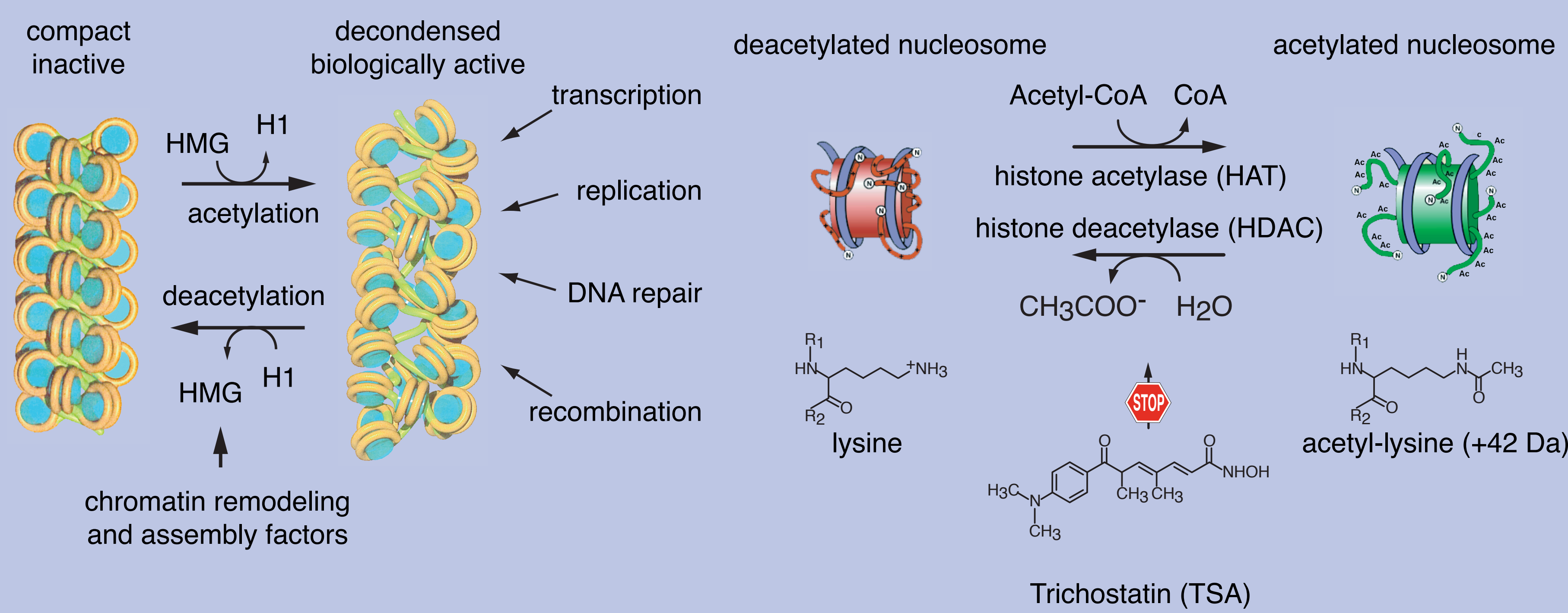


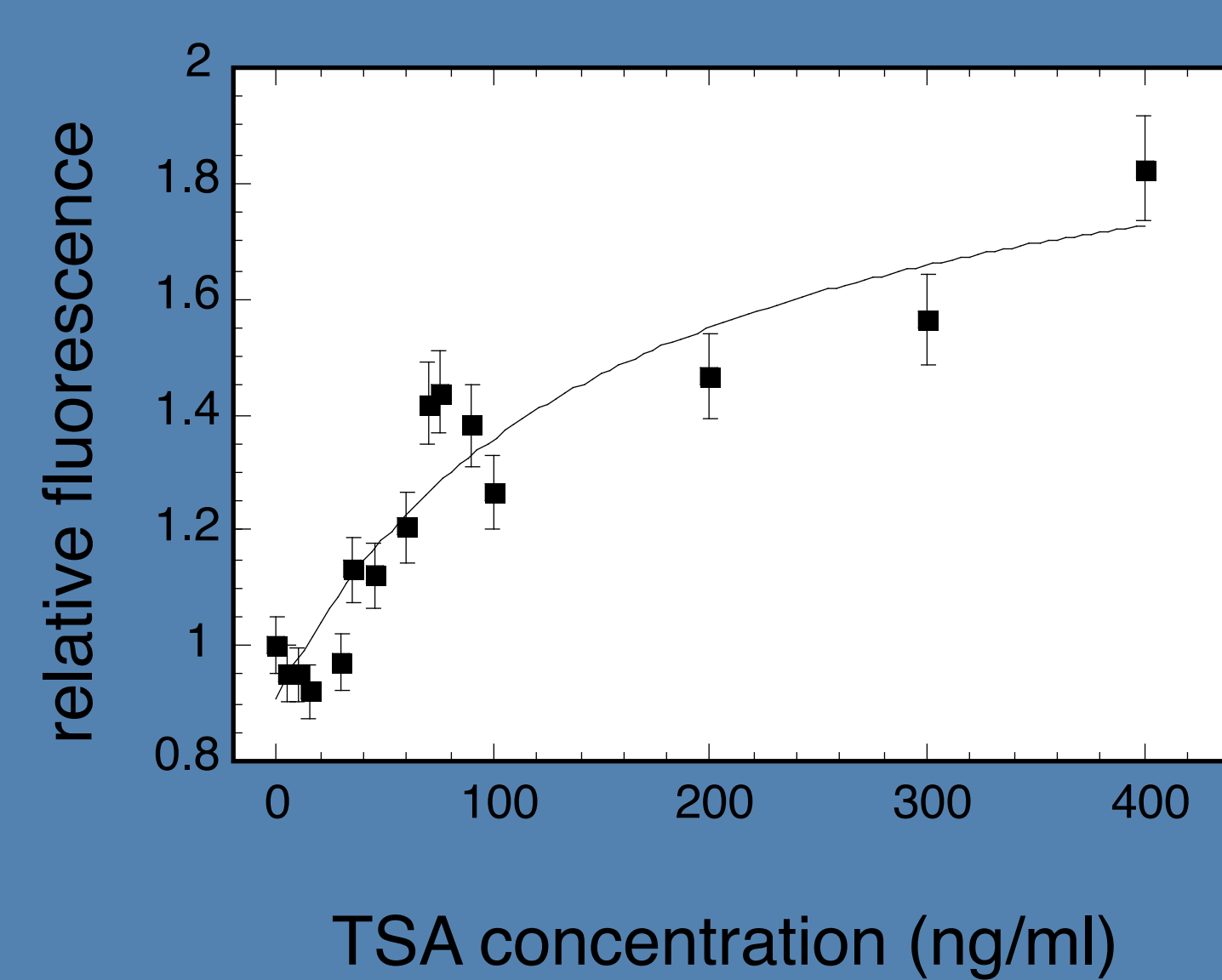
