The Control of Oxidant Stress at Fertilization

BENNETT M. SHAPIRO*

Metazoan eggs alter their coats after fertilization to protect the early embryo. In sea urchins, this modification consists of a rapid, coordinated set of noncovalent macromolecular assembly steps that are stabilized by protein cross-linking. The sea urchin egg uses an oxidative cross-linking reaction that requires hydrogen peroxide and a secreted peroxidase and thus faces the challenge of oxidant stress at the beginning of its development. Protection from the deleterious effects of this oxidative mechanism is afforded by regulation of the production and utilization of oxidizing species. This regulation requires a specific protein kinase C–activated oxidase and ovothiol, an intracellular antioxidant.

WHEN PARENTAL HAPLOID GENOMES JOIN TO FORM A unique individual at fertilization, the destiny of a species may be recast. Because fertilization occupies a central place in biology, it has been studied at different levels of resolution since antiquity. The union of sperm and egg involves a series of signaling mechanisms that leads to species-specific gamete interaction, resulting in the activation of an egg by a single sperm. The biochemical pathways of fertilization are complex because sperm behavior is modified by egg components, and egg activation is effected by sperm attachment and fusion. With the tools of molecular biology, the discrete pathways and regulatory principles that govern fertilization are now being clarified (*1*).

The sea urchin may be the most facile experimental system for studying molecular mechanisms of fertilization. Each female produces as many eggs as do millions of mice. Mammalian fertilization is difficult to study, but enormous populations of sea urchin eggs can be fertilized synchronously in vitro, allowing the biochemistry of the attendant phenomena to be explored. These techniques have provided insights into guanylate cyclase–mediated signaling (*2*), use of a phosphocreatine shuttle for energy transport (*3*), rapid modulation of sperm behavior by ionic fluxes (*4*), a membrane potential–mediated rapid block to polyspermy (*5*), and intracellular alkalinization in egg activation (*6*). In this article I discuss another such molecular mechanism, the biochemistry of egg surface modifications that occur in response to sperm entry.

Animal eggs change their coats after fertilization to protect the embryo from noxious agents, including additional sperm. A dramatic example of this is seen with sea urchin eggs (Fig. 1). Within 5 min after fertilization, a spherical envelope is produced that protects the egg during the early cell divisions, until the blastula stage when the embryo hatches to become an independent organism. Harvey (7) showed that cytolytic agents like H_2SO_4 and NaOH destroy the egg but leave the fertilization envelope intact. The property of the envelope to resist disaggregation by protein denaturants and detergents is achieved by an extracellular cross-linking reaction that occurs within 10 min of fertilization. This morphogenesis is different from bacteriophage or ribosome assembly, which take place within the intracellular milieu of enzymes, adenosine triphosphate (ATP), and metabolites, where multiple regulatory cascades are operative. For this reason, I speculated that interesting and perhaps novel control mechanisms would be used to effect rapid envelope assembly outside of the cell. The reaction is, indeed, highly regulated: specific, topologically constrained protein aggregates associate under the influence of the divalent cations in seawater and then this matrix is stabilized by a hierarchy of cross-linking reactions, the ultimate one using H_2O_2 as an extracellular oxidant.

The Cortical Reaction

The fertilization envelope is formed by the mixing of two cellular compartments: a vitelline layer, which is tightly apposed to the outside of the plasma membrane, and the contents of intracellular secretory vesicles, which are located beneath the membrane (Fig. 2). Gamete membrane fusion elicits a wave of Ca^{2+} release from intracellular stores (*8*) that initiates at the point of sperm entry and causes exocytosis from some 15,000 vesicles (cortical granules), releasing about 5% of the egg protein. The secreted components modify the vitelline layer by addition of new material and catalytic alterations of the assembled matrix. The initiating rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is secondary to the production of inositol 1,4,5-trisphosphate (IP_3), which increases along with production of diacylglycerol (DAG) within 20 s of fertilization (*9*). Although the microanatomy of cortical granule exocytosis and envelope assembly has been known for over 30 years (*10*), biochemical mechanisms have been refractory to analysis because the highly cross-linked fertilization envelope impedes study of its composition and process of assembly. When the cross-linking reaction was shown (*11*) to be catalyzed by a peroxidase that coupled tyrosyl residues between adjacent polypeptide chains, the path was cleared for a study of the assembly mechanism.

