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Fluorescent labelling of in situ hybridisation probes through the copper-catalysed azide-alkyne cycloaddition reaction

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Abstract In situ hybridisation is a powerful tool to investigate the genome and chromosome architecture. Nick translation (NT) is widely used to label DNA probes for fluorescence in situ hybridisation (FISH). However, NT is limited to the use of long double-stranded DNA and does not allow the labelling of single-stranded and short DNA, e.g. oligonucleotides. An alternative technique is the copper(I)-catalysed azide-alkyne cycloaddition (CuAAC), at which azide and alkyne functional groups react in a multistep process catalysed by copper(I) ions to give 1,4-distributed 1,2,3-triazoles at a high yield (also called 'click reaction'). We successfully applied this technique to label short single-stranded DNA probes as well as long PCR-derived double-stranded probes and tested them by FISH on plant chromosomes and nuclei. The hybridisation efficiency of differently labelled probes was compared to those obtained by conventional labelling techniques. We show that copper(I)-catalysed azide-alkyne cycloaddition-labelled probes are reliable tools to detect different types of repetitive sequences on chromosomes opening new promising routes for the detection of single copy gene. Moreover, a combination of FISH using such probes with other techniques, e.g. immunohistochemistry (IHC) and cell proliferation assays using 5-ethynyl-deoxyuridine, is herein shown to be easily feasible.

Keywords FISH · Chromosomes · In situ labelling · Interphase · DNA sequence detection

Abbreviations

FISH	Fluorescence in situ hybridisation
NT	Nick translation
dNTP	Deoxynucleoside triphosphates
CuAAC	Cu(I)-catalysed azide-alkyne cycloaddition
EdU	5-Ethynyl-deoxyuridine
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid

Introduction

In situ hybridisation using fluorescence-based detection is a powerful tool to physically map high- and single copy sequences. Initially, the detection of hybridised sequences was possible only by using radioisotopic labels and autoradiography. Nowadays, two approaches, i.e. direct and indirect labelling, are widely used for fluorescent labelling of specific target sequences. The direct labelling is carried out by the use of DNA probes possessing fluorochrome-conjugated dNTPs for hybridisation (Wiegant et al. 1991). Indirect labelling implies the use of non-fluorescent modified nucleotides, which are detected later, e.g. by fluorochrome-

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conjugated antibodies (Kelly et al. 1970a, b; Pinkel et al. 1986a, b).

The labelling of DNA probes for fluorescence in situ hybridisation (FISH) can be achieved by a variety of different methods, like nick translation, random priming, PCR, end labelling, or tailing. Of these, the most feasible (popular) approach is the widely used nick translation (Rigby et al. 1977). However, nick translation requires double-stranded template DNA and therefore does not allow labelling of single-stranded and/or short probes like oligonucleotides (Kelly et al. 1970a, b). Moreover, the efficiency of nick translation labelling strongly depends on DNase I activity and the type of modified deoxynucleotides (Rigby et al. 1977).

Here, we tested an alternative technique to label double- and single-stranded DNA probes by the use of the Cu(I)-catalysed azide-alkyne cycloaddition (CuAAC) for its suitability for FISH. CuAAC, which is based on a classical Huisgen 1,3-dipolar cycloaddition, probably represents the best example of the so-called click chemistry reactions (Kolb et al. 2001). In the presence of copper(I), azide and alkyne functional groups react 10^7 to 10^8 time faster compared to the uncatalysed 1,3-dipolar cycloaddition leading only to 1,4-distributed 1,2,3-triazoles at a high yield (Fig. 1a) (Rostovtsev et al. 2002; Tornoe et al. 2002). This technique benefits from a simple setup of the reaction conditions and neither interacts with nor interferes with a biological system (high bioorthogonality (Sletten and Bertozzi 2011)). Thereby—among several other applications—the bioconjugation (formation of complexes by covalently linking functional molecules to molecules of biological origin) of complex biomolecules such as nucleic acids is enabled (Rozkiewicz et al. 2007; Gramlich et al. 2008; Salic and Mitchison 2008). For FISH probe preparation, alkyne-bearing deoxynucleotides are first incorporated into the DNA strand either enzymatically (e.g. PCR) or via oligonucleotide solid phase synthesis. In a second step, fluorochrome-coupled azides are used to label alkyne-bearing DNA probes either before hybridisation for direct labelling or after hybridisation for indirect labelling via a CuAAC reaction (Fig. 1b). Here, we show that CuAAC-labelled probes are a reliable tool to detect different types of repetitive sequences on plant chromosomes. Moreover, we found that this technique is combinable with immunohistochemistry and cell proliferation assays using labelling of replication via 5-ethynyldeoxyuridine (EdU).

