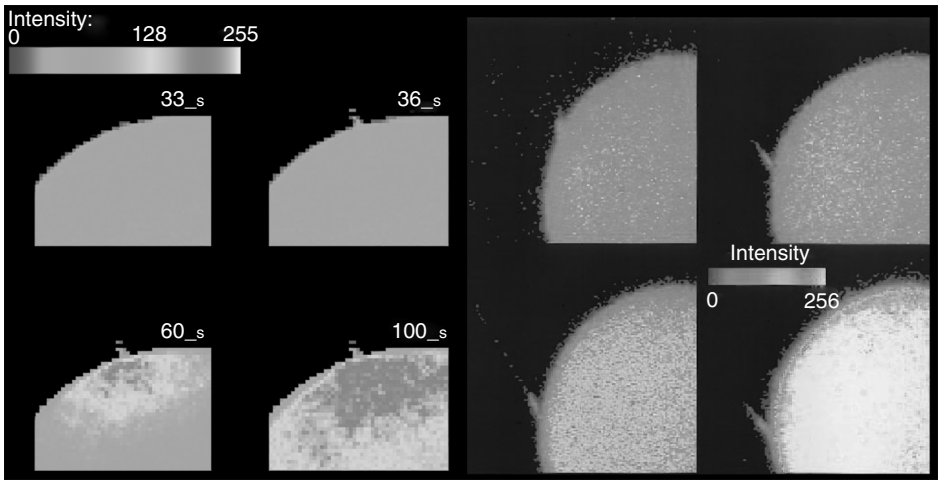

Fig. 5 Sperm–egg fusion in sea urchin and mouse eggs. The eggs contain a fluorescent dye sensitive to calcium concentration. When the sperm fuses with the egg, dye enters the sperm. Sperm–egg fusion triggers an increase of calcium within the egg. The calcium increase occurs many seconds after sperm–egg fusion. Left: sea urchin. Right: mouse.

of the sperm into the egg relies on the egg itself (Fig. 5).

Immediately after sperm–egg fusion, the connection between egg and sperm is tenuous. In many species, fusion occurs at the tip of an acrosomal process little more than 100 nm in diameter. Even during mammalian sperm–egg fusion where it is the side of the sperm head that fuses with the egg plasma membrane, the area of contact is no more than 1 μm^2. Movement of the sperm head (and eventually sperm tail) into the egg is due to actin-based traction. Actin is a force generating protein found in all cells whose acme is rapid and coordinated force generation in skeletal muscle. Actin filaments surround the sperm head, forming an extrusion from the egg surface known as a fertilization cone.

Actin filaments drag the sperm nucleus into the egg. Mitochondria also enter the egg cytoplasm. These degenerate; it is clear by observation and from the genetics of mitochondrial inheritance that all mitochondria in offspring are inherited from the egg and so from the mother. The sperm tail is incorporated. Its components dissolve in the egg cytoplasm, save for the centriole, an organelle that in the sperm organizes the tail microtubules but that, incorporated into the egg, takes over the role of organizing the mitotic spindle microtubules.

Once inside the egg, the behavior of the sperm nucleus depends upon the cell cycle stage of the egg that the sperm has fertilized.

2.5 Syngamy

The blind aim of sexual reproduction is to ensure that one sperm pronucleus coalesces with one female pronucleus. Coalescence of more than the complementary pair of pronuclei leads to genetic chaos. The penetration of more than one sperm nucleus into an egg is known as polyspermy and its consequences are usually fatal to the embryo.

Mechanisms exist to exclude supernumerary sperm. The electrical block to polyspermy (depolarization), found in echinoderms and amphibians, has already been mentioned: the fertilizing sperm depolarizes the egg, making it (for unknown reasons) much harder for subsequently arriving sperm to fuse with the egg. The electrical block is very fast and can come into play within 10 ms of sperm–egg fusion. Other polyspermy blocks are effective, but slower, requiring tens of seconds to take effect. A common mechanism comprises the secretion of the contents of secretory vesicles from the egg after fertilization. In many cases, this leads to the elaboration of a shell (vitelline envelope) around the fertilized egg that sperm cannot penetrate. In mammalian eggs, the secretions lead to inactivation of the glycoprotein that triggers the acrosome reaction in incoming sperm; sperm that have not undergone the acrosome reaction cannot fuse with the egg. In fish eggs, sperm must enter in single file down a narrow canal in the egg investments (the micropyle). Once the egg is activated by the fertilizing sperm, the secretions plug the canal, obstructing the progress of further sperm.

Not all strategies to ensure one-to-one fusion of the sperm and egg nucleus rely on sperm exclusion. Bird eggs are usually polyspermic. Once a lucky sperm nucleus has fused with the egg nucleus, all the other sperm nuclei present in the egg degenerate. In ctenophores, more than luck seems to be in play. The ctenophore egg is naturally polyspermic. The egg nucleus roams from one sperm nucleus

to the next, eventually fusing with one of them; the basis on which this choice is made is unknown.

Once the sperm nucleus has entered the egg it becomes surrounded by a nuclear envelope membrane and is known as the male pronucleus; its counterpart in the egg is the female pronucleus.