Fertilization Envelope Assembly

The protein complexes released from the cortical granules after fertilization deposit on the vitelline layer with an appropriate orientation to effect vicinal placement of tyrosyl residues (*12*). This divalent cation–dependent assembly results in a soft, noncross-linked fertilization envelope. The noncovalently associated structure is hardened by ovoperoxidase, a secreted, monomeric, heme-con-

Department of Biochemistry, University of Washington, Seattle, WA 98105.

*Present address: Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

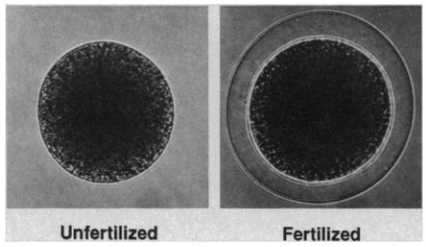

Fig. 1. Sea urchin egg before and after fertilization. The refractile fertilization envelope that surrounds the egg is formed within 3 min of fertilization and is hardened by 10 min. The envelope is retained until the multicellular blastula hatches to continue its development as a freely swimming larval form.

taining protein of mass 70 kD (*11, 13*) that catalyzes dityrosine formation. The oxidant for this extracellular cross-linking reaction is H_2O_2, which is produced in a burst after fertilization (*14*) (Fig. 2).

Evidence for this cross-linking mechanism includes the following observations: (i) dityrosine residues are present in hard fertilization envelopes (*11*) and absent from soft envelopes (*12*); (ii) inhibitors of ovoperoxidase inhibit hardening (*11*); and (iii) the soft envelope can be cross-linked in vitro concomitant with the production of dityrosine on addition of H_2O_2 to the isolated structure (*12*). Ovoperoxidase is found in cortical granules before fertilization (*15*), and over 80% of the secreted enzyme is incorporated into the fertilization envelope. Ovoperoxidase has an unusual pH dependence (*16*) that keeps the enzyme inactive in the acidic milieu surrounding the egg immediately after secretion but allows it to activate slowly on reaching the equilibrium pH of seawater. This hysteresis phenomenon (*16, 17*) could serve as a timing delay for prevention of peroxidative chemistry at the plasma membrane where damage would occur to the embryo. Once assembled into the fertilization envelope, ovoperoxidase no longer exhibits this pH-dependent hysteresis, but instead is locked into an active form (*17*). Thus, there is matrix-dependent regulation (*17*) of ovoperoxidase activity when it is positioned to effect envelope cross-linking.

Ovoperoxidase is translocated with high efficiency from the cortical granules to the fertilization envelope along with proteoliaisin (PLN), another cortical granule protein. PLN binds ovoperoxidase in a Ca^{2+}-dependent interaction (*18*) with 1:1 stoichiometry [apparent dissociation constant (K_d) = 1.1×10^{-6} M]. PLN also binds to the egg vitelline layer (*18*) in the presence of Mg^{2+} and Ca^{2+} at concentrations found in seawater. This binding by PLN is necessary and sufficient to attach ovoperoxidase to the egg surface.

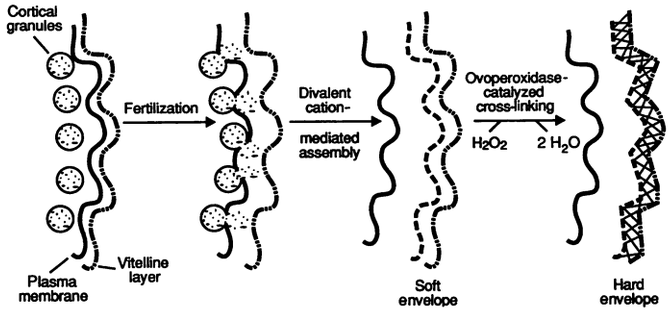

Fig. 2. Schematic diagram of fertilization envelope assembly. In response to an increase in $[Ca^{2+}]_i$, exocytosis of cortical granules releases proteins that interact with the vitelline layer to form a noncovalent aggregate (the soft fertilization envelope). The soft envelope is cross-linked in an ovoperoxidase-catalyzed reaction that consumes H_2O_2 to form dityrosyl residues. If ovoperoxidase is inhibited, the soft envelope may be isolated. When divalent cations are removed from this soft fertilization envelope, it disaggregates to leave a residual structure, called a "wraith," and releases the proteins that had been added from the cortical granules (*12*). The intact soft fertilization envelope may be hardened and cross-linked by addition of H_2O_2 because it contains ovoperoxidase in the appropriate locations.