Material and methods

Plant materials

Rye (*Secale cereale* L. cv. Petkuser Sommerroggen), barley (*Hordeum vulgare* L. cv. Morex and Emir) and hexaploid wheat (*Triticum aestivum* L. cv. Kanzler) were grown at greenhouse conditions (16 h light, 22 °C day/16 °C night). *Arabidopsis thaliana* ecotypes Pro-0 (Proaza, Asturios) (ID no. 8213 (Fulcher et al. 2015)) and Hov1-10, (ID no. 6035), were grown until rosette stage at short day (8 h light, 21 °C) afterwards at long day conditions (16 h light, 21 °C) at greenhouses.

Preparation of mitotic chromosomes for FISH

Rye, wheat and barley seeds were etiolated for 2–3 days. Root tips were cut, mitotic metaphases were accumulated by overnight treatment in ice-cold water, fixed in 3:1 ethanol/glacial acid (Carl Roth, cat. no. 9165; Merck, cat. no. 100066, respectively) and kept at −20 °C until use. For mitotic chromosome preparation, root tips were washed in ice-cold water and digested (50–60 min, 37 °C) in an enzyme cocktail (1 % cellulose (Calbichem, cat. no. 219466), 1 % pectolyase Y-23 (Sigma-Aldrich, cat. no. P3026), 1 % cytohelicase (Sigma-Aldrich, cat. no. C8274) in citrate buffer (0.01 M *tri*-sodium citrate dihydrate (Carl Roth, cat. no. 4088) and 0.01 M citric acid (Carl Roth, cat. no. 6490); pH 4.5–4.8)). Afterwards, root tips were washed in 0.01 M citrate buffer and ice-cold ethanol consecutively. For preparation of mitotic chromosome spreads, root tips were transferred to glacial acid/ethanol 3:1 (200 μl/25 root tips) and disrupted with a dissection needle. Eight microlitres of this mitotic cell suspension was dropped on glass slides placed on ice, air-dried and stored in 100 % ethanol at 4 °C (Aliyeva-Schnorr et al. 2015). *A. thaliana* slide preparation was performed according to Armstrong et al. (1998) with minor modifications. Flower buds were fixed in ethanol/glacial acid (3:1), washed and digested in an enzyme cocktail (0.07 % cellulase, 0.1 % pectolyase, 0.1 % cytohelicase in 0.01 M citric buffer). After washing, flower buds were disrupted on a slide in 10 μl of 60 % acetic acid and placed on a hot plate (1 min, 43 °C). The cell suspension was covered with fixative and air-dried. For FISH, *A. thaliana* slides were treated with pepsin (2 min, 38 °C; 0.05 mg/ml; Sigma-Aldrich, cat. no. 10108057001), washed and fixed (10 min, RT, 4 % formaldehyde (Carl Roth, cat. no.

