

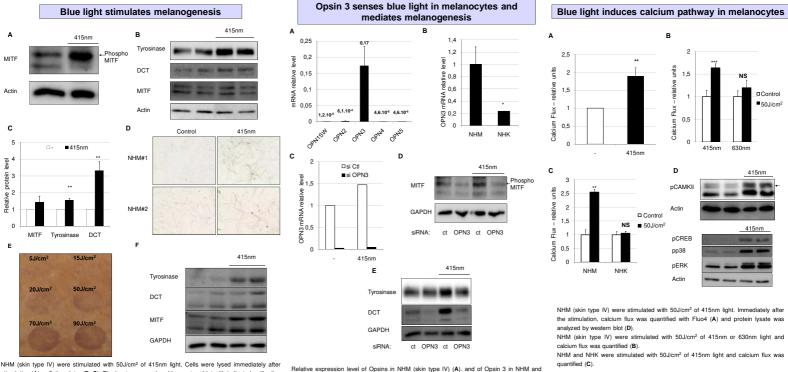


Melanocytes sense blue light and regulate pigmentation through the Opsin3

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The shorter wavelengths of the visible light have been recently reported to induce a long lasting hyperpigmentation but only in melano-competent individuals. Here, we provide evidence demonstrating that opsin 3 (OPN3) is the key sensor in melanocytes responsible for hyperpigmentation induced by the shorter wavelengths of the visible light. The melanogenesis induced through OPN3 is calcium-dependent and further activates CAMKII followed by CREB, ERK, and p38 leading to the phosphorylation of MITF and ultimately to the increase of the melanogenesis enzymes: tyrosinase and dopachrometautomerase (DCT). Furthermore, blue light induces the formation of a protein complex that we demonstrated to be formed by tyrosinase and DCT. This multimeric tyrosinase / Tyrp complex is mainly formed in dark-skinned melanocytes and induces a sustained tyrosinase activity, thus explaining the long-lasting hyperpigmentation that is observed only in skin type III and higher after blue light irradiation.OPN3 thus functions as the sensor for visible light pigmentation.



NHK (B) analyzed by qPCR. OPN levels were normalized with actin level

mined by qPCR (C)

siRNA

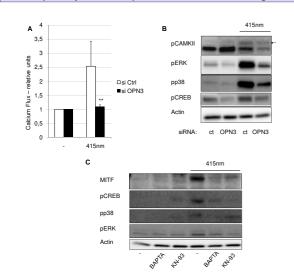
NHM (skin type IV) were transfected with siRNA directed against OPN3 or control, 2 days

later, cells were stimulated with $50J/cm^2$ of 415nm light and lysed immediately (D) or 3 days later (E). The lysate was analyzed by western blot with indicated antibodies. The efficiency of

stimulation (A) or 3 days later (B, C). The lystate was analyzed by western blot with indicated antibodes (A,B) and the relative protein level quantified (n=5) (C).

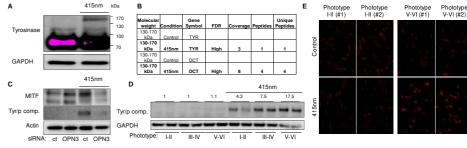
NMI (skin type V) from 2 different donors were stimulated one time with 50J/cm²of 415nm light. After 10 days cells were observed with brightfield microscopy (**D**), Skin abdominoplasty was stimulated with 5 to 90J/cm² of 415nm light (**E**), biopsied and placed in *ex vivo* culture for 5 days. Biopsies were lysed and proteins were analyzed by western blot with indicated antibodies (**F**).





NHM transfected with siRNA directed against OPN3 or control were stimulated with 50J/cm² of 415nm light, calcium flux was quantified with Fluo4 (A) and protein lysate was analyzed by western blot (B). NHM treated with calcium pathway inhibitors : BAPTA (3µM) or KN-93 (5µM) were stimulated with 50J/cm² of 415nm light and protein lysate was analyzed after stimulation(C).

Blue light induces formation of tyr/p complex leading to a sustained tyrosinase activity in dark phototype melanocytes



NHM (skin type IV) were stimulated with 50mJ/cm² of 415nm light. Multimeric tyrosinase / Tyrp protein was observed on Tyrosinase western blot (A) and analysed in proteomic experiment (B).

NHM were transfected with siRNA directed against OPN3 or control. 2 days later, cells were stimulated with 50J/cm² of 415nm light and lysed immediately (C). NHM from phototypes I-II or V-VI (2 lots' conditions) were stimulated with 50J/cm² of 415nm light, the lysate after stimulation was analyzed by western blot (D) and 2 weeks later, ryosnase activity was visualized by immunofluorescence (E).

Human melanocytes of melano-competent skins are able to see the blue light and respond by inducing a potent and long-lasting pigmentation through the OPN3 sensor. OPN3, its downstream pathway and the multimeric TYR/P proteins appear as new potential targets for regulating melanogenesis and in protection of dark skinned individuals against blue light in physiological conditions and in pigmentary disorders