Thus, PLN is a carrier protein that translocates ovoperoxidase from an intracellular compartment to a specific extracellular matrix site. PLN (230,000 kD) is a monomeric, asymmetric, acidic protein with an unusual amino acid composition [six amino acids (Asp/Asn, Glu/Gln, Gly, and Cys) account for 53% of the total residues] (*19*). Along with other released proteins of >100 kD that assemble into the envelope, PLN becomes cross-linked into the final structure (*12*). The vitelline layer and ovoperoxidase binding domains of proteoliaisin are clustered in a 50-kD region near, but not at, the NH_2-terminus (*20*). At the COOH-terminus, there is a long tail that contains tyrosyl residues that are activated by ovoperoxidase and are potential targets for cross-linking. Cross-linking is likely to be a stochastic process, in that appropriately positioned tyrosyl radicals from PLN and other proteins would form *o,o*-dityrosines on contact.

A Hierarchy of Cross-Linking Reactions

Exocytosis from the cortical granules releases PLN-ovoperoxidase complexes, which interact with the vitelline layer to form an appropriate envelope, but only if a plasma membrane transglutaminase executes an earlier event. This additional level of complexity was first indicated by experiments with the transglutaminase inhibitor, glycine ethyl ester (*21*), where envelope hardening was impaired. Glycine ethyl ester, a primary amine, blocks a morphologic change (*22*) of the assembling envelope (the I-T transition). In the presence of primary amines, PLN-ovoperoxidase complexes pass through the nascent fertilization envelope, which becomes less refractile and lacks its normal lamellar appearance (*23*). Plasma membrane transglutaminase activity increases in a transient burst during the first 5 min after egg activation (*23*). On fertilization in the presence of Zn^{2+} [median inhibition concentration (IC_{50}) = 15 μM], which inhibits egg transglutaminase, the vitelline layer never assembles into a fertilization envelope. Thus, an early transglutaminase-catalyzed event is necessary for subsequent noncovalent protein assembly, which produces a soft fertilization envelope with its I-T transition. This event is then followed by dityrosine cross-linking.

Respiratory Burst and H_2O_2 Synthesis

Eighty years ago, Warburg discovered that sea urchin eggs consume oxygen after fertilization (*24*). This, the archetype of cellular metabolic activation, was later shown to involve a burst of O_2 uptake that is not inhibited by CN^- or other classic respiratory chain inhibitors (*25*). The so-called "respiratory burst of fertilization" produces H_2O_2 (*14*) and accounts for the O_2 consumed in the burst.

The oxidase responsible for the burst (*26*) uses NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$), as a cofactor:

$$NADPH + H^+ + O_2 \rightarrow NADP^+ + H_2O_2$$

The enzyme requires Ca^{2+} and Mg^{2+}-ATP (MgATP). Inhibitors of protein kinases, such as H7 and staurosporine, inhibit the respiratory burst in vivo and the NADPH oxidase in vitro. When the reaction is initiated with Ca^{2+} or MgATP, there is a 60-s lag period before the final rate of H_2O_2 production is achieved. However, when the enzyme preparation is preincubated with Ca^{2+} and MgATP and catalysis is initiated with NADPH, the reaction begins instantaneously (*26*). Taken together, these data suggest that ATP modifies the oxidase.

Crude NADPH oxidase may be fractionated into a membrane fraction and a soluble fraction, both of which are required for activity (*27*). The partially purified soluble fraction has protein kinase C (PKC) activity and can be replaced in the oxidase assay by purified mammalian PKC. Other protein kinases cannot substitute for the NADPH oxidase. The catalytic subunit of protein kinase A, casein kinase II, calmodulin-dependent kinase II, or myosin light chain kinase will not suffice for activation (*27*). A specific inhibitory peptide from the NH_2-terminal regulatory domain of PKC inhibits the oxidase reaction (*27*), whether the assay is activated by the sea urchin egg kinase or purified rat brain PKC. Moreover, phorbol esters cause a 40-fold decrease in the concentration of Ca^{2+} required for activating the burst oxidase. These data indicate a specific function for PKC in the respiratory burst oxidase of eggs; at present, this is the only enzyme-catalyzed reaction with an obligatory requirement for PKC for activation in vitro.

