CHAPTER

10 TIRF assays for real-time observation of microtubules and actin coassembly: Deciphering tau effects on microtubule/actin interplay

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CHAPTER OUTLINE

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Abstract

Microtubule and actin cytoskeletons are key players in vital processes in cells. Although the importance of microtubule–actin interaction for cell development and function has been highlighted for years, the properties of these two cytoskeletons have been mostly studied separately. Thus we now need procedures to simultaneously assess actin and microtubule properties to decipher the basic mechanisms underlying microtubule–actin crosstalk. Here we describe an in vitro assay that allows the coassembly of both filaments and the real-time observation of their interaction by TIRF microscopy. We show how this assay can be used to demonstrate that tau, a neuronal microtubule-associated protein, is a bona fide actin–microtubule cross-linker. The procedure relies on the use of highly purified proteins and chemically passivated perfusion chambers. We present a step-by-step protocol to obtain actin and microtubule coassembly and discuss the major pitfalls. An ImageJ macro to quantify actin and microtubule interaction is also provided.

1 INTRODUCTION

Microtubule and actin cytoskeletons are key elements of fundamental cellular processes such as intracellular architecture, cell division, organelle transport, and cell migration (De Forges, Bouissou, & Perez, 2012; Pollard & Cooper, 2009). Accumulative evidences show that these processes not only rely on the specific properties of each type of filament, but also require a tight coordination between their organization and dynamics (Akhshi, Wernike, & Piekny, 2014; Huber, Boire, López, & Koenderink, 2015; Rodriguez et al., 2003). Indeed actin and microtubule crosstalk has been observed in various cell types in yeast, plants, and animals, illustrating their key role in basic cell functions (Akhshi et al., 2014; Petrásek & Schwarzerová, 2009). Cytoskeletal coordination is mediated by common signaling pathways that reciprocally control microtubule and actin networks. These so-called regulatory interactions (Rodriguez et al., 2003) include the well-characterized Rho GTPase signaling pathway that regulates cell polarization and migration (Akhshi et al., 2014; Fu, Gu, Zheng, Wasteneys, & Yang, 2005). In addition, cross-linkers establish physical interactions between microtubule and actin filaments and several candidates have been identified (Rodriguez et al., 2003). Many of them work as multiprotein complexes containing at least one microtubule-associated protein (MAP) and one actin-binding protein (ABP) that interact to link actin filaments and microtubules. The best

characterized complexes involve microtubule plus-end tracking proteins (+TIPs) that recruit ABPs at microtubule plus ends to couple microtubule growth and actin fibers in the neuronal growth cone (Akhmanova & Steinmetz, 2015; Cammarata, Bearce, & Lowery, 2016). A few bifunctional cross-linkers bearing both microtubule- and actin-binding activities have also been identified (Elie et al., 2015; Lee & Kolodziej, 2002; Roger, Al-Bassam, Dehmelt, Milligan, & Halpain, 2004; Sanchez-Soriano et al., 2009). However, up to now, direct evidence showing that these cross-linker candidates simultaneously interact with both polymers remains rare. In this context, in vitro experiments with purified proteins are of particular interest to assess the physical interaction between a defined set of components (actin, tubulin, and cross-linker(s)). They also provide quantitative data describing this interaction (e.g., filament coalignment and/or deformation, influence on polymer dynamics, K_D, dwell time), thus giving insights into the molecular mechanisms that govern the interactions. Such assays reconstituting microtubule/actin interplay in cell-free systems are still rare and mostly describe interactions between preformed filaments (Griffith & Pollard, 1982; Henty-Ridilla, Rankova, Eskin, Kenny, & Goode, 2015; Huang et al., 2007; Preciado López et al., 2014; Sider et al., 1999). Here we describe an in vitro approach to assess actin and microtubule coassembly and to demonstrate that tau is a direct bifunctional microtubule–actin cross-linker.