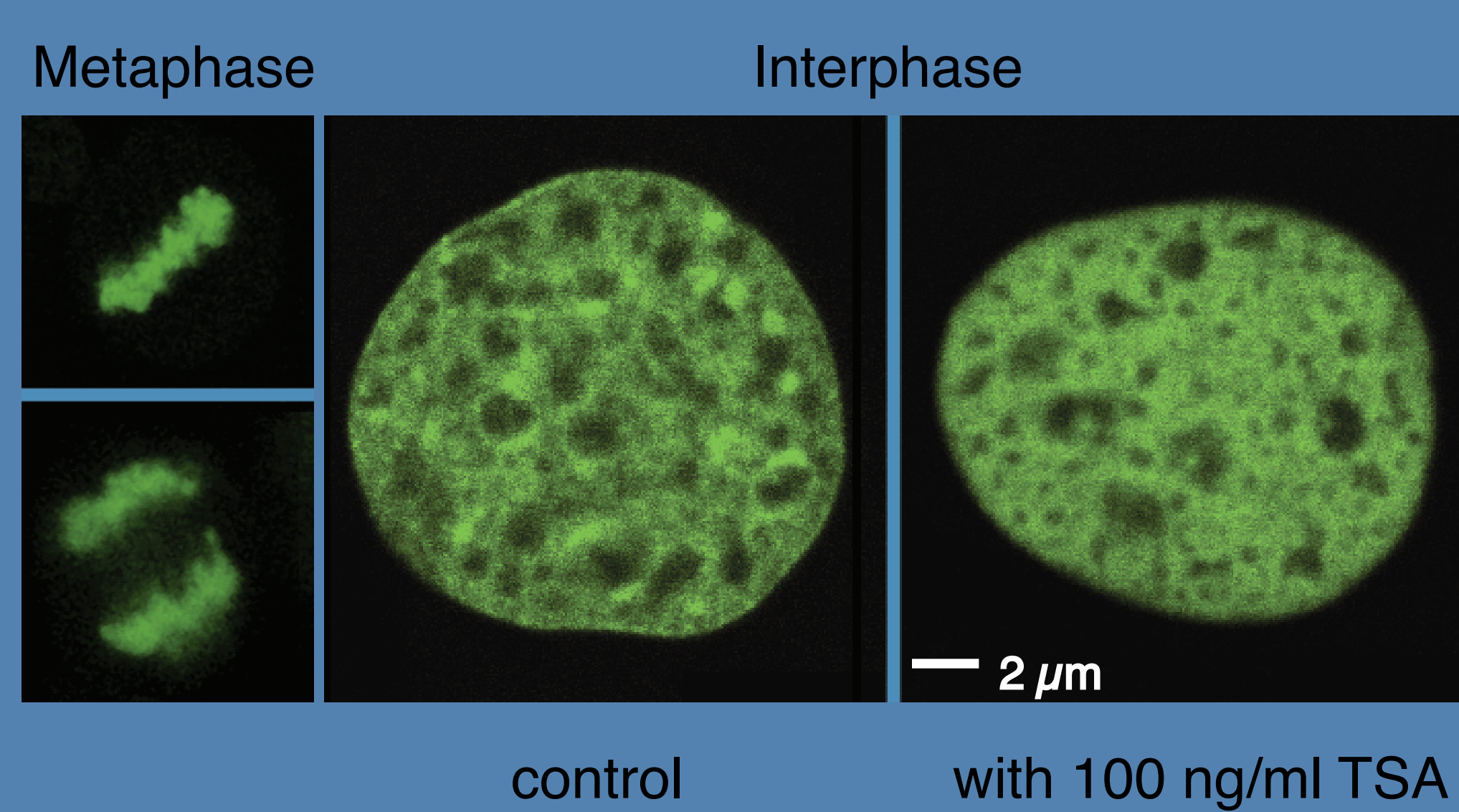
# TSA HAS A COMPLEX EFFECT ON CELL CYCLE PROGRESSION