The participation of a PKC-like activity is compatible with what is known about the egg activation cascade, because a presumed phospholipase C leads to the production of IP_3 and DAG (*9*) after fertilization (Fig. 3). The resultant increased $[Ca^{2+}]_i$ and DAG may activate PKC, which would subsequently initiate the respiratory burst. The function of NADPH oxidase in the respiratory burst is consistent with earlier observations made concerning egg metabolism after fertilization (*28*). For example, glucose-6-phosphate dehydrogenase, as well as flux through the pentose phosphate pathway, is activated along with NAD kinase. The net effect of these changes is to produce NADPH, the substrate for the oxidase as well as the primary reductant in a second pathway that protects the egg from oxidant stress (see below). Activation of the oxidase by protein phosphorylation provides a mechanism for controlling oxidant stress by allowing the synthesis of H_2O_2 to be coordinated temporally with other events in envelope assembly.

Ovothiol and Protection from Oxidative Stress

H_2O_2, which is presumably produced at or near the plasma membrane, is used in the extracellular space by ovoperoxidase for cross-linking the envelope. However, if it diffused into the egg, H_2O_2 could be toxic, both directly and because it provides the promiscuously reactive hydroxyl radical:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$$

The Fe^{2+} required for this reaction may be generated by superoxide anion (or other intracellular reducing agents):

$$Fe^{3+} + {}^{\cdot}O_2^- \rightarrow Fe^{2+} + O_2$$

This Fe^{2+}-Fe^{3+}–dependent process constitutes the Haber-Weiss reaction, with continued production of ·OH:

$$H_2O_2 + {}^{\cdot}O_2^- \rightarrow {}^{\cdot}OH + O_2 + OH^-$$

The requirement for superoxide in generating Fe^{2+} in this reaction is thought to be a principal reason why superoxide dismutase protects cells from oxidant stress (*29*).

The egg has a powerful mechanism for dealing with oxidative damage by H_2O_2 in the form of an intracellular amino acid called ovothiol. Ovothiols constitute a family of 1-methyl-4-mercaptohistidines (*30*) differing in methylation at the α-amino group. Depending on the state of N-methylation, they are called ovothiol A, B, or C (Fig. 4). As aromatic thiols, the mercaptoimidazoles have pK_a's of 2.3 (*31*) and thus exist at neutral pH in the zwitterionic form, as imidazolium thiolates. Ovothiols are present at high (3 to 5 mM) concentrations in marine invertebrate eggs (*30*) and are also found in the eggs of teleost fish (*32*), which use similar peroxidase chemistry to modify their surfaces after fertilization (*33*).

On consumption of H_2O_2, ovothiol is oxidized to the disulfide (*32*). Then ovothiol disulfide may be reduced to the imidazolium thiolate by reduced glutathione, the equilibrium for this reaction being far in the direction of ovothiol reduction (*34*). Although sea urchin eggs lack glutathione peroxidase, they have a high glutathione reductase activity (*32*) and glutathione remains reduced (*35*) after fertilization. Thus, ovothiol will be kept in the reduced form and be available to consume any H_2O_2 that enters the egg. In this process, ovothiol acts as a regenerable trap for H_2O_2, its continued reduction being driven by NADPH consumption (Fig. 3).

The utility of ovothiol as an antioxidant is not limited to the two-electron reduction of H_2O_2; ovothiol is distinguished from glutathione in its reactivity in one-electron reductions as well. For example, glutathione does not reduce cytochrome c at an appreciable rate, whereas the second-order rate constant for the ovothiol reduction of cytochrome c is $10^4\ M^{-1}\ s^{-1}$ (*31*). In experiments with either ovothiol or an imidazole analog, 1,5-dimethyl-4-mercaptoimidazole, Holler and Hopkins (*31*) found dramatic differences between these aromatic thiols and glutathione in rates of reduction of certain relatively stable free radicals and photochemically produced tyrosyl radicals. In each case, glutathione was relatively ineffective, whereas ovothiol functioned nearly as well as ascorbic acid and vitamin E, two biological radical reductants. Despite the reactivity of ovothiol as a radical reductant, its aromatic thiol is resistant to