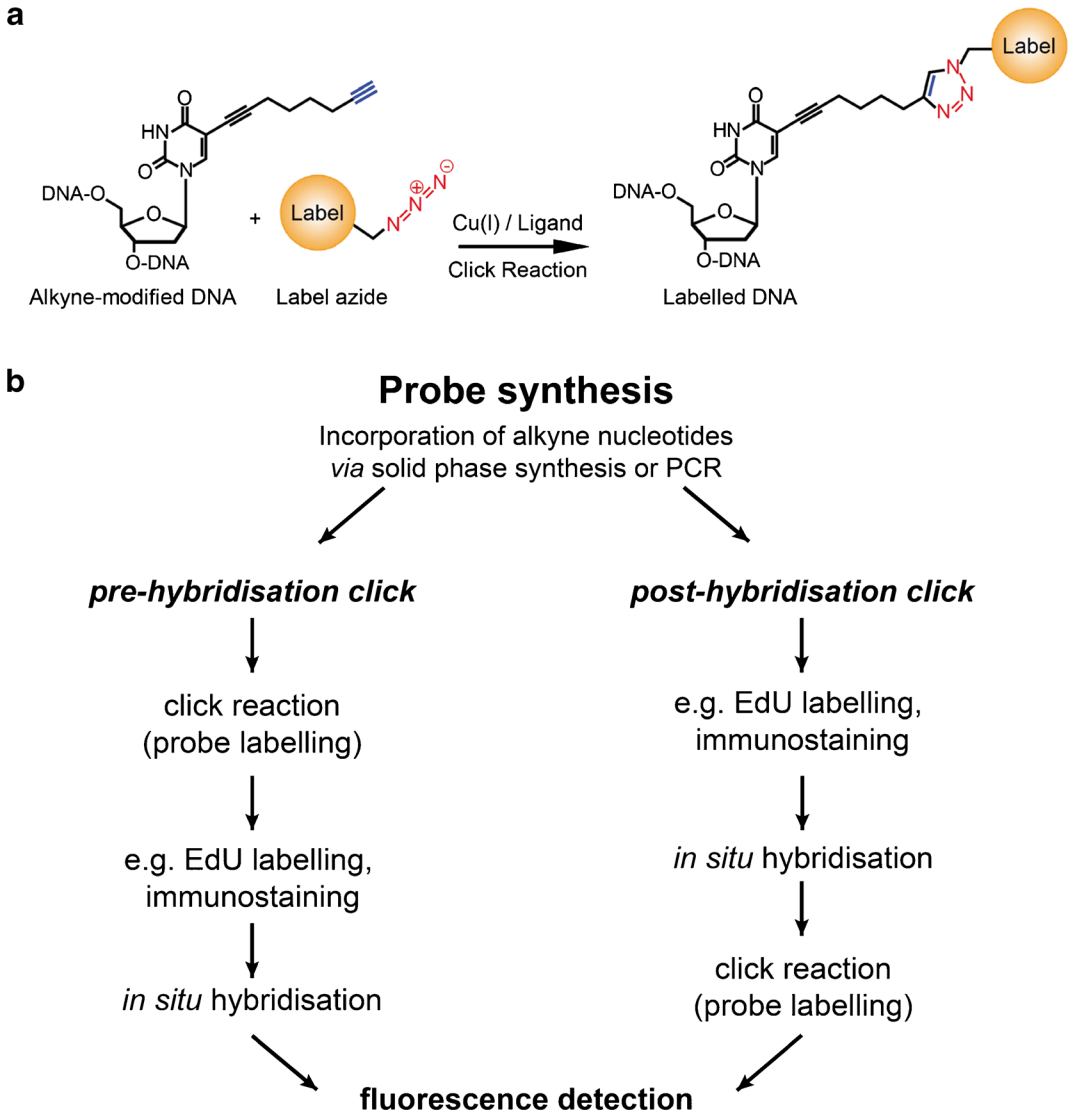

Fig. 1 The CuAAC reaction can be used to functionalise alkyne-modified DNA nucleobases. **a** The CuAAC is a Huisgen 1,3-dipolar cycloaddition where alkyne-labelled DNA (*blue*) and azide-coupled dyes (*red*) react to provide labelled DNA fluorescence in situ hybridisation (FISH) probes. This reaction is catalysed by copper(I) ions, which are stabilised by polytriazole ligands. **b** Possible applications of click chemistry for FISH by pre- or post-hybridisation click reaction labelling in combination with immunostaining or EdU labelling

4979)). After washing and dehydration in ethanol, the slides were used for FISH.

Preparation and sorting of isolated nuclei

Isolation of nuclei was performed according to Dolezel et al. (1989) with slight modifications. Approximately 0.1 g leaf material was fixed in 4 % formaldehyde in Tris buffer (10 mM Tris (Carl Roth, cat. no. 5429); 10 mM Na_2EDTA (Carl Roth, cat. no. 8043); 100 mM NaCl (Carl Roth, cat. no. 9265); 0.1 % Triton X-100 (AppliChem, cat. no. A 1388); pH 7.5) on ice for 20 min in a centrifugal vacuum concentrator (Eppendorf, model 5301). After washing twice in ice-cold Tris buffer, leaves were chopped in 1 ml isolation buffer (15 mM Tris; 2 mM Na_2EDTA; 0.5 mM Spermine tetrahydrochloride (Serva); 80 mM KCl (Carl Roth, cat. no. 6781); 20 mM NaCl; 15 mM β-mercaptoethanol (Carl Roth, cat. no. 4227); 0.1 %

Triton X-100; pH 7.5). The resulting suspension was filtered in a 5-ml polystyrene round-bottom tube with 35 μm cell strainer snap cap (Falcon, product # 352235). Nuclei were stained with 1.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI; Thermo Fischer, cat. no. 1306) and flow-sorted into Eppendorf tubes using a BD FACS Aria II (BD Biosciences). For barley 2C nuclei and for *A. thaliana* ecotype Pro-0 and Hov1-10 endopolyploid 4C nuclei were collected. Equal amounts (12–15 μl) of sucrose solution (40 % sucrose (Carl Roth, cat. no. 4621) in Tris buffer; pH 7.5) and flow-sorted nuclei suspension (approx. 450 nuclei/μl) were pipetted on glass slides, gently mixed, air-dried overnight and kept at −20 °C.

FISH probe preparation

Arabidopsis-type telomeric and microsatellite alkyne-bearing oligonucleotides (Table 1) were synthesised using an ABI 394 DNA/RNA Synthesiser (Applied Biosystems) and C8-Alkyne-dU phosphoramidites at baseclick GmbH (Neuried, Germany). The subsequent click reaction was performed using the OligoClick labelling Kit (cat. no. BCK-FISH, baseclick GmbH) according to the provided manual. Alkyne-bearing oligonucleotides and fluorescent-labelled oligonucleotides were analysed using RP-HPLC (Waters) equipped with a photodiode array detector (Waters) and a reversed phase column (XBridge OST C18, 4.6 mm × 50 mm, Waters) using a gradient method (from 45 to 85 % acetonitrile buffer). Correct masses were measured with an Auto-Flex II MALDI-TOF (Brucker Daltonics).

