Zygote 8 (Supplement), pp. S3–S4. © 2000 Cambridge University Press Printed in the United Kingdom

SPECIAL LECTURE: Regulation of mitochondrial respiration in eggs and embryos of sea urchin

Ikuo Yasumasu
Department of Biology, School of Education, Waseda University, Tokyo, Japan

It is well known that sea urchin eggs, which exhibit quite a low rate of respiration before fertilisation, undergo a sudden increase in the rate of respiration followed by its gradual decrease in about a 15 min period after fertilisation (Ohnishi & Sugiyama, 1963; Epel, 1969), in which the respiration is mediated mainly by Ca^{2+}-activated non-mitochondrial respiratory systems (Foerder *et al.*, 1978; Perry & Epel, 1985*a*,*b*). During this short period the rate of mitochondrial respiration gradually increases (Yasumasu *et al.*, 1988) and stabilises at a higher rate than before fertilisation (Warburg, 1908, 1910; Whitaker, 1933; Yasumasu & Nakano, 1963), when the respiration due to non-mitochondrial respiratory systems is turned off. The rate of mitochondrial respiration, once enhanced upon fertilisation, increases further in the period between hatching and the gastrula stage, without any changes in the number of mitochondria or the capacity of electron transport in the mitochondrial respiratory chain (Fujiwara & Yasumasu, 1997; Fujiwara *et al.*, 2000). It is likely that the respiratory rate is reduced by regulation of electron transport in the mitochondrial respiratory chain and increases due to the release of electron transport from the regulation upon fertilisation and after hatching.

A marked increase in the respiratory rate after hatching is accompanied by an evident decrease in the ATP level without any change in the levels of ADP and AMP (Mita & Yasumasu, 1984). In isolated mitochondria, the rate of respiration, estimated in the presence of ADP at the same concentration as in embryos, is reduced by a high concentration of ATP as found in embryos before hatching but is not affected at a concentration as low as in gastrulae (Fujiwara & Yasumasu, 1997; Fujiwara *et al.*, 2000) ATP at a high concentration probably blocks ATP release from mitochondria and consequently inhibits ADP uptake coupled to ATP release in the ATP/ADP translocation reaction in the mitochondrial membrane, causing a shortage of intra-mitochondrial ADP. The ADP shortage strengthens acceptor control and reduces the rate of mitochondrial respiration. After hatching, the decrease in the ATP level probably releases mitochondrial respiration from acceptor control, which have been strengthened by the high ATP level, to enhance the respiratory rate in embryos. A marked increase in the rate of mitochondrial respiration following fertilisation, which also occurs without any change in the capacity of electron transport in the mitochondrial respiratory chain (Selak & Scandella, 1987; Yasumasu *et al.*, 1996), does not seem to result from release of electron transport from acceptor control in the mitochondrial respiratory chain. The ATP level, as well as the levels of ADP and AMP, in fertilised eggs is the same as in unfertilised eggs (Epel, 1964) and is sufficient to make acceptor control strong (Fujiwara *et al.*, 2000). In unfertilised eggs, the rate of mitochondrial respiration under acceptor control is probably made further reduced by forms of regulation of electron transport other than acceptor control.

In isolated mitochondria, Ca^{2+} inhibits electron transport in the section of the mitochondrial respiratory chain between flavoproteins and cytochrome *c*. In unfertilised eggs, depression of electron transport in the same section is found to make the respiratory rate quite low (Yasumasu *et al.*, 1984, 1996). In isolated mitochondria exposed to Ca^{2+}, the respiratory rate decreases in relation to the increase in the rate of ATP-dependent, H^+-gradient-mediated Ca^{2+} uptake, which probably enhances the level of intra-mitochondrial Ca^{2+}. Ruthenium red, which inhibits Ca^{2+} uptake in mitochondria in the same manner as FCCP and CN^-, cancels Ca^{2+}-produced inhibition of respiration in isolated mitochondria. Pulse treatments with ruthenium red, FCCP and CN^- exert no effect on the respiration in fertilised eggs but enhance the rate of respiration in unfertilised eggs to the same level as in fertilised eggs. Probably, Ca^{2+}-produced inhibition of electron transport makes the respiratory rate quite low in unfertilised eggs.