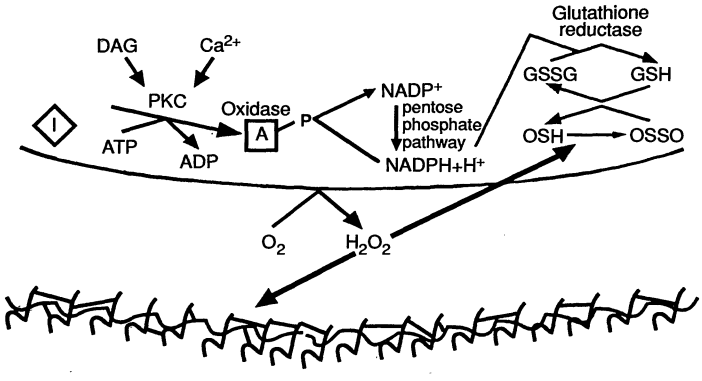

Fig. 3. Biochemistry of the respiratory burst. The reactions involved in the production of, and protection from, H_2O_2, are shown. A specific protein kinase C–dependent event leads to an active NADPH oxidase, which reduces molecular oxygen and forms H_2O_2. H_2O_2 is used as the oxidant extracellularly to cross-link the fertilization envelope; however, if it diffuses into the cell, it may be consumed by a regenerable trap in the form of ovothiol, glutathione (GSH), and glutathione reductase. See text for elaboration of this scheme. (I, inactive form of the oxidase; A, active form of the oxidase; OSH, reduced ovothiol; OSSO, oxidized ovothiol; GSSG, oxidized glutathione; and ADP, adenosine diphosphate.)

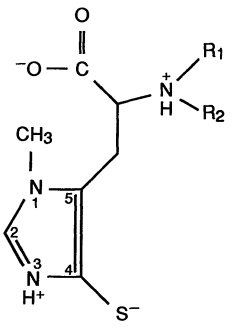

Fig. 4. Family of ovothiols. These 1-methyl-4-mercapto-histidines are derivatized to different extents at the α-amino group. Ovothiol A, R_1 = H and R_2 = H; ovothiol B, R_1 = H and R_2 = CH_3; ovothiol C, R_1 = CH_3 and R_2 = CH_3.

oxidation (*31*) in the presence of Fe^{3+}, a property it shares with the aliphatic thiol, glutathione, whereas other aromatic thiols like thiosalicylate are rapidly oxidized. Thus, relative stability of ovothiols to air oxidation may account for the fact that they are well tolerated by cells, as inferred from the fact that the high concentration in eggs (5 mM) is compatible with normal development. Ovothiol appears to be the evolutionary solution to the problem posed by an egg that developed a potent oxidizing mechanism to harden its protective fertilization envelope.

Conclusions

When sea urchin eggs use such extreme oxidative chemistry to cross-link their extracellular coats, several potential problems arise. These difficulties are obviated by careful orchestration of the biochemical pathways that activate the egg and result in fertilization envelope assembly and cross-linking. The sea urchin has a highly regulated sequence of metabolic reactions to form the shell that will serve as its "microincubator" during early development. Although the sea urchin egg provides the best-studied example, most metazoan eggs change their coats after fertilization. Why do eggs make such a highly developed fertilization envelope? The egg must be vulnerable to the first sperm but protected from subsequent ones; therefore, several sequential processes including envelope formation help the egg to achieve protection from the entrance of additional sperm.

An additional reason for the ubiquitous fertilization coats of metazoans may be a need for protection during early development when the embryonic axes are being determined. Initiation of the overall body plan depends on intercellular communication, and a fertilization envelope isolates blastomeres from extraembryonic influences. The sea urchin fertilization envelope also provides a sterile environment: in the presence of the H_2O_2 in the ocean (*36*), the envelope can act as a chemical shield containing ovoperoxidase. Thus, the fertilization envelope is central to normal development and constitutes an elegant problem in morphogenesis. As a multidimensional biochemical construction project that is amenable to analysis, it also provides a model for chemists interested in understanding mechanisms that govern complex cellular behaviors.