In neurons, microtubule/actin crosstalk is essential for the elongation of neurites and synaptic activity (Geraldo & Gordon-Weeks, 2009; Hoogenraad & Bradke, 2009). Interestingly, recent work indicates that some neuronal MAPs, known to bind the microtubule lattice, are also able to associate with and regulate actin (He et al., 2009; Lee & Kolodziej, 2002; Roger et al., 2004; Tsukada, Prokscha, & Eichele, 2006), thus providing potential direct links between the two networks. Tau is a neuronal MAP required for the normal activity of neurons. Misregulation of tau due to its abnormal phosphorylation, mutation, and/or cleavage is associated with Alzheimer's disease and other neurodegenerative disorders. Tau is long known for its ability to stimulate tubulin polymerization, to stabilize microtubules, and to organize them into bundles, in cells, and in cell-free systems (reviewed in Morris, Maeda, Vossel, & Mucke, 2011). Tau has also been identified as an ABP involved in actin organization in neurons and recently showed to act as a direct molecular linker between actin and microtubule networks (Elie et al., 2015; Frandemiche et al., 2014; He et al., 2009).

In this chapter, we describe a TIRF-based biomimetic system that uses highly purified proteins and functionalized perfusion chambers to reconstitute real-time copolymerization of microtubules and actin filaments. We first address the effects of tau on the assembly and organization of dynamic actin filaments. We next reconstitute the concomitant assembly of microtubule and actin filaments in the presence of tau and describe how tau mediates the spatial and temporal coorganization of the two networks. We further provide a new macro tool in ImageJ to visualize and quantify the interaction between growing polymers.

2 PURIFICATION AND LABELING OF TUBULIN, ACTIN, AND TAU

Use of highly purified proteins is a critical aspect for biomimetic assays and TIRF microscopy. Lacking high purity, the dynamics of microtubules and actin filaments could be greatly modified due to aggregation and/or nonspecific protein adsorption. In such cases, the signal-to-noise ratio could also be significantly lowered during image acquisition. We use proteins that are freshly prepared in our laboratory (tubulin and tau) or in collaborator's groups (profilin and actin, from Laurent Blanchoin laboratory, CEA, Grenoble, France). The quality of each protein preparation is verified by functional assays (turbidity and TIRF assays).

(a) Tubulin is purified from bovine brain by cycles of polymerization and depolymerization and cation-exchange chromatography (Shelanski, Gaskin, & Cantor, 1973), labeled with ATTO-488 (ATTO TEC Gmbh, Germany; Hyman et al., 1991), and stored in liquid nitrogen. See the detailed procedures in the accompanying chapter (Ramirez-Rios et al., 2017).

(b) Actin protein is purified from rabbit skeletal muscle acetone powder (Spudich & Watt, 1971) and subsequent gel filtration (MacLean-Fletcher & Pollard, 1980; Wiesner, 2006). For visualization, actin is labeled with Alexa-568 as previously described (Ciobanasu, Faivre, & Le Clainche, 2015; Isambert et al., 1995). Unlabeled actin is stored at 4°C and Alexa-568 actin is kept at −20°C.

(c) His-tagged human tau is purified from bacteria as described in the accompanying chapter (Ramirez-Rios et al., 2017) and stored as small aliquots at −80°C.

(d) Human profilin and fascin are purified as described previously (Reymann et al., 2011; Vignjevic et al., 2003).

3 FABRICATION AND FUNCTIONALIZATION OF PERFUSION CHAMBERS

In this section, we describe the procedures to clean glass surfaces and to functionalize them with hydrophobic silane to prevent nonspecific interactions (adapted from Portran, 2014). This procedure is critical for the success of the TIRF experiments.

3.1 EQUIPMENT

- Sonication bath.
- Centrifuge with two slide drying cassettes (VWR, Galaxy mini array, REF 93000-204). Alternatively, you can dry the slides and the coverslips with airflow, but be careful to use high quality filtered air.
- Glass staining jar (ultra clean; best if dedicated to glass cleaning).
- Polypropylene slide holder (see, for example, Dutscher, REF 391058).
- Rack for coverslips (Sigma Aldrich, REF Z688568-1EA).
- Orbital shaker.