As the male pronucleus forms, it swells. The increase in pronuclear volume is due to chromatin decondensation consequent on the replacement of the polyamines surrounding sperm DNA with egg histones.

Ultimately, male and female pronuclei congress and fuse to realize syngamy. Congression of the pronuclei occurs because the male pronucleus is closely associated with the sperm centrosome: it organizes a microtubule array (the aster) that spreads through the egg cytoplasm and captures the female pronucleus, drawing it toward the male pronucleus, while the growing aster pushes against the egg cortex to move both male and female pronucleus toward the center of the egg.

3
Fertilization Calcium Signals

3.1
Calcium Ions are a Universal Egg Activator at Fertilization

In the early part of the twentieth century, Jacques Loeb experimented with various ways of stimulating the egg to undergo development in the absence of sperm – parthenogenesis. Treatment of echinoderm eggs with butyric acid followed by hypertonic seawater was effective, proving Loeb's antivitalist point that life was chemistry but offering no insight into the underlying mechanisms that governed the onset of development.

In the 1970s, a sharp tool became available that enabled these mechanisms to be defined: a calcium ionophore with the designation A23187. Calcium ionophores are small molecules that can carry calcium ions across lipid membranes. It was shown that treatment of unfertilized eggs of both vertebrates and invertebrates with A23187 led to many of the sequelae of fertilization: secretion of cortical secretory granules, DNA synthesis, and migration of the female pronucleus. The eggs had been activated, but did not divide. What was missing? It turned out to be the centrosomes that are required to form the mitotic spindle and that are donated to the egg by the sperm at fertilization. When centrosomes were induced to form in the echinoderm egg using hypertonic seawater, full development ensued in many cases and these parthenotes could be raised to adulthood.

These investigations set calcium ions center stage at fertilization.

3.2
Calcium Signaling

Calcium ions are the bearers of messages delivered by universal signaling systems found in all higher eukaryotes. Calcium ion concentrations are very low in the cytoplasm of all cells and are below micromolar concentrations at rest. It is postulated that calcium ion concentrations must of necessity be very low in the cytoplasm because of the existence of high concentrations of inorganic phosphate, itself a key component of intermediary metabolism. High concentrations of calcium and phosphate cannot coexist because of the low solubility product of calcium phosphate salt. On the other hand, calcium concentrations are in the millimolar range in the seas and in the body fluids of metazoans,

leading to very large gradients of calcium concentration across cell membranes. The existence of these gradients has two consequences. One is that calcium ion pumps driven by the cell's metabolism must constantly eject calcium from the cell since no lipid membrane is completely impermeant to calcium. The second is that relatively small calcium fluxes across the plasma membrane can give rise to large and rapid changes in the intracellular calcium concentrations. The latter consequence makes the cytoplasmic calcium concentration very suitable as an energy-efficient cell signal.

Calcium fluxes across plasma membranes control neurotransmitter release and are the basis of all neuronal activity. In skeletal muscle, calcium causes contraction but is released into the cytoplasm not from the extracellular fluid but from a pervasive network of reticular internal membranes that form a compartment known as the sarcoplasmic reticulum. This arrangement allows calcium ion concentrations to increase rapidly throughout the cytoplasm without the diffusion delays that arise when calcium enters through the plasma membrane. The reticular network is present in other cell types, where it is known as endoplasmic reticulum (ER). It exists not solely as a source of calcium signals; in fact, its primary purpose is to permit the folding and export of proteins destined for the plasma membrane either as integral membrane proteins or as secreted products. The concentration of calcium ions in ER is closer to the concentrations in extracellular fluid than to that of the cytoplasm and is maintained by a calcium pump. We know that folding and export of ER proteins is compromised when the calcium concentration in the ER falls, so it is reasonable to suppose that the compartment's high calcium concentration exists to provide an environment for proteins comparable to that they will experience once they leave the cell. But, as with the plasma membrane calcium gradient, the ER calcium gradient has been exploited in evolution as a calcium signaling system.

3.3 Fertilization Calcium Waves

When the calcium ionophore is used to activate an unfertilized egg, it is causing the release of calcium from the internal calcium stores of the ER. This is easily demonstrated by removal of all calcium external to the egg: this has no effect on egg activation. It is also possible, though rather more complicated, to demonstrate that calcium ion concentrations increase at fertilization.

The first demonstrations of fertilization calcium increases employed a protein (aequorin) that emits light in response to calcium in a graded way. The increase in calcium at fertilization could be seen as a ring of light travelling through the peripheral cytoplasm of a large medaka fish egg. This established the basic features of the fertilization calcium signal as a large increase in cytoplasmic calcium that arises at the point of sperm–egg fusion and traverses the egg as a constant velocity reaction-diffusion wave. Similar waves were observed in frog, echinoderm, nematode, and mammalian eggs using both aequorin and more convenient fluorescent calcium indicators developed by Dr Roger Tsien (Fig. 6).