Probes for detection of the rye centromeric retrotransposon *Bilby* (Francki 2001) were generated by PCR from pSC-A vector containing a 583-bp fragment of *Bilby* using the PCR Labelling Kit (cat. no. PCR-Click 555, baseclick GmbH) according to the manual. TTTGCGACAATGACTCAAGC and TGTAGCTCATCGTGGAGTCG were used as forward and reverse primers, respectively. The annealing temperature was 58 °C. In the triphosphate solution (dATP, dCTP, dGTP, dTTP), dTTP was substituted to 100 % by C8-Alkyne-dUTP (baseclick GmbH). PCR products were purified using QIAQuick PCR purification Kit (Qiagen). The 5′-labelled *Arabidopsis*-type telomere probe $(CCCTAAA)_4$ was produced by Eurofins Genomics (Ebersberg, Germany). Nick translation-labelled telomere probes were produced via PCR according to Ijdo et al. (1991) with minor changes. PCR was accomplished using primers $(CCCTAA)_3$ and $(TTAGGG)_3$ without template (Eurofins Genomics) and *Taq* DNA polymerase (Qiagen, cat. no. 201207). The annealing temperature was 60 °C. Nick translation was performed using a NT Labelling Kit (Jena Bioscience GmbH, Jena Germany) according to the manual with the following triphosphates: 0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.25 mM dTTP and 0.25 mM aminoallyl-dUTP-5/6-TAMRA (Jena Bioscience GmbH).

Table 1 Synthesised alkyne-modified FISH probes

Name	Sequence
4PTel4	CCC TAA ACC CTAAAC CCT AAA CCC TAA A
4PTel2	CCC TAA ACC CTAAAC CCT AAA CCC TAA A
3PTel3	CCC TAA ACC CTAAAC CCT AAA
3PTel2	CCC TAA ACC CTAAAC CCT AAA
CTT	CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT

Underlined letters stand for the alkyne-modified bases (C8-Alkyne-dU)

Fluorescence in situ hybridisation using pre- or post-hybridisation click probes

Selected preparations of mitotic chromosomes were post-fixed (4 % formaldehyde in 2× SSC (300 mM NaCl; 30 mM *tri*-sodium citrate dehydrate, pH 7.0); 10 min, RT) and washed in 2× SSC. After dehydration (70, 90, 100 % ethanol) slides were air-dried. Denaturation was performed on a heating plate (2 min, 80 °C). Hybridisation was done in a moisture chamber (overnight, 37 °C). Alkyne-labelled or fluorochrome-labelled probes in DS 20 (20 % dextran sulfate (Sigma-Aldrich, cat. no. D 8906), 50 % deionised formamide (Sigma-Aldrich, cat. No. 4767), 300 mM NaCl, 30 mM *tri*-sodium citrate dehydrate, 50 mM phosphate buffer, pH 7.0) were used for post- and pre-hybridisation, respectively. Washed slides were mounted in Vectashield (Vector Laboratories, cat. no. H1000) containing DAPI (10 ng/μl; Thermo Fischer, cat. no. D1306).

Combined EdU-based DNA replication analysis and FISH using pre-clicked probes

Germinated seeds were grown for 2 h in darkness on filter paper (Macherey Nagel, cat. no. MN616) soaked with 15 μM 5-ethynyl-deoxyuridine (BCK-EdU555, baseclick GmbH, Neuried) and afterwards placed for

2.5 h on deionised water only. Root tips were cut and mitotic metaphases were accumulated by treatment with ice-cold water overnight. Mitotic slides were prepared as described above. To detect 5-ethynyl-deoxyuridine, the CuAAC reaction using 5-TAMRA-Azide was performed according to manufacturer's protocol (BCK-EdU555, baseclick GmbH). After washing, slides were dehydrated in ethanol and used for FISH with pre-hybridisation CuAAC probes as described above.

Combined immunohistochemistry and hybridisation of pre-hybridisation CuAAC-labelled FISH probes

Root tips were fixed (4 % paraformaldehyde in 1× PBS (137 mM NaCl, 2.7 mM KCl; 10 mM Na_2HPO_4) (Carl Roth, cat. no. 4984); 1.8 mM KH_2PO_4 (Carl Roth, cat. no. 3904; pH 7.4)), washed in ice-cold 1× PBS and digested in an enzyme cocktail (see above). After washing in ice-cold 1× PBS, single root tips were transferred to glass slides and squashed in 1× PBS + 0.001 % Tween-20 using cover slips (Th. Geyer, cat. no. 7695024). After removal of the cover slip by liquid nitrogen, slides were stored in 1× PBS. Incubation with primary (rabbit anti-grass CENH3 (Sanei et al. 2011); 90 min, 37 °C) and secondary antibodies (donkey anti-rabbit coupled to Alexa 647 (Dianova, cat. no. 711-606-152); 60 min, 37 °C) was performed. Slides were washed, dehydrated (70, 90 and 100 % ethanol), air-dried and fixed in ethanol/glacial acid (3:1; 24–48 h in dark). Subsequently, the slides were air-dried and incubated with DS20 (12 h; 37 °C). After short washing (2× SSC containing 0.1 % Triton X100), slides were dehydrated and air-dried. For denaturation, slides were incubated in 0.2 N NaOH (in 70 % ethanol; 10 min, RT), dehydrated and air-dried. Alkyne-modified probes were heated up (5 min; 95 °C) in DS20 before applying on slides. FISH was performed at 37 °C overnight using pre-hybridisation-labelled CTT and 4PTel4 probes. Slides were washed and afterwards mounted in Vectashield containing DAPI (10 ng/μl).