The rate of Ca^{2+} uptake in mitochondria is markedly larger at pH 6.4–6.8 than at pH 7.0–7.4. The respiratory rate in isolated mitochondria is reduced by Ca^{2+} at acidic pH more strongly than at neutral pH. The respiratory rate in mitochondria exposed to Ca^{2+} at neutral pH is higher by about 2-fold than at acidic pH. In the presence of ATP at a high concentration as in eggs, the

https://doi.org/10.1017/S0967199400130011 Published online by Cambridge University Press

rate of respiration in Ca^{2+}-exposed mitochondria is reduced at acidic pH more strongly than at neutral pH and the respiratory rate at neutral pH becomes higher by about 3.5-fold than the rate at acidic pH. In sea urchin eggs, the respiratory rate, which is maintained quite low by a high level of intra-mitochondrial Ca^{2+} at acidic pHi (intracellular pH) before fertilisation, is released from Ca^{2+}-caused inhibition by a decrease in intra-mitochondrial Ca^{2+} at neutral pHi. Fertilisation-induced cytosolic Ca^{2+} level, activating the Na^+/H^+ antiporter by its phosphorylation catalysed by Ca^{2+}/calmodulin-dependent protein kinase, enhances pHi to release respiration from the inhibition caused by intra-mitochondrial Ca^{2+}. Na^+ is also a candidate to release mitochondrial respiration from Ca^{2+}-caused inhibition. Injection of compounds expected to alter pHi and the intra-mitochondrial Ca^{2+} level, such as Tris buffer, Ca^{2+}-EGTA, EGTA and Na^+, is successful in causing the expected changes in the respiratory rate, estimated on single egg by a micro-electrochemical method.

Co-investigators in this work are Drs A. Fujiwara, A. Hino, Y. Kamata, S. Kusunoki, M. Mita, and E. Tazawa.

References

Epel, D. (1964). *Biochem, Biophys. Res. Commun.* **17**, 62–8.
Epel, D. (1969). *Exp. Cell Res.* **58**, 312–19.
Foerder, C.A., Klebanoff, S.J. & Shapiro, D.M. (1978). *Proc. Natl. Acad. Sci. USA* **75**, 3183–7.
Fujiwara, A. & Yasumasu, I. (1997). *Dev. Growth Differ.* **39**, 179–89.
Fujiwara, A., Kamata, Y., Asami, K. & Yasumasu, I. (2000). *Dev. Growth Differ.* **42**, 155–165.
Mita, M. & Yasumasu, I. (1984). *Comp. Biochem. Physiol.* **78(B)**, 33–6.
Ohnishi, T. & Sugiyama, M. (1963). *Embryologia* **8**, 79–88.
Perry, G. & Epel, D. (1985*a*). *Dev. Biol.* **107**, 58–65.
Perry, G. & Epel, D. (1985*b*). *Dev. Biol.* **107**, 58–65.
Selak, M.A. & Scandella, C.J. (1987). *Exp. Cell Res.* **169**, 369–78.
Warburg, O. (1908). *Hoppe-Seyler's Z. Physiol. Chem.* **57**, 1–16.
Warburg, O. (1910). *Hoppe-Seyler's Z. Physiol. Chem.* **66**, 305–40.
Whitaker, D.M. (1933). *J. Gen. Physiol.* **16**, 497–528.
Yasumasu, I. & Nakano, E. (1963). *Biol. Bull.* **125**, 182–7.
Yasumasu, I., Fujiwara, A., Hino, A. & Asami, K. (1984). *Zool. Sci.* **1**, 725–36.
Yasumasu, I., Hino, A., Fujiwara, A., Tazawa, E., Nemoto, S. & Asami, K. (1988). *Comp. Biochem. Physiol.* **90B**, 69–75.
Yasumasu, I., Tazawa, E., Asami, K. & Fujiwara, A. (1996). *Dev. Growth Differ.* **38**, 359–71.

https://doi.org/10.1017/S0967199400130011 Published online by Cambridge University Press