Fertilization calcium waves are the largest and longest calcium signals measured. They rise to around 5–10 μM and last for many minutes. Preparation for these large explosive signals takes place

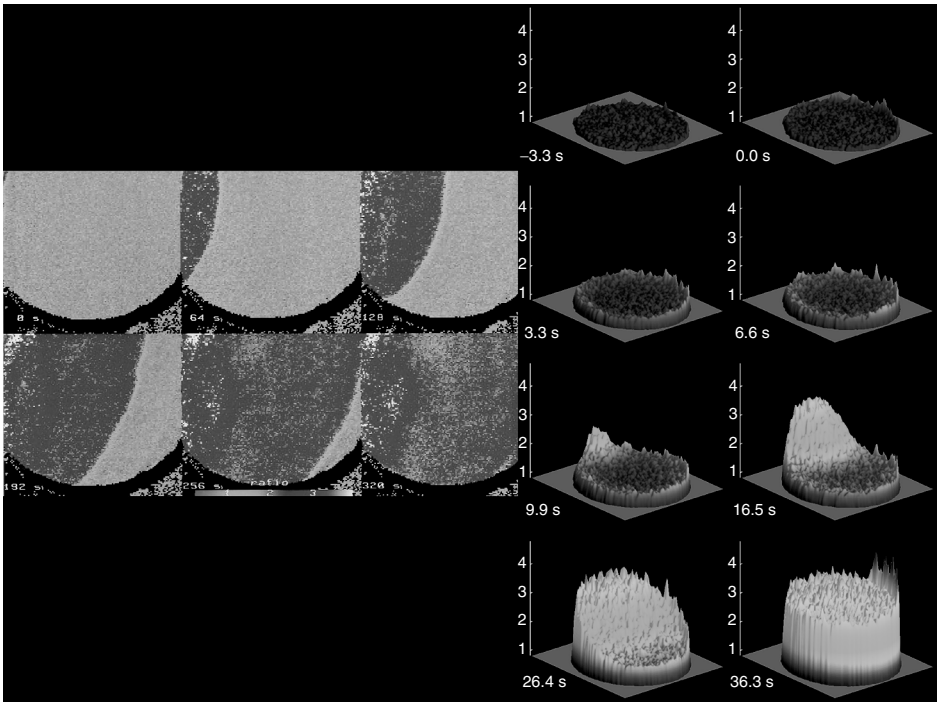

Fig. 6 Fertilization calcium waves in sea urchin and frog eggs. Calcium sensitive dyes are used to detect calcium waves. Warm colors reflect high calcium concentrations. The calcium wave crosses the sea urchin egg in around 15 s with a velocity of around 5 μm s^{-1}. The wave has a similar velocity in the frog egg, but here it takes 5 min to traverse the egg as it is over 1 mm in diameter. Left: frog egg. Right: sea urchin egg. A small early increase in calcium concentration can be seen at the egg periphery, a consequence of a calcium action potential that is triggered by sperm–egg fusion.

during meiosis. As meiosis progresses, the oocyte becomes competent to generate a large calcium wave; after fertilization, this competence disappears. The calcium ionophore experiments tell us that the waves' purpose is to initiate development and it is reasonable to suppose that their extent, magnitude, and duration are necessary to ensure that each part of the egg cytoplasm receives the message that fertilization has occurred. They ensure that the small and local interaction that occurs between egg and sperm is amplified and transmitted throughout the egg.

3.4 Repetitive Fertilization Calcium Transients

Both ascidian and mammalian oocytes manifest multiple large calcium transients at fertilization. In ascidian oocytes, the locus of initiation of the calcium wave migrates with the male pronucleus as it moves toward the oocyte's vegetal pole. In mammalian oocytes, the first few calcium transients originate at the point of sperm – egg fusion, but thereafter, each calcium transient appears to rise homogeneously within the oocyte cytoplasm. This behavior can be explained by proposing

that each transient is generated by a factor associated with the sperm and male pronucleus. In ascidian oocytes, it is supposed that this factor remains closely associated with the male pronucleus, while in mammalian oocytes, the factor slowly diffuses away from the male pronucleus to become uniformly distributed in the cytoplasm. We shall consider the likely purpose of the repetitive transients when we come to discuss the link between fertilization and the cell division cycle.

3.5 Signal Transduction during the Fertilization Calcium Wave

The question before us is how sperm–egg fusion, an event that initially involves a cytoplasmic bridge as little as 100 nm wide, is able to trigger the large fertilization calcium wave. An analogy is that the fertilization calcium wave is an explosion and that the sperm lights a fuse to set it off. This leads to two further questions: what gives the calcium wave its explosive properties and how is the fuse lit? We shall answer them in turn.

3.5.1 Reaction Diffusion Waves

Explosions occur because the products of a chemical reaction further increase the rate of the reaction itself in a process of positive feedback. The fuel for the calcium explosion at fertilization is the calcium stored within the ER. Positive feedback occurs because of the particular properties of calcium channels that exist in the ER membrane: the probability of their opening increases when cytoplasmic calcium increases. Once calcium rises at a point within the cytoplasm, a wave of calcium release will propagate from that point (Fig. 7). There is good evidence for this mechanism in heart cells made to propagate artificial calcium waves.