Quantification of telomeric FISH signals

Acquisition of FISH signal was carried out using an Olympus BX61 microscope equipped with an Orca ER CCD camera (Hamamatsu). For quantification of telomeric FISH signals, flow-sorted endopolyploid nuclei (4C) of *A. thaliana* were used. For each FISH probe, 300 nuclei were analysed. Statistical analysis was done using Kruskal-Wallis ANOVA followed by median test (STATISTICA data analysis software system, Statsoft, USA). Factorial effects and differences between groups were considered as significant at $P < 0.05$.

Results and discussion

To examine whether the CuAAC is suitable for the synthesis of FISH probes, we designed oligonucleotides recognising the *Arabidopsis*-type telomeric sequence ($(TTTAGGG)_n$). The solid phase-synthesised alkyne-modified oligonucleotides were labelled before hybridisation with TAMRA fluorochrome at defined positions using the CuAAC technique (Table 1). FISH of mitotic barley and *A. thaliana* chromosomes resulted in telomere repeat-typical signals at distal parts of the chromosomes, demonstrating that CuAAC-labelled probes are suitable for the in situ detection of telomeric sequences by FISH (Fig. 2a).

To investigate whether the length of probes and the quantity of fluorochromes conjugated to the probe influences the performance of CuAAC-labelled FISH probes, oligonucleotides consisting of either three or four 5′-CCCTAAA-3′ repeats each labelled with two to four fluorochromes were synthesised and compared to conventional nick translation and 5′ end-labelled probes. Regardless of oligonucleotide length and fluorochrome number, the telomere signals were detected by all probes on flow-sorted barley interphase nuclei (Fig. 2b). Quantification of fluorescence signal intensities of telomere probes after FISH was not feasible due to the telomere length variation of individual chromosomes as has been shown by Wang et al. (1991). For a precise signal quantification, telomeres of identical size are a prerequisite. Unfortunately, an identification of telomeres of the individual chromosomes was not possible. Next, we evaluated whether the CuAAC-labelled telomere probes show differences in their detection properties by quantification of telomeric FISH signals detected on flow-sorted endopolyploid nuclei (4C) of *Arabidopsis* ecotypes having either short or long telomeres. *A. thaliana* ecotype Pro-0 is characterised by rather long telomeres (~9.3 kb) while Hov 1-10 is possessing ~1 kb long telomeres only (Fulcher et al. 2015). Comparison of the mean number of detected telomere signals revealed that the performance of all probes was best in the ecotype having long telomeres (Fig. 2c). Significantly lower number of detected FISH signals in the Hov 1-10 ecotype possibly reflects a

