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Light-controlled gene expression in yeast using photocaged Cu^{2+}

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ABSTRACT

The manipulation of cellular function, such as the regulation of gene expression, is of great interest to many biotechnological applications and often achieved by the addition of small effector molecules. By combining effector molecules with photolabile protecting groups that mask their biological activity until they are activated by light, precise, yet minimally invasive, photocontrol is enabled. However, applications of this trendsetting technology are limited by the small number of established caged compound-based expression systems. Supported by computational chemistry, we used the versatile photolabile chelator DMNP-EDTA, long-established in neurobiology for photolytic Ca^{2+} release, to control Cu^{2+} release upon specific UV-A irradiation. This permits light-mediated control over the widely used Cu^{2+}-inducible *pCUP1* promoter from *S. cerevisiae* and thus constitutes the first example of a caged metal ion to regulate recombinant gene expression. We screened our novel DMNP-EDTA-Cu system for best induction time and expression level of eYFP with a high-throughput online monitoring system equipped with an LED array for individual illumination of every single well. Thereby, we realized a minimally invasive, easy-to-control, parallel and automated optical expression regulation *via* caged Cu^{2+} allowing temporal and quantitative control as a beneficial alternative to conventional induction *via* pipetting $CuCl_2$ as effector molecule.

1. Introduction

In the past decade, the regulation of cellular functions by light, known as optogenetics, has attracted increasing attention since light as the functional switch offers strikingly high temporal, quantitative and sometimes even spatial controllability. Particularly exciting examples include rapid switching of cellular functions within milliseconds and regiospecific manipulation in cellular compartments or in multicellular organisms that could not be achieved with methods relying on diffusion processes (Brieke et al., 2012; Deiters, 2009; Drepper et al., 2011; Ellis-Davies, 2007; Kaplan and Ellis-Davies, 1988). For the model organism *S. cerevisiae* numerous canonical expression systems, mainly regulated by primary carbon sources, are state of the art with a broad portfolio of dynamic range, mode of action, effector molecule, timing of induction, etc. (reviewed in Da Silva and Srikrishnan, 2012; Maya et al., 2008). Furthermore, for optical regulation of (recombinant) gene expression, a subfield of optogenetics, engineering of photoreceptors, i.e., light responsive proteins from microorganisms and plants, into molecular photoswitches for controlling cellular processes has successfully been applied (Kennedy et al., 2010; Shimizu-Sato et al., 2002; Tabor et al., 2011). For alternative optical regulation, the biological activity of known effector molecules – e.g., canonical inducers like IPTG or arabinose – can be masked by attaching photoprotection groups well known from synthetic organic chemistry (Bier et al., 2017; Binder et al., 2016; Young and Deiters, 2007). Once the protection group is cleaved off with light, the biological activity of the effector is restored. These photosensitized molecules, known as “caged compounds”, are thus equipped with the spatial and temporal controllability of light (reviewed and compared in Young and Deiters, 2007). Advances in synthetic chemistry extended the range of available caged compounds and thus significantly expanded both the research field and the application areas (Horspool and Lenci, 2004; Klan et al., 2013). However, many of these compounds are not yet commercially accessible and may be difficult to synthesize for non-specialists, hampering

Abbreviations: DMNP1-EDTA, 1-(2-nitro-4,5-dimethoxyphenyl)-*N*,*N*′,*N*′-tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine i.e. DM-nitrophen; *pCUP1*, CUP1 promoter

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their routine application. Among the most successful applications of a commercially available caged compound is the photorelease of calcium ions from DMNP-EDTA (1-(2-nitro-4,5-dimethoxyphenyl)-*N*,*N*,*N′*,*N′*-tetrakis[(oxycarbonyl)methyl]-1,2-ethanediamine, DM-nitrophen) (Kaplan and Ellis-Davies, 1988). DMNP-EDTA-Ca has been used extensively in neurobiology, e.g., for the detailed investigation of synapse functionality (Ellis-Davies, 2003). Its success can be traced to the fact that it combines several features desirable for using caged compounds in biological environments, which include (i) no (or low) toxicity, (ii) stability in aqueous solutions, (iii) low premature release of the effector, biocompatible release wavelengths ($\lambda > 300$ nm), (iv) large extinction coefficients and high quantum yields, and (v) quantitative release of the effector molecule. Since EDTA binds many types of metal cations, the photocage DMNP-EDTA is not limited to the photorelease of calcium. In fact, it has been used to release Mg^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Mn^{2+}, and Co^{2+} as well (Ellis-Davies, 2006; Kishimoto et al., 2001). So far, DMNP-EDTA has not been applied for light-induced gene expression despite its ready availability and desirable features, possibly because of a lack of suited promoters for the cations listed above.

For the copper(II) cation, Cu^{2+}, which – to our knowledge – has not been photoreleased from DMNP-EDTA before, the inducible promoter *pCUP1* is well established and widely used in *S. cerevisiae* (Da Silva and Srikrishnan, 2012; Farhi et al., 2006; Fujita et al., 1990; Shen et al., 2012). We therefore anticipated that by combining the promoter *pCUP1* with photorelease of Cu^{2+} from DMNP-EDTA, a non-invasive optical control over protein production in yeast could become a viable scenario. We recently reported the first application of a caged compound for optically controlled protein production in *S. cerevisiae* using a methionine-repressible promoter and photocaged methionine, which already showcased the great flexibility and responsiveness of light-regulated gene expression (Kusen et al., 2016). Here, we report on the applicability of DMNP-EDTA-Cu with the inducible promoter *pCUP1*. Initially, the capability of DMNP-EDTA for Cu^{2+} release was assessed *in vitro* using UV–VIS absorption spectroscopy and *in silico* employing quantum chemical calculations. Subsequently, production of eYFP in a newly developed reporter strain was photoinduced and monitored *in vivo* in parallelized microscale cultivations. The combination of *pCUP1* and DMNP-EDTA-Cu in the present study constitutes the first example for a light-regulated expression system based on a caged metal effector.