REFERENCES AND NOTES

1. For recent reviews see *The Cell Biology of Fertilization*, H. Schatten and G. Schatten, Eds. (Academic Press, New York, 1988); B. M. Shapiro, *Cell* **49**, 293 (1987); J. S. Trimmer and V. D. Vacquier, *Annu. Rev. Cell Biol.* **2**, 1 (1986); P. Wassarman, *Annu. Rev. Biochem.* **57**, 415 (1988).
2. S. Schulz, M. Chinkers, D. L. Garbers, *FASEB J.* **3**, 2026 (1989); S. Singh *et al.*, *Nature* **334**, 708 (1988); J. K. Bentley, D. J. Tubb, D. L. Garbers, *J. Biol. Chem.* **261**, 14859 (1986).
3. R. Tombes and B. M. Shapiro, *Cell* **41**, 325 (1985); R. Tombes, C. J. Brokaw, B. M. Shapiro, *Biophys. J.* **52**, 75 (1987); R. Tombes and B. M. Shapiro, *J. Biol. Chem.* **262**, 16011 (1987); D. D. Wothe, H. Charbonneau, B. M. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5203 (1990).
4. R. Christen, R. W. Schackmann, B. M. Shapiro, *J. Biol. Chem.* **257**, 14881 (1982); *ibid.* **258**, 5392 (1983); R. W. Schackmann, R. Christen, B. M. Shapiro, *ibid.* **259**, 13914 (1984); H. C. Lee, C. Johnson, D. Epel, *Dev. Biol.* **95**, 31 (1983); H. C. Lee and D. L. Garbers, *J. Biol. Chem.* **261**, 16026 (1986); A. Guerrero and A. Darszon, *ibid.* **264**, 19593 (1989); A. Darszon *et al.*, *Eur. J. Biochem.* **144**, 515 (1984).
5. L. A. Jaffe, *Nature* **261**, 68 (1976); _____ and N. L. Cross, *Annu. Rev. Physiol.* **48**, 191 (1986).
6. D. Epel, *Curr. Top. Dev. Biol.* **12**, 185 (1978); M. J. Whitaker and R. A. Steinhardt, *Quant. Rev. Biophys.* **15**, 4 (1982); F. Dube, T. Schmidt, C. H. Johnson, D. Epel, *Cell* **40**, 657 (1985).
7. E. N. Harvey, *J. Exp. Zool.* **8**, 355 (1910).
8. M. J. Whitaker and R. A. Steinhardt, in *The Fertilization Response of the Egg*, vol. 3 of *Biology of Fertilization*, C. B. Metz and A. Monroy, Eds. (Academic Press, Orlando, FL, 1985), pp. 168–220; L. A. Jaffe, *Dev. Biol.* **99**, 265 (1985).
9. P. R. Turner, M. P. Sheetz, L. A. Jaffe, *Nature* **310**, 414 (1981); L. C. Kamel, J. Bailey, L. Schoenbaum, W. Kinsey, *Lipids* **20**, 350 (1985); D. L. Clapper and W. C. Lee, *J. Biol. Chem.* **260**, 13942 (1985); B. Ciapa and M. J. Whitaker, *FEBS Lett.* **195**, 137 (1986).
10. E. S. Kay and B. M. Shapiro, in *The Fertilization Response of the Egg*, vol. 3 of *Biology of Fertilization*, C. B. Metz and A. Monroy, Eds. (Academic Press, Orlando, FL, 1985), p. 45; J. Runnstrom, *Adv. Morphol.* **5**, 231 (1966).
11. C. Foerder and B. M. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4214 (1977); H. G. Hall, *Cell* **15**, 343 (1978).
12. E. Kay, E. M. Eddy, B. M. Shapiro, *Cell* **29**, 867 (1982); E. S. Kay and B. M. Shapiro, *Dev. Biol.* **121**, 325 (1987).
13. T. Deits *et al.*, *J. Biol. Chem.* **259**, 13525 (1984).
14. C. A. Foerder, S. J. Klebanoff, B. M. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3183 (1978).
15. C. E. Somers, D. Battaglia, B. M. Shapiro, *Dev. Biol.* **131**, 226 (1989); S. J. Klebanoff, C. A. Foerder, E. M. Eddy, B. M. Shapiro, *J. Exp. Med.* **149**, 938 (1979).
16. T. Deits and B. M. Shapiro, *J. Biol. Chem.* **260**, 7882 (1985).