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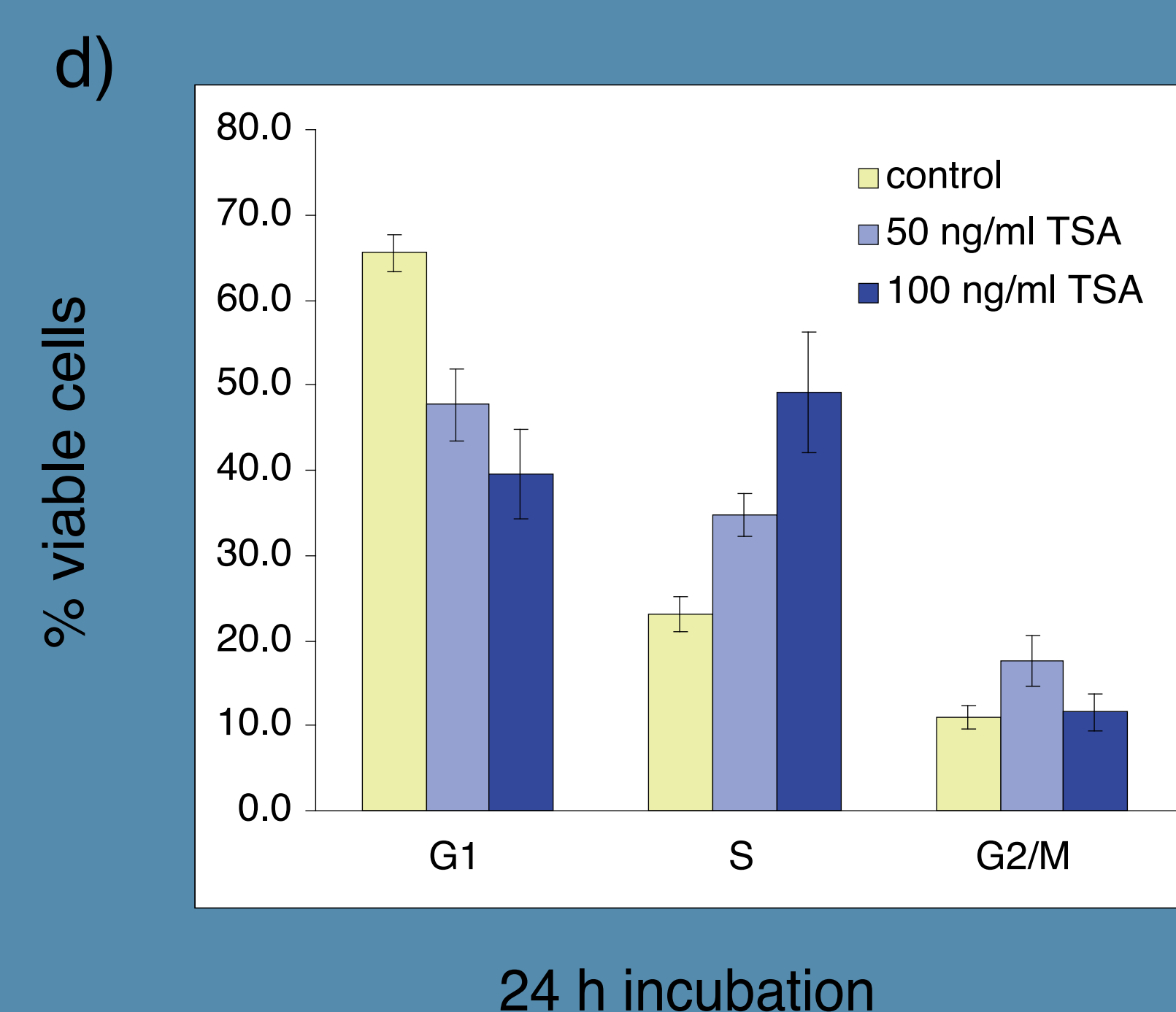
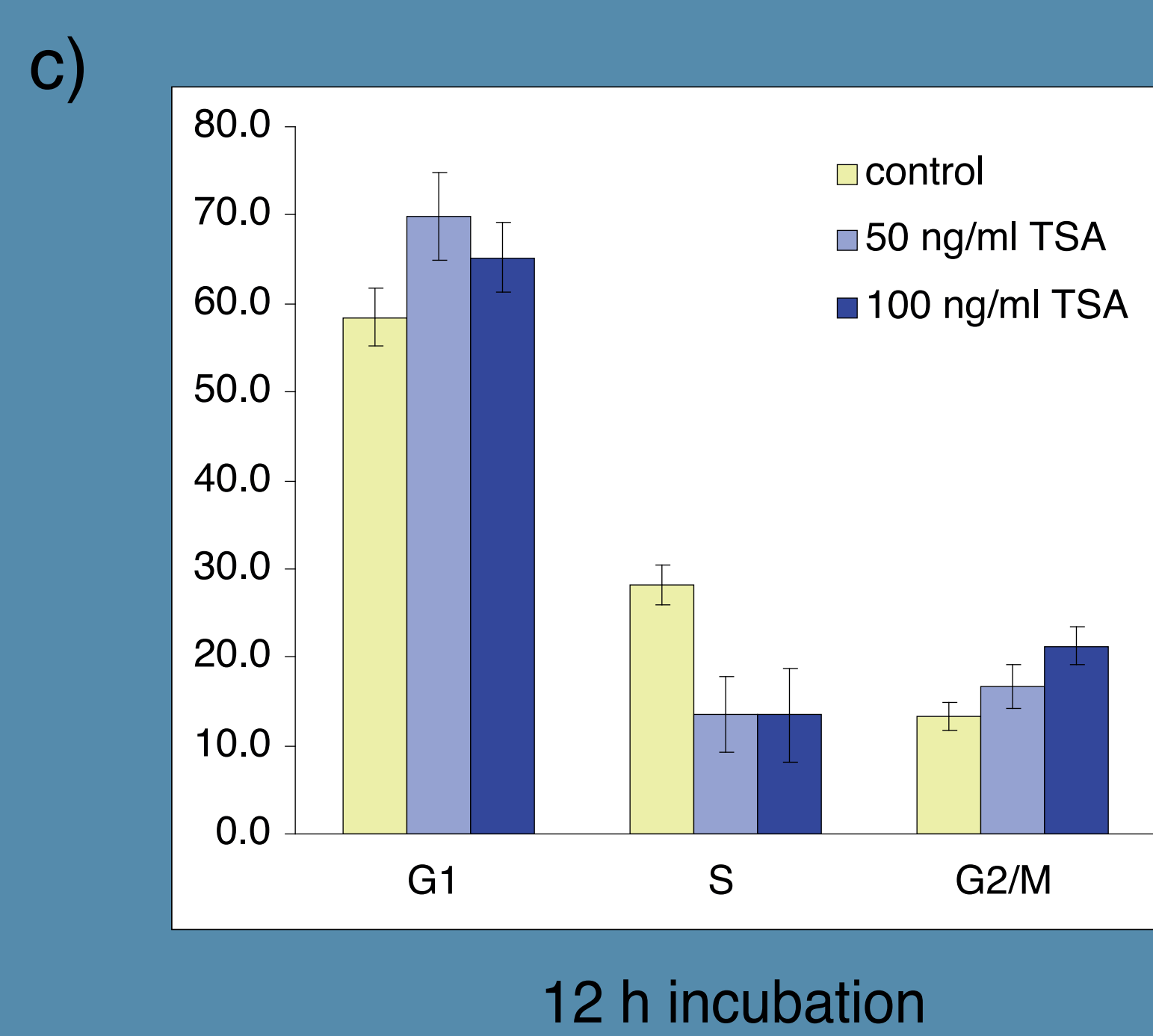
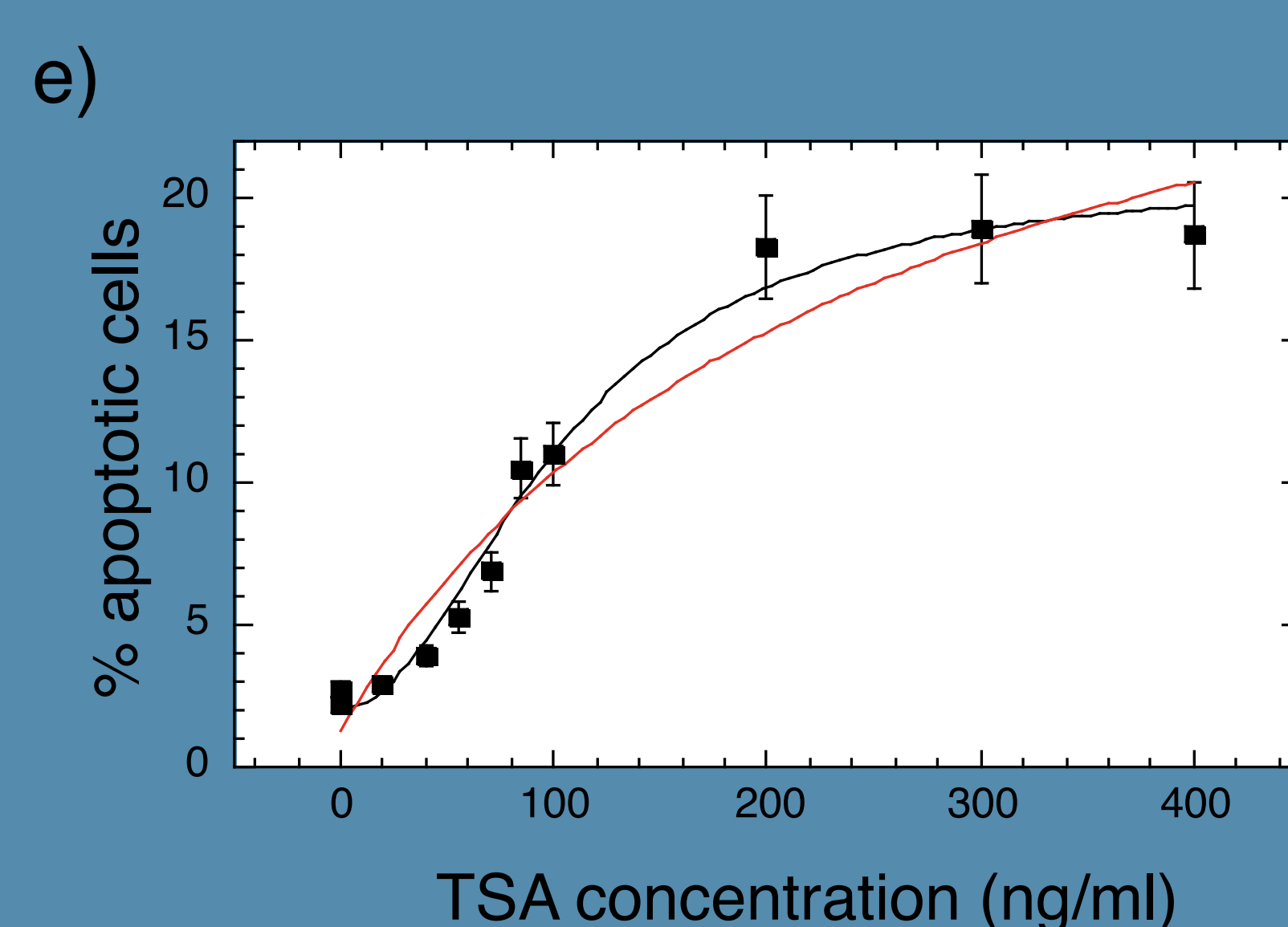
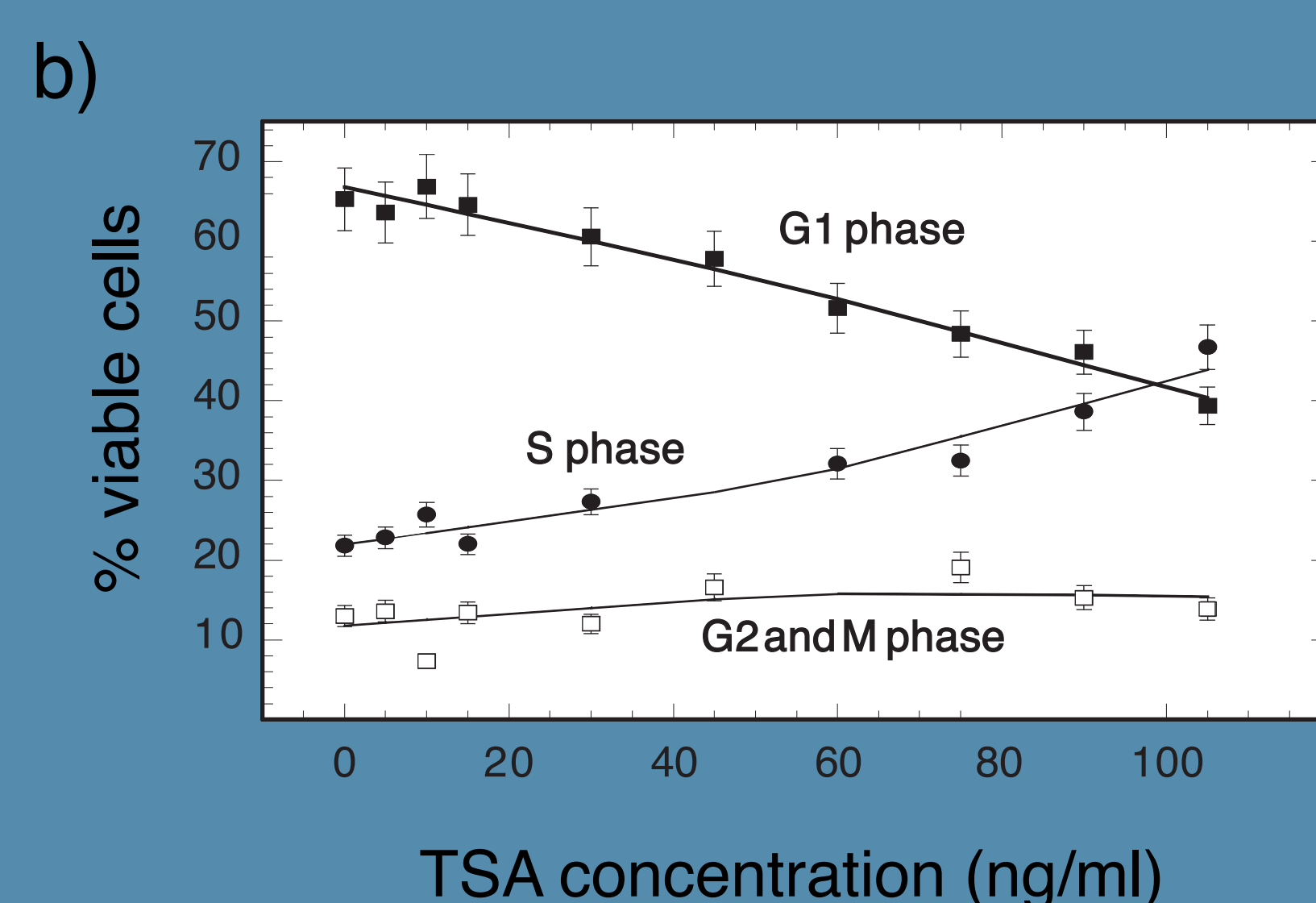