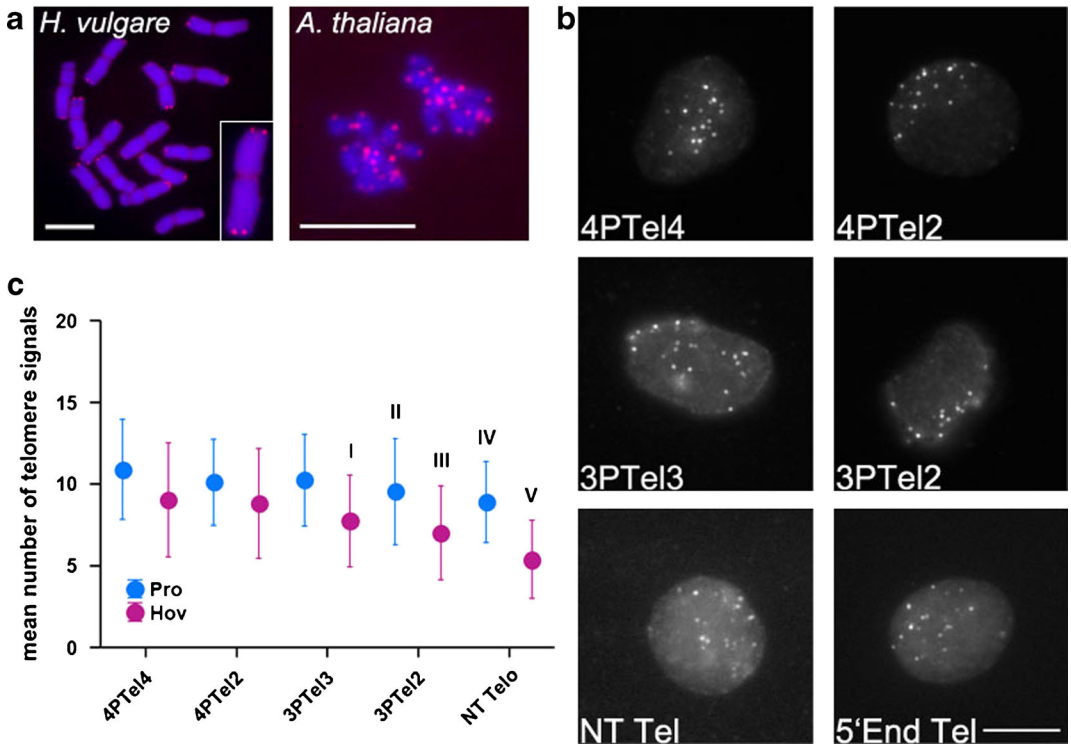

Fig. 2 CuAAC-labelled DNA probes are suitable for FISH. **a** Representative images of CuAAC-labelled *Arabidopsis*-type telomere ($(TTTAGG)_n$) probes (in *red*) hybridised on mitotic chromosomes of barley and *A. thaliana*. *Inset* shows a further enlarged chromosome. **b** Exemplary images of CuAAC-labelled probes (4PTel2, 4PTel2, 3PTel3, 3PTel2, nick translation-labelled telomere probe and 5′ end-labelled probe) hybridised on flow-sorted 2C barley nuclei. All *scale bars* represent 10 μm (**a**, **b**). **c** Quantification of detected telomeric FISH signals on flow-sorted endopolyploid 4C nuclei of *Arabidopsis* ecotypes possessing long (Pro-0, ~9.3 kb) and short (Hov 1-10, ~1 kb) telomeres by CuAAC-labelled telomere probes and nick translation-labelled telomere probe. Statistical analysis revealed that performance of all probes was markedly better in the Pro ecotype, as compared with the Hov ecotype. Significant differences ($P < 0.05$; protected two-way ANOVA followed by Bonferroni post hoc test) are labelled with Roman numerals I–V at which (*I*, *III*) means significant different to 4PTel4, 4PTel2, nick translated telomere probes, (*II*) to 4PTel4, (*IV*) to 4PTel4, 4PTel2, 3PTel3, (*V*) to 4PTel4, 4PTel2, 3PTel3 and 3PTel2

different clustering of telomeres in interphase. Furthermore, the short telomere length of the Hov 1-10 ecotype could potentially be below the detection limit of the telomeric probes. The lower performance of the nick translated-labelled probes in both ecotypes was most likely due to better hybridisation abilities of the oligonucleotide probes (Bradley et al. 2009). Moreover, the efficiency of DNA polymerase I—used in the nick translation assay—to incorporate fluorescently labelled deoxynucleotides is low reaching around 2–4 % (Yu et al. 1994). Possessing two to four fluorochromes, CuAAC-labelled telomere probes have a higher labelling rate than nick translated-labelled probes, which could also account for a better probe performance.

Further, we addressed the question whether the CuAAC reaction can also be performed after probe hybridisation, similarly to indirect labelling approaches. This would be advantageous because alkyne-labelled probes can be labelled after in situ hybridisation with various fluorochromes depending on specific needs or experimental settings. To investigate this, the same set of alkyne-modified telomeric probes (Table 1) were hybridised to mitotic wheat chromosomes, followed by an on-slide CuAAC reaction (post-hybridisation click, Fig. 1b). For comparison, a conventional nick translation-labelled telomere probe was co-hybridised. Figure 3a shows that post-hybridisation labelling of CuAAC probes indeed resulted in clearly detectable telomeric signals similar to the signals of the nick translation-labelled control probes.

To validate whether pre- and post-hybridisation click-labelled probes target the same chromosomal sites,