3.2 MATERIAL

- Microscope slides (Menzel-Gläser Superfrost, 76 mm × 26 mm; Thermo-scientific).
- Coverslips. The quality of the glass is important to minimize noise during observation; we use coverslips from Menzel-Gläser (18 mm × 18 mm #1; Thermo-Scientific). Check that the thickness of your coverslips is adapted to your microscope objective.
- Double-face precut adhesive tape (70 µm thick, 3 mm wide) purchase at LIMA company (France).
- Acetone 100%.
- Ethanol 96%.
- Hellmanex III (Hellma) 2% in water.
- 1 M KOH in water (freshly prepared).
- Deionized and filtered water.
- mPEG-silane, MW 30 k (Creative PEGWorks, PSB-2014). Dissolve 200 mg of PEG-silane in 200 mL of 96% ethanol with 0.2% (v/v) HCl to obtain a solution at 1 mg/mL final. Stir the PEG-silane solution at 50°C until the powder is completely dissolved. Store in the dark at room temperature.
- Biotin-PEG-SIL, MW 3400 (Laysan BIO, BIOTIN-PEG-SIL, MW 3400). Dissolve 200 mg of PEG-silane in 200 mL of 96% ethanol with 0.2% HCl to obtain a solution at 1 mg/mL. Do not warm the solution to dissolve the powder. Store in the dark at room temperature.

3.3 METHOD

3.3.1 Glass cleaning

Cleaning is performed in glass-staining jars at room temperature. Slides and coverslips are placed in polypropylene holder and rack, respectively, so that they can be easily transferred from one jar to another during the various steps of cleaning. Ensure that the entire glass surface is in contact with the solutions. Unless necessary (as for water cleaning) avoid touching glasses with fingers, wear gloves, and use clean tweezers. Washing steps in deionized and filtered water are achieved in large volume to ensure the complete removal of chemicals. If not, residual traces of chemicals will interfere with silane PEG during the functionalization step and proteins of the reaction mix might adsorb on the glass.

– Submerge glass slides and coverslips with acetone and sonicate for 30 min (sweep mode).
– Transfer racks in acetone and agitate on an orbital shaker (30 rpm) for 30 min.
– Transfer racks in ethanol and agitate on an orbital shaker (30 rpm) for 15 min.
– Extensively wash the glass slides and coverslips in 1 L of water (by dipping each slide and coverslip in and out water at least 10 times).
– Incubate slides and coverslips for 2 h in a 2% Hellmanex solution on an orbital shaker (30 rpm).

- Extensively wash the glass slides and coverslips in 1 L of water.
- Immerge glass slides and coverslips in a fresh solution of 1 M KOH and sonicate in a sonication bath for 4 cycles of 5 min sonication/2 min pause. This step induces the activation of the "OH" groups on the slide surface.
- Extensively rinse cover glass slides and coverslips in 1 L of water.
- Dry glass slides and coverslips using filtered airflow or minicentrifuge. Be careful as moisture will impair the next functionalization step.

3.3.2 Glass functionalization

- Functionalize slides and coverslips separately by incubation in PEG-silane and PEG-silane biotin, respectively. Incubation is performed in the dark, overnight (less than 18 h) on an orbital shaker (30 rpm).
- The next day, extensively rinse slides and coverslips in 1 L of ethanol 96% then 1 L of water, by dipping each slides/coverslips in the solutions at least 10 times.
- Dry slides and coverslips with filtered airflow or minicentrifuge.
- Store slides and coverslips at 4°C in the dark in clean boxes. As a general rule, slides and coverslips should not be stored for long period and should be used rapidly (within a few days).

3.3.3 Assembly of perfusion chamber

Fix a coverslip on the slide surface with double-face adhesive tape spaced 1 cm apart. If using a 70-μm thick tape, you will obtain a perfusion chamber of about 12–14 μL. Take care that the tape makes a flat and continuous contact with the glass to avoid leaks and to ensure that the coverslip makes a perfect plane surface.

4 SAMPLE PREPARATION FOR ACTIN AND MICROTUBULE COASSEMBLY IN THE PRESENCE OF TAU

4.1 EQUIPMENT

Perfusion chambers prepared as described in Section 2.