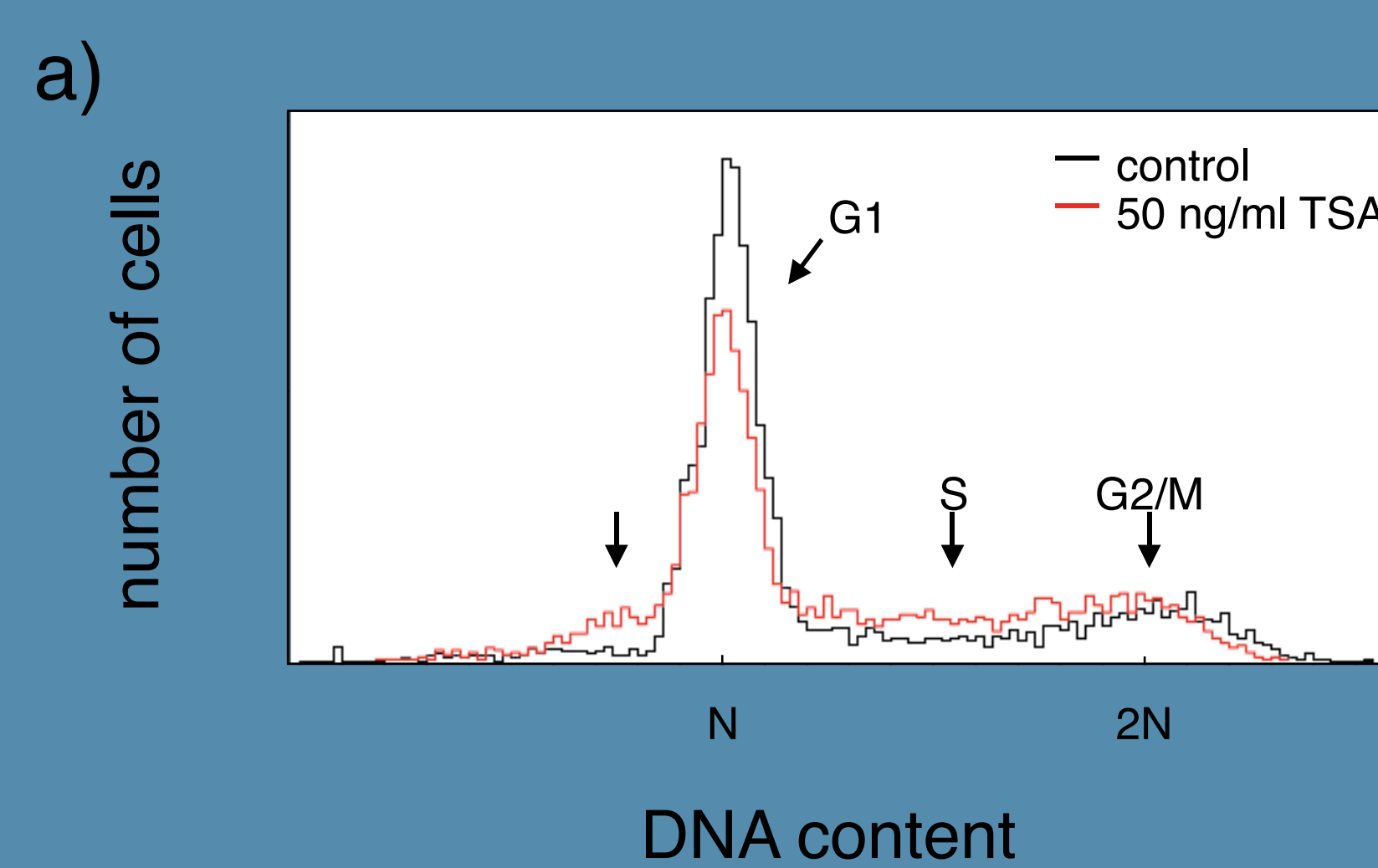
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It has been shown for a number of genes that the reversible (de)acetylation of the N-terminal histone tails by specific histone acetylases (HAT) and deacetylases (HDAC) regulates gene expression. Acetylation is thought to decrease the affinity of histones to DNA as well as the interactions between nucleosomes within the chromatin fiber. A number of HDACs can be inhibited by Trichostatin A (TSA) to induce a state of global chromatin acetylation. We have started to study the effect of TSA on the in vivo chromatin organization and on cell cycle progression.