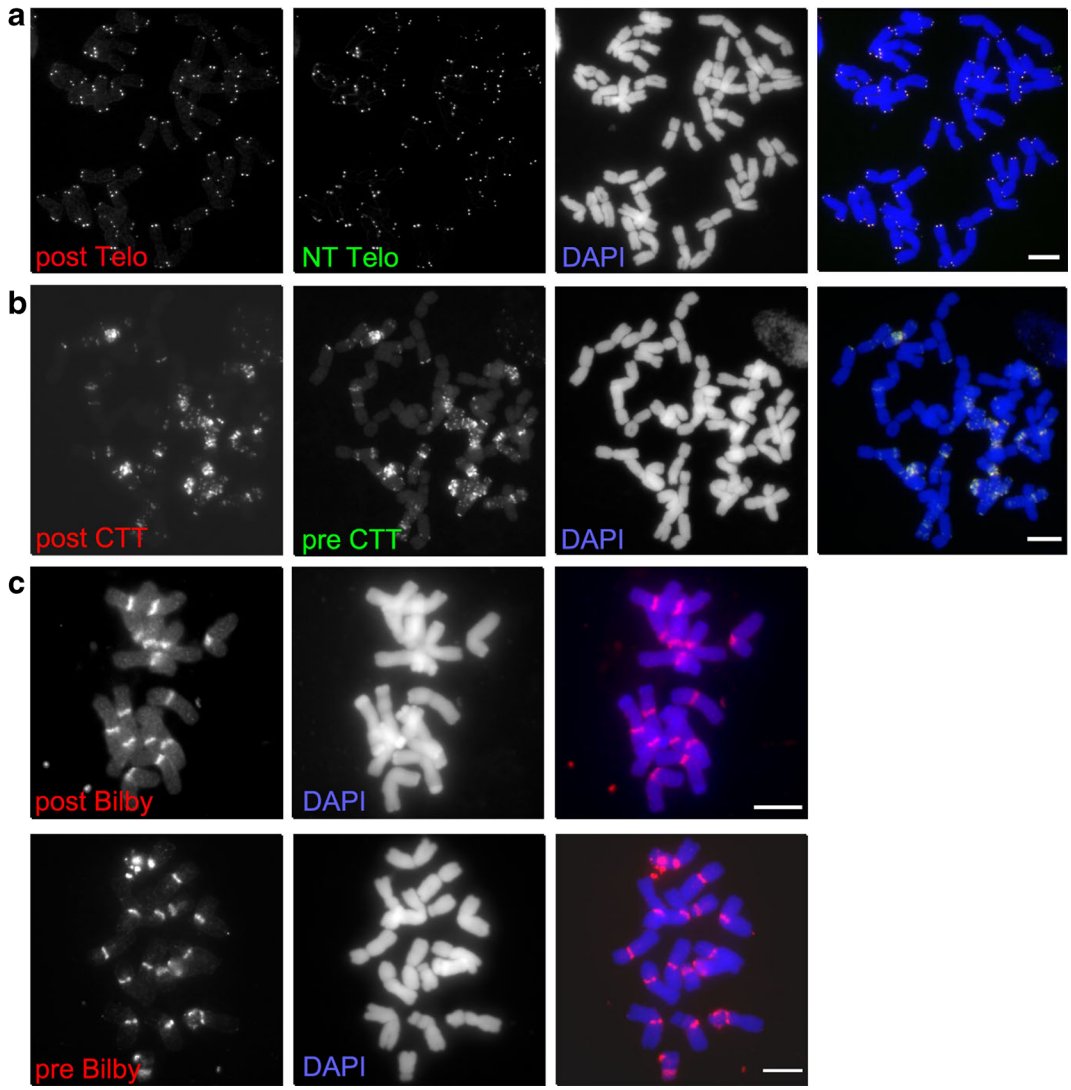

Fig. 3 Combination of pre- and post-hybridisation click-labelled probes on **a**, **b** wheat and **c** rye metaphase chromosomes. Alkyne-conjugated oligonucleotides probes were hybridised to the specimen and on-slide labelled via CuAAC. **a** Detection of post-hybridisation-labelled 4PTel2 and nick translation-labelled *Arabidopsis*-type telomere probes showing co-localised hybridisation signals. **b** Pre- and post-hybridisation click-labelled microsatellite $(CTT)_{10}$ probes showed a comparable distribution of hybridisation signals. **c** Pre- and post-hybridisation click labelling of rye centromere-specific probe (*Bilby*) synthesised via the CuAAC-based PCR assay (PCR-Click555 Kit). All *scale bars* represent 10 μm

we hybridised the pre-hybridisation fluorescently labelled microsatellite $(CTT)_{10}$ probes simultaneously with $(CTT)_{10}$ probes that were modified with alkyne only. Subsequently, the latter probe was labelled on-slide by a CuAAC reaction. We found that both types of probes resulted in comparable hybridisation patterns (Fig. 3b). The same applies for rye centromere-specific probe (*Bilby*) synthesised by PCR (Fig. 3c).

To explore a range of potential applications of click chemistry-based probes, we combined FISH with two standard cytological techniques, i.e. immunohistochemistry and labelling of DNA replication via EdU uptake. After performance of immunohistochemistry detecting the centromeric variant of histone H3 (CENH3) and click chemistry-based cell proliferation assay, pre-hybridisation click-labelled telomere and microsatellite probes (4PTel4; $(CTT)_{10}$) were successfully hybridised on barley metaphase chromosomes (Fig. 4). Note that post-hybridisation labelling of both probes via CuAAC reaction resulted in