4.2 MATERIAL

4.2.1 Proteins

- Profilin: keep profilin aliquots at 4°C on ice.
- Actin: keep actin aliquots (unlabeled and labeled actin) at 4°C on ice.
- Tau: centrifuge aliquoted samples (5 min at 210,000 × g, 4°C) to remove aggregates. After centrifugation, calculate tau concentration at 595 nm by Bradford assay. Keep tau samples on ice and use within 2 h to prevent a loss of activity.
- Tubulin: centrifuge tubulin (10 min at 280,000 × g, 4°C) to remove aggregates. Check the tubulin concentration by measuring the absorbance at 280 nm (1 OD_{280} = 10 μM). The concentration of ATTO-488-tubulin is determined at 280 and 500 nm according to the following formula:

$$\left(A_{280}-\left(A_{500}\times \mathrm{CF}^{\mathrm{atto-488}}\right)\right)/\epsilon_{280}{}^{\mathrm{tubulin}}.$$

with $\mathrm{CF}^{\mathrm{atto\text{-}488}}=0.09$ and $\epsilon_{280}{}^{\mathrm{tubulin}}=115{,}000\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ (see the detailed procedure in the accompanying chapter Ramirez-Rios et al., 2017). Make small aliquots (1 aliquot per assay) and keep in liquid nitrogen until use.

4.2.2 Chemicals and buffers

- PLL-PEG (Jenkem, PLL20K-G35-PEG2K) store at −20°C. Make aliquots of 10 mg under atmospheric argon (preferentially). Stock solution: Dilute the powder at 1 mg/mL in filtered Hepes buffer 10 mM pH 7.4 and store at −20°C. Before use, dilute to 0.1 mg/mL in 10 mM Hepes pH 7.4.
- BRB80 buffer 5 ×: 400 mM PIPES, 5 mM EGTA, 5 mM $MgCl_2$, pH 6.85 (KOH). Store at −20°C.
- Actin polymerization (AP) buffer 10 ×: 500 mM KCl, 10 mM $MgCl_2$, 10 mM EGTA, 100 mM imidazole, pH 7.0 (HCl). Store at −20°C.
- Tris–Ca^{2+} (TC) buffer: 2 mM Tris–HCl pH 8, 0.1 mM $CaCl_2$.
- ATP (SIGMA A-2383): 100 mM ATP in water. Dilute the powder in cold water, mix on ice, and immediately adjust the pH to 7 with KOH. Store at −20°C.
- BSA (SIGMA A-7030): Prepare a BSA 10% stock solution in PBS (w/v). Filter (0.2 µm filters) and store at −20°C.
- DTT (SIGMA-DO632): 100 mM in water. Filter and store at −20°C.
- Glucose (SIGMA G8270): Prepare a stock solution of 450 mg/mL in BRB80. Store at −80°C. Before use, dilute to 45 mg/mL in water.
- Antiphoto bleaching cocktail: Prepare a mix of 3.5 mg/mL catalase (SIGMA C9322-1G) and 25 mg/mL glucose oxidase (SIGMA G6766-10KU) in BRB80. Do not vortex the solution. Filter the solution (0.2 µm filter), snap freeze aliquots in liquid nitrogen, and store at −80°C. Thawed aliquots should not be frozen again. Before use, keep aliquots at 4°C. Note that the cocktail will lose efficiency after 1 or 2 h, and this may impede microtubule dynamics. Do not hesitate to thaw fresh aliquots.
- Methylcellulose (1500 cp, SIGMA M-0555). Dissolve the powder in deionized and filtered water prewarmed at 60°C to obtain a final concentration of 1% (w/v). Gently agitate until complete dissolution (it can take some time). Store at 4°C in the dark. We recommend using fresh methylcellulose solution (no more than one week old).
- High vacuum grease.

4.3 ACTIN ASSEMBLY

We describe later the in vitro TIRF-based assay that we use to study the influence of tau on actin assembly and organization (based on Michelot et al., 2006). The specific effects of tau on microtubule dynamics in cell-free systems is well described in the literature, in particular by video-DIC light microscopy methods (Panda, Goode, Feinstein, & Wilson, 1995). We also describe in the accompanying chapter from

Ramirez-Rios et al., a reconstitution assay to study the microtubule-related properties of tau by TIRF microscopy.