HeLa cells which express a fusion protein of histone H2A and a yellow fluorescent protein (YFP) were analyzed. H2A-YFP is incorporated into chromatin resulting in an in vivo fluorescence label. Cells are viable and divide normally (left, confocal image of metaphase cells). Upon treatment with 100 ng/ml TSA for 24h the chromatin distribution within the nucleus changes (middle, confocal image of interphase cell nuclei) and the fluorescence increases in a concentration dependent manner (right).



The effect of TSA on the cell cycle was examined by flow cytometry (FACS). Cells were stained with DAPI to determine the DNA content which correlates with the cell cycle stage. Upon treatment with TSA for 24 h the number of cells in S phase increased (a). The extend of the S block correlated with TSA concentration (b). Further experiments showed that the effect of TSA on the cell cycle was dependent on both the incubation time and the TSA concentration: After 12 h we detected mainly a G1 arrest at 50 ng/ml and a G2/M arrest at 100 ng/ml TSA (c). In contrast after 24 h a strong S block was observed that has not been reported in the literature so far (d).

In the FACS analysis the induction of apoptosis by TSA was investigated. The percentage of apoptotic cells increased in a concentration dependent manner after incubation for 24 h (e). A fit of the data to a model in which TSA binds reversibly to a single class of binding sites showed a systematic error (red curve in e). In contrast, a good fit of the data was obtained by including a second type of binding sites with different affinity (black sigmoidal curve in e). This result supports the view that the various types of HDACs show a different response with respect to a concentration dependent TSA inhibition.