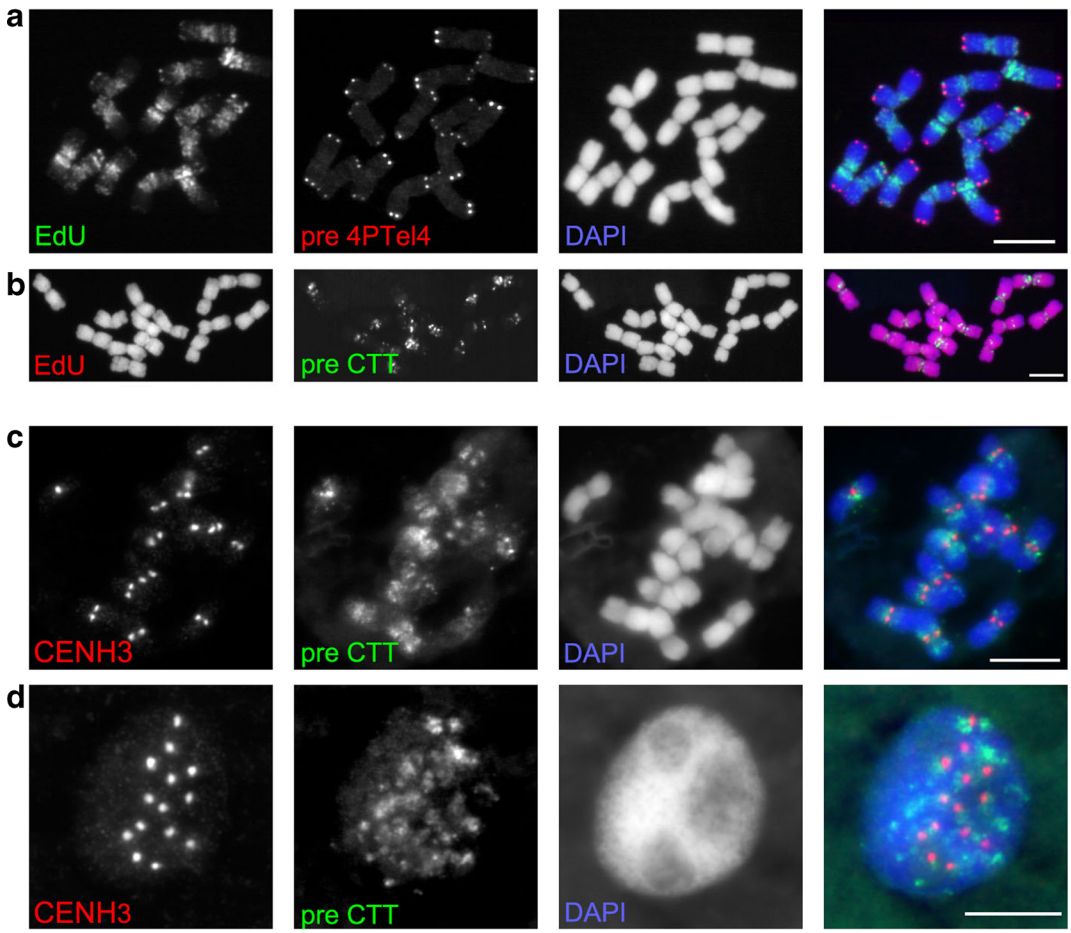

Fig. 4 Combination of CuAAC-labelled microsatellite probes with immunohistochemistry and labelling of replication via 5-ethynyl-deoxyuridine (EdU). **a** After labelling of early (*upper panel*, low amount of EdU incorporation) and **(b)** late DNA replication (*lower panel*, high amount of Edu incorporation) via click chemistry-based EdU uptake, FISH using pre-hybridisation click-labelled microsatellite $(CTT)_{10}$ (**b**) and telomeric probes (**a**) was performed on barley metaphase chromosomes. Immunolabelling of CENH3 was successfully combined with FISH using (**c**) pre-hybridisation click-labelled microsatellite probes ($(CTT)_{10}$) on metaphase chromosomes and **d** interphase nucleus of barley. All *scale bars* represent 10 μm

additional labelling of unreacted alkyne groups of EdU. Moreover, the CuAAC reaction was insufficient on specimens previously fixed by paraformaldehyde most likely due to penetration problems.

Thus, we showed that CuAAC-labelled probes represent a reliable tool to detect different types of repetitive sequences on chromosomes and nuclei. Importantly, we demonstrated that CuAAC reaction-based labelling technique can be combined with immunohistochemistry and cell proliferation assays via EdU without loss of sensitivity. This renders CuAAC reaction-based labelling technique as a valid and flexible tool in cytogenetics and cell biology with possible applications in the detection of single copy genes by CuAAC probes. In addition, this method allows for labelling of any type of FISH probe, e.g. long or short, single- or double-stranded DNA probes. Moreover, CuAAC labelling works in a modular manner. Depending on the experimental needs, one and the same alkyne-labelled probe can be detected with different kinds of label to suit the single experiments. Comparison between different labelling techniques revealed a similar sensitivity of CuAAC probes to that of conventionally labelled probes by comparable costs.

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Authors' contributions SH conceived and designed the study, performed experiments, analysed data and wrote the manuscript. AM and VC synthesised and provided all of the alkyne-modified probes, conceived the study and assisted with preparing the manuscript. LM and NR performed some experiments. JF conceived the study and assisted with preparing the manuscript. AH conceived the study and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All performed experiments comply with the current laws of the country in which they were performed. This article does not contain any studies with human or animal subjects performed by any of the authors.

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