17. _____, *ibid.* **261**, 12159 (1986).
18. P. Weidman, E. Kay, B. M. Shapiro, *J. Cell Biol.* **100**, 938 (1984); P. Weidman and B. M. Shapiro, *ibid.* **105**, 561 (1987).
19. P. J. Weidman, D. C. Teller, B. M. Shapiro, *J. Biol. Chem.* **262**, 15076 (1987).
20. C. E. Somers and B. M. Shapiro, *ibid.*, in press.
21. R. Lallier, *Exp. Cell Res.* **63**, 460 (1970); *Experientia* **27**, 1323 (1971).
22. M. Veron, C. Foerder, E. M. Eddy, B. M. Shapiro, *Cell* **10**, 321 (1977); C. F. Chandler and J. Heuser, *J. Cell Biol.* **84**, 618 (1980); C. F. Chandler and C. J. Kazilek, *Cell Tissue Res.* **246**, 153 (1986).
23. D. E. Battaglia and B. M. Shapiro, *J. Cell Biol.* **107**, 2447 (1988).
24. O. Warburg, *Z. Physiol. Chem.* **57**, 1 (1908).
25. C. Shearer, *Proc. R. Soc. London Ser. B* **93**, 213 (1922); H. Laser and L. Rothschild, *ibid.* **126**, 539 (1939); E. Turner and B. M. Shapiro, in *The Respiratory Burst and Its Physiological Significance*, A. J. Sbarra and R. R. Strauss, Eds. (Plenum, New York, 1988), pp. 385–403.
26. J. W. Heinecke and B. M. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1259 (1989).
27. J. Heinecke, K. E. Meier, J. Lorenzen, B. M. Shapiro, *J. Biol. Chem.* **265**, 7717 (1990).
28. N. Isono and I. Yasumasu, *Exp. Cell Res.* **50**, 616 (1968); S. Backstrom, *ibid.* **18**, 347 (1959); N. Isono, *J. Fac. Sci. Univ. Tokyo Sect. IV Zool.* **10**, 67 (1963); R. R. Swezey and D. Epel, *J. Cell Biol.* **103**, 1509 (1986); S. M. Krane and R. K. Crane, *Biochim. Biophys. Acta* **43**, 369 (1960); D. Epel, *Exp. Cell Res.* **58**, 213 (1969); _____, C. Patton, R. W. Wallace, W. Y. Cheung, *Cell* **23**, 543 (1981).
29. I. Fridovich, *Adv. Enzymol. Relat. Areas Mol. Biol.* **58**, 61 (1986); H. Sies, Ed., *Oxidative Stress* (Academic Press, Orlando, FL, 1985); A. E. Taylor, D. Matalon, P. Ward, Eds., *Physiology of Oxygen Radicals* (Williams and Wilkins, Baltimore, MD, 1986).
30. E. Turner, R. Klevit, P. B. Hopkins, B. M. Shapiro, *J. Biol. Chem.* **261**, 13056 (1986); E. Turner, R. Klevit, L. J. Hager, B. M. Shapiro, *Biochemistry* **26**, 4028 (1987); A. Palumbo, M. d'Ischia, G. Misuraca, G. Prota, *Tetrahedron Lett.* **23**, 3207 (1982); A. Palumbo *et al.*, *Comp. Biochem. Physiol.* **78**, 81 (1984).
31. T. P. Holler and P. B. Hopkins, *J. Am. Chem. Soc.* **110**, 4837 (1988); *Biochemistry* **29**, 1953 (1990).
32. E. Turner, L. Hager, B. M. Shapiro, *Science* **242**, 939 (1988).
33. B. M. Shapiro, C. E. Somers, P. J. Weidman, in *The Cell Biology of Fertilization*, H. Schatten and G. Schatten, Eds. (Academic Press, New York 1988), pp. 251–276.
34. B. M. Shapiro and E. Turner, *Biofactors* **1**, 85 (1988).
35. R. C. Fahey, S. D. Mikalajcryk, B. P. Meier, D. Epel, E. J. Carroll, *Biochim. Biophys. Acta* **437**, 445 (1976).
36. C. von Baalen and J. E. Marler, *Nature* **211**, 951 (1966); R. G. Petasne and R. G. Zika, *ibid.* **325**, 516 (1987).

37. Supported by grants from the NIH and the NSF. I thank J. Heinecke and J. Campbell for comments on this paper and to M. Patella and J. Cacace for preparing the manuscript.