2. Material and methods

2.1. Strains and media

For the host *S. cerevisiae* Y00000 (BY4741, MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*, Euroscarf) uracil-lacking synthetic complete (SC) medium was used (20 g/L glucose, 6.9 g/L yeast nitrogen base without copper sulfate, 0.77 g/L complete supplement mixture without uracil (DSC0169, Formedium), pH 6). *E. coli* DH5α Mach1 strains used for cloning procedures according to (Hanahan, 1983) were cultivated in lysogeny broth (LB) medium (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, 100 µg/mL ampicillin) at 37 °C.

2.2. Chemicals

DMNP-EDTA, i.e. 1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane-*N*,*N*,*N′*,*N′*-tetraacetic acid, was obtained from ThermoFisher Scientific (D6814). All other chemicals, if not mentioned separately, were purchased at Carl Roth or Sigma Aldrich in analytical grade.

2.3. Spectroscopic characterization of DMNP-EDTA-metal complexes

Absorption spectra of DMNP-EDTA (80 µM) and DMNP-EDTA-metal complexes (80 µM DMNP-EDTA, 100 µM $CaCl_2$, $MgCl_2$, or $CuCl_2$) were measured as defined by the irradiation times or until the curves were steady (Fig. 2 and Fig. S4) with a UV1800 Photometer (Shimadzu) or a UVIKON 922 spectrophotometer (Kontron; only Fig. 2A and S4A) with buffer subtraction (10 mM MOPS, pH 7). Molar extinction coefficients were determined by the slope from different DMNP-EDTA-metal-complex concentrations (10–100 µM) plotted versus their absorbance. Photocleavage by UV-A irradiation ($\lambda = 375$ nm, $I = 11$ mW/cm^2; Lumos 43 (Atlas Photonics)) was followed *via* absorption change at 370 nm. Times for half-maximum uncaging were calculated from exponential fits of absorbance over time, and quantum yields were calculated using DMNP-EDTA-Ca as a chemical actinometer (Willett and Hites, 2000); detailed calculations in Supporting information 2.

2.4. Construction of Cu^{2+} reporter strain

The *pCUP1* promoter (−57 to −292 position of the CUP1 gene, YHR055C, (Labbé and Thiele, 1999; Labbé et al., 1997)) was amplified from genomic DNA of *S. cerevisiae* Y00000 by primers atat agagctcAGTTAGAAAAAGACATTTTTGCTGTCAGTCACTGTCAAGA and tatatactagtGATGACTTCTATATGATATTGCACTAACAAGAAGATATTAT-AATGCAATT [55 °C annealing temperature, Phusion Polymerase (Thermo Fisher Scientific)]. The amplificate was cloned into the vector pIE3-pMET17-YFP (Kusen et al., 2016) *via* *Sac*I and *Spe*I thus replacing the *pMET17* promoter by the *pCUP1* promoter. The resulting vector pIE3-pCUP1-YFP was verified *via* sequencing (GATC Biotech AG). *S. cerevisiae* Y00000 cells were transformed (Gietz and Schiestl, 2007) and plated for selection on uracil-lacking SC medium agar plates (20 g/L agar, pH 6).

2.5. Cultivation

Precultivations in 10 mL SC medium buffered with 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES, pH 6.0) were inoculated to an OD_{600} of 0.1 from cryogenic stocks stored at −80 °C (70% v/v glycerol). Precultivations were conducted in 250 mL shake flasks at a shaking frequency of 350 rpm and a shaking diameter of 50 mm (LS-X, Kuhner) at 30 °C for 16 h to an OD_{600} of 3–4. Main cultivations were then carried out in 48-round well plates (MTP-R48-B, lot 1309, m2p-labs) with a filling volume of 800 µL at 1000 rpm and a shaking diameter of 3 mm under otherwise identical cultivation conditions (0.1 M MES-buffered SC medium, starting $OD_{600} = 0.1$, 30 °C). The well plates were sealed with UV-A transparent polyolefin sealing foils (900371, HJ-Bioanalytik) as a sterile barrier that reduces evaporation and allows sufficient gas transfer (Sieben et al., 2016).

2.6. Conventional and light-induced Cu^{2+} induction

Conventional Cu^{2+} induction of *S. cerevisiae* pIE3-pCUP1-YFP was achieved by manually pipetting 8 µL $CuCl_2$ solutions from concentrated stocks into the respective culture well for final concentrations of 0–300 µM Cu^{2+}. For light-triggered Cu^{2+} induction, DMNP-EDTA-Cu (52.6 µM DMNP-EDTA, 50 µM $CuCl_2$) was added at the start of the cultivation together with the other medium components from a stock solution (20x) stored in the dark at −20 °C. Cu^{2+} was then uncaged with UV-A irradiation (0–450 s, $\lambda_{max} = 368$ nm, $I = 52.7$ mW/cm^2) from an in-house constructed array of 48 high intensity LEDs positioned directly above the microtiter plate. For each well there is one corresponding LED that can be individually controlled *via* a microcontroller (Arduino Uno R3, Arduino LLC) and a control software based on LabVIEW (LabVIEW v14, National Instruments). This setup allows automated and individual illumination patterns for each well without interrupting the shaking motion and online monitoring (Wandrey et al., 2016a).

2.7. Monitoring of reporter protein and biomass (BioLector)

Production of the fluorescent reporter protein (eYFP) and biomass formation were monitored non-invasively through the transparent

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