- Flow the perfusion chamber with 50 µL PLL-PEG to cover potential defects in the silane-PEG coating. Incubate 30 s at room temperature.
- Wash with 3 × 100 µL of the 1% BSA solution in TC buffer.
- Prepare 30 µL of elongation mix in TC buffer containing:
  - AP buffer 1 ×
  - 1% BSA
  - 0.2 mM ATP
  - 4 mM DTT
  - 1 mg/mL glucose
  - 80 µg/mL catalase and 580 µg/mL glucose oxidase
  - 0.3% (v/v) methylcellulose
  - 0.4 µM G-actin mix (30% Alexa-568 labeled G actin/70% unlabeled actin)
  - 0.4 µM profilin
- Carefully mix (methylcellulose can induce inhomogeneity) while avoiding bubbles.
- Perfuse the mixture and seal the chamber with grease to avoid evaporation.
- Observe under the microscope at room temperature. The initiation of actin polymerization occurs within a few minutes (see Fig. 1, top row).
- To study the effect of tau on actin assembly, repeat the same procedure as above adding 0.1 µM tau in the reaction mix. Tau induces the formation of actin filament bundles as shown on Fig. 1 (bottom row) and should not influence the growth rate of actin filaments (Elie et al., 2015).

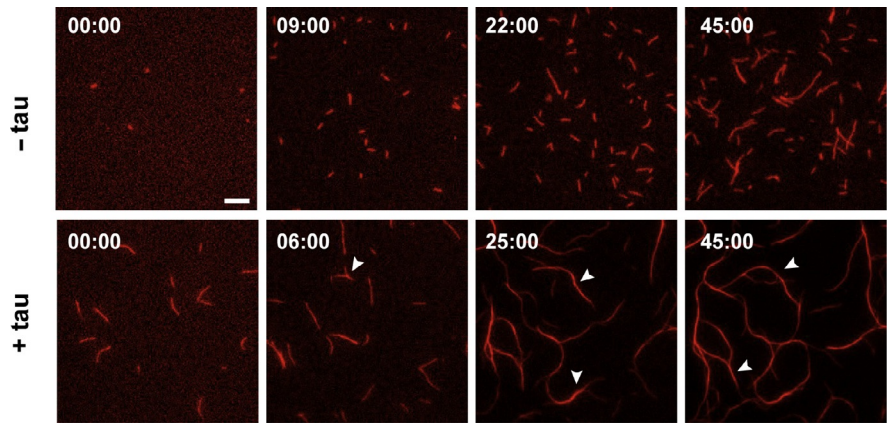

FIG. 1

TIRF image sequences of actin polymerized without or with tau. Only single actin filaments polymerize in the control without tau (*upper panel*). Tau induces actin bundles formation (*lower panel*). *White arrowheads* show an example of actin bundles. Scale bar = 10 µm.

Notes

1) Actin growth initiates at room temperature; do not wait to observe the polymerization under the TIRF microscope.
2) Adjust the actin concentration to obtain a suitable density of actin filaments.

4.4 ACTIN AND MICROTUBULE COASSEMBLY

4.4.1 Actin and microtubule coassembly without tau

– Flow the perfusion chamber with 50 µL of PLL-PEG. Incubate 30 s at room temperature.
– Wash with 3 × 100 µL of 1% BSA in BRB80.
– Prepare 30 µL of reaction mix on ice containing:
  - BRB80 1 ×
  - 50 mM KCl
  - 1% BSA
  - 1 mM GTP
  - 4 mM DTT
  - 1 mg/mL glucose
  - 80 µg/mL catalase and 580 µg/mL glucose oxidase
  - 0.2% (v/v) methylcellulose
  - 0.4 µM profilin
  - 0.4 µM G-actin mix (30% Alexa-568 labeled G actin/70% unlabeled actin)
  - 20 µM of tubulin mix (30% ATTO-488 labeled tubulin/70% unlabeled tubulin)
– Carefully mix (methylcellulose can induce inhomogeneity) while avoiding bubbles.
– Perfuse the reaction mix, seal the chamber and observe.