The identity of the calcium channel in the ER responsible for the fertilization calcium wave was determined by

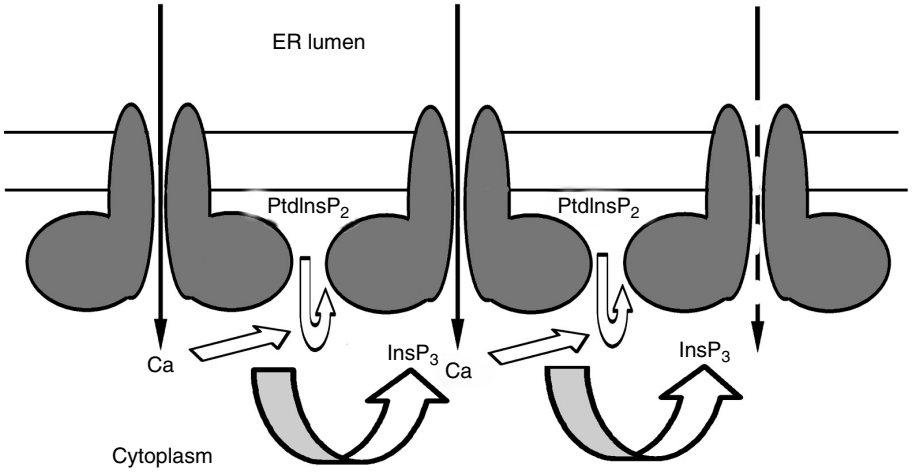

Fig. 7 Mechanism of propagation of the fertilization calcium wave. The calcium wave propagates via calcium-dependent production of $InsP_3$ and diffusion of calcium from one release channel to the next. Both $InsP_3$ and calcium are required in the vicinity of the channel to trigger channel opening and movement of calcium from the ER into the cytoplasm.

investigation of the phosphoinositide messenger pathway, a ubiquitous signaling pathway in cells of higher eukaryotes. The substrate of this pathway is phosphatidylinositol bisphosphate ($PtdInsP_2$). A signaling event, for example, the binding of a hormone or neurotransmitter to its receptor, leads to activation of a specific phospholipase C that hydrolyzes $PtdInsP_2$ to produce two products, inositol trisphosphate ($InsP_3$) and diacylglycerol. $InsP_3$ is a small sugar that causes calcium release from ER. It achieves this by binding to and allosterically activating an ER calcium channel known as the $InsP_3$ receptor ($InsP_3R$), causing it to open. Calcium in the cytoplasm is a coactivator of the $InsP_3R$. The sequence in which $InsP_3$ and calcium are presented to the channel is crucial: if calcium binds after $InsP_3$, then the channel is activated, but if it binds before, $InsP_3$ is unable to open the channel.

Calcium waves can be triggered in eggs by injecting very small amounts of $InsP_3$ into unfertilized eggs and can be blocked by agents that prevent $InsP_3$ from interacting fruitfully with the $InsP_3R$. These observations demonstrate that the $InsP_3R$ is the calcium channel responsible for propagation of the fertilization calcium wave, but they also suggest that the mechanism by which the wave propagates is more complex than that established for calcium waves in heart cells.

The properties of the $InsP_3R$ are not immediately consistent with the observation that very small quantities of $InsP_3$ are sufficient to trigger a calcium wave since once the locally injected $InsP_3$ is diluted by diffusion into the large volume of the egg, its concentration is insufficient to activate the $InsP_3R$ which, as we have seen, requires coactivation by both $InsP_3$ and calcium. There is evidence, in fact, that increases in calcium induce further hydrolysis of $PtdInsP_2$, generating $InsP_3$ locally as the wave propagates. The mechanism of wave propagation thus relies on two intertwined positive feedback pathways, calcium-induced production of $InsP_3$ and coactivation of the $InsP_3R$ by $InsP_3$ and calcium (Fig. 7).

3.5.2 Initiation of the Calcium Wave by the Sperm

The simplest idea is that the sperm generates a small amount of $InsP_3$, so setting off the calcium wave in much the same way as happens when $InsP_3$ is microinjected. This is almost certainly the case, though there is no direct evidence for an increase in $InsP_3$ in the tiny volume close to the point of sperm–egg fusion.

The evidence that an increase in $InsP_3$ triggers the fertilization calcium wave is more subtle. A significant amount of time elapses after sperm–egg fusion before the increase in cytoplasmic calcium that marks the onset of the wave is seen. In echinoderms, around 10–20 s elapses, comparable to the time it then takes for the wave to cross the egg; in mammalian eggs, several minutes pass. What is found is that an agent that interferes with the activation of the $InsP_3R$ increases this latency.

$InsP_3$ is generated by activation of $PtdInsP_2$-specific phospholipases. There has been a debate about which of these phospholipases is responsible for triggering the calcium wave. It was initially thought that an interaction of the sperm with an egg receptor might activate the β-form of phospholipase C (PLCβ); PLCβ is known to be coupled to transmembrane receptors in all cell types examined. However, as it became clearer that sperm–egg fusion was the key activating event at fertilization, attention shifted to another form of phospholipase, PLCγ, that is activated

by phosphorylation on tyrosine. Protein fragments that were able to compete with and block either PLCγ or tyrosine (Src family) kinases were found to increase the latency of the fertilization response and at higher concentrations, to block it completely. Mammalian eggs were, however, an exception, being completely insensitive to these inhibitors. On the assumption that the activating phospholipase would be present in sperm, the mammalian testis was screened for the existence of appropriate phospholipases. A novel phospholipase found only in testis (PLCζ) was shown to induce repetitive calcium transients when expressed in unfertilized mouse eggs.

