

Measurement of NADPH generation in single mammalian rod photoreceptors

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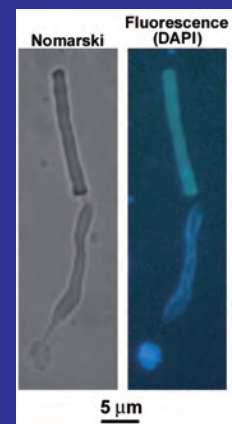
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ABSTRACT: NADPH is the primary source of reducing equivalents in the cytosol. Its major source is considered to be the pentose phosphate pathway, but cytosolic NADP⁺-dependent dehydrogenases using intermediates of mitochondrial pathways for substrates have been known to contribute. Mammalian rod photoreceptors provide a unique model for measuring the functional utilization of NADPH at the single cell level. In these cells, NADPH availability can be monitored from the reduction of the all-trans retinal generated by light to all-trans retinol using single cell fluorescence imaging. We have used mouse rod photoreceptors to investigate the generation of NADPH by different metabolic pathways. We find that in rod photoreceptors mitochondria-linked pathways can generate substantial amounts of NADPH, and do so even when the pentose phosphate pathway is operational. Furthermore, at limiting metabolic substrate concentrations, there is competition between ATP- and NADPH-generating pathways.



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