Notes

1) Methylcellulose concentration is kept lower in the presence of microtubules and actin (0.2%) compared to the assay with actin alone (0.3%) to avoid nonspecific microtubule bundle formation. At this lower methylcellulose concentration, single actin filaments hardly remain in the field of the TIRF observation (Fig. 2, top row) unless they are bundled (Fig. 2, middle and bottom) or coaligned with microtubules (Fig. 2, bottom).
2) It is important that actin filaments and microtubules initiate assembly at the same time so that the two networks expand with comparable kinetics. The concentrations we indicate worked fine in our hands, but you may need to adjust them to your own proteins and equipment.
3) Functionalization of coverslips with biotin-PEG-SIL opens the possibility to grow microtubules from biotinylated microtubule seeds attached to the coverslips surface (protocol described in accompanying chapter, Ramirez-Rios et al., 2017). This is a possible alternative to the self-assembly conditions described here.

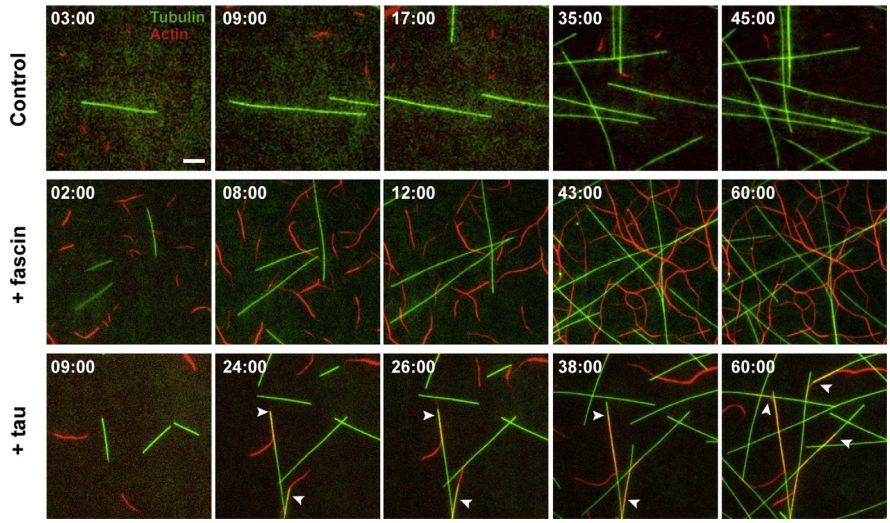

FIG. 2

TIRF image sequences of growing microtubules and actin filaments. In the control, most actin filaments float out of the TIRF field and do not coalign with microtubules (*upper panel*). Fascin (*middle panel*) induces the formation of actin bundles, distributed around the microtubules. There is no coalignment between actin filaments and microtubules. In the presence of tau (*lower panel*), growing microtubules and actin filaments coalign (*white arrowheads*). Thus tau coordinates microtubules and actin networks. Scale bar = 10 μm.

Adapted from Elie, A., Prezel, E., Guérin, C., Denarier, E., Ramirez-Rios, S., Serre, L., et al. (2015). Tau co-organizes dynamic microtubule and actin networks. Scientific Reports, 5, *9964–9974.*

4.4.2 Actin and microtubule coassembly with tau

Proceed as before, and perfuse the following reaction mix in the chamber:

- BRB80 1 ×
- 50 mM KCl
- 1% BSA
- 1 mM GTP
- 4 mM DTT
- 1 mg/mL glucose
- 80 μg/mL catalase and 580 μg/mL glucose oxidase
- 0.2% (v/v) methylcellulose (see Note 2)
- 0.4 μM profilin
- 0.7 μM of tau
- 0.4 μM G-actin mix (30% Alexa-568 labeled G actin/70% unlabeled actin)
- 5 μM of tubulin (30% ATTO-488 labeled tubulin/70% unlabeled tubulin) (see Note 1).