The picture we have of signal transduction at fertilization is that a protein (Src kinase, PLCγ, PLCζ, depending on species) passes into the egg when sperm and egg fuse. The presence of this protein in the egg leads to the very local production of $InsP_3$. As the latent period progresses, a concentration of $InsP_3$ builds up sufficiently to trigger the onset of the explosive fertilization calcium wave (Fig. 7). Observation of the repetitive fertilization calcium transients in ascidians and mammals suggests that in these species, the activating protein is bound to a component of the sperm head. In ascidian oocytes, the association persists as the male pronucleus forms, while in mammalian eggs, the protein (PLCζ) is thought to dissociate from the male pronucleus (see Sects. 3.4 and 4.3.1).

4
Fertilization and the Cell Division Cycle

4.1
Fertilization Occurs at Different Stages of the Cell Division Cycle in Different Species

It is striking given the ubiquity of calcium signals at fertilization that there are significant differences between eggs of different species in the cell cycle stage in meiosis at which fertilization occurs. Meiosis comprises two cell divisions without an intervening S-phase. Fertilization can occur before the first meiotic division begins, at metaphase of first meiosis, at metaphase of second meiosis, and in interphase once meiosis is complete (Fig. 8). With very few exceptions, this is because at the time that the egg is most receptive to sperm, its cell cycle has arrested at one of the above stages, a stage specific to the species in question.

4.2
Maturation Promoting Factor Plays a Part in Meiosis and in Meiotic Arrest

Classic experiments in the field of cell cycle control showed that cytoplasm from oocytes arrested and awaiting fertilization in meiosis I or meiosis II would induce the maturation of immature oocytes. Similarly, classical experiments in the genetics of yeast cell cycle control genes determined that two key components of

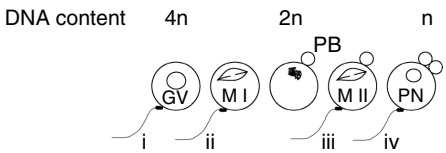

Fig. 8 Cell cycle arrest before fertilization. Oocyte of different species arrest before fertilization at different points of the meiotic division cycle. Some are fertilized as immature oocytes (i: clam), some during first meiotic metaphase (ii: other molluscs, ascidian), some during second meiotic metaphase (iii: frog, starfish, mammals) and some after meiosis at the start of the first mitotic cell division cycle (iv: sea urchin).

maturation promoting factor activity were cdk1, a protein kinase, and its activating partner, cyclin B.

Cdk1/cyclin B activation linked to accumulation of cyclin B drives the cell into meiosis or mitosis, causing condensation of chromatin and formation of the mitotic or meiotic spindle. Oocytes (and other cells) arrested at metaphase show high levels of cdk1/cyclin B activity, but what marks them out is the persistence of this normally transient activity: the persistence leads to cell cycle arrest.

Stabilization of cdk1/cyclin B activity during metaphase cell cycle arrest is due to what appear to be the two other components of maturation promoting factor, a protein termed mos and its target, MAP kinase. Mos is a protein unique to meiotic maturation. Its transcription early during maturation leads to increasing levels of MAP kinase activity. MAP kinase activity remains high throughout meiosis until fertilization occurs. It is responsible for the persistence of cdk1/cyclin B activity (Fig. 9) and, incidentally, for the suppression of S-phase between meiosis I and meiosis II.

Persistent activation of cdk1/cyclin B accounts for the two metaphase cell cycle arrest points during meiosis observed in various species, but other explanations are necessary to explain cell cycle arrest before meiosis begins or after it is completed.

Cell cycle arrest before the onset of meiosis is seen in most organisms. In most species, premeiotic arrest is a distinct stage preceding the prefertilization arrest that occurs later in meiosis and that has already been discussed earlier. Ovaries contain many immature oocytes, a proportion of which are recruited by hormonal stimulation to begin maturation. It is thought that hormonal stimulation leads to a fall in the second messenger cyclic adenosine monophosphate (cAMP). In species whose oocytes are fertilized when immature, it is the fertilization calcium signal that triggers the onset of maturation and reinitiation of meiosis (In some molluscs fertilized at this stage, calcium influx across the plasma membrane takes the place of a calcium wave.).

Cell cycle arrest in interphase of the first mitotic cell cycle occurs in some echinoderms. This arrest can be explained by the suppression of protein synthesis by a relatively acidic cytoplasmic pH and the maintenance of a redox state that lead to low levels of the synthetic nicotine-adenine dinucleotide phosphate (NADPH).