## Conclusions and perspectives

First experiments with cells expressing H2A-YFP indicate that TSA induces changes in the chromatin organization of the interphase nucleus and leads to an increase of the YFP fluorescence.

The FACS analysis revealed that the effect of TSA on cell cycle progression was strongly dependent on TSA concentration and incubation time. In addition to the G1 and G2/M arrest described previously in the literature we detected a strong block in S phase under certain conditions.

TSA caused apoptosis in a concentration dependent manner. The data suggest that various HDACs and/or multiple pathways are involved in the induction of apoptosis.

The molecular origin of the TSA induced S block and apoptosis is currently examined by expression profiling.



# TSA has a Complex Effect on Cell Cycle Progression

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## *Abstract*

It has been shown for a number of genes that the reversible (de)acetylation of the N-terminal histone tails by specific histone acetylases (HAT) and deacetylases (HDAC) regulates gene expression. Acetylation is thought to decrease the affinity of histones to DNA as well as the interactions between nucleosomes within the chromatin fibre. A number of HDACs can be inhibited by Trichostatin A (TSA) to induce a state of global chromatin acetylation. We have started to study the effect of TSA on the in vivo chromatin organization and on cell cycle progression. First experiments with cells expressing H2A-YFP indicate that TSA induces changes in the chromatin organization of the interphase nucleus and leads to an increase of the YFP fluorescence. The FACS analysis revealed that the effect of TSA on cell cycle progression was strongly dependent on TSA concentration and incubation time. In addition to the G1 and G2/M arrest described previously in the literature we detected a strong block in S phase under certain conditions. TSA caused apoptosis in a concentration dependent manner. The data suggest that various HDACs and/or multiple pathways are involved in the induction of apoptosis. The molecular origin of the TSA induced S block and apoptosis is currently examined by expression profiling.

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## Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

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