– Seal the chamber and observe with TIRF microscope at 37°C. The initiation of microtubule and actin polymerization should start simultaneously.

Notes

1) In this assay, the concentration of tubulin is lower compared to the control conditions (actin/microtubules without tau, see earlier) to avoid extensive microtubule nucleation induced by tau. This is not necessary for actin as actin nucleation is not affected by tau. In our experiments, we used 20 μM tubulin for the control and 5 μM tubulin with tau. This also ensured that actin and microtubules initiate their polymerization simultaneously.
2) As a control experiment, you can use a specific actin bundler such as fascin (1 μM final concentration in the reaction mix). Fascin induces the formation of actin bundles, but does not alter microtubule organization nor induce the coalignment of actin filaments and microtubules (Fig. 2, middle row).

5 TIRF IMAGING AND RECORDING

- Imaging is done on an inverted microscope (Eclipse Ti, Nikon) with an Apochromat 60 ×/1.49 numerical aperture (NA) oil immersion objective (Nikon). Our microscope is equipped with an Ilas2 illuminator (Roperscientific), a cooled charged-coupled device camera (EMCCD Evolve 512; Photometrics), and a warm stage controller (LINKAM MC60). The imaging station is controlled by Metamorph® software (version 7.7.5, Molecular Devices).
- We use dual-view system (Roperscientific) or multidimensional acquisition function (Metamorph) for simultaneous observation of microtubule and actin at 488 and 561 nm, respectively.
- We record movies from 45 min to 1 h, at 0.2 fps and 100 ms exposure time.

Notes

1) Ensure that the temperature of the microscope stage is stable to avoid fluctuation of actin and microtubule polymerization. We use a warm stage controller (LINKAM MC60). We recommend that you check the temperature of the reaction chamber when mounted on the microscope and adjust the temperature with the controller stage. The temperature in the reaction chamber should be around 26°C for actin polymerization and around 32°C for microtubule assembly (either alone or with actin).
2) The initiation of actin and microtubule polymerization occurs within 2–5 min. A too long delay most probably reflects a problem in the reaction mix.
3) Actin stored for a long time (more than 1 month at 4°C) has a tendency to form nonspecific bundles. Start each set of experiments with a control reaction of actin polymerization. If you observe bundles, you should use a new fresh actin sample.
4) Always use the minimum power laser.

6 DATA ANALYSIS AND RESULTS

In vitro reconstitution of microtubule and actin coassembly in the presence of tau allows the determination of several parameters characterizing this crosstalk: (1) transient or persistent coalignment between the growing polymers and (2) influence of

one network on the organization and/or growth parameters of the other one. For instance, tau induces the coalignment of growing microtubules and actin filaments and forces the two networks to adopt the same global organization and growth direction (Figs. 2 and 3). To measure the overlap between actin and microtubule networks, we developed a macro in ImageJ (Schneider, Rasband, & Eliceiri, 2012) to