4.3 Calcium Signals and Cell Cycle Progression at Fertilization

4.3.1 Metaphase Arrest

Persistent cdk1/cyclin B activity driven by mos/MAP kinase is the cause of the cell cycle arrest at metaphase in oocytes. Inactivation of these kinases by removal of both cyclin B and mos is how the

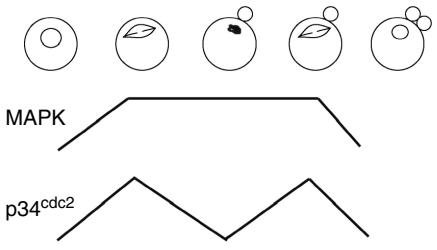

Fig. 9 MAP kinase and cdk1 during meiotic maturation. MAP kinase activity remains high throughout meiosis, while cdk1 activity dips between meiosis I and meiosis II.

fertilization calcium signal causes meiosis to resume.

Frog oocytes are arrested during meiosis II. The fertilization calcium wave triggers cyclin proteolysis by the proteasome through activating calmodulin and so calmodulin-dependent protein kinase II (CamKII). CamKII in turn activates another molecular machine known as the *anaphase promoting complex* (APC) that targets cyclin for destruction by the proteasome. A parallel mechanism, activation of the calcium-dependent protease calpain, leads to proteolysis of mos. In frog oocytes, the proximal cause of anaphase onset is the destruction of cyclin; destruction of mos occurs later than cyclin destruction. Mouse oocytes are also arrested at meiosis II awaiting fertilization. In mammals, 10 to 12 h elapses between fertilization and formation of the nuclear membrane around male and female pronuclei, the lengthy period coinciding with the process of imprinting, found only in mammals. During this period, calcium transients occur every few minutes and cyclin B is slowly degraded, the rate extent of cyclin degradation determined by the frequency of calcium signals. Formation of the pronuclear membranes marks the end of meiosis and coincides with the final disappearance of cyclin B. Mos, on the other hand, is degraded within a few hours. Ascidian oocytes are arrested before fertilization at metaphase of meiosis I. A first set of calcium transients occurs immediately after fertilization, leading to degradation of cyclin B and exit from meiosis I. Mos/MAP kinase activity is not affected by this first set of transients (remember that a role of mos/MAP kinase is to suppress S-phase in the interval between the two meiotic divisions). The repetitive transients begin again as the oocyte enters meiosis II, again leading to degradation of cyclin B and, on this occasion, degradation of mos too. The events described in this paragraph are summarized in Fig. 10.

The reader will have noted a correlation between the state of the nucleus and the occurrence of repetitive calcium transients: the transients are present during meiotic divisions when chromatin is condensed and the nuclear envelope is absent, and are absent during interphase when chromatin in decondensed and a nuclear envelope surrounds the membrane. The transients may indeed be triggered by a factor that is sequestered in the nucleus and released when the nuclear envelope breaks down as the oocyte enters meiotic division. The evidence for this is that nuclei isolated from first cell cycle interphase in mouse embryos will break down under the influence of cdk1/cyclin B when transferred to unfertilized mouse oocytes and induce repetitive calcium transients. The simplest explanation is that PLCζ, the egg activator from the sperm, is sequestered into the nucleus as the nuclear envelope reforms. This conjecture is consistent with observations that repetitive calcium transients continue indefinitely when import of proteins into the nucleus though the nuclear pores are blocked.

4.3.2 Interphase Arrest

Little more is known about how calcium lifts the cell cycle blockade after fertilization of immature oocytes than that calcium signals are known to be capable of antagonizing signaling by cyclic AMP.

Sea urchin eggs are arrested after completion of meiosis, in interphase of the first cell cycle. The fertilization calcium wave stimulates a calcium-dependent NAD^+ kinase to generate NADP, which is rapidly reduced in the cell to NADPH, providing fuel for protein synthesis. Activation of phospholipase C is known to generate

