

TSA Treatment of Mammalian Cells (PROT09)



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Introduction

Prolonged treatment of proliferating mammalian cells with low doses of a histone deacetylase inhibitor, trichostatin A, specifically affects pericentric heterochromatin. Relocation of these regions towards the nuclear periphery and loss of HP1 proteins ensue after several divisions. Meanwhile, DNA methylation state and core centromeric markers are maintained. Subsequent defects in centromeric function arise in mitosis. Remarkably, removal of the drug rapidly reverses all these changes (see [comment 1](#)).

Procedure

High dose TSA treatment is toxic to the cells and triggers cell cycle arrest in G1 and G2. In these conditions, the effect of TSA treatment on pericentric heterochromatin cannot be observed. It is thus of major importance to first determine the range of TSA doses that still allows progression through several cell cycles. The capacity to proliferate is essential to detect the effect.

Definition of optimal treatment conditions

- We use TSA (Trichostatin A) from SIGMA (T8552). Stock solution at 1mg/ml in ethanol (storage at -20°) (see [comment 2](#)).
- The cells (20% of confluence) are allowed to grown for 24 hours attached on the plate.
- Add the drug in complete medium.
- Various doses are tested to define optimal conditions enabling progression through multiple cell divisions. We usually use the following range: 5, 15, 25, 50 and 100ng/ml diluted in culture media.
- Drug and medium are renewed every 2 days (TSA is unstable) (see [comment 3](#)).
- We follow the cell culture over a week and we choose the dose just below lethality.
- Optimal concentrations were determined for specific cell types:

CELL LINE	CELL TYPE	TSA CONCENTRATION
L929 ATCC : CCL-1	Fibrosarcoma, Mouse	25ng/ml
HeLa ATCC : CCL-2	Cervix Adenocarcinoma, Human	40ng/ml
U2OS (GFP-CENPB stably transfected) Provided by Kevin Sullivan	Osteogenic Sarcoma, Human	50ng/ml

Detection of TSA effect on pericentric heterochromatin

For L929

- In mouse cells, pericentric heterochromatin domains are easily visualized by DAPI staining (4',6-diamidino-2-phenylindole, SIGMA). After 5 days of TSA treatment at the optimal concentration, almost every cell displayed an altered DAPI staining with a relocation of DAPI dense spots (corresponding to pericentric heterochromatin domains) towards the nuclear periphery.
- HP1 domain disruption upon TSA treatment is monitored by immunofluorescence using an antibody against HP1alpha (2HP1H5) from EUROMEDEX. Reversibility of the drug effect was obtained within 20 hours after TSA removal. ([Figure 1](#))
- Centromeric DNA relocation towards the nuclear periphery (after TSA treatment) can be detected by FISH with mouse Pan centromeric probe (refer to Taddei *et al.*, 2001).
- ChIP (native conditions) analysis revealed an increase of H3-K9 acetylation after TSA treatment whereas H3-K9 methylation was not affected (refer to Maison *et al.*, 2002).

For HeLa

- Perinuclear relocalization of centromeric regions (after TSA treatment) can be followed by IF with antibodies against centromeric proteins such as ACA and CENPC (refer to Taddei *et al.*, 2001).

For U2OS-GFP-CENP-B

- Perinuclear relocalization of centromeric regions (after TSA treatment) is conveniently followed directly on fixed or live cells by GFP signal ([Figure 2](#)).

Materials & Reagents

Reviewer Comments

Reviewed by: [Bryan Turner](#), Institute of Biomedical Research, University of Birmingham Medical School, Birmingham

1. This is a general comment: The effects of TSA, or other deacetylase inhibitors, on cell function are complex. As there are at least 18 different histone deacetylases in mammals, acting on a variety of histones and non-histone proteins, and as most of these are inhibited by TSA *et al.*, this is not surprising. TSA causes at least two cell cycle blocks, one in G1 and another in G2M, it drives some cells into apoptosis and others down differentiation pathways. These effects are dependent on cell type and on the concentration of the drug.
2. We also use Sigma TSA, but the source of the drug is important and the behaviour of different batches can differ. When treating mouse ES cells with low concentrations of TSA in order to maintain cell growth, we renew the drug and medium every day.
3. In our hands, TSA in the culture medium has fallen to below effective concentrations within 24 hours (i.e. the initial dramatic effect on histone acetylation has been completely reversed, Travers *et al. Exp.Cell Res.*, 280: 149-158, 2002). We have seen this with several cell types.

Figures

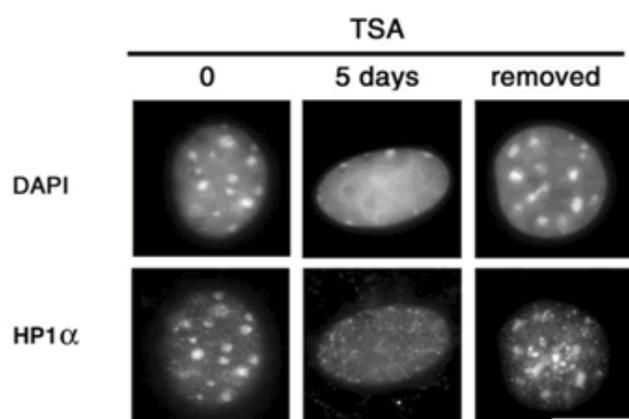


Figure 1: TSA treatment reversibly disrupts HP1alpha domains at pericentromeric regions. Immunostaining on L929 mouse cells untreated (0) or treated with TSA (25ng/ml) for 5 days, or 20 hours after TSA removal following 5 days treatment. HP1 alpha was localized using a specific monoclonal antibody (lower panels) and DNA was visualized by DAPI counterstaining (top panels). Scale bar 10µm.

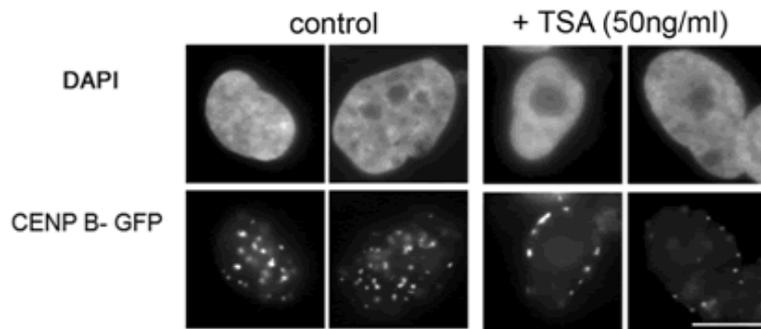


Figure 2: TSA treatment induces relocalization of centromeric proteins to the nuclear periphery. U2OS cells stably expressing GFP-CENPB were untreated (control) or treated with TSA (50ng/ml) for 5 days. Relocalization of centromeres to the nuclear periphery is analysed on fixed cells by following GFP signal.

References

1. Maison C, Bailly D, Peters A, Quivy JPQ, Roche D, Taddei A, Lachner M, Jenuwein T and Almouzni G (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nature Genetics*, **30**: 329-334
2. Taddei A, Maison C, Roche D and Almouzni G (2001) Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases in mammalian cells. *Nature Cell Biology*, **2**:114-120
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