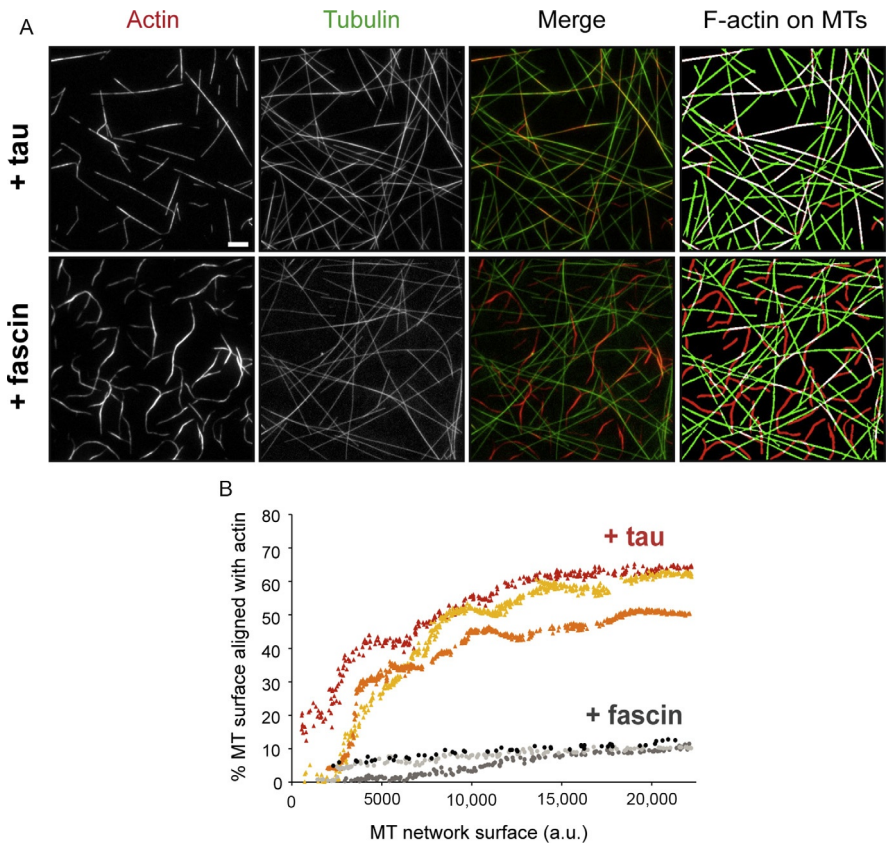

FIG. 3

Tau crosslinks growing microtubules and actin filaments. (A) Examples of TIRF microscopy images used to quantify the proportion of microtubule surface that coaligns with actin filaments in the presence of tau (*upper panel*) or in the presence of fascin (*lower panel*). The coalignment between actin filaments and microtubules is represented by white areas in the masked images (*right panel*). Microtubules are represented in *green* and actin filaments in *red*. Scale bar = 10 μm. (B) Proportion of the microtubule surface associated with actin filaments vs the total microtubule network surface in the presence of tau or fascin (three representative curves for each condition). a.u., arbitrary units; MTs, microtubules.

Adapted from Elie, A., Prezel, E., Guérin, C., Denarier, E., Ramirez-Rios, S., Serre, L., et al. (2015). Tau co-organizes dynamic microtubule and actin networks. Scientific Reports, 5, *9964–9974.*

quantify the proportion of the microtubule surface that coaligns with actin filaments. Masks were created from both sequences of microtubule and actin networks using a FFT band pass filter and automatic triangle thresholding (Fig. 3A). Surfaces of actin and microtubule networks are calculated from the masks (Fig. 3B). The intersections of the two masks give the surface of actin filaments on microtubules. This ImageJ macro (available upon request to eric.denarier@univ-grenoble-alpes.fr) is adapted to process the entire time-lapse movies (Fig. 3C).

7 CONCLUSION

We described here an in vitro TIRF-based assay enabling the observation and analysis of actin and microtubule coassembly. Conditions for the coassembly of the two polymers are difficult to obtain because the traditional buffer used for actin polymerization is not compatible with microtubule assembly. The assay we developed works well with tau proteins, but you may need to modify the buffer when working with another MAP (see for instance, Farina et al., 2016).

TIRF assays represent a powerful approach complementary to other commonly used assays, such as spectrofluorimetry or sedimentation assays. First, they allow a direct visualization of interactions between actin filaments and microtubules, in real time and at the level of single polymers. Thus rare events can be detected together with more frequent ones. Second multiple parameters can be used to describe the evolution of the reaction (e.g., surface of coalignment, angle for zippering between filaments, dynamic parameters of growing polymers), providing tools for an accurate understanding of the reaction. Finally many experimental parameters can be controlled, and in particular the concentrations of all reactants, favoring reproducible experiments. One of the main limitations of these biomimetic assays is that partners of the reaction or regulatory elements (e.g., kinases, phosphatases, gradients) might be missing. In this respect, coupling TIRF assays with microfluidics will allow sequential addition of effectors to the reaction and will provide excellent tools to further explore cytoskeletal properties and regulation (Gressin, Guillotin, Guérin, Blanchoin, & Michelot, 2015).

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