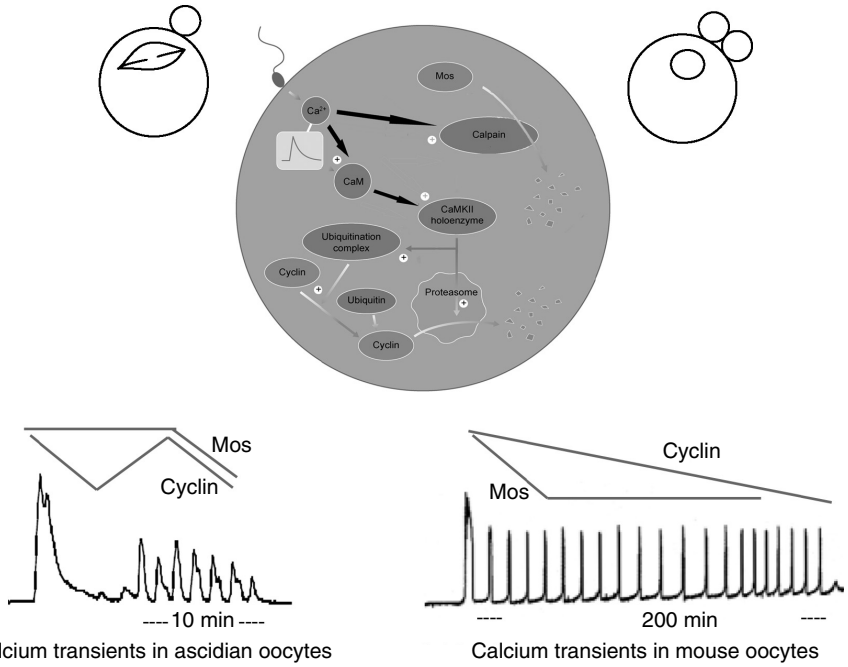

Fig. 10 Destruction of mos and cyclin B at fertilization. Two calcium-dependent pathways for proteolysis at fertilization. One degrades mos by activating calpain. The other activates CaMKinase II to stimulate the APC and cyclin degradation by the proteasome. Calcium dependent proteolysis is rapid in ascidians; in mouse and other mammals mos is rapidly degraded, but cyclin degradation takes several hours.

a diacylglycerol from $PtdInsP_2$ in addition to $InsP_3$ (Fig. 7). At fertilization in the sea urchin egg, the diacylglycerol so generated activates a protein kinase C (no relation of phospholipase C) in the egg plasma membrane in turn stimulating a sodium–hydrogen antiporter that ejects hydrogen ions from the egg, returning the cytoplasm to its normal pH of 7.2. The suppression of the protein synthetic machinery is lifted, cyclin synthesis begins, and the cell cycle proceeds.

4.4 The Cell Cycle and Syngamy

The behavior of the male pronucleus once inside the oocyte or egg after fertilization depends on the point at which fertilization occurs: during or after meiosis. Oocytes fertilized during meiosis must complete meiosis to generate a haploid female pronucleus with which the male pronucleus can undergo syngamy. In this case, the male pronucleus remains separate with its chromatin in a condensed state. Once meiosis II is completed, cyclin B/cdk1 activity falls and the male pronucleus develops a nuclear envelope, as does the female pronucleus. Syngamy comprises the mixing of male and female genomes and occurs through fusion of the pronuclei's nuclear envelopes. S-phase of the first mitotic cell cycle often begins before syngamy. In an extreme case, in mouse

zygotes, S-phase is completed and the nuclear envelope breaks down as the zygote enters the first mitotic division before syngamy occurs. Syngamy occurs on the metaphase plate as male and female chromosomes are captured by spindle microtubules.

5
Calcium and Cell Cycle Control

5.1
Cell Cycle Checkpoints

It is noteworthy that the same cytoplasmic calcium signal, the wave generated by the sperm, operates to trigger resumption of the cell cycle at three distinct points in the cell cycle in different organisms: G2/M, metaphase of MI and MII (which are formally equivalent) and G1. The question arises as to whether these fertilization mechanisms are specific examples of a more general requirement for calcium during cell cycle progression of all cell types.

More general study of the regulation of the cell cycle in yeast and in mammalian somatic cells as well as in embryos has led to the concept of cell cycle checkpoints. The concept is straightforward: before a cell makes a crucial step through the cell cycle, a checkpoint mechanism is invoked that is designed to ensure that everything is correctly in place, a preflight checklist as it were. Two examples of this are the DNA damage checkpoint and the mitosis exit checkpoint.

DNA replication is not a faultless process. Errors are introduced during copying of the DNA strands that are then repaired during late S-phase. The point here is that if mitosis were to occur before repair had taken place, then one or both daughter cells would inherit mutations that resulted from errors introduced at S-phase. The DNA damage checkpoint mechanism is invoked by damaged DNA; while it is active, the cell cannot proceed into mitosis.

The mitosis exit checkpoint is invoked during mitosis to ensure that all chromosome pairs are captured and properly aligned on the metaphase plate before they are separated by the processes of anaphase. A single misaligned chromosome will prevent mitosis. The checkpoint machinery monitors attachment of two sets of microtubules to the chromosome and also verifies by measuring tension in the microtubules that they are correctly attached to the spindle poles. In this instance, the checkpoint mechanism aims to avoid an unequal distribution of chromosomes into the daughter cells. This condition known as *aneuploidy* leads to cell death or on occasion to uncontrolled cell proliferation. A third checkpoint exists at the beginning of the cell cycle in G1. Here, a cell decides, on the basis of signals from its environment, whether it will differentiate, commit suicide (apoptosis), or progress into the cell cycle and divide. The cell cycle stage at which oocytes are found to arrest in the cell cycle correspond to the stages at which these checkpoints act.

Oocytes arrested in G2 are stopped at the stage at which the DNA damage checkpoint acts, those arrested at metaphase are stopped at the mitosis exit checkpoint stage, and those arrested in G1 resemble those cells making choices about whether to divide, die, or differentiate. It is thus very likely that cell cycle arrest before fertilization makes use of underlying cell cycle checkpoint mechanisms.

5.2 The DNA Damage Checkpoint

The DNA damage checkpoint operates by preventing activation of the mitotic kinase cdk1/cyclin B. It does this by preventing a phosphatase (cdc25) from removing a phosphate group on cdk1 that inhibits kinase function. It has been shown in mouse oocytes that activation of cdc25 is necessary and sufficient to bypass the G2/M block and trigger resumption of meiosis. In most oocytes, as mentioned earlier, resumption of meiosis is triggered by a fall in cyclic AMP (Fig. 11). In mouse oocytes, it has been shown that cdc25 is downstream of the reduction in cyclic AMP levels. The calcium transient in oocytes fertilized at G2/M may activate cdc25 via calmodulin-dependent protein kinase II, as it has been shown that progression through the G2/M checkpoint in mammalian somatic cells involves activation of this kinase.

5.3 The Mitosis Exit Checkpoint

The mitosis exit checkpoint operates by preventing activation of the APC that targets key proteins for destruction including securin and cyclin B. Loss of securin leads to loss of adhesion between paired chromosomes on the metaphase plate, allowing them to separate and spring to the poles; loss of cyclin B leads to inactivation of the cdk1/cyclin B kinase, permitting decondensation of chromatin and reformation of the nuclear envelope. These events are triggered by the fertilization calcium transient in oocytes arrested at metaphase meiosis I and meiosis II before fertilization.

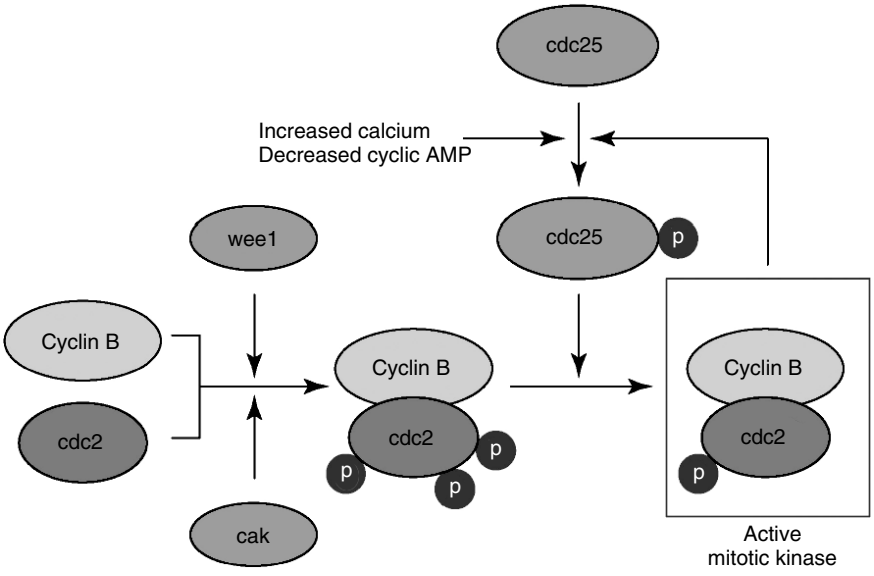

Fig. 11 Activation of mitotic kinase by the cdc25 phosphatase. Cdk activating kinase (cak) and wee1 kinase phosphorylate the cdk1/cyclin complex. Cak phosphorylation activates the mitotic kinase and wee1 phosphorylation is inhibitory. Cdc25 phosphatase removes the inhibitory phosphates. Cdc25 is controlled by cAMP and calcium.

5.4 The Checkpoint at Cell Cycle Onset

There are analogies between fertilization in species such as echinoderms that are fertilized in G1 and the G1 decision point in mammalian somatic cells. One striking example is starfish oocytes: if they do not sense a fertilization calcium transient, they proceed to apoptosis. Other similarities are the activation of an MAP kinase signaling cascade, stimulation of the sodium-hydrogen antiporter by protein kinase C, and stimulation of the protein synthetic machinery. Nonetheless, the comparison is less precise at the molecular level.

5.5 Calcium and Cell Cycle Checkpoints

The existence of identical molecular mechanisms in arrested oocytes and in the checkpoint mechanisms of somatic cells strongly suggests that the arrest before fertilization uses the checkpoint machinery and raises a question: is calcium signaling a component of general cell cycle regulation or is control of the checkpoint machinery by calcium unique to fertilization?

That calcium is a cell cycle regulatory signal is clear in some cases, and less clear in others. In some embryos, calcium signals can be identified at the times at which the checkpoint mechanisms operate; the amplitude of the signals is small, less than one-tenth of the size of the fertilization calcium signal and signals are often local to the nucleus. The calcium-calmodulin-calmodulin kinase II pathway has been shown to control entry into mitosis and calcium and calmodulin control separation of the chromosomes at mitosis. Embryos do not possess a G1 decision point mechanism.

In somatic cells, it has been shown, for example, that calmodulin-dependent protein kinase II must be activated at mitosis entry and that it phosphorylates cdc25, the activator of cdk1/cyclin B. It has also been shown that rapidly raising intracellular calcium can induce anaphase. Checkpoint-associated calcium signals can be detected, but they are not present in all circumstances. The jury is out on the universality of cell cycle calcium signaling, but if the hypothesis turns out to be true, then the calcium signals central to fertilization will have pointed the way.

See also Calcium Biochemistry; Female Reproduction System, Molecular Biology of; *In vitro* Fertilization; Male Reproductive System: Testis Development and Spermatogenesis.

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